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

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

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Chromosome numbers for 23 taxa of *Hieracium* L. from Bulgaria, Greece, Poland and Slovakia are given and their metaphase plates are illustrated. The ploidy level of 8 taxa was also confirmed by flow cytometry. Chromosome numbers are published for the first time for *Hieracium bracteolatum* subsp. *koracis* (Boiss.) Zahn (4x), *H. marmoreum* Pančić & Vis.

(3x), *H. ossaeum* Zahn (3x), *H. sartorianum* Boiss. & Heldr. (3x), *H. sericophyllum* Nejčeff & Zahn (3x) as well as for five other undescribed species.

Keywords: Asteraceae, chromosome number, Europe, *Hieracium*, karyotype, ploidy level

INTRODUCTION

We are continuing karyological studies on *Hieracium* L. as the knowledge of the ploidy level indicates the possible mode of reproduction and is useful for feature taxonomic research. To date, we have analyzed chromosome numbers of 65 taxa from more than 80 populations in Central and Southeastern Europe (Musiał and Szeląg, 2015, 2019; Musiał et al., 2016, 2017, 2018). This paper presents the chromosome numbers, complemented in part by the ploidy level estimated using flow cytometry, for 23 taxa of *Hieracium* from 29 populations in Bulgaria, Greece, Poland and Slovakia, including six most probably undescribed species, and a very interesting hybrid which originated spontaneously in garden culture, which will be the subject of separate studies.

MATERIAL AND METHODS

The seeds for karyological investigations were collected from plants in nature or cultivated in an experimental garden. Then they were 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. 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. 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 10 – 16 well-spread mitotic metaphase plates in meristematic cells of roots.

The ploidy level was estimated by flow cytometry in plants collected in the field and cultivated in the experimental garden of the Institute of Biodiversity and Ecosystem Research in Sofia, following the method described by Szeląg and Vladimirov (2019). Estimates of the level of ploidy are given by the formula ”2n ≈“.

RESULTS AND DISCUSSION

Hieracium atratum s. lat.; 2n = 4x = 36 (Fig. 1a)
Poland, Western Sudetes, Karkonosze Mts., Mały Śnieżny Kocioł glacial cirque, 1390 m a.s.l. grassy slope on granite (counted by KM).

The analyzed plants are morphologically similar to *H. atratum* subsp. *atrellum* Zahn for which a triploid chromosome number was given by Chrtek (1994).

Hieracium barbatum Tausch; 2n = 4x = 36 and 2n ≈ 4x (Fig. 1b)
Bulgaria, Stara Planina Mts., *Fagus sylvatica* and *Carpinus betulus* forest margin along the road from Etropole to Pravets towns, 660–670 m a.s.l. (counted by VV *V18088*).

This is the first chromosome number report of this species from Bulgaria which confirms the number published in plants from Poland (Musiał et al., 2018).

Hieracium bifidum s.lat.; 2n = 3x = 27 and 2n = 4x = 36 (Fig. 1c)
1. Poland, Wyżyna Krakowsko-Częstochowska upland, Skałka hill near Jerzmanowice village, 500 m a.s.l., calcareous rock with *Festuca pallens*, 2n = 3x = 27 (counted by KM).
2. Poland, Wyżyna Krakowsko-Częstochowska upland, Ostatnie Skałki hill near Przeginia village, 2n = 3x = 27 (counted by KM).
3. Poland, Western Carpathians, Pieniny Mts., Wąwóz Sobczański gorge, 600 m a.s.l., calcareous rock with *Festuca pallens* and *Saxifraga paniculata*, 2n = 3x = 27 (counted by KM).
4. Bulgaria, Stara Planina Mts., Mt. Ispolin, 1500 m a.s.l., calcareous scree in *Fagus sylvatica* forest on north-facing slope, 2n = 4x = 36 (Fig. 1c) (counted by KM).

This is the first tetraploid chromosome number report of this species from Bulgaria. Our results well match those previously published for *H. bifidum* s.lat. from different parts of SE Europe (Ilnicki and Szeląg, 2011; Musiał et al., 2016, 2017, 2018; Musiał and Szeląg, 2019).

Hieracium bohatschianum agg.; 2n = 3x = 27 and 2n = 4x = 36 (Figs. 1d & 1e)
1. Bulgaria, Rila Mts., Malyovitsa tourist complex, 1700 m a.s.l., *Picea abies* forest margin on eroded siliceous slope, 2n = 3x = 27 (Fig. 1d) (counted by KM).
2. Bulgaria, Rila Mts., along a lift to Rilski Ezera Hut (хижа Рилски езера) 1800 m a.s.l., *Picea abies* forest margin on eroded siliceous slope, 2n = 4x = 36 (Fig. 1e) (counted by KM).

The analyzed plants probably represent new species of the morphological formula *H. sparsum* − *H. schmidtii* and *H. sparsum* – *glaucinum*, respectively, and will be the subject of future studies.

Hieracium bracteolatum subsp. *koracis* (Boiss.) Zahn; 2n = 4x = 36 and 2n ≈ 4x (Fig. 1f)
Greece, Giona (Γκιώνα) Mts., along a dirt road above Stromi (Στρόμη) village, 960 m, *Abies cephalonica* forest margin on silicate N38.73037, E22.35392 (counted by VV *V19111*).

This is the first chromosome number for this Greek endemic. Previously tetraploid chromosome numbers were found in *H. bracteolatum* subsp. *reinholdii* (Schuhwerk and Lippert, 1998).

Hieracium heldreichii agg. sensu Szeląg (2018); 2n = 4x = 36 and 2n ≈ 4x (Fig. 1g)
Bulgaria, Stara Planina Mts., by the road from Karnare village to Beklemeto pass, 1280 m a.s.l., siliceous bedrock, margin of *Pinus nigra* plantation (counted by VV *V18081*).

The same chromosome number for a different taxon of *H. heldreichii* agg. was published from the Rila Mts. in Bulgaria (Musiał et al., 2018).

Hieracium laurinum Arvet-Touvet 2n = 3x = 27 (Fig. 1h)
Bulgaria, town of Samokov, 900 m a.s.l., grassy places in the municipal park (counted by KM).

This is the first triploid chromosome number for this species from Bulgaria. Previously the diploids were found in Poland (Szeląg and Vladimirov, 2005).

Hieracium marmoreum Pančić & Vis.; 2n = 3x = 27 and 2n ≈ 3x (Fig. 2a)
Bulgaria, Stara Planina Mts., Sinite Kamani Nature Park above Sliven town, N42.71583, E26.37863, ca. 950 m a.s.l., eroded slope on quartz-porphyry bedrocks (counted by V. V. *V18032*).

This is the first chromosome number report for this species known from Bulgaria and Serbia.

Hieracium naegelianum Pančić; 2n = 3x = 27 (Fig. 2b)
1. Greece, Vardousia (Βαρδούσια) Mts., Mt. Korakas (Κόρακας), 2150 m a.s.l., calcareous scree on western slope (Fig. 2b) (counted by KM).
2. Greece, Olimbos (Όλυμπος) Mts., Mt. Agios Antonios (Άγιος Αντώνιος), 2600 m a.s.l., calcareous scree on southern slope with *Potentilla deorum* and *Ranunculus brevifolius* (counted by KM).
3. Greece, Taygetos (Ταΰγετος) Mts., Mt. Profitis Ilias (Προφήτης Ηλίας) 2250 m a.s.l., calcareous scree on north-eastern slope with *Acantholimon graecum* (counted by KM).

Hieracium naegelianum is known from triploid populations in different parts of the Balkan Peninsula (Merxmüller, 1975; Grau and Erben, 1988; Buttler, 1991; Vladimirov and Szeląg, 2001; Niketić et al., 2006; Chrtek et al., 2007; Ilnicki and Szeląg, 2011) whereas diploid populations were found only in North Macedonia (Szeląg and Ilnicki, 2011).

H. naegelianum Pančić x *H. wiesbaurianum* s.lat.; 2n = 3x = 27 (Fig. 2c)., 2n = 4x = 36 (Fig. 2d).
Greece, Olimbos (Όλυμπος) Mts., Mt. Agios Antonios (Άγιος Αντώνιος), 2600 m a.s.l., calcareous scree on southern slope with *H. naegelianum* (see above) (counted by KM).

Two cytotypes were found in the randomly collected seeds. The analyzed plants probably belong to a new species being the subject of current studies.

Hieracium oistophylum Pugsley 2n = 4x = 36 (Fig. 2e).
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 KM).

A new chromosome number for this species widely distributed in Scandinavia and British Islands. A triploid chromosome number was given from Sweden (Tyler & Jönsson, 2009). The Polish plants were recognized as *H. fuscocinereum* Norrl. (Zahn 1923).

Hieracium ossaeum Zahn 2n = 3x = 27 (Fig. 2f).
Grecce, Mt. Ossa (Όσσα) (*locus classicus* of the species), 1100 m a.s.l., north-eastern slope of the mountain, eroded places in *Abies cephalonica* forest on silicate bedrock (counted by KM).

This is the first chromosome number for this Greek endemic species.

Hieracium pannosum Boiss.; 2n = 3x = 27 and 2n ≈ 3x (Fig. 2g)
Bulgaria, Stara Planina Mts. Sinite Kamani Nature Park above Sliven town, 1030 m a.s.l., eroded slope on quartz-porphyry bedrocks (counted by V. V. *V18034*).

Confirmation of the triploid chromosome number for the species from other localities in Bulgaria (Chrtek et al., 2007).

Hieracium pannosum subsp. *parnassi* Nägeli & Peter; 2n = 3x = 27 (Fig. 2h).
Grecce, Giona (Γκιώνα) Mts., along a road from Kaloskopi (Καλοσκοπή) village to Mt. Piramida (Πυραμίδα), 30°39’40” N, 22°16’53’’E, 1750 m a.s.l. (counted by KM).

The triploid plants were also found in the neighboring Parnassus Mts. (Musiał and Szeląg, 2019).

H. pojoritense Woł. x *H. oistophyllum* Pugsley 2n=5x=45 (Fig. 3a).
The analyzed plants originated in garden culture from spontaneous hybridization between diploid *H. pojoritense* Woł. from the *locus classicus* of the species in Romania and tetraploid *H. oistophyllum* from Poland (see above) and will be subject to genetic analysis, the results of which will be presented separately (counted by KM).

Hieracium rohacense 2n = 4x = 36 (Fig. 3b).
Slovakia, Western Carpathians, Nízke Tatry Mts., Mt. Panska hoľa, 1610 m a.s.l., south facing subalpine grasslands on granite (counted by KM).

The same chromosome number was given by Mráz (2001) from several localities in the Western Carpathians.

Hieracium sartorianum Boiss. & Heldr.; 2n = 3x = 27 (Fig. 3c).
Greece, Parnassos (Παρνασσός) Mts., Mt. Gerontovrachos (Γεροντόβραχος), calcareous scree on western slope along a tourist path (counted by KM).

This is the first chromosome number for this Greek endemic species.

Hieracium sericophyllum Nejčeff & Zahn; 2n = 3x = 27 and 2n ≈ 3x (Fig. 3d)
Bulgaria, Stara Planina Mts., upper part of the Kurudere gorge, 1690 m a.s.l., limestone scree (counted by VV *V18064*, *V18071*).

This is the first report on the chromosome number for this species.

Hieracium sudeticum 2n = 4x = 36 (Fig. 3e).
1. Poland, Western Sudetes, Karkonosze Mts., Kocioł Łomniczki glacial cirque, 1250 m a.s.l.; grasslands among *Pinus mugo* thickets on granite (Fig. 2d) (counted by KM).
2. Poland, Western Sudetes, Karkonosze Mts., Łabski Kocioł glacial cirque, 1270 m a.s.l., grassy places along a tourist path to Śnieżne Koły glacial cirques (counted by KM).

The same chromosome number for this endemic to the Karkonosze Mts. was published by Chrtek (1994).

Hieracium tommasinianum K. Malý; 2n = 4x = 36 and 2n ≈ 4x (Fig. 3f)
Bulgaria, Valley of River Struma floristic region, along the road from Boboshevo town to St. Dimitar Monastery above the town, 660 m a.s.l., (counted by VV *V18187*).

The tetraploid chromosome number has already been published by Musiał and Szeląg (2019) from a nearby locality.

Hieracium wiesbaurianum (*H. hypochoeroides*) agg.; 2n = 4x = 36 and 2n ≈ 4x (Fig. 3g)
Bulgaria, Stara Planina Mts., Sinite Kamani Nature Park above Sliven town, rocky places above the road from Sliven to the TV-tower, 950–1000 m a.s.l. (counted by VV *V5488*).

The first report of the chromosome number of this species from Bulgarian accession. Most likely, the collected plants belong to an agamic species not described yet.

Hieracium villosum Jacq. 2n = 3x = 27 (Fig. 3h).
Bulgaria, Stara Planina Mts., Mt. Ispolin, 1510 m a.s.l., calcareous rocks on north-facing slope (counted by KM).

The south-easternmost locality of the species in Europe. This is the first chromosome number of the species from Bulgaria which corresponds to data from other parts of the European range (Chrtek et al., 2004; Schuhwerk, 2010).

AUTHORS' CONTRIBUTION

KM – karyological analysis of 15 taxa, preparation of figures and interpretation of results; VV – sampling, karyological analysis and flow cytometry of 8 taxa; ZS – sampling of 15 taxa 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* s.lat. 2n=4x=36, (**b**) *H. barbatum* 2n=4x=36, (**c**) *H. bifidum* s.lat. 2n=3x=27 and 2n=4x=36, (**d**) *H. bohatschianum* agg. 2n=3x=27, (**e**) *H. bohatschianum* agg. 2n=4x=36, (**f**) *H. bracteolatum* s.lat. 2n=4x=36, (**g**) *H. heldreichii* s.lat. 2n=4x=36, (**h**) *H. laurinum* 2n=3x=27. Scale bars = 10 μm.

Fig. 2. Metaphase plates of: (**a**) *Hieracium marmoreum* 2n=3x=27, (**b**) *H. naegelianum* 2n=3x=27, (**c**) *H. naegelianum* x *H. wiesbaurianum* s.lat. 2n=3x=27, (**d**) *H. naegelianum* x *H. wiesbaurianum* s.lat.; 2n=4x=36, (**e**) *H. oistophylum* 2n=4x=36, (**f**) *H. ossaeum* 2n=3x=27, (**g**) *H. pannosum* 2n=3x=27, (**h**) *H. pannosum* subsp. *parnassi* 2n=3x=27. Scale bars = 10 μm.

Fig. 3. Metaphase plates of: (**a**) *H. pojoritense x H. oistophyllum* 2n=5x=45, (**b**) *H. rohacense* 2n=4x=36, (**c**) *H. sartorianum* 2n=3x=27, (**d**) *H. sericophyllum* 2n=3x=27, (**e**) *H. sudeticum* 2n=4x=36, (**f**) *H. tommasinianum* 2n=4x=36, (**g**) *H. wiesbaurianum* agg. 2n=4x=36, (h) *H. villosum* 2n=3x=27. Scale bars = 10 μm.

Figure 1

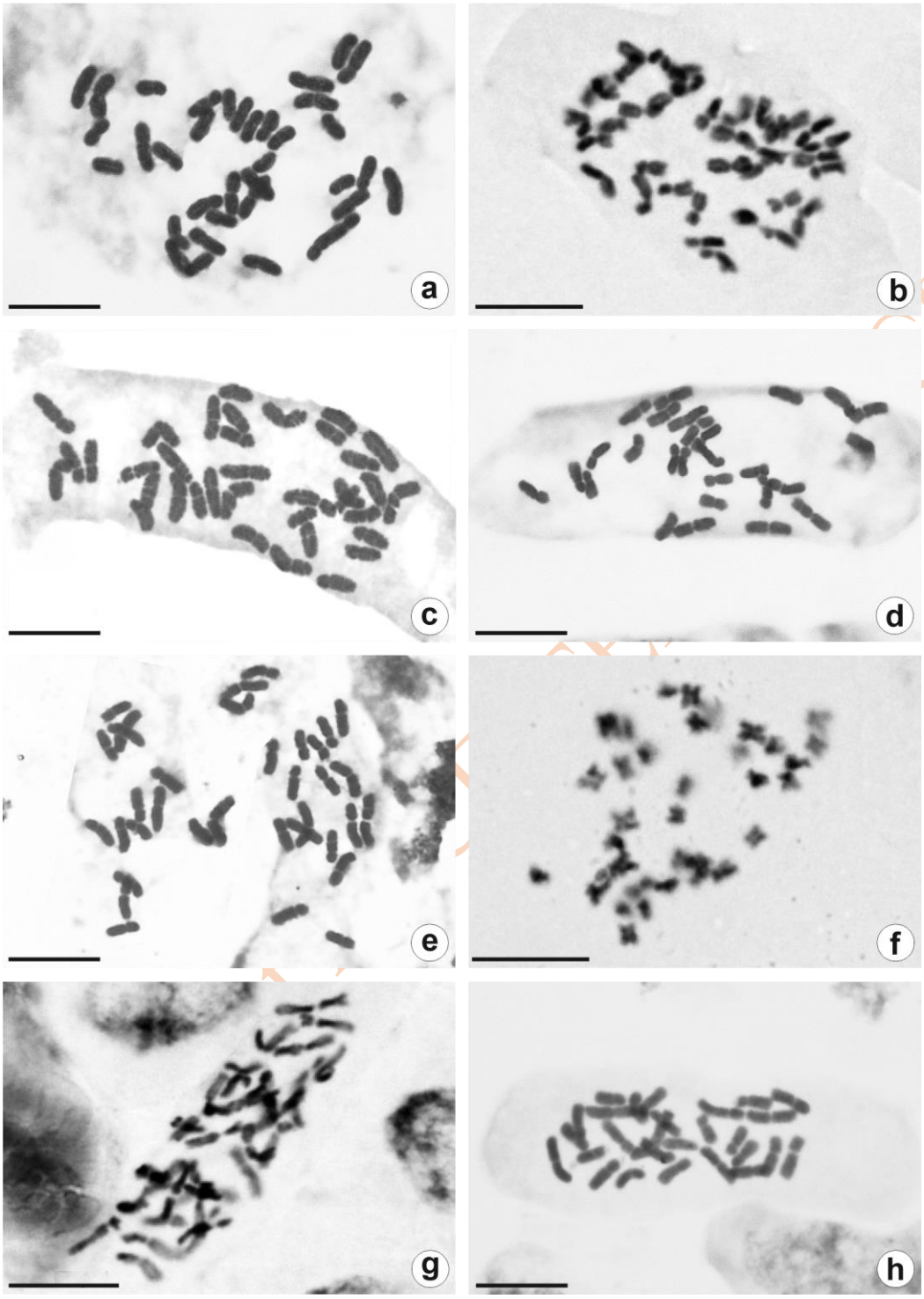

Figure 2

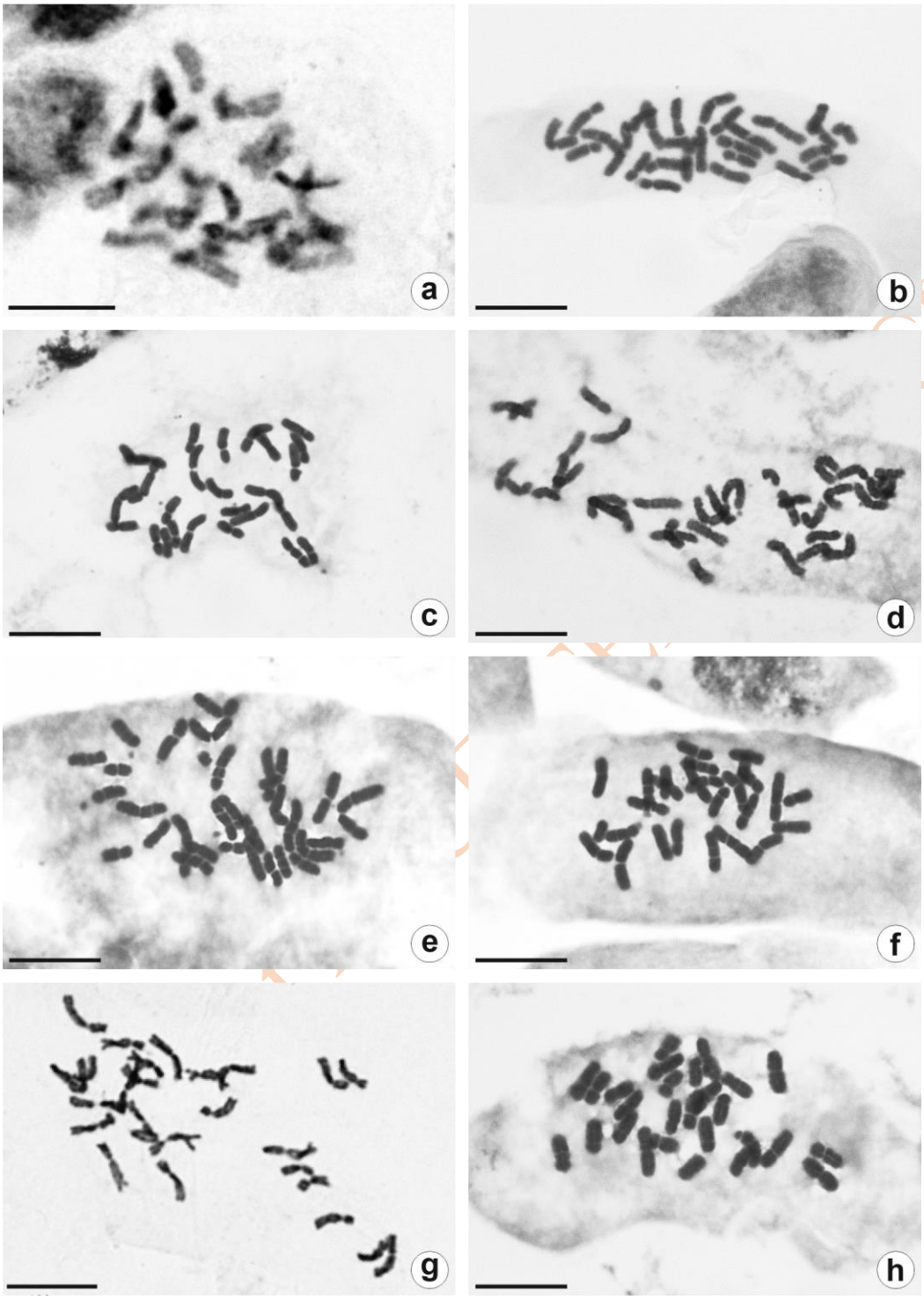

Figure 3