

A Comparative Study of Spore Morphology of Some *Pteridoideae* Subfamily Genera

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Using the method of scanning electronic microscopy (SEM), a comparative study of ten representatives of subfamily *Pteridoideae* C.Chr. ex Crabbe, Jermy & Mickel family *Pteridaceae* E.D.M.Kirchn. was carried out. A comparative study of morphological characters of investigated spores has revealed characters that allow considering the relatedness of the studied species to one subfamily – *Pteridoideae*. Those characters include spore form – triangular-roundish, laesura rays are straight, merged into sporoderm (laesura lips), tubercles on the spore surface, and in some cases “cerebriform” folds, exosporium surface without excrescences. For species of the genera *Afropteris* and *Taenitis*, we described characters that allow considering them apart from the complex of characters belonging to the species of genera *Actiniopteris*, *Anopteris*, *Onychium*, *Jamesonia* and *Pteris*.

Key words: subfamily Pteridoideae, family Pteridaceae, Actiniopteris, Jamesonia, Onychium, Pteris, Anopteris, Afropteris, Taenitis, morphology of the spores, scanning electronic microscopy (SEM).

Family *Pteridaceae* E.D.M. Kirchn. is considered one of the most complex families according to the amount of unresolved issues in the systematics of its taxonomic ranks. Partly due to insufficient knowledge of this family, as well as the lack of evidential techniques, the final volume of the family is not determined. Members of the family *Pteridaceae* are spread across all continents

except Antarctica. The maximum species diversity is observed in tropical and subtropical latitudes of the Old and New Worlds.

The system of the family *Pteridaceae* was repeatedly reprocessed based on the ferns systems being developed. In the modern period, the use of molecular genetics to solve systematic problems in a number of major works¹⁻³ allowed to prove relatedness of five subfamilies to a family *Pteridaceae* based on in-depth analysis of previously proposed systems: *Cryptogrammoideae* S. Linds.; *Pteridoideae* C.Chr. ex Crabbe, Jermy a. Mickel; *Ceratopteridoideae* (J. Sm.) R.M. Tryon;

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Vittarioideae (C. Presl) Crabbe, Jermy a. Mickel; *Cheilantheroideae* W.C. Shieh. To construct a system of the family, the information on individual genes of the chloroplast and nuclear DNA were used to recreate the intergenetic family connections in the system *Pteridaceae* family by constructing phylogenetic trees.

Subfamilies *Ceratopteridoideae* and *Cheilantheroideae*, being brought by a number of authors to the rank of families are considered the most stable system of ferns in the world. In particular, due to the presence of stable characters of external morphology. In subfamilies *Vittarioideae*, *Cryptogrammoideae* and *Pteridoideae*, a reverse situation is observed – so far, there is no definitive evidence to prove the inclusion into specified subfamilies of the number of ferns. In the course of studying ferns, for historical reasons, genera, which could not be reasonably related to a particular taxonomic category for various reasons, were included in the subfamily *Pteridoideae*.

As a result, in the work on fern spores of *Pteridaceae* family from the territory of Formosa (Taiwan)⁴ due to the use of “raw” and incomplete system of ferns of *Pteridaceae* family, as well as a misunderstanding of its volume, an erroneous spore morphology classification was offered. Consequently, for the family *Pteridaceae* was proposed a classification with two types of spores – bilateral and trilete-tetrahedral spores. In the light of the above, it is necessary to correlate the results of one’s anatomical and morphological and molecular genetic studies with new information on the taxonomy of any given taxonomic group.

Apart from the information obtained by methods of molecular genetics, in order to determine the phylogenetic boundaries of genera and establish a clear distinction between them, the results of microphotography scanning microscope have been often used over the last years. Micrographs of spores obtained with a scanning electron microscope are more informative; provide more information to resolve disputes in the systematics of complex fern groups.

Spore morphology of individual members of the subfamily *Pteridoideae* at some point were paid enough attention in large reports devoted to morphology of fern spores⁴⁻⁸. However, these works provide the descriptions only for some

members of the subfamily *Pteridoideae*. Photographs of the ferns in the works of Belling and Heusserl, as well as in Nayar with Devi’s can only be used to describe the form and carry out very “rough” measurements for biometric research. It is impossible to identify and describe the detailed morphological structures, laesura structures, equatorial ridges, tubercles on the sides of a spore and describe the surface of exosporium based on the photographs given in those papers. The exact determination of the size of all spore structures and their description is required to identify the common characters on intergeneric, specific and intersectional levels. Thus, in the works dated last century, the description of spores, as the photographs themselves do not contain enough information about the spore morphology of this complex group of ferns and require modern high-tech equipment and new additional data.

However, survey works on the morphology of fern spores of *Pteridaceae* family allow yet to receive general information on a number of stable characters common for subfamily *Pteridoideae*: tetrahedral spores with thirradial laesura, triangular-roundish or roundish-triangular, have a clear exosporium with a distinct surface ornamentation and total absence of perisporium, the surface of exosporium varies from rough to having large tubercles and roller-like thickenings.

MATERIALS AND METHODS

Spores for the study were selected from herbarium materials stored in the herbarium of the National Museum of Natural History (R), the Botanical Garden and Botanical Museum Berlin-Dahlem (B), the V.L. Komarov Botanical Institute of the Russian Academy of Sciences (LE) and the National Research Tomsk State University (TC).

Spores were investigated using scanning electron microscope “Philips SEM 525-M”, and electron-ion scanning microscope “Quanta 200 3D” situated in Tomsk Material Testing Center for collective use of the National Research Tomsk State University, a scanning electron microscope “Hitachi – S 3400 N” (Tomsk) in the laboratory of the Institute of water and Ecological Problems (Barnaul) and JEOL JSM-6390LA Analytical Scanning Electron Microscope of Center for collective use of the V.L. Komarov Botanical

Institute of the Russian Academy of Sciences (Saint-Petersburg). Spores were fixed using a carbon adhesive tape; to reduce the influence of the charge a method of thermal spraying by chromium, carbon or gold-palladium mixture in a vacuum sputtering plant. Spore surface was scanned in high vacuum at an accelerating voltage of 2 kV and a magnification of 1000 to 7000 times, and 10,000 to 16,000 times. The measurements of spores were carried out using “SIAMS MesoPlant” and “Photometer” programs.

An analysis was performed on the following spore morphological characters: 1 – equatorial diameter, μm ; 2 – polar axis; 3 – laesura length; 4 – laesura width; 5 – the width of the equatorial ridges; 6 – roller-like thickening width; 7 – roller-like fold thickness on the distal side of the spore; 8 – roller-like fold thickness on the proximal side of the spore; 9 – roller-like thickening width around laesura; 10 – diameter of tubercles on the proximal side of the spore; 11 – excrescences diameter on the proximal side of the spore; 12 – excrescences diameter on the distal side of the spore; 13 – roller-like thickening width along the contour of the distal side of disjuncts; 14 – roller-like thickening width along the contour of the proximal side of spore; 15 – transverse fold at the corners of a spore. Measurements were carried out in 25 replications.

RESULTS

Actiniopteris australis (L. f.) Link, 1841, Fil. Sp.: 80. – *Acrostichum australe* L. f. 1781, Suppl.: 444

SEM-description. Spores in a polar position are triangular-roundish, with straight sides (non lociniate), seldom – roundish-triangular with concave sides (lociniate). The equatorial diameter is of (46.4) 49.35 (53.4) μm . The distal side in an equatorial position is hemispherical, the proximal one convex. Equatorial ridge is distinctly expressed, (2.6) 3.75 (4.9) μm broad. The laesura rays are straight, (23.2) 26.1 (29.0) μm long, (1.1) 2.5 (3.9) μm broad. Some spores have irregular thickened laesura rays, up to 3.0 μm broad near the pole, to 1.6 μm near the ends of the laesura rays. The laesura lips are absent or occasionally weakly expressed. The region near the ends of the laesura rays border sharply on to the equatorial collar, or rarely merge with equatorial collar. The distal side

is rugose, with folds (3.0) 5.4 (7.8) μm broad. The proximal side has tuberculum-like ornamentation, tubercles are small, (1.1) 2.75 (4.4) μm in diam. The folds and tubercles have a distinct outline: the tubercles are roundish in outline, dense beside the pole and sparse on the margins. The surface of the exosporium is coarse-granulate, with sparse roundish excrescences (0.2) 1.25 (2.3) μm in diam. at the distal side, and (0.2) 0.7 (1.2) μm in diam. at the proximal side. Investigated specimen: Nilghiris, Mardas. Legit. Boord of Reueneue (P).

Actiniopteris dimorpha Pichi-Serm., 1962, *Webbia* **17**, **1**: 18, f. 2.

SEM-description. Spores in a polar position are triangular-roundish or irregular-triangular-roundish with straight sides (non lociniate). The equatorial diameter is (46.5) 54.5 (62.5) μm . The distal side in equatorial position is hemispherical, the proximal side is convex, with an acute pole. Equatorial ridge is distinctly expressed, (2.2) 3.4–4.9 (6.1) μm broad. The rays of laesura are straight (25.8) 27.5 (31.1) μm long, raising along the all length above the surface of the spore because of roller-like bulges of the sporoderm (‘laesura lips’). The breadth of the laesura rays is (2.1) 2.2 (2.3) μm , but in the most cases the rays do not have a clear outline because they are merged in full or in fragments in the ‘laesura lips’ (5.6) 6.7 (7.8) μm broad. The distal side is sinuate-rugose, folds are (3.4) 4.45 (5.5) μm broad. The proximal side has a tuberculum-like ornamentation, with tubercles (1.4) 3 (4.6) μm in diam. The tubercles have more or less clear outline, the tubercles more or less fused into folds. The surface of the exosporium at the distal side is coarse-granulate, the granularity is distinctly expressed, rarely it is fine-granulate with numerous roundish excrescences (0.3) 1.45 (2.6) μm in diam. The surface at the proximal side is coarse-granulate with sparse roundish excrescences (0.1) 0.9 (1.7) μm in diam.

Investigated specimens: “Universite de Lubumbashi (Zaire), Mont Mubwe (29°45' E, 13°20' S), Pente rocheuse, Alt=1400 m. 6 IV 1987. No. 2514 (P); Madagascar, Prov. Fianarantsoa, Vicinity of Zazafotzy on Route 07 between Ambalavao and Ihotay, open forest and prairie, elev. 710 m. In dense, solid stand, ca 1 m across. 2 II 1975. Thomas B. Crost, No. 30373” (P).

***Actiniopteris pauciloba* Pichi-Serm., 1962, *Webbia* 17, 1: 21, f. 3.**

SEM-description. Spores in a polar position are roundish-triangular or irregularly-roundish-triangular with straight sides (non lociniate). The equatorial diameter is (50.7) 58.4 (66.1) μm . The distal side in an equatorial position is hemispherical, the proximal one is flat, raising only along laesura rays. Equatorial ridge is distinctly expressed, (3.9) 5.7 (7.5) μm broad. The rays of laesura are straight, (23.1) 25.35 (27.6) μm long, raising above the spore surface along the whole length or only up to its middle with margins thickened into thick 'lips'. In the first case astride both sides of the region near the ends of the laesura ends (the 'laesura lips') and the equatorial collar does not have distinct borders. In the second case the ends of laesura rays clearly border astride both sides with the equatorial collar. The breadth of the laesura rays is (1.4) 1.7 (2.0) μm . The 'laesura lips' have smooth margins along the whole length, 4.7–5.3 μm broad. The distal side is sinuate-rugose, the folds are (2.8) 5.3 (7.8) μm broad. The proximal side has tuberculum-like ornamentation, tubercles are (1.4) 2.4–4.1 (4.9) μm in diam. The folds and tubercles have distinctive outlines. The surface of the exosporium at the distal side is fine-granulate with sparse roundish excrescences of (0.05) 3.15 (0.9) μm diam.

Investigated specimen: "Flora Congolana. Prov. Katanga, Territ. Manono, Loc. Kayiba, Alt=1140 m, Statio Foret katangaise. Février. 1948. Coll. G.F. de Witte, No. 3343" (B).

***Actiniopteris radiata* (Koenig ex Sw.) Link. 1841, *Fil. Sp. Hort. Berol.*: 80. – *Asplenium radiatum* Koenig ex Sw. 1801, *Journ. Bot.* 1800, 2: 50.**

SEM-description. Spores in a polar position are triangular-roundish with straight or slightly convex sides (non lociniate), sometimes roundish-triangular, with slightly concave sides. The equatorial diameter is (36.6) 40.15 (43.7) μm . The distal side in an equatorial position is hemispherical, the proximal one is broadly conical with straight sides and acute pole. The distal and proximal surfaces are separated by a clearly expressed equatorial ridge (3.2) 4.5 (5.8) μm broad. The rays of laesura are straight, (14.8) 16.8 (18.8) μm long, (0.5) 0.95 (1.4) μm broad. Bulges of sporoderm along the rays of laesura are absent. The plots near the ends of the laesura rays are not

connected with the thickened parts of the equatorial collar or abruptly border upon it. The distal side of spore is tuberculate-rugose, the folds are sinuous, (2.1) 3.7 (5.3) μm broad. The proximal side has tuberculum-like ornamentation, tubercles are (0.8) 2.55 (3.3) μm in diam. The folds and tubercles have a clear outline. The surface of the exosporium at the distal side of the spore is fine-granulate with roundish excrescences (0.2) 0.8 (1.4) μm in diam. The surface of the exosporium at the proximal side is fine-granulate with sparse roundish excrescences (0.1) 0.45 (0.8) μm in diam. Investigated specimen: [the inscription on the label is unintelligible] "No. 62" (B).

***Actiniopteris semiflabellata* Pichi-Serm., 1962, *Webbia* 17, 1: 24, f. 4.**

SEM-description. Spores in a polar position are triangular-roundish or irregularly-triangular-roundish, with straight or slightly convex sides (non lociniate). The equatorial diameter is (55.0) 60.85 (66.7) μm . The distal side in equatorial position is hemispherical, the proximal one convex or nearly flat. Equatorial ridge is (3.5) 4.15 (4.8) μm broad. The laesura rays are straight, (26.8) 31.2 (35.6) μm long and (1.3) 2.25 (3.2) μm broad. The margins of laesura are not thickened. The segments near the laesura ray ends are vague and do not have exact borders with the equatorial collar. The distal side is tuberculate-rugose, the folds are (3.4) 4.6 (5.8) μm broad. The proximal side has tuberculum-like ornamentation, tubercles are (1.0) 2.3 (3.6) μm in diam. The folds and tubercles have not exact outlines. The surface of the exosporium is coarse-granulate, with roundish excrescences (0.7) 1.3 (1.9) μm in diam. at the distal side, and with a single roundish excrescences (0.3) 0.85 (1.4) μm in diam. at the proximal side.

Investigated specimens: "Herbier, A. Baudouin, Fougerel, Reunion, "Juale", No. 584 (B); Centre O.R.S.T.O.M. de Tananarive, Reunion, Sur rocks an solue rote de alaos. 5.3.71. Coll. F. Friedmann, No. 1097" (B).

***Onychium japonicum* (Thunb.) Kunze, 1848, *Bot. Zeit.* 6: 507. – *Caenopteris japonica* Thunb. 1795, *Nova Acta Acad. Sci. Petropol.*, 9: 161.**

SEM-description. Spores in a polar position are triangular-roundish, non lociniate. The equatorial diameter is (30.7) 36.4 (42.1) μm . The distal side of the spore in an equatorial position is hemispherical, the proximal one convex. The

equatorial ridge is distinctly expressed (2.2) 2.8 (3.4) μm broad. The rays of laesura are straight, (13.0) 14.4 (15.8) μm long, (1.1) 1.6 (2.1) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (1.4) 2.25 (3.1) μm broad are formed on both sides of laesura rays. The distal side is tuberculate-pleated with single tubercles and short sinuous folds (1.3) 2.45 (3.6) μm in thickness. The ornamentation of proximal side includes besides laesura and 'laesura lips' single tubercles and sinuous folds which are arranged parallel to the spore margins. The surface of the exosporium at the proximal side is fine-grainulate, with sparse roundish excrescences (0.3) 0.85 (1.4) μm in diam. on the distal side and (0.6) 2.1 (3.6) μm in diam. on the proximal side.

Investigated specimen: "Hinghwa and vicinity, No. 6172, Lin Pi, July 24 1926" (TK).

***Onychium lucidum* (D. Don) Spreng., 1827, Syst. Veget. 4: 66. – *Leptostegia lucida* D. Don, 1825, Prodr. Fl. Nepal.: 14.**

SEM-description. Spores in a polar position are triangular-roundish, non lociniate. The equatorial diameter is (33.8) 36.35 (38.9) μm . Both the distal and proximal sides of the spore in an equatorial position are convex. The equatorial ridge is distinctly expressed (3.0) 4 (5.0) μm broad. The rays of laesura are straight, (12.2) 14.8 (17.4) μm long, (0.8) 1.25 (1.7) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (2.1) 2.95–3.11 (3.8) μm broad are formed on both sides of laesura rays. The distal side is sinuate-pleated, folds are (2.4) 2.95 (5.4) μm in thickness. The ornamentation of proximal side includes besides laesura and 'laesura lips' elongate tubercles and sinuous folds (2.3) 3.2 (4.1) μm in thickness which are arranged on the spore margins. The surface of the exosporium is coarse-grainulate with sparse roundish excrescences: (0.6) 1.15 (1.7) μm in diam. on the distal side and (0.6) 1.45 (2.3) μm in diam. on the proximal side.

Investigated specimen: "Hunan, Xinning, Ziyunshan, Li Zhen yu et Al., No. 1100, 14 Sept. 1984, Alt. 950 m, By roadside in gully" (sub nom. *Onychium contiguum*) (LE).

***Onychium moupinense* Ching, 1934, Lingnan Sci. Journ. 13: 500.**

SEM-description. Spores in a polar

position are triangular-roundish, non lociniate. The equatorial diameter is (31.45) 36.69 (41.93) μm . Both the distal and proximal sides of the spore in an equatorial position are convex. The equatorial ridge in polar position is distinctly, (2.82) 3.93 (5.03) μm broad. The rays of laesura are straight, (16.79) 17.29 (17.79) μm long, (1.28) 1.67 (2.06) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (3.39) 3.71 (4.02) μm broad are formed on both sides of laesura rays. The distal side with short folds (1.95) 2.41 (2.86) μm broad, which form irregularly shaped cells. The ornamentation of proximal side includes besides laesura and 'laesura lips' three large folds (1.92) 2.49 (3.05) μm broad which arranged parallel to the spore margins. The surface of the exosporium is nearly smooth with roundish excrescences: 0.56–0.70 μm in diam. on the distal side, and (0.41) 0.77 (1.12) μm in diam. on the proximal side.

Investigated specimen: "China (Thibet oriental). – Prov. de Moupin M. Pabbe David. 1870" (P).

***Onychium plumosum* Ching, 1934, Lingnan Sci. Journ. 13: 499.**

SEM-description. Spores in a polar position are triangular-roundish, non lociniate. The equatorial diameter is (35.1) 39.3 (43.5) μm . The distal sides of the spore in an equatorial position is hemispherical, the proximal one convex. The equatorial ridge is distinctly expressed (1.4) 2.75 (4.1) μm broad. The rays of laesura are straight, (12.5) 14.75 (17.0) μm long, (0.8) 1.5 (2.2) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (1.8) 2.45 (3.1) μm broad are formed on both sides of laesura rays. The distal side with sparse folds (1.4) 2.7 (4.0) μm in thickness, which form irregularly shaped cells with small roundish tubercles. The ornamentation of proximal side includes besides laesura and 'laesura lips' single tubercles and three sinuous folds (1.6) 2.8 (4.0) μm in thickness which are arranged parallel to the spore margins. The surface of the exosporium is fine-grainulate with sparse roundish excrescences: (0.8) 2.4 (4.0) μm in diam. on the distal side and (0.5) 1.25 (2.0) μm in diam. on the proximal side.

Investigated specimen: "Yun-nan (Chine), No.7668, Kou fy Region de Pin tchaian leg. Yean

Py. 1910. Fr. Ducloux" (P).

***Onychium siliculosum* (Desv.) C.Chr. 1906, Ind. Fil.: 468. – *Pteris siliculosum* Desv. 1811, Ges. Nat. Freunde Berlin Mag. 5: 324**

SEM-description. Spores in a polar position are triangular-roundish, non lociniate. The equatorial diameter is (47.11) 50.95 (54.78) μm . Both the distal and proximal sides of the spore in an equatorial position are convex. The equatorial ridge in polar position is distinctly expressed (3.96) 4.68 (5.40) μm broad. Two more folds (3.63) 4.14 (4.65) μm broad are formed on both sides of equatorial ridge. The rays of laesura are straight, (18.58) 19.2 (19.81) μm long, (1.19) 1.47 (1.74) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (2.85) 3.72 (4.58) μm broad are formed on both sides of laesura rays. The distal side with thick folds (3.71) 4.87 (6.02) μm in thickness located on the perimeter of the spore. The ornamentation of proximal side includes besides laesura and 'laesura lips' three large folds which arranged parallel to the spore margins. The surface of the exosporium is nearly smooth with multiple roundish excrescences (0.28) 1.44 (2.6) μm in diam.

Investigated specimen: "India, No. 332 (Doune par la Direction du Jardin Royal de KEW, 1867)" (P).

***Onychium contiguum* (Wall.) Hope, 1901, Journ. Bombay Nat. Hist. Soc. 13: 444. – *Cheilanthes contiguum* Wall., 1828, List. No. 72.**

SEM-description. Spores in a polar position are triangular-roundish, non lociniate. The equatorial diameter is (58.2) 65.1 (72.0) μm . The distal side of the spore in an equatorial position is hemispherical, the proximal one flat. The equatorial ridge is distinctly expressed, (4.4) 5.4 (6.4) μm broad. The rays of laesura are straight, (24.7) 26.5 (28.3) μm long, (1.4) 1.6 (1.8) μm broad, elevated along their whole length above the surface of the spore. The roller-like bulges of the sporoderm ('laesura lips') (3.2) 3.9 (4.6) μm broad are formed on both sides of laesura rays. The distal side is tuberculate-pleated with elongate tubercles and sinuous folds (2.6) 4.5 (6.4) μm in thickness, which locate on the periphery of the distal spore side. The ornamentation of proximal side includes elongate tubercles besides laesura and 'laesura lips' and sinuous folds on the periphery. The

surface of the exosporium has sparse roundish excrescences (0.8) 1.55 (2.3) μm in diam. on the distal side and (0.6) 1.1 (1.6) μm in diam. on the proximal side.

Investigated specimen: "Yun-nan, No. 3374, En... de Yun nan ... Rochers de la martagne. 11.8.1905. F. Ducloux" (P).

***Pteris aspericaulis* Wallich ex J. Agardh, 1839, Recens. Spec. Pter. 22.**

SEM-description. Spores in the proximal-polar and distal-polar positions are triangular-roundish, not lociniate. Equatorial diameter is (52.2) 56.34 (59.7) μm . Polar axis is (33.2) 35.0 (37.4) μm . In the equatorial position, the distal side is hemispherical proximal – convex. Laesura rays are straight (16.77) 18.7 (19.8) μm long, (1.1) 1.24 (1.4) μm wide, slightly merged on both sides into roller-like thickenings of the sporoderm with breadth of (1.9) 2.28 (2.9). Outside the laesuras and roller-like thickening on the proximal side of the spore there are tubercles of (1.4) 1.96 (2.6) μm in diameter. The width of the equatorial roller-like thickening is (6.1) 6.9 (7.8) μm . Roller-like folds on the distal side of the spore is (2.5) 3.56 (4.9) μm wide, tubercles – (1.0) 2.6 (3.3) μm in diameter. Slightly rough exosporium surface, without excrescences.

Investigated specimen: Nepalia. D 'Candolle (isotypus, LE).

***Pteris biaurita* L., 1753, Sp. Pl.: 1076.**

SEM-description. Spores in the proximal-polar and distal-polar positions are triangular-roundish, not lociniate. Equatorial diameter is (38.3) 42.34 (44.6) μm . In the equatorial position, the distal side is hemispherical proximal – convex. Laesura rays are straight (14.4) 15.2 (18.7) μm long, (0.8) 0.98 (1.2) μm wide, merged on both sides into roller-like thickenings of the sporoderm with breadth (1.7) 2.06 (2.3). Outside the laesuras and roller-like thickening on the proximal side of the spore there are tubercles of (0.9) 1.52 (2.3) μm in diameter. The width of the equatorial roller-like thickening is (5.1) 5.9 (6.7) μm . Roller-like folds on the distal side of the spore are (2.6) 3.07 (3.4) μm wide, tubercles – (3.7) 3.97 (4.9) μm in diameter. Slightly rough exosporium surface, without excrescences.

Investigated specimen: "China, Yunnan, Syemov, A. Henry, No. 12957A" (LE).

***Pteris tenera* Kaulf. 1824, Enum. Filic. 191**

SEM-description. Spores in the proximal-polar and distal-polar positions are triangular-

roundish, not lociniate. Equatorial diameter is (40.4) 41.1 (41.6) μm . Polar axis is (26.8) 28.0 (29.8) μm . In the equatorial position, the distal side is hemispherical proximal – convex. Laesura rays are straight (16.4) 18.77 (20.4) μm long, (1.1) 1.38 (1.7) μm wide, merged on both sides into roller-like thickenings of the sporoderm with breadth of (2.3) 2.86 (3.5). Outside the laesuras and roller-like thickening on the proximal side of the spore there are tubercles of (1.5) 2.22 (3.1) μm in diameter, as well as roller-like thickenings – (2.1) 2.38 (2.5) μm wide. The width of the equatorial roller-like thickening (3.0) 3.48 (3.9) μm . On both sides of the equatorial roller-like thickening there are equatorial folds (1.8) 3.04 (3.7) μm wide. Roller-like fold near the proximal side is (2.5) 3.38 (4.2) μm wide, and near the distal side – (3.1) 3.36 (3.9) μm wide. Tubercles on the proximal side are (1.5) 2.22 (3.1) μm in diameter. Exosporium surface has tuberculum-like ornamentation, without excrescences.

Investigated specimen: “Kaulf Enum sp. 191. Hb. Cha, Chile leg Cha” (typus, LE).

***Pteris umbrosa* R. Br., 1810, Prodr. Fl. Nov. Holland. 154.**

SEM-description. Spores in the proximal-polar and distal-polar positions are triangular-roundish, not lociniate. Equatorial diameter is (35.6) 36.65 (38.1) μm . Polar axis is (25.1) 26.8 (27.9) μm . In the equatorial position, the distal side is hemispherical proximal – convex. Laesura rays are straight (16.0) 16.8 (17.6) μm long, (0.7) 0.96 (1.3) μm wide, merged on both sides into roller-like thickenings of the sporoderm with breadth are (2.2) 2.5 (2.7). Outside the laesuras and roller-like thickening on the proximal side of the spore there are tubercles (1.3) 1.46 (1.8) μm in diameter. The width of the equatorial roller-like thickening is (3.8) 4.7 (5.6) μm . Roller-like folds on the distal side of the spore are (1.5) 2.3 (3.1) μm wide. Exosporium surface is almost smooth, without excrescences.

Investigated specimen: “Herb. H. Christ. Bale” (isotypus, LE).

***Anopteris hexagona* (L.) C. Chr., 1905, Ind. Fil.: 59. – *Pteris heterophylla* L., 1759, Syst. Nat. ed. 10(2): 1322.**

SEM-description. Spores in a polar position are triangular-roundish, usually with straight sides (non lociniate). The equatorial diameter is (41.0) 44.9 (48.8) μm . The rays of laesura

are straight (14.9) 16.5 (18.1) μm long, (1.0) 1.4 (1.8) μm broad, at the top raised above the surface of the spore. The distal side is non-rugose. The surface of the exosporium at the distal and proximal sides is uneven, coarse-granulate, with sparse roundish excrescences of 0.2–0.5 μm in diam.

Investigated specimen: “34 Jamaica 392 *Pteris heterophylla* L., West Indies” (LE).

***Anopteris strictum* (Kunze) A. Vaganov et. Shmakov, 2010, Turczaninowia, 1: 9. – *Onychium strictum* Kunze. 1848, in Schkuhr’s Fil. Suppl. 2: 11.**

SEM-description. Spores in a polar position are triangular-roundish, with faintly concave sides (slightly lociniate). The equatorial diameter is (35.7) 39.25 (42.8) μm . The distal side in an equatorial position is hemispherical, the proximal one flat and elevated at the pole. The rays of the laesura are straight, with smooth margins, (16.0) 17.35 (18.7) μm long, (1.7) 2.8 (3.9) μm broad, raised along their whole length above the surface of the spore. The distal surface is plicate, with a sparse folds (1.9) 2.5 (2.8) μm in thickness. The surface of the exosporium at the distal and proximal sides is even, faintly granulate, with sparse roundish excrescences of 0.3–0.8 μm in diam.

Investigated specimen: “Rio Abajo State Forest: Along hwy. 621, near end of asphalt road. 18°19’N, 66°40’W; elev. 360–390 m. Epipetric. 2 IV 1985. Thomas B. Croat. 60885 Missouri Botanical Garden Herbarium (MO)” (LE).

***Anopteris intermedia* (Morton) A. Vaganov et. Shmakov, 2010, Turczaninowia, 1: 10. – *Anopteris hexagona* subsp. *intermedia* Morton, 1957, Bull. Jard. Bot. Etat 27: 583.**

SEM-description. Spores in a polar position are triangular-roundish, with slightly concave sides (slightly lociniate). The equatorial diameter is (40.2) 41.95 (43.7) μm . The distal side of the spore in an equatorial position is hemispherical, the proximal one flat, raised at the pole. The rays of laesura are straight, weakly visible, (16.8) 18.55 (20.3) μm long, (2.5) 3.2 (3.9) μm broad, slightly elevated along their whole length above the surface of the spore. The distal surface is plicate, with sparse low folds (2.6) 3.25 (3.9) μm in thickness. The surface of exosporium at the distal side is fine-granulate, at the proximal side is coarse-granulate, with sparse roundish excrescences of 0.6–1.8 μm diam.

Investigated specimen: "Heiti, "Tlanrs du Mirne trnshant 1700 m, Borts du chemin", 8 Aout, "Dira..." No. 745" (B).

***Afropteris repens* (C. Chr.) Alston, 1956, Bol. Soc. Brot. 2(30): 5. – *Pteris repens* C. Chr., 1906, Ind.: 606.**

SEM-description. Spores in a polar position are triangular, with straight or slightly concave. The equatorial diameter is (37.63) 43,31 (48.99) μm . The distal side of the spore in an equatorial position is flat, the proximal one – convex. The rays of laesura are straight, (14.6) 18.14 (21.67) μm long, (1.29) 1.9 (2.51) μm broad, elevated above the surface of the spore near pole. The roller-like bulges of the sporoderm ('laesura lips') (4.4) 5.28 (6.16) μm broad are formed on both sides of laesura rays. The distal side in the center is tuberculate, tubercles are roundish in outline and different in size, on the periphery – flat. The ornamentation of proximal side besides laesura and 'laesura lips' is tuberculate; tubercles are roundish, sometimes fused together. The surface of the exosporium on the both sides is arachnoid.

Investigated specimen: "3 km E of KM 21 of road Yabassi-Douale, Alt. 50–100 m, det. K.U. Kramer, 17.08.1965, 6414" (P).

***Jamesonia auriculata* A.F. Tryon, 1962, Contr. Gray Herb. 191: 143, f. 1.**

SEM-description. Spores in the proximal-polar and distal-polar positions are triangular-roundish, lociniate. Equatorial diameter is (40.9) 42.38 (44.6) μm . Polar axis is (23.4) 25.7 (28.1) μm . In the equatorial position, the distal side is flat, proximal – slightly convex. Laesura rays are straight (15.8) 16.77 (17.6) μm long. (1.0) 1.28 (1.5) μm wide, merged on both sides into roller-like thickenings of the sporoderm with breadth (2.4) 2.72 (2.9). Outside the laesuras and roller-like thickening on the proximal side of the spore there are tubercles, slightly raised above the surface of spores (1.2) 2.02 (2.7) μm wide. The width of the equatorial folds is (4.6) 6.03 (7.6) μm . On both sides of the equatorial folds on the proximal side, the roller-like fold over the entire contour is (5.2) 7.16 (8.4) μm wide, and from the distal side – (6.5) 7.52 (8.3) μm wide. Tubercles on the distal side are slightly above the surface and have a diameter of (2.7) 4.3 (4.9). Exosporium surface is slightly rough, without excrescences.

Investigated specimen: "Venezuela, pra

Merida, paramu de la Negra, above la Lanada. 14.II.1939, No. 7050, Alston" (isotypus, LE).

***Taenitis blechnoides* (Willd.) Sw., 1806, Syn. Fil. (Swartz) 24: 220.**

SEM-description. Spores in the proximal-polar position are roundish-triangular, not lociniate, and in distal-polar position triangular-roundish, lociniate. Equatorial diameter is (39.0) 40.36 (42.7) μm . Polar axis is (25.5) 26.2 (27.7) μm . In the equatorial position, the distal side is hemispherical, proximal – flat. Laesura rays are straight (13.8) 14.7 (16.0) μm long, (0.8) 0.95 (1.1) μm wide, merged on both sides in the rough tubercles of (0.9) 1.2 (1.5) μm in diameter. Outside the laesura and rough tubercles area, the exosporium surface is covered with small tubercles are (0.2) 0.3 (0.5) μm in diameter. On the distal side there are oblong tubercles of (1.8) 2.2 (2.5) μm long, (1.1) 1.24 (1.4) μm wide. Exosporium surface is rough, without excrescences. Investigated specimen: "Hainan. Shan Mong, Fairly common: dry, gentle slope, clay, tricket, erect. Coll. Lau S.K., No. 2935" (LE).

CONCLUSIONS

Our studies and eventually received original spore micrographs revealed common characters of external morphology of spores that are distinctive to the subfamily *Pteridoideae*: spores are triangular-roundish, laesura rays are straight and merged into roller-like sporoderm thickening, there are tubercles on the surface of a spore, and in some cases "cerebriform" folds, exosporium surface without excrescences. The study of spores' morphology of species of the genera *Afropteris*, *Jamesonia* and *Taenitis*, revealed the characters belonging exclusively to these species. Trapezoidal form of *Afropteris repens*, clearly observed in the equatorial position of the spore, and justifies its indication by Alston as a separate genus⁹. Exosporium of *Afropteris repens* spores has a coralloidal structure that is not found in other species of the subfamily *Pteridoideae*. A description of the *Taenitis blechnoides* spore morphology allowed detecting large granular formations on the distal surface. The proximal side of the studied spores has a double exosporium pattern and carries elongated tubercles. Unique characters in the morphology of *Taenitis blechnoides* spore together with small size of the

length and width of the laesura allow making assertions about its independent position among studied specimens of the subfamily.

As a result of our study of the morphology of *Pteridoideae* subfamily fern spores, analysis of scientific papers on the morphology of fern *Pteridaceae* family spores⁴⁻⁸. and previous studies¹⁰⁻¹³ it was found that the *Pteridoideae* subfamily's independent position in the *Pteridaceae* family system based on Christenhusz et al system³ is justified. Data on the on the *Cryptogrammoideae* spore subfamily morphology allow to comprise *Cryptogramma*, *Llavea*, *Coniogramma* genera, which is consistent with the *Pteridaceae* family system based on Christenhusz et al system³. The boundaries between morphology character complex of *Cryptogrammoideae* and *Pteridoideae* away subfamilies' spores are clearly traced from three subfamilies *Ceratopteridoideae*, *Cheilanθοideae* and *Vittarioideae*. Full detailed descriptions of the spore morphology based on micrographs, as well as the exact measurements of spore structures are required for further study on the relationships on intergeneric, specific and intersectional levels of the representatives of the *Pteridoideae* subfamily and *Pteridaceae* family as a whole.

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REFERENCES

- Schuettpelz, E., H. Schneider, L. Huiet, M. Windham and K. Pryer, A molecular phylogeny of the fern family Pteridaceae: Osseing overall relationships and the affinities of previously unsampled genera. *Molecular Phylogenetics and Evolution*, 2007; **44**: 1172-1185.
- Smith, A, M. Kathleen, E. Schuettpelz, P. Korall, H. Schneider and P. Wolf, A classification for extant ferns. *Taxon*, 2006; **3**(55): 705-731.
- Christenhusz, M., X.-Ch. Zhang and H. Schneider, A linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa*, 2011; **19**: 7-54.
- Chen, S.-H. and T-Ch. Huang, Spore morphology of Formosan Pteridaceae. *Taiwania*, 1974; **19**(2): 179-229.
- Belling, A. and C. Heusserl, Spore morphology of the Polypodiaceae of northeastern North America. II. *Bulletin of the Torrey Botanical Club*, 1957; **102**(3): 115-127.
- Nayar, B. and S. Devi, Spore Morphology of the Pteridaceae I. The Pteridoid Ferns. *Grana Palynologica*, 1966; **6**(3): 476-502.
- Nayar, B. and S. Devi, Spore Morphology of the Pteridaceae II. The Gymnogrammoid Ferns. *Grana Palynologica*, 1967; **7**(2-3): 568-600.
- Wagner, W., Structure of Spores in Relation to Fern Phylogeny. *Annals of the Missouri Botanical Garden*, 1974; **61**(2): 332-353.
- Alston, A., New African Ferns. Boletim da Sociedade Broteriana, 1956; **30**(2): 5-27.
- Kusnetsov, A, I. Gureyeva, A. Vaganov and A. Shmakov, Morphological analysis of spores of the species of the genus *Actiniopteris* Link (Cryptogrammaceae). *Turczaninowia*, 2009; **12**(1-2): 5-16.
- Vaganov, A, A. Shmakov, A Kusnetsov and I. Gureyeva, Morphology of spores of the species *Cryptogramma* R.Br. ex Richards (Cryptogrammaceae). *Turczaninowia*, 2010; **13**(3): 50-58.
- Vaganov, A, A Kusnetsov and A Shmakov, Systematic and morphology of *Llavea cordifolia* Lag. (Cryptogrammaceae). *Turczaninowia*, 2011; **14**(1): 19-22.
- Vaganov, A., A. Shmakov, A. Kusnetsov, I. Gureyeva and L. Babeshina, Morphological analysis of spores of the species *Onychium* Kaulf. (Cryptogrammaceae). *Turczaninowia*, 2012; **15**(3): 59-67.