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Equisetum ×*meridionale* (Milde) Chiov. – a new hybrid taxon in the flora of Poland

Dariusz Tlałka[1]*, Elwira Sliwinska[2] & Jerzy Kruk[3]

[1]Independent/non-affiliated researcher; ORCID: DT https://orcid.org/0000-0001-5607-0719
[2]Laboratory of Molecular Biology and Cytometry, Department of Agricultural Biotechnology, Bydgoszcz University of Science and Technology, Kaliskiego 7, 85-796 Bydgoszcz, Poland; ORCID: ES https://orcid.org/0000-0001-6597-5309
[3]Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland; ORCID: JK https://orcid.org/0000-0003-4969-4907
* corresponding author (e-mail: tlalkadariusz@gmail.com)

Abstract. A new hybrid within the *Hippochaete* subgenus of *Equisetum* genus, *Equisetum* ×*meridionale* (Milde) Chiov. was discovered in Poland during the research conducted in 2022 at two locations in Kotlina Żywiecka (the Żywiec Basin) and Beskid Wyspowy (Island Beskids – Western Beskids) in the anthropogenic habitats in close proximity to *Equisetum ramosissimum*. The identity of the hybrid was unequivocally confirmed by macro-morphological observations, microscopic analysis and flow cytometry. The most pronounced macroscopic characteristics of the hybrid were the intermediate size of the shoots and leaf sheaths, which are black with long black teeth. The microscopic observations revealed that the hybrid has silica tubercles in the form of cross-bands, similarly as in the case of *E. ramosissimum,* but not in the form of two rows as in the case of *E. variegatum.* The measurements of the nuclear DNA content (2C values) revealed that the putative hybrid had a nuclear DNA content intermediate to that of the parental species, i.e. *E. ramosissimum* (56.13 pg) and *E. variegatum* (63.80 pg) obtained in the present studies and within the values previously reported for *E.* ×*meridionale* (60.7-61.2 pg).

Keywords: *Equisetum* ×*meridionale*, new hybrid taxon, Poland, Western Beskids

1. Introduction

Among the 15 *Equisetum* species known worldwide 9 of them occur in Poland (Mirek *et al.* 2020). Besides these species several hybrids have been recorded in our country. Within the *Equisetum* subgenus *E.* ×*litorale* Kühlew. ex Rupr. (*E. arvense* × *E. fluviatile*)*, E.* ×*robertsii* T. D. Dines (*E. arvense* × *E. telmateia*) and *E.* ×*font-querii* Rothm. (*E. palustre* × *E. telmateia*) have been found (Wróbel 2013a). The first nothotaxon is supposed to be widespread in Europe (Wróbel 2013a) but its distribution in Poland is poorly known (Tlałka & Rostański 2012; Wróbel 2013a). *E.* ×*robertsii* is known from the 3 contemporary and 2 historical records from the Carpathians (Foothill and Beskid Niski – Low Beskids) (Wróbel 2013b), while *E.* ×*font-querii* was recorded at only one, already historical stand in the northwestern part of Beskid Niski (Wróbel 2013a). Within the *Hippochaete* Milde subgenus *E.* ×*moorei* Newman (*E. hyemale* × *E. ramosissimum*) and *E.* ×*trachyodon* (A. Braun) W. D. J. Koch (syn. *E. mackayi* (Newman) Brihan) (*E. hyemale* × *E. variegatum*) were reported in Poland (Tlałka & Rostański 2012; Wróbel 2013a; Kalinowski *et al.* 2016). However, the taxonomic identity of the individuals representing the latter hybrid is uncertain (Tlałka, pers. comm.). *E.* ×*moorei* was found at five contemporary and eleven historical localities in the present territory of Poland, scattered throughout the country (Kalinowski *et al.* 2016). As another nothotaxon of the *Hippochaete* subgenus *E.* ×*meridionale* (Milde) Chiov. was identified in many European countries (de Winter & de Somer 2021; Hassler 2022), the aim of this study was to find this taxon in the Western Beskids (Beskidy Zachodnie).

2. Materials and Methods

The field studies in Beskidy Zachodnie were conducted in 2022. The morphological characteristics of the taxa, presented in Table 1, were compiled using

Table 1. Diagnostic characteristics of *Equisetum ramosissimum*, *E.* ×*meridionale* and *E. variegatum*

Trait	*Equisetum ramosissimum*	*Equisetum* × *meridionale*	*Equisetum variegatum*
Stem height and diameter	20-100 × 0.2-0.9 cm	20-50 × 0.2-0.3 cm	10-30 × 0.1-0.3 cm
Stem branching	at the base or regularly along the entire length of the stem	unbranched or irregularly branched	unbranched or rarely at the base of the stem
Stem sheaths	without transverse black stripe, with short black serration at the top	with black or with a transverse black stripe, with long black teeth	with a transverse black stripe, with short black teeth
Appearance and number of sheath teeth	without white margins, 8-20	with broad or narrow white margins, 6-10	with broad white margins, 4-10
Size of the central channel	3/4 of the stem diameter	1/3 of the stem diameter	1/3 of the stem diameter
Spores	normally developed	abortive	normally developed

literature data (Jepson *et al.* 2013) and own observations.

A Bresser Advance ICD 10×-160× microscope (Meade Instruments Europe GmbH & Co. KG, Germany) was used for the microscopic observations and the photographs of the shoots.

Flow cytometry (FCM) was applied for the nuclear DNA content estimation. Plants from the following

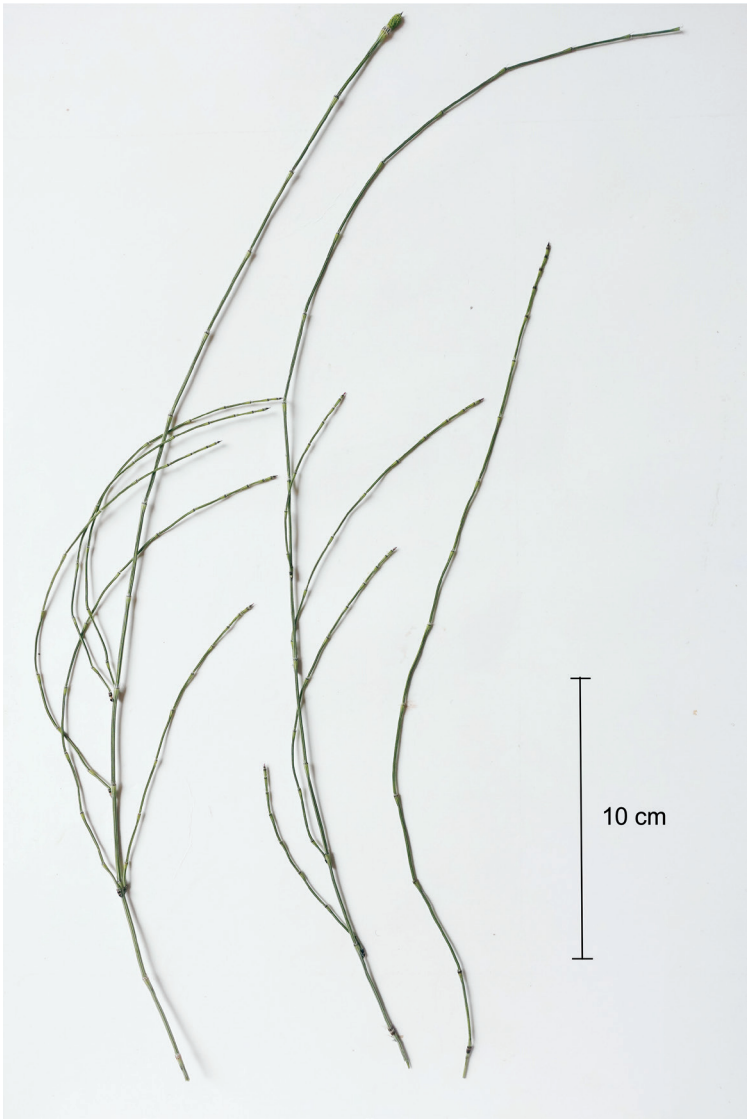

Fig. 1. Herbarium specimen of *Equisetum* ×*meridionale* from Pewel Mała (Koszarawa River Valley, Kotlina Żywiecka) (photograph by D. Tlałka, July 23, 2022)

locations were used: *E. ramosissimum* and *E. ×meridionale* from Pewel Mała as well as *E. variegatum* from an abandoned quarry in Kozy. The samples were prepared and analyzed as previously described (Kalinowski *et al.* 2016). *Allium cepa* 'Alice' (34.89 pg/2C; Doležel *et al.* 1998) was used as the internal standard. From four to five *Equisetum* shoots, collected from each population, were individually analyzed using the CyFlow SL Green flow cytometer (Partec GmbH, Münster, Germany). For each sample 3000-5000 nuclei were used to determine the DNA content. The histograms were analyzed using the FloMax software (Partec GmbH, Münster, Germany). The coefficient of variation (CV) of the G_0/G_1 peak of the *Equisetum* samples ranged from 4.86 to 7.12%. The nuclear DNA content was calculated using linear relationship between the ratio of the 2C peak positions (*Equisetum/Allium*) on a histogram of fluorescence intensities.

3. Results

During the research, conducted by the first author in 2022, small populations of plants (morphologically intermediate between *E. ramosissimum* and *E. variegatum*) were found at locations, where *E. ramosissimum* had been reported from Beskidy Zachodnie, i.e. in Pewel Mała (Fig. 1) and Raba Niżna (Wróbel 2008). Detailed morphological analysis of these plants suggested, that they represent the hybrid *E. ×meridionale*, which shows intermediate characteristics between the parent species *E. ramosissimum* and *E. variegatum*. The most pronounced macroscopic characteristics of the hybrid are the intermediate size of the shoots and the appearance of the leaf sheaths, which are black with long black teeth (Fig. 2). The morphological characteristics of *E. ×meridionale* and its parent species, *E. ramosissimum* and *E. variegatum*, are presented in Table 1.

Fig. 2. Sheaths of A) *Equisetum ramosissimum* (Pewel Mała), B) *E. ×meridionale* (Pewel Mała), C) *E. variegatum* (Pogoria I Water reservoir, Katowice Upland). Microscopic details of the stem ridges are shown in the lower panel

The microscopic observations of the individual shoots from Pewel Mała revealed, that the hybrid has silica tubercles in the form of cross-bands, similarly as in the case of *E. ramosissimum,* but not in the form of two rows as in the case of *E. variegatum* (Fig. 2). All these data suggest that the plants represent *E.* ×*meridionale*.

Table 2. Nuclear DNA content (pg/2C) of the investigated *Equisetum* taxa. The data are means ± SD (*n* = 4-5)

Taxon	DNA content (pg/2C)
E. ramosissimum	56.13 ± 0.36
E. ×*meridionale*	60.83 ± 0.84
E. variegatum	63.80 ± 0.81

Besides, the morphological characteristics – the most unequivocal evidence discriminating species and hybrids is the chromosome number or the nuclear DNA content. The FCM measurements of the nuclear DNA content (2C values) revealed, that the putative hybrid has a nuclear DNA content, intermediate to that of the parental species, i.e. *E. ramosissimum* and *E. variegatum* (Table 2). The obtained 2C values for these two species are rather close to those published previously; for *E. ramosissimum* 52.5 pg (for *E. debile*, Obermayer *et al.* 2002), 54.56 pg (Kalinowski *et al.* 2016) and 56.3 pg (Bennert *et al.* 2005); for *E. variegatum* 60.8 pg (Obermayer *et al.* 2002) and 63.3 pg (Bennert *et al.* 2005). Moreover, the obtained 2C value for the putative hybrid was within the values previously reported for *E.* ×*meridionale* (60.7-61.2 pg) (Bennert *et al.* 2005). All these data indicate that the analyzed plants unequivocally belong to *E.* ×*meridionale.*

Unlike other hybrids of the *Hippohaete* subgenus which were reported to occur in Poland (*E.* ×*moorei* and *E.* ×*trachyodon*), *E.* ×*meridionale* is more branched. Another morphological difference between *E.* ×*meridionale* and *E.* ×*trachyodon* is the presence of silica cross-bands on the surface of the shoots of the former, but not the latter hybrid (Jepson *et al.* 2013). *E.* ×*meridionale* differs from *E.* ×*moorei* in the height and the diameter of the stems, as well as in the appearance of the sheaths.

A key to the discussed taxa from the *Hippohaete* subgenus which can be found in Poland is given below.

1 Spores; properly developed, green, spherical 2
1* Spores; abortive, colorless, irregularly shaped – intermediate level of these traits in the plants compared with the ones of theparentspecies 4
2 Stems; branched at the base or along the entire length of the stem, no frost resistance in the plants (*E. ramosissimum*)

Fig. 3. *Equisetum* ×*meridionale* at Pewel Mała (Koszarawa River Valley, Kotlina Żywiecka) (photograph by J. Kruk, September 10, 2022)

2* Stems; unbranched or rarely branched at the base, winter-hardy 3
3 Stems; 40-100 cm tall, sheath teeth not persistent (*E. hyemale*)
3* Stems; unbranched or rarely branched and 10-30 cm tall, sheath teeth persistent or at least partly so with narrow dark centers and very broad white margins (*E. variegatum*)
4 Stem ridges; with silica tubercles in two distinctly separated rows (*E. ×trachyodon*)
4* Stem ridges; with silica tubercles forming cross-bands or sometimes merging into two indistinctly separated rows 5
5 Stems; 20-50 cm tall and 0.2-0.3 cm wide in diameter, sheaths usually blackish with rather long black teeth and white margins (*E. ×meridionale*)
5* Stems; 50-120 cm tall and 0.4-0.6 cm wide in diameter, sheaths light-brown without (in older shoots) or with long dark-brown teeth and narrow light borders (*E. ×moorei*)

The location of the new hybrid from the flora of Poland is as follows:

- Pewel Mała, Koszarawa River Valley, Kotlina Żywiecka, on the railway track near a willow-alder forest (Figs. 3-4), several dozen clumps, 380 m a.s.l., DG0444 square (ATPOL grid 2 × 2 km) (Zając 1978), ca. 150 m west of the railway crossing with Malownicza Street, not far from *E. ramosissimum*.
- Raba Niżna, Raba River Valley, Beskid Wyspowy, on the railway track, 430 m a.s.l., EG1011, near the railway station, close to *E. ramosissimum*.

4. Discussion

In Poland, hybrids of the *Hippochaete* subgenus (*E. ×moorei* and *E.* c.f. *×trachyodon*) have been recorded so far only in the Polish lowlands and the highlands (Wróbel 2013a; Kalinowski *et al.* 2016). *E. ×meridionale* has been presently identified at low altitudes in Kapraty Zachodnie (the Western Carpathians), but its occurrence in Poland can be expected throughout the country, especially where both its parent species co-occur (Zając & Zając 2001). Besides that, *E. ×meridionale* has been previously recorded in the countries neighboring Poland: Germany, the Czech Republic and Slovakia (de Winter & de Somer 2021; Hassler 2022). Moreover, this nothotaxon has been identified throughout Europe where both parent species occured, i.e. excluding Southern and Northern Europe (de Winter & de Somer 2021; Prelli & Boudrie 2022).

At both locations in Beskidy Zachodnie *E. ×meridionale* grows in similar, anthropogenic habitats. The hybrid was found on the railway track, close to a riverside forest (Fig. 4) and not far from *E. ramosissimum*.

Fig. 4. Habitat of *Equisetum ×meridionale* at Pewel Mała (Koszarawa River Valley, Kotlina Żywiecka) (photograph by D. Tlałka, July 12, 2022)

These stands are endangered by their close location to the places of direct human activity. It is highly probable that further occurrences of *E.* ×*meridionale* will be discovered in Poland at similar habitats and in the vicinity of its parent species.

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Research concept and design: D. Tlałka
Collection and/or assembly of data: D. Tlałka, E. Sliwinska, J. Kruk
Data analysis and interpretation: D. Tlałka, E. Sliwinska, J. Kruk
Writing the article: D. Tlałka, E. Sliwinska, J. Kruk
Critical revision of the article: D. Tlałka, J. Kruk
Final approval of article: D. Tlałka

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