CHROMOSOME NUMBERS IN Hieracium (Asteraceae) FROM CENTRAL AND SOUTHEASTERN EUROPE I.

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Chromosome numbers for 16 Hieracium s.str. species from Bosnia and Herzegovina, Bulgaria, Macedonia, Montenegro, Poland, Romania and Serbia are given and their metaphase plates are illustrated. Chromosome numbers are published for the first time for H. filarszkyi Jáv. & Zahn 2n = 3x = 27, H. fritschianum Hayek & Zahn 2n = 3x = 27, H. fritzeiforme Zahn 2n = 3x = 27, H. hercegovinicum (Freyn & Vandas) Szelaż 2n=3x=27, H. nitimontis (Oborny & Zahn) Chrtek fil. 2n = 4x = 36, H. vagneri Pax 2n = 4x = 36, as well as three undescribed species of hybrid origin between H. olympicum Boiss. and H. sparsum Friv. 2n = 4x = 36, H. naegelianum Panč. and H. scardicum Bornm. & Zahn 2n = 3x = 27, and H. transylvanicum Heuff. and H. umbellatum L. 2n = 4x = 36.

Key words: Asteraceae, chromosome number, Europe, Hieracium, karyotype.

INTRODUCTION

During several years of studies on Hieracium L. in Central and Southeastern Europe, the second author collected many living plants and transplanted them to an experimental garden for future taxonomic investigations. To date, many of them have never been analysed karyologically. Knowledge of ploidy level, which especially in Hieracium s.str. indicates the mode of reproduction, is of particular interest in understanding taxonomic and phylogenetic relationships within the genus.

The present paper gives chromosome numbers for 16 species of Hieracium s.str. including three undescribed species of hybrid origin. The ploidy levels of 9 species are published for the first time.

This study is a continuation of karyological investigations of Hieracium L. in Europe conducted at the Department of Plant Cytology and Embryology of the Jagiellonian University (Szeląg et al., 2007; Ilnicki et al., 2010; Ilnicki and Szeląg, 2011; Szeląg and Ilnicki, 2011).

MATERIAL AND METHODS

For cytological studies, seeds of the investigated species were collected from natural populations. Then they were germinated on moistened filter paper in Petri dishes. Karyological analysis was performed as described by Marciniuk et al. (2012) with some modifications. Briefly, 3- or 4-day-old seedlings were incubated in saturated aqueous solution of 8-hydroxychinoline for 4 h at room temperature. Subsequently they were fixed in a mixture of absolute ethanol and glacial acetic acid (3:1, v/v) for 24 h. Fixed material was stained in 2% acetic orcein for 4 days at room temperature. Stained seedlings were transferred to 45% acetic acid and heated to boiling over a flame. For slide preparation, root tip meristems were cut off and squashed in a drop of 45% acetic acid. The coverslip was removed after freezing in liquid nitrogen and the slide was thoroughly air-dried, and mounted in Entellan. The metaphase chromosomes were counted and photographed using Nikon Eclipse E400 microscope equipped with CCD camera.
RESULTS AND DISCUSSION

Hieracium atratum s.l.; 2n = 4x = 36 (Fig. 1a)
Poland, Eastern Carpathians, Bieszczady Mts, Mt. Tarnica 1330 m a.s.l., rocky places with Empetrum hermaphroditum.

This is the first chromosome number report for this species from the Eastern Carpathians and from Poland which confirms the number previously published by Chrtek et al. (2004) from the Western Carpathians. Chrtek (1994) also published tri- and tetraploid chromosome numbers for two species belonging to the Hieracium atratum aggregate from the Western Sudetes.

Hieracium chrysostyloides (Zahn) Chrtek fil.; 2n = 5x = 45 (Fig. 1b)
Poland, Eastern Sudetes, Mt. Śnieżnik Kłodzki, 1410 m a.s.l., alpine grassy places on western slope.

This is the first chromosome number report for this species from Poland which confirms the number previously published from the Hrubý Jeseník Mts, Eastern Sudetes (Chrtek, 1996). Hieracium chrysostoloides is endemic to the Eastern Sudetes and is one of three pentaploid Hieracium s.str. species known so far. The second one, H. pentaploideum P.D. Sell & D.J. Tennant, grows in Scotland and also belongs to the H. nigrescens agg. (Sell and Murrell, 2006; Tennant and Rich, 2008). The third one is H. virosum Pall. from Siberia in Russia (Pulkina and Tupitsyna, 2000).

Hieracium filarszkyi Jáv. & Zahn; 2n = 3x = 27 (Fig. 1c)
Romania, Southern Carpathians, Rețeza Mts, Mt. Butea, 1820 m a.s.l., grassy slope with Pinus mugo communities on granite.

This is the first chromosome number report for this endemic to the Rețeza Mts species.

Hieracium fritschianum Hayek & Zahn; 2n = 3x = 27 (Fig. 1d)
Bosnia and Herzegovina, Prenj Mts, Mt. Otiš, 1900 m a.s.l., calcareous rocks on eastern slope.

This is the first chromosome number report for this species known until now only from Albania.

Hieracium fritzeiforme Zahn; 2n = 3x = 27 (Fig. 1e)
Romania, Southern Carpathians, Rețeza Mts, Saua ciuriuia pass, 1800 m a.s.l., grassy slope with Pinus mugo communities on granite.

This is the first chromosome number report for this endemic to the Rețeza Mts species.

Hieracium heldreichii s.l. sensu Zahn; 2n = 3x = 27 (Fig. 1f)
Serbia, Mt. Besna Kobila, 1840 m a.s.l., eroded slope along road on the summit, surrounded by pastures with Festuca valida, Festuca nigrescens and Deschampsia caespitosa, on schist.

It is quite a frequent on the Balkan Peninsula collective species of the morphological formula H. pannosum < H. racemosum. The same chromosome number was earlier reported in plants of unknown origin (most probably from Bulgaria) by Gentscheff (1938). This is the first chromosome number report for this species from Serbia.

Hieracium hercegovinicum (Freyn & Vandas) Szeląg; 2n = 3x = 27 (Fig. 1g)
Montenegro, Prokletije Mts, Mt. Maja Rosave, below Zastan Koliba military checkpoint, 1430 m a.s.l., Fagus sylvatica forest margin on calcareous bedrock.

This is the first karyological data for this species of presumably hybrid origin between H. prenanthoides Vill. and H. gymnocephalum Griseb. ex Pant., morphologically closer to the former species (Szeląg, 2002). The plants analysed here were collected in the area in which diploid, sexual H. gymnocephalum grows (Szeląg and Ilnicki, 2011). The same chromosome number was reported by Niketić et al. (2003) in plants referred to as H. calophyllum aggregate (morphological formula H. gymnocephalum – H. prenanthoides).

Hieracium nigritum R. Uechtr.; 2n = 4x = 36 (Fig. 1h)
Poland, Eastern Sudetes, Mt. Śnieżnik Kłodzki, Hala pod Śnieżnikiem glade, 1260 m a.s.l., sub-alpine meadows with Festuca supina.

This is the first chromosome number report for this species from Poland which confirms the number previously published by Chrtek (1996) from the Hrubý Jeseník Mts, Eastern Sudetes, and from the Western Carpathians (Chrtek et al., 2004).
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Metaphase plates of:

- (a) *H. atratum*, 2n = 36.
- (b) *H. chrysostyloides*, 2n = 45.
- (c) *H. filarszkyi*, 2n = 27.
- (d) *H. fritschianum*, 2n = 27.
- (e) *H. fritzeiforme*, 2n = 27.
- (f) *H. heldreichii*, 2n = 27.
- (g) *H. hercegovinicum*, 2n = 27.
- (h) *H. nigritum*, 2n = 36. Bar = 10 μm.

**Fig. 1.** Metaphase plates of: (a) *H. atratum*, 2n = 36. (b) *H. chrysostyloides*, 2n = 45. (c) *H. filarszkyi*, 2n = 27. (d) *H. fritschianum*, 2n = 27. (e) *H. fritzeiforme*, 2n = 27. (f) *H. heldreichii*, 2n = 27. (g) *H. hercegovinicum*, 2n = 27. (h) *H. nigritum*, 2n = 36. Bar = 10 μm.
Hieracium nivimontis (Oborny & Zahn) Chrtek fil.; 2n = 4x = 36 (Fig. 2a)
Poland, Eastern Sudetes, Mt. Śnieżnik Kłodzki, 1380 m a.s.l., alpine grassy places on western slope.
This is the first chromosome number report for this species known only from Mt. Śnieżnik Kłodzki.

Hieracium schustleri Zlatník; 2n = 4x = 36 (Fig. 2b)
1. Poland, Eastern Sudetes, Mt. Śnieżnik Kłodzki, 1400 m a.s.l., alpine grassland on western slope (population with dark styles).
2. Poland, Eastern Sudetes, Mt. Śnieżnik Kłodzki, 1410 m a.s.l., rocky places on north-western slope (population with yellow styles) (Fig. 2b).
The same chromosome number was reported in plants from the Karkonosze/Krkonosé Mts (Chrtek, 1994).

Hieracium sudetotubulosum Szeląg; 2n = 4x = 36 (Fig. 2c)
Poland, Central Sudetes, Góry Stołowe Mts, upper verge of Mt. Szczeliniec Wielki, NW cliff next to the lookout, 900 m a.s.l.

Newly validated species (Szeląg, 2014) known from the Western Sudetes and recently discovered also in the Góry Stołowe Mts (Szeląg and Wójcik, 2014).
The same chromosome number was reported in plants from the Karkonosze/Krkonosé Mts by Chrtek (1994).

Hieracium vagneri Pax; 2n = 4x = 36 (Fig. 2d)
Poland, Eastern Carpathians, Bieszczady Mts, Mt. Bukowska Kopa, 1310 m a.s.l., rocky places on sandstone with calcicolous plants.

This is the first chromosome number report for this species of morphological formula H. alpinum – H. caesium which comprises a few taxa at the subspecies rank occurring in the Carpathians and Sudetes (Zahn, 1938).

Hieracium vulgatum Fries; 2n = 3x = 27 (Fig. 2e)
Poland, Wyżyna Miechowska upland, Kalina Lisiniec, 330 m a.s.l., thermophilous chalk grassland Inuletum ensifolii.

This is the first chromosome number report for this species from Poland. Triploid chromosome number was reported in plants from Great Britain (Morton, 1974). Information from Belarus (Dmitrieva, 1987) refers to H. lachenalii Suter.

Hieracium sp. 'Botev'; 2n = 4x = 36 (Fig. 2f)
Bulgaria, Stara Planina Mts, Kamenlivica on the southern slope of Mt. Botev, 1200 m a.s.l., eroded, siliceous rocks along touristic road from Panicite to the chalet Raj.

A new, undescribed species of presumably hybrid origin between H. olympicum Boiss. and H. sparsum Friv.

Hieracium sp. 'Ljuboten'; 2n = 3x = 27 (Fig. 2g)
Macedonia, Šarplanina Mts, Mt. Ljuboten, 2350 m a.s.l., calcareous rock crevices.

A new, undescribed species of presumably hybrid origin between H. naegelianum Panč. and H. scardicum Bornm. & Zahn. The hybrid grows together with diploid H. naegelianum (Szeląg and Ilnicki, 2011) and tetraploid H. scardicum (Ilnicki and Szeląg, 2011).

Hieracium sp. 'Raušor'; 2n = 4x = 36 (Fig. 2h)
Romania, Southern Carpathians, Retezat Mts, above Raušor ski centre, 1520 m a.s.l., grassy slope on Picea abies forest margin.

A new, undescribed species of presumably hybrid origin between sexual H. transylvanicum Heuff. and sexual H. umbellatum L. An emascula-
tion experiment in the garden-cultivated plants shows that the new species reproduces apomictically.
Detailed information on the experimental method followed here is given in Szeląg (2004).
The diploid chromosome number was stated in the artificial F1 hybrid received in experimental crosses between both diploid, parental species (Mráz and Paule, 2006).
The three above-enumerated undescribed species are the subject of ongoing studies to be pre-
sented separately (Szeląg, in prep.).

AUTHORS’ CONTRIBUTION

KM – karyological analysis, figures preparation and interpretation of results; ZS – idea, sampling and drafting of manuscript. The authors have declared that there is no conflict of interest.

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Metaphase plates of: (a) *H. nivimontis* 2n = 36. (b) *H. schustleri* 2n = 36. (c) *H. sudetotubulosum* 2n = 36. (d) *H. vagneri* 2n = 36. (e) *H. vulgatum* 2n = 27. (f) *Hieractum* sp. 'Botev'; 2n = 36. (g) *Hieractum* sp. 'Ljuboten'; 2n = 27. (h) *Hieractum* sp. 'Raušor', 2n = 36. Bar = 10 μm.

**Figs. 2.** Metaphase plates of: (a) *H. nivimontis* 2n = 36. (b) *H. schustleri* 2n = 36. (c) *H. sudetotubulosum* 2n = 36. (d) *H. vagneri* 2n = 36. (e) *H. vulgatum* 2n = 27. (f) *Hieractum* sp. 'Botev'; 2n = 36. (g) *Hieractum* sp. 'Ljuboten'; 2n = 27. (h) *Hieractum* sp. 'Raušor', 2n = 36. Bar = 10 μm.
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