Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2017, 7(4), 290–294, doi: 10.15421/2017_118

ORIGINAL ARTICLE *582.394*

Spore morphology of *Haplopteris* C. Presl species (*Vittarioideae*, Pteridaceae) from China

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Submitted: 02.11.2017. Accepted: 14.12.2017

A comparative study of spores of four species of *Haplopteris* C. Presl, *H. amboinensis* (Fée) X.C. Zhang, *H. forrestiana* (Ching) E.H. Crane, *H. linearifolia* (Ching) X.C. Zhang, and *H. mediosora* (Hayata) X.C. Zhang, from China was performed using the method of scanning electronic microscopy (SEM). Spores of *Haplopteris* species are bilateral monolete ellipsoidal or ellipsoidal, but slightly narrowed in the middle. Perispore thin, easily breakable, smooth; exospore smooth, its surface is finely granulate or finely undulate (as seen at higher magnification). *Haplopteris amboinensis* have the largest spores (79.5×34.3×40.9 μм), *H. mediosora* – the smallest one 49.2×24.5×24.9 μм).
Key words: *Haplopteris, Vittarioideae;* Pteridaceae; spore morphology; scanning electronic microscopy (SEM)

The genus *Haplopteris* C. Presl was re-defined by E.H. Crane (1997) to accommodate nearly all the Old World species formerly recognised as '*Vittaria*' (Lindsay, 2003). M. Christenhusz et al. (2011) included the genus *Haplopteris* together with eleven more genera (*Adiantum* L., *Ananthacorus* Underw. & Maxon, *Anetium* Splitg., *Antrophyum* Kaulf., *Hecistopteris* J. Sm., *Monogramma* Schkuhr, *Polytaenium* Desv., *Radiovittaria* (Benedict) E.H. Crane, *Rheopteris* Alston, *Scoliosorus* T. Moore, *Vittaria* Sm.) in subfamily *Vittarioideae* (C. Presl) Crabbe, Jermy & Mickel of large family Pteridaceae. E. Schuettpelz et al. (2016) on the rezults of large molecular-phylogenetic study based on plastid markers (atpA, chlN, rbcL, rpoA) also support the recognition of eleven vittarioid genera, some of them are another in compare with M. Christenhusz's classification: *Ananthacorus, Antrophyopsis* (Benedict) Schuettp., *Antrophyum, Haplopteris, Hecistopteris, Polytaenium, Radiovittaria, Rheopteris, Scoliosorus, Vaginularia* Fée, and *Vittaria*. According to molecular-phylogenetic data, *Haplopteris* is close related with *Radiovittaria* and *Hecistopteris* (Schuettpelz et al., 2016). Genera *Hecistopteris* and *Radiovittaria* occur only in tropical America; the genus *Haplopteris* includes about 40 species, most of them occur in tropical Asia and the Pacific Ocean islands, about ten species grows in tropical and subtropical Africa and the Indian Ocean islands (Schuettpelz et al., 2016; GBIF). Flora of China includes 13 species (Zhang et al., 2013).

Our previous studies of spores of some Pteridaceae groups are shown that spore morphology studied using SEM-method and molecular-phylogenetic results are congruent for different genera such as *Onichium* (Vaganov et al., 2017a), *Pityrogramma* (Vaganov et al., 2017b), *Vaginularia* (Vaganov et al., 2017c). Morphology of spores is very important for systematics of subfamilies Pteridoideae (Kuznetsov, 2014), Cryptogrammoideae (Vaganov, 2016) and Ceratopteridoideae (Vaganov et al., 2017c).

Spores of several species of *Haplopteris* were investigated by Chen Ch.-W. et al. (2013, 2014, 2017). SEM-micrographs of some *Haplopteris* species are provided in the large monograph by A. Tryon and B. Lugardon (1991) under the genera *Antrophyum* (*Antrophyum* (*Haplopteris*) *ensiforme* Hk. in Benth. and *Vittaria* (*Vittaria* (*Haplopteris*) *zosterifolia* Willd. and *V.* (*Haplopteris*) *scolopendrina* (Bory) Thwaites). According to these studies, spores of *Haplopteris* are monolete and ellipsoidal or bean-shaped. The perispore are either smooth or slightly papillate and scattered with spherules and rodlets, high variation in spore size is observed in some *Haplopteris* species (Tryon, Lugardon, 1991; Chen et al., 2017).

The aim of this study is to provide details of spore morphology of *Haplopteris* species using scanning electronic microscopy (SEM) to reveal features useful for systematics and phylogenetics.

Materials and methods

Spores were obtained from herbarium specimens of four species of *Haplopteris*: *H. amboinensis* (Fée) X.C. Zhang (Hunan Province), *H. forrestiana* (Ching) E.H. Crane (Yunnan Province), *H. linearifolia* (Ching) X.C. Zhang и *H. mediosora* (Hayata) X.C. Zhang (Tibet Autonomous Region), stored in PE (Herbarium of Institute of Botany, Chinese Academy of Sciences (Beijing). Only

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 electronion 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× to 15000× magnification. The length of major and minor equatorial diameter and polar axis, length and width of laesura were used as the main morphometrical characters. All measurements were made on SEM-micrographs of spores in distal (major and minor equatorial diameter), proximal (length and width of laesura) and equatorial positions (polar axis) using the computer program "Image J". For terminology, we primarily followed A. Tryon and B. Lugardon (1991).

Investigated specimens:

H. amboinensis: "Pingjiang County, Hunan Province. Coll.: Gu Jia, Qi Shixin, Wang Lanying. No 124, 2008.01.26, No 01782709".

H. forrestiana: "Yunnan Province. Mt. Huanglian Lvchun County Reservoir. Alt. (m): 1780. Col.: Qi Xinping. No Q091. Dt.: 2008.10.02. No 01757772".

H. linearifolia: "Zayu County, Tibet Autonomous Region. Alt.: 2500 m. Coll.: X.C. Zhang. 97°04´N 28°37´E. №4945. 2007.10.03. №01792281".

H. mediosora: "Zayu County. Tibet Autonomous Region. On rocks. Alt.: 2800 m. Coll.: X.C. Zhang. 97°01´N 28°46´E. No 4978. 2007.10.05. No 01792121".

Results and discussion

Haplopteris has bilateral monolete spores. The spores of all examined taxa of *Haplopteris* are bean-shaped in lateral view, ellipsoidal or ellipsoidal compressed laterally in the middle part in distal and proximal view, and round in polar view. Perispore is thin, smooth, and easily breakable, exospore without sculpture.

1. *H. amboinensis* (Fig. 1, A–D; table 1). Spores in distal and proximal view are ellipsoidal, slightly narrowed in the middle. Major equatorial diameter 73.9–88.2 μм, minor equatorial diameter 33.5–36.2 μм, polar axis 40.0–41.5 μм, laesura length 51,5–54,7 μм, laesura width 2.6–3.1 μм. Exospore surface is finely granulate.
2. *H. forrestiana* (Ching) E.H. Crane (Fig., E–H; table 1). Spores in distal and proximal view are ellipsoidal. Major equatorial diameter 71.5–76.8 μм, minor equatorial diameter 36.9–40.9 μм, polar axis 31.4–38.6 μм, laesura length 52.0–59.0 μм, laesura width 1.8–2.6 μм. Exospore surface is finely granulate.
3. *H. linearifolia* (Fig. 2, A–D; table 1). Spores in distal and proximal view are ellipsoidal. Major equatorial diameter 51.7–61.3 μм, minor equatorial diameter 29.2–32.9 μм, polar axis 21.7–32.9 μм, laesura length 40.2–43.5 μм, laesura width 1.3–1.8 μм. Exospore surface is finely undulate.
4. *H. mediosora* (Fig. 2, E–H; table 1). Spores in distal and proximal view are ellipsoidal, slightly narrowed in the middle. Major equatorial diameter 47.7–51.3 μм, minor equatorial diameter 23.1–26.3 μм, polar axis 24.6–25.2 μм, laesura length 32.1–37.6 μм, laesura width 1.1–2.5 μм. Exospore surface is finely granulate.

Table 1. Spore size of *Haplopteris* species, mean (min–max)

Species	Major equatorial diameter, μm	Minor equatorial diameter, μm	Polar axis, μм	Laesura length, μм
H. amboinensis	79.5 (73.9–88.2)	34.3 (33.5–36.2)	40.9 (40.0–41.5)	53.1 (51.5–54.7)
H. forrestiana	74.7 (71.5–76.8)	38.7 (36.9–40.9)	34.7 (31.4–38.6)	55.2 (52.0–59.0)
H. linearifolia	58.1 (51.7–61.3)	30.6 (29.2–32.9)	27.5 (21.7–32.9)	41.6 (40.2–43.5)
H. mediosora	49.2 (47.7–51.3)	24.5 (23.1–26.3)	24.9 (24.6–25.2)	34.9 (32.1–37.6)

In contrast with most Pteridaceae species having tetrahedral trilete spores with very distinctive sculpture, that were described in multiple papers (Sladkov, 1961; Nayar, Devi, 1967; Tryon, Lugardon, B., 1991; Demske et al., 2013; Kuznetzov et al., 2014; Vaganov, 2016; Vaganov et al., 2017a, b, c, d), *Haplopteris* species has bilateral monolete spores. Spores of four examined species are very similar in shape to each other. Spores of *Haplopteris forrestiana* and *H. linearifolia* are ellipsoidal in distal and proximal position, spores *H. amboinensis* and *H. mediosora* are ellipsoidal, slightly narrowed in the middle. It should be noted, that monolete spores of Pteridaceae species, particularly *Haplopteris*, are different with monolete spores of species from other families of leptosporangiate ferns by length-to-width ratio (major-to-minor equatorial diameter) of the spore. In *Haplopteris* length of spore is 2–2.5 times larger, than its width, in *Cystopteris* Bernh. and *Gymnocarpium* Newman (Cystopteridaceae) length-to-width ratio is 1.5–1.8 (according to data, given by Gureyeva & Kuznetsov, 2015), i.e. spores of *Haplopteris* species are longer and subtler.

Spores of examined species demonstrate differences in size: spores in *H. amboinensis* and *H. forrestiana* are 1.3–1.6 times larger than those in *H. linearifolia* and *H. mediosora*. Differentiation in spore size may reflect the different ploidy levels of these species: *H. amboinensis* and *H. forrestiana* may have a higher ploidy level than others. The largest spores are characteristic for *H. amboinensis*, the smallest spores are peculiar to *H. mediosora*. Similar results were obtained by Chen et al. (2017) for the same species of *Haplopteris* (*H. forrestiana, H. linearifolia, H. mediosora*, as seen on the figure 5 (Chen et al., 2017, p. 552). Two of studied species, *H. forrestiana* and *H. linearifolia*, belongs to *H. taeniophylla* complex, which comprises of closely related species exhibiting a little genetic differentiation (Chen et al., 2017). Spores of these species are similar in shape but different in size.

Acknowledgements

We are grateful to curators of the Herbarium of Institute of Botany, Chinese Academy of Sciences (Beijing) (PE) for the possibility of obtaining of spores from the herbarium material. The study was partly supported by RFBR (project No 16-04-00513 A) and Tomsk State University competitiveness improvement program.

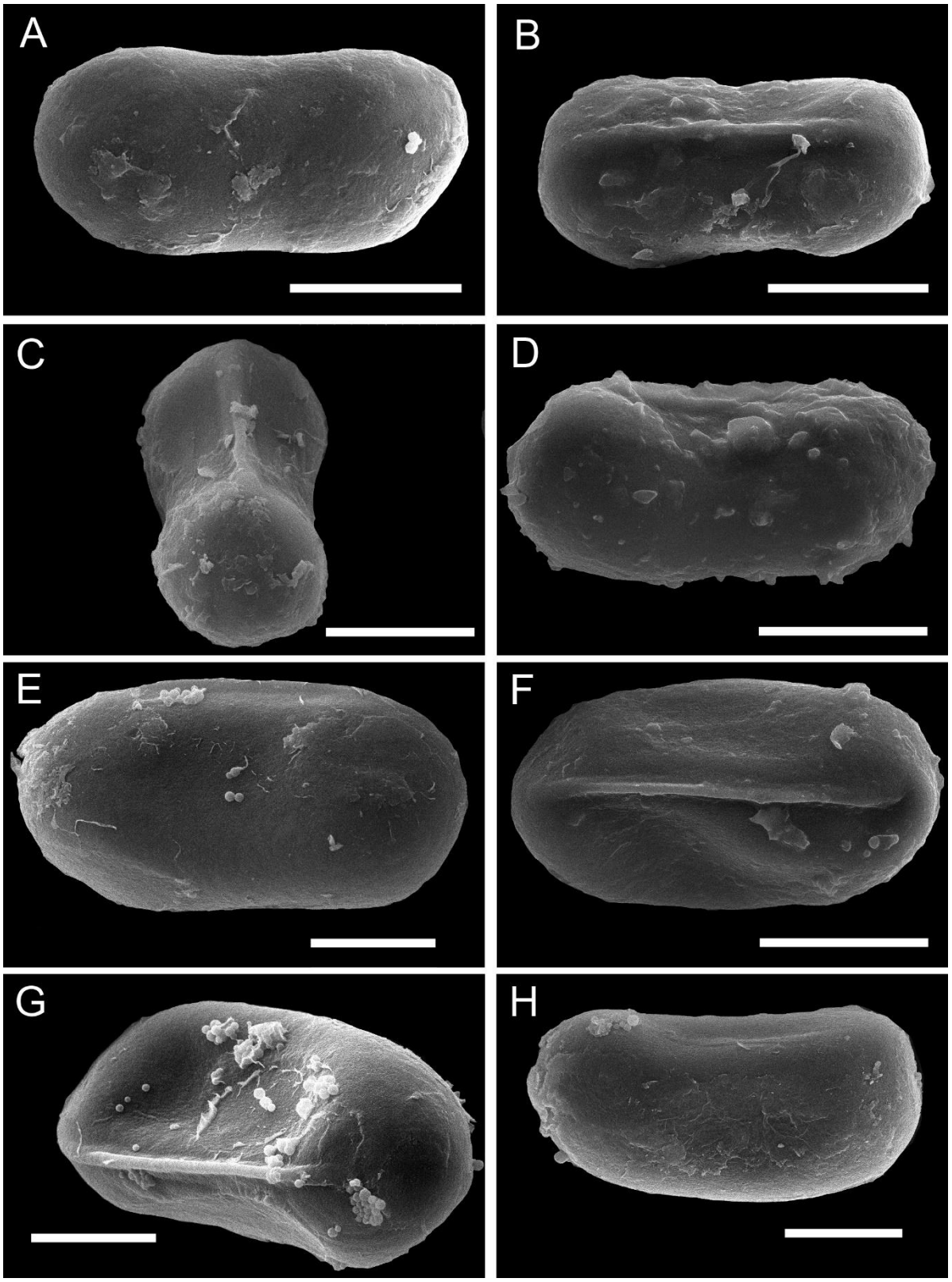

Fig. 1. SEM-micrographs of spores of *Haplopteris amboinensis* (Fée) X.C. Zhang (A–D) and *H. forrestiana* (Ching) E.H. Crane (E–H)

A, E – distal side of spore; B, F – proximal side of spore, laesura in the centre; C, G – equatorial-proximal view of spore; D, H – lateral view of spore. All spores have fragmented perispore. Spherical spores of fungy on the surface of spores (E, G, H). Scale bars: A–D, F – 30 μм; E, G, H – 20 μм.

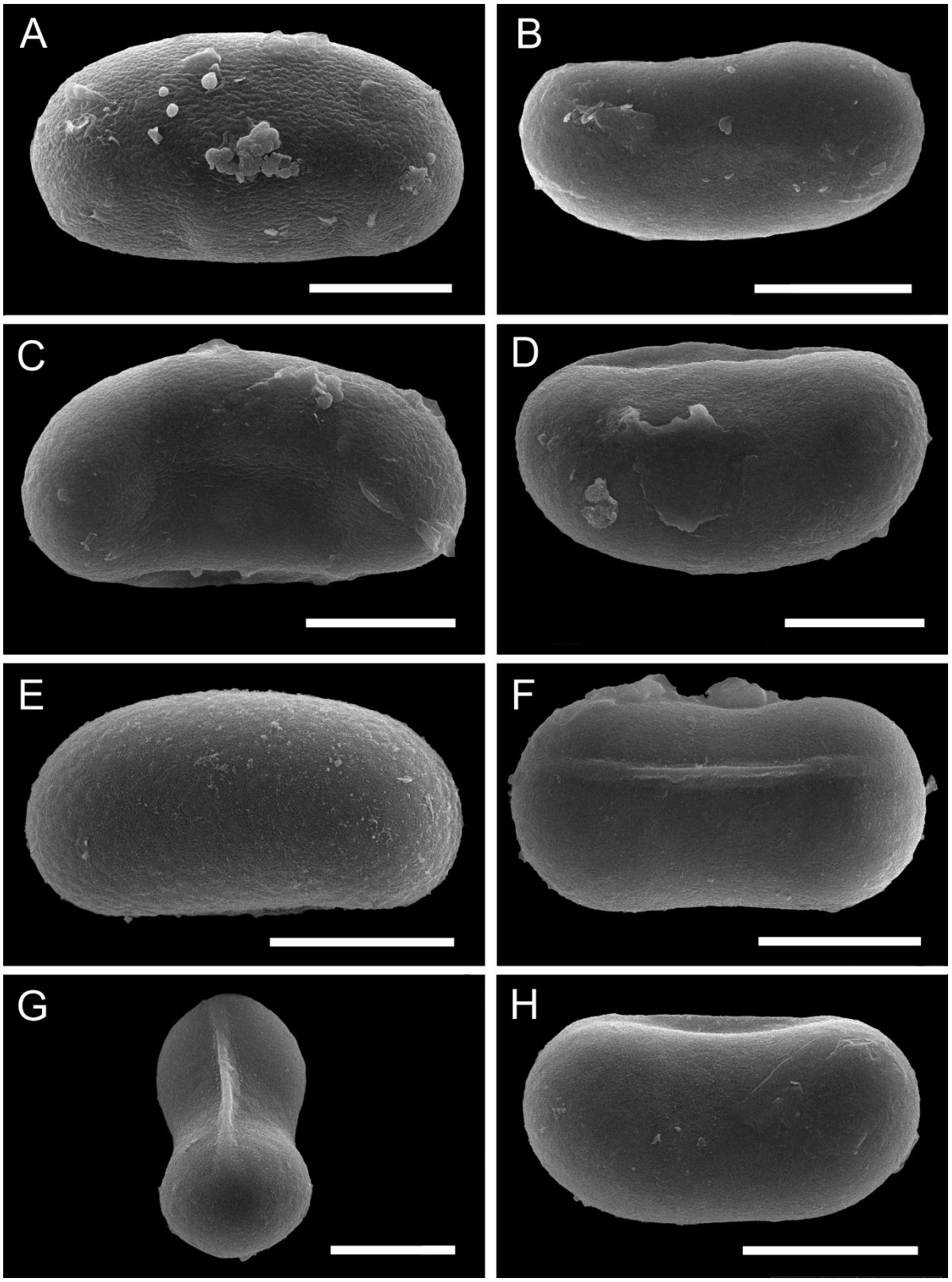

Fig. 2. SEM-micrographs of spores of *Haplopteris linearifolia* (Ching) X.C. Zhang. (A–D) and *H. mediosora* (Hayata) X.C. Zhang (E-H)
A, E – distal side of spore; B –equatorial-distal view of spore; C –equatorial-proximal viev of spore; D, H – lateral view of spore, laesura from above; E – distal side of spore; F – proximal side of spore, laesura in the centre; G, H – spore in equatorial position. A–D – fragmentes of perispore are visible. Spherical spores of fungy on the surface of spores (A, C). Scale bars: A–H – 20 μм.

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Citation:
Vaganov, A.V., Gureyeva, I.I., Kuznetsov, A.A., Romanets, R.S. (2017). Spore morphology of Haplopteris C. Presl species (Vittarioideae, Pteridaceae) from China. *Ukrainian Journal of Ecology, 7*(4), 290–294.