Wulfenia **25** (2018): 131–156

Wulfenia
Mitteilungen des
Kärntner Botanikzentrums
Klagenfurt

Siliceous sporoderm of hornworts: an apomorphy or a plesiomorphy?

Vladimir R. Filin & Anna G. Platonova

Summary: Silicification of the sporoderm is well known in extant ligulate Lycopodiophyta and some Pteridophyta, but it has been unknown among Bryophyta until now. We have discovered a thin outermost siliceous layer in the sporoderm of *Phaeoceros laevis* and *Notothylas* cf. *frahmii* by means of EDX analysis. Silicon plays an important multifunctional role in plant life – structural, protective and physiological. The siliceous layer could protect the spores from injury by soil microorganisms and invertebrates, from UV radiation, desiccation and other unfavorable environmental forces. Data on biology and ecology of *Ph. laevis* suggest that this species as well as probably *Notothylas*, possesses characters of both shuttles and fugitives, and these taxa will be referred to sprinters. *Anthoceros agrestis* has a very similar biology, but as well as *A. caucasicus* it lacks the siliceous layer in the sporoderm. These two groups (with and without siliceous layer) belong to two sister clades according to molecular phylogenetic data. In this regard, the question appears: Is the siliceous sporoderm an apomorphy or plesiomorphy for hornworts? Hypothesizing on environment where the ancestor of embryophytes and, in particular of hornworts, likely appeared, we do not exclude that silicified sporoderm would give significant advantages to the first land plants, and silicification of sporoderm may be lost in some clades during the evolution of hornworts. It is possible that further researches will discover many instances of loss and subsequent re-gain of siliceous sporoderm in different clades of hornworts.

Keywords: Anthocerotophyta, *Anthoceros*, *Notothylas*, *Phaeoceros*, sporoderm, silica, ecology, life strategy

Most authors now consider bryophytes (including liverworts, mosses and hornworts) as a paraphyletic group, and only few investigators (Renzaglia et al. 2000; Nishiyama et al. 2004; Cox et al. 2014) show the monophyly of bryophytes. Cox et al. (2014) concluded that the support of paraphyly of bryophytes appears to be a phylogenetic artefact and that the lineage of bryophytes is rather monophyletic. Whether bryophytes are regarded as a taxon or as a paraphyletic group, the position of hornworts within the system of higher plants is still uncertain (Goffinet 2000; Shaw & Renzaglia 2004; Antonov 2006; Duff et al. 2007; Qiu 2008; Villarreal & Renner 2013; Wickett et al. 2014; Villarreal & Renzaglia 2015; Villarreal et al. 2015). Antonov (2006) supposes that the most ancient group are liverworts, but he notes that it cannot be denied that it is still impossible to establish phylogenetic relationships between liverworts and hornworts by molecular methods reliably. Various morphological characters including spore structure are involved in the discussion on the relationships of hornworts.

Brown & Lemmon (1988) inferred in their early work that the initiation of exine in hornworts *Notothylas orbicularis* (Schwein.) Sull. ex A. Gray and *Phaeoceros laevis* (L.) Prosk. is not associated with the development of tripartite lamellae that are characteristic for hepatics and mosses. A long time later, Brown et al. (2015) revealed these lamellae at early stages in sporogenesis of *Leiosporoceros dussii* (Stehani) Hässel (Leiosporocerotopsida, Anthocerotophyta). It is possible that Brown & Lemmon (l.c.) did not find tripartite lamellae in sporogenesis of *N. orbicularis* and *Ph. laevis* in their early investigation due to difficulties with fixation and resin infiltration of spores mentioned by them. What could impede the infiltration of hornwort spores with a resin?

Millington & Gawlik (1967) faced difficulties with pigments extracting from cells of colonial green alga *Pediastrum boryanum* (Turp.) Menegh. (Hydrodictyaceae) using organic solvents. In order to ascertain the cause of the problem, they examined the chemical composition of algal cell wall and established that the narrow outer layer of the wall contains silica. Later, Millington & Gawlik (1970) discovered plaques deposited outside the plasma membrane in *P. boryanum*. These plaques have some similarities with the three appressed membranes (the plasma membrane and two membranes of the vesicle with silica) in the diatom *Amphipleura*, however, the authors did not observe vesicles with silica in *P. boryanum*. Parker (1969) detected silica in *Hydrodictyon reticulatum* (L.) Bory, *P. boryanum*, *P. tetras* (Ehrenberg) Ralfs, *Pediastrum* sp. and *Tetraëdron bitridens* Beck-Mannagetta (Hydrodictyaceae). The silica (largely isotropic non-crystalline opal) predominates in the cell walls of at least *Pediastrum* and *Tetraëdron*. Parker concluded that silica constitutes a rigid external layer of the cell wall.

Biogenic or hydrated silica ($SiO_2 \cdot nH_2O$) does not release water until it is heated up to 60°C owing to very narrow spaces between silica gel particles (Iler 1979). Therefore, we assume that silicification of sporoderm may be the cause of poor infiltration with a resin of hornwort spores reported by Brown & Lemmon (1988).

Materials and methods

Sporogonia of *Phaeoceros laevis* (L.) Prosk. subsp. *carolinianus* (Michx.) Prosk. [1] (Anthocerotopsida, Notothyladaceae [2]) were collected in vicinity of Kalistovo station (Pushkino District, Moscow Region) by V.R. Filin in October 2001 [MW 9110003]. The material was fixed and stored in 75% ethanol. Filin did not notice wether the plants of *Ph. laevis* were monoecious or dioecious. Nevertheless, the spores from sporogonia of these plants possess papillae in the center of each proximal triradiate face (Fig. 1A, B) which is characteristic of subsp. *carolinianus* [3]. *Ph. laevis* specimens collected in Odintsovo District by V.R. Filin and A.G. Platonova [MW 9110046] and in outskirts of Moscow by M.V. Remizova and D.D. Sokoloff [MW 9110047] in autumn 2017 are monoecious like in subsp. *carolinianus*.

Sporogonia of *Anthoceros agrestis* Paton [4] (Anthocerotopsida, Anthocerotaceae) were collected in vicinity of Skorotovo settlement (Odintsovo District, Moscow Region) in November 2011 by V.R. Filin [MW 9110002]. The material was fixed and stored in 75% ethanol.

---

1 Proskauer formerly distinguished two species: dioecious *Ph. laevis* (L.) Prosk. and monoecious *Ph. carolinianus* (Michx.) Prosk. (Proskauer 1951). Later, he considered these species as subspecies of *Ph. laevis* (Proskauer 1954). He wrote that "*Phaeoceros laevis* is a species in a phase of intense evolution" (Proskauer 1957: 129) and "It appears that subsp. *carolinianus* is beginning to differentiate local races, which as yet are quite ill defined" (Proskauer 1957: 127). Grolle (1976, 1983), Düll (1983), Arts (1984), Hasegawa (1984), Hässel de Menéndez (1989), Konstantinova et al. (1992), Cargill & Fuhrer (2008), Potemkin & Sofronova (2009) and Villarreal & Renner (2013) accepted Proskauer's first point of view, whereas Smith (1990) and Schumacker & Váňa (2005) followed the second one, but Váňa accepted the first point of view earlier (Söderström et al. 2002).

2 Authors follow the classification of Duff et al. (2007)

3 Proskauer (1957) noted, that he never observed a cluster of coarse granules in the center of each proximal spore face in the type subspecies, but such feature occurs in subsp. *carolinianus*. Cargill & Fuhrer (2008) also reported that verrucae numbers are low and they are confined to the center of each triradiate face in spores of *Ph. carolinianus*.

4 Paton (1979) described *Anthoceros punctatus* var. *cavernosus* sensu Prosk. as *A. agrestis* sp. nov. According to Smith (1990), some authors consider *A. agrestis* as a variety of *A. punctatus* L., but geographical distribution of these species would justify the recognition of them as subspecies.

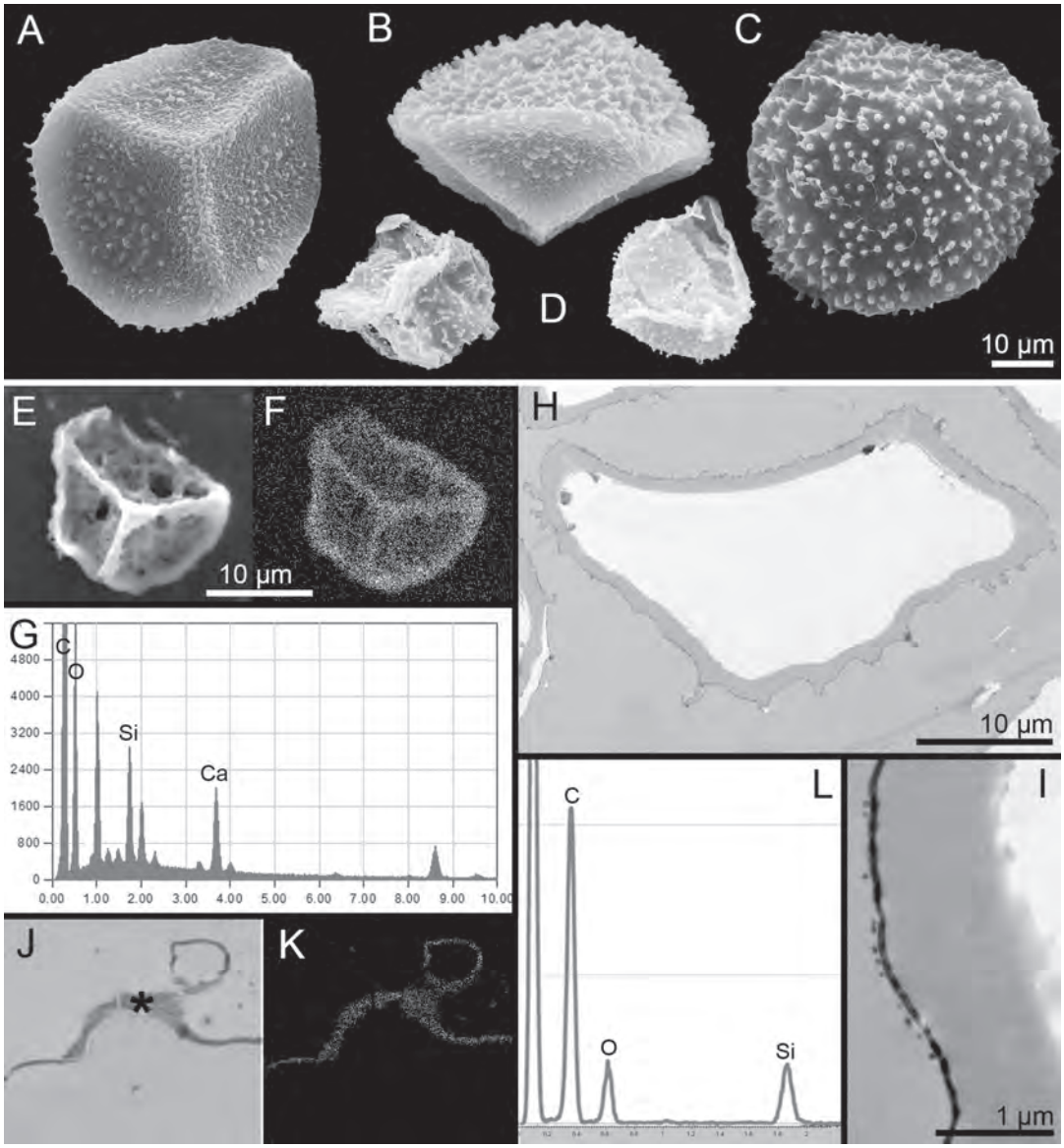

Figure 1. *Phaeoceros laevis* subsp. *carolinianus.* Proximal (A), equatorial (B) and distal (C) views of intact spores and burnt spores (D) in the same scale (SEM); burnt spore (E) together with silicon elemental map (F) and elemental spectrum (G) of the same area obtained using X-ray analysis (SEM); thin sections of the spore (the protoplast absent, H) and a part of sporoderm (I) with thin electron-dense black outer layer (TEM); thin section of sporoderm (J) together with silicon elemental map of the same area (K) and elemental spectrum (L) obtained using X-ray analysis from the point matched with * in J (TEM).

Thalli of *A. caucasicus* Steph. with sporogonia were collected in the Batumi Botanical Garden (Georgia) in December 1977 by I.N. Volkov and were fixed and stored in 75% ethanol [MW 9110001].

Thalli of *Notothylas* cf. *frahmii* Chantanaorr. (Anthocerotopsida, Notothyladaceae) with sporogonia were collected on wet soil in the campus of Shivaji University (Kolhapur, Maharashtra, India) in August 2016 by D.D. Sokoloff, S.M. Patil and R.A. Lavate and were determined as *Notothylas levieri* Schiffn. ex Steph. by S.M. Patil and R.A. Lavate [MW 9110004]. However, the

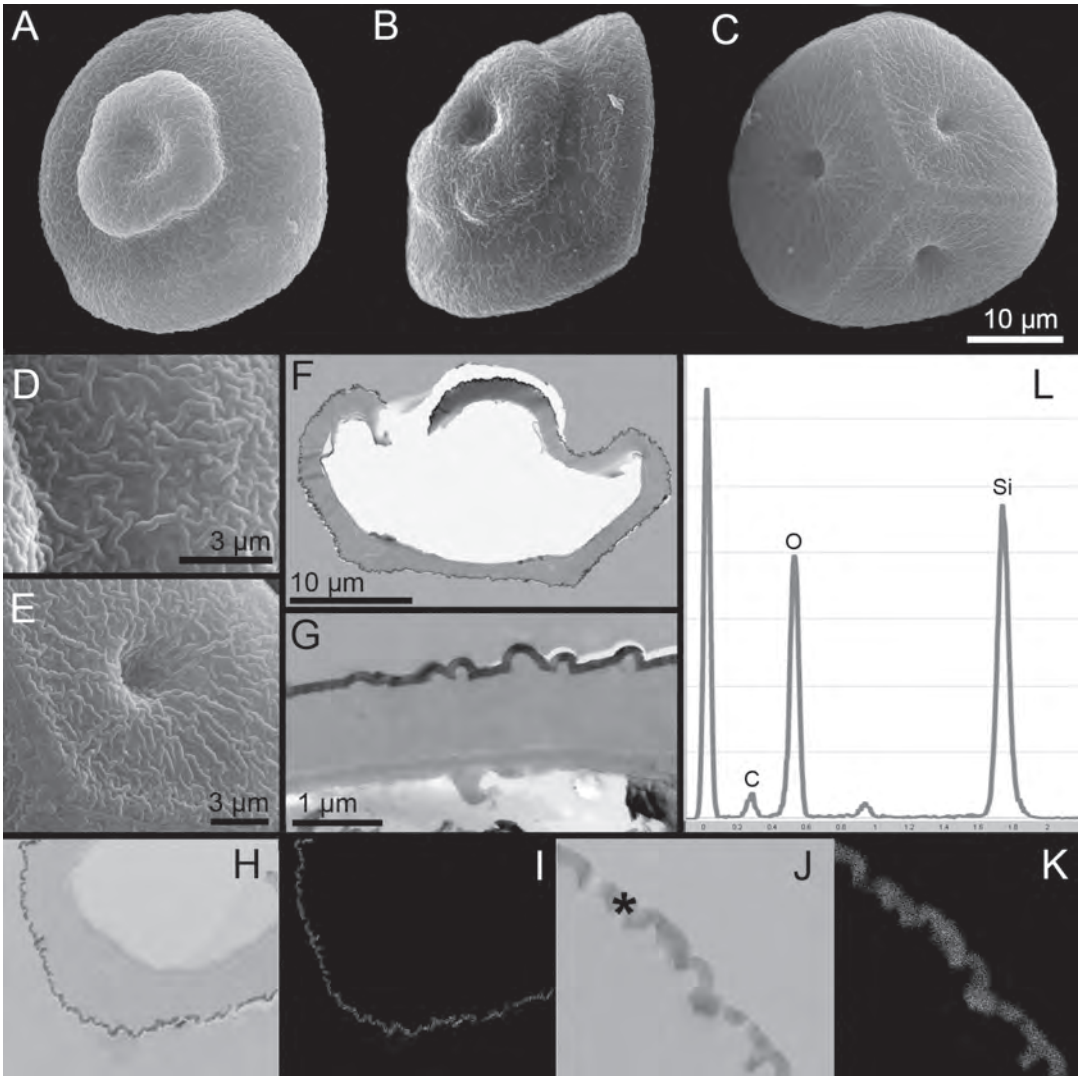

Figure 2. *Notothylas* cf. *frahmii*. Distal (A), semi-equatorial (B) and proximal (C) views of intact spores, vermiculate distal surface of spore (D) and a depression in the middle of one triradiate proximal face (E) (SEM); thin sections of the spore (the protoplast absent, F) and a part of sporoderm (G) with thin electron-dense black outer layer (TEM); thin sections of sporoderm (H, J) together with silicon elemental maps of the same area (I, K) and elemental spectrum (L) obtained using X-ray analysis from the point matched with * in J (TEM).

specimen has no dehiscence lines consisting of 4–8 rows of thick-walled cells in capsule epidermis characteristic for *N. levieri* (Udar & Singh 1978; Chantanaorrapint 2015). Spores of this specimen are yellow, but not dark brown to blackish as in *N. levieri* (Chantanaorrapint 2015); microsculpture of the spore surface is finely vermiculate (Fig. 2A, D), but not tuberculate and each triradiate face possesses a central depression (Fig. 2C, E) which is absent in spores of *N. levieri* (Chantanaorrapint 2015). According to spore micromorphology (Fig. 2A–E), this specimen belongs most probably to *N. frahmii*, but an additional study of *Notothylas* from the campus of Shivaji University is required. For the further investigation, capsules from the herbarium specimens were rehydrated in water and then infiltrated in a series of increasing concentrations of ethanol up to 75% ethanol.

For scanning electron microscopy (SEM), the capsules with intact spores of all four species were dehydrated through absolute acetone and critical point dried using a Hitachi HCP-2 critical point dryer. For EDX analysis on SEM, material was prepared by dry ashing procedure. Capsules of *Ph. laevis*, *A. agrestis* and *A. caucasicus* were put into porcelain crucible and burnt in muffle furnace at 700°C for 8 hours. Both intact and burnt material was mounted on SEM stubs and coated with gold and palladium using an Eiko IB-3 ion-coater. The material was observed using a CamScan 4 DV in the laboratory of electron microscopy at the Biological Faculty of Lomonosov Moscow State University.

For EDX analysis, burnt material was mounted with carbon adhesive discs on SEM stubs and coated with carbon using a JEE-4C ion-coater. The material was investigated using JSM-6380LA with EDX analyzer JED-2300 in the laboratory of electron microscopy at the Biological Faculty of Lomonosov Moscow State University and using TeScan with EDX analyzer at the Paleontological Institute of Russian Academy of Sciences. The elemental spectrum was received from the whole visual field containing a burnt spore. Then, the elemental map of Si was constructed to confirm that silicon is contained exactly in a spore but not in foreign particles, which could stick to the spore.

For transmission electron microscopy (TEM), capsules of *Ph. laevis*, *A. agrestis* and *A. caucasicus* were dissected; capsule of *N.* cf. *frahmii* was taken as a whole. Capsules of all four species without any counterstains (including OsO_4) from 75% ethanol through absolute acetone and acetone-Epon mixtures were embedded into Epon, placed in embedding molds and polymerized ca 24 h at 60°C. Ultrathin sections were prepared in the laboratory of electron microscopy at the Biological Faculty of Lomonosov Moscow State University and then studied using JEM-1011. The same sections were analyzed using JEM-2100 with EDX analyzer Oxford INCA in the laboratory of electron microscopy at the Biological Faculty of Lomonosov Moscow State University. Firstly, the elemental spectra were received for points in different layers of sporoderm and in pure resin. In case of significant differences in the amount of Si between different points, the Si distribution map was constructed.

Results

Wholly preserved mineralized 'skeletons' of spores were found in the burnt material of *Ph. laevis*, *A. agrestis* and *A. caucasicus* among the destroyed remnants. Hereafter, these 'skeletons' are called burnt spores. Burnt spores were ca three times smaller than intact spores, but the main characteristics of sporoderm micromorphology were preserved in the burnt spores of all three species (Figs 1A–D, 3A–C, 4A–C). The mentioned decrease of spore volume after burning is a result of burning-out of organic compounds as well as of loss of water.

The EDX analysis of burnt spores of *Ph. laevis* revealed a great amount of Si in the spores (Si peak on spectrogram is comparable to Ca peak [5], Fig. 1G); the localization of the main amount of silica within spore was confirmed by the Si distribution map (Fig. 1E, F). The EDX analysis of the ultrathin section of the *Ph. laevis* spore confirms the localization of silica within the sporoderm (Fig. 1L). Comparison of the Si EDX map of the part of the section (Fig. 1K) with the micrograph of the same part (Fig. 1J) suggests that Si is localized in the outermost thin

5 Large amount of Ca in the spectrogram of a burnt spore appears to be localized in the sporoderm. We suppose that Ca from the sporoderm will be utilized by a growing young thallus.

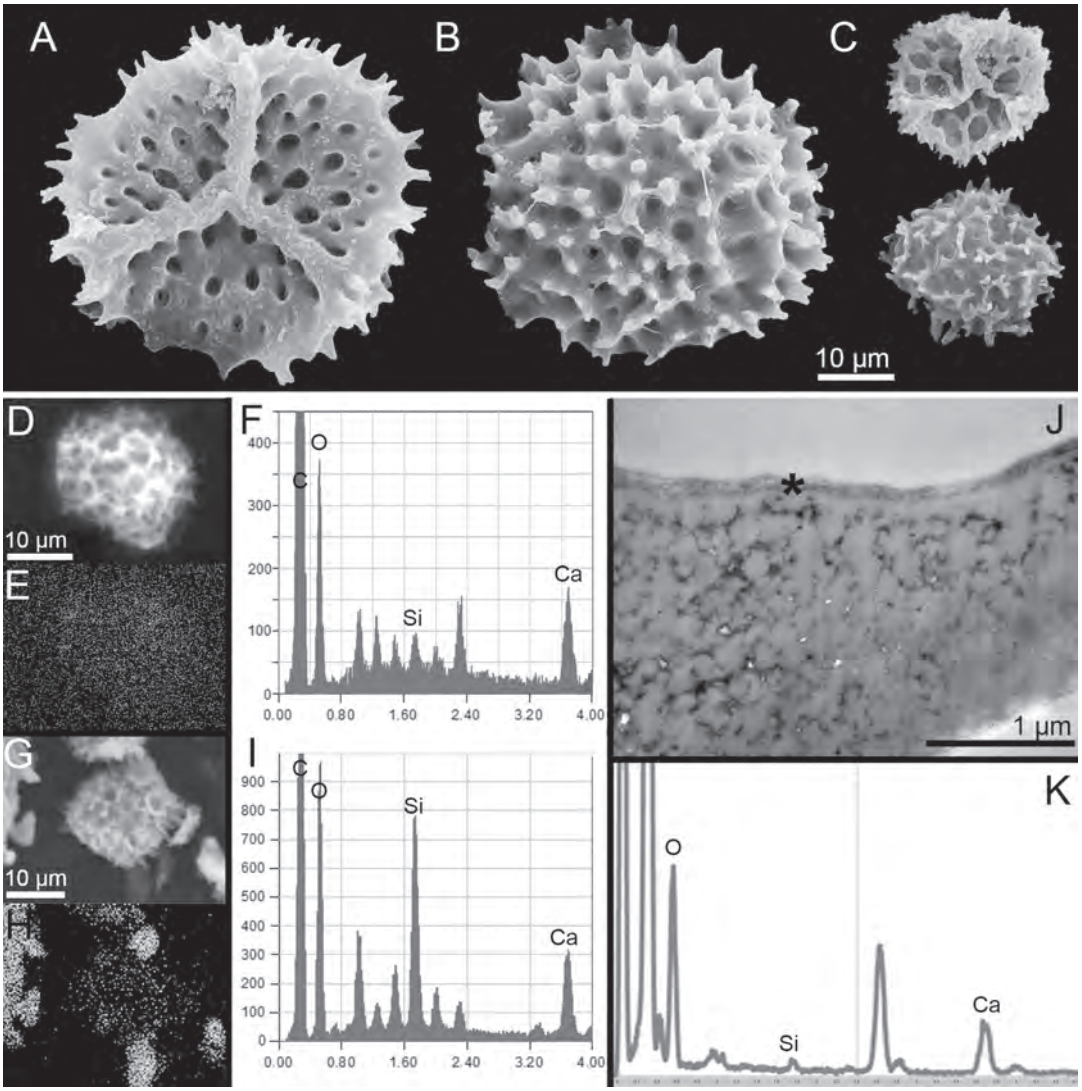

Figure 3. *Anthoceros agrestis*. Proximal (A) and distal (B) views of intact spores and burnt spores (C) in the same scale, SEM; burnt spores (D, G) together with silicon elemental maps (E, H) and elemental spectra (F, I) of the same areas respectively obtained using X-ray analysis (SEM); thin sections of a part of sporoderm (J) without electron-dense black outer layer (TEM); elemental spectrum (K) obtained using X-ray analysis from the point matched with * in J (TEM).

black electron-dense layer of the sporoderm of *Ph. laevis* (Fig. 1H, I). Similar results were received for the ultrathin section of the mature spore of *N.* cf. *frahmii*. Its sporoderm also possesses the outermost thin black layer (Fig. 2F, G) where large amount of silicon was detected (Fig. 2H–L). Apparently, the electron-density of the outer layer is caused by the crystallin-like structure of silica (no OsO_4 treatment of material, mentioned earlier, was conducted).

In contrast to *Ph. laevis* and *N.* cf. *frahmii*, the spores of both *A. agrestis* and *A. caucasicus* contained trace amount of Si registered by SEM with EDX analyzer (the peak of Si on spectrograms is much smaller than the peak of Ca: Figs 3D–F; 4D–F). Si detected on spectrograms of burnt spores of *A. agrestis* (Fig. 3I) originated most probably from the extraneous particles, what was confirmed by the Si distribution map (Fig. 3G, H). There is no outermost black layer in ultrathin sporoderm

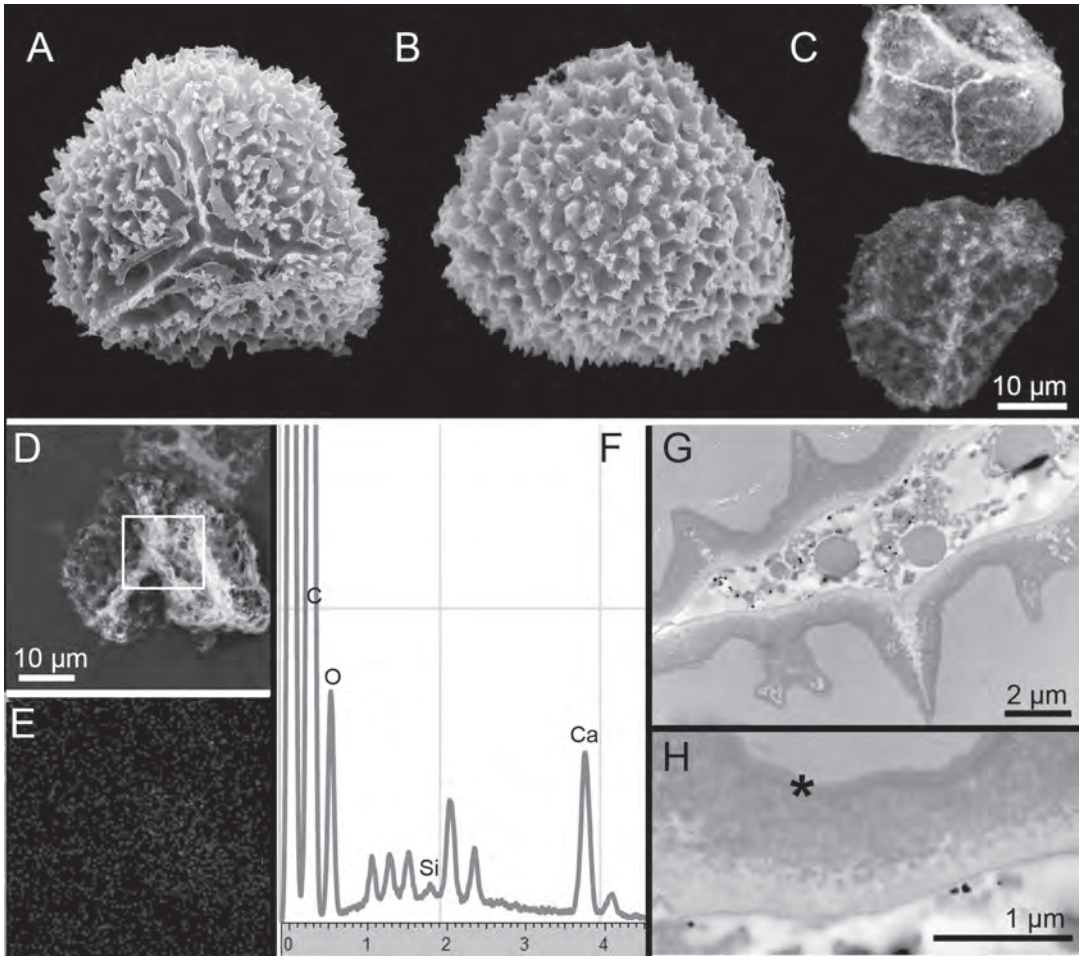

Figure 4. *Anthoceros caucasicus*. Proximal (A) and distal (B) views of intact spores and burnt spores (C) in the same scale, SEM; burnt spore (D) together with silicon elemental map of the same area (E) and elemental spectrum from the square area in D (F) obtained using X-ray analysis (SEM); thin sections of a part of spore (G) and sporoderm (H) without electron-dense black outer layer (TEM).

sections of these two species (Figs 3J; 4G, H) and no significant amount of Si was detected in the outer sporoderm layer by means of TEM with EDX analyzer (Fig. 3K).

Discussion

Silicon in spores and pollen of land plants

Silicification is widespread in nature. It is not surprising, taking into account that each sixth atom in the lithosphere is silicon / silicium (Si) (Voronkov & Kuznetsov 1984). Silicon poorly dissolves in water forming a silicic acid. When concentration of the acid in solution exceeds the solubility of the amorphous solid phase, the autopolycondensation reaction takes place and a hard amorphous hydrated oxide of Si ($SiO_2 \cdot nH_2O$) is formed in the specific physical and chemical conditions. Many plants are able to absorb silicic acid actively and to form biosilica particles in their body. Form, dimensions and packing of the particles depend on many factors. Biosilica particles can occur inside the cell, in the cell wall and in intercellular spaces (Barber & Shone

1966; Raven 1983; Perry 2003; Coradin et al. 2006; Currie & Perry 2009; Bauer et al. 2011; etc.). Sometimes, silicon binds mucopolysaccharides and polyuranyl molecules (Schwarz 1973).

Silicon in the sporodem of land plants was discovered by Tchistiakoff (1873) for the first time. He believed that the exosporium of *Isoëtes durieui* Bory (Isoëtaceae) entirely consists of pure silica. Later, silicon was found in the sporoderm of other quillworts as well as in spores of another ligulate lycopsid, *Selaginella* (one of the latest articles on this subject: Moore et al. 2006). Silicon was discovered in sporoderm of various pteridophytes, in ferns (Edman 1932; Parkinson 1995; Moore 2005) and in horsetails (Perry 2003; Currie & Perry 2009). Illustrations of spore ultrastructure of *Lygodium japonicum* (Thunb.) Sw. (Schizaeaceae) from Edman (1932) show that silica is located in the sporoderm layer which corresponds to perisporium in the paper of Tryon & Lugardon (1991). Parkinson (1995) discovered that the siliceous surface of the outer perisporium of *Schizaea pectinata* (L.) Sw. spores is formed by composite bodies consisting of partially polymerized sporopollenin associated with silicon and phenolics.

Silicon was detected in the pollen wall of *Lychnis alba* Mill. (Caryophyllaceae) and *Impatiens sultanii* Hook. f. (Balsaminaceae) (Crang & May 1974). Crang & May (l.c.) believed that silicic acid is derived from tapetal cells, and Si is a part of orbicules with silica gel deposited on the surface of exine and tapetum. They supposed that Si may serve in a binding capacity for organic compounds or may be an integral part of organosilicon molecules.

As for bryophytes, the investigation of Taylor et al. (1976) on *Conocephalum conicum* (L.) Dum. is noteworthy. The authors detected only traces of silicon in spores, but rather much silicon was found in elaters by means of EDX analysis. Like Crang & May (1974), they proposed that Si may serve in binding capacity for organic compounds and noted that the precise role of this element in spores and elaters has yet to be determined. Moore (2005) found traces of Si by means of EDX analysis in spores of three mosses: *Brachythecium velutinum* (Hedw.) B.S.G., *Bryum capillare* Hedw. (Bryaceae) and *Funaria hygrometrica* Hedw. (Funariaceae). She pointed out that it is difficult to identify the localization of Si within the spore, and therefore it is not clear whether Si is restricted to a particular sporoderm layer or it is evenly distributed among the sporoderm and the spore lumen. Moore also noticed that functions of Si in spores are unknown. We also detected traces of Si by means of SEM with EDX analysis in burnt spores of *Conostomum tetragonum* (Hedw.) Lindb. (Bartramiaceae) and *F. hygrometrica*, but taking into consideration that "silica is present in significant quantities even in the best-purified nutrition salt, water and air" (Werner & Roth 1983: 683), we shall not insist at present that the sporoderm of all above mentioned mosses as well as spores and elaters of *Conocephalum* contain silicon. The EDX analysis of ultrathin sporoderm sections using TEM is necessary to rule the sporoderm silicification out.

Thus, the silicified outer layer of sporoderm of *Ph. laevis* and *N.* cf. *frahmii* is the first precisely documented record of sporoderm silicification among bryophytes. This layer in *Ph. laevis* sporoderm corresponds to the electron-dense layer resembling perine of the same species in the work of Brown & Lemmon (1988) and the Si-containing layer in the sporoderm of *N.* cf. *frahmii* corresponds to the perine-like layer of *N. temperata* Hasegava (Renzaglia et al. 2009). We have not encountered difficulties with resin infiltration of investigated spores with a resin; the artifacts of our sections caused by the absence of glutaraldehyde fixation. Obviously, our assumption that the difficulties Brown & Lemmon (1988) reported about were caused by the presence of silica

in sporoderm was false. While testing this hypothesis, we discovered the silicified sporoderm in hornworts.

To better understand the role of the silicified outer layer of exosporium in the life of *Phaeoceros* and *Notothylas*, we will discuss ecological and biological peculiarities of these plants.

Ecology and biology of *Phaeoceros laevis*

Phaeoceros laevis subsp. *laevis* is restricted to Europe, where it has a Mediterranean-Atlantic distribution and tends to be the more common subspecies there. Subsp. *carolinianus*, on the other hand, is essentially a world-wide taxon characteristic of the temperate zone. It is the most common subspecies in central, northern and eastern Europe (Proskauer 1958). Smith (1990) reported that both subspecies are widespread in the world, but Cargill & Fuhrer (2008) believe that *Ph. laevis* s.str. is probable confined to the northern hemisphere. Campbell (1981) cultivated thalli from tubers of *Phaeoceros* originated from New Zealand and distribution of sex organs on these samples shows that they belong to subsp. *carolinianus*. Potemkin & Sofronova (2009) consider that both taxa are rare in European Russia. Probably, the occurrence of these plants is estimated incorrectly, and actually they may occur much more often. They are rarely collected in herbaria because this group is usually of interest to specialists only. These hornworts often grow in peculiar habitats, and investigators who know their biology may more likely find these plants there. *Ph. laevis* is a pioneer species; it grows on clay moist acid soil in naturally or artificially disturbed habitats or damp turfaceous rock. It may be found on cultural lands, by roadside and ditch banks, wood ridges, very rarely on fine earth cliffs in shady habitats subjected to constant trickle water.

The two subspecies of *Ph. laevis* are widely distributed in the subtropical humid deciduous forests of Western Himalayas. They occur from 1900 m to 2412 m altitude on lime rock soil and the plants grow at 7–32°C in summer and 1–7°C in winter with average annual rainfall up to 2400 mm (Asthana & Nath 1994). Piippo (1993) reports that *Ph. laevis* subsp. *carolinianus* occurs in New Guinea mainly in rainforests, occasionally in gardens, open grasslands or very open forests at 500–2000 m. It grows mostly on rocks, boulder and soil, rarely on stump or rotten logs. Warny et al. (2012) reported about numerous spores of hornworts (including the *Ph. laevis* type) found in horizon with charcoals from cores of the sea coast of the Gulf of Mexico. We suppose that the presence of charcoals suggests that hornworts (and particularly *Ph. laevis*) may appear after forest fires (possibly anthropogenous). At the same time, Warny et al. (2012) believe that the site was a freshwater swamp or an area with permanently moist soil at the time of hornworts' occurrence.

In Switzerland, *Ph. carolinianus* can be found on arable fields in June–July; thalli may appear from spores, which were kept in the soil bank 2–3 years after the thalli were discovered for the first time on the control plots (Bisang 1999). Observations of Cailliau & Price (2007) bear also evidence of an important role of soil bank in maintenance of the populations of *Ph. carolinianus*. Thalli of *Phaeoceros* sp. appear on soil, which was taken from a depth of 4–8 cm in 20 years old broad-leaved forest with moss cover in Illinois, USA (Edwards 1994). Spores of *Ph. laevis* may germinate and form normal thalli after 13 years keeping plants in herbarium (Proskauer 1957). Normal thalli of *Ph. bulbiculosus* (Brother) Prosk. developed from spores of more than 8 years old herbarium specimens (Herguido & Ron 1989).

Phaeoceros laevis is an annual or pauci-perennial plant (Proskauer 1948a; Smith 1990). The gametophytes die after spore liberation from the capsule. Because thalli form subterminal or ventral tubers (Proskauer 1948a, 1969), the plant may be named 'monocarpic' conditionally. According to several authors (e.g. Chopra & Kumra 1988), thalli produce gametangia at a short day and in a wide range of temperatures (5–27°C); photoperiod is the critical factor in gametangia initiation, whereas temperature has no appreciable effect. However, Proskauer (1969) reported that the dependence on photoperiod is related to intraspecific variation. Similar situation was observed in a number of widespread angiosperms (Voroshylov 1960). In the same time, *A. agrestis* demonstrated the most effective induction of gametangia under long day (16 h light) or continuous light conditions after dropping the growth temperature from 23° to 16°C (Szövényi et al. 2015).

The thallus forms several sporophytes with non-synchronous spore production. Spore diameter is 30–60 µm (Proskauer 1957; Smith 1990). Sporophytes of *Ph. carolinianus* develop 3–4 weeks after formation of gametophytes that persist on thalli up to October–November in arable fields in Switzerland (Bisang 2003). In Northern Ireland, *Ph. laevis* produces spores from March to December (usually in June–September) (Proskauer 1948a). Sporophytes of *Ph. laevis* are 0.7–9 cm (usually 3–4 cm) long (Proskauer 1948a). Bisang (1995) cultivated gametophytes of *Ph. carolinianus* (collected in the end of September in Switzerland) in laboratory on their original substrates in Petri dishes that were kept in a north facing window at 20–22°C under natural light conditions. All samples persisted during the whole period of experiment (18 months) and young sporophytes were observed from April to May 1993. She believes that gametophytes seem to have the potential for a longer life span than they exhibit under the climate conditions of central and northern Europe. A 1.5 cm dehisced capsule of *A. agrestis* contains an average of 11,000 spores (Bisang 2001). It is obvious that spore output is much higher in the longer capsule of *Ph. laevis*.

Sydow & Sydow (1903) discovered spores of smut fungus *Tilletia abscondita* Syd. & P. Syd. in capsules of *Anthoceros dichotomus* Raddi (now *Ph. dichotomus* (Raddi) Prosk. (Proskauer 1951)) collected on Corfu island. Ligrone (1988) found the vesicular arbuscular mycorrhiza in gametophytes of *Ph. laevis* from Italy. The absence of fungi in the British samples of this species examined by Pokock & Duckett (1984) seems to indicate that the *Ph. laevis* mycorrhizal fungus association is not obligate as Ligrone supposed.

Phaeoceros laevis is an anemochorous plant, but it is very likely that short mature capsules liberate spores only after capsule decay following lasting rainy weather, as it was noted for *Anthoceros punctatus* (Proskauer 1948b). In any case, spores can remain on soil surface or later be buried in soil not very far from the source as a result of activities of water, people and animals (rodents, slugs and earthworms). Spores from soil surface may be taken away by wind in the dry season.

Longton & Schuster (1983) believe that hornwort spores are too large and may be transferred only over short distances, except for bird or water dispersal. Bisang (1999, 2001) agrees with these authors. Indeed, populations of hornworts are surrounded by herbs and trees in the present time, and aerial spore dispersion on long distances is unlikely. Schuster noticed that *Anthoceros laevis* L. (now *Ph. laevis*) and *Aspiromitus punctatus* (L.) Schlyakov (now *Anthoceros punctatus* L.) have been widely dispersed in the last 3–4 centuries by the activity of people and the very 'weedy' nature of these species "suggests a long and involved association with agricultural man, and most populations of these species are as 'domesticated' as a dog and house fly" (Schuster 1984: 1089).

In this connection, it is worth reminding the words of Grime & Pierce: "It seems reasonable to conclude therefore that, prior to advent of agriculture, the majority of arable weeds had already evolved in habitats subjects to more 'natural' form of distribution" (Grime & Pierce 2012: 83).

Proskauer pointed to a remarkable resistance and longevity of the spores of *Ph. laevis* and wrote: "I cannot see why, even should their viability be decreased by radiation factors in the atmosphere, spores of *P. laevis* should not *occasionally* be able to drift around the world even several times and still land in viable condition" (Proskauer 1957: 126). But it is usually believed that expansion of a species (especially for dioecious species) by means of a single diaspore happens very rarely, and the 'step by step' expansion mostly occurs.

Bisang (2003) considers *Ph. carolinianus* as an annual shuttle species according to the life strategy system proposed by During (1979, 1992). Initially, During (1979) attributed annual-pauciennial species to this group, but later he (During 1992) attributed only species with a potential life span less than one year to annual shuttles. Annual shuttles possess few large (>20 µm) spores. Asexual reproduction (reproduction by gemmae sensu During 1979: 9) in these species is absent (During 1979). But Proskauer (1948a) described tubers in *Ph. laevis*. Summarizing data on tubers in other species of *Phaeoceros*, Proskauer came to a conclusion: "It is quite possible that the capacity to produce them is inherent in all the species of the genus" (Proskauer 1951: 335). Ligrone & Lopes (1989) found slime on the surface of the thick outer wall of the epidermal cells in tubers of *Ph. laevis*. Perennating tubers of hornworts can tolerate unfavorable conditions, especially desiccation, and allow the plant to be perennial (Campbell 1981; Schofield 1985). Cargill & Fuhrer (2008) wrote about ventral unstalked brown and flat tubers full of starch granules in *Ph. laevis* subsp. *carolinianus*, but they do not consider these tubers as propagules. Arts (1984), Risse (1987) and Bisang (2003) wrote nothing about tubers in *Ph. carolinianus*. Penjor et al. (2016) believed that the absence of tubers in *Ph. carolinianus* from Thailand is caused by the habitat which is the most well-suited for plants. Paton reported that "In temporary habitat subsp. *laevis* is generally an annual but it is facultative perennial in permanent sites which are sufficiently moist to permit continuous vegetative growth or where the production of tubers enable it to survive periods of desiccation. Subsp. *carolinianus* is probably an obligatory annual on denuded soils in temporary habitats such as arable fields" (Paton 1973: 542). During believed that large spores have a low dispersal capacity, "but probably better chances of successful establishment and a longer life span in the diaspore bank" (During 1992: 16, 17). He notes that species with larger spores have adapted to microhabitats, which predictably disappear at varying rates but reappear, frequently within the same community, whereas colonists or short-lived (few years) shuttle species are found in somewhat longer-lasting microsites.

During (1992) also discerns fugitives (*Funaria hygrometrica* type), which resemble shuttles. They have a short life span (ephemeral-annual) and very light (<20 µm in diameter) spores (During 1992). The fugitive strategy fits in highly unpredictable environments that exist for only a short time. "The fugitives are often the first species in a secondary succession series" (During 1979: 12). Pianka (1981) considered the fugitive species as an interesting special case of an opportunistic species. These species are always locally excluded by interspecific competition, but they remain and colonize newly disturbed areas by virtue of a high dispersal ability. Obviously, *Ph. laevis* possess characters of both shuttles and fugitives. According to the system of Yurtsev

(1986), *Ph. laevis* may be attributed to sprinters. Sprinters achieve maximal photosynthetic rate in a short period of time. They correspond to explerents in the system of Ramensky (1938) or ruderals in a system of Grime (1979, 2001).

Ecology and biology of *Notothylas*

Chantanaorrapint (2015) reports that *Notothylas frahmii* is known only from the type locality (Thailand, Tak prov.), but this species may possibly occur also in other areas of Thailand with similar climatic conditions. Chantanaorrapint does not report anything about the biology of this plant.

The genus *Notothylas* includes 24 species, and it is the fifth-largest genus in the Anthocerotophyta (Villarreal et al. 2010; Peng & Zhu 2014; Chantanaorrapint 2014, 2015). Species of this genus occur from tropic to temperate latitudes in both Old and New World; the center of the diversity of the genus is the Indian subcontinent. *Notothylas* occurs in plains or in mountains up to 3030 m above sea level. *Notothylas* is often found in rainy season on moist disturbed soil along walking trails and roads, in gardens and farms, on damp soil or mud, on drained rice-fields, in undisturbed areas on moist soil and wet sandy rocks in shaded environments. Thalli are likely annual (Breil 1970; Hasegawa 1979; Udar & Singh 1979a,b; 1981a,b; Singh 1988; Asthana & Srivastava 1991; Schuster 1992; Peng & Zhu 2014; Chantanaorrapint 2015). Udar & Singh (1979a) correctly indicate that *Notothylas* species inhabiting microenvironmental niches are often better resistant against macro-climatic variations. The plants are often found growing in association with other hornworts, particularly with *Ph. laevis* subsp. *carolinianus* (Chantanaorrapint 2015). Tubers were found in some species. *Nostoc* colonies were not observed in *N. nepalensis* Singh (Singh 1988) and in *N. frahmii* (Chantanaorrapint 2015). *N. orbicularis* is a short-day plant (Chopra & Kumra 1988). The short (0.7–0.5 cm long) numerous prostrate capsules are completely enclosed in involucre or slightly protrude from the mouth of involucre. Pseudoelaters are very short.

The capsule of most species possesses a dehiscence line and the dry capsule usually dehisces like a follicle from apex downwards into two valves along the only suture (Pandé 1934; Müller 1951; Asthana & Srivastava 1991). The capsule of *N. vitalii* Udar & Singh has no dehiscence lines, and it is reported to dehisce by a transversal separation of the apical portion (Oliveira et al. 2017). *N. breutelii* Gottsche possesses a well-marked dehiscence line, but capsules of this species are described as indehiscent (Lang 1907; Bartlett 1928). *N. pfleidereri* Udar & D.K. Singh has a very small capsule without a line of dehiscence and it is cleistocarpous (Asthana & Srivastava 1991).

Despite the capsules of most species are able to dehisce, the capsules of *Notothylas* are short prostrate, with thick-walled cells of the capsule wall and they are almost entirely included in the involucre. Considering this, it is unlikely that spores liberate from capsule of *Notothylas* as effectively as from long and erect, distally free from the involucre capsule of *Phaeoceros*. Spores of *Notothylas* seem often liberated only after the decay of involucre and capsule wall from gametophytes buried in soil. Undoubtfully, the spores of this genus remain viable for a long time. It is no coincidence that Oliveira et al. (2017) based on the peculiarities of spores' germination in culture concluded that spores of *N. vitalii* could be included in soil bank. We suppose that spores of *Notothylas* are dispersed by water, wind, animals and also by men. The sporogonium of *Notothylas* is considered to be the result of a reduction of the sporogonium of *Anthoceros* type

(Lang 1907) or as the result of progenesis (Filin 2009). We believe that life strategy of *Notothylas* is similar to that of *Ph. laevis*.

Possible role of the siliceous layer of sporoderm in life of *Phaeoceros laevis* and *Notothylas* cf. *frahmii*

It is known that silicon plays such an important role in plant life that Epstein & Bloom (2005) considered that it should be classified as an essential element for plants. This role is multifunctional: structural, protective and physiological (Lewin & Reimann 1969; Raven 1983; Epstein 1999, 2001; Sangster et al. 2001; Perry 2003; Richmond & Sussman 2003; Coradin et al. 2006; Currie & Perry 2009; Liang et al. 2007; Bauer et al. 2011). Considering these data, we may assume that a continuous siliceous layer on the sporoderm surface of *Ph. laevis* and *N.* cf. *frahmii* increases mechanical strength of spores against wind or water transfer above surface soil. It prevents injury of spores by soil microorganisms and invertebrates (nematodes, earthworms [6], tardigrades, proturans, collembolans, pincers). The siliceous layer protects the spores from UV-B radiation, desiccation and very low or high temperatures, when the spores are on the surface of the soil or in the air. It reduces injurious action of Al, Fe, Mg, Zn ions on the germinating spores. This layer promotes formation of phytoalexins by the germinating spores and the young thalli. Organic compounds of the biosilica particles may be used for nourishment by the young thallus.

Ecology and biology of *Anthoceros agrestis*

The ecology and biology of *A. agrestis* is similar in many aspects to that of *Ph. laevis* subsp. *carolinianus* (Proskauer 1948a; Paton 1979; Bisang 1992, 1995, 1999, 2001, 2003). We also observed repeatedly in autumn 2011 and 2017 in Moscow region that *A. agrestis* and *Ph. laevis* subsp. *carolinianus* often grew side by side on disturbed soil and not infrequently with *Fossombronia wondraczekii* (Corda) Lindb. (Fossombroniaceae). The similar association is illustrated in the work of Delwiche & Cooper (2015: Fig. 2E). The joint growth of different hornworts must be taken into account in sporogonia selecting for sporogenesis studying.

Paton (1979) believed that *A. agrestis* occurs in northern, eastern and central Europe only. She indicated that the type sample of the species was collected on arable field in England. Söderström et al. (2002) marked that *A. agrestis* and *A. punctatus* are often confused especially in older literature, therefore, the occurrence of the two taxa in other parts of Europe is unclear and several reports (e.g. all samples from Japan) may belong to *A. agrestis*. By the way, Bakalin et al. (2007) found *A. agrestis* in the north of Kamchatka peninsula (Russia) near hot springs. This locality is ca 2600 km north from the nearest locality in Primorski Territory. These authors believe that the Kamchatka locality is a relict of the earlier epoch when thermophilous hornworts were distributed much more broadly in the North-East of Russia. On the other hand, Gambaryan (1992), studying the hepaticoflora of the Far East for more than 10 years, collected this species only once at the side of a road in the broad-leaved forest near a settlement on Russky Island (Primorski Territory, Russia). We suppose that this species was not a relict in Kamchatka, but brought to by birds or men.

6 Duthie (1929) reports that micro- and megaspores of *Isoëtes* species passed through the digestive duct of earthworms without injuries.

On the British Isles, *A. agrestis* generally grows on recently disturbed moist or wet, circumneutral or moderate base-rich clay or loam. It is a summer annual with sporophytes 8–30 mm long, maturing from July to November. *A. punctatus* is an annual or facultative perennial, depending on habitat conditions (Paton 1979). In central and northern Europe, *A. agrestis* and *Ph. carolinianus* are almost exclusively confined to secondary habitats such as cultivated fields (Bisang 1992). Many samples of *A. agrestis* which were cultivated in laboratory lived considerably longer (up to 11 months) than in field (3–4 months) and sporophytes were observed in several cultures at every screening during the 18 months of monitoring (Bisang 1995). Bisang remarks that no attempts have been made yet to distinguish the origin of thalli (sporeling or regenerant). Spores of *A. agrestis* are rather large (38–62 µm) (Paton 1979). In Switzerland, thalli of *A. agrestis* may appear on arable fields in June–July from spores which obviously have been kept in soil bank for five years (Bisang 1999). Therefore, we believe that this species is not a real annual shuttle sensu During (1992). In the same time, it is a real sprinter according to the system of Yurtsev (1986). *A. caucasicus* was collected on disturbed soil, and we know nothing about its biology and environment.

Indisputable fossil hornworts are unknown from Paleozoic, nevertheless, the most ancient hornworts are assumed to have existed in Silurian (Kenrick & Crane 1997) and even in Cambrian (Schlyakov 1975). Speculating if the silicification of the sporoderm plays a role in the origin and evolution of hornworts, we will attempt to reconstruct the habitat and environment, where the oldest embryophytes (including the ancestor of hornworts) appeared and diversified.

Terrestrial environment and biota in Proterozoic and early Paleozoic

Bryophytes have originated from the ancestral form related to extant charophyte algae. However, long before charophyte algae settled, the land cyanobacteria had lived there. Wellman & Strother (2015) gave a very detailed review of the evidence of the terrestrial biota prior to the origin of embryophytes. We will mention only several papers on this issue.

Blank & Sánchez-Baracaldo (2010) used a phylogenomic approach coupled with ancestral state reconstruction and demonstrated that cyanobacteria firstly appeared in the terminal Archaean and were restricted to freshwater ecosystems until at least 2.4 Ga. In this time, cyanobacteria lived in freshwater and were unicellular and probably planktonic or endolithic. Blank (2013) believed that the last common ancestor of the photosynthetic eukaryotes inhabited freshwater environment and their initial diversification also took place in terrestrial environments. Strother et al. (2011) reported about large populations of diverse organic-walled microfossils from the non-marine Torridonian (1.2–1.0 Ga) of Scotland. These fossils demonstrate the presence of eukaryotes in freshwater and sometimes in subaerial habitats during the Neoproterozoic era, although authors remark that the extent to which they lived subaerially cannot be ascertained. Rozanov (2009) did not preclude the appearance of photosynthetic eukaryotes and fungi in the Archean. He pointed out that the appearance of groups of organisms and their wide invasion did not coincide and these two events can be distant from each other for hundred million years. Heckman et al. (2001) indicated that green algae and major lineages of fungi were present 1000 Ma and they suppose that land plants appeared by 700 Ma.

Lewis & McCourt (2004) distinguished the divisio Chlorophyta (green algae s.str.) with 4 classes and the divisio Charophyta (charophyte algae and embryophytes) with the classes

Mesostigmatophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnemophyceae, Coleochaetophyceae and the subdivision Streptophytina [7] with the classes Charophyceae and Embryophyceae. Some authors considered Charophyceae or Coleochaetophyceae or both as the closest extant algal relatives of the embryophytes (Graham & Wilcox 2000; Raven 2000; Lewis & McCourt 2004; Becker & Marin 2009). But others considered Zygnematophyceae or both Zygnematophyceae and Coleochaetophyceae as the sister group of embryophytes (Bremer 1985; Wodniok et al. 2011; Laurin-Lemay et al. 2012; Timme et al. 2012; Zhong et al. 2013; Delwiche & Cooper 2015; Harholt et al. 2016; Vries de et al. 2016).

Becker (2013) believes that Chlorophyta/Streptophyta split was related to glaciation events in the Neoproterozoic Sturtian glaciation (about 720 Ma, a hard snow ball state of Earth). This glaciation seems to have selected only two strains of green flagellates which were physiologically best preadapted to terrestrial existence. Later, these strains gave rise to chlorophytes and charophytes. During Marinoan glaciation (about 630 Ma), the Earth probably represented a hard snow ball too, but in Gaskier glaciation (approx. 580 Ma) it probably was a soft snow ball and ice coverage did not reach low latitudes. Therefore, Becker (l.c.) believes that Gaskier glaciation events might have been the trigger for the colonization on the terrestrial habitats by charophyte algae, as climate became drier in the glaciation conditions. In this time, many freshwater bodies might have dried out and forced charophyte algae to adapt to a terrestrial habitat. Apparently, chlorophyte algae inhabited freshwater pools and bodies in Neoproterozoic as well as charophyte algae. We suppose that chlorophytes, as well as charophytes, colonized aquatic interstices in moist soil and, on the whole, they might be more powerful competitors than charophytes.

Selosse & Le Tacon suppose that lichens involving cyanobacteria and not-septate fungi can be expected to have existed until 1.0 Ga, and "that one could wonder whether lichens were the first the land colonizer" (Selosse & Le Tacon 1998: 15). Plant-mycorrhizal fungus symbiosis might have played an important role in the origin of land plants. It is supposed that the initial step of land plant evolution may be the partnership between two basically aquatic organisms, a green alga and a 'phycomycetose' fungus, similar to modern *Geosyphon pyriforme* von Wettstein, the only known example of endocytobiosis between a fungus (Zygomycotina, Glomales; macrosymbiont) and a cyanobacterium (*Nostoc*; microsymbiont) (Pirozynski & Malloch 1975; Gehrig et al. 1996; Heckman et al. 2001). Based on the mycorrhizal genes found in nearly all major land plant lineages including hornworts (*A. agrestis* and *Ph. laevis*), Wang et. al. (2010) supposed that these genes were present in the common ancestor of land plants, and the symbiosis likely played a key role in process of land colonization by plants by assisting them to absorb water and nutrients from soil.

The hypothetic ecotope where primary embryophytes evolved

Thus, in the Proterozoic, the Earth was already inhabited by algae, cyanobacteria and fungi, which were linked with various symbiotic associations. It is known that at present time microbiotic crusts cover soil surface in many arid and semiarid regions of the world. These crusts consist of lichens, cyanobacteria, algae, fungi and mosses which bind soil particles together and make the soil surface resistant to erosion (Evans & Johansen 1999). A similar association called 'green

7 The divisio Streptophyta (Anthocerotophyta) with subdivisions Zygophytina, Charophytina and Embryophytina (= Anthocericae, land plants, embryophytes) was introduced by Jeffrey (1967) for the first time.

horizon' was discovered in sandy deserts and on sandy shores of rivers in Russia almost 90 years ago by Zasukhin (1930). It represents a surface layer (ca 1 mm) of dump sand, inhabited by cyanobacteria, chlorophyte algae and invertebrates and buried under a thin layer of dried sand. Zasukhin called the population of living things in this horizon 'psammon'. This horizon combines traits of aquatic and aero-terrestrial ecosystems due to the physical properties of sand.

Heckman et al. (2001) did not exclude that lichens and free-living cyanobacteria may have formed biological crusts similar to modern ones in the Neoproterozoic (900 to 544 Ma) or even earlier. We agree with Heckman et al. (l.c.) and believe that similar crusts or psammon, consisting of algae, cyanobacteria, fungi and lichens, had existed on the largest part of the land at the time of the first embryophyte's appearance. But we do not think that such algae-lichens crusts had been the favorable ecotope for embryophytes originated there.

Modern biological crusts are extremely susceptible to surface disturbance and fire (Evans & Johansen 1999). Without doubt, the Palaeozoic ones were even more susceptible to wind and water erosion in the absence of the land plants with rhizomoids. Water streams from thawing snow or heavy showers changed direction from time to time and easily destroyed the climacteric algae-lichens communities and new bars, banks and shallow depressions with no deep bedding of ground fresh water appeared. Damp sand of these spaces is covered with a very thin layer of dried-up sand. This thin layer transmits the light and prevents water loss of damp sand. We suppose that the charophytes settling in this new oligotrophic sandy ecotope might have evolved into embryophytes.

Stebbins & Hill (1980) considered that archegoniates may have started with the unicellular charophytes, which inhabited aquatic interstices in moist soil. Graham & Gray (2001) suppose that charophyte ancestors of embryophyte most likely occurred in the ephemeral freshwater habitats characterized by unpredictable water availability. We leave the sophisticated question about the appearance of charophytic ancestor of embryophytes aside of the discussion.

Like sandy sea ecotope or psammal is considered as a peculiar 'workship' for the animal phyla (Schönborn 1987), the land psammal seems to be an ecotope, where the first embryophytes with their characteristic features appeared. Certainly, a series of traits inherited from the charophytic ancestor, such as a cellulosic cell wall, capacity to produce a mucous matrix extracellular and a large vacuole was the preadaptation for terrestrial life. Graham & Gray (2001) consider the desiccation tolerance of various diaspores and also vegetative cells as preadaptation to unpredictable water availability in terrestrial environment. Such adaptation to life on the land was modeled on the example of *Coleochaete*. Cultivated on quartz sand under conditions similar to aeroterrestrial habitat *Coleochaete* produced desiccant-tolerant cells that resemble some Paleozoic microfossils (Graham et al. 2012).

The multicellular complicated gametophytes of embryophytes might have evolved only if the apical cell was protected from desiccation. Such protection might be not only physiological (e.g. mucilage secretion), but also be caused by ultrastructural changes of the apical cell. The mutualistic associations with fungi (as source of phosphorus) and/or cyanobacteria (as source of nitrogen) had a great significance for the colonization of oligotrophic sandy ecotopes by the ancestor of embryophytes. Selosse & Le Tacon (1998) noted that a mutualistic association of phototrophs with fungi also allowed an evolutionary jump by creating new entities and new

abilities for different terrestrial constraints. For instance, the presence of a fungus may facilitate the emergence of thick parenchymatous tissue in plants.

The multicellular ancestor of embryophytes might have possessed a plagiotropic thallus. Rhizoids on the ventral side of the thallus should have became positively geotropic for fixing the thallus on the soil surface and for absorption of water with minerals. The appearance of a comparatively large gametophyte allowed this ancestor to become competitive with green algae. The appearance of the antheridium and the archegonium with the only egg cell and with the embryo developing within venter of the archegonium led to the appearance of the first embryophyte with characteristic alternation of generations. It is believed that the earliest embryophytes possessed bryophyte-like anatomy and physiology (Gray 1985; Wellman & Gray 2000).

Brown & Lemmon (2011) assumed that protoembryophytes had no sporophytes. They suppose that a delay in entry of the zygote into the meiotic pathway allowed intercalation of mitoses and had firstly served to increase the number of cells entering meiosis and later gave rise to embryo/sporophyte development. Unfortunately, the authors did not explain the construction of the female gametangium of protoembryophytes and the formation of archegonium in embryophytes. Wellman & Strother noticed too: "The question remains as to the vegetative nature of the green algae that produced these spores" (Wellman & Strother 2015: 617).

Obviously, the first embryophyte gave rise to all lineages of bryophytes comparatively quickly. We assume that the most ancient hornworts possessed weakly specialized thalloid gametophytes, unstable to drying out; they inhabited moist quartz sand with periodically dried upper horizon. In this ecotope, plant sprinters of the *Ph. laevis*-type would be most successful. Such *Phaeoceros*-like bryophytes can be classified as pioneer species (in the sense of Rabotnov 1995). These plants should have a very short life cycle which would allow them to produce numerous spores in an ephemeral sandy ecotope. Their spores, on the contrary, should be long-lived to remain viable after repeated washing, throwing and burying in the upper layer of sand. If the favorable conditions appear, these spores should readily germinate into the gametophyte. Spores with a silicified sporoderm certainly would be successful in such conditions. Silicified spores were less damaged when scattered by wind or water on long distance. If such spores fell in primitive soil of algae-lichens mat, soil invertebrates could not damage them and spores could again appear on the surface and form thalli occupying the gap.

Siliceous sporoderm in the hornworts evolution

Various algal groups (including the Charophyceae) produce sporopollenin (Graham & Wilcox 2000; Wellman 2004). Main functions of sporopollenin in algae are considered to protect reproductive structures against microbial and fungal attack and mechanical damage, and if these cells are exposed to the subaerial environment, as well as against desiccation and/or UV-B radiation. Wellman (2004) believes that production of sporopollenin in algae is almost certainly preadaptation to life on land. Brown & Lemmon (2011) assumed that zygote of protoembryophytes possessed sporopollenin wall. During evolution, deposition of sporopollenin in the wall of the zygote was delayed and sporopollenin began to arise in meiosis and mature spores became 'units of sexual reproduction'.

Various extant green algae produce siliceous walls, too (Graham & Wilcox 2000) and for example the outer wall layer of *Pediastrum boryanum* contains both silicon and sporopollenin (Millington 1981). We consider that if silicified wall arose in a charophyte ancestor of embryophytes, it would

be a preadaptation to aeroterrestrial life on land, too. Like the sporopollenin, this feature would be transferred from vegetative cells to meiospores which appeared in the life cycle of embryophytes. Undoubtfully, the siliceous sporoderm could give the advantages for the hornworts ancestor in survival and spreading, but was the sporoderm of this ancestor really silicified? Unfortunately, studying the mineralization of spores during fossilization with standard protocol and processing extraction of microfossils from rocks with HF (Traverse 2007) do not permit to find silica in the sporoderm of first hornworts.

The question arises: which character of the sporoderm (with or without siliceous layer) should be considered as plesiomorphic for hornworts? Biology and ecology of *A. agrestis* is very similar to that of *Ph. laevis*. Nevertheless, the sporoderm of the former species as well as the sporoderm of *A. caucasicus* lacks the siliceous layer.

Based on combined molecular and morphological analysis, Duff et al. (2007) introduced the taxonomic hierarchy of hornworts with two classes: monotypic Leiosporocerotopsida (with the only species *Leiosporoceros dussii*) and Anthocerotopsida including other hornwort genera. Anthocerotopsida is subdivided into three subclasses: Anthocerotidae, Notothylatidae and Dendrocerotidae. *Anthoceros* belongs to the former subclass whereas *Phaeoceros* and *Notothylas* with silicified sporoderm belong to Notothylatidae. Due to the absence of data on sporoderm silicification in *Leiosporoceros dussii* and all members of Dendrocerotidae, we are not able to discuss the evolutionary state of these feature.

However, the sporoderm of *Phaeomegaceros fimbricatus* (Gottsche) Duff et al. (Dendrocerotidae) possess an electron-dense, perine-like layer (Villarreal & Renzaglia 2006: Fig. 12D) similar to the silicified layer in *Ph. laevis* and *N.* cf. *frahmii*. Certainly, the electron-density of the outer layer may be caused by other reasons than silicification of the sporoderm and the EDX-analysis is necessary to test our hypothesis. Notably, Duff et al. (2007) segregated the genus *Phaeomegaceros* from *Phaeoceros* based on molecular and morphological features.

Hasegawa (1988) initially considered *Leiosporoceros dussii* and *Phaeoceros hirticalyx* (Steph.) Haseg. as vicarious species differentiated in the Neotropic and Paleotropic regions, respectively. He supposed that it is reasonable to consider these two species in genus *Phaeoceros* at a subgeneric rank. However, Duff et al. (2007) separated *Leiosporoceros* in the monotypic class. The 'outer exine' in spores of this species is not electron-dense (Brown et al. 2015: Pl. IX,2). At the same time, Brown et al. (2015) studied nearly mature tetrads, where the wall of the tetrad mother cell still remains. Obviously, the silicification of sporoderm takes place at the latest period of sporogenesis. We observed no Si in the outermost layer of *N.* cf. *frahmii* in the late tetrad stage with all layers having developed; the outermost layer was grey, but not black as in the mature spores. It is not improbable that *L. dussii* possesses silicified sporoderm, too, but only mature spores should be studied for this purpose.

Nevertheless, we assume that silicification of sporoderm will be discovered in other genera and species of hornworts. At this stage of the study, we suppose that the silicified sporoderm was a plesiomorphy of hornworts, but it is possible that loss and subsequent re-gain of siliceous sporoderm took place in evolution of certain hornwort clades. It will be appropriate to recall the words of Proskauer: "In the hornworts we are simply facing a situation in which certain species are extremely ancient, but these coexist with other which at the other extreme may be as recent as any species in the flowering plants" (Proskauer 1969: 58).

Acknowledgements

We thank the staff of the laboratory of electron microscopy at the Biological Faculty of Lomonosov Moscow State University and R.A. Rakitov from the Paleontological Institute of the Russian Academy of Sciences for their assistance and for providing SEM and TEM facilities. We also thank V.N. Figurovskaya and S.V. Polevova for their help in the work. We are grateful to D.D. Sokoloff for critical reading and advices on the manuscript and for providing the plant material; to V.M. Ivanov for invaluable comments; to E.E. Demidova for correction of the manuscript and to I. Bisang, H.J. During and J.C. Villareal for supplying their publications. This research was performed in the framework of a government order to Lomonosov Moscow State University (AAAA-A16-116021660045-2).

References

Antonov A. S. (2006): Plant genotypes systematics [Genosistematika rastenij]. – Moscow: IKC Akademkniga. [In Russian]

Arts T. (1984): *Phaeoceros carolinianus* (Michx.) Prosk. (Hepaticae), nieuw voor België. – Dumortiera **29–30**: 13–15.

Asthana A. K. & Nath V. (1994): Distributional pattern of *Phaeoceros* Prosk. in Kumaon and Garhwal region: Western Himalayas. – Cryptog. Bryol. Lichénol. **15**: 129–134.

Asthana A. K. & Srivastava S. C. (1991): Indian hornworts. (A taxonomic study). – Bryophyt. Biblioth. **42**: 1–158.

Bakalin V. A., Chernyagina O. A. & Kirichenko V. E. (2007): Anthocerotophyta – a new divisio of plants for the flora of Kamchatka (North-West Pacific). – Arctoa **16**: 153–156.

Barber D. A. & Shone M. G. T. (1966): The absorption of silica from aqueous solutions by plants. – J. Exper. Bot. **17**: 569–578.

Bartlett E. M. (1928): A comparative study of the development of the sporophyte in the Anthocerotaceae, with especial reference to the genus Anthoceros. – Ann. Bot. **42**: 409–430.

Bauer P., Elbaum R. & Weiss I. M. (2011): Calcium and silicon mineralization in land plants: transport, structure and function. – Pl. Sci. **180**: 746–756.

Becker B. (2013): Snow ball earth and the split of Streptophyta and Chlorophyta. – Trends Pl. Sci. **18**: 180–183.

Becker B. & Marin B. (2009): Streptophyte algae and the origin of embryophytes. – Ann. Bot. **103**: 999–1004.

Bisang I. (1992): Hornworts in Switzerland – endangered? – Biol. Cons. **59**: 145–149.

Bisang I. (1995): On the phenology of *Anthoceros agrestis* (Anthocerotae, Anthocerotaceae), with special reference to Central Europe. – Fragm. Florist. Geobot. **40**: 513–518.

Bisang I. (1999): Welche Faktoren bestimmen das Vorkommen von Hornmoosen (Anthocerotales) in intensiv genutzten Agrarökosystemen des Schweizer Mittellandes? – Stuttgarter Beitr. Naturk., A **594**: 1–10.

Bisang I. (2001): An estimate of mature spore production in *Anthoceros agrestis* in Switzerland. – J. Bryol. **23**: 142–145.

Bisang I. (2003): Population development, demographic structure, and life cycle aspects of two hornworts in Switzerland. – Lindbergia **28**: 105–112.

Blank C. E. (2013): Origin and early evolution of photosynthetic eukaryotes in freshwater environments: reinterpreting Proterozoic paleobiology and biogeochemical processes in light of trait evolution. – J. Phycol. **49**: 1040–1055.

Blank C.E. & Sánchez-Baracaldo P. (2010): Timing of morphological and ecological innovations in the cyanobacteria – a key to understanding the rise in atmospheric oxygen. – Geobiology **8**: 1–23.

Breil D.A. (1970): Liverworts on the Mid-Gulf Coastal Plain. – Bryologist **73**: 409–491.

Bremer K. (1985): Summary of green plants phylogeny and classification. – Cladistics **1**: 369–385.

Brown R.C. & Lemmon B.E. (1988): Sporogenesis in bryophytes. – Advances Bryol. **3**: 159–223.

Brown R.C. & Lemmon B.E. (2011): Spores before sporophytes: hypothesizing the origin of sporogenesis at the algal-plant transition. – New Phytol. **190**: 875–881.

Brown R.C., Lemmon B.E., Shimamura M., Villarreal J.C. & Renzaglia K.S. (2015): Spores of relictual bryophytes: diverse adaptations to life on land. – Rev. Palaeobot. Palynol. **216**: 1–17.

Cailliau A. & Price M.J. (2007): Hornworts in the agricultural fields of Geneva: new findings, the soil diaspore bank and ex situ soil cultures. – Candollea **62**: 165–172.

Campbell E.O. (1981): Notes on some Anthocerotae of New Zealand. – Tuatara **25**: 7–13.

Cargill D.C. & Fuhrer B.A. (2008): Chapter twenty: taxonomic studies of the Australian Anthocerotophyta II: the genus *Phaeoceros*. – Fieldiana Bot. N.S. **47**: 239–253.

Chantanaorrapint S. (2014): *Notothylas irregularis* (Notothyladaceae, Anthocerotophyta), a new species of hornwort from northern Thailand. – Acta Bot. Hung. **56**: 269–274.

Chantanaorrapint S. (2015): Taxonomic studies on Thai Anthocerotophyta II. The genus *Notothylas* (Notothyladaceae). – Cryptog. Bryol. Lichénol. **36**: 251–266.

Chopra R.N. & Kumra P.K. (1988): Biology of bryophytes. – New Delhi: Wiley Eastern Limited.

Coradin T., Desclés J., Luo G.-Z. & Lopez P.J. (2006): Silicon in the photosynthetic lineages: molecular mechanisms for uptake and deposition. – In: Teixeira da Silva J.A. [ed.]: Floriculture, ornamental and plant biotechnology: advances and topical issues. Vol. 4: 238–244. – London: Global Science Books.

Cox C.J., Li B., Foster P.G., Embley T.M. & Civáň P. (2014): Conflicting phylogenies for early land plants are caused by composition biases among synonymous substitutions. – Syst. Biol. **63**: 272–279.

Crang R.E. & May G. (1974): Evidence for silicon as a prevalent elemental component in pollen wall structure. – Can. J. Bot. **52**: 2171–2174.

Currie H.A. & Perry C.C. (2009): Chemical evidence for intrinsic 'Si'within *Equisetum* cell walls. – Phytochemistry **70**: 2089–2095.

Delwiche C.F. & Cooper E.D. (2015): The evolutionary origin of a terrestrial flora. – Current Biol. **25**: R899–R910.

Duff R.J., Villarreal J.C., Cargill D.C., & Renzaglia K.S. (2007): Progress and challenges toward developing a phylogeny and classification of the hornworts. – Bryologist **110**: 214–243.

Düll R. (1983): Distribution of the European and Macaronesian liverworts (Hepaticophytina). – Bryologische Beiträge **2**: 1–114.

During H.J. (1979): Life strategies of bryophytes: a preliminary review. – Lindbergia **5**: 2–18.

During H.J. (1992): Ecological classifications of bryophytes and lichens. – In: Bates J.W. & Farmer A.M. [eds]: Bryophytes and lichens in a changing environment: 1–31. – Oxford: Clarendon Press.

Duthie A.V. (1929): The method of spore dispersal of three South African species of *Isoëtes*. – Ann. Bot. **43**: 411–412.

Edman G. (1932): Verkieselung und Verholzung der Sporenmembran bei *Lygodium japonicum* Sw. – Svensk Bot. Tidskr. **26**: 313–326.

Edwards D. (1994): The bryophyte diaspore bank in the soil of an upland deciduous forest. – Amer. J. Bot. **81** (Suppl.): 7.

Epstein E. (1999): Silicon. – Ann. Rev. Plant Physiol. Plant Mol. Biol. **50**: 641–664.

Epstein E. (2001): Silicon in plants: facts vs. concepts. – Studies in Plant Science **8**: 1–15.

Epstein E. & Bloom A. J. (2005): Mineral nutrition of plants: principles and perspectives. [2nd ed.] – Sunderland, MA: Sinauer Associates.

Evans R. D. & Johansen J. R. (1999): Microbiotic crusts and ecosystem processes. – Critical Rev. Pl. Sci. **18**: 183–225.

Filin V. R. (2009): Divisio Anthocerotophyta. – In: Timonin A. K. [ed.]: Botanika. Tom 4, Kniga 1. Sistematika vysshih rastenij: 95–103. – Moscow: Akademia. [In Russian]

Gambaryan S. K. (1992): Anthocerotae and Hepaticae of the Southern Primorye [Antocerotovye i pechenochniki Yuzhnogo Primor'ya]. – Vladivostok: Dal'nauka DO RAN. [In Russian]

Gehrig H., Schüssler A. & Kluge M. (1996): *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (Cyanobacteria), is an ancestral member of the Glomales: Evidence by SSU rRNA analysis. – J. Mol. Evol. **43**: 71–81.

Goffinet B. (2000): Origin and phylogenetic relationships of bryophytes. – In: Shaw A. J. & Goffinet B. [eds]: Bryophyte biology: 124–149. – New York: Cambridge University Press.

Graham L. E., Arancibia-Avila P., Taylor W. A., Strother P. K. & Cook M. E. (2012): Aeroterrestrial Coleochaete (Streptophyta, Coleochaetales) models early plant adaptation to land. – Amer. J. Bot. **99**: 130–144.

Graham L. E. & Gray J. (2001): The origin, morphology, and ecophysiology of early embryophytes: neontological and paleontological perspectives. – In: Gensel P. G. & Edwards D. [eds]: Plants invade the land: evolutionary and environmental perspectives: 140–158. – New York: Columbia Univ. Press.

Graham L. E. & Wilcox L. W. (2000): Algae. – London: Prentice Hall.

Gray J. (1985): The microfossil record of early land plants: advances in understanding of early terrestrialization, 1970–1984. – Philos. Trans. Roy. Soc. London, Ser. B **309**: 167–195.

Grime J. P. (1979): Plant strategies and vegetation processes. – Chichester, New York: John Wiley & Sons.

Grime J. P. (2001): Plant strategies, vegetation processes, and ecosystem properties. [2nd ed.] – Chichester: John Wiley & Sons.

Grime J. P. & Pierce S. (2012): The evolutionary strategies that shape ecosystems. – UK: John Wiley & Sons.

Grolle R. (1976): Verzeichnis der Lebermoose Europas und benachbarter Gebiete. – Feddes Repert. **87**: 171–279.

Grolle R. (1983): Hepatics of Europe including the Azores: an annotated list of species, with synonyms from the recent literature. – J. Bryol. **12**: 403–459.

Harholt J., Moestrup Ø. & Ulvskov P. (2016): Why plants were terrestrial from the beginning? – Trends Pl. Sci. **21**: 96–101.

Hasegawa J. (1979): Taxonomical studies on Asian Anthocerotae I. – Acta Phytotax. Geobot. **30**: 16–30.

Hasegawa J. (1984): Distribution of Japanese species of Anthocerotae. – J. Hattori Bot. Lab. **56**: 21–28.

Hasegawa J. (1988): A proposal for new system of the Anthocerotae, with a revision of the genera. – J. Hattori Bot. Lab. **64**: 87–95.

Hässel de Menéndez G. G. (1989): Las espécies de *Phaeoceros* (Anthocerothophyta) de América del Norte, Sud y Central; la ornamentación de sus esporos y taxonomia. – Candollea **44**: 715–739.

Heckman D. S., Geiser D. M., Eidell B. R., Stauffer R. L., Kardos N. L. & Hedges S. B. (2001): Molecular evidence for the early colonization of land by fungi and plants. – Science **293**: 1129–1133.

Herguido P. & Ron E. (1989): Sobre la germinación de la espora en *Phaeoceros bulbiculosus* (Brothero) Prosk. – Cryptog. Bryol. Lichénol. **10**: 353–359.

Iler K.R. (1979): The chemistry of silica. Solubility, polymerization, colloid and surface properties and biochemistry of silica. – New York: John Wiley & Sons.

Jeffrey C. (1967): The origin and differentiation of the archegoniate land plants: a second contribution. – Kew Bull. **21**: 335–349.

Kenrick P. & Crane P.R. (1997): The origin and early diversification of land plants. A cladistic study. – Washington & London: Smithsonian Inst. Press.

Konstantinova N.A., Potemkin A.D. & Schljakov R.N. (1992): Check-list of the Hepaticae and Anthocerotae of the former USSR [Spisok pechenochnikov i antocerotovyh territorii byvshego SSSR]. – Arctoa **1**: 87–127. [In Russian]

Lang W.H. (1907): On the sporogonium of *Notothylas*. – Ann. Bot. **21**: 201–210.

Laurin-Lemay S., Brinkman H. & Philippe H. (2012): Origin of land plants revisited in the light of sequence contamination and missing data. – Current Biol. **22**: R593–R594.

Lewin J. & Reimann B.E.F. (1969): Silicon and plant growth. – Ann. Rev. Pl. Physiol. **20**: 289–304.

Lewis L.A. & McCourt R.M. (2004): Green algae and the origin of land plants. – Amer. J. Bot. **91**: 1535–1556.

Liang Y., Sun W., Zhu Y.-G. & Christie P. (2007): Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. – Environm. Pollut. **147**: 422–428.

Ligrone R. (1988): Ultrastructure of a fungal endophyte in *Phaeoceros laevis* (L.) Prosk. (Anthocerotophyta). – Bot. Gaz. **149**: 92–100.

Ligrone R. & Lopes C. (1989): Ultrastructure, development and cytochemistry of storage cells in the 'tubers' of *Phaeoceros laevis* Prosk. (Anthocerotophyta). – New Phytol. **112**: 317–325.

Longton R.E. & Schuster R.M. (1983): Reproductive biology. – In: Schuster R.M. [ed.]: New manual of bryology 1: 386–462. – Nichinan, Japan: Hattori Bot. Laboratory.

Millington W.F. (1981): Form and pattern in *Pediastrum*. – In: Kiermayer O. [ed.]: Cytomorphogenesis in plants: 99–118. – Vienna, New York: Springer-Verlag.

Millington W.F. & Gawlik S.R. (1967): Silica in the wall of *Pediastrum*. – Nature **216**: 68.

Millington W.F. & Gawlik S.R. (1970): Ultrastructure and initiation of wall pattern in *Pediastrum boryanum*. – Amer. J. Bot. **57**: 552–561.

Moore S.E.M. (2005): Studies of lower embryophyte spore walls with particular reference to the perispore. – Ph.D. Thesis: Cardiff University.

Moore S.E.M., Hemsley A.R. & Borsch T. (2006): Micromorphology of outer exospore coatings in *Selaginella* megaspores. – Grana **45**: 9–21.

Müller K. (1951): Die Lebermoose Europas (Musci hepatici). – In: L. Rabenhorst's Kryptogamen-Flora. 3 Aufl., B. 6, Lief. 2: 161–319. – Leipzig: Akademische Verlagsgesellschaft.

Nishiyama T., Wolf P.G., Kugita M., Sinclair R.B., Sugita M., Sugiura C., Wakasugi T., Yamada K., Yoshinaga K., Yamaguchi K., Ueda K. & Hasebe M. (2004): Chloroplast phylogeny indicates that bryophytes are monophyletic. – Mol. Biol. Evol. **21**: 1813–1819.

Oliveira B.A., Pereira A.F. de N., Pôrto K.C. & Maciel-Silva A.S. (2017): Spore germination and young gametophyte development of the endemic Brazilian hornwort *Notothylas vitalii* Udar & Singh (Notothyladaceae-Anthocerotophyta), with insights into sporeling evolution. – Acta Bot. Brasil. **31**: 313–318.

Pandé S.K. (1934): On the morphology of *Notothylas levieri* Schiff. Ms. – Proc. Indian Acad. Sci. B **1**: 205–217.

Parker B.C. (1969): Occurrence of silica in brown and green algae. – Canad. J. Bot. **47**: 537–540.

Parkinson B.M. (1995): Spore wall development in *Schizaea pectinata* (Schizaeaceae: pteridophyta). – Grana **34**: 217–228.

Paton J.A. (1973): *Phaeoceros laevis* (L.) Prosk. subsp. *carolinianus* (Michaux) Prosk. in Britain. – J. Bryol. **7**: 541–543.

Paton J.A. (1979): *Anthoceros agrestis*, a new name for *A. punctatus* var. *cavernosus* sensu Prosk. 1958, non (Nees) Gottsche et al. – J. Bryol. **10**: 257–261.

Peng T. & Zhu R.-L. (2014): A revision of the genus *Notothylas* (Notothyladaceae, Anthocerotophyta) in China. – Phytotaxa **156**: 156–164.

Penjor P., Chantanaorrapint S. & Meesawat U. (2016): Morphological and anatomical features of cosmopolitan hornwort: *Phaeoceros carolinianus* (Michx.) Prosk. – Walailak J. Sci. Technol. (WJST) **13**: 769–779.

Perry C.C. (2003): Silicification: the processes by which organisms capture and mineralize silica. – Rev. Mineral. Geochem. **54**: 291–327.

Pianka E.R. (1981): Evolutionary ecology. [2nd ed.] – New York: Harper & Row.

Piippo S. (1993): Bryophyte flora of the Huon Peninsula, Papua New Guinea. LIV. Anthocerotophyta. – Acta Bot. Fenn. **148**: 27–51.

Pirozynski K.A. & Malloch D.W. (1975): The origin of land plants: a matter of mycotrophism. – BioSystems **6**: 153–164.

Pocock K. & Duckett J.G. (1984): A comparative ultrastructural analysis of the fungal endophytes in *Cryptothallus mirabilis* Malm. and other British thalloid hepatics. – J. Bryol. **13**: 227–233.

Potemkin A.D. & Sofronova E.V. (2009): Liverworts and hornworts of Russia. Vol.1 [Pechenochniki i antocerotovye Rossii. T. 1]. – St. Petersburg, Yakutsk: Boston Spektr. [In Russian]

Proskauer J. (1948a): Studies on the morphology of *Anthoceros*. I. – Ann. Bot. **12**: 237–265.

Proskauer J. (1948b): Studies on the morphology of *Anthoceros*. II. – Ann. Bot. **12**: 427–439.

Proskauer J. (1951): Studies on Anthocerotales. III. – Bull. Torrey Bot. Club **78**: 331–349.

Proskauer J. (1954): The European Anthocerotaceae. – In: VIII Congrès International de botanique. Paris. Rapports et communications. Sect. 16: 68–69.

Proskauer J. (1957): Studies on Anthocerotales V. – Phytomorphology **7**: 113–135.

Proskauer J. (1958): Nachtrag zur Familie Anthocerotaceae. – In: Müller K. [ed.]: Die Lebermoose Europas; Rabenhorst's Kryptogamen-Flora [3rd ed.]: 1303–1319. – Vienna, New York: Springer.

Proskauer J. (1969): Studies on Anthocerotales VIII. – Phytomorphology **19**: 52–66.

Qiu Y.-L. (2008): Phylogeny and evolution of charophytic algae and land plants. – J. Syst. Evol. **46**: 287–306.

Rabotnov T.A. (1995): Phytozönologie. Struktur und Dynamik natürlicher Ökosysteme. – Stuttgart: Eugen Ulmer.

Ramensky L.G. (1938): Introduction to complex soil-geobotanical investigation of lands [Vvedenie v kompleksnoe pochvenno-geobotanicheskoe issledovanie zemel']. – Moscow: Selhozgiz. [In Russian].

Raven J.A. (1983): The transport and function of silicon in plants. – Biol. Rev. **58**: 179–207.

Raven J.A. (2000): Land plant biochemistry. – Phil. Trans. Roy. Soc. London, Ser. B **355**: 833–846.

Renzaglia K.S., Duff R.J., Nickrent D.L. & Garbary D.J. (2000): Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. – Phil. Trans. Roy. Soc. London, Ser. B **355**: 769–793.

Renzaglia K.S., Villarreal J.C. & Duff R.J. (2009): New insights into morphology, anatomy, and systematics of hornworts. – In: Goffinet B. & Shaw A.J. [eds]: Bryophyte biology. [2nd ed.]: 139–171. – Cambridge: Cambridge University Press.

Richmond K.E. & Sussman M. (2003): Got silicon? The non-essential beneficial plant nutrient. – Curr. Opinion Pl. Biol. **6**: 268–272.

Risse S. (1987): *Phaeoceros carolinianus* (Michx.) Prosk. in Nordrhein-Westfalen. – Natur & Heimat **47**: 121–129.

Rozanov A. Yu. (2009): Life conditions on the Early Earth after 4.0 Ga [Usloviya zhizni na rannej Zemle posle 4 mlrd. let nazad]. – In: Problemy proiskhozhdeniya zhizni: 185–201. – Moscow: PIN RAN. [In Russian].

Sangster A. G., Hodson M. J. & Tubb H. J. (2001): Silicon deposition in higher plants. – In: Datnoff L. E., Snyder G. H. & Korndörfer G. H. [eds]: Silicon in agriculture: 85–113. – Amsterdam: Elsevier.

Schlyakov R. N. (1975): Hepaticae. Morphology, phylogeny, classification [Pechenochnye mhi. Morfologiya, filogeniya, klassifikaciya]. – Leningrad: Nauka. [In Russian].

Schofield W. B. (1985): Introduction to bryology. – New York, London: Macmillan.

Schönborn W. (1987): Theorie des Urbiotopes der Tierwelt [O pervichnyh biotopah zhivotnogo mira]. – Zhurn. Obshch. Biol. **48**: 27–40. [In Russian with German abstract]

Schumacker R. & Váňa J. (2005): Identification keys to the liverworts and hornworts of Europe and Macaronesia (Distribution and Status).[2nd rev. ed.] – Poznań: Sorus.

Schuster R. M. (1984): Morphology, phylogeny and classification of the Anthocerotae. – In: Schuster R.M. [ed.]: New manual of bryology. Vol. 2: 1071–1009. – Nichinan, Japan: Hattori Bot. Laboratory.

Schuster R. M. (1992): The Hepaticae and Anthocerotae of North America east of the hundredth meridian. Vol. VI. – Chicago: Colombia Univ. Press.

Schwarz K. (1973): A bound form of silicon in glycosaminoglycans and polyuronides (polysaccharide matrix/connective tissue). – Proc. Natl. Acad. Sci. **70**: 1608–1612.

Selosse M.-A. & Le Tacon F. (1998): The land flora: a phototroph-fungus partnership? – Trends Ecol. Evol. **13**: 15–20.

Shaw J. & Renzaglia K. (2004): Phylogeny and diversification of bryophytes. – Amer. J. Bot. **91**: 1557–1581.

Singh D. K. (1988): A new species of *Notothylas* Sull. (Bryophyta) from Nepal. – J. Bombay Nat. Hist. Soc. **84**: 649–653.

Smith A. J. E. (1990): The liverworts of Britain and Ireland. – Cambridge: Cambridge University Press.

Söderström L., Urmi E. & Váňa J. (2002): Distribution of Hepaticae and Anthocerotae in Europe and Macaronesia. – Lindbergia **27**: 3–47.

Stebbins G. L. & Hill G. J. C. (1980): Did multicellular plants invade the land? – Amer. Naturalist **115**: 342–353.

Strother P. K., Battison L., Brasier M. D. & Wellman C. H. (2011): Earth's earliest non-marine eukaryotes. – Nature **473**: 505–509.

Sydow H. & Sydow P. (1903): Die Microsporen von *Anthoceros dichotomus* Raddi, *Tilletia abscondita* Syd. nov. spec. – Ann. Mycol. **1**: 174–176.

Szövényi P., Frangedakis E., Ricca M., Quandt D., Wicke S. & Langdale J. A. (2015): Establishment of *Anthoceros agrestis* as a model species for studying the biology of hornworts. – BMC Plant Biol. **15**: 98.

Taylor J., Le Vee G. J., Hollingsworth P. J. & Bigelow W. C. (1976): Sporopollenin and major mineral constituents of the spores, elaters, and capsule wall of *Conocephalum conicum*. – Michigan Botanist **15**: 167–172.

Tchistiakoff J. (1873): Notice préliminaire sur l'histoire du développement des sporanges et des spores de *l'Isoetes durieui* Bory. – Nuovo Giorn. Bot. Ital. **5**: 207–212.

Timme R. E., Bachvaroff T. R. & Delwiche C. F. (2012): Broad phylogenomic sampling and the sister lineage of land plants. – PLoS One **7**: e29696.

Traverse A. (2007): Paleopalynology. [2nd ed.] – In: Landman N. H. & Jones D. S. [eds]: Topics in geobiology. Vol. 28: 1–813. – New York: Springer.

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

Udar R. & Singh D. K. (1978): Thickened bands in the capsule wall of *Notothylas levieri*. – Bryologist **81**: 575–577.

Udar R. & Singh D. K. (1979a): *Notothylas dissecta*, a hornwort new to India. – Bryologist **82**: 625–628.

Udar R. & Singh D. K. (1979b): On a new species of *Notothylas* Sull. from Western Ghats, India. 1. – Rev. Bryol. Lichenol. **45**: 201–208.

Udar R. & Singh D. K. (1981a): A new species of the genus *Notothylas* Sull., *N. himalayensis* Udar et Singh, from India. – J. Bryol. **11**: 451–457.

Udar R. & Singh D. K. (1981b): *Notothylas khasiana* Udar et Singh sp.nov. from Shillong, India. – J. Indian Bot. Soc. **60**: 112–117.

Villarreal J. C., Cargill D. C., Harborg A., Söderström L. & Renzaglia K. S. (2010): A synthesis of hornwort diversity: Patterns, causes and future work. – Phytotaxa **9**: 150–166.

Villarreal J. C., Cusimano N. & Renner S. S. (2015): Biogeography and diversification rates in hornworts: The limitations of diversification modeling. – Taxon **64**: 229–238.

Villarreal J. C. & Renner S. S. (2013): Correlates of monoicy and dioicy in hornworts, the apparent sister group to vascular plants. – BMC Evol. Biol. **13**: 239.

Villarreal J. C. & Renzaglia K. S. (2006): Sporophyte structure in the neotropical hornwort *Phaeomegaceros fimbriatus*: implications for phylogeny, taxonomy, and character evolution. – Int. J. Pl. Sci. **167**: 413–427.

Villarreal J. C. & Renzaglia K. S. (2015): The hornworts: important advancements in early land plant evolution. – J. Bryol. **37**: 157–170.

Voronkov M. G. & Kuznetsov I. G. (1984): Silicon in living nature [Kremnij v zhivoj prirode]. – Novosibirsk: Nauka. [In Russian]

Voroshylov V. N. (1960): The rhythm of development of plants [Ritm razvitija u rastenij]. – Moscow: Izd-vo AN SSSR. [In Russian]

Vries de J., Santon A., Archibald J. M. & Gould S. B. (2016): Streptophyte terrestrialization in light of plastid evolution. – Trends Pl. Sci. **21**: 467–476.

Wang B., Yeun L. H., Xue J.-Y., Liu Y., Ané J.-M. & Qiu Y.-L. (2010): Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. – New Phytol. **186**: 514–525.

Warny S., Jarzen D. M., Evans A., Hesp P. & Bart P. (2012): Environmental significance of abundant and diverse hornwort spores in a potential submerged Paleoindian site in the Gulf of Mexico. – Palynology **36**: 234–253.

Wellman C. H. (2004): Origin, function and development of the spore wall in early land plants. – In: Hemsley A. R. & Poole I. [eds]: The evolution of plant physiology. Vol 21: 43–63. – Oxford: Elsevier.

Wellman C. H. & Gray J. (2000): The microfossil record of early land plants. – Phil. Trans. Roy. Soc. London, Ser. B **355**: 717–732.

Werner D. & Roth R. (1983): Silica metabolism. – In: Läuchli A. & Bieleski R. L. [eds]: Inorganic plant nutrition. Vol. 2: 682–694. – Berlin: Springer.

Wellman C. H. & Strother P. K. (2015): The terrestrial biota prior to the origin of land plants (embryophytes): a review of the evidence. – Palaeontology **58**: 601–627.

Wickett N. J., Mirarab S., Nguyen N., Warnow T., Carpenter E., Matasci N., Ayyampalayam S., Barker M. S., Burleigh J. G., Gitzendanner M. A., Ruhfel B. R., Wafula E., Der

J.P., Graham S.W., Mathews S., Melkonian M., Soltis D.E., Soltis P.S., Miles N.W., Rothfels C.J., Pokorny L., Shaw A.J., DeGironimo L., Stevenson D.W., Surek B., Villarreal J.C., Roure B., Philippe H., dePamphilis C.W., Chen T., Deyholos M.K., Baucom R.S., Kutchan T.M., Augustin M.M., Wang J., Zhang Y., Tian Z., Yan Z., Wu X., Sun X., Wong G.K.-S. & Leebens-Mack J. (2014): Phylotranscriptomic analysis of the origin and early diversification of land plants. – Proc. Nat. Acad. Sci. **111**: E4859–E4868.

Wodniok S., Brinkmann H., Glöckner G., Heidel A.J., Philippe H., Melkonian M. & Becker B. (2011): Origin of land plants: do conjugating green algae hold the key? – BMC Evol. Biol. **11**: 104.

Yurtsev B.A. (1986): Production strategies and plant life-forms [Produkcionnye strategii i zhiznennye formy rastenij]. – In: Serebryakova T.I. & Sokolova T.G. [eds]: Zhiznennye formy v ehkologii i sistematike rastenij: Mezhvuzovskij sbornik nauchnyh trudov: 9–23. – Moscow: Nauka. [In Russian]

Zasukhin D.N. (1930): Materialien zur Frage über die Organismen des Flugsandes in den Kirgisensteppen. – Hidrobiol. Zhurn. S.S.S.R. **9**: 121–130. [In Russian with German abstract]

Zhong B., Xi Z., Goremykin V.V., Fong R., Mclenachan P.A., Novis P.M., Davis C.C. & Penny D. (2013): Streptophyte algae and the origin of land plants revisited using heterogeneous models with three new algal chloroplast genomes. – Mol. Biol. Evol. **31**: 177–183.

Address of the authors:

Vladimir R. Filin
Anna G. Platonova (corresponding author)
Department of Higher Plants
Faculty of Biology
Moscow State University
Leninskie Gory 1 (12)
119234, Moscow, Russia
E-mail: platon-anna@yandex.ru