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Polystichum caucasicum sp. nov. (Dryopteridaceae), a new, surprisingly hexaploid fern species from the Caucasus

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Abstract

A new fern species, *Polystichum caucasicum sp. nov.*, is described from the Caucasus region and adjacent areas of Georgia, Armenia, Turkey and Russia. The species was likely overlooked due to its morphological similarity to *P. braunii* and *P. aculeatum*, but it can be distinguished by a combination of characters, including a densely scaly rachis, enlarged basal acroscopic pinnules, and long-pointed pinnae. *P. caucasicum* could also be misidentified as *P. kadyrovii* in this region; however, a revision of the type material confirms that *P. kadyrovii* is conspecific with *P. aculeatum* and therefore not related to the newly described taxon. Genome size analyses (flow cytometry) of 20 individuals revealed an average 2C genome size of 44.7 pg, indicating a hexaploid cytotype, which is also supported by guard cell size measurements. In addition, a comprehensive comparative analysis of genome size characteristics was conducted across the majority of European and Caucasian *Polystichum* species, providing estimates of genome size, monoploid genome size (1Cx), and genomic GC content. The species produces well-developed fertile spores. *Polystichum caucasicum* appears to be an endemic of the Caucasus region and adjacent areas, growing in a wide ecological range in humus-rich and rocky beech, ravine or alluvial forests dominated by *Acer*, *Fagus*, *Tilia* and *Alnus*. Its habitat preferences are documented through four phytosociological relevés and realized climatic niche of the species at known locations was performed using the Chelsa Bioclim dataset.

Key words: Caucasus, ferns, flow-cytometry, genome size, guard cells, habitat preference, ploidy, *Polystichum*, taxonomy

Introduction

The genus *Polystichum* Roth (1799: 69) belonging to Dryopteridaceae comprises about 400–500 species worldwide (Jorgensen & Barrington 2017, Hassler 2025) with the main diversity centers in southeastern Asia and Central-Southern America (Zhang & Barrington 2013). It is notorious for being taxonomically complex with many similar and highly variable species, frequent allopolyploidy, hybridization and apomixis (Little & Barrington 2003; Morero *et al.* 2015). Besides frequent diploid and tetraploid taxa, only a few hexaploid and octoploid taxa are known so far (Barrington 2006, Morero *et al.* 2015).

So far, six species of the genus *Polystichum* are known from the region of the Caucasus (Askerov 2001, 2022). There are two diploid species—*Polystichum lonchitis* (L.) Roth (1799: 71), *P. setiferum* (Forssk.) Woynar (1913:

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181), and three tetraploids—*P. aculeatum* (L.) Roth (1799: 79), *P. braunii* (Spenn.) Fée (1852: 278), prominently Caucasian—*P. woronowii* Fomin (1911: 21), and one, *P. kadyrovii* Askerov & A. E. Bobrov (1972: 1297), of unknown cytotype. This taxon was described from the Talysh Mts of Azerbaijan.

During a botanical excursion to the Caucasus region in Georgia an unknown, still not recognized type of *Polystichum* was discovered. It is morphologically very similar to *P. braunii* or the hybrid *P. braunii* × *aculeatum* – *P.* × *luerssenii* Hahne (1904: 103). However while *P. braunii* and *P.* × *luerssenii* are tetraploids, this type was found to be hexaploid. In addition, plants in the Arctic-Alpine Garden in Chemnitz, Germany, bearing the name *P. braunii* from two collections from the Caucasus by J. Hemmerling and W. Meusel in 1965 and 1966 (see Appendix 1) are also hexaploid and belong to the new taxon. We describe this new species as *Polystichum caucasicum*, which has thus far only been found in the Caucasus and its surroundings. Its species specificity is demonstrated through comparisons of morphology and genome size with European and Caucasian *Polystichum* species.

Material and methods

Material used

The present taxonomical examination is based on fieldwork in Georgia, Armenia and Turkey during 2022–2025. Some herbarium revisions were carried out in herbarium CBFS, JE, PR, PRC (abbreviations follow Thiers 2025). The majority of the collections examined by us are deposited at CBFS and BRNU and some representatives are planted in The Arctic-Alpine Garden in Chemnitz (Germany, S. Jessen) and the private garden in Telč (Czech Republic, L. Ekrt). For the complete list of specimens seen or collected see Appendix 1.

Flow cytometry

Flow cytometry was carried out for identification of cytotype and for estimation of genome size of representatives of genus *Polystichum*. We used only fresh material in the two-step protocol with Otto buffers (Otto 1992). Individual plants were chopped with the internal standard in 400 µl of Otto I buffer using a sharp razor blade in a Petri dish and filtered through 42 µm nylon mesh. After 3–5 min 800 µl of Otto II buffer with 2-mercaptoethanol (2 mg/ml) and fluorochrome DAPI (4,6-diamidino-2-phenylindole, final concentration 4 µl/ml) staining AT basis was added into the sample (relative genome size = RGS). Stained samples were analysed using a CyFlowSpace instrument (Sysmex-Partec) equipped with 365 nm UV-LED as a light source. Fluorescence intensity of 3000 particles was recorded. Total genome size was determined using propidium iodide (PI) staining, which stains all bases (AT + GC) indiscriminately. The sample preparation was identical to DAPI stain, only using PI at final concentration 50 µg/mL as fluorochrome instead of DAPI. Fluorescence intensity of 5000 particles was analyzed using a CyFlowSpace instrument (Sysmex-Partec) equipped with a green solid-state laser (Cobolt Samba 532 nm, 100 mW). In PI analyses, a mean value of three measurements on different days was used for genome size calculation. Flow cytometry histograms were evaluated using Flowing Software 2.5.1 (P. Terho, University of Turku, freeware available at https:// bioscience.fi/services/cell-imaging/flowing-software/). As internal standards *Pisum sativum* 'Ctirad' (2C DNA = 9.09 pg; Doležel *et al.* 1998), *Chlorophytum comosum* (2C DNA = 24.14 pg; Hornych *et al.* 2019) and *Vicia faba* 'Inovec' (2C DNA = 26.90 pg; Doležel *et al.* 1992) were used for the flow-cytometric measurements. Proportion of AT/CG basis was performed following Šmarda *et al.* (2008). The relationship between sample relative fluorescence (i.e., ratio of sample/standard mean fluorescence) and ploidy level was extrapolated from comparison with other reference taxa with known ploidy level: *Polystichum aculeatum* (4*x*), *P. braunii* (4*x*), *P. lonchitis* (2*x*), *P. setiferum* (2*x*), *P. woronowii* (4*x*), *P. setigerum* (6*x*), *P. monticola* (6*x*); see Appendix 1.

Phytosociological relevés and climate data

We recorded phytosociological relevés to characterize habitat preferences of *Polystichum caucasicum*. For evaluation of coverage of plants in phytosociological relevés was used the 9-degree Braun-Blanquet scale (Dengler *et al.* 2008). The plots were stored in the Transcaucasian Vegetation Database (Novák *et al.* 2023). Vascular plant names (except ferns) follow Euro+Med (2006–Present). Nomenclature for ferns was unified according to PPG1 (2016), and for mosses according to Hodgetts *et al.* (2020). To outline the realized climatic niche of the species, we utilized the CHELSA BIOCLIM dataset (Karger *et al.* 2017, https://chelsa-climate.org/bioclim/). We included the following variables: Mean annual temperature (BIO1), Temperature seasonality (BIO4), Minimal temperature of the coldest month (BIO6), Annual precipitation (BIO12) and Precipitation seasonality (BIO15).