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MORPHOLOGY AND PECULIAR FEATURES OF SPORES OF FERN SPECIES OCCURRING IN POLAND

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Abstract

The morphology of fern spores collected from natural sites in Poland was examined under light microscopy. Spore samples represented 44 species in 18 genera and in 13 families. Only spores of Ophioglossaceae were obtained from the herbarium of the Adam Mickiewicz University in Poznań while the remaining samples were obtained from living plants. Spore size ranges between 20 to 75 µm and the spores of *Osmunda regalis* and *Polypodium interjectum* were found to have remarkably large dimensions. The spores are ellipsoidal, tetrahedral and spherical/globoid in shape. Their apertures are monolete or trilete types. The exine surface patterns are baculate, cristate, granulate, reticulate, tuberculate and verrucate. Pictures of the analyzed spores are collected in a contrasting (size, colour) table to make it easier to distinguish between species.

The peculiar characters of fern spores are described after a review of major articles concerning the allergenic features of fern spores with special attention to *Pteridium aquilinum* whose spores and vegetative tissues revealed mutagenic and carcinogenic activity.

Key words: fern spores, shape/size, surface ornamentation, allergenic effects

INTRODUCTION

Studies to determine spores of fern species native to Poland have not been extensive (Pięknoś 1974, Pięknoś 1975a, 1975b, Pięknoś-Mirkowa, 1979; Kałuzinska, 1994). In the accessible literature there are some valuable morphological studies of living and fossil fern spores (Tyron and Lugardon, 1991). In such specialist, multithematic works however, which contain data relating to 1480 taxa, it is difficult to find the necessary information needed to identify fern genus or even species with the speed that is sometimes desired. A solution could be provided by compendia of the local pteridoflora, with a comparative combination of pictures belonging to native fern spores. The data included in such a compendium would enable interspecific differences between size, shape and surface sculpture of fern spores to be observed quickly and efficiently.

Information concerning morphological data of fern spores may be useful not only to botanists, but also for palynologists and allergologists. Monitoring atmospheric fern spore loads has been initiated in airstream over Edinburgh UK (Caulton et al. 1995). When the fern stands are 1.6 km away from the city center in a long-term volumetric spore trap placed on a roof-top (21m above the ground) 2-3 spores on m^3 was found. Other analysis revealed that fern spores made up 6.2-8.6% of the total airspora sampled (Lacey and McCartney, 1994). Very light spores overcome long distances and numerous fern species inhabited urban-areas eg. Gdańsk (Buliński, 2002), Poznań (Jackowiak, 1998) or Wrocław (Szczęśniak, 2009)

Detailed study indicated that fern spore diurnal patterns showed a late morning (09.00 and 11.00 hrs.) the peak period of dissemination, highly correlated with local meteorological conditions. The seasonal distribution of airborne fern spores includes late summer and early autumn. They are not abundantly represented in the atmosphere and are counted together with pollen grains and fungal spores. Between August and September the daily mean concentrations exceeded 750 spores on $m^{-3}$of air 1 m from the stand of ferns (Lacey and McCartney, 1994; Siman et al. 1999). With regard to personal observations some species disseminate spores much earlier e.g. *Osmunda regalis* spores are

ready to disperse at the beginning of June (from 2-3rd to 12th of June). Moreover, *Matteuccia struthiopteris* a typical rheophyte – at the end of February/ beginning of March (very often during snow coverage). Dispersed spores of some fern species have consequences for human health through inhalation and ingestion, especially in areas where e.g. *Pteridium aquilinum* (bracken fern) population has increased. In England newspapers regularly print information to warn readers about the peak of the dissemination of spores e.g. *Pteridium aquilinum* which are known to contains toxic and carcinogenous substances, (Page, 1997; Siman et al. 1999). The most alarming information about the carcinogenic activity of the extracts from spores and vegetative tissues of bracken has been obtained from experiments that have demonstrated the induction of adducts in the DNA of cultured human (K562) cells in vitro. But the possible carcinogenic effects of spores other than bracken so far are unknown (Siman et al. 2000). Using extracts from spores of five fern species for the nasal test: *Anemia phyllitidis*, *Dicksonia antarctica*, *Pteridium aquilinum*, *Pteris vittata* and *Sadleria pallida*, it was shown that damage was induced after 2 h of exposure in atopic adult volunteers (Geller-Bernstein et al. 1987). In Poland such an analysis concerning our most frequent native fern species has not yet been undertaken.

MATERIALS AND METHODS

The majority of spores were collected from living plants growing in the field, only spores of Ophioglossaceae were obtained from the herbarium of the Adam Mickiewicz University in Poznań. Sites of fern spore collection are listed in Table 1. Spore size ranges were measured in 30 samples of each species for the length of the polar and equatorial axes. The size classes followed Erdtman (1957): very small <10µm; small 10-25µm; medium 25-50µm; large 50-100µm; very large 100-200µm. The descriptive terms of the spore surface followed Tryon and Lugardon (1991). The main morphological microcharacters of spores of 44 species of ferns are summarized in Table 1 which contains their description, useful to distinguish between the taxa. Spore shape and sculpturing terminology is based on Tryon and Lugardon (1991). For slide preparation, spores were mounted in glycerol, viewed under 250 x magnification and photographed under a Jenamed Carl Zeiss Jena microscope with a Practica camera.

The preparation of fern spores from the herbarium specimens for LM observations was made according the method improved by Hu et al. (2010). Usually spores collected from the herbarium are covered by debris (scales and glandular hairs) which affects the analysis of their surface. Therefore, Hu proposed a new technique for the removal of contamination from spores using an ultrasonic wave (50-60Hz, 90W) bath with 70% of ethanol for 10 min. before the standard fixation (Hu et al. 2010).

Thick-walled fern spores are stable in a vacuum which enables SEM analysis of *Pteridium aquilinum* spores without dehydration techniques. Their structural characters provide stable and highly evident criteria for species determination.

RESULTS AND DISCUSSION

In the present study, the spore morphology of homosporous fern species was investigated by light microscopy. This contribution is a part of a broader study on fern spores obtained from species native to Polish pteridoflora. The group covered in this study consists of 44 taxa from approximately 53 mentioned in the Polish Check List (Mirek et al. 2002).

The size of the majority of spores was rather medium (between 25-40 µm) in length or diameter; only spores of *Osmunda regalis* and *Polypodium interjectum* had a wide range in dimension. The size of spores may correlate with ploidy level and the number of spores per sporangium. Three spore size groups, which contained small, medium and large spores, were distinguished (Table 1).

Under LM, the spores revealed differences in their morphological characters (e.g. size, shape, colour, and the surface pattern of ornamentation). The aperture types of the spores were trilete and monolete (triradiate or simple leasural) and they were correlated with spore shape. When the shape was globose/spheroidal or tetrahedral, the aperture was trilete, and when it was ellipsoidal in the polar view, the aperture was monolete. The trilete spores have radial symmetry and were flat or convex at the distal face, while monolete, ellipsoidal or reniform spores have bilateral symmetry (Fig. 2).

The colour of trilete spores of Ophioglossaceae (a rare and threatened species in Poland) is white to light yellow, with small spheroid-tuberculate elements on the surface. Monolete spores, medium sized in the majority, ranged from light to dark brown, except for the bright yellow dye of *Polypodium vulgare* and *P. interjectum* spores. Only two species have chlorophyll-bearing spores, which were manifested as the clear green colour of *Osmunda* and the greenish-brown of *Matteuccia struthiopteris* (Fig. 2).

The sporoderm ornamentation depends on the exospore and perispore multifoliate substructure. Notably, an exospore larger than the perispore forms characteristically long, undulate folds or wings contiguous to the lower lying strata.

Under SEM analysis, tetrahedral spores of *P. aquilinum* (relatively small) were not distinguished from other species by any special morphological character (Fig. 1 A, B). Their apertures are commisures, linear, narrow, and trilete. The exine is thick and densely structured. Ornamentation is similar on the distal and proximal face. The surface is irregularly covered by mild, small verrucae and bacula, without any sharp structures. The spores are heteropolar with a rounded distal face and a flattened proximal face, while their ambs are rounded to triangular. *Pteridium aquilinum* has the potential to realase large numbers of relatively small spores which are dispersed by wind between August and October. Bracken spore density was mainly measured in the proximity of fern populations where spore concentrations are often very high. One person may inhale 50,000 bracken spores in 10 min. (De la Cruz et al. 2010).

According to literature data, fern spores may contain a variety of carcinogens such as: ptaquiloside (norsesquiterpene glucoside); L-ecdysone and 20-hydroxy ecdysone; shikimic acid; aquilide A and quercetin, whose presence and activity have also been analysed in bracken spores (Siman et al. 1999 and literature cited there). The DNA-damaging potential of *Pteridium aquilinum* spores and aqueous extract of fronds was demonstrated when mice were force--fed with bracken and were seen to have developed lung, intestinary or mammary tumours and died (Siman et al. 2000). Spores of other homosporous ferns, *Anemia phyllitidis*, *Pteris vittata* and *Sadleria pallida*, also induce DNA adducts, i.e. covalent modifications of the normal bases present in the DNA and detected by 32 P-post-labelling analysis (Siman et al. 2000).

The seasonal distribution of airborne fern spores includes late summer and early autumn. They are not abundantly represented in the atmosphere and are counted together with pollen grains and fungal spores.

Positive skin test reactions to aqueous extracts from fern spores were observed in a group of patients suffering from hay-fever (Geller-Bernstein et al. 1987). Common household decorative ferns, such as *Adiantum capillus-veneris, Cyrtomium falcatum* and *Pteris vittata*, induced milder skin reactions, but *Dryopteris setigera* induced a much stronger one. When spore extracts of *Acrostichum aureum* were used in nasal provocation tests, 60-70% of patients suffering from allergic health problems reacted to this factor (Bunnag et al. 1989).

Exposure to *Lycopodium* spores caused a case of asthma in a dental technician, as reported by Kobayashi (in Siman et al. 1999). The published literature contains some information relating to the adverse effects of fern spores (skin rashes, respiratory problems, sneezing and watering of the eyes) in fern spore collectors and herbarium curators. A preventative measure against spore inhalation is to wear a face mask so as to avoid contact with dangerous particles (Siman et al. 1999).

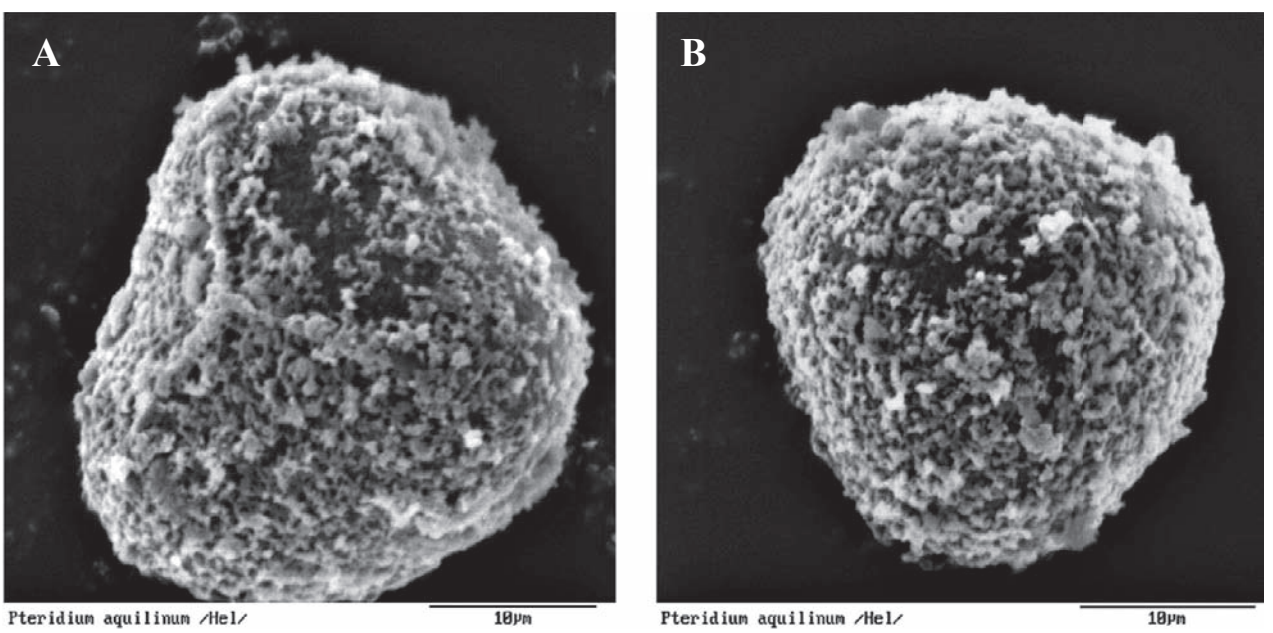
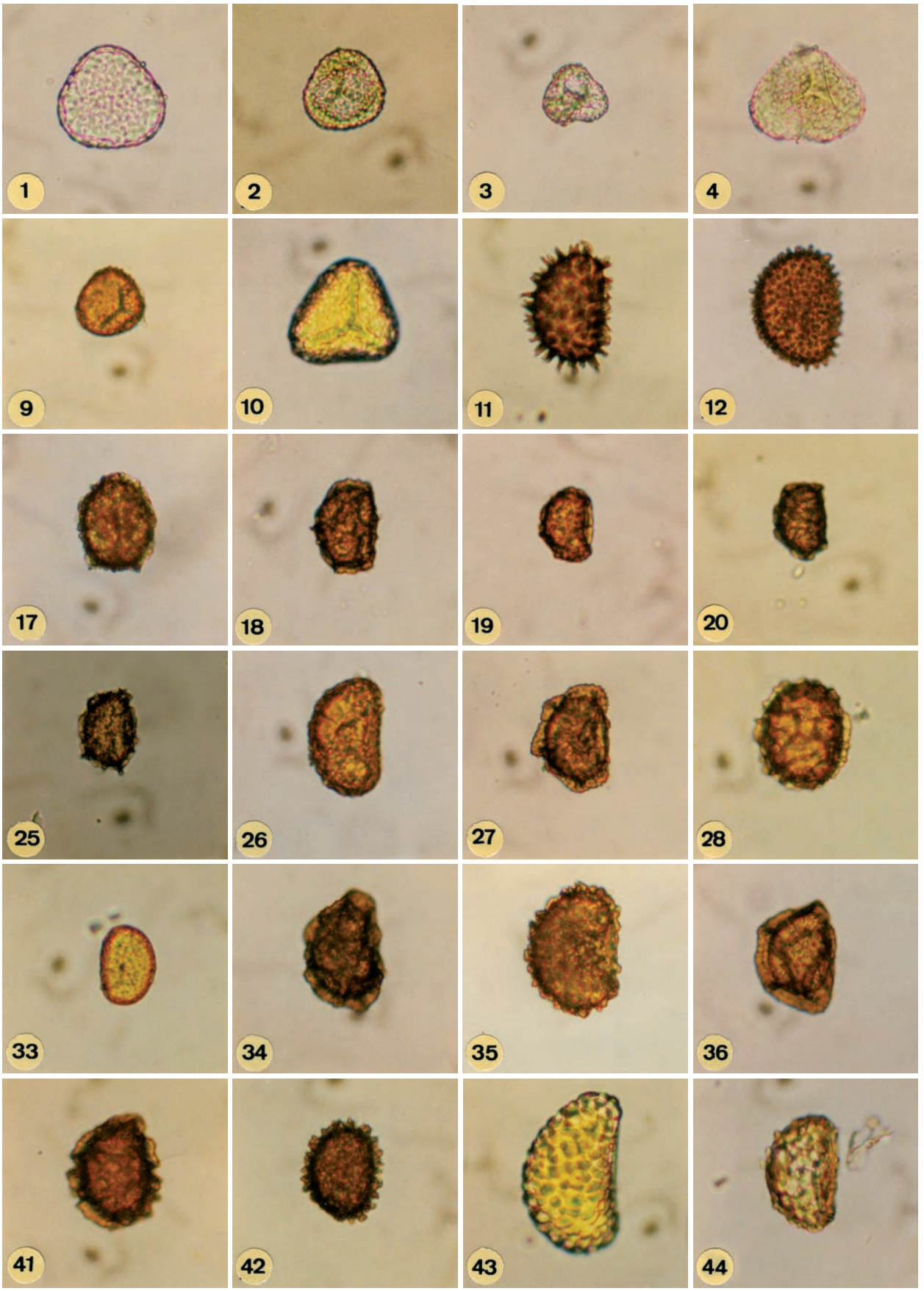


Fig. 1. A, B – A – Proximal and B – distal faces of trilete, tetrahedral spores of *Pteridium aquilinum.* SEM, Bar 10 µm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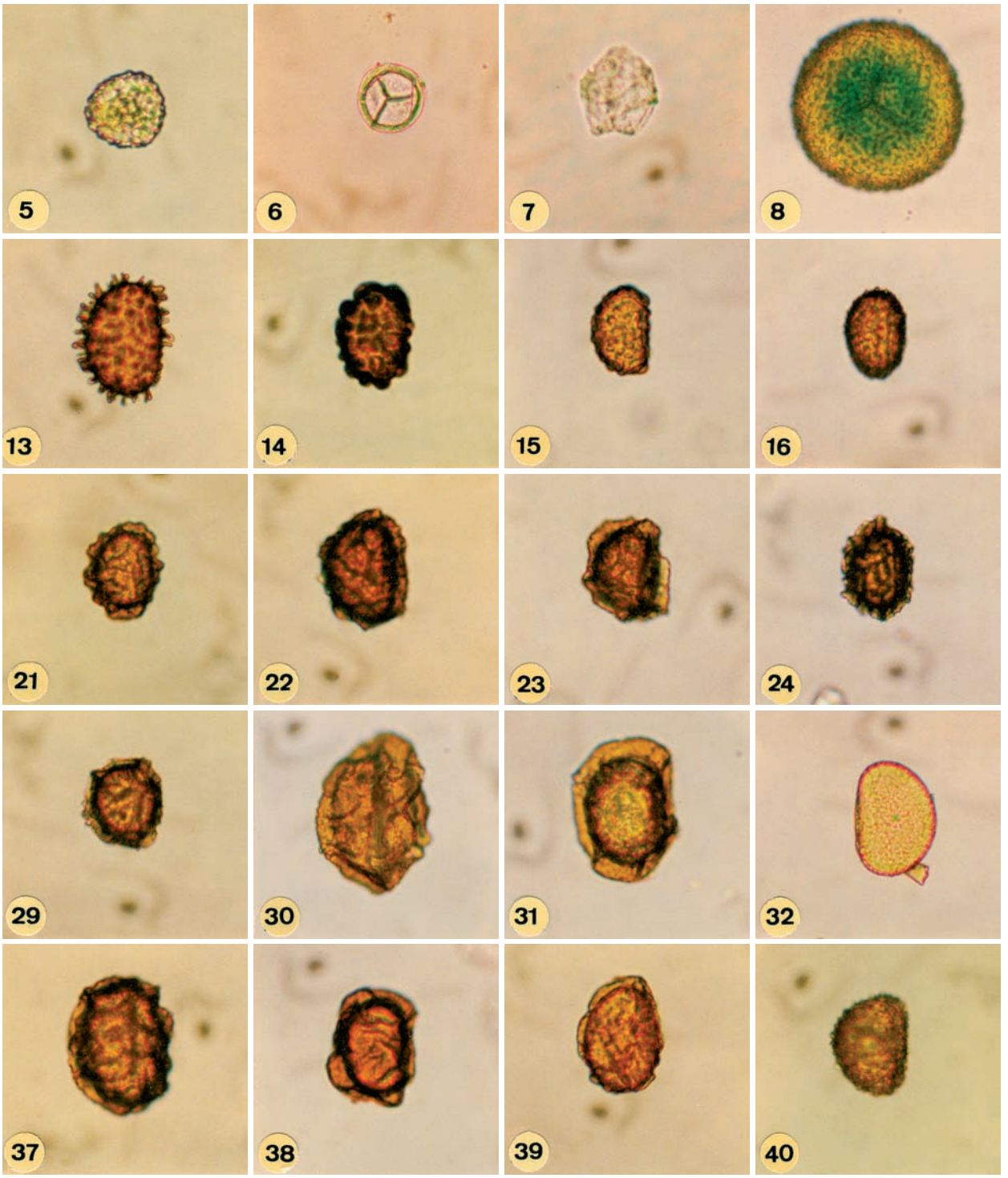

Fig. 2. Shape, size, colour and surface pattern of analyzed spores of 44 species of ferns 1. *Botrychium lunaria;* 2. *B. matricariifolium;* 3. *B. multifidum;* 4. *B. simplex;* 5. *B. virginianum;* 6. *Ophioglossum azoricum;* 7. *O. vulgatum;* 8. *Osmunda regalis;* 9. *Pteridium aquilinum;* 10. *Cryptogramma crispa;* 11. *Cystopteris fragilis;* 12. *C. montana;* 13. *C. regia;* 14. *C. sudetica;* 15. *Gymnocarpium dryopteris;* 16. *G. robertianum;* 17. *Asplenium adiantum-nigrum;* 18. *A. adulterinum;* 19. *A. cuneifolium;* 20. *A. onopteris;* 21. *A. ruta-muraria;* 22. *A. septentrionale;* 23. *A. trichomanes;* 24. *A. viride;* 25. *Phyllitis scolopendrium;* 26. *Thelypteris palustris;* 27. *Phegopteris connectilis;* 28. *Woodsia alpina;* 29. *W. ilvensis;* 30. *Matteuccis struthiopteris;* 31. *Blechnum spicant;* 32. *Athyrium distentifolium;* 33. *A. filix-femina;* 34. *Dryopteris carthusiana;* 35. *D. cristata;* 36. *D. dilatata;* 37. *D. expansa;* 38. *D. filix-mass;* 39. *D. villarii;* 40. *Polystichum aculeatum;* 41. *P. braunii;* 42. *P. lonchitis;* 43. *Polypodium vulgare;* 44. *P. interjectum*

Table 1.
The main morphological characters of spores of 44 species of ferns occurring in Poland.

Family/Species	Chromosome number	Sites	Type / Shape / Colour	Size (μm)	Surface
			OPHIOGLOSSACEAE		
Botrychium lunaria (L.) Sw.	2n = 90	Śnieżne Kotły, Karkonosze Mts	Tetrahedral-globose /White	31-32 x 30- 33 Small	Irregular papillate-verrucate
Botrychium matricariifolium (Retz) A. Braun ex W.D.J. Koch	2n = 180	Florianki, Zamość County	Tetrahedral-globose/ White	31-35 x 35-38 Small	Irregular verrucate
Botrychium multifidum	2n = 90	Borzyszkowo, Chojnice County	Tetrahedral-globose/ White	26-28 x 22-45 Medium	Irregular papillate-verrucate
Botrychium simplex E. Hitchc.	2n = 90	Lipnica, Chojnice County	Tetrahedral-globose/ White	22-25 x 20-38 Small	Irregular papillate-verrucate
Botrychium virginianum (L.) Sw.	2n = 90	Kotłów, Złoczów County	Tetrahedral-globose/ White	18-22 x 25-32 Small	Coarsely-verrucate
Ophioglossum azoricum K. Presl	2n =720	Zemborzyce near Lublin	Globose/White	23-26 x 30-32 Small	Rugulate / reticulate
Ophioglossum vulgatum L.	2n = 480	Lower Silesia	Globose/White	29-26 x 30-31 Small	Rugulate / reticulate
			OSMUNDACEAE		
Osmunda regalis L.	2n = 44	Brzeziny, Kalisz Forest District	Spheroidal / White green chloroplasts	60-65 Large	Coarsely tuberculate with echinate tubercles *ryptogramma*
			DENNSTAEDTIACECE		
Pteridium aquilinum (L.) Kuhn	2n = 104	Puszcza Zielonka near Poznań	Tetrahedral-globose	22-25 x 25-28 Small	Irregularly granulate
			PTERIDACEAE		
Cryptogramma crispa (L.) R. Br.	2n = 120	Duży Kocioł Śnieżny Karkonosze	Tetrahedral-globose	35- 38 x 40-45 Large	Verrucate
			CYSTOPTERIDACEAE		
Cystopteris alpina (L.)	2n = 252	scree slope, M.Oko, Tatra Mts	Ellipsoidal/Brown	26- 28 x 44-55 Medium	Echinate
Cystopteris fragilis (L.) Bernh.	2n = 252	scree slope, M. Oko, Tatra Mts	Ellipsoidal/Brown	26- 28 x 34-42 Medium	Coarsely echinate
Cystopteris montana (Lam.) Desv.	2n = 168	scree slope, M. Oko, Tatra Mts	Ellipsoidal/Brown	22- 26 x 42-58 Medium	Coarsely echinate
Cystopteris sudetica A. Braun et Milde	2n = 168	Białka Valley, Tatra Mts	Ellipsoidal/Brown	18-23 x 26-35 Medium	Coarsely echinate
Gymnocarpium dryopteris (L.) Newman	2n = 80	Czarny Staw, Tatra Mts	Ellipsoidal/Brown	18-22 x 29-32 Small	Inflated folds
Gymnocarpium robertianum	2n = 80	Łysa Polana	Ellipsoidal/Brown	18-25 x 24-36 Small	Inflated folds
			ASPLENIACEAE		
Asplenium adiantum-nigrum L.	2n = 72	Winna Góra near Sobótka	Ellipsoidal/ Spheroidal/Brown	23-35x 38-52 Medium	Alate/ prominent long wings/ cristate
Asplenium adulterinum Milde	2n = 144	Winna Góra near Sobótka	Ellipsoidal/Brown	21-31 x 30-40 Small	Echinulate, winged folds
Asplenium cuneifolium V iv. ristate	2n = 72	Kamieniołom, Kielczyn	Ellipsoidal/Brown	25-34 x 35-41 Small	Echinulate, winged folds
Asplenium onopteris L.	2n = 72	Winna Góra near Sobótka	Ellipsoidal/Brown	22-28 x 32-35 Small	Echinulate, winged folds
Asplenium ruta-muraria L.	2n = 144	viaduct, Wielkopolska National Park	Ellipsoidal/Brown	32-34 x 35-42 Medium	Echinulate, winged folds /cristate

Asplenium septentrionale (L.) Hoffm	2n = 144	Radunia, Sobótka Forest District	Ellipsoidal/Brown	25-34 x 35-46 Medium	Echinulate, winged folds
Asplenium trichomanes L.	2n = 72	Radunia, Sobótka Forest District	Ellipsoidal/Brown	20-25 x 29- 38 Small	Echinulate, low criste
Asplenium viride Huds.	2n = 72	Winna Góra near Sobótka	Ellipsoidal/Brown	25- 32 x 34-44 Medium	Echinulate, winged folds
Phyllitis scolopendrium (L.) Newm.	2n = 72	Wąwóz Myśliborski, Jawor Forest District	Ellipsoidal/Brown	35-44 x 50-65 Medium	Folds cristate-fenestrate
		THELYPTERIDACEAE			
Thelypteris palustris Schott	2n = 70	Strzeszyńskie Lake near Poznań	Ellipsoidal/Brown	25-28 x 35-33 Small	Echinulate with winged folds
Phegopteris connectilis (Michx.) Watt	2n = 90	Szrenica, Karkonosze Mts	Ellipsoidal/Brown	31-43 x 35-48 Medium	Echinulate
		WOODSIACEAE			
Woodsia alpina (Bolton) Gray	2n = 164	Western Tatra Mts	Ellipsoidal/Brown	35-38 x 41-58 Medium	Papillate wing-like folds
Woodsia ilvensis (L.) R. Br.	2n = 82	Western Tatra Mts	Ellipsoidal/Brown	21-25 x 32-42 Small	Echinulate, winged folds
		ONOCLEACEAE			
Matteuccia struthiopteris (L.) Tod.	2n = 80	Skoczów on Vistula River	Ellipsoidal/Brown	38-42 x 50-65 Large	Echinulate wing-like folds
		BLECHNACEAE			
Blechnum spicant (L.) Roth	2n = 68	Śnieżnik Massif	Ellipsoidal/Brown	27-34 x 34-48 Medium	Rugulate, irregularly granulate
		ATHYRIACEAE			
Athyrium distentifolium Tausch ex Opiz	2n = 80	Szrenica, Karkonosze Mts	Ellipsoidal/Yellow	22-27 x 30-44 Small	Rugate
Athyrium filix-femina (L.) Roth	2n = 80	Kobylnica near Poznań	Ellipsoidal/Yellow	29-35 x 48-65 Medium	Rugulate-papillate folds
		DRYOPTERIDACEAE			
Dryopteris carthusiana (Vill.) H.P. Fuchs	2n = 164	Kobylnica, near Poznań	Ellipsoidal/Brown	26-30 x 39-42 Medium	Inflated folds -echinulate tubercles
Dryopteris cristata (L.) A. Gray	2n = 164	Przytór Peninsula	Ellipsoidal/Brown	23-29 x 40-44 Medium	Compresed folds with cristae
Dryopteris dilatata (Hoffm.) A. Gray	2n = 164	Kobylnica near Poznań	Ellipsoidal/Dark brown	28-34 x 30-47 Medium	Short inflated folds
Dryopteris expansa (C.Presl) Fraser-Jenk.& Jermy	2n = 82	Smokowiska, Low Beskids	Ellipsoidal/Dark brown	26-33 x 31-44 Medium	Short inflated folds
Dryopteris filix-mass (L.) Schott	2n = 164	Kobylnica near Poznań	Ellipsoidal/Brown	25-31 x 36-45 Medium	Inflated folds
Dryopteri villarii (Bellardi) Woyn. ex Schnitz&Thell.	2n = 82	Mountain Botanical Garden Zakopane	Globoid	20-28 x 34-45 Medium	Inflated folds
Polystichum aculeatum (L.) Roth	2n = 164	Bukowica, Low Beskids	Ellipsoidal/Dark brown	27-36 x 42-49 Medium	Inflated folds , cristae
Polystichum brauni (Spenn.) Fee	2n = 164	Smokowiska, Low Beskids	Ellipsoidal/Brown	26-35 x 30-45 Medium	Fenestrate folds, cristae
Polystichum lonchitis (L.) Roth	2n = 82	Belianskie Tatras	Ellipsoidal/Brown	30-38 x 40-48 Medium	Echinate cristae
		POLYPODIACEAE			
Polypodium interjectum Shivas	2n = 222	Międzyzdroje	Fusiform/Ellipsoidal/ Yellow	39-45 x 53-78 Large	Coarsely-verrucate
Polypodium vulgare L.	2n = 148	Międzyzdroje	Ellipsoidal/Yellow	35-40 x 43-55 Medium	Coarsely-verrucate granulate

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Morfologia i szczególne własności zarodników paproci występujących w Polsce

Streszczenie

Morfologię zarodników paproci pochodzących ze stanowisk naturalnych w Polsce analizowano w mikroskopie świetlnym. Zarodniki należały do 44 gatunków z 18 rodzajów i 13 rodzin. Jedynie zarodniki Ophioglossaceae pochodziły ze zbiorów zielnikowych UAM w Poznaniu, pozostałe zebrano w terenie z żywych roślin. Pomiary wielkości zarodników wykazały, że mieściły się one w przedziale od 25 do 70 µm, a do największych należały spory *Osmunda regalis* i *Polypodium interjectum.* Większość zarodników miała kształt elipsoidalny, część – tetrahedralny, a tylko dwa gatunki paproci tworzyły zarodniki sferyczne lub kuliste. Apertura zarodników należała do dwu typów – szczelinowego (monolete) lub trójdzielnego (trilete). Najczęściej występującym wzorem ornamentacji egzyny były oskrzydlenia, okienkowate pofałdowania oraz drobne lub większe uwypuklenia typu: baculate, cristate, granulate, reticulate, tuberculate lub verrucate. Fotografie zarodników 44 gatunków paproci zebrano w tablicę, ułatwiającą rozróżnienie gatunków m. in. na podstawie ich barwy i wielkości. Szczególne własności zarodników paproci podano za literaturą wskazującą na alergogenny charakter wodnego wyciągu z zarodników, zwłaszcza *Pteridium aquilinum,* wykazujących ponadto własności mutagenne i karcinogenne. Dodatkowo zamieszczono elektronogram SEM zarodników *Pteridium aquilinum*, których szkodliwość dla zdrowia ludzi została udowodniona.