

## Spore morphology of *Vaginularia* Fée species (Pteridaceae) from South-Eastern Asia

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A comparative study of spores of two species of *Vaginularia* (Vittarioideae, Pteridaceae) from South-Eastern – *Vaginularia paradoxa* (Fée) Mett. ex Miq. and *V. trichoidea* Fée – was performed by the method of scanning electronic microscopy (SEM). Spores are tetrahedral, trilete; they are very similar to each other in smooth surface and relatively narrow laesura arms. Differences between species are in shape of spores in proximal position (triangular with narrowly-rounded corners and concave sides in *V. paradoxa* and triangular with broadly-rounded corners and slightly concave sides in *V. trichoidea*), in length of laesura arms relative to the radius of the spore (laesura arms are about 0.75 of spore radius in *V. paradoxa*, and riches corners of spore in *V. trichoidea*) and in size of spore (spore of *V. paradoxa* are about 1.5 times more in equatorial diameter and 1.7 times more in polar axis length).

**Key words:** *Vaginularia*; Vittarioideae; Pteridaceae; spore morphology; scanning electronic microscopy (SEM)

The *Vaginularia* Fée species, which previously treated in *Monogramma* Schkuhr, occur on the Mascarene Islands eastward to south India and Ceylon; in Malesia eastward to New Guinea, northward to the Philippines and China, and southward to Queensland, Australia; in the Pacific New Hebrides and Solomon Islands, Fiji, eastward to Samoa (Tryon, Lugardon, 1991). Two species of *Vaginularia* – *V. paradoxa* (Fée) Mett. ex Miq. and *V. trichoidea* Fée occur in China (Zhang et al., 2013).

R. E. G. Pichi Sermolly (1977) had treated *Monogramma* and *Vaginularia* as the separate genera. A. R. Smith et al. (2006), as well as M. Christenhusz et al. (2011) considered *Vaginularia* as a synonym of *Monogramma* and included *Vaginularia* species to *Monogramma*. Morphological and molecular-phylogenetic studies of B. Ruhfel et al. (2008), based on plastid rbcL sequence data, support the separation of *Vaginularia* from *Monogramma* s.str., and advocated for the application of the name *Vaginularia* Fée to species formerly treated in *Monogramma* that were most closely related to *Rheopteris* Alston. According to recent molecular-phylogenetic study, based on four plastid markers (atpA, chlN, rbcL, rpoA) (Schuettpelez et al., 2016), the name *Vaginularia* is applied to the most reduced species previously treated in *Monogramma*. These species occur exclusively in Asia and the Pacific Ocean. Together with monotypic *Rheopteris*, they form on the phylogramm the smaller clade, sister to the rest vittarioid ferns.

Some time ago, SEM-method was used to make a detailed analysis of the spore morphology of *Vaginularia* species on samples from New Guinea (*Monogramma* (*Vaginularia*) *emarginata* (Brause) K.I. Goebel) and Fiji (*V. angustissimus* (Brack.) Mett.) (Tryon, Lugardon, 1991). They described spore as tetrahedral-globose in shape, with trilete aperture, the laesura arm length from 1/2 to 3/4 of the radius of spore, surface of spore is plain. The micrographs of the spores of *Vaginularia junghuhnii* Mett. and *V. trichoidea* (J. Sm.) Fée are given by Ch.-W. Chen et al. (2017). Spores of the both species are tetrahedral-globose, with smooth surface. Spores of *V. trichoidea* was studied by Yi-H. Chang et al. (2015). It was showed on numerous example that spore morphology and molecular-phylogenetic results are congruent for different genera such as *Gymnocarpium* and *Cystopteris* (Gureyeva, Kuznetsov, 2015), *Onichium* (Vaganov et al., 2017 a, b, c), *Pityrogramma* (Vaganov et al., 2017b) therefore the morphology of spores has phylogenetic signification. Spore morphology is very important for systematics of Pteridaceae subfamilies: Cryptogrammoideae (Vaganov, 2016) and Ceratopteridoideae (Vaganov et al., 2017c).

The aim of this study is to provide details of spore morphology of two *Vaginularia* species – *V. paradoxa* (Fée) Mett. ex Miq. and *V. trichoidea* (J. Sm.) Fée – from South-Eastern Asia using scanning electronic microscopy (SEM) to reveal features useful for systematics and phylogenetics.

### Materials and methods

Spores were obtained from herbarium specimens of two species: *Vaginularia paradoxa* (Java Island, Indian Ocean Islands southwards of India) and *V. trichoidea* (Hainan Island) stored in PE (Herbarium of Institute of Botany, Chinese Academy of

Sciences (Beijing). 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 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 400× to 15000× magnification.

The length of equatorial diameter and polar axis, length and width of laesura arms 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". For terminology, we primarily followed A. Tryon and B. Lugardon (1991).

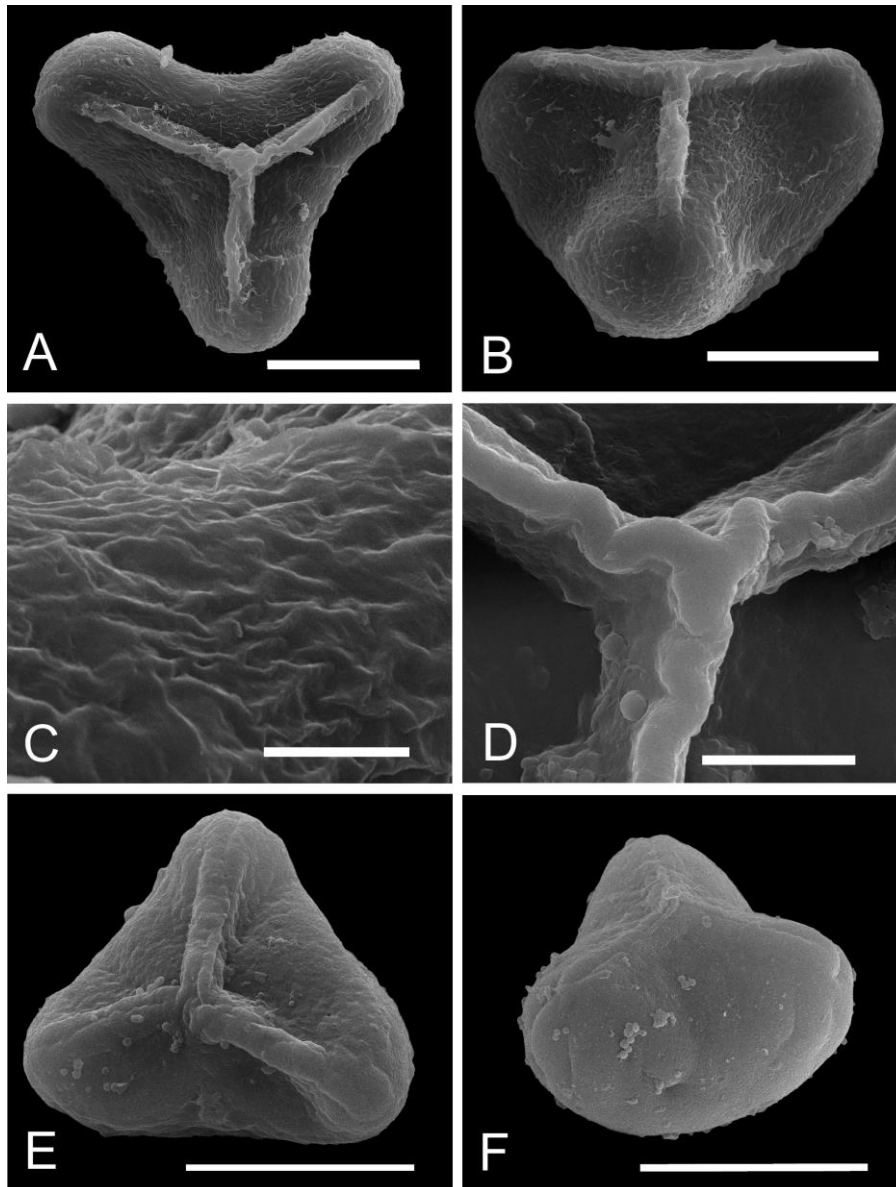
Investigated specimens:

*Vaginularia paradoxa*: "Southsea Islands ex Herb. H.F. Sun. No. 01715346";

*Vaginularia trichoidea*: "Hainan. Po-ting. Alt.: 1400 ft. On rocks in shaded forested ravine. July 8, 1935. No. 73143. Coll. F.C. How".

## Results and discussion

Spores of both studied *Vaginularia* species are tetrahedral, trilete.



**Fig. 1.** SEM-micrographs of spores of *Vaginularia paradoxa* (Fée) Mett. ex Miq. (A–D) and *V. trichoidea* Fée. (E, F) A, E – proximal side of spore; B, F – spore in equatorial-proximal position; C – fragment of proximal surface; D – fragment of proximal side of spore and laesura. Scale bars: A, B, E, F – 20µm; C – 3 µm; D – 5 µm.

1. *Vaginularia paradoxa* (Fée) Mett. ex Miq. (Fig. 1, A–D). Spores are trilete, tetrahedral, in the proximal-polar and distal-polar positions are triangular with narrowly-rounded corners and concave sides (lociniate). Contour of spore is subcrenate. In equatorial position, the distal side of the spore is hemispherical, the proximal side is slightly conical. The equatorial diameter is 45.0 (43.2–46.1) µm, polar axis is 34.4 (32.0–36.6) µm. Laesura arms are straight or slightly undulated, to  $\frac{3}{4}$  of the radius of spore, 20.6 (18.8–22.0) µm long and 3.0 (2.5–3.2) µm width, elevated above the spore surface, they margins are unthickened.

The exosporium surface is smooth, but appears fine-wrinkled at higher magnification.

2. *Vaginularia trichoidea* Fée. (Figure, E–F). Spores in the proximal-polar and distal-polar positions are triangular with broadly-rounded corners and slightly concave sides. Contour of spore is smooth. In equatorial position, the distal side of the spore is hemispherical, the proximal side is slightly conical. The equatorial diameter is 31.1 (29.8–32.6)  $\mu\text{m}$ , polar axis is 20.3 (19.0–21.2)  $\mu\text{m}$ . Laesura arms are straight, stretch to the corners of spore, 14.6 (13.4–15.4)  $\mu\text{m}$  long and 3.0 (2.6–3.3)  $\mu\text{m}$  width, highly elevated above the spore surface, they margins are slightly thickened, especially near corners of spore. The exosporium surface is smooth or slightly rough.

In general, *Vaginularia* species have typical for the Pteridaceae family tetrahedral spores with trilete laesura and distinctly expressed exosporium. Spores of two studied species – *Vaginularia paradoxa* and *V. trichoidea* – are very similar to each other in smooth surface, relatively narrow laesura arms. Differences between these species are in shape of spores in proximal position (triangular with narrowly-rounded corners and concave sides in *V. paradoxa* and triangular with broadly-rounded corners and slightly concave sides in *V. trichoidea*), in length of laesura arms relative to the radius of the spore (laesura arms are about  $\frac{3}{4}$  of spore radius in *V. paradoxa*, and riches corners of spore in *V. trichoidea*) and in size of spore (spore of *V. paradoxa* are about 1.5 times more in equatorial diameter and 1.7 times more in polar axis length). Differences in size may indicate different ploidy of species. The spores of *Vaginularia* species are close in characteristics to spore of *Rheopteris* that were described by A.F. Tryon and B. Lugardon (1991) as tetrahedral-globose, with trilete aperture, laesura arms to  $\frac{3}{4}$  of spore radius, surface is plain or finely granulate at high magnification; size is 40–50  $\mu\text{m}$ . Close similarity of spores of *Vaginularia* and *Rheopteris* species confirms their close relationship, identified by molecular-phylogenetic study, carried out by B. Ruhfel et al. (2008) and E. Schuettpelz et al. (2016).

## Acknowledgements

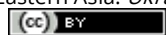
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