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## Spore morphology of *Parahemionitis arifolia* (Cheilanthoideae, Pteridaceae)

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**Summary.** A study of spores of the single species of the genus *Parahemionitis* Panigrahi was performed using the method of scanning electronic microscopy (SEM). Spores of *Parahemionitis arifolia* (Burm. f.) Panigrahi are tetrahedral trilete, roundish-triangular in polar position, with micro-wrinkled exospore and sculptured perispore. Sculpture of perispore is cristate-reticulate, cristae are quite regularly distributed and form reticulum with small mostly closed polygonal luminae of different shape. Laesura arms are often obscured by numerous cristae. Size of spores is 53–63 × 40–42 μm. Spores of *P. arifolia* are similar in perispore sculpture with those of species of some cheilanthoid ferns.

## Морфология спор *Parahemionitis arifolia* (Cheilanthoideae, Pteridaceae)

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**Ключевые слова:** Китай, сканирующая электронная микроскопия (СЭМ), скульптура периспория, споры, Cheilanthoideae, *Parahemionitis*, Pteridaceae.

**Аннотация.** Методом сканирующей электронной микроскопии (СЭМ) проведено исследование спор единственного вида рода *Parahemionitis* Panigrahi. Споры *Parahemionitis arifolia* (Burm. f.) Panigrahi тетраэдрические, трехлучевые, округло-треугольные в полярном положении, с мелкоморщинистым экзоспорием и скульптурным периспорием. Скульптура периспория гребенчато-сетчатая. Гребни (крысты) расположены упорядоченно и образуют густую сеть из ячеек небольшого размера различной формы. Лезура большей частью скрыта многочисленными кристами. Размер спор 53–63 × 40–42 мкм. Споры *P. arifolia* сходны по скульптуре периспория со спорами некоторых видов хейлантаидных папоротников.

The genus *Parahemionitis* Panigr. was described by G. Panigrahi in 1993 on the base of single species *Asplenium arifolium* Burm. f. selected as the type of this new genus. In turn, *Asplenium arifolium* was first proposed by N. L. Burmann in “Flora Indica” (Burmann, 1768). Later, it was transferred to *Hemionitis* L. by T. Moore (1859), who suggested

a new combination *Hemionitis arifolia* (Burm. f.) T. Moore. In 1974 C. V. Morton and then in 1997 C. R. Fraser-Jenkins rejected the name *Asplenium arifolium* as based on the type specimen determined by A. Alston (Aug. 1952, *in schedae*) as belonging to another species – *Acrostichum aureum* L. If so, C. V. Morton (1974) suggested *Hemionitis cordata* Roxb.

ex Hook. et Grev. as a correct name for this taxon. C. R. Fraser-Jenkins (1997) used this name as basionym for new suggested combination *Parahemionitis cordata* (Roxb. ex Hook. et Grev.) Fras.-Jenk.

The problem with correct name for discussed species was resolved by J. Mazumdar (2015). He found out, that figures by Petiver's (1702, t. 50, f. 12) and van Rhede tot Drakestein's (1692–1703, Tabula 10, Fig.) showing auriculate sinuous fronds were mentioned in the N. L. Burmann's protologue and his diagnosis was based on these figures. Therefore J. Mazumdar (2015) selected figure by van Rhede tot Drakestein's from "Hortus Indicus Malabaricus" (1703) as lectotype. On the cause of no collection data is available for the specimen on which this drawing was based, J. Mazumdar (2015) selected epitype; it is the herbarium specimen collected by him: "India. West Bengal: Burdwan, Kanchannagar, near bridge above canal, 30 m, 21 Nov 2007, J. Mazumdar 59" (CAL). In this case, the name *Asplenium arifolium* Burm. f. is correct basionym for considered taxon, and the genus *Parahemionitis* based on this species is valid.

C. R. Fraser-Jenkins (1997) in his large publication "New species syndrome in Indian pteridology and the ferns of Nepal" wrote that J. T. Mikel (1974) had suggested the possibility of description of a new genus for this species, but considered this inappropriate and related it to the genus *Hemionitis*. But T. Ranker (1989) has shown the Indian fern definitely does not belong to true *Hemionitis*. J. T. Mikel and T. Ranker had not formalized their idea about separate genus by publishing a new genus. More reasons for description of a new genus on the base of "*Hemionitis*" *arifolia* gave R. Tryon. He has written that it is "a morphologically isolated species of uncertain affinity" (Tryon, 1986, p. 186) and "not readily included in any recognized genus" (Tryon et al., 1990, p. 245). Later A. Tryon and B. Lugardon (1991, p. 173) mentioned that "the widely distributed Asiatic *Hemionitis arifolia* (Burm.) Moore is an apogamous tetraploid that is excluded [from *Hemionitis*] on the basis of its distinctive morphology". They considered also that specific of flavonoid composition of this species, found out by D.E. Giannasi and J.T. Mikel (1979), was the important reason for its excluding from *Hemionitis* (Tryon and Lugardon, 1991). '*Hemionitis*' *arifolia* contains only three of the 16 flavonoid compounds recognized in other species of *Hemionitis* and only four flavonoids that occur in "*Hemionitis*" *arifolia* are absent in others (Giannasi, 1974; Giannasi, Mikel, 1979; Tryon, Lugardon, 1991).

All of suggestions were realized by G. Panigrahi, who undertook three attempts of description of new genus on the base of *Asplenium arifolia* (Panigrahi, 1991, 1992, 1993). In the first two descriptions (Panigrahi, 1991, 1992) he did not follow "The International code of botanical nomenclature", publication of the description of the genus *Parahemionitis* in 1993 (Panigrahi, 1993) was valid.

In the last Pteridophyta systems *Parahemionitis* is considered as a member of the Pteridaceae (Smith et al., 2006). M. J. M. Christenhusz et al. (2011) consider *Parahemionitis* as synonym of the large genus *Hemionitis*, which is attributed to the subfamily *Cheilantheae* of the large family Pteridaceae. "A community-derived classification for extant lycophytes and ferns" (Pteridophyte Phylogeny Group, 2016) includes *Parahemionitis* as the separate genus belonging to subfamily *Cheilantheae* of Pteridaceae family.

E. Schuetzel et al. (2007) determined phylogenetic relationships within the Pteridaceae using three plastid genes (*rbcL*, *atpB*, and *atpA*). *Parahemionitis arifolia* is nested in the large cheilanthoid clade, which corresponds to subfamily *Cheilantheae*, and is sister to the clade formed by *Hemionitis* species. Similar results were obtained by G.-M. Zhang et al. (2009). They conducted special study of the systematic position of *Parahemionitis arifolia* (under the name *Parahemionitis cordata*) based on the analysis of *rbcL*-gene sequence. Their study confirmed removing discussed species from *Hemionitis* to the separate genus *Parahemionitis*.

Thus, the nomenclatural citation for this genus is: *Parahemionitis* Panigrahi, 1993, Amer. Fern J. 83(3): 90. Type: *Parahemionitis arifolia* (Burm. f.) Panigrahi (*Asplenium arifolium* Burm. f.). The genus *Parahemionitis* is monotypic, represented by the single species – *P. arifolia*, growing in India and Sri Lanka throughout continental South-East Asia to southern China, Taiwan and the Philippines (Winter, Amoroso, 2003). The nomenclatural citation for the species is the following: *Parahemionitis arifolia* (Burm. f.) Panigrahi, 1993, Amer. Fern J. 83(3): 90. – *Asplenium arifolium* Burm. f., 1768, Fl. Ind.: 231. – *Hemionitis arifolia* (Burm. f.) T. Moore, 1859, Index Fil.: 114. – *Hemionitis cordata* Roxb. ex Hook et Grev., 1828, Icon. Fil. 1: 64. – *Parahemionitis cordata* (Roxb. ex Hook. et Grev.) Fras.-Jenk., 1997, New species syndrome in Indian pteridology and the ferns of Nepal: 187. Lectotype (Mazumdar, 2015: 91): "van Rhede tot Drakestein, Hortus Indicus Malabaricus 12: 21, tabula 10, 1703 (Fig. 2)"; Epitype (Mazumdar, 2015: 91): "India.

West Bengal: Burdwan, Kanchannagar, near bridge above canal, 30 m. 21 XI 2007. J. Mazumdar 59”.

The aim of this study is to provide details of morphology and ornamentation of spores of *Parahemionitis arifolia* using scanning electronic microscopy (SEM) to reveal features useful for systematics and phylogenetics.

### Materials and methods

Spores were obtained from herbarium specimen of *Parahemionitis arifolia*, stored in PE (Chinese National Herbarium, Chinese Academy of Sciences, Beijing). Mature spores 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 the scanning electron microscope “Mini-SEM SNE-4500M” in the Laboratory of Structural and Molecular Analysis of Plants (Tomsk State University, Tomsk, Russia). Spore surface was scanned in a high vacuum at voltage of 20 kV, through 2000–2500 $\times$  magnification. Equatorial diameter (distal or proximal position of spore), polar axis (equatorial position of spore), length and width of laesura arms (proximal position of spore) were measured. All measurements were made on SEM-micrographs of spores using the computer program “Image J”. For terminology of sculpture elements, we primarily followed A. Tryon and B. Lugardon (1991), shape of the distal and proximal sides was described using terms by B. K. Nayar and S. Devi (1966). Spelling of the names of taxa and authors is given according to “The International Plant Name Index” (<http://www.ipni.org>).

### Results and discussion

*Parahemionitis arifolia* forms typical for Pteridaceae tetrahedral trilete spores (Fig. 1). Spores are roundish-triangular in polar position, with convex sides and wide-rounded corners. In equatorial position, the distal side is hemispherical, proximal side is broadly conical. Spores have plain exospore and sculptured perispore. Exospore is micro-wrinkled, visible in case of destruction of fragile perispore. Perispore on the proximal and distal sides is cristate-reticulate, the cristae are more or less long and thin (1.5  $\mu$ m in mean), with uneven upper margin, quite regularly distributed and form reticulum with small mostly closed polygonal luminae of different shape. Equatorial diameter ranges from 53.2 to 63.5  $\mu$ m (mean 56.9  $\mu$ m), polar axis is 40.3–41.5  $\mu$ m (mean 41.4  $\mu$ m), inner size

of luminae is 3.4–8.1  $\mu$ m (mean 4.6  $\mu$ m). Laesura arms are straight, 20.0–23.5  $\mu$ m (mean 21.3  $\mu$ m) in length and 0.9–2.2  $\mu$ m (mean 1.5  $\mu$ m) in width, often obscured by numerous cristae.

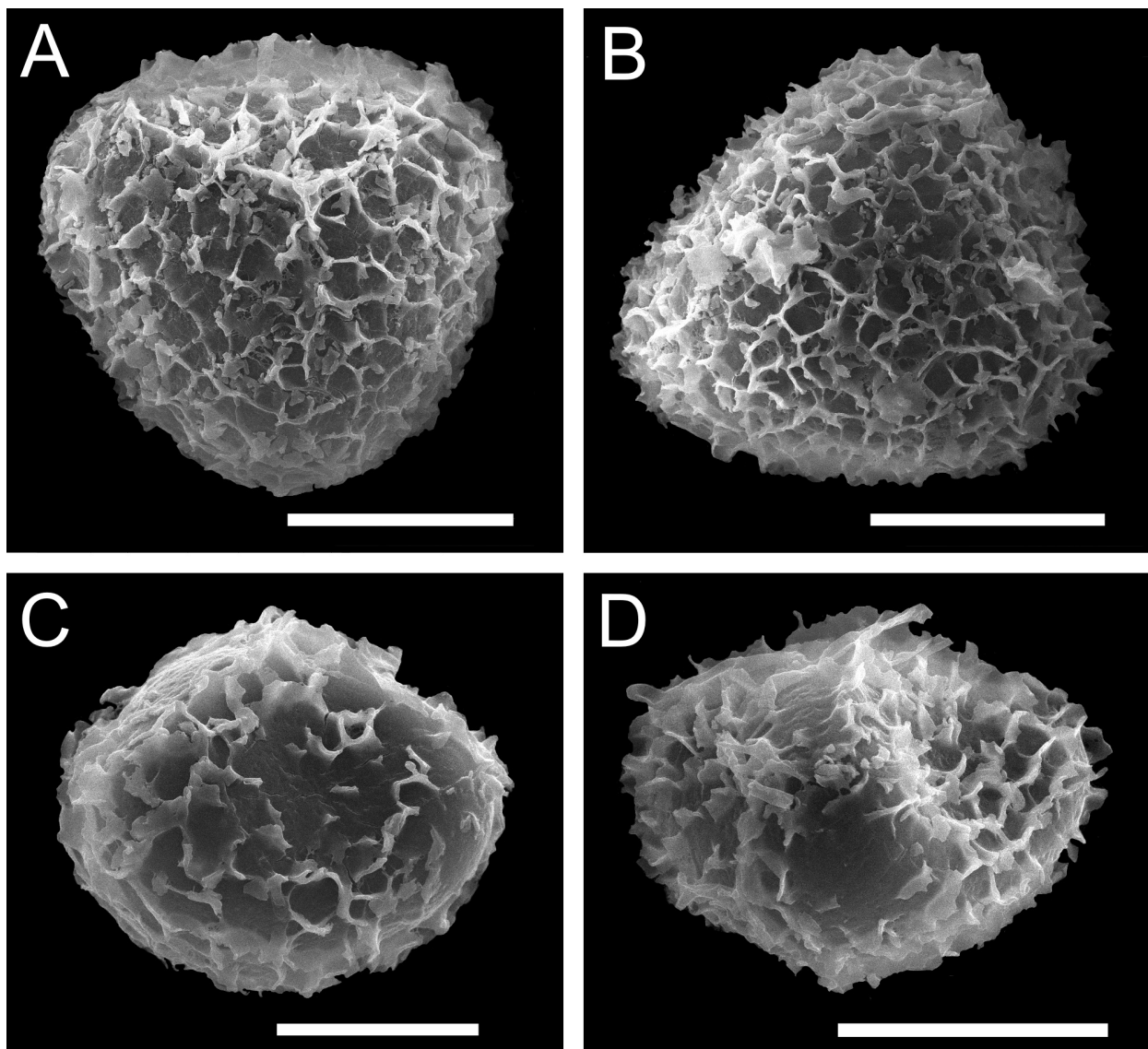
Investigated specimen: China, Hainan. Coll. Mr. W. Hancock. 1898. № 117B. № 01644177 (PE).

Spore having cristate perispore are characteristic for cheilanthoid ferns, in particular for some species of *Bommeria* E. Fourn., *Cheilanthes* Sw., *Doryopteris* J. Sm., *Hemionitis* L., *Notholaena* R. Br., *Paraceterach* Copel., *Pellaea* Link, *Trachypteris* André ex Christ (Tryon, Lugardon, 1991). Judging by the given photos by Tryon and Lugardon (1991), cristae are different in dimensions and distribution on the surface of spore. Spores of *Parahemionitis arifolia* despite of mentioning of this species were not used in Tryon–Lugardon’s publication, but they supposed in this species “cristate spores similar to those of several genera in the subfamily *Cheilanthoideae*” (Tryon, Lugardon, 1991, p. 173). B. K. Nayar and S. Devi (1967), who studied spores of this species collected in India under the light microscope, described them as having “perine closely adherent to the exine and skin-like, smooth and wrinkled into thin, apparently solid folds 4–6 mm tall, 1.5 mm thick with irregular crest and coalesced to form a reticulate pattern” (p. 583). They indicated the dimensions of spores as 36  $\times$  48  $\mu$ m, length of laesura as 14  $\mu$ m. Spores of *Parahemionitis arifolia* are most similar in dimensions and sculpture of perispore to spores of *Bommeria ehrenbergiana* (Kl.) Underw. and *B. hispida* Underw. judging by the photos given by A. Tryon and B. Lugardon (1991) for these species in their monograph. In contrast with it, *Parahemionitis arifolia* and species of *Bommeria* belongs to different clades in the molecular study (Schuettelpelz et al., 2007; Zhang et al., 2009).

Thus, spores of *Parahemionitis arifolia* differs from the spores of other cheilanthoid ferns having cristate-reticulate perispore by more densely arranged cristae, forming reticulum with small closed polygonal luminae.

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**Fig. 1.** SEM-micrographs of spores of *Parahemionitis arifolia* (Burm.) Panigrahi: A – proximal side of spore, laesura arms are obscured by numerous cristae; B – distal side of spore; C, D – spore in equatorial position, laesura above. Spores on C and D have fragment of destroyed perispore, where wrinkled exospore is visible. Scale bars: A, B, D – 30µm, C – 20 µm.

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