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CHROMOSOME NUMBERS AND DNA PLOIDY LEVELS OF SELECTED SPECIES OF *HIERACIUM* S.STR. (*ASTERACEAE*)

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Abstract: Chromosome numbers and /or ploidy levels are reported for 44 species and subspecies of *Hieracium* s.str. from the following European countries: Andorra, Austria, Bulgaria, Czech Republic, France, Italy, Montenegro, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland and Ukraine. The chromosome numbers/DNA ploidy levels of *H. bocconei* (2n ~ 4x), *H. bupleuroides* subsp. *leviceps* (2n = 27), *H. caesioides* subsp. *caesioides* (2n = 27), *H. basifolium* (*H. caesium* agg., 2n = 36), *H. plumbeum* (*H. caesium* agg., 2n = 36), *H. glaucum* subsp. *nipholepium* (2n = 27), *H. gouanii* (2n = 18), *H. gymnocerinthe* (2n = 27), *H. ramondii* (2n = 27), *H. recoderi* (2n = 18), *H. stelligerum* (2n = 18), and *H. tomentosum* (2n = 18, 2n ~ 2x, 2n ~ 3x) were determined for the first time. New ploidy levels are reported for *H. cerinthoides* s.str. (2n = 27), *H. humile* (2n = 36), and *H. tommasinianum* (2n = 27).

Keywords: *Compositae*, Europe, Flow cytometry, Polyploidy

INTRODUCTION

Hieracium L. subgen. *Hieracium* (*Hieracium* s.str.) is one of largest and taxonomically most difficult groups of the *Asteraceae*. It comprises perennial herbs distributed in temperate regions of Europe, Asia, northernmost Mediterranean Africa, and North America (and introduced to several other regions, e.g. to New Zealand). *Hieracium* subgen. *Hieracium* forms an immense agamic complex with a base chromosome number of x = 9. Generally, polyploid taxa (triploids, tetraploids, and very rarely pentaploids) prevail in this subgenus (SCHUHWERK 1996, CHRTEK et al. 2004). Diploid species (or diploid cytotypes of otherwise polyploid species) are much less frequent and mostly confined to certain geographical areas. They have been mostly reported from SW Europe, the Eastern Carpathians and Balkan Peninsula (e.g. MERXMÜLLER 1975, CHRTEK 1996, SCHUHWERK & LIPPERT 1998, MRÁZ 2003b, VLADIMIROV 2003, VLADIMIROV & SZELĄG 2006, CASTRO et al. 2007, SZELĄG et

al. 2007). Recently, hexaploids (2n = 54) and heptaploids (2n = 63) were found in *Hieracium virosum* PALL. (PULKINA & TUPITSYNA 2000).

Until now all examined diploid species are sexual and self-incompatible (SI). However, the SI system can fail under the influence of heterospecific pollen on the stigma (mentor effect; MRÁZ 2003b, MRÁZ & TOMČÍKOVÁ 2004, MRÁZ & PAULE 2006). In contrast, triploids, tetraploids and pentaploids are agamospermous. Development of unreduced embryo sac follows the "*Antennaria* type" of diplospory, i.e., the female meiosis is fully omitted (e.g. NOGLER 1984). All hitherto studied plants showed autonomous endosperm development, i.e., the plants are not dependent on pollination. However, some irregularities have been reported showing remnants of sexual processes in the female meiosis and development of the female gametophyte (BERGMAN 1941, SKAWIŃSKA 1963).

Although chromosome counts for many *Hieracium* species have been published (see e.g. SCHUHWERK 1996), there are still considerable gaps in our knowledge of karyological diversity and its geographic pattern. As ploidy level is well correlated with the mode of reproduction (see above), detailed knowledge of chromosome numbers in particular species plays an important role in forming hypotheses about evolutionary potential of the species and evolutionary processes in the genus. In this paper we report chromosome numbers and/or ploidy levels for 111 accessions (91 accessions for chromosome counts, 19 for flow cytometry, and one for both of them) from 45 taxa from Europe. In most cases we adopted the taxonomic concept proposed by ZAHN (1921–1923). In *Hieracium alpinum* L., *H. caesium* FR., *H. nigrescens* WILLD., *H. rohacsense* KIT. and *H. waldsteinii* TAUSCH (all s.l.) the narrow species concept is accepted (microspecies grouped in an aggregate species/group). Species concept of the section *Cerinthoidea* follows MATEO (2005).

MATERIAL AND METHODS

Plants

Plants were collected between 1996 and 2006 from their natural habitats and transplanted in the experimental gardens in Průhonice near Praha and in Košice, except of *H. eriophorum* ST.-AMANS, which was grown from seeds collected in the field. Pot-grown plants were kept in either field conditions or in an unheated greenhouse. Voucher specimens are deposited in the Herbarium of the Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice (PRA, plants counted by JC and JZ) and Herbarium of the Institute of Biology and Ecology, P.J. Šafárik University, Košice (KO, plants counted or measured by flow cytometry by PM). The numbers given in parentheses after each locality refer to cultivation numbers.

Chromosome numbers

The studies were made on the root-tip meristems of pot-grown plants. Two different methods were used:

(i) actively growing roots were placed into pretreatment solution of saturated p-dichlorbenzene and kept for 3–4 hours at room temperature, then fixed in a mixture of ethanol and glacial acetic acid (3:1) overnight at 4 °C and stored in cold 70% ethanol at 4 °C

until used. The squash method and staining by lacto-propionic orceine were used (DYER 1963). (Method used by JC and JZ).

(ii) root tip cuttings were pre-treated with 0.5% solution of colchicine for 1.5–3 hours at room temperature, fixed as above, stored in 70% ethanol at 4 °C and hydrolyzed for 7–10 minutes in 1N HCl at 60 °C. The squash and smear method with cellophane replacing the glass covers followed MURÍN (1960). Giemsa solution in phosphate buffer was used as a stain. (Method used by PM).

DNA ploidy level estimation

Analysis of relative nuclear DNA content was performed with a FACSCalibur instrument (Becton Dickinson, USA) equipped with an argon-ion laser exciting at 488 nm in the Laboratory of Flow Cytometry, Institute of Biology and Ecology, P.J. Šafárik University, Košice. Sample preparations were carried out in a two-step procedure (OTTO 1990, DOLEŽEL & GÖHDE 1995). Approximately 1 cm^2 of leaf tissues of both the sample and the reference internal standard were chopped together for about 30 s in a Petri dish containing 1 ml of ice-cold Otto I buffer (0.1 M citric acid monohydrate + 1 ml 0.5% Tween 20 adjusted to 200 ml and filtered through a 42 µm filter). The filtration of chopped sample through 42 µm nylon mesh was followed by centrifugation at 150 g for 5 min. The supernatant was removed and 100 µl of fresh Otto I buffer was added. The nuclei in the pellet were resuspended and stored for 30 min at room temperature for incubation. For DNA staining 1 ml of Otto II buffer (0.4 M disodium hydrogenphosphate dodecahydrate) including propidium iodid (PI, final concentration 50 µg/ml), ribonuclease (Ribonuclease A R5000, Sigma, final concentration 50 µg/ml), and 2 µl mercaptoethanol was used. As an internal reference standard we used leaves of *Zea mays* CE-777 (2C = 5.43 pg). To estimate DNA ploidy level of analyzed plants, the exact position of peaks of previously counted di-, tri- and tetraploid *Hieracium* taxa to the peak of standard was measured. As diploid taxa we used *H. umbellatum* L. (cult. no. UMB12JP, chromosome number published in MRÁZ 2003b, and further ca. 10 plants of this species cf. TOMČÍKOVÁ 2006 and unpubl.) and *H. tomentosum* (this paper), as triploid *H. sabaudum* s.l. (cult. no. 872, CHRTEK et al. 2004) and tetraploid *H. carpathicum* BESS. (cult. no. 1150, CHRTEK et al. 2004). The ratios between nuclei fluorescence intensity of internal reference (*Zea mays*) and above mentioned *Hieracium* taxa were as follows: *H. umbellatum* (2x) 1.4–1.5, *H. tomentosum* (2x) 1.33–1.37, *H. sabaudum* s.l. (3x) 1.95–2.0 and *H. carpathicum* (4x) 2.5. Coefficient of variations (CV) of the peaks of internal standard adjusted at channel 100 ranged from 3.5% to 6.9% (with the most frequent value about 5.5%), and CV of peaks of measured samples varied between 3.0% and 5.6%. Only plants with clear peak positions corresponding to distinct ploidy level were included in the present study. DNA ploidy level estimations are given by the following formula "2n ~".

RESULTS AND DISCUSSION

***Hieracium alpinum* agg.**

***Hieracium halleri* VILL.** **2n ~ 3x**

Localities

1. Switzerland, cant. Valais, Alpes valaisannes: Col du Grand Saint Bernard, E of the col, 7°10′19″ E, 45°52′02″ N, 2560 m, 25 August 2005, leg. P. MRÁZ (H1823, analyzed by PM, peak ratio 2.05).
2. Switzerland, cant. Wallis, Walliser Alpen: Furkapass, ca. 200 m S of the col, exposed slopes with *Salix herbacea*, 2457 m, 8°24′49″ E, 46°34′22″ N, 24 August 2005, leg. P. MRÁZ (H1839, analyzed by PM, peak ratio 2.03).
3. Switzerland, cant. Graubünden, Albula Alpen: Davos, Flüelapass col, 2354 m, 9°57′54″ E, 46°44′36″ N, 22 August 2005, leg. P. MRÁZ (H1829, analyzed by PM, peak ratio 1.94).

Triploid chromosome counts were reported from the Western Carpathians (CHRTEK 1997b, MRÁZ 2001, ŠTORCHOVÁ et al. 2002) and by SCHUHWERK & LIPPERT (1999) from the Austrian Alps.

***Hieracium amplexicaule* L.** **2n = 36**

Locality

1. Italy, Trentino-Alto Adige, Gruppo dell′ Adamello: Passo del Tonale, Mt. Monticello, E slope, near the road, 2 km SE of the col (monument), 2180 m, 29 August 2005, leg. J. CHRTEK & P. MRÁZ (H1073/1b, H1073/2, counted by JC).

Hieracium amplexicaule* subsp. *amplexicaule **2n = 27**

Localities

1. Austria, Carinthia, Hohe Tauern, Goldberggruppe: Innerfragant, near the old (not marked) path to the Fraganter Hütte, ca. 1 km SW of the village, rocks above the brook, 1233 m, 28 July 2005, leg. J. CHRTEK & P. MRÁZ (H 1050/3a, H1050/4, counted by JC).
2. Austria, Carinthia, Hohe Tauern, Ankogelgruppe: Stappitz near Mallnitz, Seebachtal valley, Zelenigleiten, 4 km NE of the village, wet rocks, 1360 m, 13°10′45″ E, 47°01′12″ N, 29 July 2005, leg. J. CHRTEK (H1055/2, counted by JC).
3. Spain, Catalunya, prov. Gerona, Pirineos Mts.: Queralbs, along the road to Casa dels Plaus, near the brook of Torrent dels Plaus, 1480 m, 27 August 1996, leg. K. MARHOLD (H346, H347, counted by PM).
4. Spain, Catalunya, prov. Gerona, Pirineos Mts.: Queralbs, along the tourist path from Daió de Baix to Refugi Manelic, near the brook of Freser, 1750–1950 m, 24 August 1996, leg. K. MARHOLD (H352, counted by PM; H354, counted by JZ).

Three chromosome numbers, i.e., 2n = 18, 2n = 27 and 2n = 36 have been reported in this collective species. Triploids (2n = 27) referred to subsp. *amplexicaule* were found in the Pyrenees (SCHUHWERK & LIPPERT 1998) and in the Sierra de Baza Mts. in southern Spain (CUETO ROMERO & BLANCA LÓPEZ 1986), triploid counts without an exact identification of subspecies were reported from the Alps (GADELLA & KLIPHUIS 1970), Morocco (cf. subsp. *olivicolor* JAHAND. et ZAHN; VOGT & OBERPRIELER 1994), Spain and Balearic Islands

(CASTRO et al. 2007). Chromosome number 2n = 27 was also found in plants of subsp. *berardianum* (ARV.-TOUV.) ZAHN from the Austrian Alps (SCHUHWERK & LIPPERT 1999) and of subsp. *speluncarum* (ARV.-TOUV.) ZAHN from the Serra do Geres Mts. in Portugal (FERNANDES & QUIERÓS 1971). Tetraploids (2n = 36) of *H. amplexicaule* were reported by GENTCHEFF & GUSTAFSSON (1940) and QUÉZEL (1957). The same chromosome number has been found in plants referred to *H. pulmonarioides* VILL. from British Isles (MILLS & STACE 1974) and from botanical gardens (ROSENBERG 1927, GENTCHEFF 1937). Recently, CASTRO et al. (2007) revealed for the first time a diploid cytotype (2n = 18) in plants from Spain.

***Hieracium bocconei* GRISEB.** **2n ~ 4x**

Locality

1. Italy, Alto Adige, Deferegger Alpen: Passo Stalle (Staller Sattel), S slopes, 1954 m, 12°11′46″ E, 46°53′24″ N, 30 July 2005, leg. P. MRÁZ & J. CHRTEK (H1816, analyzed by PM, peak ratio 2.41).

To our best knowledge this is the first record on ploidy level for this species.

Hieracium bupleuroides* C.C. GMEL. subsp. *bupleuroides **2n = 27, 2n ~ 3x**

Localities

1. Austria, Vorarlberg, Allgäuer Alpen: Baad, between Starzel Joch (1867 m) and Hochstarzel (1974 m), 20 July 2002, leg. P. MRÁZ (H1235, counted by PM).
2. Slovakia, distr. Ilava, Biele Karpaty Mts.: Vršatské Podhradie, castle ruin of Vršatec, 770 m, 15 June 2005, leg. J. CHRTEK (H1033/2, H1033/3, 2n = 27, counted by JC).
3. Slovakia, distr. Tvrdošín, Chočské vrchy Mts.: Kvačianska dolina valley, upper part, 2 km SW of the church in the village of Huty, limestone rocks, 765 m, 19°32′58″ E, 49°12′16″ N, 17 August 2006, leg. J. CHRTEK & S. CHRTKOVÁ (H1209/2, 2n = 27, counted by JC).
4. Slovakia, distr. Poprad, Slovenský raj region: Stratená, calcareous rocks and small screes above the road near the upper end of the road tunnel, ca. 840 m, 9 July 2005, leg. P. MRÁZ (H1755, 2n ~ 3x, analyzed by PM, peak ratio 2.0).
5. Slovakia, distr. Gelnica, Volovské vrchy Mts.: Kojšov, Turniská in the massif of Murovaná skala, 1 July 2005, leg. P. MRÁZ & V. MRÁZOVÁ (H1752, 2n = 27, counted by PM).

***Hieracium bupleuroides* subsp. *leviceps* NÄGELI & A. PETER** **2n = 27**

Locality

1. Austria, Oberösterreich, Dachstein massif: Vorderer Gosausee mountain lake, rocks on NW bank, 6 km SSW of the village of Gosau, 940 m, 13°29′55″ E, 47°31′53″ N, 13 August 2005, leg. J. CHRTEK, det. F. SCHUHWERK (H1063/2, counted by JC).

***Hieracium bupleuroides* subsp. *tatrae* (GRISEB.) NÄGELI & A. PETER** **2n = 27, 2n ~ 3x**

Localities

1. Slovakia, distr. Poprad, Slovenský raj region: Vernár, S part of the village, limestone slopes above the road, 780 m, 20°16′07″ E, 48°54′50″ N, 5 August 2005, leg. J. CHRTEK & K. CHRTKOVÁ (H1062/1, 2n = 27, counted by JC).

2. Slovakia, distr. Poprad, Slovenský raj region: Stratená, calcareous rocks and small screes above the road near the upper end of the road tunnel, ca. 840 m, 9 July 2005, leg. P. MRÁZ (H1754, 2n ~ 3x, analyzed by PM, peak ratio 1.95).

Triploids seem to prevail in this rather variable taxon. CHRTEK et al. (2004) reported 2n = 27 for plants identified as subsp. *gmelinianum* (= subsp. *bupleuroides*) and subsp. *tatrae*. The same number was found in *H. bupleuroides* from the Slovenský kras region (southern Slovakia) by Murín and Uhríková (in MÁJOVSKÝ 1970). Further triploid counts come from the Bavarian Alps (SCHUHWERK & LIPPERT 1999), the Austrian Alps (POLATSCHEK 1966, SCHUHWERK & LIPPERT 1999 – plant referred to subsp. *bupleuroides*) and from northern Bavaria (SCHUHWERK & LIPPERT 1999). CHRISTOFF & POPOFF (1933) also published the same chromosome number, but the locality was not given. A tetraploid chromosome number was given for plants from Montenegro (NIKETIĆ et al. 2003, 2006; *H. bupleuroides* subsp. *pseudoschenkii* ROHLENA & ZAHN), Slovakia (CHRTEK et al. 2004), and southern Poland (SZELĄG & VLADIMIROV 2005).

Hieracium caesioides* ARV.-TOUV. subsp. *caesioides **2n = 27**

Localities

1. France, dépt. Alpes maritimes, valley of Roya: Tende, along the old road to the Col de Tende, ca. 0.5 km above the tunnel, 6 km NNW of the village, 1331 m, 07°33′57″ E, 44°08′19″ N, 28 August 2005, leg. J. CHRTEK & P. MRÁZ (H1067/4, H1067/5, H1067/6, H1067/10, counted by JC and JZ).
2. France, dépt. Alpes maritimes, valley of Roya: Tende, Mt. Cime de Salante, S slopes near the marked path, 8 km NW of the village, 2080 m, 28 August 2005, leg. J. CHRTEK & P. MRÁZ (H1068/2, counted by JC).

This is the first chromosome number record for *H. caesioides*.

***Hieracium caesium* agg.**

***Hieracium basifolium* (FR. ex ALMQ.) LÖNNR.** **2n = 36**

(*H. caesium* subsp. *basifolium* sensu ZAHN)

Locality

1. Sweden, prov. Gästrikland, par. Hille: dry road/forest margin at Oslättfors, ca. 15 km NW of Gävle, July 2006, leg. et det. T. TYLER (H1227/2, counted by JC).

***Hieracium plumbeum* FR.** **2n = 36**

(*H. caesium* subsp. *caesium* s. str. sensu ZAHN)

Locality

1. Sweden, prov. Gotland, par. Hall: open limestone scree by the sea 1.3 km SE of Hallshuk (close to the NW point of the island of Gotland ca. 40 km NNE of Visby), July 2006, leg. et det. T. TYLER & A. SENNIKOV (H1231/3, counted by JC).

The presented numbers are the first for the respective taxa. Chromosome number 2n = 36 has been reported by SCHUHWERK & LIPPERT (1999) for subsp. *caesium* from Bavaria (Germany) and subsp. *carnosum* (WIESB. ex DICHTL) ZAHN from Austria.

***Hieracium cerinthoides* L. s.str.** **2n = 27**

(*H. cerinthoides* subsp. *cerinthoides*)

Locality

1. Spain, Catalunya, prov. Lérida: Os de Civís, 1 km WSW of the village, margin of a pasture, 1720 m, 21 July 2006, leg. J. CHRTEK, G. MATEO & J. A. ROSSELLÓ, det. G. MATEO (H1176/1, counted by JC).

DELAY (1969) found 2n = 18 in *Hieracium cerinthoides*. However, taxonomic identity of the counted plants is not clear; they might belong to another taxon within the *H. cerinthoides* species group.

***Hieracium cordifolium* LAPEYR. s.str.** **2n = 18**

Locality

1. Andorra, Pirineos Mts.: near Bixessarri (NW of Sant Julia de Loria), valley of Torrent dels Llimois, rocks and margins of a path ca. 100 m from the street, 1.5 km NW of the village, 1305 m, 21 July 2006, leg. J. CHRTEK, G. MATEO & J. A. ROSSELLÓ, det. G. MATEO (H1177/2, counted by JC).

CASTRO et al. (2007) determined diploid and triploid cytotypes in this species. Previously, SCHUHWERK & LIPPERT (1998) reported 2n = 18 for *H. cordifolium* subsp. *neocerinthe* (FR.) ZAHN and 2n = 27 for *H. c.* subsp. *eriocerinthe* (FR.) ZAHN.

***Hieracium eriophorum* ST.-AMANS** **2n = 18**

Localities

1. France, dépt. Landes, Labenne: plage de Labenne Océan Sud, 27 September 2006, leg. E. FOREY (H1221, counted by JC).
2. France, dépt. Landes, Seignosse-le-Penon: plage de Estagnols Seignosse, 27 September 2006, leg. E. FOREY (H1222, counted by JC).
3. France, dépt. Landes, Vieux-Boucau-les-Bains: plage de Vieux-Boucau, 27 September 2006, leg. E. FOREY (H1223, counted by JC).

The same number was published by MERXMÜLLER (1975).

***Hieracium glaucum* ALL.** **2n ~ 3x**

Locality

1. Austria, Carinthia, the Karawanken Mts.: Bad Eisenkappel, limestone rocks and pine forest (alliance *Erico-Pinion*) near the road to Bad Vellach, 4.5 km SSW of the town, 658 m, 14°34′20″ E, 43°27′07″ N, 26 July 2005, leg. J. CHRTEK & P. MRÁZ (H1757, analyzed by PM, peak ratio 1.96).

***Hieracium glaucum* subsp. *nipholepium* NÄGELI & A. PETER** **2n = 27**

Localities

1. Slovenia, Primorska region, Julijske Alpe Mts.: Trenta valley, ca. 1 km W of the village of Podklanec, near the road Vršič – Bovec, 425 m, 9 November 2005, leg. B. VREŠ & J. CHRTEK (H1081/2, counted by JC).
2. Slovenia, Primorska region, Julijske Alpe Mts.: near the road from the col of Vršič to the Trenta valley, 1020 m, 9 November 2005, leg. B. VREŠ & J. CHRTEK (H1083/3, counted by JC).
3. Slovenia, Primorska region, Julijske Alpe Mts.: Zadnjica valley, along the marked path to the Luknja col, 4 km E of the village of Trenta, ca. 1100 m, 8 August 2005, leg. V. ZAVADIL (H1232/2, counted by JZ).

The same chromosome number, corresponding to the triploid level, has been recorded in plants of *H. glaucum* from Styria (Austria) (POLATSCHEK 1966), Italy (SCANNERINI 1971) and Germany (SCHUHWERK & LIPPERT 1999, plant corresponding to subsp. *isaricum* (NÄGELI ex J. HOFM.) NÄGELI & A. PETER).

***Hieracium gouanii* ARV.-TOUV.** **2n = 18**

(*H. cordifolium* subsp. *gouani* (ARV.-TOUV.) ZAHN)

Locality

1. Spain, Catalunya, prov. Gerona: rocks at the road between Ripoll and Ribes de Fresser, 24 July 2006, leg. J. CHRTEK (H1171/2, H1171/5, H1171/6, counted by JC).

The first karyological record for the species, treated by most authors at the subspecific level as *H. cordifolium* subsp. *gouanii* (ARV.-TOUV.) ZAHN. It clearly differs from *H. cordifolium* s.str. from the Central Pyrenees mainly by the taller stem and longer glabrescent leaves (MATEO 2005). It is confined to Catalunya and eastern part of the Pyrenees Mts. (NE Spain, S France).

***Hieracium gymnocerinthe* ARV.-TOUV. & GAUT.** **2n = 27**

Locality

1. Spain, Catalunya, prov. Lérida, distr. La Seu d´Urgell: Adraén, Serra del Cadí mountain ridge, NW slopes, 1 km SE of the village, 1600 m, road margin in a pine forest with dominating *Arctostaphylos uva-ursi*, 1°30′34″ E, 42°16′15″ N, 23 July 2006, leg. J. CHRTEK, G. MATEO & J. A. ROSSELLÓ, det. G. MATEO (H1172/4, counted by JC).

It differs from morphologically similar *H. ramondii* mainly in the indumentum of the phyllaries (numerous simple eglandular hairs in *H. ramondii* and numerous glandular hairs in *H. gymnocerinthe*); leaves are glabrous. It has been often treated as a subspecies of *H. cerinthoides*, which possesses (regarding the indumentum of the phyllaries) an intermediate position between *H. ramondii* and *H. gymnocerinthe*.

***Hieracium humile* JACQ.** **2n = 36**

Locality

1. Austria, Oberösterreich, Dachstein massif: Vorderer Gosausee mountain lake, rocks on NW bank, 6 km SSW of the village of Gosau, 940 m, 13°29′55″ E, 47°31′53″ N, 13 August 2005, leg. J. CHRTEK (H1064/2, H1064/3, counted by JC and JZ).

This is the first tetraploid (2n = 36) chromosome number ascertained for this species. SCHUHWERK & LIPPERT (1999) reported 2n = 27 for *H. humile* subsp. *pseudocottetii* (ZAHN) ZAHN from the Bavarian Alps (Karwendelgebirge).

***Hieracium intybaceum* ALL.** **2n = 18**

Locality

1. Italy, Trentino-Alto Adige, Gruppo dell´ Adamello: Passo del Tonale, valley of the Presena rivulet, glacial cirque above the Lago Presena mountain lake, 3 km SSE of the col (monument), 2270 m, 10°35′49″ E, 46°13′42″ N, 29 August 2005, leg. J. CHRTEK & P. MRÁZ (H1069/1, counted by JC).

Three ploidy levels, namely diploid, triploid and tetraploid have been reported in this species. The first published count (2n = 27) comes from a plant cultivated by C.H. Ostenfeld in the Botanical Garden in Copenhagen (ROSENBERG 1927). Later LARSEN (1954) reported the same chromosome number in plants from the Swiss Alps (Schynige Platte, Oberland Bernois). Nevertheless, the most common ploidy level seems to be the diploid one, although it remained unrevealed until the 1990s (DOBEŠ et al. 1997, FAVARGER 1997). Only two tetraploid populations have been published until now, namely from the Alpes valaisannes (FAVARGER 1997).

***Hieracium kittaniae* VLADIMIR.** **2n = 18**

Locality

1. Bulgaria, Central Rhodope Mts.: Trigrad gorge, limestone rocks near the natural entrance to Dyavolskoto garlo cave, September 2005, leg. P. IGNATOVA (H1228/2, counted by JC).

Hieracium kittanae is a distinct relict species restricted to crevices of limestone rock in the Central Rhodope Mts. in southern Bulgaria. Our counts coincide with previously published data (VLADIMIROV 2003).

***Hieracium lachenalii* SUTER** **2n = 27**

Localities

1. Czech Republic, Bohemia, distr. Rakovník: forest between the villages of Roztoky and Křivoklát, 350 m, 29 May 2006, leg. J. ZAHRADNÍČEK (H1150/4, counted by JC).
2. Czech Republic, Bohemia, distr. Praha-východ: Tehov, forest margin 1.8 km NE of the village, 430 m, 14°42′36″ E, 49°59′01″ N, 25 July 2002, leg. J. CHRTEK & H. CHAPMAN (H831/1, counted by JC).
3. Czech Republic, Moravia, distr. Znojmo: Lukov, forest 1.3 km SSW of the village, 410 m, 15°54′27″ E, 48°51′04″ N, June 2006, leg. J. ZAHRADNÍČEK (H1160/4, counted by JC).

Hieracium lachenalii represents a taxonomically very difficult entity. Our counts well match most of the previously published data (2n = 27; cf. CHRTEK et al. 2004). LAVRENKO & SERDITOV (1987) reported the tetraploid level (2n = 36) and ROSTOVTSEVA (1983; ut *H. tilingii* JUXIP) the hypertriploid one (2n = 28) .

Hieracium laevigatum* WILLD. subspecies group *laevigatum **2n = 27**

Localities

1. Czech Republic, Bohemia, distr. Rokycany: Strašice, N part of the village, margin of a forest, 550 m, 13°45′07″ E, 49°44′51″ N, 29 June 2005, leg. J. CHRTEK (H1031/5, H1031/11, counted by JC).
2. Czech Republic, Bohemia, distr. Hradec Králové: Hradec Králové, forest 1.2 km SE of the church in Nový Hradec Králové, 270 m, 15°52′16″ E, 50°10′17″ N, 1 July 2006, leg. J. CHRTEK & K. CHRTKOVÁ (H 1165/1, counted by JC).

Triploids (2n = 27) seem to be most frequent in this collective species. In addition, diploid counts (2n = 18) were published (e.g. SCHUHWERK 1996 and other standard reference manuals, e.g. Missouri Botanical Garden (2007)).

***Hieracium murorum* L.** **2n = 27**

Localities

1. Czech Republic, Bohemia, distr. Plzeň: Plzeň, village of Koterov, the street "V závrtku", 0.6 km SSW of the railway station "Plzeň-Koterov", slopes along the street, ca. 320 m, 13°25′07″ E, 49°43′02″ N, 12 August 2003, leg. M. KRÁL (H875/4, H875/6, H875/8, H875/16, counted by JC and JZ).
2. Czech Republic, Bohemia, distr. Beroun: Svatý Jan pod Skalou, oak-hornbeam forest 0.8 ENE of the village, 350 m, 14°08′30″ E, 49°58′15″ N, 28 May 2005, leg. J. CHRTEK (H1030/1, H1030/2, counted by JC).
3. Czech Republic, NW Bohemia, Doupovské hory Mts.: Stružná, 2 km N of the village, along the road, 650 m, 13°02′ E, 50°18′ N, 1 June 2006, leg. J. ZAHRADNÍČEK (H1152/2, counted by JC).
4. Czech Republic, Bohemia, distr. Domažlice: Kdyně, forest margin near a parking place 2.4 km NE of the town, 640 m, 13°04′08″ E, 49°24′35″ N, 12 June 2006; leg. J. CHRTEK (H1156/3, counted by JC).

The chromosome number of 2n = 27 is the most common one among the karyological data on *H. murorum*. Tetraploids (2n = 36) seem to be rare (e.g. SCHUHWERK 1996 and other standard reference manuals).

Hieracium naegelianum* PANČIĆ subsp. *naegelianum **2n = 27**

Locality

1. Montenegro, Durmitor Mts.: Mt. Veliki Međed, alpine grassland on limestone, 2050 m, 19°04′13″ E, 43°03′31″ N, 1 August 2006, leg. and det. Z. SZELĄG (H1208/1, H1208/3, counted by JC).

This chromosome number confirms the previous counts from the Durmitor Mts. (NIKETIĆ et al. 2003, 2006) and from other Balkan localities (MERXMÜLLER 1975, GRAU & ERBEN 1988, VLADIMIROV & SZELĄG 2001).

***Hieracium nigrescens* agg.**

***Hieracium decipientiforme* (WOŁ. & ZAHN) SCHLJAKOV** **2n = 36**

Locality

1. Ukraine, Oblast´ Zakarpatska, Marmaros´ki Al´py Mts.: Dilove, at the foot of Mt. Berlebashka, SE exposition, ca. 1600 m, 30 July 1996, leg. P. MRÁZ (H81, counted by PM).

CHRTEK (1997a) reported the same chromosome number for this morphologically very distinct species.

Hieracium olympicum* BOISS. subsp. *olympicum **2n = 27**

Locality

Bulgaria, Stara Planina Mts., Kaloferska Planina Mts.: Valley of Vidima River, 2 km NE of the Kaloferski Monastyr, eroded slope in the *Carpinus orientalis* forest, 870 m, 24°58´42″ E, 42°40´36″ N, 9 August 2006, leg. and det. Z. SZELĄG (H1206/3, counted by JC).

VLADIMIROV & SZELĄG (2001) reported a triploid chromosome number for this species.

Hieracium pannosum* BOISS. subsp. *pannosum **2n = 27**

Locality

1. Bulgaria, Stara Planina Mts., Trojanska Planina plateau: Mt. Kozja stena, grassy slope on limestone, 1570 m, 24°34′06″ E, 42°47′27″ N, 8 August 2006, leg. and det. Z. SZELĄG (H1205/1, counted by JZ).

This is the first triploid chromosome count from Bulgaria. Earlier, the tetraploid count for *H. pannosum* was reported from Greece (PAPANICOLAOU 1984) and Bulgaria (VLADIMIROV & SZELĄG 2001). The triploid chromosome number was reported from Greece (STRID & FRANZÉN 1981, PAPANICOLAOU 1984, SCHUHWERK & LIPPERT 1998).

***Hieracium petrovae* VLADIMIR. & SZELĄG** **2n = 18**

Locality

1. Bulgaria, Central Rhodope Mts.: Trigrad gorge, crevices of limestone rock (locus classicus), 750–800 m, 24°21′50″ E, 41°39′55″ N, 15 October 2005, leg. V. VLADIMIROV (H1229, counted by JC).

Hieracium petrovae is the only known diploid representative of the *H. pannosum* agg. It is closely related to a number of presumably descendent taxa in the *H. pannosum*, *H. pilosissimum* and *H. heldreichii* collective species. It is a calciphilous chasmophyte confined to several localities in the Central Rhodope Mts. in southern Bulgaria (VLADIMIROV

& SZELĄG 2006). Our count confirms the previously published data (VLADIMIROV & SZELĄG 2006).

***Hieracium piliferum* HOPPE** **2n ~ 3x**

Localities

1. Italy, Alpi lepontine, Spluga: Passo dello Spluga (Splügenpass), 2120 m, 9°1′54″ E, 46°30′21″ N, 23 August 2005, leg. P. MRÁZ (H1845, H1846, analyzed by PM, peak ratio 1.93).
2. Austria, Kärnten, Hohe Tauern: Kobnitz, Reißeck Hütte, S of the tunnel, 2225 m, 13°21′41″E, 46°55′59.8 ″N, 29 July 2005, leg. P. MRÁZ (H1802, analyzed by PM, peak ratio 1.98).

Hieracium piliferum* subspecies group *piliferum **2n = 27, 2n ~ 3x**

Localities

1. France, dépt. Hautes Alpes: SE of Col du Galibier, 2570 m, 6°24′09″ E, 45°04′59.8″ N, 4 July 2003, leg. P. MRÁZ (H1344, 2n = 27, counted by PM).
2. Switzerland, cant. Bern, Berner Alpen: Interlaken, ca. 1 km NE of Mt. Schillthorn, 2230 m, 7°52′35″ E, 46°34′05″ N, 19 July 2006, leg. P. MRÁZ (1 plant without no., 2n ~ 3x, analyzed by PM, peak ratio 1.93).

Hieracium piliferum* subspecies group *glanduliferum **2n ~ 3x, 4x**

Localities

1. Switzerland, cant. Valais, Alpes valaisannes: Col du Grand Saint Bernard, ca. 2600 m, 25 August 2005, leg. J. KOŠÚT (H1856, 2n ~ 3x, analyzed by PM, peak ratio 1.99).
2. France, dépt. Puy de Dome, Massif Central Mts.: Mt. Puy Sancy, very steep slope below the top exposed to E, 1875 m, 27 June 2006, leg. P. MRÁZ (H06/23, 2n ~ 4x , analyzed by PM, peak ratio 2.69).

The triploid level seems to prevail in *Hieracium piliferum*. It was first published by SCHOLTE (1977) from Switzerland. Later on, SCHUHWERK & LIPPERT (1999) found tetraploid plants in the same country. Isolated Western Carpathian populations of *H. piliferum* were also revealed to be tetraploid (MRÁZ 2003a). Tetraploid plants from the Massif Central were collected in the early stage (rosette leaves) and determined as *H. piliferum* s.l. only. However, according to local specialist F. Billy, only *H. glanduliferum* subsp. *glanduliferum* occurs at the locality (BILLY 1977).

***Hieracium porrifolium* L.** **2n = 18, 2n ~ 2x**

Localities

1. Italy, Trentino-Alto Adige: Villini dell´ Alpe, calcareous rocks along the road to Pianizza di Sopra (direction to Bolzano), 2 km E of the village, 30 August 2005, leg. J. CHRTEK & P. MRÁZ (H1075/2, H1075/5, 2n = 18, counted by JC).
2. Austria, Carinthia, the Karawanken Mts.: Bad Eisenkappel, limestone rocks and pine forests (alliance *Erico-Pinion*) near the road to Bad Vellach, 4.5 km SSW of the town,

658 m, 14°34′20″ E, 43°27′07″ N, 26 July 2005, leg. J. CHRTEK & P. MRÁZ (H1052/6, H1052/9, 2n = 18, counted by JC; H1756, 2n ~ 2x, analyzed by PM, peak ratio 1.39).
3. Slovenia, Primorska region, Julijske Alpe Mts.: Trenta valley, Trnovo ob Soči, near the road to Kobarid, 335 m, 9 November 2005, leg. B. VREŠ & J. CHRTEK (H1080/1, H1080/2, 2n = 18, counted by JC).

The species appears to be invariable in chromosome number. Diploids (2n = 18) have been reported by FAVARGER (1965) from the Julijske Alpe Mts. (Julian Alps), and by MARCUCCI & TORNADORE (1999) from the Treviso region in NE Italy.

Hieracium prenanthoides* VILL. subspecies group *prenanthoides **2n = 27**

Locality

1. Andorra, Canillo, SE margin of the village, 1530 m, 22 July 2006, leg. J. CHRTEK, G. MATEO & J. A. ROSSELLÓ (H 1187/2, counted by JC).

This is only the second reference on ploidy level in *H. prenanthoides* from the Pyrenees and it confirms the previous count stated on plants originating from the same region (cf. CASTRO et al. 2007). Three ploidy levels, namely diploids (2n = 18), triploids (2n = 27), and tetraploids (2n = 36) have been reported in this collective species. However, triploids strongly prevail among the examined plants (cf. CHRTEK 1996, SCHUHWERK 1996, and other chromosome number indexes). Diploids were only reported from the French Alps (Hautes Alpes; FAVARGER 1969a, FAVARGER 1969b); tetraploids were found by CHRISTOFF & POPOFF (1933, cultivated plant of unknown origin) and by LÖVE (1970) in plants from Iceland.

***Hieracium ramondii* GRISEB.** **2n = 27**

Locality

1. Andorra, Pirineos Mts.: Encamp, valley of Riu de les Deveses, NW slopes of Mt. Alt del Griu, 3.8 km E of the town, rocky outcrops in a light mountain forest, 2040 m alt., 1°37′52″ E, 42°32′07″ N, 22 July 2006, leg. J. CHRTEK, G. MATEO & J. A. ROSSELLÓ, det. G. MATEO (H 1173/4, H 1173/5, counted by JC).

This is the first chromosome number record for the species.

***Hieracium rohacsense* agg.**

***Hieracium rauzense* MURR** **2n ~ 3x**

Locality

1. Austria, Osttirol, distr. Lienz: Staller col, E of the Obersee glacial lake, 7.5 km WSW of Mariahilf, along the road, 2043 m, 12°12′33″ E, 46°53′23″ N, 29 July 2005, leg. P. MRÁZ & J. CHRTEK (H1759, H1760, H1761, analyzed by PM, peak ratio 1.83–1.89).

The same ploidy level based on chromosome counts was found by MRÁZ (2001) in plants originating from Vorarlberg (the Austrian Alps). Due to some level of morphological convergence, *H. rauzense* is traditionally treated within the *H. rohacsense* group, or even it is

given as a synonym of *H. rohacsense* s.str. However, the taxa are rather different with respect to their morphology, ploidy level, distribution range and allozyme pattern (MRÁZ & MARHOLD 1999, MRÁZ 2001, MRÁZ et al., unpubl.). Both taxa likely have different origins.

***Hieracium recoderi* DE RETZ** **2n = 18**

Locality

1. Spain, Catalunya, prov. Barcelona: Berga, monastery of Queralt, rocks ca. 200 m below the parking place, 24 July 2006, leg. J. CHRTEK (H1174/4, counted by JC).

This is the first karyological record for this species. The accession comes from the locus classicus (DE RETZ 1978). The species is only known from several localities in north-central Catalunya.

***Hieracium schmidtii* TAUSCH** **2n = 27**

Localities

1. Czech Republic, Bohemia, distr. Litoměřice: Boreč, the Boreč hill, NW slope, 800 m NW of the village, 350 m, 13°59′15″ E, 50°30′54″ N, 15 May 2005, leg. J. CHRTEK (H1024/6, H1024/7, counted by JC).
2. Czech Republic, Bohemia, distr. Litoměřice: Boreč, the Boreč hill, E slope, 500 m N of the village, 370 m, 13°59′25″ E, 50°30′53″ N, 15 May 2005, leg. J. CHRTEK (H1025/3, H1025/5, counted by JC).

Our chromosome counts well match those published by CHRTEK (1996) from the Krkonoše Mts. (Czech Republic), by CASTRO et al. (2007) from Spain and by SCHUHWERK & LIPPERT (1999) for *H. schmidtii* subsp. *comatulum* (BOREAU) GOTTSCHL. and *H. schmidtii* subsp. *kalmutinum* (ZAHN) GOTTSCHL. from Bavaria. Chromosome number 2n = 36 was reported for plants referred to as *H. schmidtii* agg. from Central Bohemia, Czech Republic (KIRSCHNER & ŠTĚPÁNEK in MĚSÍČEK & JAROLÍMOVÁ 1992) and for plants of *H. schmidtii* from Spain (CASTRO et al. 2007).

***Hieracium stelligerum* FROEL.** **2n = 18**

Locality

1. France, dépt. Ardeche, Vallon Pont d'Arc: crevices of the calcareous rocks along the road D 390, just on the opposite side of the "le Pont d'Arc", ca. 3.5 km SE of the village, October 2006, leg. P. MRÁZ (H06/38, 39, counted by PM).

This is the first chromosome number record for *H. stelligerum*.

***Hieracium tomentosum* L.** **2n ~ 3x**

Locality

1. France, dépt. Hautes Alpes: Briançon, 1300 m, 2005, leg. R. DOUZET (plant without no., 2n ~ 3x, analyzed by PM, peak ratio 1.98).

Hieracium tomentosum* subsp. *tomentosum **2n = 18, 2n ~ 2x**

Locality

1. France, dépt. Alpes maritimes, valley of Roya: Tende, along the old road to the Col de Tende, ca. 0.5 km above the tunnel, 6 km NNW of the village, 1331 m, 7°33′57″ E, 44°08′19″ N, 28 August 2005, leg. J. CHRTEK & P. MRÁZ (H1066/1, H1066/2, H1066/6, H1066/8, 2n = 18, counted by JC and JZ; H1852, 2n = 18, counted by PM; H1851, H1852, H1853, 2n ~ 2x, analyzed by PM, peak ratio 1.33–1.37).

Surprisingly, these are the first chromosome counts in *H. tomentosum*. While in the Alpes Maritimes we found a diploid cytotype, one plant from a northerly situated population from Briançon was triploid.

***Hieracium tommasinianum* MALY** **2n = 27**

(*H. tommasinii* RCHB. f., nom. illeg.)

Locality

1. Serbia, SW part, distr. Zlatibor: Mileševka river gorge, 10 km SW of Prijepolje, 19°44′53″ E, 43°21′31″ N, 30 April 2006, leg. M. NIKETIĆ (H1224, counted by J.Z.)

SCHUHWERK & LIPPERT (1998) found 2n = 36 in plants from Montenegro.

***Hieracium transylvanicum* HEUFF.** **2n = 18**

Locality

1. Ukraine, Oblast´ Zakarpatska, Marmaros´ki Al´py Mts.: Mt. Berlebashka (1480 m), NW slope along the trail (red marked), E of the village of Dilove, 19 September 2005, leg. J. ZAHRADNÍČEK (H1077/2, H1077/10, counted by JC and JZ).

The species appears to be invariable in chromosome number; only diploids have been reported so far. The published counts come from the Ukrainian Eastern Carpathians (PASHUK 1987, CHRTEK 1996, MRÁZ et al. 2005), Romanian Eastern Carpathians (MRÁZ 2003b, MRÁZ & SZELĄG 2004, MRÁZ et al. 2005), Durmitor Mts. in Montenegro (SZELĄG et al. 2007) and from the Stara Planina Mts. in Bulgaria (VLADIMIROV 2000, YURUKOVA-GRANCHAROVA et al. 2006). The first chromosome number in this species was reported by ROSENBERG (1927) but without indication of the exact locality (garden plant).

***Hieracium umbellatum* L.** **2n = 18**

Localities

1. Czech Republic, Bohemia, Praha: Praha-Troja, "Pustá vinice", heathland along a path, 240 m, 14°24′14″ E, 50°07′18″ N; 28 June 2006, leg. J. CHRTEK (H1162/3, H1162/4, counted by JC).
2. Ukraine, Oblast´ Zakarpatska, Skhidni Beskidi Mts.: Scherbovets, southern slopes of Mt. Pikui, 1300–1400 m, 22°59′45″ E, 48°49′52″ N, 21 July 2003, leg. P. MRÁZ & J. CHRTEK (H1420, H1422, H1427, H1430, counted by PM).

Table 1. Summary of the analyzed taxa and their chromosome numbers/DNA ploidy levels. DNA ploidy level estimations are given by the following formula "~". First record(s) for a taxon is/are marked in bold.

Taxon	Chromosome number/DNA ploidy level
H. alpinum agg.	
H. halleri	~ 3x
H. amplexicaule	36
H. amplexicaule subsp. *amplexicaule*	27
H. bocconei	**~ 4x**
H. bupleuroides subsp. *bupleuroides*	27, ~ 3x
H. bupleuroides subsp. *leviceps*	**27**
H. bupleuroides subsp. *tatrae*	27, ~ 3x
H. caesioides subsp. *caesioides*	**27**
H. caesium agg.	
H. basifolium	**36**
H. plumbeum	**36**
H. cerinthoides s.str.	27
H. cordifolium s.str.	18
H. eriophorum	18
H. glaucum	~ 3x
H. glaucum subsp. *nipholepium*	**27**
H. gouanii	**18**
H. gymnocerinthe	**27**
H. humile	36
H. intybaceum	18
H. kittaniae	18
H. lachenalii	27
H. laevigatum subspecies group *laevigatum*	27
H. murorum	27
H. naegelianum subsp. *naegelianum*	27
H. nigrescens agg.	
H. decipientiforme	36
H. olympicum subsp. *olympicum*	27
H. pannosum subsp. *pannosum*	27
H. petrovae	18
H. piliferum	~ 3x
H. piliferum subspecies group *piliferum*	27, ~ 3x
H. piliferum subspecies group *glanduliferum*	~ 3x, 4x
H. porrifolium	18
H. prenanthoides	27
H. ramondii	**27**
H. rohacsense agg.	
H. rauzense	~ 3x
H. recoderi	**18**
H. schmidtii	27
H. stelligerum	**18**
H. tomentosum	**~ 3x**
H. tomentosum subsp. *tomentosum*	**18, ~ 2x**
H. tommasinianum	27
H. transylvanicum	18
H. umbellatum	18
H. waldsteinii agg.	
H. plumulosum	18

Both sexual diploids (2n = 18) and apomictic triploids (2n = 27) are known in this species (for references see e.g. MÁJOVSKÝ et al. 1987, SCHUHWERK 1996, and other standard chromosome number indexes).

***Hieracium waldsteinii* agg.**

***Hieracium plumulosum* A. KERN.** **2n = 18**

Locality

1. Montenegro, Canyon of the Mrtvica river, 35 km SW of Kolasin, halfway through the canyon, around the bridge, 1000 m, 19°48′59″ E, 42°28′40″ N, August 2006, leg. J. ZAHRADNÍČEK, det. Z. SZELĄG (H1218/1, H1218/2, counted by JC).

Three chromosome numbers have been published for *H. waldsteinii* s.l. by SCHUHWERK & LIPPERT (1998). They reported 2n = 18 for plants referred to *H. w.* subsp. *suborieni* ZAHN from Montenegro, 2n = 27 for those identified as subsp. *plumulosum* (A. KERN.) ZAHN from Serbia and Bosnia and Herzegovina and 2n = 36 for plants of subsp. *baldaccianum* (FREYN) ZAHN from Montenegro. The first diploid (2n = 18) count in *H. plumulosum* was reported by SZELĄG et al. (2007) from the Sinjajerina Mts. in Montenegro.

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