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

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Running title: *Musiał et al. …… Chromosome numbers in* Hieracium

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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* Szeląg (4x), *H. pseudobifidum* var. *bucuranum* Nyárady & Zahn (3x), *H. umbellonigritum* Szeląg (3x), *H. wiesbaurianum* subs. *herculanum* Zahn (3x), as well as five undescribed species *H. umbrosum* agg. (3x) and *H. vulgatum* agg. (3x belonging to the *H. bifidum* agg. (3x), *H. caesium* agg. (4x),) 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 Szeląg, 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 Bulgaria, 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., *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. oistophyllum*, *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 Szeląg, 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 Szeląg, 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 Szeląg, 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; 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).
This is the first chromosome number for this rare species known from the Eastern Sudetes 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 (Szeląg, 2023a).

Hieracium oistophyllum Pugsley; 2n = 3x = 27 (Fig. 2b)

Poland, Wyżyna Krakowsko-Częstochowska upland, Dalnica hill near Jerzmanowice village, 470 m a.s.l., shadowed thickets with *Carpinus betulus* on the northern calcareous slope (counted by AG-J).

Previously, plants from the same locality were found to be tetraploid (Musiał et al., 2020), but this was due to a misinterpretation of the metaphase plates. This species, widespread in Scandinavia, is represented only by triploid populations (Tyler and Jönsson, 2009).

Hieracium piliferum agg.; 2n = 4x = 36 (Fig. 2c)

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. 2d)

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. 2e)

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 (Szeląg, 2023b).

Hieracium umbrosum agg.; 2n = 3x = 27 (Fig. 2f)

Poland, Western Carpathians, Babia Góra Massive, Złotnica ridge, 1500 m a.s.l. on margins of *Pinus mugo* thickets along a tourist path (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.

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 Szeląg, 2015, 2019).

Hieracium wiesbaurianum subsp. *herculanum* Zahn; 2n = 3x = 27 (Fig. 2h)
Romania, Mehedinți Mts., Cheile Țăsnei 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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FIGURES:

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

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

Figure 1

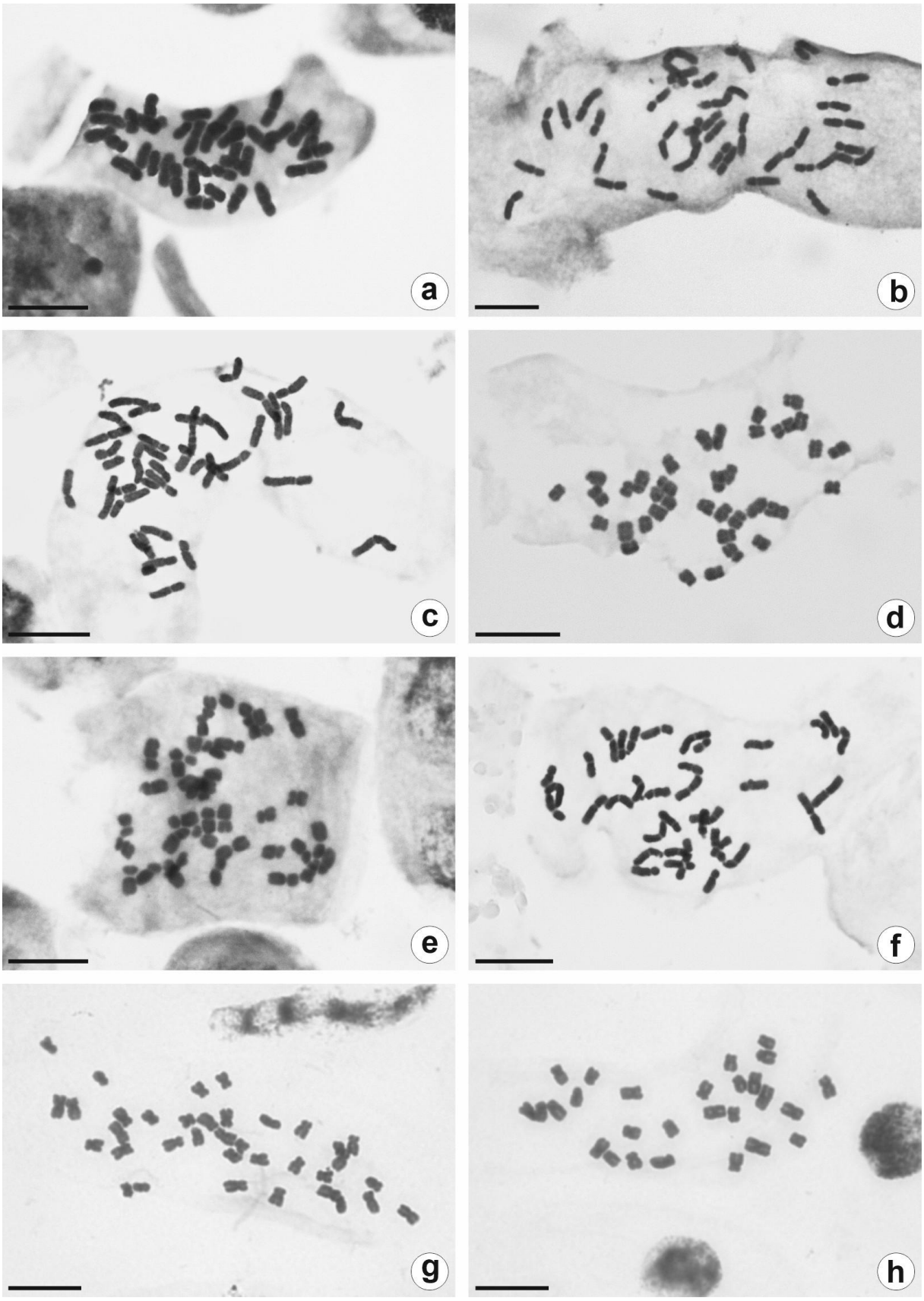

Figure 2