Biodiv. Res. Conserv. 67: 1-8, 2022

BRC
www.brc.amu.edu.pl

DOI 10.2478/biorc-2022-0010

Submitted 10.08.2022, Accepted 30.09.2022

Characteristics of indusia and sori in the two subspecies of *Pteridium aquilinum* (L.) Kuhn. occurring in Poland

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Abstract. Two subspecies of *Pteridium aquilinum* (L.) Kuhn: subsp. *aquilinum* and subsp. *pinetorum* occurring in Poland have been studied with respect to their micromorphological traits, in order to facilitate their differentiation. The micromorphology of marginal sori typical to *Pteridium* are described. Differences were found in the width of the outer membrane of indusium, the features of its edge (straight/notched) and the size and shape of the indusium cells. Moreover, the differentiating features turned out to be the frequency and length of the cillia on the edge of the indusium.

Key words: Bracken Fern, cilia, marginal sori, membranous indusium, Poland, *Pteridium aquilinum*

1. Introduction

The genus *Pteridium* Gled. ex Scop. has worldwide distribution and is present in diverse habitats (Page 1976, 1989). It includes three diploid species, two tetraploid species, a number of subspecies and some hybrid forms. Diploid species (2n = 104) differ between each other in their ranges: *P. aquilinum* (L.) Kuhn dominates the Northern Hemisphere; yet, *P. esculentum* (G. Forst.) Cockayne (Australia, New Zealand, New Caledonia) and *P. arachnoideum* (Kaulf.) Maxon (Venezuela, Brazil, Costa Rica) belongs to the species of the South Hemisphere (Thomson 2004).

The data resulting from morphometric measurements, cytological and DNA-based tests prove that *P. aquilinum* as a species complex comprises: subsp. *latiusculum* (Desv.) Underw. ex A. Heller and subsp. *pubescens* Underw. from North America; subsp. *aquilinum* Page & Mill and subsp. *pinetorum* (C.N. Page & R.R. Mill) J.A. Thomson from Europe, as well as subsp. *japonicum* (Nakai) Love & Love and subsp. *sibiricum* Gureyeva & Page from Asia (Thomson *et al.* 2008). The above classification confirms the results of diagnostic usage of variable regions of the chloroplast genome trnS-rpS4 and additionally reveals that subsp. *aquilinum* remains haplotype B and subsp. *pinetorum* – haplotype A (Der *et al.* 2009).

Nowadays in Europe the presence of subsp. *aquilinum* is not controversial but the presence of subsp. *pinetorum* requires further determination. The taxon was found in Scotland (Page 1989) and described as a subspecies (Page & Mill 1995a, 1995b); since then there has been a growing interest in the subspecies and attempted determination of its former presence in Europe. Subspecies *pinetorum* was confirmed in Finland (Harmaja 1990), in the European parts of Russia (Ershova 1990; Shorina & Perestronina 2000), in Siberia (Gureyeva & Page 2005; 2008), in Belarus (Tikhomirov 2009), in Ukraine (Vasheka & Bezsmertna 2012) and in Poland (Zając & Zając 2019; Zenkteler & Nowak 2019). At present the subspecies are known, but still insufficiently recognized in the field.

This paper provides a successive confirmation of the occurrence of two bracken subspecies in Poland, i.e. *P. aquilinum* subsp. *aquilinum* and *P. aquilinum* subsp. *pinetorum*. They are still functioning as one species. Individuals of the subsp. *pinetorum* are hence treated as an irregular form of subsp. *aquilinum.* Due to the fact that there is lack of knowledge about the occurrence of such abundant and common subspecies as *pinetorum* we do not have any data concerning subspecies *pinetorum* in synoptic tables, phytosociological registers or characteristics of plant communities. As a result it is difficult to complete the maps in the plant distribution atlases.

The earliest mention of the occurrence in *Pteridium,* so-called false indusium (as a cover by a revolute margin of a leaf) comes from a drawing of the frond with a part of the rhizome by Leonhart Fuchs in The New Herbal in 1543 (after Eddington 2013). Detailed data on the structure of *Pteridium* indusia consisting of two elements: a wider and more fringed outer indusium and a narrower and sparsely fringed inner one, was given by Hyde & Wada (1954). After many years, Schölch (2000), based on a comparative analysis of the initiation and development of marginal and submarginal sori in the Hypolepidaceae and Dennstaedtiaceae families explained the structural and developmental patterns of the indusia. According to *Pteridium* developmental type, marginal sori non-vein-related, contains a convex receptacle (vascular commissure) which arises from initials of the pinnula marginal meristem, and membranous indusia (outer and inner) formed at the base of the receptacle. For this reason the Author used the term indusium (as the true indusium) which has been confirmed by her SEM research (Schölch 2000, 2003).

Our paper provides a description of the macromorphology of marginal sori and some data concerning detailed micromorphology of indusium in order to facilitate the recognition of both *Pteridium* subspecies occurring in Poland.

2. Material and methods

2.1. Subspecies identification

As a result of the studies by Thomson (2000, 2004), Thomson *et al*. (2008) to identify *P. aquilinum* subsp. *aquilinum* and by Page (1989, 1995a, 1995b) to identify *P. aquilinum* subsp. *pinetorum* we have obtained the main sources of data concerning the taxonomy and morphology of both European subspecies. The accepted name of the subspecies has been determined following The Plant List (2013). The previous studies allowed us to precisely identify the populations of both subspecies of *Pteridium* in our country (Fig. 1).

Fig. 1. Phenotype of *Pteridium* subspecies from natural stands in Poland

Explanations: A – *Pteridium aquilinum* subsp. *aquilinum* from oak forest vicinity to Katowice (Upper Silesia), B – *P. aquilinum* subsp. *pinetorum* from mixed forest along to Wełna River (Great Poland)

Fig. 2. Adaxial and abaxial pinnula surface of *Pteridium aquilinum* subsp. *aquilinum* (A) and *P. aquilinum* subsp. *pinetorum* (B), for isolation of indusia. Scale bar = 1 cm

Pteridium aquilinum (L.) Kuhn subsp. *aquilinum* C. N. Page & R. R. Mill, in Bot. J. of Scotland, 47: 233(1995); *P. aquilinum* (L.) Kuhn var. *typicum* Tryon, in Rhodora, 43(505): 15 (1941).

Description: Geophyte. Long/short creeping rhizome system, brown to dark brown, spreading underground (below 20 cm). Fronds singular, growing alternately from the rhizome, expand slowly (4-5 weeks). Stipe long, blade vertically raised, rigid, elongate-lanceolate, almost oval, 3-4-pinnate-pinnatifid. Pinnae with upper surface green and lower dim, fastened to rachis at right angles. Second pinna from the base is the largest. Pinnnule with distinct stipites, lanceolate, dissected, veins free, indusium ciliate, (Fig. 2A). Chloroplast haplotype B. The examined specimens are shown in Table 1A.

Pteridium aquilinum subsp. *pinetorum* (C. N. Page & R. R. Mill) J. A. Thomson; *Pteridium pinetorum* C. N. Page & R. R. Mill subsp. *pinetorum* 1995, Bot. J. Scotland 47:2-243

Description: Geophyte. Long/short creeping rhizome system, brown to dark brown, spreading underground (below 10 cm). Fronds singular, growing alternately from the rhizome, expand rapidly (2-3 weeks). Stipe short, blade deviated from stipe at 45 degree angles, triangular, evidently tripartite, 3-pinnate. The surface of the lamina is glabrous, grassy and dim, with lower surface light. Basal pinnae are the largest. Pinnules on short stipites, oblong, veins free, indusium ciliate (Fig. 2B). Chloroplast haplotype A. The examined specimens are shown in Table 1B.

Table 1. Samples of *Pteridium aquilinum* subsp. *aquilinum* (A) and *P. aquilinum* subsp. *pinetorum* (B) collected by E. Zenkteler and K. Michalak. The number of pinnulae measured in each accession is given in brackets

A1: (10) Katowice vicinity, Valley of Three Ponds, edge of oak forest, along A4, sparse stand
A2: (10) Dąbrowa-country Myślenice community, edge of strongly disturbed mixed wood
A3 (10) Gorzyczki coutryside, Wodzisław Sląski vicinity

B1: (10) Mixed forest alongside the Wełna River
B2: (10) Grzymiszew, roadside in forest
B3 (10) Raduńskie Small Lake, Kaszubski National Park, edge of mixed forest

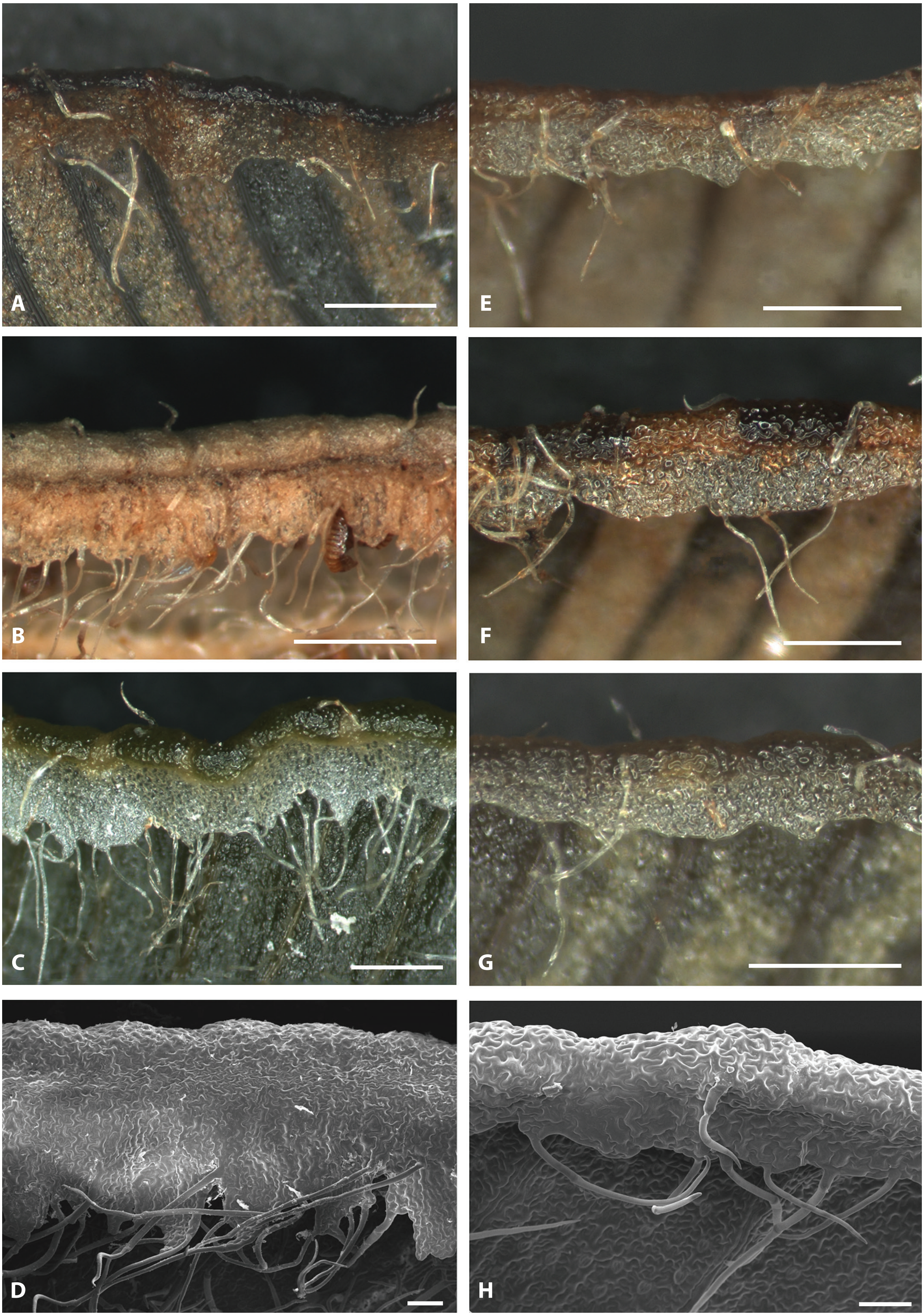

Fig. 3. Outer membrane of marginal sori on pinnula of *Pteridium aquilinum* subsp. *aquilinum* A-C stereo microscope, D – scanning (stands A1-A3 Table 1) and *P. aquilinum* subsp. *pinetorum* E-G stereo microscope, H – scanning (stands B1-B3 Table 1). Scale bars: A-C, E-G = 500 µm; D, H = 100 µm

2.2. Material collection

The 3 samples of *P. aquilinum* subsp. *aquilinum* (30 shoots) and 3 samples of *P. aquilinum* subsp. *pinetorum* (30 shoots) were collected from habitats shown in Table 1. Due to the clonal growth of bracken we collected 3 fronds from one population.

2.3. Micromorphological analyses and numeric data in subspecies delimitation

The observation was performed for subspecies identification on fresh, mature pinnules of both subspecies collected in the field during summer 2021, and transported in plastic bags. The material was analysed as fresh or soon after desiccation. Membranous outer indusia were analysed using a stereo microscope Zeiss StereoLumar V 12 (Carl-Zeiss Jena, Germany) equipped with a Colour View camera AxioCam MRc5 to photograph the membrane surface and measure the width, the number and the length of the cilia attached to the lower and upper edge of the membrane. Due to the fact that the cilia became electrified and easily deformed the obtained measurements were approximate.

Marginal sori images were obtained using scanning electron microscopy with a Zeiss EVO40. Hand-sectioned material underwent dehydration in an ethanol series, then an acetone series, and finally, critical-point air-drying. Specimens were mounted on double sided adhesive tapes and gold-coated.

Descriptive statistics of quantitative characteristics were calculated for each subspecies based on the entire data set. Histogram and box plot were used to display the data.

3. Results

3.1. Sori and indusia morphology

We have analysed marginal sori based on the samples of *P. aquilinum* subsp. *aquilinum.* Both analysed subspecies represent the true type of marginal sori described by Schölch (2000). Sori were fused in an elongate synangium, situated along the margin of the pinnula and covered by outer and inner indusium. The main feature of the outer indusium was its width, prominent in subsp. *aquilinum* and significantly narrower in subsp. *pinetorum*. The width was influenced by its shape (undulating and frilly proturberant) e.g. in subsp. *aquilinum*, especially during sporogenesis. The indusia in subsp. *pinetorum* was rather flat and even. Stereoscopy measurements and observations indicated that the most remarkable micromorphological differences between the indusia of the two subspecies were the width and shape of their outer membrane (Fig. 3A-C, Fig 3E-G, Fig. 4A).

Cilia (setaceous hairs) on the upper margin of the outer indusium showed variation in number and size for the two studied subspecies. The cilia on the upper margin of subsp. *aquilinum* were few in number and relatively short, while in subsp. *pinetorum* their absence was marked. In contrast, the lower margin of subsp. *aquilinum* was mostly undulating, and densely covered with long, stiff cilia, while in subsp. *pinetorum* the cilia were short and there were far fewer of them. A comparison of the abundance of cilia on lower margin of the indusium (entire or erose) revealed that on an over 100 µm segment of subsp. *aquilinum* were 8-10 cilia, mean length of 242 µm, and for subsp. *pinetorum* about 2-3 cilia, mean length of 133 µm. In both subspecies cilia were bicellular: a short cell at the base and an elongated second cell with a hooked constriction at the top (Table 2).

Scanning observations show differences between subsp. *aquilinum* such as incised, wedge edge and wavy membrane surface, contrary to the straight edge of subsp. *pinetorum* (Fig. 3D and 3H). Moreover, the shape of the cells in the outer membrane (similar to puzzle elements) were different: starry in subsp. *pinetorum* and elongate-meandrous in subsp. *aquilinum* (Fig. 5). Differences also concern regular or irregular arrangement of cells, regular along the width of the indusium (subsp. *aquilinum*) and irregular across the width of

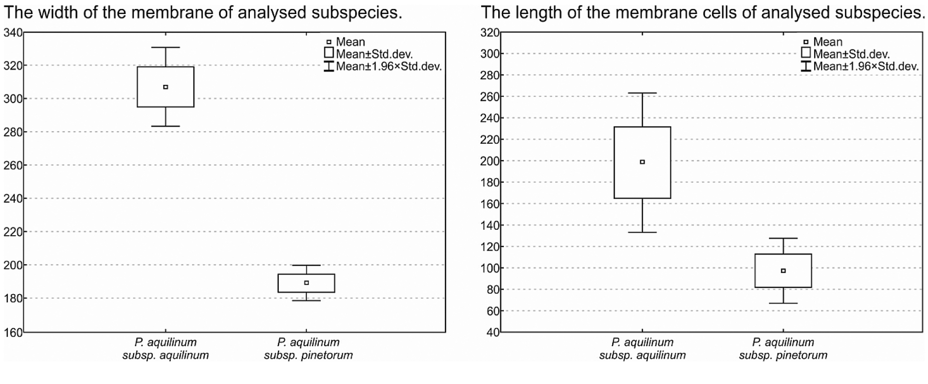

Fig. 4. Histogram and box plot of width of the outer membrane (A) and size of cells (B) in analysed subspecies of *Pteridium*. Indication of two differentiated characters for subsp. *aquilinum* and subsp. *pinetorum*

Table 2. Comparative summary of suitability of cilia to distinguish of *Pteridium* subspecies

Subspecies	Stands	Mean length of cilia [µm]	Cilia complexity		Cilia frequency		
			2 cells	3 cells	low edge	upper edge	surface
P. aquilinum	1	178	+	-	rare	rare	-
subsp.	2	286	+	-	dense	rare	-
aquilinum	3	530	+	+	numbers	dense	+
P. aquilinum	1	125	+	-	rare	-	-
subsp.	2	165	+	-	rare	v. rare	-
pinetorum	3	111	+	-	rare	-	-

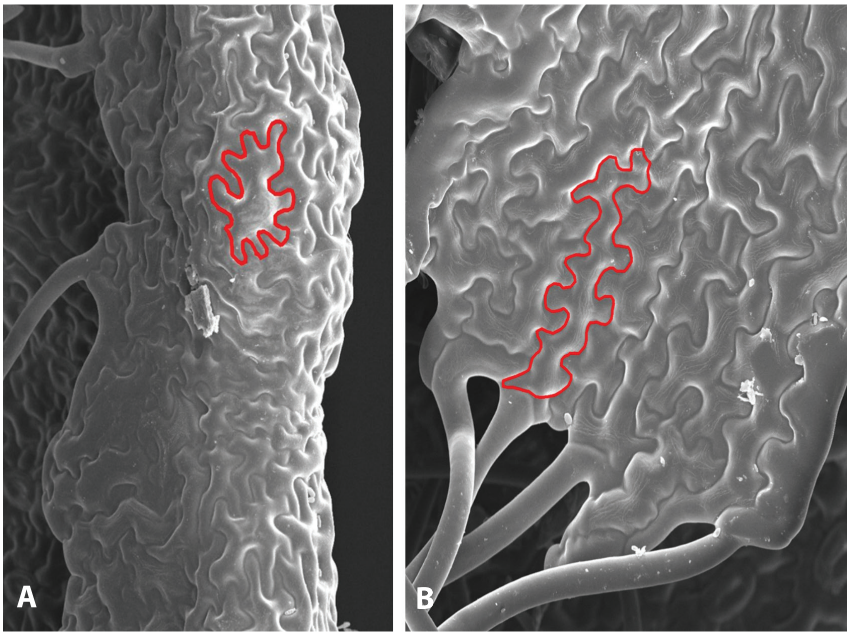

Fig. 5. The shape and length of the membrane cells in two analysed subspecies of *Pteridium* subsp. *pinetorum* (A), subsp. *aquilinum* (B)

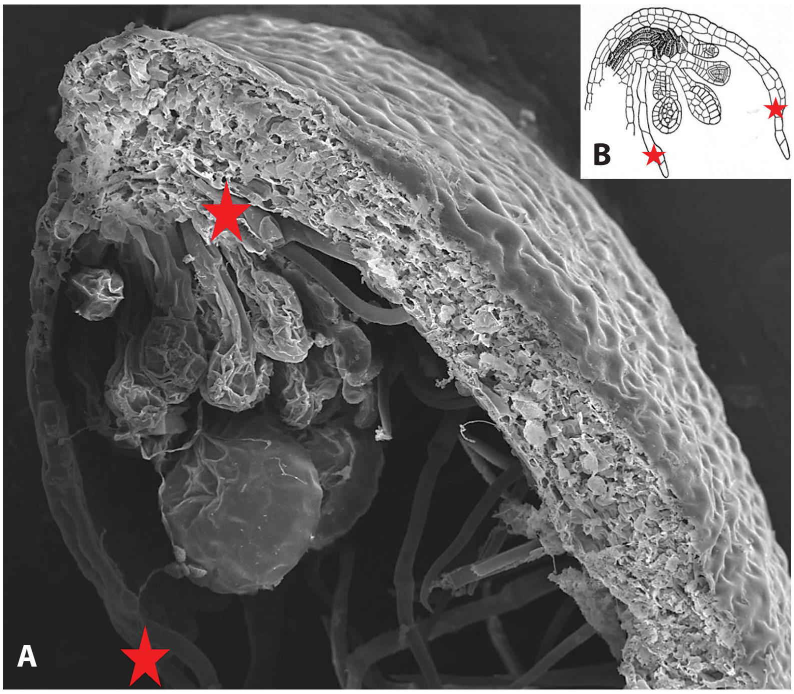

Fig. 6. Structure of marginal sori of *Pteridium aquilinum* subsp. *aquilinum*

Explanations: A – view on transversal section on pinnula margin with part of the lamina tissues and sori elements as convex receptacle with sporangia on basipetal location and outer and inner membranes of indusium, B – scheme of marginal sori structure (according to Schölch 2000, changed). Stars indicating both membranes, inner one on the scheme only. Scale bar = 100 µm the indusium (subsp. *pinetorum*). The most significant differences were found in the length of cells in subsp. *aquilinum* which are 3-5 times longer than the cells of subsp. *pinetorum* (Fig. 6).

The cross section of marginal sori shows the position of the rod-shaped receptacle (vascular commissure) on the straight edge of the pinnula (Fig. 6). Outer membranous indusium derive from the receptacle base and have an upper margin (on the border with pinnula), often without cilia, and the lower margin covered densely with cilia. Sporangia are attached to the receptacle, its initiation and maturation was basipetal and gradual (Fig. 6).

4. Discussion

The study aimed at comparing the sori and indusia of the two subsp. of *Pteridium aquilinum*. According to *Pteridium* developmental type (Schölch 2000) marginal sori arise from the initials of the pinnula marginal meristem and membranous indusium (outer and inner) formed at the base of the receptacle. For this reason, Schölch does not use the term pseudo-indusium, understood previously as a cover by a revolute margin of a leaf. The submarginal sori development in *Cibotium* and *Dicksonia* are morphologically different from *Pteridium*, but in terms of their developmental phases are similar (Churchill *et al.*1998). The major patterns of soral morphogenesis indicate homology at the receptacle and indusia level in marginal and submarginal sori (Schölch 2003). A particular function conducts the indusia of submarginal sori in *Adiantum* by actively participating in spore release, together with mechanical protection, and preventing the sporangia from premature dessication through loss of water (Poppinga *et al.* 2015). In *Pteridium* indusium there is a permanent structure (it does not fall off after the spores are dispersed). It develops despite the developmental disorders of sporangia (e.g. on leaves damaged by spring frosts). In non-sporulating populations (even for several seasons), the presence of indusia has been found (Marrs & Watt 2006). In addition to significant phenotypic variation in *Pteridium* some influence on the width of the indusia in both subsp. was exerted by the size of the fronds (pinna and pinnula) (Zenkteler & Michalak 2022).

A comparative summary of cell wall outlines of the indusia of five American *Pteridium* subsp. highligted the chracteristic differences in the length, width and intensity of folds of these walls (Thompson *et al.* 2008). The tendency of cells towards longitudinal growth was determined by the serial and dense arrangement of membrane-initial cells, as shown in the scanning study of Schölch (2000, 2003). When growing longitudinally side by side, these cells are only able to meander narrowly or broadly, which determines their shape. These features, together with the characteristics of cilia occurring at the lower edge, top edge or both edges of the membrane, enable differentiation between the Polish taxa in our study. The indusium and cilia present in Polish populations of subsp. *aquilinum* turned out to be very similar to the features of the Danish-origin subsp. *aquilinum* from Thomson's study (2008). Furthermore, the high similarity to the cell arrangement of indusia in the American subsp. *latiusculum* was shown by the cell arrangement of the subsp. *pinetorum* examined in this study.

The features such as size and shape of the indusia of the North American subspecies of *Pteridium aquilinum* underscore their importance for taxon discrimination. The three following traits: width, margin of membrane, and the median length of membranous cells were the most informative in evaluating *Pteridium* subspecies (Thomson *et al.* 2008). Gureeva's research also shows that the form of pinnulets and character of indusium could be used for the differentiation of the *Pteridium* taxa. From her analyses, it appears that the features of *Pteridium aquilinum* subspecies such as elongate, acute pinnulets with relatively wide indusium, and ragged margins with dense long hairs evidently contrast with *P. pinetorum* subspecies with broad pinnulets rounded at the apex, and narrow indusium with a fairly smooth margin and very sparse hairs (Gureyeva *et al.* 2014). So far only one paper has noted that cilia are also adequate for *Pteridium* subspecies identification purposes. Its postulates taking into account the number and the length of cilia attached to lower edge of membrane (Thomson *et al.* 2008).

5. Conclusions

Evidence from the distinctness of the micromorphological features between the analysed subspecies, supports the recognition of subspecies *pinetorum* which differs from subspecies *aquilinum* in having: a narrow membrane of indusium, with a nearly linear edge; short median length of membrane cells; simple, two celled, shorter cillia with low frequency attachment to the lower edge of the membrane.

Author Contributions:
Research concept and design: E. Zenkteler
Collection and/or assembly of data: E. Zenkteler, K. M. Michalak
Data analysis and interpretation: E. Zenkteler, K. M. Michalak, O. Nowak
Writing the article: E. Zenkteler
Critical revision of the article: E. Zenkteler
Final approval of article: E. Zenkteler

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