

CHROMOSOME NUMBERS IN HIERACIUM (ASTERACEAE) FROM CENTRAL AND SOUTHEASTERN EUROPE VIII

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Chromosome numbers of 16 taxa of *Hieracium* s.str. from Bulgaria, Poland and Romania are specified and their metaphase plates are illustrated. Chromosome numbers are published for the first time for *H. camkorijense* subsp. *rilae* Rech. fil. & Zahn (4x), *H. grabowskianum* Nägeli & Peter (4x), *H. haematopodum* var. *sudeticum* Schack & Zahn (3x), *H. lomniczkianum* Szelag (4x), *H. pseudobifidum* var. *bucuranum* Nyárady & Zahn (3x), *H. umbellonigritum* Szelag (3x), *H. wiesbaurianum* subs. *herculanum* Zahn (3x), as well as six undescribed species belonging to the *H. bifidum* agg. (3x), *H. caesium* agg. (4x), *H. umbrosum* agg. (3x, 4x) and *H. vulgatum* agg. (3x) and a new supposed hybrid between *H. bifidum* s.lat. and *H. murorum* s.lat. (3x).

Keywords: Asteraceae, Europe, Hieracium, karyotype, polyploidy, somatic chromosomes

INTRODUCTION

This work is a continuation of the series devoted to karyological research on *Hieracium* L. in Central and Southeastern Europe. Knowledge of the ploidy level, which in *Hieracium* s.str. indicates the possible mode of reproduction, is of particular importance for understanding of taxonomic and phylogenetic relationships within the genus. Hitherto, we have examined the chromosome numbers of 105 taxa from over 130 populations (Musiał and Szelag, 2015, 2019, 2023; Musiał et al., 2016, 2017, 2018, 2020).

This paper presents the chromosome numbers of 16 taxa of *Hieracium* s.str. from Bulgaria, Poland and Romania, including an undescribed species of presumably hybrid origin between *H. bifidum* s.lat. and *H. murorum* s.lat. from Bul-*H. atratum, H. bifidum* s.lat., *H. caesium* s.lat.,

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garia, as well as a new species of the *H. umbrosum* aggregate from Poland.

MATERIAL AND METHODS

Seeds for karyological investigations were collected directly from plants in nature or from plants previously collected and grown in an experimental garden by the third author (ZS). They were then germinated on moistened filter paper in Petri dishes. The 3- or 4-day-old seedlings were incubated in saturated aqueous solution of 8-hydroxychinoline for 4 h at room temperature. They were subsequently fixed in a mixture of absolute ethanol and glacial acetic acid (3:1, v/v) for 24 h. In the case of *H. atratum*, *H. bifidum* s.lat., *H. caesium* s.lat.,

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H. camkorijense subsp. rilae, H. crassipedipilum, H. vulgatum s.lat. and the hybrid species (H. bifidum s.lat. x H. murorum s.lat.) the fixed material was stained in 2% acetic orcein for 4 days at room temperature. The 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. For the remaining species, i.e., H. grabowskianum, H. haematopodum var. sudeticum, H. lomniczkianum, H. piliferum, H. pseudobifidum var. bucuranum, H. umbellonigritum, H. umbrosum, H. wiesbaurianum subsp. *herculanum* chromosome staining with 0.1% aqueous solution of toluidine blue was performed after a preliminary procedure according to Grabowska-Joachimiak et al. (2021). The metaphase chromosomes were counted and photographed using a Nikon Eclipse E400 microscope equipped with a CCD camera. At least 10 seedlings were analyzed for each taxon and, depending on the species, the somatic chromosome number was established on 9-15 well-spread mitotic metaphase plates in the meristematic cells of roots. The vouchers of the analyzed taxa are deposited in KRAM.

RESULTS AND DISCUSSION

Hieracium atratum Fries; 2n = 4x = 36 (Fig. 1a) Poland, Sudetes, Karkonosze Mts., Łomnica valley, 1140 m a.s.l., *Picea abies* forest near the Domek Myśliwski chalet (counted by KM).

Despite high morphological diversity of the *H. atratum* agg. in the Karkonosze Mts. (Zlatník 1938, 1939), all of the previously analyzed populations were represented by tetraploid plants (Chrtek, 1994; Musiał and Szelag, 2019; Musiał et al., 2020).

Hieracium bifidum s.lat.; 2n = 3x = 27 and 2n = 4x = 36 (Fig. 1b) Poland, Wyżyna Krakowsko-Częstochowska upland, Dolina Brzoskwinki valley, 290 m a.s.l., calcareous rocks on the left slope in *Fagus sylvatica* forest (counted by KM).

Our results well match those previously published for *H. bifidum* s.lat. from different parts of the Wyżyna Krakowsko-Częstochowska upland (Musiał et al., 2016, 2017, 2018; Musiał and Szelag, 2019, 2023; Grabowska-Joachimiak et al., 2023). This morphologically very variable collective species is known from triploid and tetraploid populations, while the occurrence of two different ploidy levels in the same population was rarely found.

Hieracium bifidum s.lat. – H. murorum s.lat.; 2n = 4x = 36 (Fig. 1c) Bulgaria, Stara Planina Mts., Mt. Ispolin, 1500 m a.s.l., calcareous scree in *Fagus sylvatica* forest on north-facing slope (counted by KM).

The analyzed plants grow in a mixed population with tetraploid *H. bifidum* s.lat. (Musiał et al., 2020) and probably belong to a new hybridogenous species.

Hieracium caesium s.lat.; 2n = 4x = 36 (Fig. 1d) Poland, Wyżyna Krakowsko-Częstochowska upland, along a road from town of Skała to Ojców National Park, 260 m a.s.l., north-facing slope with *Pinus sylvestris* thicket (counted by KM).

Previously, a tetraploid chromosome number for this collective species was reported from Austria, Germany, Poland and Sweden (Schuhwerk and Lippert, 1999; Chrtek et al., 2007; Musiał and Szelag, 2023).

Hieracium camkorijense subsp. rilae Rech. fil. & *Zahn*; 2n = 4x = 36 (Fig. 1e) Bulgaria, Rila Mts., Monastirska reka valley, 1370 m a.s.l., open places in *Picea abies* forest on granite (counted by KM).

This is the first chromosome number for this endemic to the Rila Mts., a taxon known only from the type locality in the Monastirska reka valley (Rechinger, 1933).

Hieracium crassipedipilum (Pawł. & Zahn) Chrtek fil.; 2n = 4x = 36 (Fig. 1f) Poland, Western Carpathians, Babia Góra Massive, Mt. Diablak, 1690 m a.s.l. (counted by KM).

This is the first karyological data for the species from Poland and the first data on its occurrence on Mt. Babia Góra. Previously, a triploid and tetraploid chromosome counts were given by Chrtek et al. (2004) from Slovakia.

Hieracium grabowskianum Nägeli & Peter; 2n = 4x = 36 (Fig. 1g). Poland, Western Carpathians, Babia Góra Massive, Mt. Kościółek Zachodni, 1570 m a.s.l., in the *Saxifrago-Festucetum versicoloris* association on the NE slope (counted by AG-J).

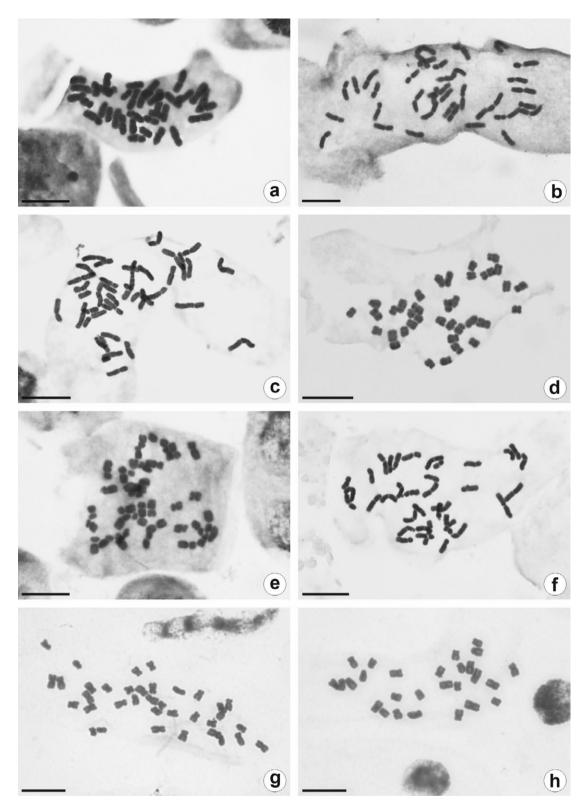


Fig. 1. Metaphase plates of: (a) *Hieracium atratum* 2n=4x=36, (b) *H. bifidum* s.lat. 2n=4x=36, (c) *H bifidum* s.lat. - H. *murorum* s.lat. 2n=4x=36, (d) *H. caesium* s.lat. 2n=4x=36, (e) *H. camkorijense* subsp. *rilae* 2n=4x=36, (f) *H. crassipedipilum* 2n=4x=36, (g) *H. grabowskianum* 2n=4x=36, (h) *H. haematopodum* var. *sudeticum* 2n=3x=27. Scale bars = 10 µm

This is the first chromosome number for tes and Western Carpathians.

Hieracium haematopodum var. sudeticum Schack & Zahn; 2n = 3x = 27 (Fig. 1h) Poland, Sudetes, Karkonosze Mts., Czarny Kocioł glacial cirque, 1170 m a.s.l., rocky slope on granite (counted by AG-J).

This is the first chromosome number for this taxon endemic to the Karkonosze Mts.

Hieracium lomniczkianum Szeląg; 2n = 4x = 36(Fig. 2a) Poland, Sudetes, Karkonosze Mts., Kocioł Łomniczki glacial cirque, 1370 m a.s.l., rocky and grassy slope with Pinus mugo along a tourist path on granite (counted by AG-J).

A recently described species of the H. wiesbaurianum agg., known only from the type locality (Szelag, 2023a).

Hieracium piliferum agg.; 2n = 4x = 36 (Fig. 2b) Bulgaria, Rila Mts., Mt. Golyam Kalin, in granite crevices on the ridge 2600 m a.s.l. (counted by AG-J).

Our data confirm the chromosome number published by Vladimirov (2021) for plants from the same population and determined as H. amphigenum Briq.

Hieracium pseudobifidum var. bucuranum Nyárady & Zahn; 2n = 3x = 27 (Fig. 2c) Romania, Retezat Mts., Bucura valley, 1650 m a.s.l., Picea abies forest on granite, along a tourist path to Bucura lake (counted by AG-J).

This is the first chromosome number for this very variable collective species of hybrid origin between H. transylvanicum Heuffel and different taxa of H. bifidum s.lat. The analyzed plants were collected in the type locality (Zahn, 1934).

Hieracium umbellonigritum Szeląg; 2n = 3x = 27(Fig. 2d) Poland, Sudetes, Karkonosze Mts., NW of Wielki Staw glacial lake, along a tourist path from Polana glade to main ridge of the mountains, 1280 m a.s.l. (counted by AG-J).

A recently described species intermediate between H. umbellatum L. and H. nigritum R. Uechtr. known only from the type locality (Szelag, 2023b).

Hieracium umbrosum agg.; 2n = 3x = 27 (Fig. 2e), 2n = 4x = 36 (Fig. 2f) 1. Poland, Western Car-

pathians, Babia Góra Massive, Złotnica ridge, this rare species known from the Eastern Sude- 1500 m a.s.l. on the margins of Pinus mugo thickets along a tourist path, 2n = 3x = 27(counted by AG-J).

> The analyzed plants belong to a new species characterized by brown-spotted leaves and will be the subject of future studies.

> 2. Poland, Western Carpathians, Babia Góra Massive, above the Markowe Szczawiny shelter, 1200 m a.s.l., Picea abies forest along a tourist path to the Brona pass, 2n = 4x = 36 (counted by AG-J).

> The analyzed plants are similar morphologically to H. umbrosum Jordan.

> *Hieracium vulgatum s.lat.*; 2n = 3x = 27 (Fig. 2g) Poland, Sudetes, Karkonosze Mts. Mt. Chojnik, 610 m a.s.l., sandstone rocks on the southern slope (counted by KM).

> The triploid chromosome number was reported in plants from Great Britain (Marton, 1974) and from Poland (Morton, 1974; Musiał and Szelag, 2015, 2019).

> Hieracium wiesbaurianum subsp. herculanum Zahn; 2n = 3x = 27 (Fig. 2h) Romania, Mehedinți Mts., Cheile Tăsnei gorge, 560 m a.s.l., shadowed calcareous scree with Pinus banatica (counted by AG-J).

> This is the first chromosome number for this rare, endemic to the Mehedinți Mts. taxon.

AUTHORS' CONTRIBUTION

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

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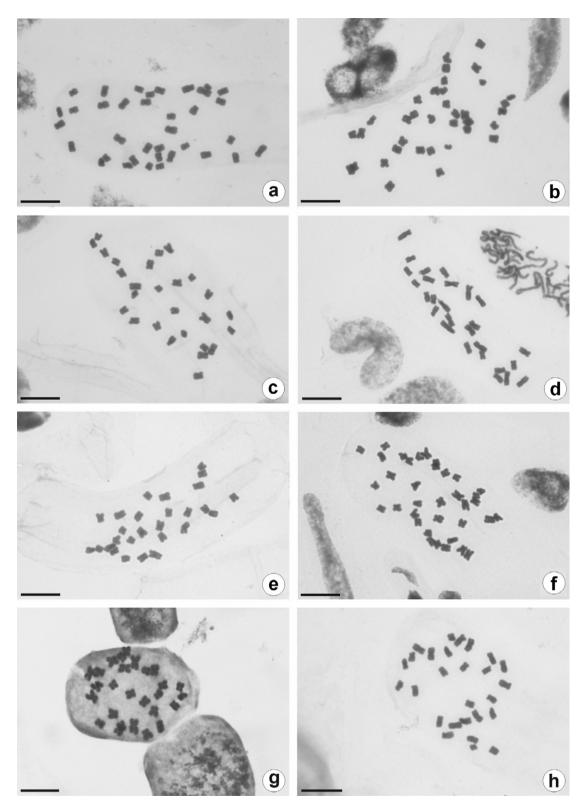


Fig. 2. Metaphase plates of: (a) *Hieracium lomniczkianum* 2n=4x=36, (b) *H. piliferum* agg. 2n=4x=36, (c) *H. pseudobifidum* var. *bucuranum* 2n=3x=27, (d) *H. umbellonigritum* 2n=3x=27, (e) *H. umbrosum* agg. 2n=3x=27, (f) *H. umbrosum* agg. 2n=4x=36, (g) *H. vulgatum* s.lat. 2n=3x=27, (h) *H. wiesbaurianum* subsp. *herculanum* 2n=3x=27. Scale bars = 10 µm

REFERENCES

- CHRTEK J, JUN. 1994. Chromosome numbers in selected *Hieracium* species in the Krkonoše Mts. (the West Sudeten). *Folia Geobotanica et Phytotaxonomica* 29: 91–100.
- CHRTEK J, JUN. MRÁZ P, and SEVERA M. 2004. Chromosome numbers in selected species of *Hieracium* s. str. (*Hieracium* subgen. *Hieracium*) in the Western Carpathians. *Preslia* 76: 119–139.
- CHRTEK J, JUN. MRÁZ P, ZAHRADNÍČEK J, MATEO G, and SZELAG Z. 2007. Chromosome numbers and DNA ploidy levels of selected species of *Hieracium* s.str. (Asteraceae). *Folia Geobotanica* 42: 411–430.
- GRABOWSKA-JOACHIMIAK A, ŻYTKOWICZ M, KWOLEK D, and SZE-LAG Z. 2021. Chromosome complex of the relict diploid species Hieracium bracteolatum. Acta Biologica Cracoviensia Series Botanica 63(2): 29–34. https://doi.org/ 10.24425/abcsb.2021.136702
- GRABOWSKA-JOACHIMIAK A, KWOLEK D, PIĘTA E, SZELAG Z, and JOACHIMIAK AJ. 2023. rDNA-FISH pattern in selected *Hieracium* species representing different ploidy levels. *Acta Societatis Botanicorum Poloniae* 92(1):1-10. doi: 10.5586/asbp/172418
- MORTON JK, 1974. Chromosome numbers of British plants, 3. Watsonia 10: 169.
- MUSIAŁ K, JANAS A, and SZELAG Z. 2016. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe II. Acta Biologica Cracoviensia Series Botanica 58(1): 119–123. https://doi.org/10.1515/ abcsb-2016-0007
- MUSIAŁ K, JANAS A, and SZELAG Z. 2017. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe III. Acta Biologica Cracoviensia Series Botanica 59(2): 83–87. https://doi.org/ 10.1515/ abcsb-2017-0013
- MUSIAL K, JANAS A, and SZELAG Z. 2018. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe IV. Acta Biologica Cracoviensia Series Botanica 60(2): 75–79. https://doi.org/10. 24425/118052
- MUSIAŁ K, and SZELAG Z. 2015. Chromosome numbers in *Hieracium* (Asteraceae) from Central and South-

eastern Europe I. Acta Biologica Cracoviensia Series Botanica 57(2): 115–120. https://doi.org/10.1515/ abcsb-2015-0020

- MUSIAŁ K, and SZELAG Z. 2019. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe V. *Acta Biologica Cracoviensia Series Botanica* 61(2): 63–68. https://doi.org/10.24425/ abcsb.2019.127748
- MUSIAŁ K, and SZELAG Z. 2023. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe VII. Acta Biologica Cracoviensia Series Botanica 65(1): 23–28. https://doi.org/10.24425/ abcsb.2023.145593
- MUSIAL K, VLADIMIROV V, and SZELAG Z. 2020. Chromosome numbers in *Hieracium* (Asteraceae) from Central and Southeastern Europe VI. Acta Biologica Cracoviensia Series Botanica 62(2): 43–50. https://doi.org/10. 24425/abcsb.2020.131672
- RECHINGER fil. K.H. 1933. Ergebnisse einer botanischen Reise nach Bulgarien. *Magyar Botaniki Lapok* 32: 5–58.
- SCHUHWERK F, and LIPPERT W. 1999. Chromosomenzahlen von *Hieracium* (Compositae, Lactuceae) Teil 3. *Sendtnera* 6: 197–214.
- SZELAG Z. 2023a. Hieracium lomniczkianum (Asteraceae), a new species in the H. wiesbaurianum aggregate from Sudetes in Poland. Phytotaxa 594(4): 251–255. https:// doi.org/10.11646/phytotaxa.594.4.2
- SZELAG Z. 2023b. Hieracium umbellonigritum (Asteraceae), a new hybridogenous species from the Sudetes in Poland. *Phytotaxa* 589(3): 289–292. https://doi. org/10.11646/phytotaxa.589.3.8
- VLADIMIROV V. 2021. The first record of *Hieracium piliferum* agg. (Asteraceae) in the Bulgarian flora. *Comptes rendus de l'Academie bulgare des Sciences* 74: 972–976. https://doi.org/10.7546/CRABS.2021.07.03
- ZAHN KH. 1934. Neue Beiträge zur Hieracium-Flora Rumäniens. Buletinul Grādinii Botanice și al Muzeului Botanic dela Universitatea din Cluj 13: 59–67.
- ZLATNÍK A. 1938. *Hieracia* Alpina Sudetorum Occidentalium. Studia Botanica Čechoslovaca 1: 37–51 & 105–242.
- ZLATNÍK A. 1939. Additamentum ad "*Hieracia* Alpina Sudetorum Occidentalium". *Studia Botanica Čechoslovaca* 2: 64–96.