Biodiv. Res. Conserv. 56: 1-12, 2019

BRC
www.brc.amu.edu.pl

DOI 10.2478/biorc-2019-0015 Submitted 14.11.2019, Accepted 31.12.2019

Application of morphometric study to discriminate *Pteridium aquilinum* (L.) Kuhn subsp. *pinetorum* (C. N. Page & R. R. Mill 1995) J. A. Thomson in Poland

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Abstract. *Pteridium aquilinum* subsp. *pinetorum* is one of two morphologically distinct taxa of the genus *Pteridium* identified in Poland. In order to confirm their distinctively recognizable morphology, nine features defining each of these taxa were subjected to a morphometric analysis. These features, including taxonomic characters of vegetative structures, were measured or counted for their most comprehensive descriptions. The measurements were also expressed as ratios of two characters (length of the first and second pairs of leaflets) to prevent the size of fronds from influencing the results. Unique features of bracken, such as: frond and pinnae shape and orientation; basal pinna structure; ultimate segment of pinnae and pinnula shape and size; frond expansion sequencing; frond lamina texture, shape and pubescence were treated descriptively. Canonical discriminant analysis was employed for a morphometric study of quantitative characters. On their basis, two taxa – *P. aquilinum* subsp. *aquilinum* and *P. a.* subsp. *pinetorum* were determined.

Key words: *Pteridium*, morphometric study, canonical discriminant analysis

1. Introduction

The genus *Pteridium* is said to have the largest cosmopolitan distribution, occurring both in temperate and tropical regions of the world. Its main member, *Pteridium aquilinum* (L.) Kuhn, is considered as a multi-variety complex comprising several closely related taxa. In Eurasia, *P. aquilinum* can be further subdivided into subsp. *aquilinum* occurring in Africa, Europe and Asia Minor and subsp. *japonicum* (Nakai) Á. Löve & D. Löve – occurring from East Asia to eastern Europe (Zhou *et al*. 2014). Formerly, according to Tryon (1941), *Pteridium aquilinum* aggr. contained 2 subspecies and 12 varieties. Revisionary concepts focused on morphological, phenological and ecological criteria of *P. aquilinum* aggr. in Britain (Page 1976; Rumsey *et al.* 1991) and, later, in Europe resulted in the identification of two morphotypes: 'tall' *aquilinum* and 'small' *latiusculum* (Thomson 2004, 2008). Subsequently, morphotype *latiusculum* was given the status of a subspecies – *P. pinetorum* ssp. *pinetorum* (Page 1976, 1986, 1989), and in 1997, raised to the rank of species – *P. pinetorum*, as being worthy of nomenclatural distinction (Page 1997). The papers of Page (Page & Mill 1995a, 1995b) are the main sources of data concerning taxonomy, distribution and ecology of *P. pinetorum* in Europe. Unfortunately, some British pteridologists remain doubtful whether *P. pinetorum* should be considered a species (Wolf *et al.* 1995). In response to those concerns, Thomson (2004) published a detailed comparative diagnostic description of *P. a.* subsp. *pinetorum*. Although the origin of *P. a.* subsp. *pinetorum,* native to continental Europe and Western Asia, remains still insufficiently examined, analyses of global chloroplast DNA sequences performed to determine patterns of genetic differentiation classified them as two separate elements inside this aggregate (Der *et al.* 2009). The authors indicated three haplotypes (A, B and C) that differed with respect to nucleotide sites, in the trnS-rpS4 spacer+gene and in the rpL16 intron. They stated that *P. a.* subsp. *pinetorum* belonged to haplotype

VARIABILITY, TAXONOMY AND PHYLOGENY

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A and *P.a.* subsp. *aquilinum* – to haplotype B (Der *et al.* 2009). New phylogenetic analyses and species delimitation tests based on three chloroplast DNA fragments (*rps4-trnS*GGA, *rpl16* and *trnS*GCU – *trnG*UCC) and three microsatellite loci were used to recognize again intra-clade structure of *P. aquilinum*. The species was divided into subsp. *aquilinum* (Africa, Europe and Asia Minor) and subsp. *japonicum* (East Asia (China, Japan, Korea, Far East of Russia/Siberia); eastern Europe (Ukraine) and northern Europe (Finland, Scotland) (Zhou *et al.* 2014). The results also confirmed that the subspecies *P. aquilinum* subsp. *japonicum* and *P. aquilinum* subsp. *pinetorum* should be treated as the same taxon. One of the recent papers concerning relationships in the genus *Pteridium* (Wolf *et al.* 2019), shows elements of genetic similarity between subsp. *pinetorum* and subsp. *japonicum* based on the ddRADseq data.

Lack of evident diagnostic features made it hard to distinguish *P. aquilinum* subsp. *pinetorum* from *P. aquilinum* in Poland. Therefore, *P. aquilinum* (L.) Kuhn was, so far, the only species according to data comprised in Polish Plants Checklist (Mirek *et al.* 2002), as well as in monograph of Rutkowski (2004). Although, when Polish authors recognised morphological differences between fronds of two peculiar populations of *Pteridium*, they assigned these differences to detrimental influence of heavy metal ions (Cr; Pb; Ni) contained in serpentinite soil in which one of the compared populations was growing (Halarewicz & Koszelnik-Leszek 2007).

P. pinetorum C. N. Page & R. R. Mill was identified, for a long time, in pteridoflora of neighbouring countries such as Russia (Shorina & Perestronina 2000; Gureyeva & Page 2005, 2008a, 2008b), Belarus (Tikhomirov 2009), Ukraine (Washeka & Bezsmertna 2012), Germany (Frank 2008 as *P. a.* subsp. *pinetorum*), Czech Republic (Petřík *et al*. 2009) and Scandinavia (Harmaja 1990; Karlsson 2000). Preliminary studies of fresh fronds of *Pteridium* specimens left little doubt that two taxa occurred in Poland (Zenkteler *et al.* 2015). Both our native ferns became a significant component of plant associations (such as *Vaccinio-Piceeta* Br.-Bl. 1939 or *Pruno-Rubion fruticosi* R.Tx. 1952 corr. Doing 1962 em.) currently identified as part of Poland's vegetation. Whilst *P. aquilinum* subsp. *aquilinum* is a common taxon found in every suitable habitat, *P. a.* subsp. *pinetorum* also has a wide distribution throughout the lowland of the country (Zenkteler *et al.* 2015, 2016). Both taxa became widespread from dominant vegetation types of pine forests and acid grasslands.

It was clear that morphological comparison of leaves of both taxa in herbarium sheets seemed easy (*P. a.* subsp. *pinetorum* with triangular blade, smaller than oval blade of *P. a.* subsp. *aquilinum*), but it was not so obvious which qualitative and quantitative characters would be sufficient in the natural stands to determine without doubt that appropriate specimens of *P. pinetorum* were found. Therefore, it was important to investigate, examine and describe features enabling accurate diagnosis of native populations of *P. a.* subsp. *pinetorum.*

2. Material and methods

2.1. Plant material

Frond samples were collected from ten different populations of Western bracken fern (*Pteridium* Kuhn) across forest districts of Oborniki, Chodzież and Piła in the Wielkopolska region. Their habitats were scattered in the forest areas located about 1 km from the road No. 11, along the both sides of the road. In order to select some features useful to distinguish between the two *Pteridium* taxa, macromorphology of fronds was examined *in situ* and *ex situ* in the laboratory of Department of General Botany, also under binocular, with the aim to estimate their details. The samples were later deposited in the Herbarium of Adam Mickiewicz University in Poznań (POZ).

In total, nine quantitative characters of fronds as well as their measure units were evaluated (Table 1). These characters were chosen on the basis of literature from comprehensive description of *Pteridium* (Gureyeva & Page 2005, 2008a, 2008b; Thomson *et al.* 2008; Thomson 2008; Tikhomirov 2009).

Quantitative characters were measured on at least 30 fronds collected from one locality, as follows: blade length (LB) was assessed along the rachis, from the top part of lamina to the base of the first pair of pinnae; stipe length (LS) was assessed from the base of the first pair of pinnae to soil level; lowermost pinnae length (LP-1) was assessed along the whole pinnae rachis; length of the second pair of pinnae (LP-2) was assessed in similar way; lowermost pinnae width (WP-1) and the second pair of pinnae (WP-2) were measured crosswise of pinnae rachis. Occurrences of pinnae pairs of the lamina (NP) were counted along the main rachis. Pinnae dissection (PD) was assessed visually. The angle between the main leaf rachis and the rachis of the second pair of pinnae (PA) was measured by a protractor.

As the analyzed material was collected from field sites, variation included environmental components. Therefore, soil samples (of at least 0.5 kg) were collected from each of the teen *Pteridium* stands and analysed in the local Soil Laboratory of Chemical and Agricultural Station (O.S.Ch.R.) in Poznań.

2.2. Data analysis

The data of morphometric evaluation was gathered in a matrix containing quantitative characters and analysed

Table 1. List of quantitative characters of *Pteridium* fronds used in morphometric analysis with respective acronyms and measurement units

No	Characters	Acronym	Unit
1	Length of the blade	LB	cm
2	Length of the stipe	LS	cm
3	Length of the lowermost pinnae	LP-1	cm
4	Length of the second pair of pinnae	LP-2	cm
5	Width of the lowermost pinnae	WP-1	cm
6	Width of the second pair of pinnae	WP-2	cm
7	Number of pinnae pairs of the lamina	NP	-
8	Pinnae dissection	PD	-
9	Pinnae at an acute/right angle	PA	^{0}

by multivariate statistical analysis using the integrated system of Statistica (StatSoft 2003). The results made it possible to employ canonical discriminant analysis. Before the analysis, quantitative data were standardized. The analysis allowed determination of quantitative characters which would serve best in discrimination of the two examined *Pteridium* taxa.

3. Results

3.1. Comparison of morphological data

P. a. subsp. *pinetorum* were recorded from the Wielkopolska region from populations scattered from the Oborniki forest district to Piła forest district (Zenkteler *et al* 2015). Seven visited populations belonging to *P. a.* subsp. *pinetorum* were found in the areas of pinewood vegetation in a wide range of plant communities. They often occurred in dense or sparse stands in open areas; in rows along roadsides. Three populations of *P. a.* subsp. *aquilinum* were recorded from mesic broadleaves to coniferous forest site types. Distribution of some recent localities of *P. a.* subsp. *pinetorum* is shown in Fig. 1.

P. a. subsp. *pinetorum* is lower growing, with horizontally inclined fronds, where more light penetrated allowing co-habitation with grasses, mosses and other species associated with pinewood communities (Fig. 2a, 2b). Stipe was short 29-91 cm, erect, rigid, with blade ascending horizontally. Blade was broadly triangular, 35-98 cm in length, bipinnate, bright green in colour on the upper surface. The number of pinnae on rachis

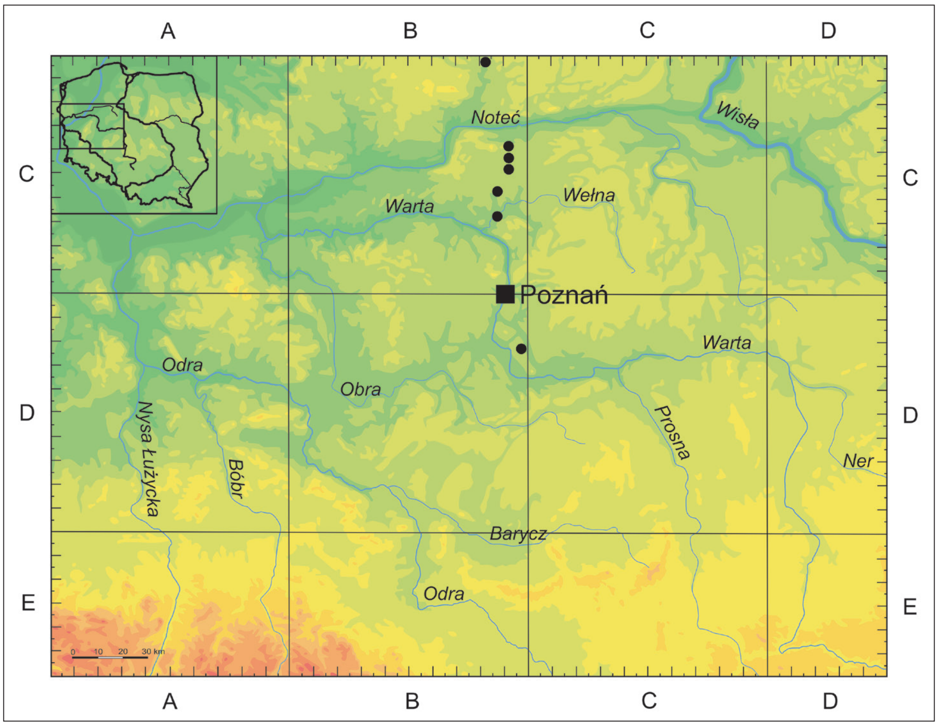

Fig. 1. Distribution map of natural localities of *Pteridium aquilinum* subsp. *pinetorum* in the Wielkopolska region

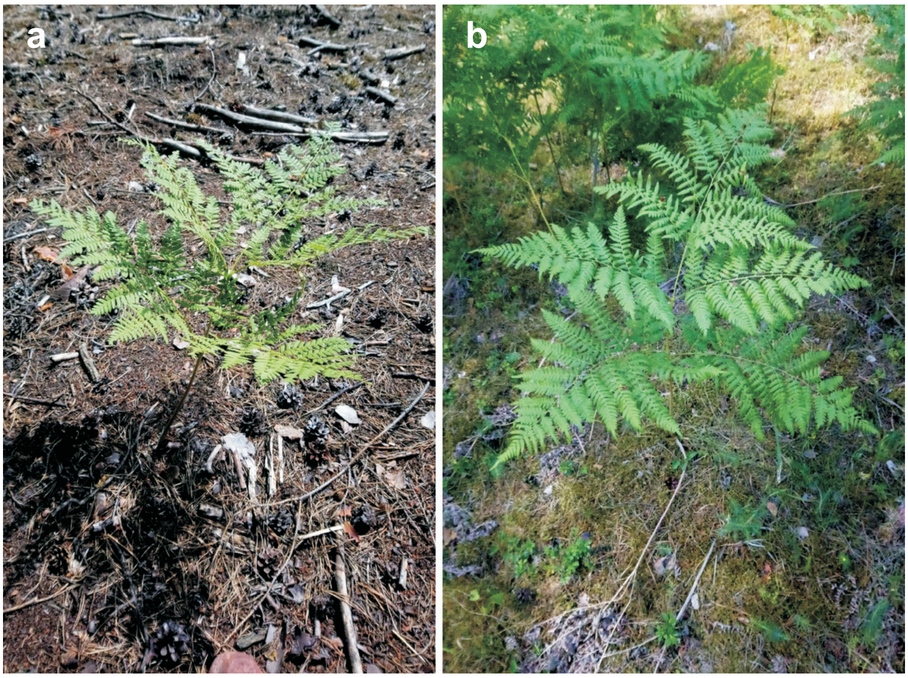

Fig. 2. Habit of *Pteridium aquilinum* subsp. *pinetorum* in the studied localities in pine forest (a) and along a forest road (b) (photograph by K. Michalak)

was 6-13, the basal half-length of the first pinna 24-54 cm and respective width 17-40 cm. The length of the second pinnae pair was 16-46 cm and respective width 15-34 cm. In autumn, fronds turned yellow-brown. The distinctive features of this subspecies included: rapid frond development rate in spring and the proportion of the length of the first pair of pinnae I-r to the smaller second pair, stiff and rigid fronds, rapid whole-frond expansion rate and triangular shape of blades.

Widespread and cosmopolitan *P. a.* subsp. *aquilinum* is a much larger and taller-growing taxon with vigorous and highly variable fronds, occurring frequently in mixed forests (Fig. 3a, 3b). Its distinctive characteristics included: slow frond expansion rate, soft frond texture

Fig. 3. *Pteridium aquilinum* subsp. *aquilinum* on the slope inside a broad-leaved forest (a) and on the forest edge (b) (photograph by E. Zenkteler and K. Michalak)

Table 2. Two *Pteridium* taxa delimitation based on their diagnostic characters in natural localities

P. a. subsp. *pinetorum*	*P. a.* subsp. *aquilinum*
Straw frond lamina	Leathery frond lamina
Rachis arcuate	Rachis upright
The first pair of pinnae are the longest	The second pair of pinnae are the longest
Pinnules ovate on stipites	Pinnules elongate, sessile
Pinnulets rounded at apex	Pinnulets acute at apex
Pinnae dissection 2	Pinnae dissection 2-3 (rare 3-4)
Glabrous, sparse, short, catenate hairs	Pubescent, with long, catenate hairs
Pseudo-indusium narrow	Pseudo-indusium vide
Acute angle between rachis and pinnae	Right angle joined pinna to rachis
Frond expansion 1-2 weeks	Frond expansion 3-4 weeks
Average frond density 6-10 m^2	Average frond density 10-15 m^2

and late seasonality. Stipe was 39-94 cm in length, erect, rigid, with blade raised vertically. Blade was oval-oblong, 60-191 cm in length, bi-, three-pinnate (2-3), dark green in colour on the surface. The number of pinnae on rachis was 8-17 and they were deflected at the top. The length of the first pinnae pair was 26-58 cm and respective width 16-34 cm. The length of the second pinnae pair was 30-70 cm and respective width 26-39 cm. Further distinctive features of *P. a.* subsp. *aquilinum* included: vertical orientation of the rachis with horizontally inserted pinnae and tight, unrolling pinna pairs during gradual development (Table 2; Fig. 3a, 3b). The indumentum of *P. a.* subsp. *aquilinum* was characterized by abundant, cinnamon-coloured hairs covering croziers at the beginning of the expansion phase (Fig. 4a). At the same time, the top of expanding lamina of *P. a*. subsp. *pinetorum* was covered by light-coloured hairs (Fig. 4b). The results of fronds comparison and their spring development rates provided evidence of morphological differences in form and phenology between the two taxa of bracken (Table 2).

Samples, that represented 'true' morphotypes of both: *P. a.* ssp. *pinetorum* and *P. a.* subsp. *aquilinum* were morphologically different during comparison of herbarium specimens (Fig. 5). Their characters revealed all features taken into account as diagnostic for the two taxa. As can be seen in Fig. 2b and 3b, stipe and blade in *P. a.* subsp. *pinetorum* were shorter than in *P. a.* subsp. *aquilinum*. The arrangement of pinna I-row and the number of side pinnae pairs were less numerous in

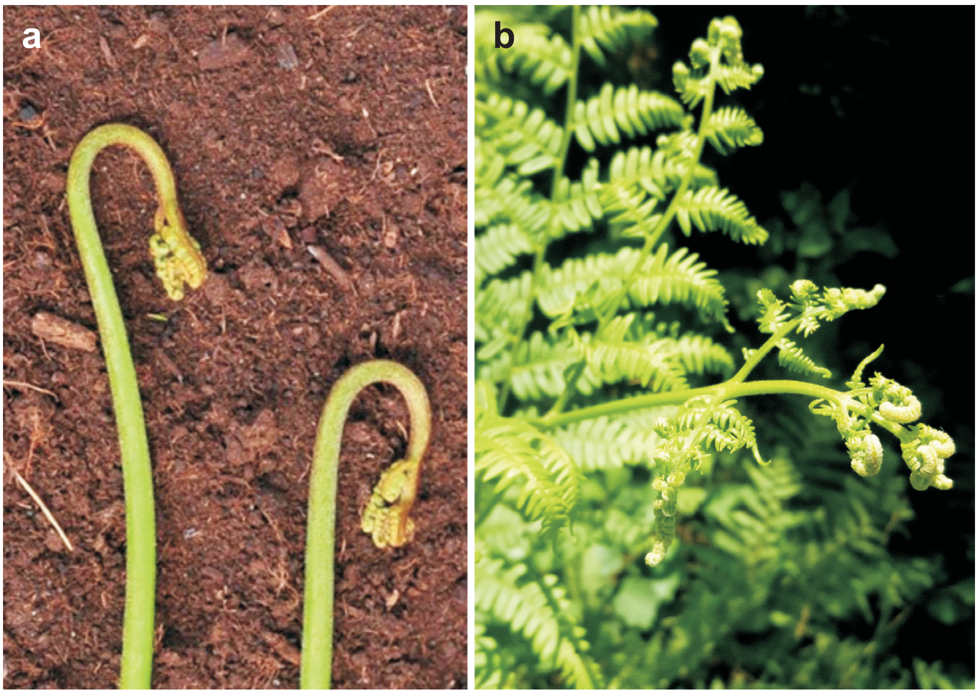

Fig. 4. Indumentum (hairy coat) of (a) croziers of *Pteridium aquilinum* subsp. *pinetorum* and (b) young leaves of *Pteridium aquilinum* subsp. *aquilinum* (photograph by E. Zenkteler)

Fig. 5. Comparison of size. shape and lamina structure of fronds of: (a) *Pteridium aquilinum* subsp. *pinetorum* and (b) *Pteridium aquilinum* subsp. *aquilinum* specimens, collected from the Wielkopolska region and deposited in the Herbarium of Adam Mickiewicz University in Poznań (POZ). Scale bars – 10 cm

P. a. subsp. *pinetorum* than in *P. a.* subsp. *aquilinum* (Fig. 5a and 5b). Morphological characters of fronds that were of taxonomic value differed remarkably in *P. a.* subsp. *pinetorum* and *P. a.* subsp. *aquilinum*. Generally, fronds of *P. a.* subsp. *pinetorum* showed smaller amount of variation of blade structure contrary to *P. a.*

Table 3. Nutrient level of soil samples collected from natural localities of two *Pteridium* taxa

No of sample	pH	Contents mg/dm³ of soil						NaCl g/dm³
		$N\text{-}NO_3$	P	K	Ca	Mg	Cl	
1	3.6	4	<2.7	25	123	23	<11.5	0.07
2	3.7	10	15	25	225	45	17	0.24
3	4.2	<2.8	4	30	163	41	<11.5	0.07
4	4.5	3	<2.7	25	123	28	<11.5	0.06
5	4.7	74	7	115	357	104	<11.5	0.54
6	5.2	4	7	50	286	71	12	0.11
7	3.8	16	15	40	153	41	<11.5	0.23
8	4.4	50	20	55	429	121	<11.5	0.37
9	4.1	38	11	20	276	34	<11.5	0.24
10	3.5	15	11	18	223	25	<11.5	0.29
Means	4.1	21.7	9.5	40.3	235.8	53.3	12.1	0.22

Explanation: samples from the localities of *Pteridium aquilinum* subsp. *aquilinum* (5, 7, 9) and *Pteridium aquilinum* subsp. *pinetorum* (1, 2, 3, 4, 6, 8, 10)

Table 4. T-test of differences in morphological characters of the fronds of *Pteridium aquilinum* subsp. *pinetorum* (Pp) and *Pteridium aquilinum* subsp. *aquilinum* (Pa)

Variable	Mean	Std. deviation	t-value	df	p
LB_Pp	**54.58**	**14.67**			
LB_Pa	**101.13**	**40.71**	**-5.30**	**25**	**0.000017**
LS_Pp	**48.67**	**16.89**			
LS_Pa	**67.35**	**14.22**	**-4.97**	**25**	**0.000040**
LP 1_Pp	38.15	7.78			
LP 1_Pa	40.95	7.75	-1.60	25	0.122291
LP 2_Pp	**31.34**	**7.67**			
LP 2_Pa	**49.38**	**9.24**	**-9.72**	**25**	**0.000000**
WP 1_Pp	28.72	5.46			
WP 1_Pa	27.58	4.60	0.78	24	0.443473
WP 2_Pp	**24.62**	**5.00**			
WP 2_Pa	**33.30**	**4.04**	**-6.40**	**24**	**0.000001**
NP_Pp	**8.92**	**1.22**			
NP_Pa	**12.40**	**2.38**	**-6.42**	**24**	**0.000001**
PD Pp	2.48	0.51			
PD Pa	2.61	0.50	-0.90	22	0.377440
PA_Pp	**37.60**	**8.25**			
PA_Pa	**50.20**	**5.49**	**-6.67**	**24**	**0.000001**

subsp. *aquilinum*, with great irregularity in the size and number of pinnae of I-row.

Field observations of *P. a.* subsp. *pinetorum* showed that the taxon preferred open areas, where competition of other plants was low, and it was rather absent in dense stands. In open conditions, their short fronds produced spores more often. In the summer of 2016, both subspecies of ferns were observed to be sterile; however, their leaves reached maturity. This study also supports *in situ* observation of the rate of spring development of both taxa. Although restricted to material from the area of Wielkopolska, the present study

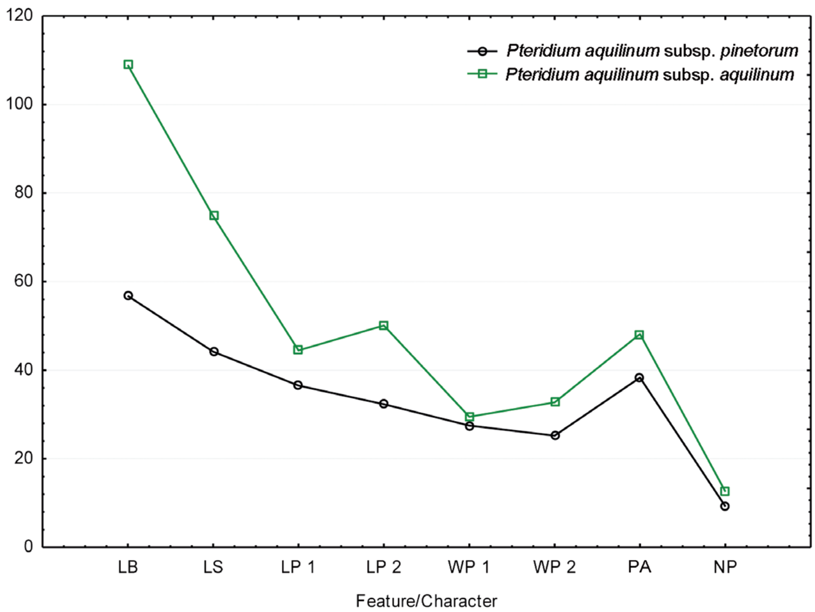

Fig. 6. Diagram of the mean values of the morphometric measurements in two studied taxa

Table 5. Results of discriminant function multivariate analysis of variance (MANOVA)

N=72	Wilks' Lambda	Partial Lambda	F remove (1.62)	p-level	Toler.	1-Toler. (R-sqr.)
LB	0.115089	0.991090	0.55739	0.458135	0.333184	0.666816
LS	0.115796	0.985037	0.94178	0.335591	0.814629	0.185371
LP 1	0.119778	0.952292	3.10609	0.082929	0.215849	0.784152
LP 2	**0.139724**	**0.816346**	**13.94815**	**0.000411**	**0.184713**	**0.815287**
WP 1	**0.188086**	**0.606443**	**40.23549**	**0.000000**	**0.165536**	**0.834464**
WP 2	**0.170410**	**0.669349**	**30.62727**	**0.000001**	**0.230694**	**0.769306**
NP	0.114326	0.997706	0.14258	0.707020	0.430611	0.569389
PD	0.114069	0.999953	0.00294	0.956921	0.868388	0.131612
PA	0.121938	0.935420	4.28042	0.042730	0.893837	0.106164

Explanations: number of variables in model: 9; Grouping: taxa (2 groups)

provides additional evidence to support the existence of two subspecies belonging to the genus *Pteridium* in our country (Fig. 5, Table 2).

Gradient of soil conditions assessed by chemical analysis revealed its relative low nutrient level. The soil in *Pteridium* localities contained sand, clay and gravel, was acidic and rather poor in nutrients (Table 3). Surface peaty layer (from decaying frond-blades) was narrow. Both subspecies occurred mainly on acid podzols. The localities of ten populations exhibited rather similar nutrient contents: N-21,7; P-9,5; K-40,3. Measurements of pH ranged from 3.5-4.7, therefore, populations of *Pteridium* were tolerant to a wide range of acidity. Frond heights were not very tall in poor nutrient local conditions (Table 3).

3.2. Comparison of morphometric data

This study re-examined the usefulness of morphometry to discriminate as separate two closely related taxa of the genus *Pteridium*. The data of morphometric measurements of the two taxa contained in Table 4 reveals differences between statistic means of nine evaluated frond characters. Statistical significance of differences in the values of frond morphometric data is visible in all cases besides characters such as: WP1 (for both subspecies) and PD (both subspecies). Diagrams of the results of T-test show differences in morphological features of fronds between the two analyzed taxa (Fig. 6). The most widely used as alternative statistics to perform a similar task to T-test is Wilks'Lambda test (Table 5). This is useful in direct measurement of the proportion of the variance in the combination of two independent variables.

Wilks' Lambda multivariate test also confirmed differences in morphology of analysed frond features. Statistical significance in different data of measured frond characters was indicated in all cases except: WP 1, WP 2 and pinnae dissection. Ordination of the fronds of *Pteridium* specimens by coefficient values represents the two axes. Table 6 contains standardized coefficients of discriminant function for canonical variables. On this level, two morphotypes are distinctly visible.

Table 6. Discriminant coefficients values for two analyzed subspecies

Character	Pa p= 0.34722	Pp p= 0.65278
LB	-0.0730	-0.1098
LS	0.1424	0.0887
LP 1	0.1720	**0.5511**
LP 2	0.1081	**-0.6547**
WP 1	**-1.2654**	0.5681
WP 2	**2.0326**	0.3630
NP	3.1544	3.3946
PD	11.1305	11.2299
PA	0.7770	0.5186
Constant	-78.0072	-49.7622

Explanations: Pa – *Pteridium aquilinum* subsp. *aquilinum*, Pp – *Pteridium aquilinum* subsp. *pinetorum*, coefficient values, taxa groupings

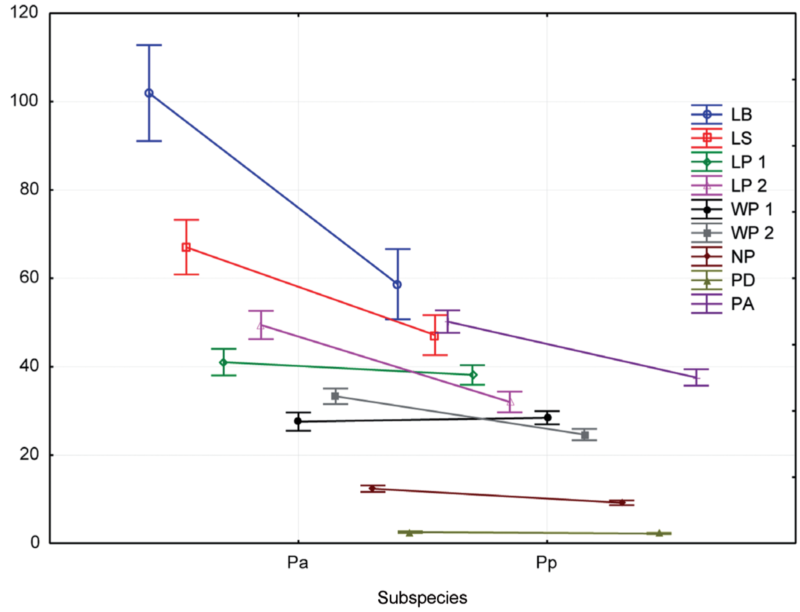

Fig. 7. Morphological characters of *Pteridium aquilinum* subsp. *aquilinum* (Pa) and *Pteridium aquilinum* subsp. *pinetorum* (Pp) analyzed using a multivariate analysis (Wilks' Lambda test)

Explanations: Wilks'Lambda = 0.11406, F (9. 62) = 53.506, p=0.0000. Vertical bars denote 95% confidence intervals

The results of the cluster analysis and canonical discriminant analysis of fronds support the recognition of *P. a.* subsp. *aquilinum* and *P. a* subsp. *pinetorum* as two separate taxa. The four most important features that discriminate these two specimens are: the length and width of the first and second pairs of pinnae (Fig. 7, Table 7).

Ordination of fronds of *Pteridium* specimens by canonical discrimination analysis was presented on two canonical axes (Table 7). This analysis revealed that population of *P. a.* subsp. *aquilinum* is evidently separated from related population of *P.a.* subsp. *pinetorum*. Eigenvalues for each canonical variable and cumulative proportion of explained variance were given as well.

The distribution chart of samples analysed by their morphological variability delimitated the two examined taxa. Mahalanobis distance squares were used to illustrate the distribution of samples, with each point representing one plant. Coordinates of points/plants on the chart – Mahalanobis distance squares – made it possible to allocate each sample to an appropriate subspecies group (Fig. 8). As shown below, a distinctive segregation of *P. a.* ssp. *pinetorum* was obtained (green ellipse) and *P. a.* subsp. *aquilinum* (red ellipse).

Table 7. Raw and standardized coefficients for canonical variables

Character	Raw coefficients	Standardized coefficients
LB	-0.006378	-0.173738591
LS	-0.009291	-0.143987461
LP 1	0.065684	0.499482373
LP 2	**-0.132151**	**-1.05937542**
WP 1	**0.317656**	**1.6381607**
WP 2	**-0.289252**	**-1.2719364**
NP	0.041600	0.0775523079
PD	0.017206	0.00785396391
PA	-0.044774	-0.285574957
Constant	3.902285	-
Eigenval.	7.767050	7.76705036
Cum. prop.	1.000000	1.000000

Explanations: values for which discriminant functions are most weighted were given in bold. Eigenvalue for each canonical variable and cumulative proportion of explained variance are included

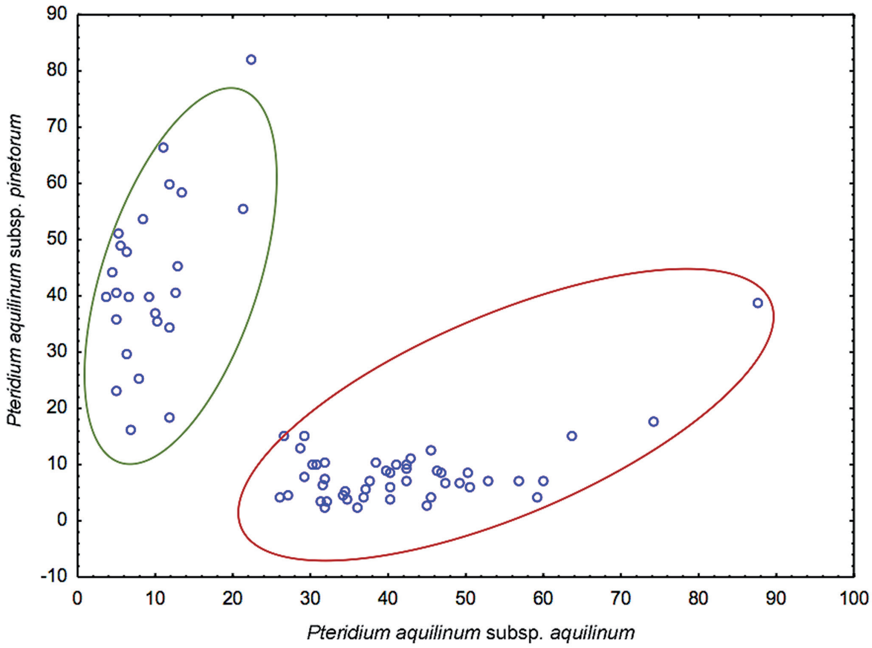

Fig. 8. Scatterplot of Mahalanobis distance squares between two analyzed taxa

4. Discussion

The occurrence of two taxa of bracken in Poland was not recognized so far. Considerable morphological variability of *P. aquilinum* was the main cause of discontinuance in attentive observation and perception of the distinct second taxon as *P. a.* subsp. *pinetorum*. This taxon was not mentioned recently in the main Polish botanical synopsis (Mirek *et al* 2002; Rutkowski 2004). Important justification of the fact is a remark: "The unknown are often unseen" (Frank 2008). The author, analysing the oldest herbarium specimens of *Pteridium*, noticed that *P. pinetorum* was formerly recorded in many locations in Germany as *P. aquilinum*. In Polish herbarium collections, the situation can be similar. *P. a.* subsp. *pinetorum,* which also occur across northern Europe (Page 1986, 1989, 1995a, 1995b); Siberia and East Asia (Gureyeva & Page 2005, 2008a, 2008b), appears to be native to our country as an ancient member of local pteridoflora. *P. a.* subsp. *pinetorum* at its locality in the Wielkopolska region (forests in Oborniki, Chodzież and Piła districts) is vigorous, forms large populations and tends to prefer the areas of pine forest, their edges and nearby open stands. As a result of measurements and morphometric analysis, morphological differces of *P. a. aquilinum* and *P. a.* subsp. *pinetorum* were not only confirmed, but some useful characters to facilitate their identification were also provided (Table 2). One of them was the rate of spring development of fronds, quite useful in discrimination between the two taxa *in situ.* Field observations allowed the conclusion that phenotypic plasticity of fronds was greater in *P. a.* subsp. *aquilinum* than in *P. a.* subsp. *pinetorum.* The explanation of this phenomenon was given by Gureyeva & Page (2008b), who noted that, in all populations of *P. a.* subsp. *aquilinum*, alongside fully developed big fronds, fronds with lesser dissection and dimension appeared, that developed later than the remaining ones (from immature croziers). Our morphometric study also revealed that morphological variability within each taxon was significantly lower than variability between them, as shown by data from Table 4 and Fig. 5.

In this study, out of nine characters of fronds used to distinguish *P. a.* subsp. *pinetorum*. from *P. a.* subsp. *aquilinum*, the highest indicative potential was recorded in a set of four: length of the lowermost pair of pinnae (LP-1): length of the second pair of pinnae (LP-2); together with length of the stipe (LS) and the number of pinna pairs of the lamina (NP) in both compared taxa. Morphometric analysis of fronds quantitative characters proved that the two taxa could be delimited on the basis of distinction of relative low numbers of morphological characters. Among 29 quantitative characters (Thompson *et al.* 2008), 23 characters chosen by Gureyeva & Page (2008a, 2008b) and 21 by Tikhomirov (2009), which were used in delimitation of *Pteridium* in their own papers, the most helpful were only a few: length of the basal pinna of the first and second pair of lamina segments.

In our study, the most reliable diagnostic characters were reduced to nine and they provided sufficient discriminating characters for separating the two taxa of bracken. According to the result of canonical discriminant analysis of both *Pteridium* taxa, the scatterplots of the compared samples formed two distinct clouds (Fig. 8) confirming our field observations and morphological comparison. Gureyeva & Page (2008b, 2015), using discriminant analysis, also confirmed the presence of two isolated bracken subspecies (*pinetorum* and *sibiricum*) in the Euro-Siberian region.

In Polish field observations, other qualitative traits, such as colour and texture of frond lamina, are less evident because they can be affected by environmental conditions (shading or water-logging). In addition, informative value have frond terminating leaflets, which are more loose in *P. a.* subsp. *aquilinum* in contrast to those in *P. a.* subsp. *pinetorum* that are found together on undivided top. Moreover, according to Thomson, additional observations of high diagnostic value are: microscopic confirmation of pseudo-indusia width (0.25 mm; 0.21-0.28 mm for *pinetorum*) and specific shape of their indusial cells (Thomson *et al*. 2008).

Although restricted to the material originating from the area of Wielkopolska and native to its flora, the presented results provide evidence that supports the presence of *P. aquilinum* subsp. *pinetorum* as a distinct subspecies in the Polish pteridoflora. The conducted research significantly expands the knowledge about the occurrence of *P. aquilinum* subsp. *pinetorum* in Poland.

Acknowledgements. The authors are grateful to mgr Kornel Michalak for his help in photographic documentation. We thank Dr hab. Piotr Szkudlarz and Dr hab. Zbigniew Celka for help in the preparation of scans of herbarium specimens.

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