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SHORT COMMUNICATION in FLORISTICS AND PLANT CONSERVATION

Polystichum setiferum at the Northeastern Limit of Its Distribution Range

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Abstract

During field studies in 2019 in the Beskid Śląski mountain range in southern Poland, one individual morphologically resembling *Polystichum setiferum* was found. Nuclear DNA content analysis confirmed the species identity. The new stand of *P. setiferum* corresponds to historical literature reports and herbarium specimens from the Czech Republic and Poland, and extends the northeastern limit of the distribution of this species. As the historical stands in both countries have not been confirmed, the newly discovered stand is at present strongly isolated, as the nearest extant locations of *P. setiferum* in Austria, southern Hungary, and western Romania are nearly 500 km away. Therefore, this unique stand deserves special attention and protection.

Keywords

distribution; Europe; native species; Poland; *Polystichum setiferum*

1. Introduction

Polystichum setiferum (Forssk.) Moore ex Woynar. is a fern species with an Atlantic and sub-Mediterranean distribution, from Canary Islands, northern Africa to Ireland in the west, to the Balkans and the Caucasus Mountains in the east (Dostál & Reichstein, 1984). Its northeastern limit of distribution in central Europe has been supposed until recently to lie in Hungary and Romania (Dostál & Reichstein, 1984). Although this species was reported from regions close to the Polish-Czech border as early as in the nineteenth century (Milde, 1855, 1865) under different names (e.g., *Aspidium aculeatum* Sw.), it was regarded not to occur in the Czech Republic and Poland by contemporary floras (Dostál & Reichstein, 1984; Šourková, 1997; Szafer et al., 1988). This was due to the lack of herbarium documentation and similarity of some forms of *Polystichum aculeatum* (L.) Roth to *P. setiferum*, leading to frequent misidentification (Dostál & Reichstein, 1984). Nevertheless, a recent detailed morphological study of herbarium materials allowed the identification of *P. setiferum* in herbarium collections dated 1881–1935 from the territory of the Czech Republic (Ekrt, 2016). These individuals came from Moravskoslezské Beskydy Mountains (Mt Kněhyně and Mt Smrk), Moravský kras (Olomučany), Jeseníky Mountains (Rýmařov, Mt Praděd, Velká kotlina), and Rychlebské hory (Lázně Jeseník) (Figure 1). Currently, *P. setiferum* has not been confirmed at these locations, and it is regarded as an extinct species in the Czech flora (Kaplan et al., 2017). The species occurs on moderately acidic, humid soil in beech and mixed forests in moderately high mountains with mild winters (Dostál & Reichstein, 1984). *Polystichum setiferum* is a diploid species ($2n = 82$), which is one of the parental species of the allotetraploid *P. aculeatum* ($2n = 164$) (Dostál & Reichstein, 1984). Therefore, these morphologically similar species can be unequivocally distinguished by chromosome count or nuclear DNA content analysis.

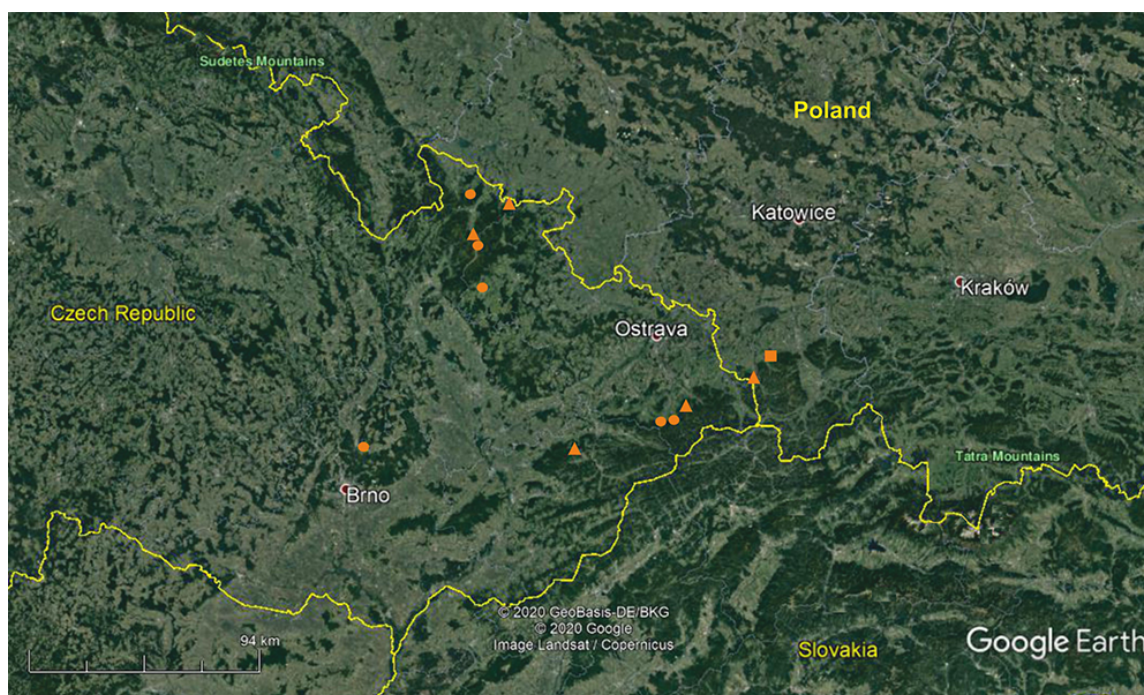


Figure 1 Distribution of *Polystichum setiferum* at the northeastern limit of its range based on verified herbarium specimens (circles), literature data (triangles), and the present study (square). Historical records were based on Ekrt (2016) and updated from Kaplan et al. (2017).

Table 1 Morphological and cytogenetic comparison of *Polystichum setiferum* and *P. aculeatum*.

Character	<i>Polystichum setiferum</i>	<i>Polystichum aculeatum</i>
Leaves	Bright green, mat, feels soft Truncate at base Petiole usually more than 1/6 leaf length	Deep green, glossy, feels stiff Tapering to base Petiole usually less than 1/6 leaf length
Pinnae	Apex with distinct, protruding teeth	Apex with indistinct, adherent teeth
Pinnules	Obtuse to acute angled base, stalked, hair-tipped The upper pinnula, nearest rachis, hardly longer or equal to the following on the lowest pinnae	Acute angled base, usually sessile, spiny-tipped Considerably longer than the following
Scales	Bright brown on young plants, bright brown with dark brown interior on adult plants	Brown
Ploidy	Diploid; $2n = 82$	Tetraploid; $2n = 164$

See Material and Methods for details.

Based on past and recent data, we undertook field surveys for *P. setiferum* in the Opawskie Mountains and in the western part of the Beskid Śląski mountain range (Silesian Beskid), areas close to the historical locations of the species in the Eastern Sudetes and the Western Carpathians, respectively. The aim of our study was to verify the presence of *P. setiferum* in Poland in light of the historical data.

2. Material and Methods

Field studies aimed at finding *P. setiferum* in the Opawskie Mountains and Beskid Śląski were conducted between 2018 and 2020. According to our observations, the best season for the search of *P. setiferum* is winter when all other species besides those of *Polystichum* are gone.

Analysis of the morphological characteristics, listed in Table 1, was based on data from literature (Dostál & Reichstein, 1984; Rich & Jermy, 1998).



Figure 2 Leaves of *Polystichum setiferum* (right) from Mt Czupel in the Beskid Śląski and *P. aculeatum* (left) from the Soła Valley in Kobiernice in the Pogórze Śląskie (Poland) (photo by D. Tlalka, January 8, 2020).

Herbarium studies were performed at the herbaria of the Jagiellonian University (KRA) and the Institute of Botany, Polish Academy of Sciences (KRAM).

Stomatal cell length ($n = 100$) was determined using leaf fragments placed between glass slides in a water drop, and was measured using a Delta Optical microscope, model Genetic Pro, with an ocular micrometer and a calibration glass slide (Opta-Tech, Poland).

For nuclear DNA content estimation, five leaf samples from one individual of putative *P. setiferum* and three samples from different *P. aculeatum* individuals, growing in the neighborhood of *P. setiferum*, were analyzed on three different days. Nuclei were released simultaneously from fresh leaves a sample species and an internal standard (*Allium cepa* 'Alice', 34.89 pg/2C; Doležel et al., 1998) by placing



Figure 3 Leaf fragments of *Polystichum setiferum* (top) and *P. aculeatum* (middle) from Mt Czupel, as well as that of *P. aculeatum* var. *aristatum* Christ. (bottom) from Mt Jasieniowa in the Pogórze Cieszyńskie (Poland).

chopped leaves in a Petri dish with 1 mL of Galbraith's buffer (Galbraith et al., 1983) supplemented with propidium iodide (PI; $50 \mu\text{g cm}^{-3}$), ribonuclease A ($50 \mu\text{g cm}^{-3}$), and 1% (v/v) polyvinylpyrrolidone (PVP). The suspension was passed through a 50- μm mesh nylon filter and analyzed using a CyFlow SL Green (Partec GmbH, Germany) flow cytometer equipped with a high-grade solid-state laser with green light emission at 532 nm, long-pass filter RG 590 E, DM 560 A, as well as side (SSC) and forward (FSC) scatters. Analyses were performed on three–four samples of each taxon. For each sample, the DNA content was established in 3,000–5,000 nuclei. Histograms were analyzed using the software FloMax (Partec GmbH). The coefficient of variation (CV) of the G_0/G_1 peak of *Polystichum* sp. ranged between 3.8% and 5.2%. Nuclear DNA content was calculated using the linear relationship between the ratio of the 2C peak positions *Polystichum/Allium* on a histogram of fluorescence intensities.

3. Results and Discussion

Field studies performed during 2018–2020 in Poland, in regions of historical occurrence of *P. setiferum*, led to the identification of one individual in the western part of the Beskid Śląski mountain range (Figure 1), morphologically corresponding well to this species (Figure 2 and Figure 3). It was found by the first author on



Figure 4 Habitat of *Polystichum setiferum* at Mt Czupel in the Beskid Śląski (Poland) (photo by D. Tlalka, July 7, 2020).

December 21, 2019, on the southwestern slopes of Mt Czupel near Brenna, in the western part of the Beskid Śląski mountain range, in one of the abandoned sandstone quarries at ca. 480 m a.s.l. (ATPOL DG0201 square), covered with a 100-year-old beech forest with an admixture of artificially planted larch trees in a half-shaded habitat (Figure 4). This plant was accompanied by several individuals of *P. aculeatum* located further apart.

The identification was based on the investigation of the morphological characteristics distinguishing *P. setiferum* and *P. aculeatum* (Table 1). The most pronounced morphological characteristics of *P. setiferum* differentiating it from *P. aculeatum* are the relatively long petiole, leaf blade truncated at the base, and stalked, hair-tipped pinnules.

Stomata length or stomatal cell (guard cell) length could also be useful in differentiating the two species (Ekrt, 2016). The results of the stomatal cell measurements of *P. setiferum* and *P. aculeatum* from the stand (Figure 5) show that the length of stomatal cells of the putative *P. setiferum* individual was evidently lower than that of *P. aculeatum* plants. The length ratio of *P. setiferum* to *P. aculeatum* stomatal cells was 0.8, which corresponds well with the stomata length ratio of specimens from the Czech Republic (0.8) (Ekrt, 2016) and those originating from southern Europe (0.76) (Ekrt, 2016).

In addition to morphological characteristics, the most unequivocal evidence discriminating the two species is the chromosome number count or the corresponding nuclear DNA content measurements. Nuclear DNA content (2C values) of the putative *P. setiferum* and *P. aculeatum* growing in the neighborhood, collected from the new stand, were 15.46 and 28.77 pg, respectively (Table 2). To the best of our knowledge, this is the first study to estimate the genome size of these species (Leitch et al., 2019). The results revealed that 2C values of the investigated species differed nearly twofold, corresponding well with their ploidy level (Dostál & Reichstein, 1984) (Table 1). Moreover, the 2C value of *P. setiferum* found in our

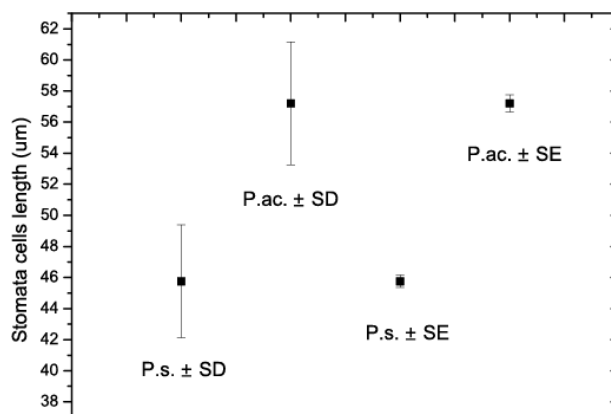


Figure 5 Length of stomata cells (guard cells) of *Polystichum setiferum* (P.s.) and *P. aculeatum* (P.ac.) from the stand in the Beskid Śląski. The means \pm SD and \pm SE ($n = 100$) are shown.

Table 2 DNA content (pg/2C) of the investigated *Polystichum* species from Mt Czupel in the Beskid Śląski.

Species	DNA content (pg/2C)
<i>Polystichum setiferum</i>	15.456 \pm 0.070
<i>Polystichum aculeatum</i>	28.773 \pm 0.199

The data are means \pm SD ($n = 5$ and 3 for *P. setiferum* and *P. aculeatum*, respectively). See Material and Methods for details.

studies is the same as that of the diploid American species, *P. acrostichoides* (Michx.) Schott (15.5 pg/2C) (Bainard et al., 2011). These data indicate that the analyzed *P. setiferum* individual indeed represents this diploid species and not tetraploid *P. aculeatum* or its triploid hybrid with *P. setiferum*, that is, *P. \times bicknellii* (Christ) Hahne, which should have a 2C value intermediate between those of the parental species.

An additional search for *P. setiferum* specimens among those of *P. aculeatum* collected in Poland in the KRA and KRAM herbaria did not result in finding *P. setiferum* even though hundreds of *P. aculeatum* sheets were deposited in these herbaria. This indicates that *P. setiferum* must have already been a very rare species in the past.

The newly identified, isolated stand of *P. setiferum* is situated approximately 10 km from the nearest historical location at Wielka Czantoria near Ustroń (Milde, 1855) and nearly 500 km from other existing populations of this species in Austria (Steiermark) (Virtual Herbaria, 2020, search term: “*Polystichum setiferum*”), southern Hungary (Mecsek Mountains) (Bátori et al., 2009), and western Romania (near Arad) (Virtual Herbaria, 2020, search term: “*Polystichum setiferum*”).

The present finding of *P. setiferum* determines its northeastern limit of distribution in Europe, which is a remnant of the more widespread occurrence of this species in the past. However, it cannot be excluded that the discussed stand is a result of a relatively new colonization by spores originating from other presently unidentified or extinct sites located in Poland or the Czech Republic.

As it is the only extant stand of *P. setiferum* in the area of the Sudetes and the Carpathian Mountains, it deserves special attention and protection.

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