Біологічний вісник МДПУ імені Богдана Хмельницького 6 (2), стор. 28–33, 2016
Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6 (2), pp. 28–33, 2016

ARTICLE UDC 582.394.742+581.33

SPORE MORPHOLOGY OF *POLYPODIUM ALEUTICUM* A.E. BOBROV (POLYPODIACEAE) AND RELATED SPECIES

A.P. Shalimov, A.I. Shmakov

Altai State University, Lenina str., 61, Barnaul, 656049, Russia. Email: ssbgbot@mail.ru

Spores of type specimens of *Polypodium aleuticum* A.E. Bobrov are studied by SEM-method. The morphology of the spores has been studied and described for comparative purposes. The results showed characteristic features of *P. aleuticum* exospores different from *P. glycyrrhiza* D.C. Eaton, which gives the basis for acknowledgment the independence of the investigated species.

Key words: *Polypodium, ferns, type species, spore, morphology, Polypodium aleuticum, Aleutian Islands.*

INTRODUCTION

Polypodium aleuticum A.E. Bobrov was described by A. E. Bobrov in 1964 from USA "Unalaschka, Langsdorf". The species is distributed in the Aleutian Islands (Attu, Kodiak, Unalaska) and Alaska (island Sitka). Since its publication, the species was not being recognized by many researchers because of its similar morphological characters with the other north-american species *P. glycyrrhiza.* In the Flora of North America (Haufler et al., 1993) *P. aleuticum* is provided as synonym of *P. glycyrrhiza*. In our opinion, these are two completely different and independent species in spite of close morphological characters (Shalimov et al., 2015). The first studies of the morphology of the spores of *P. aleuticum* were performed by A.E. Bobrov (1964) and based on the data of light microscopy (LM).

At a later date researches of the spore morphology of American and Europe-Asiatic *Polypodium* species were carried out using light and scanning electron microscopy (SEM) and presented in following articles: Belling & Heusser (1975), Lloyd (1981), Ferrarini et al. (1986), Tryon & Lugardon (1991) and Shalimov et al. (2011).

The purpose of the research is to clarify the taxonomic positon of *P. aleuticum*. To achieve this objective, we studied differences in the spore structure of the taxa, since the spore morphology and exospores structure are the characters stable enough in the fern systematics and have unique structural features at the genus and species level.

MATERIALS AND METHODS

The study material was the specimens of spores from the herbarium material of Herbarium collection LE in V. L. Komarov Botanical Institute (BIN RAS, Saint-Petersburg, Russia) and Herbarium ALTB of Altai State University (Barnaul, Russia). For the study we chose 2 specimens: *P. aleuticum* and *P. glycyrrhiza*. As well as issued species we added some representatives of *Polypodium* L. from the Russian Far East. We used morphometric parameters of *P. sibiricum* Sipl., *P. kamelinii* Shmakov and *P. fauriei* Christ studied by authors in 2011 (Shalimov et al., 2011). Studies of the spores of *P. aleuticum* were carried out in the laboratory of the Center for collective use of BIN RAS using Analytical Scanning Electron Microscope JEOL JSM-6390LA. The spores of *P. glycyrrhiza* were studied in the laboratory of the Institute for Water and Environmental Problems (IWEP SB RAS, Barnaul), on the scanning electron microscope – S 3400

Citation:
Shalimov, A.P., Shmakov, A.I. (2016). Spore morphology of *Polypodium aleuticum* A.E. Bobrov (Polypodiaceae) and related species. *Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University,* 6 (2), 28–33.

Поступило в редакцию / Submitted: 01.04.2016
Принято к публикации / Accepted: 13.05.2016

crossref **http://dx.doi.org/10.15421/201632**

N, Hitachi High Technologies Corp. Spores were applied to the double sided tape, then attached to the metal stage with a diameter of 10 mm. In both studies, the surface of spores was treated with a mixture of gold-palladium in a vacuum sputtering installation JEOL SPUTTER JFC 1100 (BIN RAS) and Emitech SC 7620 / QT S (IWEP SB RAS), deposition time was 7–10 min. All spore samples were studied in high vacuum mode. Scanning of the samples was carried out with the increase from ×400 (a general view of spores) to ×14 000 (exospores surface). The parameters of spores were measured in 20-fold repetition. Measurements were carried out on the following values: 1 – major equatorial diameter, μm; 2 – polar axis, μm; 3 – aperture length, μm; 4 – minor equatorial diameter (spore width), μm; 5 – height of tubercles on the contour of spores, μm. The morphometric data were processed using discriminant analysis in the program *Statistica 7.0* for Windows. The descriptive terms we used in this study follow the definitions used by Tryon & Lugardon (1991).

Table 1. List of *Polypodium* species whose spores were imaged for this study

Species	Voucher	Where studied
P. aleuticum A.E. Bobrov	Unalaschka, Langsdorf (typus LE)	This research
P. glycyrrhiza D.C. Eaton	California (ALTB)	This research
P. sibiricum Sipl.	Altai kr., Charysh District, 3 km above vil. Sentelek, 22.07.1993. Kamelin R. V., Shmakov A. I. Dyachenko S. Kiselev A., Kashcheyev M., Solovyov A.; Altai kr., Altai District, 5 km western vil. Altaiskoye, river B. Shemilovka, 20.07.1997. Terekhina T. A., Golyakov P., Gromova E.; Krasnoyarsk Krai, spurs of Borus ridge, right bank of the Yenisei River, near the mouth of the stream Kibik. 52°56` N, 91°30` E, 25.09.1997. Shmakov A. I., Shaulo D. N., Smirnov S. V., Tikhonov D., Golyakov P. V., Herman D.; Primorskiy Krai, Kavalerovsk District, near vil. Khrustal'nyi, a stream valley, shady rocks. 06.09.1987. Shmakov A. I., Ebel A. L. (ALTB).	Shalimov et al. (2011)
P. kamelinii Shmakov	Primorskiy Krai, Lazovsk district, Petrov island, slope of northern exposition, yew wood, 17.10.1992. Shmakov A. I., Kiselev A. Ya. (typus, isotypus, ALTB).	Shalimov et al. (2011)
P. fauriei Christ.	Kuril Islands, Kunashir. 31.08.1968. Terekhin E. (ALTB).	Shalimov et al. (2011)

RESULTS

Morphology

P. aleuticum A. Bobrov, 1964, Bot. Zhurn. 49(4): 542; Shalimov et al. 2015, Turczaninowia 18 (3): 140. – *P. ocidentale* (Hook.) Hultén, 1941, Fl. Alaska, 1: 44, p. p. – *P. vulgare* var. *occidentale* Hook 1840, Fl. Bor.-Amer. 1: 258, p. p. – *P. vulgare* var. *rotundum* Milde, 1867, Fil. Eur.-Atl.: 18, p. p. – *P. glycyrrhiza* D. C. Eaton, 1856, Amer. J. Sci., ser. 4, 22: 138, p. p.; Tzvelev, 2004, Bot. Zhurn. 89, 10: 1648, p. p. (Fig. 1: 1a, 1b).

Spores bean-shaped, in the equatorial plane of the plano-convex, symmetrical. The major equatorial diameter (49.1) 56.4–58.0 (64.4) μm. The polar axis (32.0) 33.7–37.1 (40.3) μm. Minor equatorial diameter (spore width) (33.4) 36.8–38.6 (40.8) μm. Apertura: monolete, its length (25.7) 32.8–34.4 (42.3) μm. The proximal and distal surfaces have verrucae. Form of tubercles rounded-faceted at the base, sometimes elongated, tubercles connivent raised. Tubercle size (1.2) 1.5–1.9 (2.4) μm. The surface of tubercle base from smooth to slightly rugate-grainy.

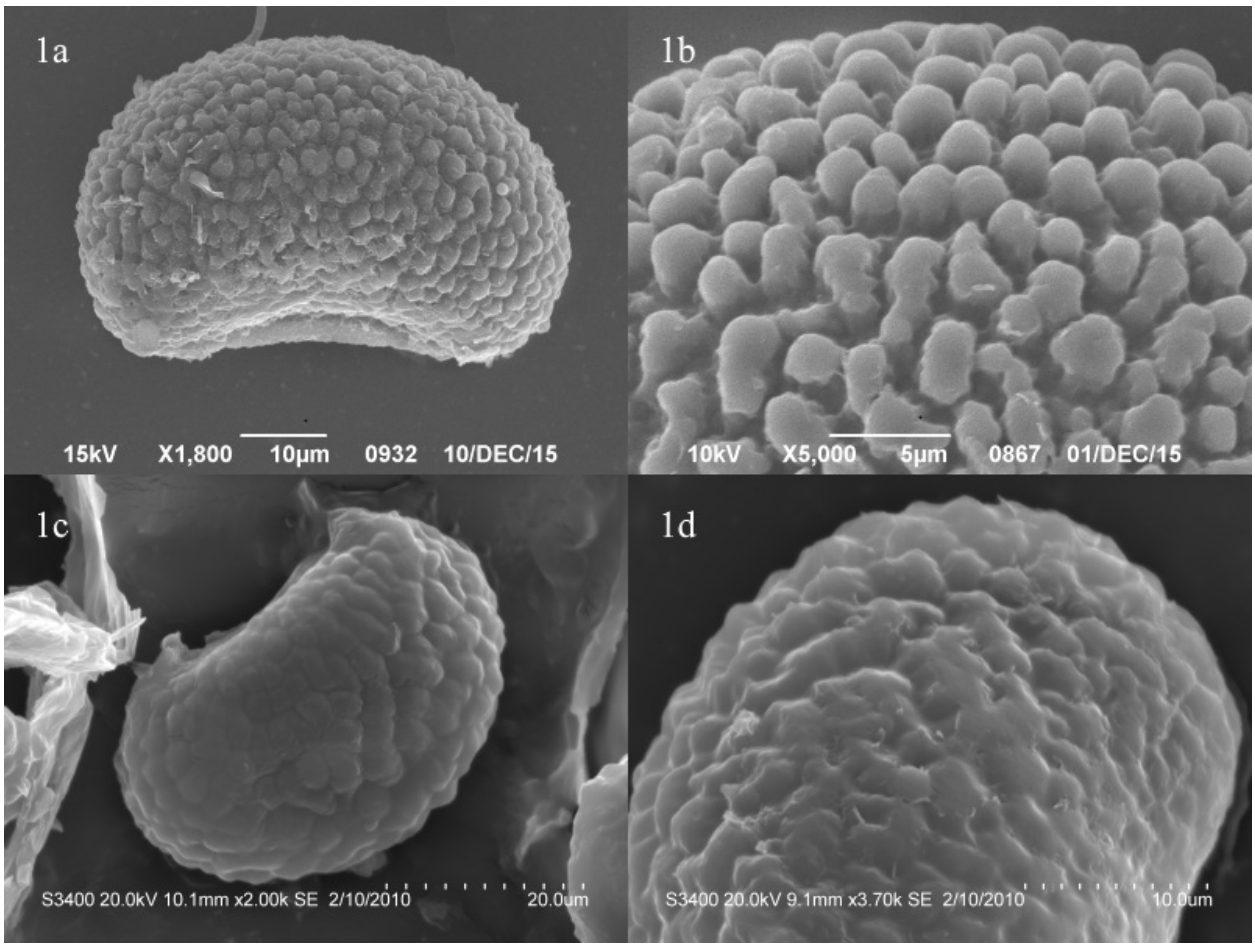

Fig. 1. SEM micrographs of spores: 1a, 1b – *Polypodium aleuticum* A.E. Bobrov; 1c, 1d – *Polypodium glycyrrhiza* D.C. Eaton (a, c – spore in equatorial position, b, d – fragment of distal surface).

DISCUSSION

The studied sample of *P. aleuticum* differs by exospores surface from *P. glycyrrhiza*. *P. aleuticum* has strongly verrucate exospores surface (Fig. 1: 1a, 1b), whereas *P. glycyrrhiza* (Fig. 1: 1c, 1d) exospores surface is low verrucate, with tubercles oblong or roundish form (Tryon & Lugardon 1991, fig 131.20.). In our opinion, it is quite distinguishable feature in the morphology of spore of these two species. At the base of tubercles of *P. glycyrrhiza* and *P. aleuticum* exospores surface is smooth, sometimes slightly rugate in *P. aleuticum*. Similar slightly rugate exospores surface at the base of the tubercles could be seen in other North-American species *P. virginianum* L. studied in Tryon & Lygardon (1991, fig. 131.19).

Spores morphology of *P. aleuticum* is clearly different from representatives of *Polypodium* from the Russian Far East. The exospores of *P. aleuticum* has verrucate form, *P. fauriei* and *P. kamelinii* have the tubercles from rounded to oblong in shape, and exospores of *P. sibiricum* is low echinate tubercles (Fig. 2). Two species: *P. kamelinii* and *P. sibiricum* have perispore; the first species has exfoliative perispore, the second one – echinate. The results of a more detailed morphological description of spores are presented in the research paper of Shalimov et al. (2011).

For the morphological studies of *Polypodium* species (*P. aleuticum*, *P. glycyrrhiza*, *P. sibiricum, P. fauriei* and *P. kamelinii*) we reviewed the typical and fund collections of herbarium specimens (LE, ALTB, TK, PE), as well as published data (Tzvelev, 1991; Haufler et al., 1993; Iwatsuki et al., 1995; Shmakov, 2001; Shalimov et al., 2015) revealed a number of morphological differences between species. The results are presented in Table 2.

Discriminant analysis of spore morphometric characters of *Polypodium* representatives from the Pacific coast of the Russian Far East and the west of United States has revealed clear differences *P. glycyrrhiza* and *P. aleuticum* (Fig. 3). The dispersal cloud of *P. aleuticum* occupies a middle position between *P. glycyrrhiza*

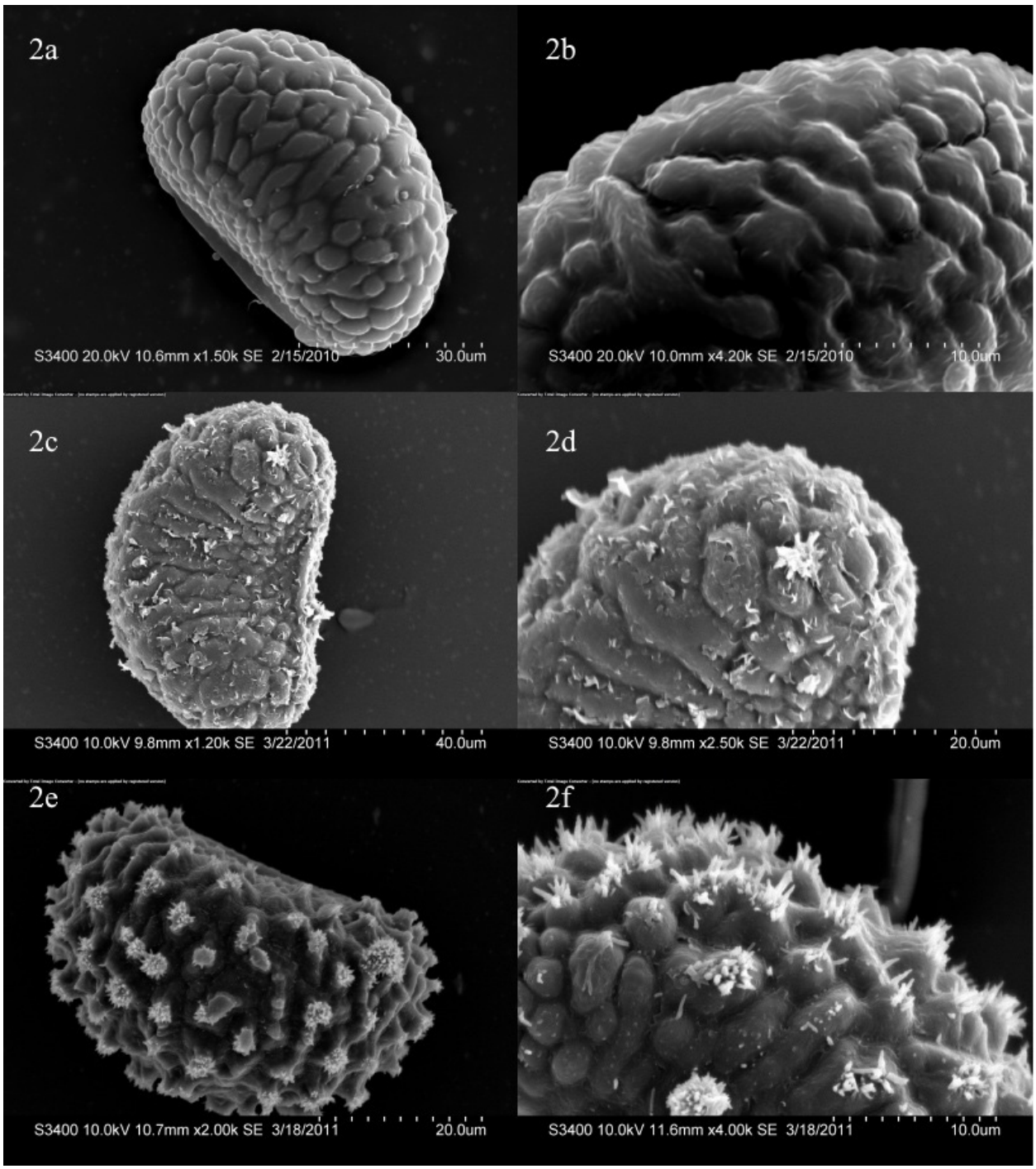

Fig. 2. SEM micrographs of spores: 2a, 2b – *Polypodium fauriei* Christ; 2c, 2d – *Polypodium kamelinii* Shmakov; 2e, 2f – *Polypodium sibiricum* Sipl. (2a, 2c, 2e – spore in equatorial position; 2b, 2d, 2f – fragment of distal surface) by Shalimov et al. (2011).

and *P. fauriei*; it may indicate the involvement of genes of both species in the formation of the genome of *P. aleuticum* in the Aleutian Islands.

The dispersal cloud of *P. sibiricum* on the sum of morphometric characters occupies a separate position from the rest of studied species. The median position of *P. kamelinii* in the species dispersal cloud between *P. fauriei* and *P. sibiricum* can talk about its possible origin from these two species, but not from *P. glycyrrhiza*.

The spores of the studied *Polypodium* species have the clear morphological differences at the species level, reflecting the graph (Fig. 3). Each species on the sum of morphometric characters has a separate cloud,

Table 2. Comparison of closely related *Polypodium* species by major morphological characters

Species	Scale color	Blade form	Lobe form and margin	Indumentum	Leaf coiled when dried
P. aleuticum	Concolored, brown	Ovate-oval	Segments oblong-lanceolate, margin entire or slightly crenate, apex obtuse	Midrib and rachis shortened puberulent adaxially	Slightly coiled
P. glycyrrhiza	Concolored, brown or slightly darker near point of attachment	Lanceolate-ovate to oblong	Segments linear to oblong, margins serrate, apex acute to attenuate	Midrib and rachis puberulent adaxially	Not coiled
P. sibiricum	Brown	Oblong-anceolate	Segments oblong, margins entire to crenulate; apex rounded to broadly acute	Glabrous on adaxial and abaxial surface	Not coiled
P. kamelinii	Concolored with slightly darker central part	Lanceolate	Segments oblong or oblong-lanceolate, margins crenate-dentate, apex obtuse to acute	Glabrous on adaxial and abaxial surface	Not coiled
P. fauriei	Concolored, pale brown	Narrowly ovate to broadly lanceolate	Segments linear-lanceolate, margins minutely dentate, apex moderately acute	Glabrous on adaxial surface, sparsely with multicellular hairs on lower surface	Coiled

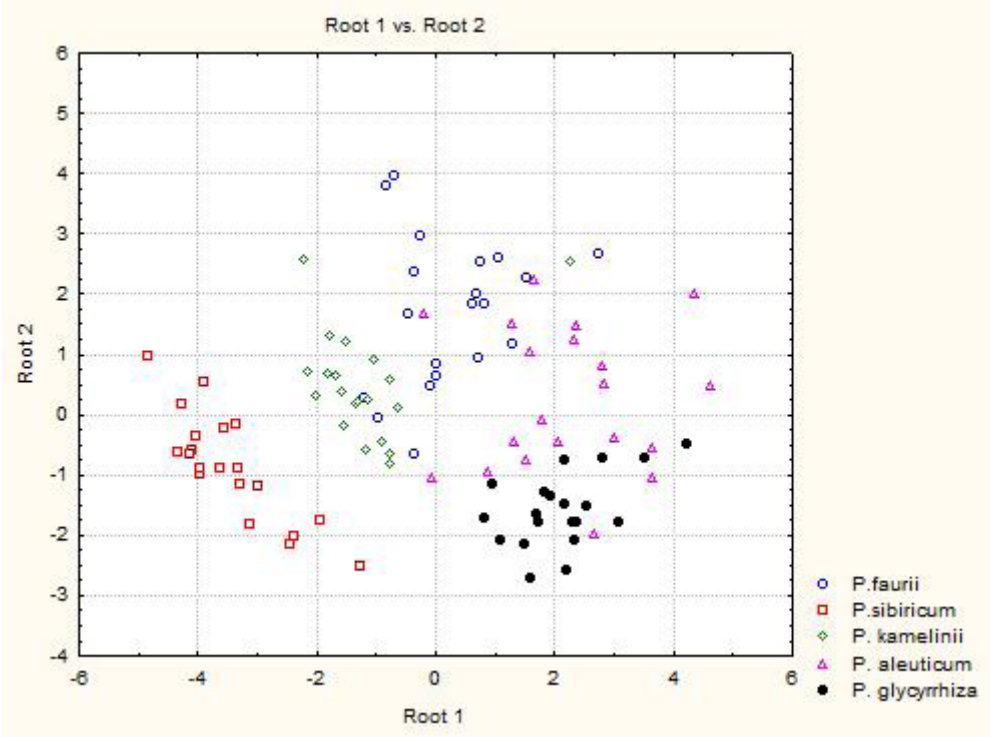

Fig. 3. The objects projection of groups *P. fauriei, P. sibiricum, P. kamelinii, P. aleuticum, P. glycyrrhiza* in the axes of the canonical variables obtained from discriminant analysis of total morphometric parameters of spores.

with a slight overlay *P. aleuticum* on *P. glycyrrhiza* and *P. fauriei*. The main characters of the differences in the spore structure of *Polypodium* species are the form and surface of exospores, the spore parameters: major and minor equatorial diameter, polar axis, aperture length.

The results of this research confirm the independence of both species, but further study of *P. aleuticum* from the Aleutian Islands and Alaska is required to establish its area of distribution.

ACKNOWLEDGEMENTS

The authors thank V. I. Dorofeev, the curator of the General Herbarium sector of BIN RAS, and assistant curator T. P. Lebedeva, the employee of Herbarium, for the opportunity to work with herbarium material. Thanks also herbarium curators I. I. Gureeva (TK), X. C. Zhang (PE). For the opportunity to conduct research on a scanning electron microscope we thank L. A. Kartseva, the engineer of Center for collective use of BIN RAS, as well as V. V. Kirillova, PhD, the head of water ecology laboratory in IWEP SB RAS, for the possibility of SEM studies, A. V. Dyachenko, the postgraduate student of water ecology laboratory in IWEP SB RAS, for help with the work on the SEM. The work was supported by The Russian Foundation for Basic Research (project 15-34-51124_mol nr) and RNF (project 14-14-00472).

REFERENCES

Bobrov, A. E. (1964). A comparative morphological and taxonomical study of the species of *Polypodium* L. of the flora of the USSR. *Botanicheskii Zhurnal, 49*(4), 514–545 (in Russian).

Belling, A.J., Heusser, C.J. (1975). Spore morphology of the Polypodiaceae of northeastern North America. II. *Bulletin of the Torrey Botanical Club*, *102*(3), 115–127.

Ferrarini, E., Climpolini, F., Pichi Sermolli, R.E.G., Marchetti, D. (1986). Iconographia palynologica pteridophytorum Italiae. *Webbia*, *40*, 1–202.

Haufler, H. C., Windtham, M. D., Lang, F. A., Whitmore, S. A. (1993). *Polypodium* L. *In: Flora of North America. North of Mexico* (pp. 315–323). Oxford: Oxford University Press.

Iwatsuki, K., Yamazaki, T., Boufford, D.E., Ohba, H. (1995). *Polypodium* L. *In: Flora of Japan, Volume 1. Pteridophyta and Gymnospermae* (pp. 251–253). Tokyo, Japan: Kodansha.

Lloyd, R.M. (1981). The perispore in *Polypodium* and related genera (Polypodiaceae). *Canadian Journal of Botany*, *59*, 175–189.

Shalimov, A. P., Vaganov, A. V., Shmakov, A. I. (2011). Spore morphology of *Polypodium* L. (Polypodiaceae J. Presl & C. Presl) species from Russia. *Turczaninowia*, *14*(2), 5–14 (in Russian).

Shalimov A. P., Vaganov A. V., Shmakov A. I. (2015). A synopsis of *Polypodium* L. (Polypodioideae, Polypodiaceae) of Russia and adjacent countries. *Turczaninowia*, *18*(3), 129–153. doi: 10.14258/turczaninowia.18.3.9. (in Russian).

Shmakov, A. I. (2001). Synopsis of the ferns of Russia. *Turczaninowia*, *4*(1–2), 36–72 (in Russian).

Tryon, A.F., Lugardon, B. (1991). *Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope studies*. Springer–Verlag.

Tzvelev, N. N. (1991). *Polypodium* L. *In:* Kharkevich S.S. (ed.). *Plantae Vasculares Orientalis Extremi Sovietici. Lycopodiophyta, Equisetophyta, Polypodiophyta, pinophyta (Gymnospermae), Magnoliophyta (Angiospermae)* (pp. 30–33). St. Petersburg, Nauka Publ. (in Russian).