

Chromosome numbers and mode of reproduction in *Picris hieracioides* s.l. (Asteraceae), with notes on some other *Picris* taxa

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Chromosome numbers for 88 plants of *Picris hieracioides* s.l. originating from 32 localities from Austria, Andorra, Croatia, France, Germany, Hungary, Italy, Slovakia and Spain are reported. All analysed plants were diploid, with $2n = 2x = 10$. In addition to *P. hieracioides* s.l., we also found diploidy ($2n = 2x = 10$) in *P. nuristanica* from Kirgizia and *P. japonica* from Japan. Our data confirmed previously published results in the literature. We also studied the mode of reproduction in all three taxa. *Picris nuristanica* and *P. japonica* s.l. were able to produce some seeds by selfing, and this is the first record of autogamy in this genus. In *P. hieracioides* s.l. only strict allogamy was found, demonstrating the presence of a self-incompatibility system in this species. Because apomixis, namely apospory, has previously been reported in *P. hieracioides*, castration experiments were performed in order to confirm or reject this report. However, no evidence of apomictic seed formation was found within this species. In the light of our results, *P. hieracioides* should be considered a strictly sexual taxon.

The genus *Picris* (tribe Lactuceae) comprises approximately 40 (Feinbrun-Dothan 1978) to 50 (Chaudhary 2000) species, depending on the taxonomic definitions applied. Representatives of the genus are wide-spread in Eurasia and their putative evolutionary centres are usually placed in the Mediterranean and Asia Minor (Lack 1974). Moreover, three species occur in eastern and tropical Africa (Lack 1979), while about ten autochthonous species are recorded from Australia and New Zealand (Holzapfel and Lack 1993, Holzapfel 1994). The genus *Picris* includes several taxonomically complicated groups of closely related species, morphologically very similar to each other, and several highly polymorphic species with large infraspecific variation.

Picris hieracioides s.l. is a typical and the best known example of such a polymorphic group. It occurs natively in Europe and extends to Asia where it is vicarianced by the morphologically very similar aggregate *Picris japonica* s.l. Significant morphological variation of *P. hieracioides* has during the last two centuries led to the description of a huge number of infraspecific taxa, mostly from the European area. However, no study using modern taxonomical tools has addressed the morphological and genetic variation of this group so far. Only diploid levels ($2n = 2x = 10$) have previously been reported for *P. hieracioides* s.l. (Table 1). However, with respect to the range of the taxon, the knowledge of its karyological pattern is rather poor. Based on our field observations and partial morphometric study, it is possible to distinguish two main morphological groups (Slovák and Marhold 2007, Slovák unpubl.). While the first

group includes morphological types that inhabit ruderal biotopes mainly in the lowlands of southern and central Europe, the second group corresponds to the morphologically variable populations confined to the mountain meadows of the high European mountain systems (Alps, Apennines, Carpathians and Pyrenees).

In this paper we prefer using a broad species concept (*P. hieracioides* s.l.) for all potential intraspecific entities, because our study on *P. hieracioides* group is still ongoing, and many taxonomic and nomenclatural problems are not yet satisfactory resolved. However, it is obvious that most of the published chromosome records refer to the former 'lower altitude morphological type' (Table 1).

Unusually for diploid taxa (cf. Asker and Jerling 1992), apospory (development of an unreduced embryo sac from somatic cells in the nucellus) was reported by Bergman in the diploid *P. hieracioides* (Bergman 1935). However, Bergman himself (1935) was aware that aposporic embryo-sac formation was observed in one plant only. Moreover, he did not find apospory in any of the other *Picris* taxa involved in his experiments, namely *P. blanchiana* Boiss., *P. echioides* L., *P. pauciflora* Guss. ex Ten. and *P. sprengeriana* Poir. Nevertheless, this record was quickly adopted and spread by several authors (Battaglia 1951, Clapham et al. 1958, Lack 1974, Czapik 1996, Štěpánek 2004). Gustafsson (1947a, 1947b) critically revised this report of apospory in *P. hieracioides* and pointed out that "agamospermy is no more than a tendency". Gustafsson himself did not consider apomictic reproduction to be present in this species, because

Table 1. Previously published chromosome numbers of *Picris hieracioides* s.l., *P. japonica* s.l., *P. nuristanica* Bornm. as well as of taxa most probably belonging to the *P. japonica* group: *P. davurica* Fisch. and *P. kamschatica* Ledeb. Records are listed in the following order: name of taxon under which the chromosome record was published (in alphabetical order), chromosome number (CN), country (in alphabetical order) and source (author and year of publication).

Published as	CN	Country and source
<i>P. davurica</i> Fisch.	2n = 10	China: Wang et al. 2003.
<i>P. hieracioides</i> L.	2n = 10	ASIA. Armenia: Nazarova 1984. China: Ge et al. 1987. India: Mehra et al. 1965. Kazakhstan: Rudyka 1990. Pakistan: Razaq et al. 1994. Country not given: Ishikawa 1911, Ishikawa 1916. EUROPE. Austria: Lack 1974, Kiehn et al. 1991, Dobeš et al. 1996, Dobeš et al. 1997. Belarus: Dimitrieva 1987. Bulgaria: Kuzmanov and Georgieva 1977. France: Natarajan 1981, 1988. Italy: Löve and Löve 1982, Rotti-Michelozzi and Serrat, 1980. The Netherlands: Van den Brand et al. 1979. Poland: Skalinska et al. 1978. Portugal: Fernandez and Queirós 1971. Russia: Volkova and Boiko 1986, Krasnikov and Lomonosova 1990. Serbia: Van Loon and Kieft 1980. Slovakia: Feráková 1971, Májovský et al. 1974, 1978, Králik and Kothajová 2000, Šimková 2007. Spain: Díez et al. 1984, Devesa and Viera 1987. Sweden: Bergman 1935, Lövkvist and Hultgård 1999. Ukraine: Kliphius 1977. United Kingdom: Morton 1977
<i>P. hieracioides</i> var. <i>glabrescens</i> (Regel) Ohwi	2n = 10	Japan: Nishikawa, 1979
<i>P. hieracioides</i> subsp. <i>grandiflora</i> (Ten.) Arcang.	2n = 10	Germany: Lippert and Heubl 1989, Albers and Pröbsting 1998
<i>P. hieracioides</i> subsp. <i>japonica</i> (Thumb.) Hand.-Maz.	2n = 10	China: Hong and Zhang 1990, Zhang 1998
<i>P. hieracioides</i> subsp. <i>jessoensis</i> (Tatew.) Ohwi	2n = 10	Japan: Nishikawa 1979
<i>P. hieracioides</i> subsp. <i>longifolia</i> (Boiss. and Reut.) P. D. Sell	2n = 10	Spain: Devesa 1983
<i>P. hieracioides</i> subsp. <i>morrisonensis</i> (Hay.) Kitam	2n = 10	Taiwan: Peng and Hsu 1977, 1978
<i>P. hieracioides</i> subsp. <i>ohwiana</i> (Kitam.) Kitam.	2n = 10	Taiwan: Peng and Hsu 1977, 1978
<i>P. hieracioides</i> subsp. <i>spinulosa</i> (Bertol.) Arcang.	2n = 10	Greece: Strid and Franzen 1981
<i>P. japonica</i> Thumb.	2n = 10, 20	Russia: Stepanov 1994, Rostovtseva 1979, Sokolovskaya et al. 1985. China: Zhang 1994
<i>P. kamschatica</i> Ledeb.	2n = 10	Russia: Volkova and Boiko 1986; Sokolovskaya et al. 1985
<i>P. nuristanica</i> Bornm.	2n = 10	Afghanistan: Podlech and Dieterle 1969

despite some observed irregularities in megasporophyte formation, further parthenogenetic development of the embryo sac was completely missing.

The main aims of our study were to: 1) evaluate the karyological variation in *P. hieracioides* s.l. and two closely related Asian taxa *P. japonica* s.l. and *P. nuristanica* Bornm., 2) clarify the mode of reproduction in the above-mentioned taxa, particularly with respect to published records on apomixis in *P. hieracioides* s.l.

Material and methods

Plants

Altogether 88 individuals from 32 populations of *P. hieracioides* and three individuals of each of *Picris japonica* s.l. and *P. nuristanica* were studied for chromosome numbers (Table 2). Seventy individuals of *P. hieracioides* s.l. from Europe and nine plants belonging to the two latter taxa were cultivated during the period 2004–2006 for determination of their breeding system. Seeds were sampled in their natural habitats (Table 2) and transferred to the Institute of Botany of the Slovak Academy of Sciences in Bratislava. Seeds of *P. nuristanica* were obtained from the Botanical garden in Osnabrück (Germany). All samples were stored at room temperature. Seeds were germinated in plastic pots in a moderately heated greenhouse and cultivated for several weeks under humid, frost-free conditions. In late spring, the seedlings were replanted to bigger plastic pots and cultivated on an experimental field. Most of the plants flowered in the second year of cultivation.

Chromosome counts

Chromosome numbers were counted from mitotic metaphases in root tips of the cultivated plants and/or germinated seeds. Seeds were germinated in petri dishes on wet filter paper at laboratory temperature (20–23°C). The root tips were pre-treated with 0.002 M hydroxyquinoline for at least six hours (best overnight) at 4°C, then fixed in a fresh mixture of concentrated ethanol (96%) and acetic acid (100%), in ratio 1:1, up to one h. The fixed material was stored in 70% ethanol at 4°C. Washed root tips (with distilled water for 10 min) were transferred into the hydrolyzation mixture of concentrated hydrochloric acid (35%) and ethanol (96%), in ratio 1:1, for 3–5 min (depending on the root size) at laboratory temperature and subsequently washed with water for 10 min again. Squashes were made under cellophane square (Murín 1960) and stained in a 10% solution of Giemsa (Riedel-deHaën Co. Germany) in 0.2 M Sörensen phosphate buffer for one h. Slides were observed using a light microscope Olympus BX 61 with oil immersion objective. Photos were taken with a digital compact camera (Olympus SP 350). Voucher specimens and selected permanent slides of analysed plants were deposited at the Institute of Botany of the Slovak Academy of Sciences in Bratislava (SAV).

Mode of reproduction

We performed three experiments to test for breeding system (allogamy, autogamy and apomixis) in *Picris hieracioides* s.l. First, we left a portion of the capitula of each studied plant for open pollination. Second, several inflorescences of each studied plant were isolated in cotton bags before and during anthesis to prevent cross-pollination (test for autogamy). To confirm or exclude an aposporic formation of seeds in *P. hieracioides* s.l. we finally performed emasculation (cf. Gadella 1984, 1987) by cutting-off the upper part of the flower head just before anthesis using a razor blade. Because styles are missing in emasculated capitulas, the pollination and fertilisation of ovules is impossible. If the flower heads produce seeds after emasculation, they are considered to be of asexual (apomictic) origin.

Results and discussion

Chromosome numbers

Exclusively a diploid chromosome number, $2n = 2x = 10$ (Fig. 1), was found in all individuals of the three studied species; *P. hieracioides* (88 plants), *P. nuristanica* (3 plants) and *P. japonica* (3 plants) (Table 2, Fig. 2). Our results are in congruence with previous karyological studies with exception of the latter species (Table 1, Fig. 2). Only one polyploid count ($2n = 4x = 20$) has been reported for a plant belonging to the *P. japonica* group, from Krasnoyarsk – Siberia (Stepanov 1994). However, all other published chromosome counts for *P. japonica* s.l. are on the diploid level; which is in accordance with our results (Table 1, 2). This result may be biased by the limited amount of available material. With regards to the previously published tetraploid cytotype from Russia (Stepanov 1994), a further larger-scale study should be done to analyse the general pattern of karyological variation of *P. japonica* s.l.

Although the diploid level ($2n = 10$) is considered prevailing in almost all taxa of the genus *Picris* (Lack 1974, 1979, Sell 1975, 1976, Holzapfel and Lack 1993, Holzapfel 1994, Smalla 2000), several polyploid counts are known as well. Besides the diploid populations, tetraploid ($2n = 4x = 20$) and hexaploid ones ($2n = 6x = 30$) of *P. hispanica* (Willd.) P. D. Sell were discovered in the Iberian Peninsula, and in the mountains of northern Africa (Humphries et al. 1978, Galland 1988, Oberprieler and Vogt 1993). For *P. aculeata* Vahl., tetraploid individuals originating in Algeria were observed by Guittonneau (1978), in addition to the diploid cytotype (Brullo et al. 1977).

Mode of reproduction

Unlike other related genera from the tribus *Lactuceae*, little attention has been given to the mode of reproduction of the genus *Picris* so far. Our work represents the first broader study focused on the mating system within *P. hieracioides*. Emasculation experiments of 62 *P. hieracioides* plants, five individuals of *P. nuristanica* and three plants of *P. japonica* revealed that none of them were able to form ripe achenes (Table 2). Thus, our data clearly exclude the presence of

Table 2. List of localities of *Picris hieracioides* s.l., *P. japonica* s.l. and *P. nuristanica* Bornm. investigated in the present study. Abbreviations used: N – number of karyologically analysed individuals per population, only diploid level ($2n=2x=10$) has been detected in all of them; IE – results of isolation experiments, number of plants with ripen seeds/number of analyzed individuals; EM – results of emasculatation experiments, number of plants with ripen achenes/total number of analyzed individuals. Localities are arranged from west to east. Collector abbreviations: FC – Fabio Conti (Barisciano), GD – Gianjanantonio Domina (Palermo), IH – Iva Hodálová (Bratislava), JL – Judita Lihová (Bratislava), MK – Martin Kolník (Bratislava), KM – Karol Marhold (Bratislava), NP – Nicodemo Pasalaqua (Cosenza), PR – Peter Repa (Bratislava), MP – Marián Perný (Bratislava), MS – Marek Slovák (Bratislava). Coordinates and altitudes given in square brackets were estimated from the maps.

Locality	N	IE	EM
<i>Picris hieracioides</i> s.l.			
Spain, Sierra Nevada Mts., prov. Granada, village of Capileira, 1470–1606 m a.s.l., 36° 57.703'N, 03° 21.457'W, coll.: MS and JL	6	0/3	0/1
Spain, Pyrenees Mts., prov. Huesca, village of Torla-Bujaruelo, 1460–1510 m a.s.l., 42° 39.766'N, 00° 06.509'W, coll.: MS and JL	3	–	–
Spain, Pyrenees Mts., prov. Huesca, village of Baños di Panticosa, 1576 m a.s.l., 42° 45.029'N, 00° 14.557'W, coll.: MS and JL	–	0/1	0/1
Spain, Pyrenees Mts., prov. Lérida, town of Vielha, 1230 m a.s.l., 42° 41.190'N, 00° 47.241'W, coll.: MS and JL	3	0/3	0/4
Spain, Pyrenees Mts., prov. Lérida, village of Espot, 1587 m a.s.l., 42° 33.547'N, 01° 05.416'E, coll.: MS and JL	3	0/1	0/1
Spain, Montseny Mts., between villages of Montseny and El Brull, 740 m a.s.l., 41° 46.702'N, 02° 23.780'E, coll.: MS and JL	1	0/2	0/1
Andorra, Pyrenees Mts., the town of Soldeu, 1900 m a.s.l., 42° 34.170'N, 01° 40.691'E, coll.: MS and JL	1	0/2	0/2
France, Pyrenees Mts., Languedoc-Roussillon, village of Estavar, 1640 m a.s.l., 42° 29.915'N, 02° 00.815'E, coll.: MS and JL	3	0/1	0/1
France, dépt. Hautes-Alpes, mountain pass Col du Lautaret, 2067 m a.s.l., 45° 02.092'N, 06° 24.239'E, coll.: MS	2	0/3	0/3
France, dépt. Savoie, villages of Valloire and Hameau la Rivine, 1556 m a.s.l., 45° 10.000'N, 06° 25.600'E, coll.: MS	2	0/1	0/1
France, dépt. Hautes-Alpes, town of Briançon, 1308 m a.s.l., 44° 55.184'N, 06° 37.261'E, coll.: MS	2	0/2	0/1
France, dépt. Doubs, village of Les Fins, 911 m a.s.l., 47° 05.181'N, 06° 38.356'E, coll.: MS and PR	5	–	–
Italy, prov. Piemonte, village of Limonneto, 1600 m a.s.l., 44° 12.000'N, 07° 34.000'E, coll.: MS, JL and KM	–	0/6	0/5
Italy, prov. Piemonte, village of Breia, 799 m a.s.l., 45° 45.897'N, 08° 18.300'E, coll.: MS and PR	2	–	–
Italy, city of Roma, ruderal area near the petrol station E of the city of Roma, 620 m a.s.l., 42° 03.944'N, 13° 02.172'E, coll.: MS and PR	2	–	–
Italy, Sicily, prov. Palermo, village of Sagana, 637 m a.s.l., 38° 04.756'N, 13° 12.620'E, coll.: GD and MS	2	–	–
Italy, Sicily, prov. Palermo, city of Palermo, Monte Cuccio Mt., 611 m a.s.l., 38° 06.938'N, 13° 14.543'E, coll.: GD and MS	1	–	–
Italy, Sicily, prov. Palermo, national park Madoniae, Piano Zucchi, [1100 m a.s.l.], [37° 54.000'N, 13° 59.300'E], coll.: GD	2	0/11	0/11
Italy, prov. Calabria, Mula Mt., [1900 m a.s.l.], [39° 45.000'N, 16° 01.000'E], coll.: NP	3	–	–
Italy, prov. Abruzzi, town of L'Aquila, 421 m a.s.l., 42° 12.103'N, 13° 24.256'E, coll.: MS	–	0/1	0/1
Italy, prov. Abruzzi, town of Pescara, 6 m a.s.l., 42° 27.489'N, 14° 12.596'E, coll.: MS	–	0/1	0/1
Italy, prov. Basilicata, village of Muro Lucano, 750 m a.s.l., 40° 15.573'N, 15° 27.420'E, coll.: MS and PR	3	–	–
Italy, prov. Calabria, town of Cosenza, 225 m a.s.l., 39° 10.340'N, 16° 32.706'E, coll.: MS and PR	3	–	–
Germany, Bavarian Alps Mts., town of Grassach, 768 m a.s.l., 47° 28.217'N, 11° 07.114'E, coll.: MS and PR	2	–	–
Austria, Tirolian Alps Mts., town of Kitsbühel, 930 m a.s.l., 47° 28.200'N, 12° 23.807'E, coll.: MS and PR	3	–	–
Austria, Eastern Alps Mts., village of Annaberg, 519 m a.s.l., 47° 54.984'N, 15° 26.399'E, coll.: MS and JL	4	0/1	0/1
Austria, Eastern Alps Mts., Schneeberg Mt., 543 m a.s.l., 47° 44.225'N, 15° 44.086'E, coll.: MS and JL	–	0/1	0/1
Austria, Eastern Alps Mts., Ötscher Mt., [1000 m a.s.l.], [47° 520'N, 15° 800'E], coll.: MS and MK	1	0/4	0/4
Croatia, Zadar county, town of Pirovac, 24 m a.s.l., 43° 49.340'N, 15° 40.140'E, coll.: MS and PR	3	–	–
Hungary, Pannonian lowlands, village of Rétszilás, 106 m a.s.l., 46° 48.734'N, 18° 38.423'E, coll.: MS, IH and JL	3	–	–
Slovakia, Velká Fatra Mts., Fačkovské sedlo saddle, 1220 m a.s.l., 48° 58.660'N, 18° 37.063'E, coll.: MS	1	0/1	0/1
Slovakia, Nízke Tatry Mts., Demänovská dolina valley, 677 m a.s.l., 49° 02.192'N, 19° 34.611'E, coll.: MS and JL	4	0/5	0/5
Slovakia, Nízke Tatry Mts., Jánska dolina valley, 771 m a.s.l., 49° 03.480'N, 19° 40.505'E, coll.: MS and JL	5	0/1	0/1
Slovakia, Západné Tatry Mts., Mačie Diery, 900 m a.s.l., 49° 15.525'N, 19° 40.234'E, coll.: MS and JL	–	0/3	0/4
Slovakia, Cerová vrchovina hills, Hájnačka hill, 212 m a.s.l., 48° 14.956'N, 19° 57.939'E, coll.: MS and JL	3	0/3	0/3
Slovakia, Belianske Tatry Mts., Ždiarska dolina valley, 890 m a.s.l., 49° 16.099'N, 20° 14.991'E, coll.: MS and JL	3	–	–
Slovakia, Popradská kotlina basin, village of Liptovský Trnovec, 588 m a.s.l., 49° 06.926'N, 20° 20.932'E, coll.: MS and JL	5	0/1	0/1
Slovakia, Slovenský kras karst, Soroška pass, 544 m a.s.l., 48° 37.053'N, 20° 37.805'E, coll.: MS and JL	–	0/1	0/1
Romania, prov. Hunedoara, town of Deva, 220 m a.s.l., 45° 50.012'N, 22° 56.341'E, coll.: MS, IH, MP and MK	2	0/2	0/2
<i>Picris nuristanica</i> Bornm.			
Kirgizia, Tian-Schan, Fergana Bergkette (Fergana Kyrka Toosu), 2800 m a.s.l., 41° 15.450'N, 73° 37.280'E, coll.: unknown	3	3/5	0/5
<i>Picris japonica</i> s.l.			
Japan, Hokkaido, Atsuta-gun, Atsuta-mura, Morai, Morai-gawa river, 66 m a.s.l., 43° 21.006'N, 141° 29.422'E, coll.: KM	3	4/4	0/3

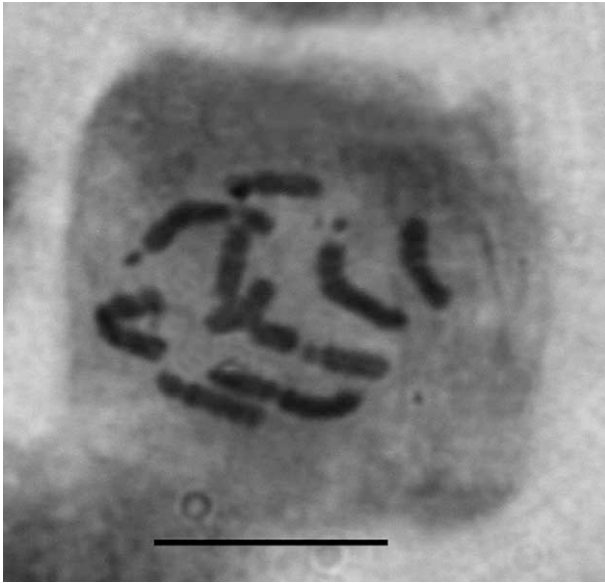


Fig. 1. Microphotograph of mitotic metaphase of *Picris hieracioides* s.l. (originating from Hungary, Pannonian lowlands, Rétság village), $2n = 2x = 10$. Scale bare = 10 μm .

apomictic reproduction in the studied taxa, including *P. hieracioides* s.l. Although Bergman (1935) recorded a tendency towards apomixis within *P. hieracioides*, neither he nor others have sufficiently resolved the question of reproduction modes in this species. His record on apospory in *P. hieracioides*, sometimes cited as a record of apomixis (or agamospermy), has been (uncritically) included in

several papers. However, already Gustafsson (1947a, 1947b) considered this record as an insufficient proof of apomixis. According to him, the extremely rare formation of an aposporic-like embryo sac in *P. hieracioides* is only an abnormality, and he clearly stated that this is just a tendency towards agamospermy. Moreover, for successful production of seeds via apomixis, two independent processes should be involved: (1) formation of an embryo sac with unreduced ploidy level (avoiding meiosis), and (2) parthenogenetic development of an unreduced embryo sac (Asker and Jerling 1992). Bergman never observed the latter phenomenon in *P. hieracioides* (Bergman 1935). Similar abnormalities in the embryo sac formation, namely early stages of apospory has been observed in other diploid taxa of the Asteraceae (Terziński et al. 1997, Yurukova-Grancharova et al. 2002, 2006). However, in none of these cases a fully developed embryo was observed. Thus, these records should be considered to document only a tendency towards apospory, but not as a proof of apomixis.

Isolation experiments excluded autogamy for *P. hieracioides* s.l. (68 plants), as no fully developed and mature fruits were produced. On the other hand, under open pollination (non-isolated inflorescences) we observed massive production of seeds during all three years of experiments.

Based on these results, *P. hieracioides* s.l. should be treated as a strictly allogamous taxon with a functional self-incompatibility system. Self-incompatibility has already been confirmed for some representatives from closely related genera of the subtribus *Hypochaeridinae*, namely in *Leontodon* (Izuzquiza and Nieto Feliner 1991, Ruiz de Clavijo 2001) and *Hypochaeris* (Parker 1975, Wells 1976).

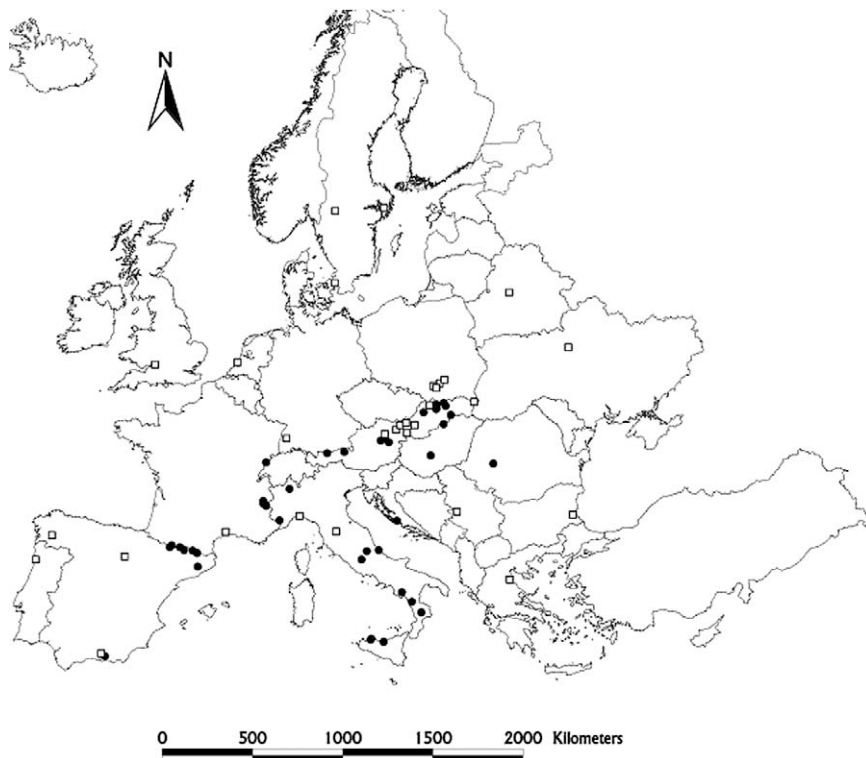


Fig. 2. Map of localities of previously published (empty squares) and new chromosome counts (solid circles) of *Picris hieracioides* s.l. from Europe.

Unlike in *P. hieracioides* s.l., isolated capitulas of *P. nuristanica* (three plants) and *P. japonica* (four plants) produced several ripe achenes (from 5 to 20 per capitula). Despite the fact that only a few seeds germinated, this is the first record of autogamy in the genus *Picris*. Because the plants produced many mature and well-developed fruits under the open pollination experiment, we consider both taxa to be partially self-incompatible. Different levels of self-compatibility and self-incompatibility have been observed in four species of *Hypochaeris* (Ortiz et al. 2006). The authors observed the full range from exclusively self-incompatible to almost self-compatible plants, and evaluated correlations between mating systems and floral and fruit parameters. In general, self-compatible species tend to possess a lower number of smaller flowers than self-incompatible ones do.

Due to the limited number of population samples of both the widely distributed Asian taxa, we cannot quantify differences in number and size of particular floral parts (ligules and involucral bracts). However, our observation of the cultivated plants suggests that *P. japonica*, and especially *P. nuristanica*, have a reduced number of floral parts that are conspicuously smaller than those of individuals in the *P. hieracioides* group.

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