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Lamprospora tuberculata, *Octospora ithacaensis*, *O. orthotrichi* and *O. affinis* – four bryoparasitic ascomycetes new to the Czech Republic

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Four bryoparasitic ascomycetes, *Lamprospora tuberculata*, *Octospora ithacaensis*, *O. orthotrichi* and *O. affinis* were recently found in the Czech Republic for the first time. For each species, basic description of macroscopical and microscopical characters, information about its habitat, distinguishing features of similar species as well as notes on the distribution in Europe are provided. Furthermore, apothecia, spores and parasitising structures are illustrated. The following parasite-host relationships were observed: *L. tuberculata* on *Pleuridium subulatum*, *O. ithacaensis* on *Marchantia polymorpha*, *O. orthotrichi* on *Orthotrichum diaphanum* and *O. affinis* on *Orthotrichum affine*. One locality of *L. tuberculata*, two of *O. ithacaensis*, eight of *O. orthotrichi* and one of *O. affinis* were found. Internal cavities in the warts of spores in *O. affinis* and *L. tuberculata* are newly reported as well as the infection of leaf cells in *O. affinis*.

Key words: bryoparasitic *Pezizales*, *Ascomycota*, *Pyronemataceae*, rhizoid galls, central Europe.

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Čtyři druhy bryoparazitických vřeckovýtrusých hub, *Lamprospora tuberculata*, *Octospora ithacaensis*, *O. orthotrichi* a *O. affinis*, byly poprvé nalezeny v České republice. U každého druhu jsou popsány makroskopické a mikroskopické znaky a uvedeny údaje o stanovišti, odlišnosti oproti podobným druhům a poznámky k rozšíření v Evropě. Jsou připojeny fotografie apothecií, výtrusů a infekčních struktur. Jako hostitelské mechorosty byly rozpoznány *Pleuridium subulatum* u *L. tuberculata*, *Marchantia polymorpha* u *O. ithacaensis*, *Orthotrichum diaphanum* u *O. orthotrichi* a *Orthotrichum affine* u *O. affinis*. *L. tuberculata* byla nalezena na jedné lokalitě, *O. ithacaensis* na dvou, *O. orthotrichi* na osmi a *O. affinis* na jedné. Nově jsou popsány kavity uvnitř bradavek výtrusů *O. affinis* a *L. tuberculata* a infekce buněk fyloidů v případě *O. affinis*.

INTRODUCTION

Lamprospora and *Octospora* are genera of operculate ascomycetes, which, together with *Neottiella*, *Filicupula* and *Octosporella*, form a closely related group of bryoparasitic *Pezizales*. They usually have tiny, disc-shaped apothecia coloured in shades of orange to red. Because of their small size of generally a few millimetres, they can easily be overlooked and our knowledge of their occurrence is therefore far from complete.

Research on bryoparasitic *Pezizales* has a long tradition in the Czech Republic. The first mention from the territory of the current Czech Republic can be found in Corda (1838, 1842) where *Neottiella ricciae* (P. Crouan & H. Crouan) Korf & W.Y. Zhuang (as *Peziza leucoloma* Reb. ß. *ricciaecola*) and *Octospora humosa* (Fr.) Dennis (as *Peziza humosa* Fr.) were reported. In the first half of the 20th century, Klika (1926a, 1926b) and Velenovský (1922, 1934) published records of bryoparasitic *Pezizales* from Czechia. The latter especially showed a good nose for this group of fungi and even described new species, although many of his type specimens were later revised by Svrček (1976). Three of Velenovský's species, *Lamprospora arvensis* (Velen.) Svrček, *L. minuta* (Velen.) Svrček and *Octospora rustica* (Velen.) J. Moravec, are still accepted taxa.

In the second half of the 20th century, research on bryoparasitic *Pezizales* was continued mainly by Svrček (1948, 1974, 1976), Svrček & Kubička (1961, 1963) and Moravec (1968, 1969, 1997). In this period, *Octospora gyalectoides* Svrček & Kubička and *O. pseudoampezzana* (Svrček) Caillet & Moyne were newly described based on Czech collections by Svrček & Kubička (1963) and Svrček (1948), respectively.

It is difficult to say how many bryoparasitic *Pezizales* are currently known from the Czech Republic. The names and taxonomy of many species have changed and, moreover, some published records could only be validated by studying the specimens, but this is not within the scope of this paper. Nevertheless, we have tried to establish the number of bryoparasitic *Pezizales* currently known from the Czech Republic by analysing the references above as well as Svrček (1981a, 1981b) and Benkert (1997, 1998c, 2007). Based on this investigation and by applying the nomenclature and the taxonomic concepts of Benkert (1987, 2009), we found 38 taxa of the genera *Lamprospora*, *Octospora* and *Neottiella* reported from the Czech Republic.

In the following account, recent collections of four species formerly not known from the Czech Republic are described and illustrated.

Material and methods

Fungi were collected in the Czech Republic between January 2014 and January 2015. The description of macro- and microscopical characters is based on fresh material. Fragments of fruitbodies were examined in water, Lugol's solution and cotton blue in lactophenol at a magnification of up to 1000× with an Olympus CX21 microscope. At least 30 values of spore size and 10 values of other structures were measured in water. To study hyphae and infections, small parts of soil and bryophytes from the vicinity of an apothecium were removed and carefully cleaned with water. Subsequently the remaining material was stained with cotton blue in lactophenol.

The nomenclature of bryoparasitic *Pezizales* follows Benkert (2009) with the exception of one case indicated in the text. Geographical coordinates and altitudes of locations were determined using a tourist map. The coordinates are given in the WGS 84 coordinate system. The MTB alphanumeric code in 'material examined' refers to the grid square of the Central European grid mapping system. Vouchers are deposited in the PRM herbarium and the private herbarium of Z. Egertová.

All relevant literature, especially Holec et al. (2013) and Velenovský (1934), was checked for earlier records of the four presented species in the Czech Republic. Additionally, the curators of the PRM, BRNM, BRNU, CB and HR herbaria (acronyms according to Thiers on-line) and Jiří Moravec were asked for potential earlier records.

Results and discussion

Lamprospora tuberculata Seaver, Mycologia 4(2): 47, 1912

Synonyms: *Octospora tuberculata* (Seaver) Caillet & Moyne, Bulletin trimestriel de la Société Mycologique de France 96(2): 185, 1980;
= *Lamprospora modestissima* Grélet, Bulletin trimestriel de la Société Mycologique de France 42(3-4): 204, 1926.

Description. Apothecia 0.3–1 mm broad, sessile, rounded, orange, margin indistinct (Fig. 1). Asci 225–315 × 17–22 µm, cylindrical, operculate, non-amyloid, containing 8 uniseriately arranged spores. Spores 14–16 µm in diameter, globose, hyaline, with one guttule (8–9 µm), ornamented with obtuse warts of different sizes (1–4 µm broad, 1.3–3 µm high), warts with small internal cavities (Fig. 7). Paraphyses straight, septate, 3.5–4.5 µm broad, with a broadened, obtuse apex (5.5–7 µm), containing orange pigment. Infections were found on strong rhizoids. The infection consists of spherical structures, probably galls, 30–150 µm wide,

attached to the rhizoid. Both, the 'galls' and the rhizoid are enveloped by a complete cover of interwoven hyphae (Fig. 2). Because of the dense hyphae cover no internal structures of the 'galls' could be observed.

Notes. *Lamprospora tuberculata* is characterised by spores ornamented with large tubercles of unequal size and by its parasitising mosses of the genus *Pleuridium*. The occurrence of small cavities inside the tubercles has not been mentioned in the literature so far. The similar *Lamprospora tuberculatella* Seaver differs microscopically in spores ornamented with smaller tubercles. Additionally, *L. tuberculatella* occurs on different host mosses, namely *Didymodon vinealis* (Brid.) R.H. Zander, *Ephemerum megalosporum* (Austin) E.S. Salmon, *Weissia controversa* Hedw. and *Paraleucobryum enerve* (Thed.) Loeske (Benkert 2002, Eckstein 2014). A similar ornamentation as in *L. tuberculata* appears also in the recently described *Lamprospora esterlechnerae* Benkert, which is so far known from the holotype locality only. Its tubercles narrow towards the apex and sometimes have two or three obtuse lobes. *L. esterlechnerae* parasitises *Dicranodontium denudatum* (Brid.) E. Britton and has been found on wood, while *L. tuberculata* is considered to be a terrestrial species (Benkert 2011). *Lamprospora maireana* Seaver has rounded tubercles of about the same size as in *L. tuberculata*, with typical internal cavities, which are much more numerous and larger than in our specimen of *L. tuberculata* (Benkert 1987). *Lamprospora rehmii* Benkert has larger spores which are almost completely covered by isodiametric tubercles and short, more or less bent, broad ridges (Benkert 1994).

Whether this is the first find of *L. tuberculata* in the Czech Republic or not is unclear. Svrček & Kubička (1963) reported *Lamprospora modestissima* Grélet, a synonym of *L. tuberculata* according to Benkert (1987), from Bohemia with the comment that their find fully agrees with the original description and illustration by Grélet (1926). However, the specimen collected by Svrček & Kubička (1963) was later revised by Benkert (1987) as *Lamprospora dicranellae* Benkert, a species having spores ornamented with irregular broad ridges sometimes forming a reticulate pattern with prominent warts among the ridges. Furthermore, Svrček & Kubička (1963) mention *Barlaea melina* Velen., which was described from Bohemia by Velenovský (1934), as a synonym of *L. modestissima*. The voucher with *B. melina* was studied by Svrček (1976), who came to the conclusion that the fungus was *Lamprospora annulata* Seaver. However, *L. annulata* has spores ornamented with large warts and thick bands often forming circles around the spore. Svrček (1976, p. 128) noted: "No apothecia were found in the type collection, but comparison of the original description together with Velenovský's manuscript has shown the identity of his species [*Barlaea melina*] with Seaver's one [*L. annulata*]." To complete the confusion, the spore illustration of *Barlaea melina* in Velenovský (1934) shows prominent tubercles, which fit well with the

current concept of *L. tuberculata*. Based on Velenovský's description and illustration, Benkert (1987) considers this find to represent *L. tuberculata*. Unfortunately, the original specimen seems to be lost (Svrček 1976) and its true identity remains unclear. Therefore, the new find from Rusava represents the first unambiguous collection of *L. tuberculata* from the Czech Republic.

Outside the Czech Republic, *Lamprospora tuberculata* is known from Slovakia (Lukáš Jánošík, pers. comm.), Germany (Engel 1984), Switzerland (Beatrice Senn-Irlet, pers. comm.), the Netherlands (Brouwer 1999), France (Caillet & Moyne 1980), Spain (Enrique Rubio, pers. comm.) and Norway (Kristiansen 2006).

Material examined

Czech Republic. Moravia. Hostýnské vrchy hills, 725 m NW of the Povýšení sv. Kříže Church in Rusava (Kroměříž District), 49°21'29" N, 17°41'32" E, MTB 6672a, 575 m a.s.l., on soil in a small orchard, with *Pleuridium subulatum* (Hedw.) Rabenh., 2 Nov. 2014, leg. et det. Z. Egertová (PRM 933815).

Octospora ithacaensis (Rehm) K.B. Khare, Norwegian Journal of Botany 22(2): 111, 1975

Basionym: *Humaria ithacaensis* Rehm, Annales Mycologici 2(1): 35, 1904.

Synonyms: *Humarina ithacaensis* (Rehm) Seaver, The North American Cup-Fungi (Operculates) 3: 124, 1928;

≡ *Neottiella ithacaensis* (Rehm) Schweers, Revue de Mycologie 10: 69, 1946.

Description. Apothecia up to 1 mm broad, pinkish orange, plane or truncate-obconical, sessile, without margin, with hairy base visible when magnified (Fig. 3). Solitary or in groups on living thalli of *Marchantia polymorpha* L. Asci 205–265 × 14–19 µm, cylindrical, operculate, non-amyloid, containing 8 uniseriately to biseriately arranged spores. Spores 19.5–23.5 × 9.5–11.2 µm (Q = 1.85–2.2), ellipsoid with narrowed poles, hyaline, warted (warts 0.2–0.8 µm broad), with 2 equal guttules of 5.5–6.5 µm (Fig. 8). Paraphyses straight, 4–4.5 µm wide, with a broadened, obtuse apex 6–8.5 µm wide, septate, containing vacuolar bodies. Orange pigment in paraphyses not observed. Hairs hyaline, septate, up to 2.5–5 µm broad, with 0.3–1 µm wide cell walls, apex obtuse. Infections were observed on surface cells of the thallus as well as on rhizoids of *M. polymorpha*. Additionally, hyphae of *O. ithacaensis* often enter the air chambers of the *Marchantia*-thallus and probably also infect the assimilation cells inside. Appressoria are only slightly differentiated and about twice as wide as adjacent cells (Fig. 4).

Notes. *Octospora ithacaensis* is one of the few species of bryoparasitic *Pezizales* growing on liverworts. *Octospora cashiae* (Gamundí) Benkert occurs on an unidentified species of *Metzgeriales*. *Lamprospora aneurae* Benkert and

Neottiella ricciae (P. Crouan & H. Crouan) Korf & W.Y. Zhuang grow on *Aneura pinguis* (L.) Dumort. and *Riccia* spp. respectively (Benkert 1998b). All three species differ from *O. ithacaensis* in spore size and ornamentation.

Although the host of *Octospora ithacaensis* is the common liverwort *Marchantia polymorpha*, the fungus is only known from a few records in Europe. Its taxonomic position is not definitely clarified (Benkert 1998b). The infection was found to be virtually identical to the description and illustrations by Döbbeler (1979: 837–838, Fig. 4).

Both in Buchlovice and Kroměříž, *O. ithacaensis* shared the substrate with *Pezoloma marchantiae* (Sommerf.) Benkert, which is also considered a rather rare and probably overlooked species: in the Czech Republic it was last collected in 1934 (Markéta Šandová, pers. comm.).

In Europe, *O. ithacaensis* is known from Germany (e.g. Benkert 2009, Eckstein & Eckstein 2009), Switzerland (Elisabeth Stöckli, pers. comm.), Denmark (Læssøe & Petersen 2010), the Netherlands (Schweers 1945) and Spain (Rubio et al. 2012).

Material examined

Czech Republic. Moravia. Buchlovice (Uherské Hradiště District), castle park, 49°04'56" N, 17°20'21" E, MTB 6970a, 255 m a.s.l., on thalli of *Marchantia polymorpha* on a crushed brick pathway, 25 Oct. 2014, leg. et det. Z. Egertová (PRM 933380). – Kroměříž (Kroměříž District), Záhlinické rybníky nature park, 49°17'13" N, 17°26'43" E, MTB 6770b, 195 m a.s.l., in cover of living *Marchantia polymorpha* beside a pheasantry, 14 Dec. 2014, leg. et det. Z. Egertová (PRM 933381).

Octospora orthotrichi (Cooke & Ellis) K.B. Khare & V.P. Tewari, Canadian Journal of Botany 56(17): 2118, 1978

Basionym: *Peziza orthotrichi* Cooke & Ellis, Grevillea 6(37): 7, 1877 (as *P. orthotricha*).

Synonyms: *Humaria orthotrichi* (Cooke & Ellis) Sacc., Sylloge Fungorum 8: 119, 1889 (as *H. orthotricha*);

≡ *Humarina orthotrichi* (Cooke & Ellis) Seaver, The North American Cup-Fungi (Operculates) 3: 127, 1928 (as *H. orthotricha*).

Description. Apothecia 0.5–1.2 mm broad, orange, rounded, plane, with a whitish fimbriate margin (Fig. 5). Asci 130–245 × 17–23.5 µm, cylindrical, operculate, non-amyloid, containing 8 spores, uniseriate or biseriate. Spores 16.5–19 × 11–13.5 µm (Q = 1.3–1.65), ellipsoid, sometimes bean-shaped, often with one side flattened, warted [warts (0.2)0.5–1(1.5) µm broad], with one guttule 7.5–10.5 µm in diameter or less often with 2 guttules (Fig. 9). Paraphyses 3–6 µm broad, straight, septate, containing orange pigment. Hairs hyaline, thick-walled, 3–9 µm broad. Infections induce spherical galls on rhizoid tips which are completely covered with interwoven hyphae. The whole structure, gall and hyphae-cover, is 50–70 µm in diameter.

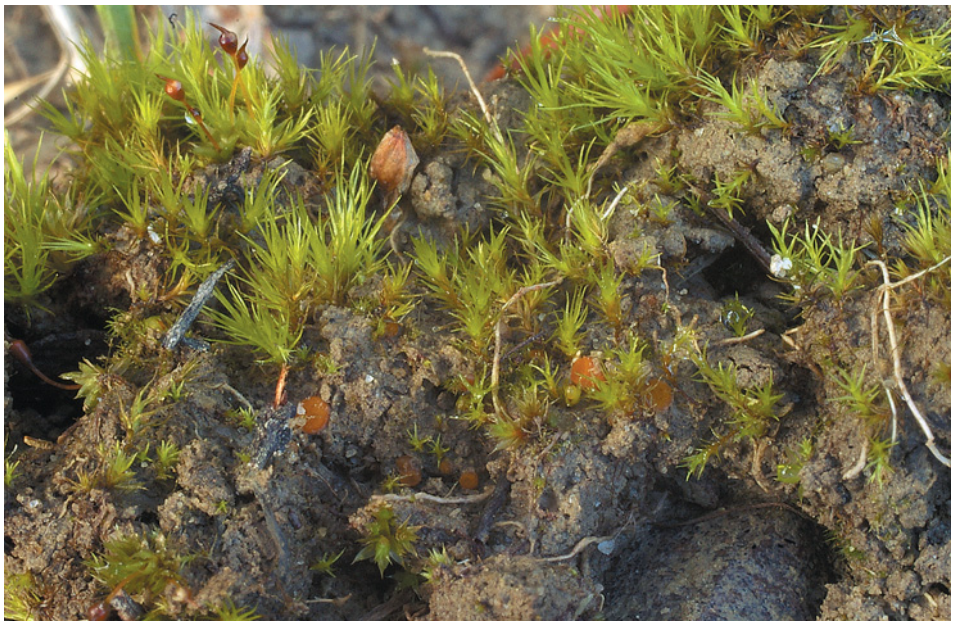


Fig. 1. Apothecia of *Lamprospora tuberculata* growing with *Pleuridium subulatum*, NW of Rusava, Czech Republic, 2 Nov. 2014 (PRM 933815). Photo Z. Egertová.

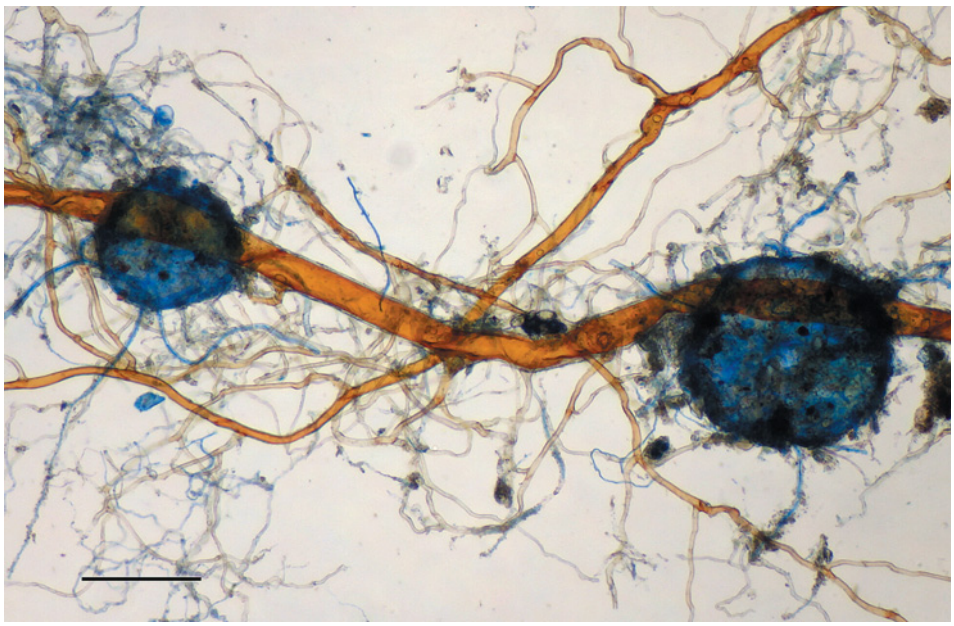


Fig. 2. Galls of *Lamprospora tuberculata* (PRM 933815) on rhizoids of *Pleuridium subulatum*. Bar = 100 µm. Photo J. Eckstein.

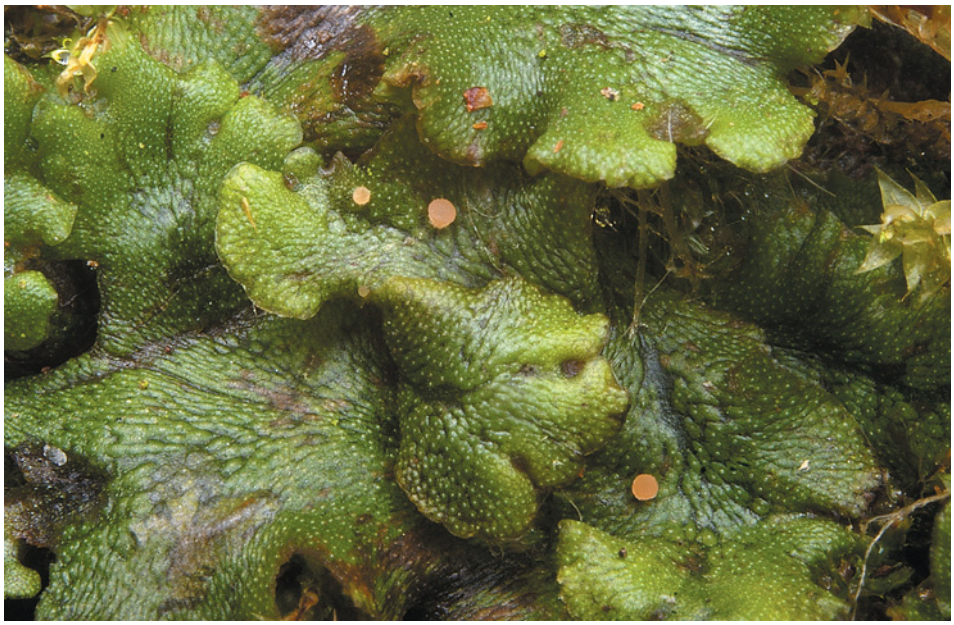


Fig. 3. Apothecia of *Octospora ithacaensis* on a thallus of *Marchantia polymorpha*, Buchlovice castle park, Czech Republic, 25 Oct. 2014 (PRM 933380). Photo Z. Egertová.

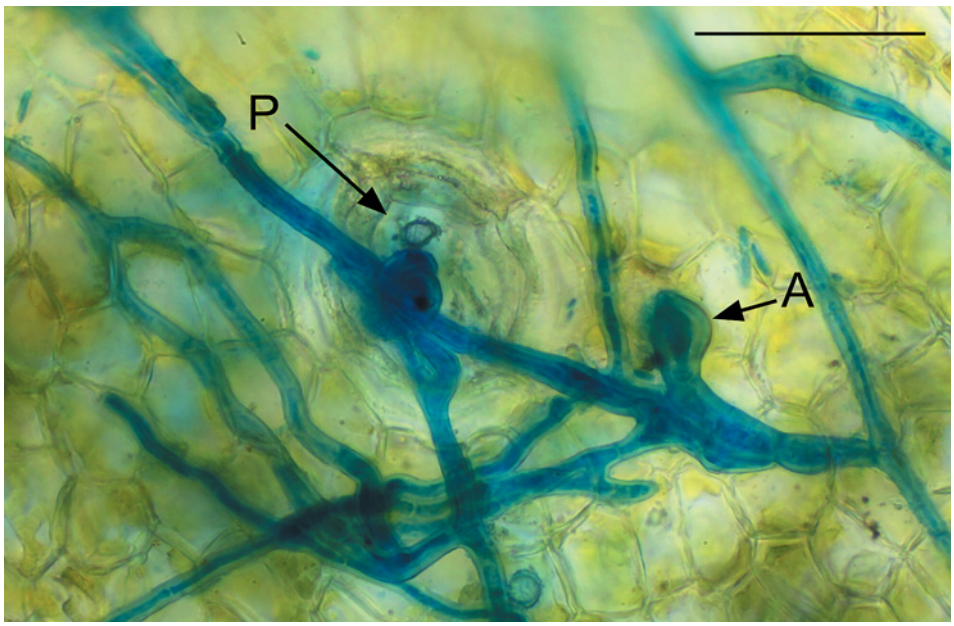


Fig. 4. Hyphae of *Octospora ithacaensis* (PRM 933380) running across the surface of a *Marchantia polymorpha*-thallus. Some hyphae enter the air chamber via a thallus-pore (P). An appressorium (A) is attached to an infected host cell. Bar = 50 µm. Photo J. Eckstein.

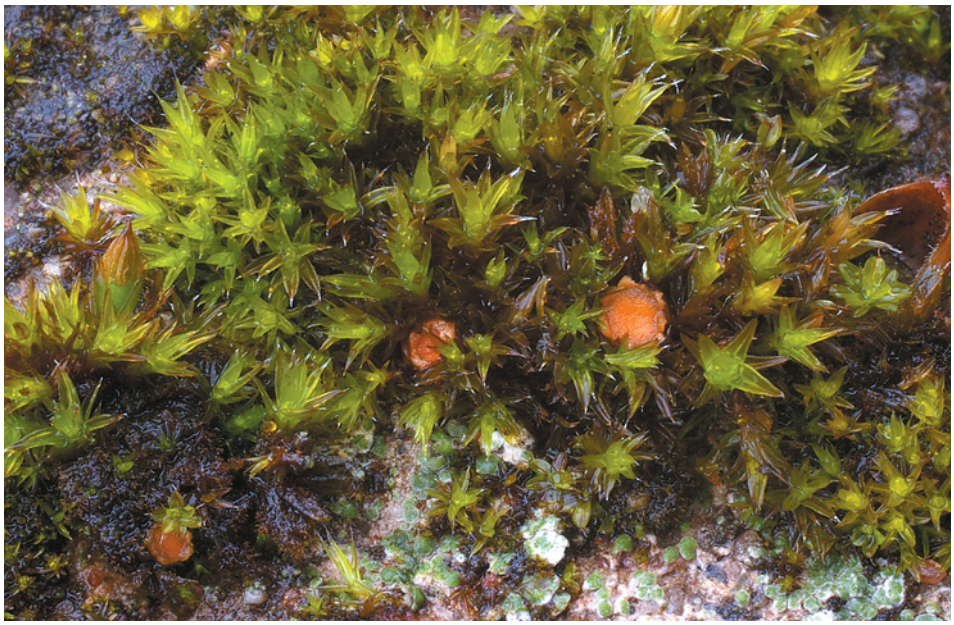


Fig. 5. Apothecia of *Octospora orthotrichi* with *Orthotrichum diaphanum*, Tyršův most bridge, Smiřice, Czech Republic, 15 Jan. 2014 (herb. Z. Egertová). Photo Z. Egertová.

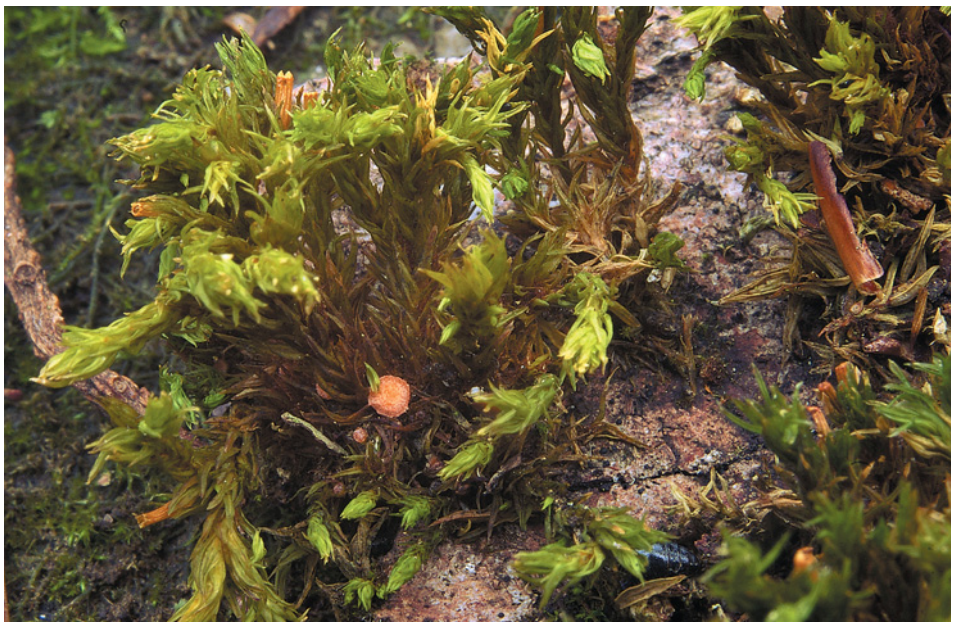
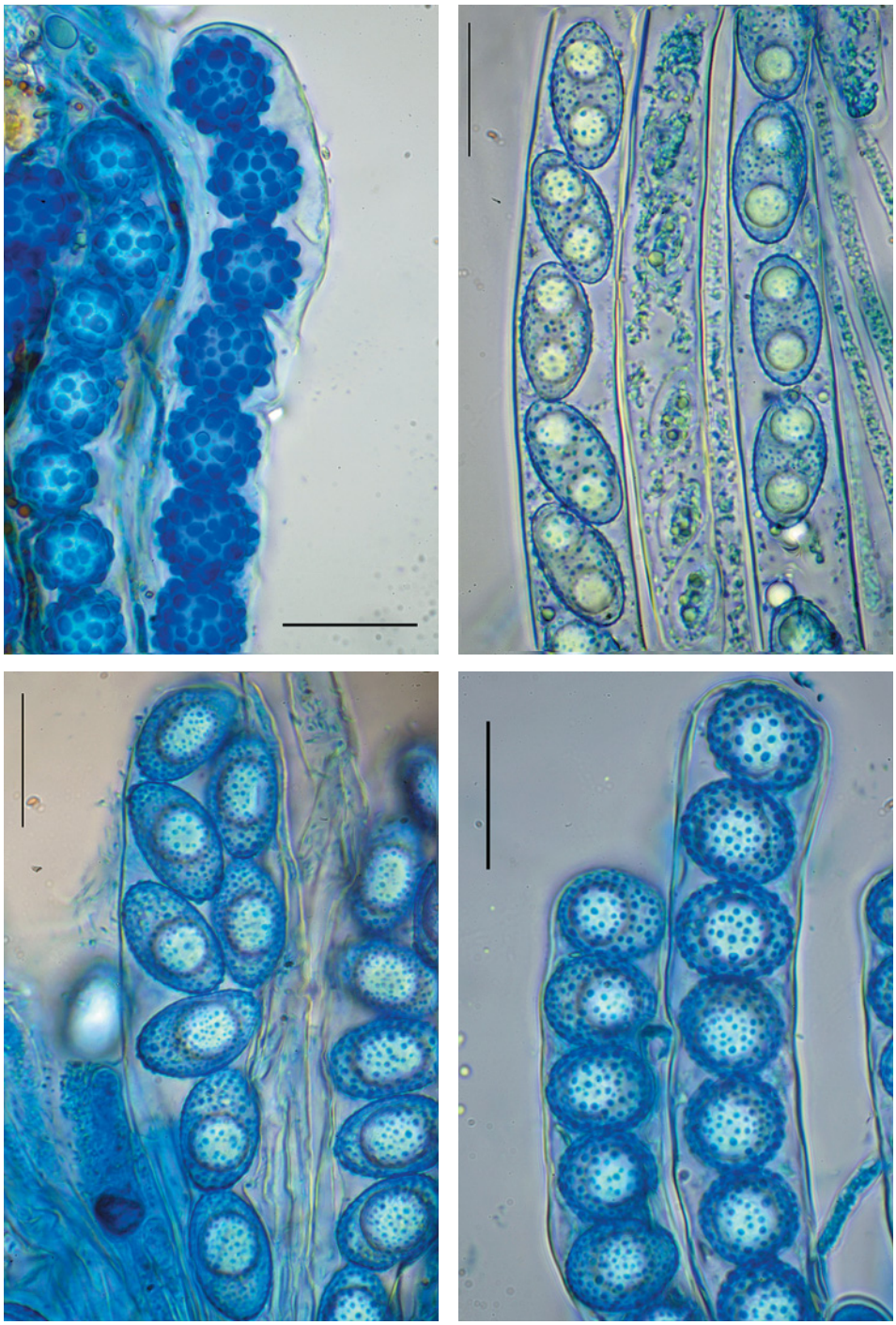


Fig. 6. Apothecia of *Octospora affinis* attached to *Orthotrichum affine*, Hostěnice, Czech Republic, 11 Jan. 2015 (PRM 933385). Photo Z. Egertová.



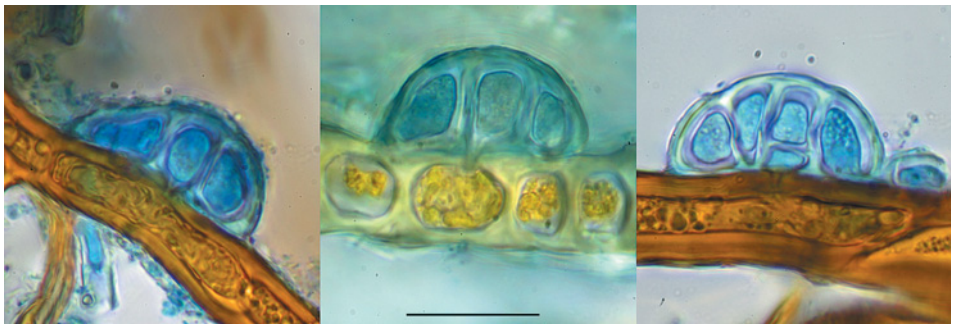


Fig. 11. Septate appressoria of *Octospora affinis* (PRM 933385) on rhizoids (left and right) and on a leaf of *Orthotrichum affine*. Bar = 20 µm. Photo J. Eckstein.

Notes. We agree with Khare (2003) that the grammatically correct specific epithet is *"orthotrichi"* (genitive of the name of the host moss *Orthotrichum*) instead of *"orthotricha"*.

Octospora orthotrichi is a member of the section *Wrightoideae*, which includes species with ellipsoid, broadly ellipsoid or subglobose, warted spores, parasitising mosses of the orders *Hypnales* and *Neckerales* and inducing galls on rhizoids of the host (Benkert 1998a). It is a species associated exclusively with *Orthotrichum diaphanum* Schrad. ex Brid., at least in Europe (Benkert 1998a). The observed infection structures correspond well with the detailed descriptions and illustrations by Senn-Irlet (1988).

Octospora orthotrichi can be found on the bark of trees or on stones, always between shoots of its host. The species does not seem to be rare. At least in neighbouring Germany it is common in some regions. The fact that it has not been recorded in the Czech Republic so far is probably due to its special habitat which is not regularly explored by mycologists. *Octospora affinis* Benkert & L.G. Krieglst., another species of the section *Wrightoideae*, also parasitises *Orthotrichum*. However, it is known to exclusively parasitise *O. affine* Schrad. ex Brid. and it also differs from *Octospora orthotrichi* in its smaller, broadly ellipsoid to subglobose spores (Benkert & Krieglsteiner 2006).

O. orthotrichi is known from Slovakia (Lukáš Jánošík, pers. comm.), Germany (Benkert 1998a), Switzerland (Senn-Irlet 1988), France (Benkert 1998a), the Netherlands (Brouwer 1999) and Norway (Kristiansen 2013).

Fig. 7 (top left). Spores of *Lamprospora tuberculata* (PRM 933815) in cotton blue.
Fig. 8 (top right). Spores of *Octospora ithacaensis* (PRM 933380) in cotton blue.
Fig. 9 (bottom left). Spores of *Octospora orthotrichi* (PRM 933816) in cotton blue.
Fig. 10 (bottom right). Spores of *Octospora affinis* (PRM 933385) in cotton blue.
Bar = 20 µm. Photo J. Eckstein.

Material examined

Specimens were collected and identified by Z. Egertová if not otherwise stated. All reported collections were found on *Orthotrichum diaphanum*.

Czech Republic. Bohemia. Smiřice (Hradec Králové District), Tyršův most bridge, 50°17'56" N, 15°52'32" E, MTB 5761a, 245 m a.s.l., on a stone bridge, 1 Jan. 2014 (PRM 933816); ibid., 15 Jan. 2014 (herb. Z. Egertová). – Moravia. Olomouc (Olomouc District), Poděbrady, 49°37'33" N, 17°13'41" E, MTB 6369c, 215 m a.s.l., on concrete panels, 4 Feb. 2014 (PRM 933817). – Olomouc, Smetanovy sady city park, 49°35'25" N, 17°14'48" E, MTB 6469a, 220 m a.s.l., on a stone bridge, 19 Jan. 2014, leg. Z. Egertová et M. Sochor (PRM 933818). – Šternberk (Olomouc District), Zelená budka vantage point, 49°44'01" N, 17°18'40" E, MTB 6269d, 400 m a.s.l., on a concrete floor, 18 Jan. 2014, leg. Z. Egertová et M. Sochor (PRM 933819). – Kroměříž (Kroměříž District), 49°17'17" N, 17°25'45" E, MTB 6770b, 190 m a.s.l., on stem of a vital *Malus domestica* tree, 10 Jan. 2015 (PRM 933384). – Hulín (Kroměříž District), Čechova Street, 49°18'59" N, 17°27'49" E, MTB 6670d, 190 m a.s.l., on bark of a vital *Juglans* tree, 19 Oct. 2014 (PRM 933820). – Holešov (Kroměříž District), castle park, 49°20'07" N, 17°35'08" E, MTB 6671d, 240 m a.s.l., on bark of a living *Malus domestica* tree, 19 Dec. 2014 (PRM 933382). – Strážnice (Hodonín District), Bzenecká Street, 48°54'18" N, 17°18'42" E, MTB 7069d, 170 m a.s.l., on a concrete panel, 20 Dec. 2014 (PRM 933383).

Octospora affinis Benkert & L.G. Krieglst., Zeitschrift für Mykologie 72(1): 54, 2006

Description. Apothecia 0.5–1.2 µm in diameter, rounded, pale orange, also salmon pink, with short whitish hairs at the margin, attached to the moss (Fig. 6). Asci 165–225 × 15.5–21 µm, cylindrical, operculate, non-amyloid, containing 8 uniseriately, rarely biseriately arranged spores. Spores 14–16 × 11.5–13.5 µm (Q = 1.1–1.33), broadly ellipsoid, hyaline, ornamented with isolated hemispherical warts 0.5–1.5 µm broad, with 1 globose guttule 8.5–9.5 µm (Fig. 10). In larger warts a small hollow inside is often visible at 1000× magnification. Paraphyses 3–4 µm wide, with a broader apex (6.5–10.5 µm), straight to slightly bent, septate, branched, containing orange pigment. Infections are located on strong rhizoids and on leaf cells. Appressoria are ellipsoid, 25–55 × 14–17 µm and 2–3-septate, rarely with an additional longitudinal wall (Fig. 11 right). The infection peg originates from the middle-cell of the appressorium and is about 1 µm wide. Thin-walled, irregular shaped haustoria were observed inside the host cells (Fig. 11).

Notes. This species was described as late as 2006 and has been reported as a common species in some countries since (e.g. Benkert & Krieglsteiner 2006, Chaillet & Moyne 2013). For more information on the host and distinguishing characters, see *Octospora orthotrichi*.

Infections were found to be consistent with the descriptions by Benkert & Krieglsteiner (2006) with the new observation, that leaf cells, besides rhizoids, can also be infected. The observations of hollow spore-warts and of the infection of leaf cells have not been mentioned neither in the description of *O. affinis* nor in other published records.

The species is known from Great Britain (Nick Aplin, pers. comm.), Spain, France, Luxembourg, Germany, Austria (Benkert & Krieglsteiner 2006), Switzerland (Elisabeth Stöckli, pers. comm.), the Netherlands (Henk-Jan van der Kolk, pers. comm.), Belgium (Bernard Declercq, pers. comm.), Slovakia (Z. Egertová, pers. observ.), Romania and Croatia (Lothar Krieglsteiner, pers. comm.).

Material examined

Czech Republic. Moravia. Hostěnice (Brno-venkov District), 49°14'28" N, 16°45'26" E, MTB 6766d, 395 m a.s.l., in tufts of *Orthotrichum affine* on trunk of a living *Acer* tree, 11 Jan. 2015, leg. et det. Z. Egertová (PRM 933385).

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