Reviewed research article

# Chromosome number, genome size and spore morphology of the Iceland-endemic fern *Struthiopteris fallax* in comparison with its related taxa

JÓHANNES BJARKI URBANCIC TÓMASSON[1, 2], EVA M. TEMSCH[3], HJÖRTUR ÞORBJÖRNSSON[4] AND KESARA ANAMTHAWAT-JÓNSSON[1*]

[1] Institute of Life and Environmental Sciences, University of Iceland, Askja, Sturlugata 7, 102 Reykjavík, Iceland

[2] Sveppafélagið ehf., Brávallagata 44, 101 Reykjavík, Iceland

[3] Department of Botany and Biodiversity Research, Faculty of Life Sciences, University of Vienna, Rennweg 14, 1030 Wien, Austria

[4] Reykjavík Botanic Garden, P.O. Box 8372, 128 Reykjavík, Iceland

* Corresponding author: Kesara Anamthawat-Jónsson, Institute of Life and Environmental Sciences, University of Iceland.
E-mail: kesara@hi.is

## ABSTRACT

*Struthiopteris fallax* (isl, *tunguskollakambur*) is an Iceland-endemic deer fern species known from a single geothermal site by the hot spring Deildartunguhver in Borgarfjörður, western Iceland. It is closely related to *Struthiopteris spicant* var. *spicant* (isl, *skollakambur*), a widespread species in northern Icelandic lowlands, typically found in areas with persistent snow cover. The present study investigated taxonomically relevant characteristics of *S. fallax*, including chromosome number, genome size and spore characteristics, in comparison with *S. spicant* var. *spicant* from Iceland and the Iberian Peninsula. Field-collected samples included fiddleheads or young frond tips for chromosome analysis with light/fluorescence microscopy (LM), and genome size estimation using flow cytometry (FCM). Fertile fronds were used for spore size measurement and morphological analysis via scanning electron microscopy (SEM). Spore size data were statistically evaluated. The LM results indicated that *S. fallax* is most likely diploid ($2n=2x=68$), like *S. spicant*. The present study is the first to document meiotic chromosomes in *S. fallax*, inferring its haploid gametic number of 34. The FCM results showed average 1C genome sizes of 6.96 pg for *S. fallax* and 6.91 pg for Icelandic *S. spicant*, with a combined average of 6.94 pg, only a 1.02% deviation from the single existing record for *S. spicant* in the Plant DNA C-values Database. The spore analysis by SEM showed that spores of *S. fallax* (mean equatorial length: 41.70 µm) are not statistically different in size from those of *S. s.* var. *spicant* from Iceland and Spain (means: 43.58 and 42.32 µm, respectively). On the other hand, the spore of *S. fallax* is clearly different in morphology from *S. spicant* in that it is oblate/elliptic in shape with smooth outline and filamentous pattern of perispore ornamentation, whereas the spore of *S. s.* var. *spicant* appears more rounded and angular in shape with irregular outline and rugulate/reticulate pattern of perispore ornamentation. These findings highlight the diagnostic value of spore morphology in fern taxonomy.

## INTRODUCTION

The genus *Struthiopteris* Scop., which belongs to the leptosporangiate fern family Blechnaceae, comprises five temperate species, mostly from China and Japan, with *Struthiopteris spicant* (L.) Weiss (type species) having a circumboreal distribution (de Gasper *et al*. 2016; PPG I 2016). Recently, one new species was added to the list, that is, the Iceland-endemic *Struthiopteris fallax* (Lange) S. Molino, Gabriel y Galán & Wasowicz, upgraded from the variety status *S. spicant* var. *fallax* (Lange) Wasowicz & Gabriel y

Galán (Molino *et al*. 2019). Species investigated in the present study are *S. spicant* and *S. fallax*.

The fern species *Struthiopteris spicant*, or the deer fern, is distributed across northern temperate and arctic regions, but concentrated in two major clusters, in western N-America (Cousens 1981; Soltis & Soltis 1988; Nauman 2025a) and western Europe (Molino *et al.* 2019; POWO 2025). Previously, *S. spicant* was placed in the genus *Blechnum* L., as *Blechnum spicant* (L.) Roth, but a series of molecular and phylogenetic studies have indicated that *Blechnum* in the traditional sense is not a monophyletic group and this has led to the transfer of the species from *Blechnum* to *Struthiopteris*, as *S. spicant* (Gabriel y Galán *et al*. 2013; Perrie *et al*. 2014; de Gasper *et al*. 2016; Wasowicz *et al*. 2017a).

*Struthiopteris spicant* has a compact erect stem with frond rosettes growing from the apex, creeping rhizomes and dimorphic fronds (de Gasper *et al*. 2016). The sterile fronds are pinnate, lanceolate in the proportions 1:5 and form a rosette. The fertile fronds are longer than the sterile fronds, erect with narrower and more dispersed pinnae which bear sporangia on the underside in continuous bands. This widely distributed *S. spicant* is quite variable in its taxonomic circumscription for which several varieties have been proposed. Three varieties are recognized to date: *Struthiopteris spicant* (L.) Weiss var. *spicant* (hereon referred to as *S. s.* var. *spicant*); *Struthiopteris spicant* var. *homophyllum* (Merino) Gabriel y Galán & R. Pino (hereon referred to as *S. s.* var. *homophyllum*); and *S. spicant* var. *pradae* S. Molino & Gabriel y Galán (Molino *et al*. 2019, 2020). The present study includes two of these varieties, *S. s.* var. *spicant* and *S. s.* var. *homophyllum*. The latter variety is only included in a small part of this study. The variety *homophyllum* comprises smaller plants than the variety *spicant*, with erect fronds up to 20 cm, usually all sporogenous, monomorphic or subdimorphic, but has several morphoanatomical features overlapping with those of the variety *spicant* (Merino *et al*. 2019). *S. s.* var. *homophyllum* is endemic to the northwest of the Iberian Peninsula, in both Spain and Portugal (Molino *et al*. 2019).

There are two species of *Struthiopteris* in Iceland: *S. s.* var. *spicant* (isl, *skollakambur*) and *S. fallax* (isl, *tunguskollakambur*) (Kristinsson *et al*. 2018; Wasowicz *et al*. 2017b; Wasowicz 2021). The former, *S. s.* var. *spicant*, is by far the dominant species found mostly in the northern part of Iceland. It generally grows to 10-50 cm (Kristinsson *et al*. 2018), in lowland areas below 200 m, often in basins or crevices frequented by heavy snows (Wasowicz 2021). Globally, *S. s.* var. *spicant* is considered LC- least concern according to the IUCN Red List Categories and Criteria. It has, however, not been evaluated regionally for Iceland.

In contrast, the latter species, *S. fallax*, has the status of EN- endangered according to the IUCN Red List Categories and Criteria regionally, and is protected nationally (Wasowicz & Heiðmarsson 2019). This species is Iceland-endemic and has very limited distribution. It has so far been found only at one location in western Iceland, that is, by the hot spring Deildartunguhver in Reykholtsdalur (Fig. 1), where the soil temperatures reach about 30°C, in mosses or on bare ground at an altitude of 34 MASL (Wasowicz 2021). *S. fallax* differs from the common variety *S. s.* var. *spicant* in many ways (Kristinsson *et al*. 2018; Wasowicz *et al*. 2017b; Molino *et al*.

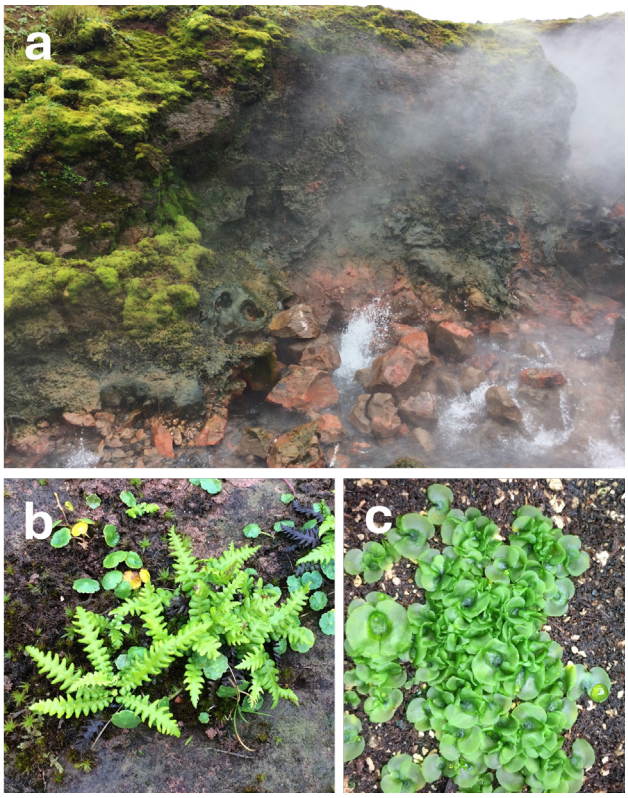


**Figure 1**. (**a**): Deildartunguhver geothermal hot spring in Reykholtsdalur, western Iceland. (**b**): *Struthopteris fallax* growing in the warm soil above Deildartunguhver. Diameter of the plant on the left is around 12 cm. (**c**): Gametophytes of *S. fallax* growing in a pot at Reykjavík Botanic Garden. Average size of the gametophytes is about 4 mm. They were germinated from spores collected in the field. These gametophytes later produced *S. fallax* sporophytes.

2019). Firstly, the environments in which the two species thrive are the opposite of one another, that is, in warm ground versus snow basins. Secondly, *S. fallax* is minuscule, measuring merely 2-8 cm. Thirdly, the fronds of *S. fallax* are prostrate but those of *S. s.* var. *spicant* have raised and slightly draping leaves. Fourthly and probably the most important, characteristic of *S. fallax* is its monomorphism. Instead of having architecturally different fertile and sterile fronds (dimorphism) like *S. s.* var. *spicant*, *S. fallax* has only one type of leaves, where the spore bearing leaves are morphologically the same as sterile leaves.

The present study investigated microscopical characteristics, including chromosome number, genome size and spore morphology and size, of *S. fallax* in comparison with *S. s.* var. *spicant* from Iceland and with reference to *S. s.* var. *spicant* and *S. s.* var. *homophyllum* from the Iberian Peninsula. The aim was to see if there would be any of such characters that can further differentiate between *S. fallax* and *S. s. var. spicant*. This could strengthen the species delineation, which so far has been based on macro- and micro-morphology, together with certain ecological and geographical preferences.

Chromosome number is the karyotypic feature most used in cytotaxonomical analyses (Guerra 2008). The chromosome base number (*x*), in particular, when constant confirms evolutionary relatedness among closely related species, but when variable can reflect evolutionary changes among species. In the traditional genus *Blechnum*, base chromosome numbers are variable, x = 28, 29, 31, 32, 34, 36 (Nauman 2025b). This is expected, however, as *Blechnum* is not monophyletic. Based on the Chromosome Counts Database (CCDB; Rice *et al*. 2015), existing records of chromosome number in *Struthiopteris* are extremely limited with only two records for *S. spicant*, one of *S. s*. var. *homophyllum* and no records on *S. fallax*. An additional reference on chromosome number of *S. spicant* from Iceland (Löve & Löve 1961) showed the same sporophytic number as in CCBD, which is diploid with $2n=2x=68$, implying $x=34$ gametic number (base number). Records on genome sizes of *Blechnum* are even more limited, i.e., with only one reference for *S. spicant*, which reported the DNA amount 1C as 6.44 pg (Pustahija *et al.* 2013). In the present study, we measured genome size and attempted chromosome extraction from leaf samples of both *S. fallax* and *S. s.* var. *spicant*. The mega analysis of genome size data by Clark *et al*. (2016) revealed strong correlation between genome size and chromosome number across all ferns. Thus, in the case of our study, genome size may be used to infer chromosome number, and vice versa. To the best of our knowledge, the present study is the first report of genome size of *S. fallax* and the first showing meiotic chromosomes from the monomorphic frond of *Struthiopteris*.

Recently, fern spores have been emphasized as a diagnostic feature of homosporous ferns, even at species specific level, in particular spore size and ornamentation (Passarelli *et al*. 2010). In the present study, we examined spores of *S. fallax* in comparison with its related taxa, using Scanning Electron Microscopy (SEM). We described morphological appearance of the spores from SEM images and statistically analysed spore sizes. The aim was to compare our data with those of Molino *et al*. (2020), the study that also included *S. fallax*. The knowledge of spores is important in understanding the taxonomy and evolutionary history of a species.

## MATERIALS AND METHODS

### *Plant material*

Plant species and varieties examined in the present study are listed in Table 1, together with their place of origin, sample identification numbers and the investigations performed. The Icelandic samples were collected during field trips in June and July 2016 and again in 2017. The *Struthiopteris* samples used in this study came from the only one location of *S. fallax*, Deildartunguhver (four samples from the field and three from RBG- Reykjavík Botanic Garden), five populations of *S. s.* var. *spicant* from diverse regions in Iceland (total nine field samples and two samples from RBG), two locations of *S. s.* var. *spicant* from Spain (one sample each) and one location of *S. s.* var. *homophyllum* also from Spain (one sample). Field samples from Iceland were collected by JBUT, KAJ and HÞ, authors of this manuscript, and they were identified by HÞ, Director of RBG. The samples for Spain were provided by JM Gabriel y Galán (University Complutense Madrid, Spain) and corroborated by P. Wasowicz (Icelandic Institute of Natural History, Akureyri, Iceland).

The Iceland-endemic species *S. fallax* was found growing on the warm ground just above the hot spring Deildartunguhver (Fig. 1). Deildartunguhver

**Table 1**. Samples used in this study.

| Species of *Struthiopteris* | Location | Origin GPS-Coordinates | Sample ID | Chromo-somes | Genome size | Spores by SEM |
|---|---|---|---|---|---|---|
| *S. fallax* (Lange) S. Molino, Gabriel y Galán & Wasowicz | Deildartunguhver Borgarfjörður W-Iceland | 64.66348°N 21.41075°W | DE 03 | X | | X |
| | | | DE-01, DE-02 & DE-04 | X | | |
| | | | RBG- Pot1S & Pot2S Sporophyte | | X | |
| | | | RBG- Pot1G Gametophyte | | X | |
| *S. spicant* (L.) Weiss var. *spicant* | Svanshóll Bjarnarfjörður NW-Iceland | 65.78971°N 21.55871°W | BJ-02 (Bjarn 02) | X | | X |
| | | | BJ-01, BJ-05 & BJ-06 | X | | |
| | Landmannalaugar (Brennisteinsalda) S-Iceland | 63.98264°N 19.08941°W | LA-01 | X | | |
| | Héðinsfjörður (Grundarkot) E-Iceland | 66.10055°N 18.81422°W | HE-01 & HE-02 | X | | |
| | Reykjanes (Trölladyngja) SW-Iceland | 63.94981°N 22.09039°W | RE-01 & RE-03 | X | | |
| | Skútudalur Siglufjörður N-Iceland | 66.123611°N 18.885556°W | ISL-03-2018-pl.1 & pl.2 | | X | |
| | Valdés, Paladeperre Spain | ~43.783331°N 6.56667°W | AS JND09 | | | X |
| | San Miguel de Valera, Salamanca Spain | ~40.55000°N 5.91667°W | SA JND03 | | | X |
| *S. spicant* var. *homophyllum* (Merino) Gabriel y Galán & R. Pino | Tabagón/Tomiño, Pontevedra Spain | ~41.93333°N 8.78333°W | GA JND04 | X | | X |

is a hot spring in the valley Reykholtsdalur in Borgarfjöður, western Iceland. Deildartunguhver is considered Europe's most powerful hot spring, providing around 180 L/sec of 97°C hot water. It provides hot water for central heating in the nearby towns of Borgarnes and Akranes. Geothermal soil is usually a few to several degrees warmer than the ground in nearby areas, due to radiated heating from geothermal water channels that are present in the bedrock. In the case of Deildartunguhver, the soil temperatures that support *S. fallax* could be up to 30°C (Wasowicz 2021). Moderate soil warming promotes plant growth through an active root system and a healthy underground network of microorganisms. It is therefore expected that many plants growing on geothermal soil are either not able to grow in colder environments or they are species particularly adapted to a geothermal habitat, so-called thermophilic species. The geothermal area around this hot spring supports a relatively rich herbaceous vegetation that is typical of moist habitats such as bogs and marshes. In this hot spring area, mosses are the most prevalent component of the vegetation. According to the survey commissioned by the Icelandic Institute of Natural History (Kristinsson *et al*. 2007), mosses and bryophytes together formed 50 – 84 % of the total vegetation. This survey recorded population size of the fern *S. fallax* as comprising 200-300 plants essentially in the main area of about 10 $m^2$, the area of the Deildartunguhver site where *S. fallax* is most abundant.

*Chromosome preparation*

Developing fiddleheads, or small pieces of frond tips, were collected in the field. The fresh samples were placed immediately in ice water and kept in there at 4°C for 24-27 hours to arrest metaphases. The samples were then transferred into freshly made

fixative composed of a 3:1 ratio of 96% ethanol and glacial acetic acid.

Chromosomes were prepared from these fixed samples using the protoplast-dropping method described in Anamthawat-Jónsson (2004). The fixed samples were first rinsed off fixative with distilled water and kept in water for 20 min. An individual sample was then prepared by trimming away older tissue with forceps leaving behind the youngest part about 1–2 mm in size. For each sample, 2–3 such pieces were placed in 100 µL of cellulase/pectinase enzyme mixture and incubated at 37°C for 16 h. The enzyme mixture contained Pectinase (30 units/mL, Merck no. 1.06021) and Cellulase Onozuka R10 (80 units/mL, Merck no. 1.02321) in buffer containing 75 mM KCl and 7.5 mM EDTA. The digested leaf tissue was then minced in its enzyme mixture into suspension using a 200-µL pipette tip. The suspension was filtered through a nylon mesh. The filtered cell/protoplast suspension was then hypotonically treated with 1.5 mL of cold 75 mM KCl solution for 15–20 min at room temperature. The suspension was spun down in a microfuge at 7,000 rpm for 5 min and the supernatant discarded. The cell/protoplast pellet was then cleaned with 1 mL of ice-cold fixative by resuspending and spinning down pellet, twice. The final, cleaned pellet was resuspended in 50-100 µL of fresh and cold fixative. The protoplast suspension was then dropped onto an ice-cold and water-wet microscopic slide from 10–20 cm height, one drop on each slide. When the drop just dried up, the slide was dipped briefly in 96% ethanol and air-dried. The slides were kept at 4 °C in a dry place until use.

The chromosomes were then stained for 1 min with a 1 µg/mL solution of blue-fluorescing DAPI (4,6-Diamidino-2-phenylindole), the fluorochrome that binds specifically to chromosomes (to the double-stranded DNA in chromosomes), not to cytoplasmic artefacts. The DAPI-stained cells were examined under 1000x magnification in the Nikon Eclipse E800 epifluorescence microscope. The images were captured with Nikon Digital Camera DXM1200F.

*Flow cytometry*

For genome size measurement by flow cytometry, fresh samples of fiddleheads or pieces of young fronds and gametophyte plants were sent by an express delivery to Vienna and kept in the meanwhile on wet tissue to avoid dehydration. Fresh sporophyte leaves (or fresh gametophyte tissue in one case) were co-chopped (Galbraith *et al*. 1983) together with an appropriate internal standard organism (*Pisum sativum*, 4.42 pg/1C, Greilhuber & Ebert 1994; *Solanum pseudocapsicum*, 1.295 pg DNA/1C, Temsch *et al*. 2010) in Otto´ buffer I (Otto *et al.* 1981) using a sharp razor blade. Subsequently, the nuclei isolate was filtered through a 30 µm nylon mesh and treated for 30 min with RNase A (0.15mg/mL, Sigma, USA) at 37°C in the water bath. For fluorescence staining, propidium iodide (PI, 50µg/ml, AppliChem GmbH, Germany) containing Otto´s buffer II (Otto *et al*. 1981) was added.

After incubation in the refrigerator, measurement was conducted on a CyFlow ML flow cytometer (Partec, Münster, Germany) equipped with a green laser (100 mW, 532 nm, Cobolt Samba, Cobolt AB, Stockholm, Sweden). Finally, genome size calculation followed the equation: ($G_{0/1}$ FI peak position$_{Object}$ / $G_{0/1}$ FI peak position$_{Standard}$)* 1C-value$_{Standard}$ = 1C-value$_{Object}$ (where FI is the mean fluorescence intensity).

*Spore analysis*

For spore analysis, whole leaves or pinnae were collected and dried in an envelope until use. In the present study, the spores were examined in SEM (Scanning Electron Microscope), model JEOL JSM 6610LA, located at the Institute of Life and environmental Sciences, University of Iceland. Spore samples were prepared by pouring loose spores from the drying envelope and carefully scraping them from the underside of dried leaf of each sample onto a sample plate, a double-sided adhesive carbon-dot (from Agar Scientific Ltd. UK). The cardon-dot with spores was placed on the specimen stub. Each set of stubs accommodated seven dots, or seven samples. They were then loaded into the SEM specimen chamber. All samples were imaged using BEIW (back-scattered electron imaging) mode with compositional contrast, low vacuum mode with pressure setting at 20 Pa (Pascal units), relatively high accelerating voltage of 10 kV, very short working distance of 8 mm between sample and detectors, zero tilt range and 45 mm spot size. For each sample, images at ×500, ×900 and ×1000 magnifications were taken from different areas on the sample dot.

SEM images were used for observing perispore patterns and for measurements of the spores. Description of the perispore pattern followed the terminology used in Passarelli *et al*. (2010). Spore

size was measured from SEM images in a computer by using pixel coordinates and Pythagoras' theorem to calculate the length of the spores. In all samples the perispore (the outermost layer of the spore) was measured. The equatorial axis was measured as in Gómez-Noguez *et al*. (2016). The equatorial axis for irregularly shaped spores was defined as the length of the spore parallel to the laesura. For statistical purposes spores were on occasion pooled into four groups: *S. fallax*, *S. s*. var. *spicant* from Iceland, *S. s.* var. *spicant* from Spain and *S. s.* var. *homophyllum* from Spain. Statistics were performed in R version 4.5.1 using RStudio version 2025.05.1+513 for Windows.

## RESULTS

### *Chromosome number by fluorescence microscopy*

The *Struthiopteris* chromosome analysis is summarized in Table 2 and selected DAPI-stained cells are shown in Fig. 2. The aim of this part of the study was to obtain 2*n* mitotic chromosome number of each of the species and varieties investigated (Table 1). We adopted the protoplast dropping method developed for trees to prepare chromosomes from shoot-tips collected in the field (Anamthawat-Jónsson 2004). Although the method was successfully applied for diverse groups of plants, such as *Betula* L. (Thórsson *et al*. 2007), *Curcuma* L. (Puangpairote *et al*. 2016) and *Sorbus* L. (Anamthawat-Jónsson & Þorbjörnsson 2016), it was not tested in ferns. As it turned out, the chromosome preparation from leaf cells of the *Struthiopteris* ferns did not yield well-spread metaphases usable for chromosome counting. The problem was likely technical, for example, the hypotonic treatment was insufficient. Some of the samples were not good either. While fiddleheads were suitable as they contained active mitosis, mature leaves/ fronds were not. They contained differentiated cells, mostly, for example, RE-03 (*S. s.* var. *spicant* from Reykjanes) was dominated by spores. Leaf pieces of GA JND04 (*S. s.* var. *homophyllum* from Spain) only contained interphase cells. The sample was probably not ice-water treated, or chemically treated, to arrest metaphases.

In some samples that were prepared from fiddleheads, metaphases were present, but they were mostly too compact to allow chromosome counting. As the mitotic chromosomes were long and slender, they tended to overlap vastly. Nevertheless, rough estimates of chromosome number were

**Table 2**. Microscopic evidence for ploidy levels of *S. fallax* and *S. spicant* samples assorted by their origin, based on CCBD (Chromosome Counts Database, Rice *et al*. 2015), whereby *S. spicant* is mitotic diploid (2*n*=2*x*=68). N/A= Not applicable (due to either the leaf sample was too mature, hence no mitosis, or some technical reasons of the chromosome preparation).

| Species | Origin | Sample ID | Ploidy | Microscopic evidence |
|---|---|---|---|---|
| *S. fallax* | Deildartunguhver Reykholtsdalur Borgarfjörður W-Iceland | DE-01 | Fronds diploid containing some gametic haploid cells | Small cells, with 1-2 nucleoli. Larger cells, with 3-4 nucleoli. |
| | | DE-02 | Meiotic I | Metaphase with ≥30 chromosome pairs (Fig. 2a). |
| | | DE-03 | N/A | Numerous nucleoli |
| | | DE-04 | Gametic haploid included | Small cells mostly. Compact metaphases. |
| *S. spicant* var. *spicant* | Landmannalaugar S-Iceland | LA-01 | Likely diploid | Long, slender (mitotitc) chromosomes. Large, compact metaphases (Fig. 2b). Nucleoli 2-4. |
| *S. spicant* var. *spicant* | Bjarnarfjörður NW-Iceland | BJ-01 | Likely diploid | Chromosomes similar to other samples. Compact metaphases (Fig. 3c). Numerous nucleoli. |
| | | BJ-02 | N/A | Compact metaphases. |
| | | BJ-05 | Likely diploid | Compact metaphases. |
| | | BJ-06 | Likely diploid | Chromosome number >50 (Fig. 2d). At least 3 nucleoli. |
| *S. spicant* var. *spicant* | Héðinsfjörður E-Iceland | HE-01 | Likely diploid | Large, compact metaphase. Nucleoli 2-4. |
| | | HE-02 | Likely diploid | Chromosome number >50 (Fig. 2e). Nucleoli 2-4. |
| *S. spicant* var. *spicant* | Reykjanes SW-Iceland | RE-01 | Likely diploid | Metaphases similar to other samples. Nucleoli 2-4. |
| | | RE-03 | N/A | N/A Spores abundant. |
| *S. spicant* var. *homophyllum* | Tabagón Spain | GA JND04 | Probably diploid | At least 3 nucleoli. |

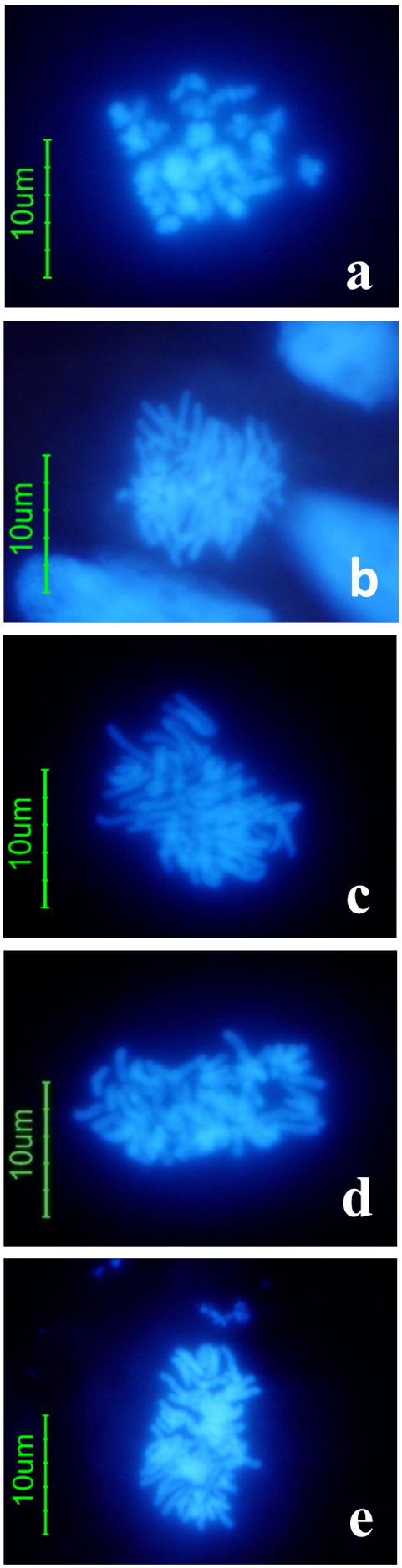


**Figure 2**. Results of the chromosome preparation from leaf tissues or fiddleheads of *Struthiopteris* sporophytes showing DAPI-stained metaphase chromosomes.

(**a**): *S. fallax* from Deildartunguhver, Iceland. Meiotic metaphase cell from sample DE-02 showing at least 30 pairs of chromosomes, which is not far from the expected gametic (1*n*) number of 34 for this species.

(**b**): *S. spicant* var. *spicant* from Landmannalaugar, S-Iceland. Mitotic metaphase cell from sample LA-01 showing a cluster of >50 chromosomes, which is the typical size and shape observed in this species/variety. It is most likely diploid with 2*n*=2*x*=68, rather than being any other ploidies.

(**c-d**): *S. spicant* var. *spicant* from Bjarnarfjörður, NW-Iceland. Mitotic metaphases from both samples, BJ-01 (**c**) and BJ-06 (**d**), are identical in both chromosome shape and cluster size, that is comprising well over 50 chromosomes. This variety/accession is most likely diploid with 2n=2x=68.

(**e**): *S. spicant* var. *spicant* from Héðinsfjörður, E-Iceland. Mitotic metaphase cell from sample HE-02 showing a cluster of >50 chromosomes, as with other accessions from Iceland, supporting that this species/variety is most likely diploid.

possible in some cases (Table 2). For example, BJ-06 and HE-02, both *S. s.* var. *spicant* from Iceland, each appeared to contain many more than 50 chromosomes. It is therefore likely that this variety is diploid (expected 2*n*=2*x*=68, as in CCDB, Rice *et al*. 2015), rather than being in other ploidy levels, as each level increases the 2*n* number by 34. One feature that is shared among all samples in the present study is that all metaphases, even partially analysable, are of the same overall size and the chromosomes are very similar in shape (see for example, Fig. 2b/ LA-01, Fig. 2c/ BJ-01, Fig. 2d/ BJ-06 and Fig. 2e/ HE-02). Furthermore, interphase nuclei, which were found abundant in all samples, are of the similar shape and size. They also showed similar number of nucleoli, 3-4 in each nucleus. It is therefore likely that all samples in the present study have the same ploidy level, which in this case is diploid with 2*n*=2*x*=68.

An interesting result coming out of this study is the discovery of cells undergoing meiosis in leaf tissues of *S. fallax* (Table 2). This is only possible because we isolated chromosomes from shoot-tips (fiddleheads or young fronds), not from root-tips. In the *S. fallax* plant DE-02, the first meiosis (meiosis I) was evident, whereby chromosomes were seen in pairs, and in this case showing at least 30 pairs (Fig. 2a). This means the gametic chromosome number of *S. fallax* is most likely 34, half of the diploid somatic number of 68. For other *S. fallax* plants investigated, such as DE-01 and DE-04, their

leaf tissues contained both large and small cells. The large ones are presumably diploid sporophytic cells (before meiosis), and these nuclei tend to have 3-4 nuclei in each, like those of *S. spicant*. The small ones are presumably haploid gametic cells (after meiosis) and these cells showed 1-2 nucleoli in each. In conclusion, the sporophytic stage of *S. fallax* is most likely diploid with $2n=2x=68$, just like *S. s.* var. *spicant*. The gametic stage of *S. fallax* is haploid with $1n=1x=34$.

*Genome size by flow cytometry (FCM)*

The results of FCM (Table 3) show that there is no notable difference in genome size between the Iceland-endemic species *Struthiopteris fallax* (as measured from two transplanted sporophyte plants, twice, and from one gametophytic plant) and *S. spicant* var. *spicant* (two sporophytic plants) from Iceland. The results here are considered preliminary, however, given low replication and small sample size.

The earlier measurements (January 2018) produced almost the same 1C values for *S. fallax*. The average value of the two sporophytic samples was 6.94 pg, whereas the value from the gametophytic sample was 6.90 pg. Theoretically, the 1C-values of sporophyte- and gametophyte- derived genome size measurement must be the same (Temsch *et al*. 2021). The 1C-value refers to the DNA content of an unreplicated haplophasic nucleus (one chromatid per chromosome, one chromosome set per nucleus, i.e., as in a gamete). This is irrespective of the tissue types used for measurement.

The later measurements of sporophytic samples from Iceland (autumn 2018) included *S. fallax* (the same two samples measured before in January) and *S. spicant* var. *spicant* (two plants originally from Siglufjörður, northern Iceland). The former had the average 1C value of 7.00 pg and the latter had the average 1C value of 6.92 pg. The values of the two taxa are similar. It can be interpreted that *S. fallax* and *S. s.* var. *spicant* from Iceland are in the same ploidy level, that is both are diploid.

The results conform well with the author EMT's own measurement in 2014 of sample from an unknown subspecies of *S. spicant* from Rettenegg, Styria, Austria, whereby 1C value was 7.17 pg (unpublished results), only 3% higher when compared to the final average 1C value in the present study (that is 6.96 pg considering taxa together). One record of 1C DNA amount in the Kew C-values database (Pustahija *et al*. 2013) shows the value for *S. spicant* to be 6.44 pg, which seems different from the average value of 6.94 pg of *S. s.* var. *spicant* from Iceland (7.7% variation). In fact, the *Pisum sativum* standard C-value used by Pustahija *et al*. (2013) was lower (4.185pg/1C) than we used for our measurements (4.42pg/1C, Greilhuber & Ebert 1994). The resulting variance was therefore only 1.02-fold.

The difference in mean 1Cx values between the two measurement periods (January vs. September/ October) is insignificant. Compared to the measurement in January, there is a higher variation among the recent samples (Table 3). This is probably due to the preparations and is not a true variation between the taxa. The peak-CV% were higher in the later measurements than in the measurements in January. The variation between the two taxa is low, only 1.0061-fold (*S. s. var. spicant*: mean = 6.9173 pg, CV% = 2.88%; *S. fallax*: mean = 6.9594 pg, CV% = 1.47%) when the January measurements of *S. fallax* samples were included, but 1.012-fold when only the autumn measurements were considered.

**Table 3**. Results of genome size measurements.

| Sample | Species/ Variety | Generation | Date of measurement | 1C value (pg) | 1C average |
|---|---|---|---|---|---|
| *Fallax* RGB-Pot1S | *S. fallax* | Sporophyte | January 2018 | 6.99 | 6.94 |
| *Fallax* RGB-Pot2S | *S. fallax* | Sporophyte | January 2018 | 6.90 | |
| *Fallax* RGB-Pot1G | *S. fallax* | Gametophyte | January 2018 | 6.90 | |
| **Mean (January)** | | | | **6.93** | |
| *Fallax* RBG-Pot1S | *S. fallax* | Sporophyte | October 2018 | 7.1243 | 7.00 |
| *Fallax* RBG-Pot2S | *S. fallax* | Sporophyte | September 2018 | 6.8761 | |
| ISL-03-2018-pl.1 | *S. s. var. spicant* | Sporophyte | October 2018 | 7.0584 | 6.92 |
| ISL-03-2018-pl.2 | *S. s. var. spicant* | Sporophyte | September 2018 | 6.7762 | |
| **Mean (autumn)** | | | | **6.9588** | |
| **Standard deviation (autumn)** | | | | **0.1607** | |
| **Coefficient of variation (autumn)** | | | | **2.3097** | |

*Spore morphology and size by Scanning Electron Microscopy (SEM)*

Spore ornamentation and morphological appearance by SEM are summarized in Table 4 and representative images are shown in Fig. 3. Spores of *S. fallax* differ from other taxa in the present study in that they were oblate/elliptic with smooth outline and the perispore ornamentation was no obvious rugate, but instead a filamentous pattern resembling non-anastomosing veins (Fig. 3a-3b). Another difference encountered between *S. fallax* and other taxa under study was that *S. fallax* spores were more concave as if having a reduced or absent protoplast (Fig. 3c). The severity of this deformity differed among spores but was noticed in a large part of the spores examined. Spore samples of *S. s.* var. *spicant*, both from Iceland and from Spain, had more rounded and full appearance (not concave), angular in shape, and the outline was irregular with rugulate/reticulate pattern of perispore ornamentation (Fig. 3d-3f and Fig. 3g-3h). The pattern varied in intensity among samples, with most having an apparently thick perispore, as compared to the *S. fallax* samples, with consistent and obvious rugate ornamentation on the perispore. The spores of *S. s.* var. *homophyllum* from Spain (GA-JND04, Fig. 3i-3j) had the same appearance as *S. s.* var. *spicant*.

Exospores of *S. s.* var *spicant* were smooth, with long laesura (images not shown). The perispore appeared thick and were easily fragmented or cracked, exposing its smooth exospore. In contrast, the perispore of *S. fallax* appeared thinner and when ripped exposing its exospore (Fig. 3b).

Spore sizes, as measured in equatorial length of spores, are summarized in Table 4. The average equatorial length of spores in this study was 43.27 µm, which ranged between groups, from 41.7 to 47.09 µm. Spore equatorial lengths were normally distributed in all sample groups, as designated by a Shapiro-Wilk test, with $p > 0.05$ for all groups (Sokal & Rohlf 2012). The Levene's test for homogeneity of variances resulted in $p = 0.983$. A one-way analysis of variance (ANOVA) of equatorial spore length was performed on four groups of *Struthiopteris* sampling origins: *S. fallax* from Deildartunguhver, Iceland (n = 40, mean 41.7 µm, median 41.82 µm); *S. s.* var. *spicant* from Svanshóll in Bjarnarfjörður, Iceland (n = 16, mean 43.58 µm, median 43.48 µm); *S. s.* var. *spicant* from Spain, one sample from Paladeperre and one from Salamanca combined (n = 30, mean 42.32 µm, median 42.84 µm); and *S. s.* var. *homophyllum* from Pontevedra, Spain (n = 12, mean 47.09 µm, median 45.02 µm). The ANOVA analysis revealed a significant difference among the means of these four groups ($p < 0.05$). The follow up Tukey's test (Table 5) showed that the *homophyllum* variety had significantly larger spores than *S. fallax* ($p < 0.05$) and *S. s*. var *spicant* from Spain ($p < 0.05$).

**Table 5**. Results of a Tukey's honest significance test for equatorial spore length presented as a pairwise comparison in relation to the sample groups. Bold numbers with asterisk denote significant differences ($p < 0.05$). The four groups tested are (1) *S. fallax* (*Struthiopteris fallax*, (2) Var. *spicant* IS (*Struthiopteris spicant* var. *spicant*, Iceland), (3) Var. *spicant* ES (*S. s.* var. *spicant*, Spain), and (4) Var. *homophyllum* (*S. s*. var. *homophyllum*, Spain).

| Group A | Group B | Mean Difference (µm) | p-value |
|---|---|---|---|
| Var. *homophyllum* | *S. fallax* | 4.25 | **0.03*** |
| Var. *spicant* ES | *S. fallax* | -0.19 | 0.10 |
| Var. *spicant* IS | *S. fallax* | 1.00 | 0.09 |
| Var. *spicant* ES | Var. *homophyllum* | -4.44 | **0.04*** |
| Var. *spicant* IS | Var. *homophyllum* | -3.25 | 0.26 |
| Var. *spicant* IS | Var. *spicant* ES | 1.19 | 0.86 |

**Table 4**. Spore morphology and size, where n stands for the number of spores examined. Spore ornamentational patterns were based on descriptions by Passarelli et al. (2010). The four groups examined are (1) *S. fallax* (*Struthiopteris fallax*, Deildartunguhver), (2) Var. *spicant* IS (*S. spicant* var. *spicant*, Bjarnafjörður, Iceland), (3) Var. *spicant* ES (S. s. var. *spicant*, Spain, SA JND03 and AS JND09), and (4) Var. *homophyllum* (S. s. var. *homophyllum*, Spain, GA JND04).

| Sample group | n | Spore size Mean µm | Spore size Median µm | Spore outline | Perispore pattern | Concave |
|---|---|---|---|---|---|---|
| S. *fallax* | 40 | 41.70 | 41.82 | Smooth | Filamentous | Yes |
| Var. *spicant* IS | 16 | 43.58 | 43.48 | Irregular | Rugulate | No |
| Var. *spicant* ES | 30 | 42.32 | 42.84 | Irregular | Rugulate | No |
| Var. *homophyllum* | 12 | 47.09 | 45.02 | Irregular | Rugulate | No |

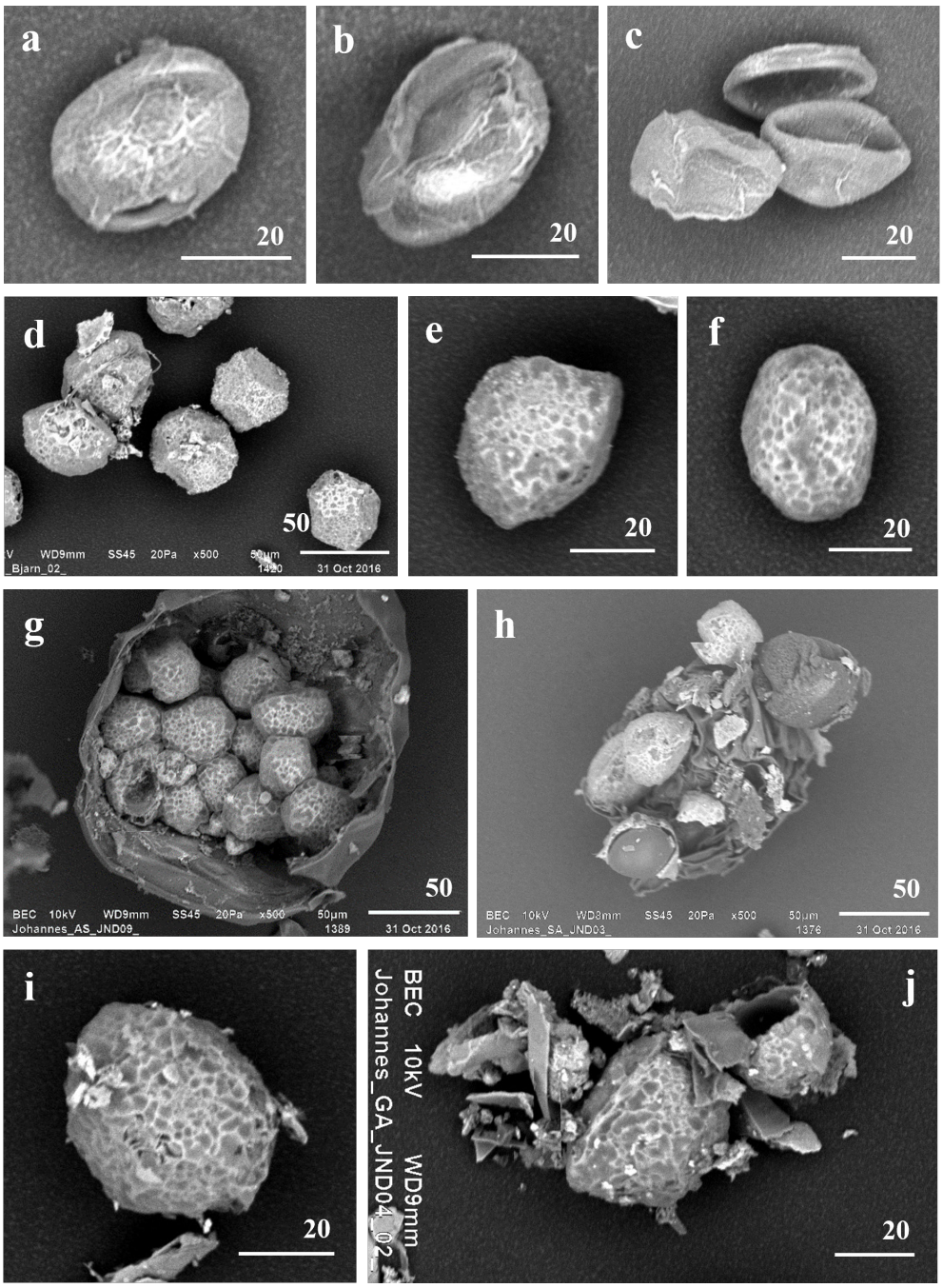


**Figure 3**. Representative SEM-images of spores from *Struthiopteris fallax* and *S. spicant*. Note different scales in µm.

(**a** – **c**): *S. fallax* from Deildartunguhver, Iceland: sample DE-03. The spores are oblate/elliptic, with relatively smooth outline and filamentous pattern of the perispore ornamentation, resembling non-anastomosing veins (**a**, **b**). The perispore appears thin and when ripped exposing its exospore (**b**). *S. fallax* spores tend to be strongly concave as if having a reduced or absent protoplast, resembling aborted spores (**c**).

(**d** – **f**): *S. spicant* var. *spicant* from Bjarnarfjörður, NW-Iceland: sample BJ-02. The spores are angular in shape but overall, they are more rounded than those of *S. fallax*. The perispore outline is irregular with rugulate or reticulate pattern of ornamentation. The perispore appears thick and brittle.

(**g** – **h**): *S. spicant* var. *spicant* from Spain: sample AS-JND09 from Paladeperre (**g**) and sample SA-JND03 from Salamanca (**h**). The spores shown here are contained in a sporangium (**g**). Spores from both accessions have similar morphology as those of *S. s.* var. *spicant* from Iceland (Bjarnarfjörður), that is, they are angular and with rugulate pattern of ornamentation. The perispore is clearly thick and when cracked exposing its smooth and filled exospore (**h**).

(**i** – **j**): *S. spicant* var. *homophyllum* from Spain: sample GA-JND04 from Pontevedra. The spores have the same morphology as those from *S. s.* var. *spicant*, both from Iceland and Spain. The perispore is also thick and brittle.

In summary, spores of *S. s. var. homophyllum* were relatively larger than spores of two of the other sample groups in the present study, but *S. fallax* had a unique appearance and ornamentation pattern of perispore compared to *S. spicant*.

## DISCUSSION

The main subject of this study is the Iceland-endemic fern species *Struthiopteris fallax*. We investigated its chromosome number and measured genome size in comparison with its closely related species *S. spicant* var. *spicant* widely distributed in Iceland. We examined spores of these ferns, described spore morphology and evaluated spore size, in comparison to the Iberian varieties of *S. spicant*, i.e., *S. s*. var. *spicant* and *S. s.* var. *homophyllum*.

### *Struthiopteris fallax has the same genome size and chromosome number as S. spicant.*

This paper is the first report of genome size of *S. fallax* and of *S. spicant* var. *spicant* from Iceland. The average genome size (1C value) of *S. fallax* is 6.96 pg, calculated from four measurements of two sporophytic samples and one measurement of gametophytic sample, whereas the average of *S. s.* var. *spicant* is 6.91 pg based on two sporophytic samples. We therefore interpret that these two taxa have the same genome size. One record of 1C DNA amount in the Plant DNA C-values Database (Leitch *et al*. 2019) shows the value of 6.44 pg for *S. spicant* (unknown variety), which is not far from the average value of *S. s*. var. *spicant* from Iceland or from that of *S. fallax*.

Current Pteridophyte genome size database covers about 2.8% of extant fern diversity (Clark *et al*. 2016; Pellicer & Leitch 2020), a significant increase from the previously low <1% taxonomic coverage by Bennett & Leitch (2012). In this new analysis (Clark *et al*. 2016), Blechnaceae (unknown number of species) has the mean ancestral a1C value of 12.06 pg, whereas Polypodiales (7192 taxa) has the mean extant 1C value of 12.19 pg. The Plant C-DNA Database (PC-DD, 2025) shows genome size of three *Blechnum*/ *Struthiopteris* species, *S. spicant* (6.44 pg, mentioned earlier), *B. microphyllum* (8.95 pg) and *B. numdum* (12.89 pg) (Leitch *et al*. 2019). This is all that is published regarding genome sizes in the polyphyletic genus *Blechnum*, far too little information to make any inference in the evolutionary context. But the most important point from the analysis is the confirmation of the positive relationship between holoploid genome size (1C) and chromosome number across all ferns (Clark *et al*. 2016), which is not the case with angiosperms or gymnosperms. Ferns are considered to have shown considerably greater stability in their chromosome structure over the last 400 million years (Haufler 2014), compared with the seed plants (Leitch & Leitch 2012). Based on this direct correlation between genome size and chromosome number among ferns, we assume that, as *S. fallax* has the same genome size as *S. spicant*, *S. fallax* also has the same chromosome number as *S. spicant*, that is, $2n=2x=68$.

Results from karyotyping in the present study were unclear as few cells from the samples taken were actively dividing and samples with visible chromosomes usually had a large and tight metaphase (see examples in Fig. 2). Precise chromosome count was therefore not possible but rough estimates could be made in some cases. As described in the result section, all the rough estimates indicated diploidy with $2n=2x=68$. This is the case of *S. spicant* var. *spicant* from all four different locations in Iceland, i.e., BJ- Bjarnafjörður (NW), HE- Héðinfjörður (E), LA- Landmannalaugar (S) and RE- Reykjanes (SW). In conclusion, *S. s.* var. *spicant* from Iceland is also diploid having 68 chromosomes like all other *S. spicant* elsewhere. All 27 records of chromosome number of *S. spicant* (registered as *Blechnum spicant*, no identification to varieties) in the CCBD-Chromosome Count Database show the sporophytic number $2n=2x=68$, where the base number $x$ for this species is 34 (Rice *et al.* 2015).

Chromosome numbers in ferns, both $2n$ and $x$ numbers, are generally higher than those among the seed plants (Leitch & Leitch 2012). The range of 2n chromosome numbers in all ferns (9118 taxa) is 18-1440, mean 121, whereas in Polypodiales (7192 taxa) the 2n range is 22-576, mean 114 (Clark *et al.* 2016). Blechnaceae of North America comprises six species in two genera, *Blechnum* (including *B. spicant* with $2n=68$) and *Woodwardia* Smith, all together the 2n numbers reported for this family are 56-72 (Nauman 2025b). For China and Asia, Blechnaceae comprises 14 species in eight genera (including *Blechnum* and *Struthiopteris*), all together the 2n numbers reported for this family are 66, 68, 74 and 136 (eFlora 2025). This 2n=136 (or 2x 68) is unique – it is *Woodwardia orientalis* Swartz, growing at relatively high altitudes in China, Taiwan, Japan and the Philippines. Overall,

the most common 2n number from these two references is 2*n*=68, the number of *Struthiopteris* species in the present study. It is considered a diploid number, 2*n*=2*x*=68, where *x*= 34, as the ferns behave as genetic diploids.

*Struthiopteris fallax undergoes meiosis early on at the fiddlehead stage.*

The present paper shows for the first time meiotic cell division in young leaf tissue (fiddlehead) of *S. fallax*. We encountered metaphase cells in an unfurling leaf, which showed the chromosome count of ≥30 (sample DE-02). The chromosomes were condensed and appeared in pairs, typical of those in meiotic metaphase I, in contrast to the long and slender mitotic chromosomes of *Struthiopteris*. We interpret this as diploid spore mother cells undergoing meiosis to produce spores. We also found that older frond tissue (sample DE-03) comprised differentiated cells and mature spores, with no meristematic cells left among them. Spores collected for SEM imaging also came from this plant DE-03. The other samples studied (DE-01 and DE-04) showed a mixed cell diversity, comprising large interphase nuclei with 3-4 nucleoli and small nuclei with 1-2 nucleoli, presumably sporophytic diploid and gametophytic haploid cells, respectively. As proposed earlier, *S. fallax* is comparatively diploid with the sporophytic chromosome number 2*n*=2*x*=68, like *S. spicant*, the discovery of meiosis here means that the gametic (and gametophytic) chromosome number of *S. fallax* is haploid with 1*n*=1*x*=34. The same gametophytic count was found in the Iberian variety *S. s.* var. *homophyllum* (Horjales et al. 1990). Eight out of 27 records of chromosome number of *S. spicant* (no identification to varieties) in the CCBD also show the gametophytic number *n*=34 (Rice *et al*. 2015). These records, however, might simply be the sporophytic number 68 divided by two, or it is the number from gametophyte plants. We believe we are the first to report the gametic number from a fertile frond.

*Struthiopteris fallax* is a monomorphic fern, that is, sterile (vegetative) frond and fertile (soriferous, spore producing) frond are architecturally the same (Wasowicz *et al*. 2017b). But are they two separate fronds? All four plants of *S. fallax* in the present study held evidence of meiosis or spore production, although the samples collected for chromosome analysis were at a very young stage (such as in the fiddlehead stage). These young fronds may have been considered non-reproductive (sterile) simply because spores are not visible to the naked eye. Microscopically, all fronds of *S. fallax* appear to be fertile. As noted by Molino et al. (2019), most fronds of *S. fallax* are fertile. This could be considered as an extreme case of fern monomorphy, whereby the frond has dual functions, both photosynthesis and reproduction. The photosynthesis is to build up energy and the reproduction spends it.

Fertile–sterile dimorphy in ferns appears to come at considerable carbon cost in dimorhic species (Watkins Jr. et al. 2016), as carbohydrates must be transported to the sori from other leaves or other parts of the plant. This same study also found that for dimorphic species there were approximately 80% more sterile fronds produced than fertile fronds, a consistent pattern both within and across individuals, but in the monomorphic taxa this phenomenon was reversed. It means that to support the expensive reproduction system, dimorphic species produce prolific vegetative growth, and that requires rich environment both above ground (light and CO2) and below ground (water and minerals). In the case of the Iceland-endemic fern *S. fallax*, the dual-functioning monomorphy must have ecological and physiological advantages, that is, it can survive limited resources. The plant study by Raven & Griffiths (2015) suggested possible evolutionary benefits of photosynthesis in reproductive structures to include decreasing the carbon cost of reproduction (due to short distance between photosynthesis and reproduction) and using transpiratory loss of water to deliver phloem-immobile minerals via the xylem (more effective transport system within plant).

*Struthiopteris fallax differs in its spore morphology from that of S. spicant.*

Although preliminarily, cytotaxonomic analysis in the present study does not differentiate *Struthiopteris fallax* from *S. spicant*. But morphologically and ecogeographically, *S. fallax* stands out, thus it received the species status (Molino *et al*. 2019). The question left is whether spore morphology can differentiate the two closely related species. Our study shows that it does. Spores have been used to separate fern taxonomic groups, thus becoming an extremely important source of character traits with taxonomic relevance, mainly the spore dimensions, the model of laesura, and the perispore ornamentation and structures (e.g., Passarelli *et al*. 2010).

Results from the measurement of spore size, based

on equatorial length of perispore from SEM images, do not indicate that there is a considerable difference between the spores of *S. fallax* (mean size 41.7 µm) and spores of *S. spicant* var. *spicant* (Iceland: mean size 43.58 µm; Spain: mean size two accessions combined 42.32 µm) (Table 4). The difference is not statistically significant either (Tables 5). Molino *et al.* (2020) measured spore size (equatorial length of exospore) under light microscope (LM) and found that *S. fallax* from Iceland had larger spore size (43.33 µm, n=20-30) than *S. s.* var. *spicant* from Spain (40.37 µm, n=20-30), but the difference was not statistically tested. In our study, we measured perispore, not the exospore, which placed the *S. fallax* on the smaller, rather than larger, end of the spectrum of our spore samples. In our results, we note that the perispore of *S. fallax* appeared thinner than that of the other taxa. Furthermore, spore sizes from different studies, using different microscopies, e.g. LM vs. SEM, cannot be compared directly.

As stated in the results, the mean spore size of all taxa in the present study combined is 44.27 µm. The measurements in this study were performed with the perispore intact, therefore 2-3 µm (estimated from SEM images) should be subtracted from the values given here, thus the mean exospore size in the present study becomes 42-43 µm. This fits well within the exospore size range of 35-50 µm reported by Passarelli *et al*. (2010) for *S. spicant*. Another paper describes *S. spicant* spores from Poland as ranging within 34-48 µm, although it is not clear whether that applies to the exospore or perispore length (Zenkteler 2012).

We also measured spore size of *S. s.* var. *homophyllum* from Spain and found mean perispore size of this variety to be 47.09 µm (Table 4), which is significantly larger than that of *S. s.* var. *spicant* from Spain and *S. fallax* (Table 5). Molino *et al.* (2020) reported the opposite, that is, the exospore size of *S. s.* var. *homophyllum* from Portugal (38.01 µm) was smaller than that of *S. s.* var. *spicant* (40.37 µm). The pattern of ornamentation on perispore is also quite different. In our study, the rugulate/reticulate pattern of this variety is the same as that of *S. s.* var. *spicant* from Spain (Fig. 3). In Molino *et al.* (2020), the spore of *S. s.* var. *homophyllum* appears less rugulate and more filamentous than that of *S. s.* var. *spicant*, somewhat similar to the spore of *S. fallax* in their study. We have no explanation for this discrepancy, but as *S. s.* var. *homophyllum* is not the focus of this paper we just leave it at that.

The most important point of the spore analysis in our study is that the spores of *S. fallax* exhibit certain differences in spore morphology and ornamental pattern from the spores of other taxa (Table 4, Fig. 3). Ornamentation on the perispore is an important diagnostic tool in fern taxonomy. *Struthiopteis spicant* s.l. has rugulate spores (Zenkteler 2012; Mazooji & Salimpour 2014; Molino *et al*. 2020), which is the predominant ornamentation pattern in Blechnaceae (Passarelli *et al*. 2010), and so do all samples in the present study with one exception. The spores of *S. fallax* are ornamented bearing filamentous and smooth pattern. This pattern appeared on the perispore of spores obtained from nature and from transplanted plants kept in a greenhouse sampled at different times. We therefore regard this perispore pattern to be consistent for *S. fallax*. Possibly, the pattern observed on the perispore of *S. fallax* is a less intense expression of the typical *S. spicant* pattern rather than a novel ornamentation. Interestingly, the perispore ornamentation of *S. fallax* shown in Molino *et al*. (2020) is also rugulate/filamentous like that discovered in our study.

*Struthiopteris fallax* also show different spore morphology from that of *S. spicant* var. *spicant*. Spores of *S. fallax* are oblate/elliptic in an overall shape, whereas spores of *S. s.* var. *spicant* are more rounded and angular in shape (Fig. 3). The angular spore shape of *S. spicant* is illustrated in Passarelli *et al*. (2010, Fig. 3D) and Molino et al (2020, Fig. 6f). Furthermore, spores of *S. fallax* often appear concave as if having a reduced protoplast. This characteristic was also consistent throughout repeated sampling of *S. fallax* over time and was not found in *S. spicant* varieties in the present study. The reason for this morphology is unknown. Wagner Jr. *et al.* (1986) mentioned that concave structure of spores is a symptom of spore abortion or that spores could have been immature when collected. While this may be the case, other symptoms of spore abortion and of immature spores are either incompatible or contrary to the morphology of the samples, for example, abortive and immature spores show great variability in spore size, perispore thickness and size and shape of the laesura (Wagner Jr. *et al*. 1986), but our samples appeared consistent in all of these traits, and even showed less variability than samples of *S. s.* var. *spicant*. We have obtained numerous gametophytes from spores of *S. fallax* in a greenhouse (e.g. Fig. 1c), clearly indicating that *S. fallax* is fertile. We have

further obtained a few sporophyte plants belonging to *S. fallax* from these gametophytes.

Nevertheless, say that the *S. fallax* spores are often abortive, the fertility of the sporophytes may not be as much compromised if the species is highly spore productive. Indeed, *S. fallax* is most likely a spore productive species. Peck *et al*. (1990) evaluated several life history attributes of temperate North American ferns, including estimating annual spore production of several species. They found a clear difference in annual spore production across dimorphic species: the monomorphic taxa in their study produced an average of 151,000 million spores per plant per year, followed by 29,000 million in holodimorphic and 7,500 million in their hemidimorphic species. Such differences are dramatic. For *S. fallax*, being a monomorphic fern gives the species the advantage of producing huge quantity of spores compared to dimorphic ferns such as *S. spicant*, hence *S. fallax* should be able to afford spore abortion due to environmental stresses. Clearly as *S. fallax* thrives in an extreme environment, i.e. warm geothermal soil and cold air above ground, the species may not be able to support full spore production, that is the production of high-quality spores. There are several examples of flowering plants that when under stress during seed development stages, such as drought, heat, salt or nutrient stress, they allocate their limited resources (due to low photosynthetic rates) to produce low number of good seeds and leave the rest empty or aborted, rather than filling all seeds halfway (e.g. Contreras *et al*. 2008 for lettuce; Farahani *et al*. 2010 for barley; Cohen *et al*. 2021 for soybean).

The diagnostic value of fern spores has been acknowledged in the taxonomic classification at the genus or species levels. Spore ornamentation has distinct diagnostic value even at specific level for *Blechnum* species (Passarelli *et al*. 2010) and novel *Blechnum* species from Brazil, such as *B. areolatum* Dittrich & Salino and *B. longipilosum* Dittrich & Salino, have been assorted into groups based on perispore ornamentation and their spore size. Their spore ornamentation was also used to support their distinction as separate species (de Oliveira Dittrich *et al*. 2012). Although *Struthiopteris* has recently been segregated from *Blechnum* (de Gasper *et al*. 2016), there are indications that spore ornamentation could also be a useful complementary tool in *Struthiopteris* taxonomy since *S. spicant* appears to have unique spores when size and ornamentation are considered together (Passarelli *et al*. 2010). The above results of *S. fallax* spore ornamentation are therefore interesting as they indicate that the spore ornamentation of *S. fallax* is not the same as that of its closely related *S. spicant* var. *spicant*. Our results of the spore analysis could thus support the species status of the Iceland-endemic *Struthiopteris fallax*.

## CONCLUSION

The aim of this paper was to investigate the spores, chromosome number and genome size of the Iceland-endemic fern *Struthiopteris fallax* and to compare it with its closely relates species *S. spicant* var. *spicant*. The results are that although the chromosome number and genome size are most likely to be the same between the two, *S. fallax* spores differ significantly in appearance. Our results are consistent with the current recognition of *S. fallax* as a distinct species, based on previously established morphological and ecological differences.

## ACKNOWLEDGEMENTS

We wish to dedicate this paper to the late Dr José María Gabriel y Galán Moris (1968 - 2020), for his knowledge of fern systematics, evolution and ecology, with focus on Blechnaceae. Via the bilateral agreement of international mobility sighed between University of Iceland and Universidad Complutense de Madrid, JMGyG and KAJ received funding from our respective universities to support the sample collection trips in Iceland in July 2016, part of which provided samples used in the study reported here. We greatly appreciate the contribution from JMGyG to this study, in the form of lecture at the University of Iceland, the field supervision and research correspondence thereafter. We would like to thank Dr Pawel Wasowicz of the Icelandic Institute of Natural History at Akureyri for initiating the collaboration with JMGyG on this *Struthiopteris* work. We thank PW for his generosity with the field trips in northern Iceland and for his botanical knowledge about the fern species under study. We thank all our colleagues at the Institute of Life and Environmental Sciences of the University of Iceland and from the Reykjavík Botanic Garden for collaboration and assistance in this project. We appreciate the LM and SEM facilities at University of Iceland and the FCM facilities at University of Vienna. The project was partially supported by the University of Iceland Research Fund.

## ETHICAL STATEMENT

Not applicable

## REFERENCES


Anamthawat-Jónsson, K., 2004. Preparation of chromosomes from plant leaf meristems for karyotype analysis and in situ hybridization. Methods Cell. Sci. 25, 91–95. https://doi.org/10.1007/s11022-004-5620-y

Anamthawat-Jónsson, K. & H. Þorbjörnsson, 2016. Úlfareynir. Skógræktarritið 2016-1, 6-16.

Bennett, M.D. & I.J. Leitch, 2012. Plant DNA C-values database (release 6.0, December 2012). http://data.kew.org/cvalues/

Clark, J., O. Hidalgo, J. Pellicer, H. Liu, J. Marquardt, Y. Robert, M. Christenhusz, S. Zhang, M. Gibby, I.J. Leitch & H. Schneider, 2016. Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny. New Phytol. 210, 1072–1082. https://doi.org/10.1111/nph.13833

Contreras, S., M.A. Bennett & D. Tay, 2008. Restricted Water Availability During Lettuce Seed Production Decreases Seed Yield per Plant but Increases Seed Size and Water Productivity. Hort. Sci. 43, 837–844. https://doi.org/10.21273/HORTSCI.43.3.837

Cohen I., S.I. Zandalinas, F.B. Fritschi, S. Sengupta, Y. Fichman, R.K. Azad & R. Mittler, 2020. The impact of water deficit and heat stress combination on the molecular response, physiology, and seed production of soybean. Physiol. Plant. 172, 41–52. https://doi.org/10.1111/ppl.13269

Cousens, M.I., 1981. *Blechnum spicant*: Habitat and Vigor of Optimal, Marginal, and Disjunct Populations, and Field Observations of Gametophytes. Bot. Gaz. 142, 251–258. https://www.jstor.org/stable/2474281

de Gasper, A.L., T.E. Almeida, V.A.O. Dittrich, A.R. Smith & A. Salino, 2016. Molecular phylogeny of the fern family Blechnaceae (Polypodiales) with a revised genus level treatment. Cladistics, 1–18. https://doi.org/10.1111/cla.12173

de Oliveira Dittrich, V.A., A. Salino & T.E. Almeida, 2012. Two New Species of the Fern Genus *Blechnum* with Partially Anastomosing Veins from Northern Brazil. Syst. Bot. 37, 38–42. http://www.jstor.org/stable/41416934

eFlora (2025). *Struthiopteris* Blechnaceae. Flora of China, vol. 2-3, pages 411–412. Published on http://www.efloras.org [accessed 28.05.2025]. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.

Farahani, S.M., D. Mazaheri, M. Chaichi, R.T. Afshari & G. Savaghebi, 2010. Effect of seed vigour on stress tolerance of barley (*Hordeum vulgare*) seed at germination stage. Seed Sci. Tech. 38, 494–507. https://doi.org/10.15258/sst.2010.38.2.21

Gabriel y Galán, J. M., C. Prada, C. Rolleri, A. Ainouche & M. Vicent, 2013. cpDNA supports the identification of the major lineages of American Blechnum (Blechnaceae, Polypodiopsida) established by morphology. Turkish J. Bot. 37, 769–777. https://doi.org/10.3906/bot-1210-49

Galbraith, D.W., K.R. Harkins, J.M. Maddox, N.M. Ayres, D.P. Sharma & E. Firoozabady, 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220, 1049–1051. https://doi.org/10.1126/science.220.4601.1049

Gómez-Noguez, F., B. Pérez-García, K. Mehltreter & A. Orozco-Segovia, 2016. Spore mass and morphometry of some fern species. Flora - Morphology, Distribution, Functional Ecology of Plants 223, 99–105. https://doi.org/10.1016/j.flora.2016.05.003

Greilhuber, J. & I. Ebert, 1994. Genome size variation in *Pisum sativum*. Genome 37, 646–655. https://doi.org/10.1139/g94-092

Guerra, M., 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenet. Genome Res. 120, 339–350. https://doi.org/10.1159/000121083

Horjales, M., N. Redondo, & J.M. Pérez Prego, 1990. Nota Citotaxonómica sobre pteridoflora del noroeste de la peninsula Ibérica. Anales del Jardín botánico de Madrid 48, 82–84 [In Spanish].

Kristinsson, H., E.G. Þorvaldsdóttir & B. Steindórsson, 2007. Vöktun válista plantna 2002 – 2006 [Monitoring endangered plant species in Iceland 2002 – 2006]. Fjölrit Náttúrufræðistofnunar 50, 50-51. [In Icelandic].

Kristinsson, H., Th.E. Thórhaldsdóttir & J.B. Hlidberg, 2018. Flóra Íslands—Blómplöntur og byrkningar [Flora of Iceland—Flowering plants and bryophytes]. Vaka-Helgafell, Reykjavík, [In Icelandic]. ISBN 978-9979-2-2486-0

Leitch, A.R. & I.J. Leitch, 2012. Ecological and genetic factors linked to contrasting genome dynamics in seed plants. New Phytologist 194, 629–646. https://doi.org/10.1111/j.1469-8137.2012.04105.x

Leitch, I.J., E. Johnston, J. Pellicer, O. Hidalgo & M.DO. Bennett, 2019. Angiosperm DNA C-values database (release 9.0, Apr 2019) https://cvalues.science.kew.org/

Löve, Á. & D. Löve, 1961. Some Chromosome Numbers of Icelandic Ferns and Fern-allies. Amer. Fern J. 51, 127–128. https://doi.org/10.2307/1546094

Mazooji, A. & F. Salimpour, 2014. Spore Morphology of 34 Species of Monilophyta from Northern Parts of Iran. Ann. Res. Rev. Biol. 4, 924–935. https://doi.org/10.9734/ARRB/2014/6708

Molino, S., J.M. Gabriel y Galán, P. Wasowicz, P. de la Fuente & E.B. Sessa, 2019. The *Struthiopteris spicant* (Blechnaceae, Polypodiopsida) complex in Western Europe, with proposals for taxonomic and nomenclatural changes. Plant Syst. Evol. 305, 255–268. https://doi.org/10.1007/s00606-019-1565-0

Molino, S., C. Prada, J.M. Gabriel y Galán, P. Wasowicz, B. Estébanez & R. Vázquez, 2020. Sporangia and Spores in the

Fern Genera *Spicantopsis* and *Struthiopteris* (Blechnaceae, Polypodiopsida). Bot. Rev. 86,76–921. https://doi.org/10.1007/s12229-020-09217-z

Nauman, C.E. (Clifton), 2025a. *Blechnum spicant* (L.) Roth. In: Flora of North America Committee, eds. Flora of North America, north of Mexico, vol. 2. [accessed 28 May 2025]. http://dev.floranorthamerica.org/Blechnum_spicant#Distribution

Nauman, C.E., 2025b. *Blechnum* L. In: Flora of North America Committee, eds. Flora of North America, north of Mexico, vol. 2. [accessed 28 May 2025]. http://dev.floranorthamerica.org/Blechnum

Otto, F., H. Oldiges, W. Göhde & V.K. Jain, 1981. Flow cytometric measurement of nuclear DNA content variations as a potential in vivo mutagenicity test. Cytometry 2, 189–191. https://doi.org/10.1002/cyto.990020311

Passarelli, L.M., J.M. Gabriel y Galán, C. Prada & C.H. Rolleri, 2010. Spore morphology and ornamentation in the genus *Blechnum* (Blechnaceae), Grana 49, 243262. https://doi.org/10.1080/00173134.2010.524245

Peck, J.H., C.J. Peck & D.R. Farrar, 1990. Influences of Life History Attributes on Formation of Local and Distant Fern Populations. Amer. Fern J. 80, 126–42. https://doi.org/10.2307/1547200

Pellicer, J. & I.J. Leitch, 2020. The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist 226, 301–305. https://doi.org/10.1111/nph.16261

Perrie, L.R., R.K. Wilson, L.D. Shepherd, D.J. Ohlsen, E.L. Batty, P.J. Brownsey & M.J. Bayly, 2014. Molecular phylogenetics and generic taxonomy of Blechnaceae ferns. Taxon 63, 745–758. https://doi.org/10.12705/634.13

PC-DD, 2025. The Plant C-DNA Database (https://cvalues.science.kew.org/).

POWO, 2025. *Blechnum spicant* (L.) Roth. Plants of the World Online. Royal Botanic Gardens, Kew. Accessed 28.5.2025 https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30079143-2

PPG I, 2016. A community-derived classification for extant lycophytes and ferns. J. Syst. Evol. 54, 563–603. https://doi.org/10.1111/jse.12229

Puangpairote, T., C. Maknoi, T. Jenjittikul, K. Anamthawat-Jónsson & P. Soontornchainaksaeng, 2016. Natural triploidy in phyto-oestrogen producing *Curcuma* species and cultivars from Thailand. Euphytica 208, 47–61. https://doi.org/10.1007/s10681-015-1497-x

Pustahija, F., S.C. Brown, F. Bogunic, N. Basic, E. Muratovic, S. Ollier, O. Hidalgo, M. Bourge, V. Stevanovic & S. Siljak-Yakovlev, 2013. Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. Plant Soil 373, 427–453. https://doi.org/10.1007/s11104-013-1794-x

Raven, J.A. & H. Griffiths, 2015. Photosynthesis in reproductive structures: Costs and benefits. J. Exp. Bot. 66, 1699–1705. https://doi.org/10.1093/jxb/erv009

Rice, A., L. Glick, S. Abadi, M. Einhorn, N.M. Kopelman, A. Salman-Minkov, J. Mayzel, O. Chay & I. Mayrose, 2015. The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytol. 206, 19–26. https://doi.org/10.1111/nph.13191

Sokal, R.R. & J.R. Rohlf, 2012. Biometry (4th ed.). New York, W.H. Freeman and Company.

Soltis, P. S. & D.E. Soltis, 1988. Genetic variation and population structure in the fern *Blechnum spicant* (Blechnaceae) from Western North America. Amer. J. Bot. 75, 37–44. https://doi.org/10.1002/j.1537-2197.1988.tb12159.x

Temsch, E.M., J. Greilhuber & R. Krisai, 2010. Genome size in liverworts. Preslia 82, 63–80.

Temsch, E.M., P. Koutecký, T. Urfus, P. Smarda & J. Doležel, 2021. Reference standards for flow cytometric estimation of absolute nuclear DNA content in plants. Cytometry 101A, 710–724. https://doi.org/10.1002/cyto.a.24495

Thórsson, Æ.Th., S. Pálsson, A. Sigurgeirsson & K. Anamthawat-Jónsson, 2007. Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. Ann. Bot. 99, 1183–93. https://doi.org/10.1093/aob/mcm060

Wagner Jr, W.H., F.S. Wagner & W.C. Taylor, 1986. Detecting Abortive Spores in Herbarium Specimens of Sterile Hybrids. Amer. Fern J. 76, 129–140. https://doi.org/10.2307/1547721

Wasowicz, P., 2021. Diversity and distribution of Icelandic ferns (Polypodiopsida). Bot. Complut. 45, 1–21. https://dx.doi.org/10.5209/bocm.72025

Wasowicz, P. & S. Heiðmarsson, 2019. A Vascular Plant Red List for Iceland. Acta Bot. Isl. 16, 31–48. https://ust.is/library/sida/Nattura/Pl%c3%b6ntufri%c3%b0un.pdf)

Wasowicz, P., J.M. Gabriel y Galán & R.P. Perez, 2017a. New combinations in *Struthiopteris spicant* for the European flora. Phytotaxa 302, 198–200. https://doi.org/10.11646/phytotaxa.302.2.11

Wasowicz, P., L. Folcik & A. Rostański, 2017b. Typification of *Blechnum spicant* var. *fallax* Lange (Blechnaceae). Acta Soc. Bot. Pol. 86, 3542. https://doi.org/10.5586/asbp.3542

Watkins J.E., A.C. Churchill & N.M. Holbrook, 2016. A site for sori: Ecophysiology of fertile–sterile leaf dimorphy in ferns. Amer. J. Bot. 103, 845–855. https://doi.org/10.3732/ajb.1500505

Zenkteler, E., 2012. Morphology and peculiar features of spores of fern species occurring in Poland. Acta Agrobot. 65, 3–10. http://dx.doi.org/10.5586/aa.2012.053