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Spore morphology of *Onychium ipii* Ching (*Pteridoideae*, Pteridaceae)

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Key words: morphology of the spores, scanning electronic microscopy (SEM), *Onychium ipii*, Pteridaceae, *Pteridoideae*.

Summary. The ultrastructure of the spore surface of *Onychium ipii* Ching (*Pteridoideae*, Pteridaceae) was investigated by using of scanning electron microscopy. Spores of *Onychium ipii* are trilete, tetrahedral, with hemispherical distal side and convex proximal one. Equatorial diameter 42.4–48.1 μm, polar axis 35.7–40.6 μm. Distal and proximal sides are separated by an equatorial flange, which protrudes on 4.3–4.8 μm all around the spore. Tubercles of different size form the interrupt "laesura lips" situated on both sides of laesura arms. Proximal side of spore with three stright ridges 3.1–4.5 μm width arranged parallel to spore margins and formed triangle in outline. Fused tubercles on distal side form sinuous folds with a few small areolae. Spores of *Onychium ipii* in compare with spores of *O. moupinense* Ching (Figure 3, D–F; Tables 1–2) are larger; their equatorial flange is more prominent; laesura arms are narrower; "laesura lips" are interrupted; stright ridges on the proximal side of spores *Onychium ipii* are broader, than the same in *O. moupinense* spores; sinuate folds on the distal side of *Onychium ipii* spores are more frequent than the same in *O. moupinense* spores. Spores of *Onychium ipii* and *O. moupinense* have the differences in the character of spore ornamentation and form. These features are the addition argument for recognition of *Onychium ipii* and *O. moupinense* as separate species.

Морфология спор *Onychium ipii* Ching (*Pteridoideae*, Pteridaceae)

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Ключевые слова: морфология спор, сканирующая электронная микроскопия (СЭМ), *Onychium ipii*, Pteridaceae, *Pteridoideae*.

Аннотация. Методом сканирующей электронной микроскопии проведено сравнительное исследование морфологии спор *Onychium ipii* Ching. Выявлены основные признаки, характерные для спор вида. Споры трехлучевые, тетраэдрические, в проксимально-полярном и дистально-полярном положениях треугольно-округлые, стороны споры слегка выпуклые. В экваториальном положении проксимальная сторона выпуклая, дистальная – полусферическая. Экваториальный диаметр 42,4–48,1 мкм, полярная ось 35,7–40,6 мкм. Проксимальная и дистальная стороны разделяются хорошо выраженной экваториальной складкой 4,3–4,8 мкм шир., с выемками в углах споры. Лучи лезуры прямые, с расположенными в ряд по обеим сторонам каждого луча дискретными бугорками разного размера. Дистальная сторона споры извилисто-складчатая, с редкими небольшими ареолами. Близ экваториальной складки бугорки и складки дистальной стороны сливаются в сплошную складку, располагающуюся вокруг споры параллельно экваториальной. Проксимальная сторона с тремя пря-

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мыми складками, расположенными близ краев споры параллельно экваториальной складке. Отличительными признаками, свойственными *O. ipii* в сравнении с *O. moupinense* Ching, являются: больший размер спор, более широкие лучи лезуры, прерывистые утолщения вдоль лучей лезуры, более широкие краевые складки на проксимальной стороне споры, извилисто-складчатая орнаментация дистальной стороны споры. Отличия в форме и орнаментации спор являются дополнительным аргументом в пользу признания самостоятельности *Onychium ipii* и *O. moupinense*.

The genus *Onychium* contains about 10 species occuring in the border of warm-temperate and tropical zones mostly in the Sikkim-Himalayan area and southwest China, in northeast Africa, eastward to India, China, and Japan; in Malesia eastward to New Guinea (Tryon, Lugardon, 1991; Zhang G.M. et al., 2013). According to the last classifications, the genus *Onychium* belongs to subfamily *Pteridoideae* C. Chr. ex Crabbe, Jermy et Mickel, family Pteridaceae E. D. M. Kirchn. (Smith et al., 2006; Christenhusz et al., 2011). Most of species grow in the mountains at an altitude of 3500 m above sea level (Ching et al., 1990). Phylogenetic analysis on the base of sequencing of the chloroplast *rbc*L gene demonstrates that *Onychium* is closely related to *Actiniopteris* Link and *Pteris* L. (Zhang G. et. al., 2005; Dou et al., 2015; Zhang L. et. al., 2017). Relationship of *Onychium* and *Actiniopteris* was confirmed also by molecular-phylogenetic studies, based on analysis of three plastid gene (*rbc*L, *atp*B, and *atp*A) data set; the genus *Onychium* is a member of one of five main clades – pteridoid clade (Schuettpelz et al., 2007). Relationship between *Onychium* and *Actiniopteris* is confirmed also by similarity in spore morphology (Kuznetsov et al., 2009, 2013; Vaganov et al., 2012).

Onychium ipii Ching was described as the separate species in 1936 (Ching, 1936; figures – Ching, 1937). This species occurs in southeast China (provinces Sichuan and Hubei) and is considered as local endemic. Most authors treat this taxon as a variation of *O. moupinense* Ching – *O. moupinense* Ching var. *ipii* (Ching) K. H. Shing (Ching et al., 1990), or do not recognize it at all (Zhang G. M. et al., 2013).

A. V. Vaganov described a new series *Moupinenses* A. Vaganov (Vaganov, Shmakov, 2012) on the base of the form and dissection of frond: frond blade is elongate-lanceolate to linear, 2–3-pinnately divided. Both *O. ipii* and *O. moupinense* were included in this new serie. *O. ipii* has the differences with *O. moupinense* in the form of frond and the form of scales in the base of the stipe (Figure 1, 2).

Several molecular-phylogenetic studies were conducted in *Pteridoideae* subfamily (Zhang L. et. al., 2017) and the genus *Onychium* (Dou et al., 2015) in the last years. But it is not possible to indicate what the specimens were used in these studies. L. Zhang et al. (2017) indicated the number of specimen of *O. moupinense* var. *ipii* as "Zhang X. C., 2999 (PE), Chongqing, China", but the correct number is 2699 (not 2999). Specimen No. 2699 was used also for sequencing by P. Dou et al. (2015). In fact, No. 2699 indicates five herbarium specimens in Herbarium PE (Institute of Botany, Chinese Academy of Sciences, Beijing). These specimens were identified by X. C. Zhang as *O. japonicum* or *O. japonicum* var. *ipii* (?); they have the same collection number (No. 2699) and different barcode. But barcode of specimen which was used for sequencing was not indicated in the study of P. Dou et al. (2015). So, we cannot say, what specimen was used for molecular-phylogenetic studies. One of the authors of this study, A. V. Vaganov, identified four of these six specimens No. 2699 (PE: 01385258; 01385256; 01385254; 01385255; 01385257; 01385259) as *O. moupinense* (PE: 01385254; 01385255; 01385257; 01385259), two of them was identified as *O. ipii* (PE: 01385258; 01385256).

Spores of some *Onychium* species were studied and described by B. K. Nayar and S. Devi (1967) (*Onychium japonicum* (Thunb.) Kunze, *O. lucidum* (D. Don.) Spreng., *O. siliculosum* (Desv.) C. Chr.), brief description and SEM-micrographs of spores of three *Onychium* species (*O. contiguum* (Wall.) C. Hope, *O. siliculosum, O. divaricatum* (Poir. in Lam.) Alston) are given by A. Tryon and B. Lugardon (1991), spores of 6 *Onychium* species (*O. siliculosum, O. contiguum, O. japonicum, O. plumosum Ching, O. lucidum, O. moupinense*) studied by using of scanning electronic microscope (SEM) were described by A. V. Vaganov et al. (2012). The most characteristic features for *Onychium* spores are the following: tetrahedral-globose shape, trilete aperture, prominent equatorial flange and coarse adjacent ridges, coarsely tuberculate or rugate proximal face, reticulate-tuberculate distal face; the outer layer with sparse granulate deposit (Tryon, Lugardon, 1991).

The aim of present work is the searching of features in spore ornamentation detectable with using of scanning electronic microscope for confirmation of the distinctness of *O. ipii* from *O. moupinense*.

Fig. 1. The type specimen of *Onychium ipii* Ching (PE-00049692).

Fig. 2. The type specimen of *Onichium moupinense* Ching (PE-00049695).

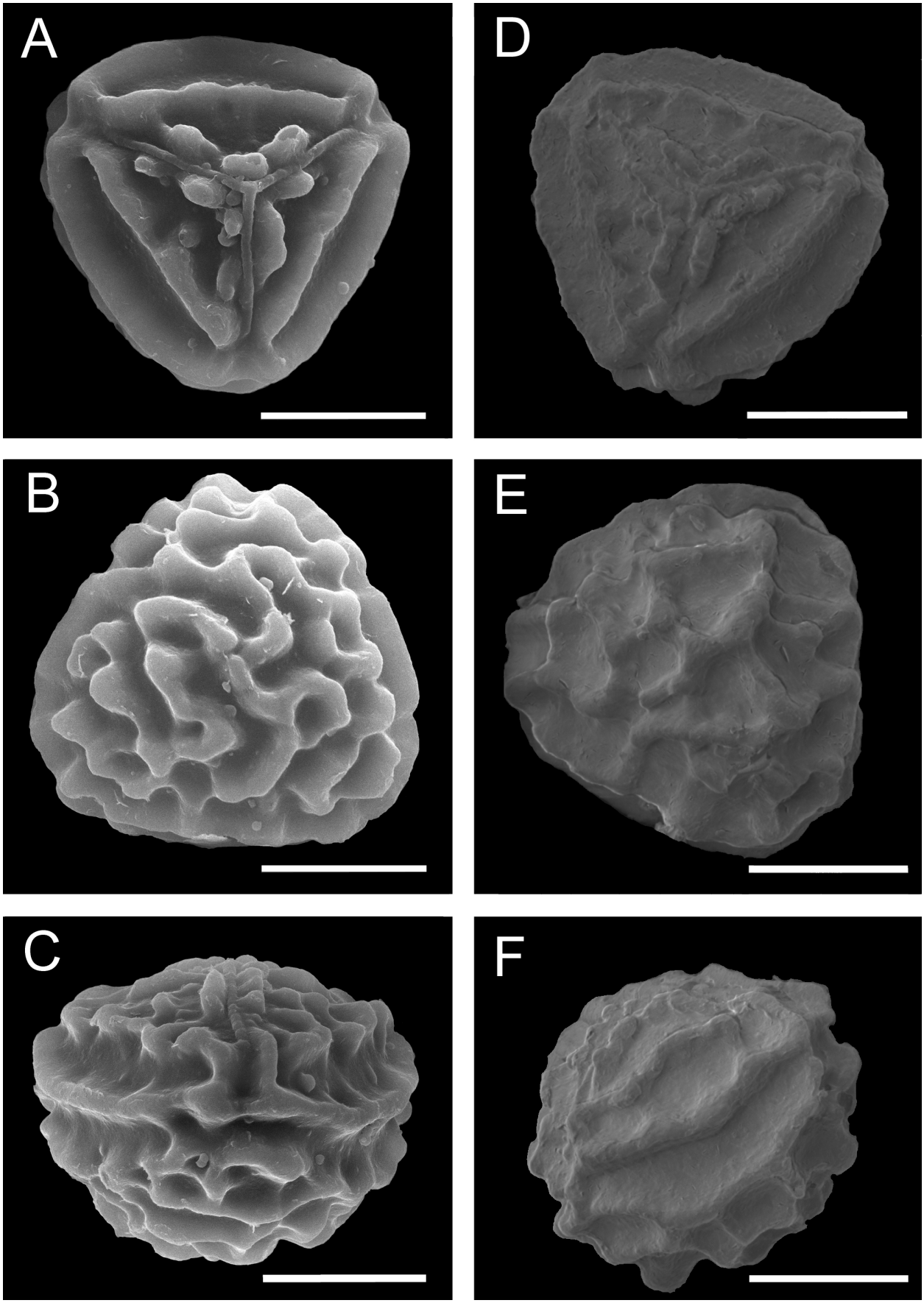

Fig. 3. SEM-micrographs of spores of *Onichium ipii* (A–C) and *O. moupinense* (D–F): A, D – proximal side of spores; B, E – distal side of spore; C, F – spores in equatorial position, proximal side at top, distal side from below, equatorial flange in the middle. Scale bars – 20 μm. Photos of *O. moupinense* from A. V. Vaganov et al. (2012) and A. A. Kuznetsov et al. (2013).

Matherials and methods

The study includes spores of *Onychium ipii* collected in Hubei province of China. Spores were obtained from herbarium specimen stored in PE (Herbarium of Institute of Botany, Chinese Academy of Sciences, Beijing). Identification of specimen was conducted by author of this article A.V. Vaganov. Identification was made on the basis of morphological features of fronds. Only mature spores from adult fronds were used for SEM observations. Spores were mounted on SEM stubs using double-sided carbon adhesive tape and coated with gold in a "Quorum Q150R S" sputter-coater. Stubs were viewed and photographed with a scanning electronion microscope "Mini-SEM SNE-4500M" in the laboratory of structural and molecular analysis of plants (Tomsk State University, Tomsk, Russia). Spore surfaces were scanned in a high vacuum at voltage of 25 to 30 kV, through 400× to 14000× magnification.

The equatorial diameter, polar axis, length and width of laesura arms, ridges and folds were used as the main morphometrical characters. All measurements were made on SEM-micrographs of spores in distal, proximal and equatorial positions using the computer program "Image J". 25 measurements of each parameter were made. For terminology, we primarily followed B. P. Nayar and S. Devi (1966, 1967), they described spores observed under light microscope; A. Tryon and B. Lugardon (1991), they described spores of some *Onichium* species observed in SEM. SEM-micrographs of *Onychium moupinense* Ching were published earlier (Vaganov et al., 2012; Kuznetsov et al., 2013).

Investigated specimen: "Shennongjia Forest District (31°30' N, 110°30' E): vicinity of Houshanping on the S side of the Hou River. Elevation 800–1050 m. In thin soul at the base of boulders and ledges. 24. 01. 1981. Sino-American Botanical Expedition to Westren Hubei Province Peoples Repubic of China, No. 1599. 21 September 1980" (PE-01385245).

Results and conclusion

Onychium ipii (Figure 2, A–C; Tables 1–2). Spores trilete, tetrahedral, in distal-polar and proximal-polar positions triangular with broadly rounded corners and convex sides. The equatorial diameter of spore 44.3 (42.4–48.1) μm, polar axis 37.7 (35.7–40.6) μm. Distal side of spore in equatorial position hemispherical, proximal side is convex. Two sides are separated by an equatorial flange, which protrudes in 4.5 (4.3–4.8) μm all around the spore as seen in polar position; equatorial flange 3.2 (2.9–3.6) μm thick in equatorial position. Equatorial flange concave in the corners as seen in proximal-polar position. Laesura arms stright, 15.8 (13.6–19.8) μm long and 1.1 (0.7–1.4) μm wide, elevated above the spore surface, with a row of regularly arranged along both sides of each laesura arm particularly fused tubercles. So, tubercles

Table 1

Comporative morphometrical characters of the spores of *Onychium ipii* and *O. moupinense*, mean (min – max)

Feature	*O. ipii*	*O. moupinense*
Equatorial diameter, μm	44.3 (42.4–48.1)	37.3 (31.4–41.9)
Polar axis, μm	37.7 (35.7–40.6)	37.1 (37.4–37.87)
Equatorial flange in polar position (width), μm	4.5 (4.3–4.8)	3.9 (2.8–5.03)
Laesura arms (width), μm	1.1 (0.7–1.4)	1.6 (1.3–2.1)
Laesura lips (width), μm	2.4 (1.8–2.9)	3.4 (3.4–4.0)
Ridges on the proximal side of spore (width), μm	3.6 (3.1–4.5)	2.5 (1.9–3.1)

Table 2

Comparative characteristics of spore ornamentation of *Onychium ipii* and *O. moupinense*

Feature	*O. ipii*	*O. moupinense*
Equatorial flange	prominent and broad	prominent and broad
Laesura arms	stright, elevated above the spore surface	stright, elevated above the spore surface
Laesura lips	interrupted, formed from fused tubercles	uninterrupted, roller-like
Ridges on the proximal side	stright	stright
Distal side	sinuate-folded with a few small areolae	with fused elongate tubercles, formed large areolae

of different size form the interrupt "laesura lips" situated on both sides of laesura arms. Proximal side of spore with three stright ridges 3.6 (3.1–4.5) μm wide, arranged parallel to spore margins and forming triangle in outline. Fused tubercles on distal side form sinuous folds 2.8 (1.9–3.5) μm wide, with a few small areolae. Fused tubercles and folds form a regular row parallel to the equatorial flange. Surface is nearly smooth with rare round excrescences 0.2–0.6 μm in diam.

In general, spores of *Onychium ipii* and *O. moupinense* have the differences in the character of spore ornamentation and shape. Spores of *Onychium ipii*, in compare with spores of *O. moupinense* (Figure 3, D–F; Tables 1–2) are larger, their equatorial flange is more prominent and broader; laesura arms are narrower, "laesura lips" are interrupted; stright ridges on the proximal side of *Onychium ipii* spores are broader, than the same in *O. moupinense* spores; sinuate folds on the distal side of *Onychium ipii* spores are more frequent then the same in *O. moupinense* spores. Differentiation in spore morphology is the addition argument for recognition of *Onychium ipii* and *O. moupinense* as separate species. Confusion in identification of these species can lead to incorrect results particularly in molecular-phylogenetic studies.

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