Palacký University Olomouc
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Diversity, ecology and evolution of bryophilous ascomycetes

Doctoral thesis

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Olomouc 2023

BIBLIOGRAPHIC IDENTIFICATION

Author's first name and surname: MDDr. Zuzana Sochorová (birth name Egertová)
Title of the thesis: Diversity, ecology and evolution of bryophilous ascomycetes
Type of thesis: Ph.D.
Department: Department of Botany, Faculty of Science, Palacký University Olomouc
Study program: P1527 Biology
Field of study: 1507V004 Botany
Supervisor: doc. RNDr. Michaela Sedlářová, Ph.D.
Co-supervisor: Dr. Jan Eckstein
Year of defence: 2024

ABSTRACT

The thesis focuses on bryophilous ascomycetes, a diverse group of fungi obligately growing on bryophytes. Six bryophilous species of the order Pezizales have been described as new to science: *Octosporopsis erinacea* Egertová & Döbbeler on *Dumortiera hirsuta* and *Octospora kelabitiana* Egertová & Döbbeler on *Riccardia* spp. from Borneo, *Octospora conidiophora* Sochorová & Döbbeler on *Trichosteleum perchlorosum* and *Sematophyllum brachycarpum* from South Africa, *Lamprospora sylvatica* Egertová & Eckstein on *Dicranum montanum* from Ukraine, Slovakia, Germany and Norway, *Octospora doebbeleri* Sochorová & Eckstein on *Dicranoweisia cirrata* from the Czech Republic and *Lamprospora aberrans* Sochorová, M. Vega, J. Hernanz & Eckstein on *Gymnostomum* spp. from Spain and Croatia. All new species, together with their infection structures on host bryophytes, were described in detail, and their phylogenetic positions based on molecular study were established. *Octosporopsis erinacea* and *Octospora kelabitiana* represent the first reports of bryophilous Pezizales from Borneo. *Octospora conidiophora* and three phylogenetically related cryptic species are the first taxa of bryophilous Pezizales with known anamorph. Together with *Lamprospora campylopodis* W. D. Buckley growing on *Campylopus pyriformis* and *Neottiella albocincta* (Berk. & M. A. Curtis) Sacc. on *Atrichum androgynum*, these represent the first records of bryophilous Pezizales from South Africa. New localities of *Octospora svrcekii* Benkert from Albania, Austria, Croatia, France, Slovakia and Spain have been published and the species has been described in detail in accordance with the principles of vital taxonomy. A phylogenetic analysis of the section *Wrightoideae* Benkert, based on the EF1α, SSU rDNA and LSU rDNA loci, showed that *Octospora svrcekii* forms a monophyletic group with *O. wrightii* (Berk. & M. A. Curtis) J. Moravec (the type species of the section), *O. erzbergeri* Benkert, *O. hygrohypnophila* Dissing & Sivertsen and *O. americana* Benkert, all of which have subglobose to broadly ellipsoid ascospores ornamented with isolated warts, and infect mosses in the order Hypnales, inducing galls on their rhizoids. On the contrary, *O. orthotrichi* (Cooke & Ellis) K. B. Khare & V. P. Tewari and *O. affinis* Benkert & L. G. Krieglst., formerly also considered members of the section *Wrightoideae*, do not belong to the group. The new genus *Bryorutstroemia* Sochorová & Baral (Helotiales) has been established for the necrotrophic parasite *Helotium fulvum* Boud. Several rarely reported species of bryophilous ascomycetes have been found in the Czech Republic, including *Octospora pseudoampezzana* (Svrček) Caillet & Moyne, which has been listed in the Red list of fungi (macromycetes) as a probably extinct species.
Keywords: bryophilous Helotiales; bryophilous Pezizales; infection; new species; *Wrightoideae*
Number of pages: 175
Language: English

BIBLIOGRAFICKÁ IDENTIFIKACE

Jméno a příjmení autora: MDDr. Zuzana Sochorová (roz. Egertová)
Název práce: Diverzita, ekologie a evoluce bryofilních askomycetů
Typ práce: doktorská
Pracoviště: Katedra botaniky, Přírodovědecká fakulta, Univerzita Palackého v Olomouci
Studijní program: P1527 Biologie
Studijní obor: 1507V004 Botanika
Vedoucí práce: doc. RNDr. Michaela Sedlářová, Ph.D.
Konzultant: Dr. Jan Eckstein
Rok obhajoby: 2024

ABSTRAKT

Disertační práce je zaměřena na bryofilní askomycety, rozmanitou skupinu vřeckovýtrusých hub obligátně rostoucích na mechorostech. Bylo popsáno šest nových bryofilních druhů z řádu Pezizales: *Octosporopsis erinacea* Egertová & Döbbeler na *Dumortiera hirsuta* a *Octospora kelabitiana* Egertová & Döbbeler na *Riccardia* spp. z Bornea, *Octospora conidiophora* Sochorová & Döbbeler na *Trichosteleum perchlorosum* a *Sematophyllum brachycarpum* z Jihoafrické republiky, *Lamprospora sylvatica* Egertová & Eckstein na *Dicranum montanum* z Ukrajiny, Slovenska, Německa a Norska, *Octospora doebbeleri* Sochorová & Eckstein na *Dicranoweisia cirrata* z České republiky a *Lamprospora aberrans* Sochorová, M. Vega, J. Hernanz & Eckstein na *Gymnostomum* spp. ze Španělska a Chorvatska. Všechny nové druhy byly detailně popsány a vyobrazeny včetně infekčních struktur na jejich hostitelských mechorostech a byla objasněna jejich fylogenetická pozice. *Octosporopsis erinacea* a *Octospora kelabitiana* představují první publikované sběry bryofilních Pezizales z Bornea. *Octospora conidiophora* a tři fylogeneticky blízké kryptické druhy jsou prvními taxony bryofilních Pezizales, u nichž byla zjištěna anamorfa. Společně s *Lamprospora campylopodis* W. D. Buckley rostoucí na *Campylopus pyriformis* a *Neottiella albocincta* (Berk. & M. A. Curtis) Sacc. na *Atrichum androgynum* představují první publikované nálezy bryofilních Pezizales z Jihoafrické republiky. *Octospora svrcekii* Benkert byla nalezena na nových lokalitách ve Španělsku, Francii, Rakousku, na Slovensku, v Chorvatsku a Albánii a detailně popsána v souladu se zásadami vitální taxonomie. Fylogenetická analýza zaměřená na sekci *Wrightoideae* Benkert, založená na lokusech EF1α, SSU rDNA a LSU rDNA, ukázala, že *Octospora svrcekii* tvoří monofyletickou skupinu s *O. wrightii* (Berk. & M. A. Curtis) J. Moravec (typovým druhem sekce), *O. erzbergeri* Benkert, *O. hygrohypnophila* Dissing & Sivertsen a *O. americana* Benkert, které všechny mají subglobózní až široce elipsoidní výtrusy ornamentované izolovanými bradavičkami a infikují mechy z řádu Hypnales, na jejichž rhizoidech indukují tvorbu hálek. Naopak *O. orthotrichi* (Cooke & Ellis) K. B. Khare & V. P. Tewari a *O. affinis* Benkert & L. G. Krieglst., které byly dříve také řazeny do sekce *Wrightoideae*, do zmíněné skupiny nepatří. Nový rod *Bryorutstroemia* Sochorová & Baral (Helotiales) byl ustanoven pro nekrotrofně parazitický druh *Helotium fulvum* Boud. Byla nalezena řada vzácně publikovaných druhů bryofilních askomycetů, včetně *Octospora pseudoampezzana* (Svrček) Caillet & Moyne, která je dosud zařazena v Červeném seznamu hub (makromycetů) jako pravděpodobně vyhynulý druh.
Klíčová slova: bryofilní Helotiales; bryofilní Pezizales; infekce; nové druhy; *Wrightoideae*
Počet stran: 175
Jazyk: anglický

DECLARATION

I hereby declare that this thesis represents my own work and that I wrote this thesis independently using cited references.

In Olomouc ……. ……

MDDr. Zuzana Sochorová

LIST OF PAPERS INCLUDED IN THE THESIS WITH STATEMENT OF AUTHORS' CONTRIBUTION

Egertová Z., Döbbeler P., Sochor M. (2018) *Octosporopsis erinacea* and *Octospora kelabitiana* (Pezizales) – two new hepaticolous ascomycetes from Borneo. Mycological Progress 17: 103–113.

ZE collected samples, performed microscopy, prepared descriptions of apothecial morphology and wrote the first draft of the manuscript. PD performed microscopy, prepared descriptions of apothecial characters and infection structures and drew illustrations. MSo collected samples, conducted sequencing and molecular analysis. All of the authors contributed to and approved the final version of manuscript.

Sochorová Z., Döbbeler P., Sochor M., van Rooy J. (2019) *Octospora conidiophora* (Pyronemataceae) – a new species from South Africa and the first report of anamorph in bryophilous Pezizales. MycoKeys 54: 49–76.

ZS collected samples, prepared description of the apothecial features, photographed the apothecia, microscopic characters, conidia and infection, participated on molecular analysis and wrote the first draft of the manuscript. PD performed microscopy, discovered the anamorph, prepared description of the mycelial characters and the anamorph and illustrated them. MSo collected samples, conducted sequencing and molecular analysis and photographed the locality in the collection ZE 11/18. JvR determined host bryophytes and prepared the text about their characteristics and distribution. All of the authors contributed to and approved the final version of manuscript.

Egertová Z., Eckstein J., Sochor M., Vega M. (2018) *Lamprospora sylvatica* (Pyronemataceae), a new bryophilous ascomycete on *Dicranum montanum*. Phytotaxa 357: 17–29.

ZE collected the samples in Ukraine and Slovakia, prepared the description of apothecial characters, participated in sequencing, photographed the apothecia and microscopic characters and wrote the first draft of the manuscript. JE collected the sample in Germany, described and illustrated the infection, took photographs of infectious structures and SEM of ascospores. MSo conducted sequencing and molecular analysis and photographed the locality in Slovakia. All of the authors contributed to and approved the final version of manuscript.

Sochorová Z., Eckstein J., Sedlářová M., Sochor M. (2021) *Octospora doebbeleri*, a new bryophilous species on *Dicranoweisia cirrata.* Sydowia 73: 233–246.

ZS collected samples, prepared the description of apothecial characters, photographed the apothecia, microscopic characters, infection and locality and wrote the first draft of the manuscript. JE photographed the ascospores, described, photographed and illustrated the infection. MSe photographed ascospores in DAPI. MSo conducted sequencing and molecular analysis. All of the authors contributed to and approved the final manuscript.

Sochorová Z., Vega M., Hernanz J., Eckstein J., Sochor M. (2023) *Lamprospora aberrans* sp. nov. – the first species of *Lamprospora* with hairy apothecia. Herzogia 36: 206–221.

ZS collected the Croatian sample, prepared the description of apothecial characters, photographed microscopic features and prepared the first draft of the manuscript. MV collected the sample from Mallorca and described the apothecial characters. JH collected the Spanish specimens, described apothecial characters and photographed the apothecia, microscopic characters and the locality. JE described and illustrated the infection and took photographs and SEM of ascospores. MSo conducted the sequencing and molecular analysis. All of the authors contributed to and approved the final version of manuscript.

Baral H. O., **Sochorová Z.**, Sochor M. (2023) *Bryorutstroemia* (Rutstroemiaceae, Helotiales), a new genus to accommodate the neglected sclerotiniaceous bryoparasitic discomycete *Helotium fulvum* Boud. Life 13: 1041.

HOB collected samples, prepared the description of the species, illustrated it and prepared the first draft of the manuscript. ZS collected samples, prepared the description of the species and photographed the apothecia, microscopic features and localities. MSo conducted sequencing and phylogenetic analysis. All of the authors contributed to and approved the final version of manuscript.

Sochorová Z., Matočec N., Kušan I., Janošík L., Eckstein J., Vega M., Mešić A., Sedlářová M., Martínez-Gil R., Sochor M. (2020) Amended description of the rarely reported bryophilous ascomycete *Octospora svrcekii* (Pyronemataceae) with notes on the phylogeny of the section *Wrightoideae*. Phytotaxa 475: 1–17.

ZS collected samples, prepared the description of apothecial features, provided photographic documentation and wrote the first draft of the manuscript. NM and IK collected the sample in Croatia and prepared the description of apothecial characters. LJ collected samples, prepared the photographic documentation, conducted sequencing and phylogenetic analysis. JE took SEM of ascospores, described and illustrated the infection. MV and RMG collected samples. MSo collected samples, conducted sequencing and phylogenetic analysis. All of the authors contributed to and approved the final version of manuscript.

Authors' abbreviations

HOB – Hans-Otto Baral
IK – Ivana Kušan
JE – Jan Eckstein
JH – Jorge Hernanz
JvR – Jacques van Rooy
LJ – Lukáš Janošík
MSe – Michaela Sedlářová
MSo – Michal Sochor
NM – Neven Matočec
PD – Peter Döbbeler
RMG – Rubén Martínez-Gil
ZE – Zuzana Egertová (= maiden name of Zuzana Sochorová)
ZS – Zuzana Sochorová

ACKNOWLEDGEMENTS

This thesis could not have been completed without the contributions of many colleagues and friends.

First of all, I am very grateful to my supervisor Michaela Sedlářová for large support I was given from her and patience during my studies, as well as bringing many valuable ideas how to improve the work.

I would like to thank my co-authors Michal Sochor, Jan Eckstein, Marcel Vega, Peter Döbbeler, Hans-Otto Baral, Jorge Hernanz, Ivana Kušan, Neven Matočec, Lukáš Janošík, Rubén Martínez-Gil, Armin Mešić and Jacques van Rooy for cooperation on our joint publications, sharing their outstanding knowledge, teaching me new methods and exchanging specimens and literature. Marcel Vega was the first one who turned my attention to bryophilous Pezizales and taught me how to find them in the field. Armin Mešić accepted me in his lab during my study stay in Zagreb, and later included me in the ForFungiDNA project. Neven Matočec repeatedly hosted me in his home in Novo Selo Okičko and together with Ivana Kušan taught me how to work according to the standards of vital taxonomy, organized joint field excursions and helped me with obtaining permits to enter Croatian protected areas.

Thanks also belong to George Greiff, Caroline Hobart, Lee Knight, Markus Wagner and Chris Yeates who helped to improve English in the papers, and to the staff of cooperating herbaria – especially Tereza Tejklová (Museum of Eastern Bohemia, Hradec Králové), Miriam Kuchaříková, Petr Zehnálek, Markéta Šandová and Jan Holec (Mycological Department of the National Museum, Prague), Miroslav Beran (South Bohemian Museum, České Budějovice), Arne Anderberg (Swedish Museum of Natural History) and Riana Jacobs-Venter (South African National Collection of Fungi).

I am grateful also to several people who shared their specimens, photographs, sequences, provided literature, identified bryophytes or helped me in various other ways: Pablo Alvarado, David Attu, Dieter Benkert, Sandra Bernhardt, Jannes Boers, Bernard Declercq, Hubert De Meulder, Günter Eckstein, Elizma Fouche, Danny Haelewaters, Michel Hairaud, Viktorie Halasů, Zbyněk Hradílek, Seppo Huhtinen, Robin Isaksson, Nedim Jukić, Roy Kristiansen, Uwe Lindemann, John Manning, Csaba Németh, Peter Otto, Zdeněk Palice, Rian John Pasan, Donald Henry Pfister, Jean-Paul Priou, Joschi Siembida, Hester Steyn, Vojtěch Taraška, Henk-Jan Van der Kolk, Nicolas Van Vooren, Petr Včelička, Lucie Zíbarová and many others. Last but not least, I would like to thank my family.

The work was supported by the Internal Grant Agency of Palacký University (IGA_PrF_2017_001, IGA PrF-2018-001, IGA_PrF_2019_004, IGA PrF-2020-003, IGA UP PrF-2023-001) and the Croatian Science Foundation under the project ForFungiDNA (IP-2018-01-1736). The research on *Lamprospora sylvatica* was partly funded by the Hainich NP and financial support of the travel expenses in South Africa was kindly provided by John Manning.

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LIST OF ABBREVIATIONS

?EX – probably extinct (a category in the Red list of fungi of the Czech Republic)
AC – acetocarmine
AH – University of Alcalá, Spain
AIC – akaike information criterion
BI – Bayesian phylogeny inference
BLAST – Basic Local Alignment Search Tool
CB – South Bohemian Museum, Czech Republic
CNF – Croatian Mycological Society, Croatia
CRB – Brilliant Cresyl Blue
CTAB – Cetrimonium Bromide
DAPI – 4′,6-diamidino-2-phenylindole
DNA – deoxyribonucleic acid
EF1α – elongation factor 1 alpha
IKI – Lugol's iodine solution
ITS – internal transcribed spacer
KOH – potassium hydroxide
LACB – Lactic Acid Cotton Blue
LPCB – Lactophenol Cotton Blue
LSU rDNA – large subunit ribosomal ribonucleic acid
ML – Maximum Likelihood
MLZ – Melzer's solution
MP – Maximum Parsimony
NaCl – sodium chloride
NNR – National Nature Reserve
NP – National Park
NR – Nature Reserve
PCR – polymerase chain reaction
PEG – polyethylene glycol
PRA – Institute of Botany, Academy of Science, Czech Republic
PRC – Charles University, Czech Republic
PRM – National Museum, Czech Republic
SAR – Department of Forestry, Malaysia
SSU rDNA – small subunit ribosomal ribonucleic acid
UPS – Museum of Evolution, Sweden
VIT – The Natural History Museum of Alava
WU – University of Vienna, Austria

1. Introduction

1.1 Bryophilous ascomycetes – inconspicuous fungi growing on bryophytes

The concept 'bryophilous ascomycetes' refers to an ecologically defined group of sac fungi obligately growing on bryophytes, i.e. mosses, liverworts and hornworts. Species of this heterogeneous group differ in several aspects such as the host selection, mode of nutrition or geographical distribution. The bryophilous lifestyle has developed multiple times within the division Ascomycota (Stenroos et al. 2010); despite rather frequent changes in their classification, it is possible to state that bryophilous ascomycetes belong to at least sixteen orders: Acrospermales, Arthoniales, Capnodiales, Chaetothyriales, Dothideales, Helotiales, Hypocreales, Lecanorales, Leotiales, Lichinales, Ostropales, Pezizales, Phacidiales, Pleosporales, Sordariales and Verrucariales (Fig. 1). Some bryophilous ascomycetes are known exclusively in their anamorphic stage (Racovitza 1959), while others produce fruit bodies, often colored black or orange. Ascomata (apothecia or perithecia) are very small in most members, reaching only tens of micrometers in some species (e.g. *Epibryon endocarpum* Döbbeler, *Bryochiton monascus* Döbbeler & Poelt or *B. perpusillus* Döbbeler; Döbbeler 1978, 1980a). The group includes also lichenized species (Poelt 1986, Obermayer & Poelt 1994, Lendemer & Harris 2016).

This thesis focuses mainly on bryophilous Pezizales, which comprise the genera *Octospora* Hedw., *Lamprospora* De Not., *Neottiella* (Cooke) Sacc., *Octosporopsis* U. Lindem. & M. Vega, *Octosporella* Döbbeler and *Filicupula* Y. J. Yao & Spooner. These form sessile apothecia which are discoid, saucer-shaped, turbinate, ovoid, ellipsoid or subglobose, sized 0.2–15 mm in diameter and coloured in shades of orange, red or pink, rarely yellow or whitish. Apothecia are smooth or bear thick-walled, hyaline hairs; some have a distinct sterile margin (Fig. 2). Microscopically, they are characterised by unitunicate, clavate to cylindrical, operculate, inamyloid asci, which are usually octosporic, but in some species of *Octospora*, *Octosporella* and *Filicupula* contain only four, three or two ascospores (Döbbeler 1980b, 1988, Döbbeler & Menjívar 1992, Benkert 1998a, Yao et al. 2006, Döbbeler & Davison 2021). Paraphyses are filiform or cylindrical, often with a broadened apex, septate, straight or bent, simple or forked and contain carotenoid pigments. A remarkable variability can be found in their ascospores, which have very diverse shapes (globose, subglobose, ellipsoid, ovoid, fusoid, cylindrical, subfiliform), size, content of lipid bodies, and ornamentation – they can be smooth or ornamented by warts, tubercles, ridges, bands or their combinations (Fig. 3).

Currently, there are around 400 species of bryophilous ascomycetes described (Janošík 2017), but the real diversity is certainly much higher; Döbbeler (1997) estimates that it is at least as high as in lichenicolous fungi in which more than 2000 taxa are known (Diederich et al. 2018).

Fig. 1: Diversity of bryophilous ascomycetes. A) *Bryostroma* sp. (Dothideales), B) *Bryocentria metzgeriae* (Hypocreales), C) *Belonium coroniforme* (Helotiales), D) *Bryoglossum gracile* (Helotiales), E) *Belonioscyphella hypnorum* (Helotiales), F) *Bryoscyphus atromarginatus* (Helotiales), G) *Pezoloma marchantiae* (Helotiales), H) *Bryorutstroemia fulva* (Helotiales), I) *Mniaecia jungermanniae* (Phacidiales).

Fig. 2: Diversity of bryophilous Pezizales. A) *Neottiella albocincta*, B) *Octospora humosa*, C) *Octospora axillaris* var. *tetraspora*, D) *Lamprospora campylopodis*, E) *Lamprospora lubicensis*, F) *Lamprospora thelespora*, G) *Lamprospora miniata* var. *parvispora*, H) *Octosporella perforata*, I) *Octosporopsis erinacea*.

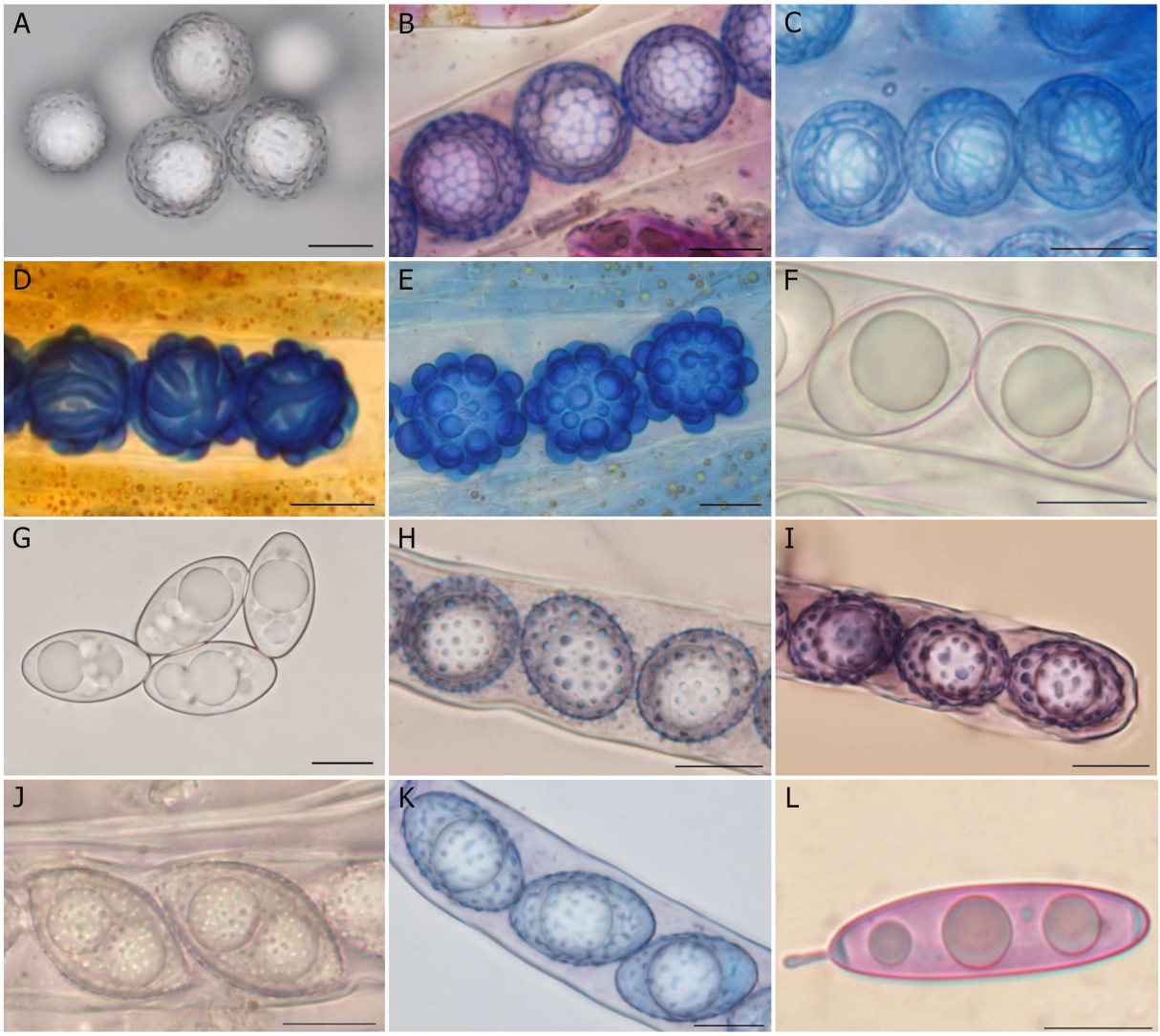

Fig. 3: Diversity of ascospores in bryophilous Pezizales. A) *Lamprospora lubicensis*, B) *Lamprospora ditrichi*, C) *Lamprospora norvegica*, D) *Lamprospora moynei*, E) undescribed *Octospora* on *Dumortiera hirsuta*, F) *Octospora oscarii*, G) *Octospora leucoloma*, H) *Octospora americana*, I) *Octospora phagospora*, J) *Octospora bridei*, K) *Neottiella albocincta*, L) *Octosporella erythrostigma.* A, F, G, J in tap water, B, H, I, L in CRB, C, D, E, K in LACB. Scale in all figures: 10 µm.

1.2 History of research on bryophilous ascomycetes

Due to their minute size, hidden lifestyle and low economic importance, bryophilous ascomycetes have never been in the centre of attention. Nevertheless, they have been noticed by scientists for a very long time. In the beginning, researchers focused mainly on describing new taxa and studying their ecology and distribution. As far back as 1789, the genus *Octospora* was established by the 'father of bryology' Johannes Hedwig (1730–1799, Germany; Hedwig 1789). Bryophilous ascomycetes appear also in works of other classic authors like Elias Magnus Fries (1794–1878, Sweden; Fries 1822), Pierre-Louis Crouan (1798–1871, France) and Hippolyte-Marie Crouan (1802–1871, France; Crouan & Crouan 1857, 1867) or Miles Joseph Berkeley (1803–1889, Great Britain; Berkeley & Broome 1865).

Giuseppe De Notaris (1805–1877, Italy) established the genus *Lamprospora* (De Notaris 1863).

Jean Louis Émile Boudier (1828–1920, France) described *Lamprospora carbonicola* Boud., *L. dictydiola* Boud. (Boudier 1907), *L. lutziana* Boud. (Boudier 1917), *Octospora hetieri* (Boud.) Dennis & Itzerott (as *Neottiella hetieri* Boud.; Boudier 1896) and *O. rubens* (Boud.) M. M. Moser (as *Humaria rubens* Boud.; Boudier 1896), but for example also the inoperculate bryophilous ascomycete *Helotium fulvum* Boud. (Boudier 1897).

Pier Andrea Saccardo (1845–1920, Italy) established the genus *Neottiella* (Saccardo 1889).

Fred Jay Seaver (1877–1970, USA) studied bryophilous ascomycetes in North America and described several new species of *Lamprospora* and *Octospora* (Seaver 1912, 1914).

Andrei Racovitza (1911–?, Romania) provided a monographic study on pyrenocarpous ascomycetes and anamorphic fungi on bryophytes (Racovitza 1959). He described more than 60 new species of bryophilous ascomycetes as new (IMA 2023).

Heinz Itzerott (1912–1983, Germany) described five species of bryophilous Pezizales, most of them with Richard William George Dennis (1910–2003, Great Britain; Dennis & Itzerott 1973, Itzerott & Thate 1974, Itzerott 1979). He also focused on studies of the infectious structures (Itzerott & Döbbeler 1982, Itzerott 1983).

Josef Poelt (1924–1995) was a botanist, bryologist and lichenologist working as professor of botany in Berlin (Germany) and Graz (Austria). He dealt with lichenized bryophilous ascomycetes (Poelt 1986).

Henry Dissing (1931–2009, Denmark) described *Lamprospora leptodictya* Dissing from Greenland (Dissing 1981) and *Octospora hygrohypnophila* Dissing & Sivertsen from Norway (Dissing & Sivertsen 1983).

Dieter Benkert (1933–2022, Germany) performed an intensive and systematic research on bryophilous Pezizales. He described (partly with colleagues Olav Aas, Emiel Brouwer, Lothar Krieglsteiner, Roy Kristiansen and Torsten Richter) more than 40 taxa of bryophilous Pezizales (Benkert 1987, 1990, 1994, 1997, 1998a, 1998b, 1998c, 2000, 2006, 2009, 2011, Benkert et al. 1991, Benkert & Brouwer 2004, Benkert & Krieglsteiner 2006, Benkert & Kristiansen 2008). His work includes the most comprehensive study on the genus *Lamprospora* so far (Benkert 1987) and study on tetrasporic taxa of *Octospora* (Benkert 1998a). He also dealt with mycofloristics focusing on bryophilous Pezizales.

James William Kimbrough (1934–2017, USA) and Yei-Zeng Wang (born 1956, Taiwan) published a monographic study on North American species of *Lamprospora* (Wang & Kimbrough 1992).

Michel Caillet (born 1934, France) and Gilbert Moyne (born 1942, France) studied bryophilous Pezizales in France (Caillet & Moyne 1980, 1987a, 1987b, 1991). They described two species infecting *Ephemerum*, *Octospora bridei* Caillet & Moyne and *O. echinospora* Caillet & Moyne (Caillet & Moyne 1987a).

Roy Kristiansen (born 1943) has studied bryophilous Pezizales mainly in Norway, resulting in discoveries of many species new to the country (Kristiansen 1999, 2006, 2007, 2013, Kristiansen & Schumacher 1993). He participated in describing *Octospora splachnophila* Benkert & R. Kristiansen (Benkert & Kristiansen 2008) and *Lamprospora norvegica* Benkert, Aas & R. Kristiansen (Benkert et al. 1991).

Peter Döbbeler (born 1946, Germany) has described almost 250 species as new and established 32 genera of bryophilous ascomycetes (IMA 2023). He discovered the induction of galls in *Octospora wrightii* (Berk. & M. A. Curtis) J. Moravec on rhizoids of *Amblystegium serpens* (Döbbeler 1980c) and described and illustrated infection structures in many other species of bryophilous Pezizales (e.g. Döbbeler 1980b, Döbbeler & Facher 2014, Döbbeler et al. 2018a). Furthermore, he found the first anamorph for bryophilous Pezizales in *Octospora conidiophora* Sochorová & Döbbeler (Sochorová et al. 2019) and later also in *Octospora bicarpa* Döbbeler, Büschlen & Eckstein (Döbbeler et al. 2021a). His work includes also discoveries of hepaticolous (Döbbeler & Menjívar 1992, Döbbeler & Carranza 1993, Döbbeler 1998, 2005, 2010a, 2010b, 2012, 2016) as well as muscicolous (Döbbeler 2011) ascomycetes inhabiting a unique habitat – the phyllosphere. Furthermore, Döbbeler identified several microniches on bryophytes inhabited by ascomycetes and highlighted the microniche selection as a typical, often diagnostic, feature of bryophilous ascomycetes (Döbbeler 2002).

Trond Schumacher (born 1949) published a study on *Lamprospora* in arctic and alpine habitats of Norway (including Svalbard) and Central Europe, in which *Lamprospora spitzbergensis* T. Schumach. was introduced (Schumacher 1993). He also described *Octospora heterosculpturata* T. Schumach. (Schumacher 1992).

Brian Martin Spooner (born 1951, Great Britain) and Yi-Jian Yao (born 1955, China) established the genus *Filicupula* Y. J. Yao & Spooner (Yao & Spooner 1996a) and described *Octosporella fusispora* Y. J. Yao & Spooner (Yao et al. 2006). Additionaly, Spooner established the genus *Bryoscyphus* Spooner (Kirk & Spooner 1984) and described *Lamprospora miniatopsis* Spooner, *Bryosphaeria megaspora* Spooner, *Epibryon chorisodontii* Spooner and *Hymenoscyphus austrobryus* Spooner (Pegler et al. 1980).

Soili Stenroos (born 1958, Finland) and her team published a robust phylogenetic study dealing with bryophilous ascomycetes (Stenroos et al. 2010). Further, she participated in other studies on this group of fungi (Huhtinen et al. 2010, Marsh et al. 2010, Wäli et al. 2014).

Marcel Vega (born 1969, Germany) has described eight species of *Lamprospora* (Eckstein et al. 2022, Vega et al. 2016, 2017, 2019, 2021a, 2021b), *Octospora pannosa* T. Richter, M. Vega & D. Savić (Vega et al. 2018) and *Octosporella microtricha* Döbbeler, Negrín & M. Vega (Döbbeler et al. 2018a). Together with Uwe Lindemann (born 1966, Germany), he

established the genus *Octosporopsis* U. Lindem. & M. Vega (Lindemann et al. 2014). In a joint paper with Gilbert Moyne, he highlighted the importance of some neglected vital characters in the taxonomy of bryophilous Pezizales (Vega & Moyne 2019).

Jan Eckstein (born 1976, Germany) is a bryologist, who has described four species of *Lamprospora* (Vega et al. 2016, 2017, Egertová et al. 2018a, Eckstein et al. 2022) and three species of *Octospora* (Döbbeler et al. 2021a, Eckstein et al. 2021, Sochorová et al. 2021). His domain are studies on infectious structures in bryophilous Pezizales. He maintains the webpage www.octospora.de.

Lukáš Janošík (born 1996, Slovakia) has participated in descriptions of five species of *Lamprospora* (Vega et al. 2019, 2021a, 2021b, Eckstein et al. 2022), *Octospora oscarii* Eckstein, Sochorová & Janošík (Eckstein et al. 2021) and *Octosporella australis* Janošík & Döbbeler (Janošík et al. 2022). He conducted a study focused on the relationship between the ascospore ornamentation, the place of infection and the host ecology (Janošík et al. 2023).

Also the Czech Republic has a long tradition in research of bryophilous ascomycetes. The first records from the country were given already by August Carl Joseph Corda (1809–1849; Corda 1838, 1842). In the first half of the 20th century, bryophilous ascomycetes from Czechia were reported by Karel Kavina (1890–1948), Jaromír Klika (1888–1957) or Josef Velenovský (1858–1949) (Kavina 1921, Klika 1926a, 1926b, Velenovský 1922, 1934). Later, the research on bryophilous ascomycetes was continued mainly by Jiří Kubička (1913–1985), Mirko Svrček (1925–2017) and Jiří Moravec (born 1942) (Svrček & Kubička 1961, 1963, Moravec 1968, 1969, Svrček 1974, 1976). In this period, *Octospora gyalectoides* Svrček & Kubička and *O. pseudoampezzana* (Svrček) Caillet & Moyne were described based on Czech collections. Furthermore, Moravec reported *Octospora leucoloma* var. *tetraspora* (Fuckel) Benkert from Egypt (sub *O. tetraspora* var. *aegyptiaca* J. Moravec; Moravec 1972) and described *Octospora kilimanjarensis* J. Moravec from Tanzania (Moravec 1997).

1.3 Bryophytes as hosts of ascomycetes

Bryophyta is a sister clade to Tracheophyta, comprising three evolutionary lineages – mosses (Bryophyta), liverworts (Marchantiophyta) and hornworts (Anthocerotophyta). They are phylogenetically ancient; the oldest known macrofossil record comes from the Middle Devonian (Hernick et al. 2008) and microfossils assigned to bryophytes even from the Middle Ordovician (Rubinstein et al. 2010). Therefore, it is assumed that bryophilous fungi belong to the most ancient plant symbionts (von Arx 1985). It is estimated that there are 11000–13000 species of moss (Magill 2010), 7000–9000 species of liverwort (von Konrat et al. 2010) and 200–250 species of hornwort (Villarreal et al. 2010), all of them representing potential hosts of bryophilous fungi.

Bryophytes are unique hosts differing from other plants in many ways. Their life cycle is characterised by alteration of generations with a dominant haploid gametophyte, while the haploid stage is reduced in vascular plants. Moreover, they are usually small and therefore offer a limited source of nutrients to the associated fungi. Bryophytes are also known for indefinite growth in dense colonies without periodic leaf-fall and poikilohydry. Coevolution thus led to adaptations in bryophilous fungi which include biochemical adaptations to the host metabolism or phenological synchronization with the bryophyte, as well as morphological adaptations such as small size of the ascomata. To prevent dessication, perithecia or perithecium-like fruit bodies are more frequent than apothecia and the ascomata are formed preferably in moist microhabitats on the host plants. Moreover, these can contain a gelatinous material for better retention of water (Döbbeler 1997).

The majority of bryophilous ascomycetes is linked to mosses and leafy liverworts, while thalloid hepatics are inhabited less frequently and hornworts only rarely. Some species are plurivorous, e.g. *Acrospermum adeanum* Höhn. (Döbbeler 1979) or *Belonioscyphella hypnorum* (Syd. & P. Syd.) Höhn. (Döbbeler 1986, Egertová et al. 2016a), while others exhibit a high host specificity (e.g. bryophilous Pezizales). Beside the host specificity, preference of a certain microsite (microniche) can be found very frequently; this means that ascomata of certain species develop preferentially or exclusively on some host organ or its part, e.g. on rhizoids, stems, leaves, hyaline hairs points of the leaves, in leaf axils, within the thalli of liverworts, on perianths or antheridial cups (Döbbeler 2002).

Representatives of bryophilous Pezizales inhabit a broad range of hosts from all the three divisions of bryophytes. Most species have been reported on mosses, especially the acrocarpous ones. Certain genera seem to be extraordinarily popular hosts, e.g. *Bryum*, *Campylopus*, *Ceratodon, Ephemerum*, *Fissidens* or *Pleuridium* (Tab. 1). The genus *Neottiella* [not counting *N. ricciae* (P. Crouan & H. Crouan) Korf & W. Y. Zhuang, which is phylogenetically distant and should not be classified in this genus] is restricted to the family Polytrichaceae. Fewer species of bryophilous Pezizales are known on pleurocarpous mosses, including the monophyletic group containing *Octospora wrightii*, known as the section *Wrightoideae* Benkert which is linked to the order Hypnales. Species of the genera *Octosporopsis*, *Octosporella* and *Filicupula* are exclusively known from liverworts, with the leafy liverwort *Frullania* being the most popular, hosting at least ten pezizalean species. No bryophilous Pezizales have been described to infect a hornwort yet, though an undescribed *Octospora* on *Anthoceros* sp. was illustrated by Vega & Moyne (2019). Altogether, it has been proven that bryophilous Pezizales possess a very high degree of host specificity, usually restricted to one bryophyte species, genus or a few closely related genera (Egertová et al. 2018a, Vega et al. 2017, Vega et al. 2019).

Comprehensive reviews on bryophilous fungi including their host range were provided by Racovitza (1959) and Felix (1988). List of bryophilous Pezizales (including their description and their hosts) can be found at www.octospora.de, a webpage maintained by Jan Eckstein since 2010.

Table 1: Examples of bryophytes and associated bryophilous Pezizales (based on Eckstein 2023)

Host	Bryophilous Pezizales
Bryum	*Lamprospora faroensis* Benkert *Lamprospora minuta* (Velen.) Svrček *Lamprospora paechnatzii* Benkert *Lamprospora rugensis* Benkert *Lamprospora seaveri* agg. *Octospora bryi-argentei* Benkert *Octospora coccinea* (P. Crouan & H. Crouan) Brumm. *Octospora gemmicola* Benkert *Octospora* aff. *gyalectoides* *Octospora heterosculpturata* T. Schumach. *Octospora leucoloma* Hedw. *Octospora similis* (Kirschst.) Benkert *Octospora subglobispora* Benkert
Campylopus	*Lamprospora angularis* M. Vega, Ribes & Janošík *Lamprospora australis* (McLennan & Cookson) Rifai *Lamprospora campylopodis* W. D. Buckley *Lamprospora verrucispora* M. Vega, Eckstein & Van der Kolk
Ceratodon	*Lamprospora arvensis* (Velen.) Svrček *Lamprospora feurichiana* (Kirschst.) Benkert *Lamprospora kristiansenii* Benkert *Lamprospora seaveri* Benkert *Octospora hetieri* (Boud.) Dennis & Itzerott *Octospora rubens* Boud. *Octospora rustica* (Velen.) J. Moravec
Ephemerum	*Lamprospora annulata* Seaver *Lamprospora moynei* Benkert *Lamprospora tuberculatella* agg. *Octospora bridei* Caillet & Moyne *Octospora echinospora* Caillet & Moyne
Fissidens	*Lamprospora bulbiformis* M. Vega & Janošík *Lamprospora gibbosa* M. Vega & Janošík *Octospora fissidentis* Benkert & Brouwer *Octospora nemoralis* Benkert & Brouwer
Frullania	*Filicupula cyanopoda* Döbbeler & P. G. Davison *Filicupula sororia* Döbbeler & P. G. Davison *Filicupula suboperculata* (Döbbeler & P. James) Y. J. Yao & Spooner *Octosporella brevibarbata* Döbbeler & P. G. Davison *Octosporella caudifera* Döbbeler & P. G. Davison *Octosporella erythrostigma* (Mont.) Döbbeler *Octosporella hemicrypta* (Döbbeler) Döbbeler *Octosporella imitatrix* Döbbeler & P. G. Davison *Octosporella microtricha* Döbbeler, Negrín & M. Vega *Octosporella nematospora* Döbbeler & F. Berger
Pleuridium	*Lamprospora annulata* Seaver *Lamprospora rehmii* Benkert *Lamprospora tuberculata* Seaver *Lamprospora pseudoarvensis* M. Vega, Eckstein, Friebes & R. Tena

1.4 Trophic strategies of bryophilous ascomycetes

The majority of bryophilous ascomycetes are biotrophic parasites which form superficial or intercellular mycelium and do not discernibly damage the host (Döbbeler 1997). This category includes also bryophilous Pezizales, infecting different organs of bryophytes – most often rhizoids, but also the aboveground organs like protonemata, leaves, stems or thalli (Janošík et al. 2023), and forming elaborate infection structures consisting of superficial appressoria, infection pegs and intracellular haustoria (Döbbeler 1980c; Fig. 4). Certain species, e.g. *Lamprospora verrucispora*, *Octospora* spp. of the section *Wrightoideae*, *O. meslinii* (Le Gal) Svrček & Kubička or *O. pseudoampezzana* modify host rhizoids by inducing formation of galls, which help to increase the absorptive area (Döbbeler 1980c, Itzerott & Döbbeler 1982, Vega et al. 2016, Sochorová et al. 2020, Németh et al. 2022).

Necrotrophic parasites, such as *Acrospermum adeanum*, *Belonioscyphella hypnorum*, *Bryoscyphus dicrani* (Ade & Höhn.) Spooner, *Lizonia* spp., *Nectria muscivora* (Berk. & Broome) Berk. or *Roseodiscus subcarneus* (Sacc.) Baral, kill their host cells and cause necrotic and chlorotic lesions exhibited as discoloured zones in otherwise healthy colonies of the bryophytes (Döbbeler 1997). In polar areas, these can reach impressive size of several meters in diameter (Wilson 1951, Hawksworth 1973, Fenton 1983). The molecular mechanism behind the interaction remains unclear and no fungal toxins have been isolated (Davey & Currah 2006). However, all these necrotrophic parasites produce intracellular mycelium; their penetration is facilitated by penetration pegs and enzymatic digestion (Davey & Currah 2006). Some species (e.g. *Acrospermum adeanum* or *Nectria muscivora*) produce additional superficial mycelium to attack the bryophyte tissues (Döbbeler 1997).

Saprotrophs are rare among bryophilous ascomycetes, probably because bryophytes contain polyphenolic, lignin-like compounds which are either unsuitable as a substrate or toxic to most microorganisms (Verhoeven & Liefveld 1997). Nevertheless, it was demonstrated that some ascomycetes are capable to degrade the cell wall of bryophytes, e.g. *Oidiodendron maius* G. L. Barron, *O. periconioides* Morrall and *Pochonia bulbillosa* (W. Gams & Malla) Zare & W. Gams can decompose the cells walls of *Sphagnum fuscum* (Tsuneda et al. 2001, Rice et al. 2006).

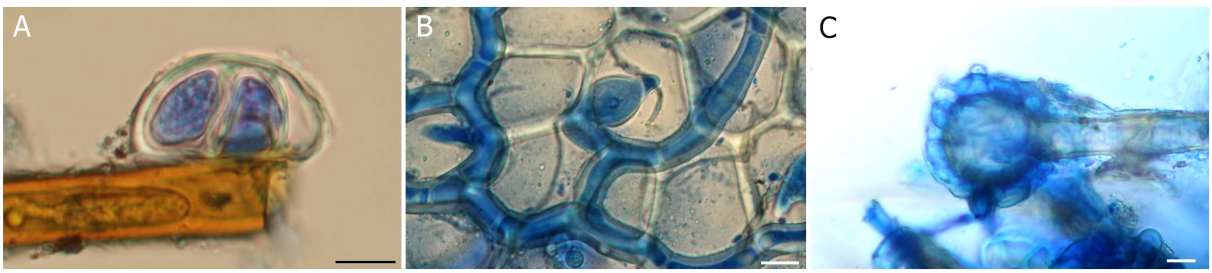

Fig. 4: Infection structures in bryophilous Pezizales. A) Appressorium of *Octospora affinis* on a rhizoid of *Orthotrichum affine*, B) superficial mycelium and appressorium (in the centre) of *Octosporella jungermanniarum* on a leaf of *Lophocolea heterophylla*, C) a gall induced by *Octospora svrcekii* on the rhizoid of *Cratoneuron filicinum*. Scales in all figures: 10 µm.

1.5 Ecology and geographical distribution

The occurrence of bryophilous ascomycetes always depends on the presence of their hosts. These grow on a broad spectrum of substrates including soil, wood, living bushes or trees, stones, boulders, rocks, houses, walls or excrements. The fungi can be found in very diverse habitats, both natural ones like forests, meadows, swamps or rivers, and man-influenced ones like cities, cemeteries or burnt places (Fig. 5).

Bryophilous ascomycetes are distributed worldwide but intensity of their research varies strongly between individual continents and regions. Most published data come from Europe (e.g. Benkert 1987, 1995, 2006, 2007, 2009, 2011, Caillet & Moyne 1980, 1987a, 1987b, Dennis & Itzerott 1973, Döbbeler 1978, 2006a, Döbbeler & Facher 2014, Döbbeler et al. 2018b, 2021a, 2021b, Eckstein & Eckstein 2009, 2013, Eckstein et al. 2020, Egertová et al. 2015, 2016a, 2016b, Huhtinen et al. 2010, Itzerott 1981, Jakobson et al. 1997, Jukić et al. 2018, Kristiansen 1999, 2006, 2007, 2013, Kristiansen & Olsen 2021, Kristiansen & Schumacher 1993, Marsh et al. 2010, Németh 2017, 2020, Racovitza 1959, Svrček 1948, Svrček & Kubička 1961, 1963, Van Vooren 2014, Vega 2017, Vega et al. 2016, 2021a, 2021b, Yao & Spooner 1996a, 1996b).

Less records are known from North America (Döbbeler & Davison 2017, 2019, 2021, Döbbeler et al. 2015, Huhtinen & Döbbeler 2018, Seaver 1912, 1914, Wang & Kimbrough 1992), South America (Döbbeler 1999, 2006b, 2007, Gamundí 1973, Suárez et al. 2023), Central America (Döbbeler & Menjívar 1992), Asia (Egertová et al. 2018b, Hosono et al. 2021, Uzun et al. 2018, Yuan et al. 2020, Berber et al. 2021), Africa (Lindemann 2013, Moravec 1972, 1997, Sochorová et al. 2019), Australia (McLennan & Cookson 1923, Rifai 1968, Janošík et al. 2022) and Antarctica (Olech & Mleczko 2000, Putzke & Pereira 2012). The lack of data on bryophilous ascomycetes in certain regions can be explained rather by the absence of mycologists and bryologists interested in them than the real absence of these often overlooked fungi. For example, Döbbeler found seven species of bryophilous Pezizales in a single collection of *Radula flaccida* from Tanzania, while only four species were known from the whole continent in that time (Döbbeler 1997). Similarly, 39 specimens of bryophilous Pezizales were collected during my three week stay in South Africa, although none had been known from the country (Sochorová et al. 2019). On the contrary, systematic surveys reveal high diversity in relatively small areas; 18 taxa of bryophilous Pezizales were reported from the Old Botanical Garden at Göttingen (Eckstein & Eckstein 2009), 28 taxa from the City of Hamburg (Vega 2017) and 57 taxa from Thuringia (Eckstein et al. 2020).

Fig. 5: Examples of habitats of bryophilous ascomycetes. A) **S**urroundings of a railway track in Istria, Croatia (locality of *Lamprospora densireticulata* on *Aloina ambigua*), B) yard in front of a house in Buzet, Croatia (*Octospora gyalectoides* agg. on *Pottia* cf. *lanceolata*), C) stone wall in Tbilisi, Georgia (*Lamprospora dictydiola* on *Tortula muralis*), D) sandstone rocks, Czech Republic (undescribed *Octospora* on *Cynodontium polycarpon*), E) stony ridge, Croatia (*Octospora leucoloma* on *Bryum argenteum*), F) forest spring, Georgia (*Pezoloma marchantiae* on *Marchantia polymorpha*), G) standing *Fagus* trunk in a beech forest, Armenia (*Octospora erzbergeri* on *Pseudoleskeella nervosa*), H) afromontane forest, South Africa (*Octospora conidiophora* on *Trichosteleum perchlorosum*), I) tropical rain forest on Borneo, Malaysia (*Octosporopsis erinacea* on *Dumortiera hirsuta*)

1.6 Threats and conservation

Basically, the threats of bryophilous ascomycetes follow the threats of their hosts. In Europe, 22.5% of bryophyte species are threatened with extinction (Hodgetts et al. 2019). The most serious factors are the habitat destruction and degradation, intensification of agriculture or climate change. Especially mountain species are sensitive to the increasing average temperature (Halda et al. 2016, Hodgetts et al. 2019).

Red list of fungi (macromycetes) of the Czech Republic includes a single species of bryophilous ascomycetes; *Octospora pseudoampezzana* (as *Hiemsia pseudoampezzana*) is listed as probably extinct (?EX) with a statement that it was not found for 50 years (Svrček 2006). Additionally, the red lists of some other countries, e.g. Austria (Dämon & Krisai-Greilhuber 2017), Switzerland (Senn-Irlet et al. 2007) or Germany (Matzke-Hajek et al. 2016) include representatives of bryophilous ascomycetes, mostly Pezizales. The recommendations for conservation of bryophytes, which are crucial also for the protection of bryophilous ascomycetes, include increased involvement of environmental agencies in the preparation of strategic plans, research and monitoring of targeted species, designation of protected areas, change of agricultural practices and land management, habitat restoration and rewilding, pollution reduction measures or *ex situ* conservation (Hodgetts et al. 2019).

2. Aims

While working on the Ph.D. thesis, I aimed to fulfill the following tasks:

- to describe new taxa based on own collections taking into account principles of vital taxonomy as well as developments in molecular biology;
- to study the diversity of bryophilous ascomycetes in Europe, especially within the Czech Republic;
- to verify whether bryophilous Pezizales occur in regions with no previous records, i.e. Borneo and South Africa;
- to describe the rarely reported species *Octospora svrcekii* Benkert based on vital characters;
- to test whether the species of the genus *Octospora* assigned to the section *Wrightoideae* form a monophyletic group.

3. Material and methods

3.1 Sample collection and observation

Publications presented in this thesis are based on the author's collections from the Czech Republic [*Bryorutstroemia fulva* (Boud.) Sochorová, Baral & Priou, *Octospora doebbeleri* Sochorová & Eckstein and all species presented in chapter 4.2], Austria (*O. svrcekii*), Germany (*B. fulva*), Poland (*B. fulva*), Slovakia (*Lamprospora sylvatica* Egertová & Eckstein), Ukraine (*L. sylvatica*), Croatia (*L. aberrans* Sochorová, M. Vega, J. Hernanz & Eckstein, *O. svrcekii*), South Africa (*Octospora conidiophora* agg.) and Borneo (*O. kelabitiana* Egertová & Döbbeler and *Octosporopsis erinacea* Egertová & Döbbeler) as well as on several specimens or documentation provided by co-authors and other colleagues.

Bryophytes were screened in the field by the naked eye or using a magnifying glass. Fungi were collected together with the host bryophytes to verify the host species and to study fungal infection structures. Macroscopic features were described from living apothecia. Whenever possible (in all species except *O. kelabitiana* and *O. erinacea*), microscopic characters were also studied in living cells and tissues in accordance with the standards of vital taxonomy (Baral 1992, Kušan 2015), as well as on rehydrated apothecia, using light microscopes with magnification up to 1600×. Media used included tap water (H_2O), 5% potassium hydroxide (KOH), Lugol's iodine solution (IKI), Melzer's solution (MLZ), Brilliant Cresyl Blue (CRB), Lactic Acid Cotton Blue (LACB), Lactophenol Cotton Blue (LPCB) and acetocarmine (AC). The solutions were bought at Myko-Service maintained by Andreas Gminder. In *Octospora doebbeleri*, nuclei were stained also in DAPI (10 µg/ml, 10 min, room temperature, dark) and fluorescence signal excited by a mercury lamp was combined with transmission light channel (50%:50%) (Olympus BX60 with attached DP73 camera). Measurements of living, freshly ejected ascospores were made on fully mature normally developed and randomly selected ascospores. Measurements were made directly with an ocular micrometer scale or in photographs using the Piximètre 5.10 software (Henriot & Cheype 2020).

Infection structures were studied in bryophilous Pezizales. This part of the work was usually performed by Jan Eckstein or Peter Döbbeler for their significant experience at this task. The bryophytes were perfectly cleaned in water, infection structures detected and observed in tap water and heated cotton blue.

The collections were dried using a drying machine, silica gel or air-dried, put in zip-lock bags and deposited in public herbaria and fungaria AH (University of Alcalá, Spain), CB (South Bohemian Museum, Czech Republic), CNF (Croatian Mycological Society, Croatia), PRA (Institute of Botany, Academy of Science, Czech Republic), PRC (Charles University, Czech Republic), PRM (National Museum, Czech Republic), SAR (Department of Forestry, Malaysia), UPS (Museum of Evolution, Sweden), VIT (The Natural History Museum of Alava, Spain) and WU (University of Vienna, Austria). All photographs presented in this thesis were taken by the author, except Fig. 5H and I by Michal Sochor.

The following research permits were used: OU-ZA-OSZP1-2017/003575-004/Ryb (Malá Fatra NP, Slovakia), 2198-1-90-02/01-16-1 (Paklenica NP, Croatia), OP 1264/2018 (South Africa) and NCCD.907.4.4(JLD.13)-337 (Borneo).

3.2 DNA extraction, PCR amplification and sequencing

DNA was extracted from fresh or dried apothecia using the CTAB method (Doyle & Doyle 1987). Apothecia were homogenised using a pestle and incubated in 300 µl of extraction buffer at 65 °C for one hour. The extract was subsequently purified in chloroform-isoamyl alcohol mixture (24:1), precipitated by isopropanol, washed in 70% ethanol, dried and finally dissolved in water and incubated with RNase (30 ug/ml) for 30 min at 37 °C. DNA quality was checked using agarose gel electrophoresis. Sequences were generated for chosen combinations of the following loci: internal transcribed spacers (ITS) of ribosomal DNA with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), the large subunit (LSU) of ribosomal DNA was amplified with primers NL1 (O'Donnell 1993) or LR0R (Vilgalys & Hester 1990) and NL4 (O'Donnell 1993) or LR6 (Vilgalys & Hester 1990); 18S subunit (SSU) of rDNA with primers NS1 and NS6 (White et al. 1990); and translation elongation factor-1alpha (EF1α) with primers EF1-983F and EF1-1567R (Rehner & Buckley 2005). PCR was performed with Kapa polymerase (Kapa Biosystems, Wilmington, Massachusetts, USA) or EliZyme FAST Taq mix (Elisabeth Pharmacon, Brno, Czech Republic), following standard protocol with 37 cycles and an annealing temperature of usually 54 °C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25M NaCl in the precipitation mixture) and sequenced in both directions using the Sanger method (Macrogen Europe, The Netherlands).

In the case of *Octospora svrcekii*, some sequences were obtained by Lukáš Janošík using a different method. For details, see Material and methods in Sochorová et al. 2020 (chapter 4.3.1).

3.3 Phylogenetic analyses

Sequences were edited and aligned in the Geneious software (Biomatters) using the MAFFT plugin. Phylogeny was reconstructed using the Maximum Likelihood (ML) or Maximum Parsimony (MP) method and tested by means of bootstrapping, using 1000 pseudoreplicates in MEGA (ver. 6.06, Tamura et al. 2011). Bayesian phylogeny inference (BI) was computed in MrBayes (ver. 3.2.4, Ronquist et al. 2012) – the parameters were set differently in each project, for details see the individual papers. The most suitable substitution model for each locus was determined in PartitionFinder 2.1.1 (Lanfear et al. 2017), using the AIC corrected for small samples (AICc). The Basic Local Alignment Search Tool (BLAST; Zhang et al. 2000) was used for searching similar sequences in publicly available sequence databases (https://blast.ncbi.nlm.nih.gov).

4. Results

4.1 Studies in bryophilous ascomycetes in Borneo and South Africa

4.1.1 *Octosporopsis erinacea* and *Octospora kelabitiana* (Pezizales) – two new hepaticolous ascomycetes from Borneo

Mycol Progress
https://doi.org/10.1007/s11557-017-1354-5

ORIGINAL ARTICLE

Octosporopsis erinacea and *Octospora kelabitiana* (Pezizales) – two new hepaticolous ascomycetes from Borneo

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Received: 6 September 2017 / Revised: 20 October 2017 / Accepted: 23 October 2017

Abstract Two new hepaticolous ascomycetes from the Kelabit Highlands (Sarawak, Borneo) are described and illustrated. Both of them infect thallose liverworts growing in damp and shaded localities. *Octosporopsis erinacea* parasitizing *Dumortiera hirsuta* (Marchantiopsida) has tiny, light yellow, rimless, setose apothecia, usually 8-spored asci and ellipsoid ascospores. Hyphae with appressoria develop superficially on, and intracellularly within, the host thallus. *Dumortiera* was recorded as a host for any bryophilous fungus for the first time. *Octospora kelabitiana* parasitizes species of the genus *Riccardia* (Jungermanniopsida). It is characterised by very small, light orange, setose apothecia, 8-spored asci, ellipsoid, biguttulate spores and very thick hyphae with conspicuous warts and ridges. Generic placement of both species was inferred based on DNA analysis of two nuclear loci (EF1α, LSU rDNA).

Keywords Bryophilous fungi · *Dumortiera* · Kelabit highlands · *Riccardia* · Sarawak

Section Editor: Roland Kirschner

This article is part of the "Special Issue on ascomycete systematics in honour of Richard P. Korf who died in August 2016".

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11557-017-1354-5) contains supplementary material, which is available to authorized users.

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Introduction

Borneo is the second largest tropical island in the world, well known for its extraordinary biodiversity (de Bruyn et al. 2014; Myers et al. 2000). Nevertheless, a large part of its biological richness still remains unknown. One such unexplored group of organisms is bryophilous Pezizales – obligate, highly adapted parasites of mosses and liverworts, comprising the genera *Octospora*, *Lamprospora*, *Neottiella*, *Octosporopsis*, *Octosporella*, and *Filicupula*. Their apothecia are coloured in shades of orange, red or yellow and reach sizes from ca 300 μm in *Octosporella* (Döbbeler 1980a) and *Filicupula* (Yao and Spooner 1996) up to over 10 mm in *Neottiella* or some *Octospora* species (e.g., Benkert 1998a; Caillet and Moyne 1987). Their hosts can be found on diverse substrates, such as soil, stones and rocks, leaves, bark, wood or excrements.

Bryophilous Pezizales have been mostly studied in Europe (e.g., Boudier 1907; Velenovský 1934; Caillet and Moyne 1980, 1987; Benkert 1987, 1998b, 2007, 2011; Benkert and Brouwer 2004; Schumacher 1993; Moyne et al. 2011), less so in the USA (Seaver 1912, 1914; Wang and Kimbrough 1992) and Australia (McLennan and Cookson 1923, 1926; Rifai 1968), while information from other parts of the world is rather sporadic. Only a few records have been published from the tropics. Seaver (1925) reported *Octospora leucoloma* Hedw. and *O. wrightii* (Berk. & M.A. Curtis) J. Moravec from

Puerto Rico. *Octosporella radulae* Döbbeler & Menjívar was described from Costa Rica (Döbbeler and Menjívar 1992), *O. epiphylla* Döbbeler & Beenken from Costa Rica and French Guiana (Döbbeler 2011). From Venezuela, *Neottiella vivida* (Nyl.) Dennis, *N. rutilans* (Fr.) Dennis (Dennis 1960), *Lamprospora carbonicola* Boud., *L. tuberculatella* Seaver (Dennis 1960, 1970) and *Octosporella hemicrypta* (Döbbeler) Döbbeler (Döbbeler 1978) are known. Moravec (1997) described *Octospora kilimanjarensis* J. Moravec from Tanzania, Lindemann (2013) reported *O. kilimanjarensis* and another undescribed species from Ethiopia. Chipp (1921) discovered *Neottiella rutilans* in the Malay Peninsula.

No data on bryophilous Pezizales have been published from Borneo, although the island has a rich bryoflora (Menzel 1988, Suleiman et al. 2006, Chuah-Petiot 2011) and, therefore, a good potential to offer suitable substrates. Recently, two new hepaticolous species were found in the Kelabit Highlands in Sarawak (Malaysian part of Borneo). They are described in this study as *Octosporopsis erinacea* sp. nov. and *Octospora kelabitiana* sp. nov.

Material and methods

Sample collection and observation

The study is based on collections from the vicinity of Pa′ Lungan and Pa′Umor villages in the Bario part of the Kelabit Highlands, Sarawak, Malaysia, sampled in February 2016 and January 2017. Fresh fungi were photographed and macroscopically described and then dried in silica gel. Fragments of rehydrated apothecia were examined using standard light microscopy. Microcharacters were measured and illustrated in lactophenol cotton-blue (CB), if not otherwise stated. Amyloidity was tested in Lugol's solution.

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried apothecia by the CTAB method (Doyle and Doyle 1987). Up to three apothecia were homogenised by a pestle, incubated in 300 μl extraction buffer at 65 °C for one hour; the extract was subsequently purified in chloroform–isoamyl alcohol mixture, precipitated by isopropanol and finally dissolved in water and incubated with RNase for 30 min. at 37 °C. DNA quality was checked on agarose gel. Molecular sequence data were generated for two loci: a large subunit of ribosomal DNA (LSU) was amplified with primers LR0R and LR6 (Vilgalys and Hester 1990), and translation elongation factor-1alpha (EF1α) with primers EF1-526F (Rehner 2001) and EF1–1567R (Rehner and Buckley 2005). PCR was obtained with Kapa polymerase (Kapa Biosystems, Wilmington, USA) following a standard protocol with 37 cycles and annealing temperature of 56 °C or 54 °C for LSU and EF1α, respectively. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced from both directions by the Sanger method at Macrogen Europe, Amsterdam, The Netherlands. Sequences were edited and aligned in GENEIOUS (version 7.1.7; Kearse et al. 2012) together with a diverse set of species of *Octospora* and related genera (Online Resource 1).

Phylogenetic analysis

The most suitable substitution model was determined in jModelTest (ver. 2.1.4; Darriba et al. 2012) using Akaike Information Criterion (AIC). Phylogeny was inferred by the Bayesian approach in MrBayes (ver. 3.2.4; Ronquist et al. 2012) with a GTR + I + G substitution model and 10^6 generations, sampling every 1000th generation, in two independent runs, each with four chains; the first 250,000 generations were excluded as burn-in. Maximum parsimony trees were computed in MEGA (ver. 5.2; Tamura et al. 2011) by the Subtree-Pruning-Regrafting method and a MP search level of 3 and tested by bootstraping with 100 replications.

Results

Phylogenetic analysis

For a total of 17 species (incl. Two outgroup species), the EF1α sequences provided a 559 bp long alignment with 270 (48%) variable sites, mostly (260) within the ingroup. The LSU locus was analysed in 23 species and provided 1095 bp alignment with 457 (42%) variable sites, of which 450 were variable within the ingroup. A small intraspecific polymorphism was observed in *O. kelabitiana* both at LSU (1.3% variable positions in the total alignment, mostly indels in microsatellite motives) and EF1α (1.4%), and in *O. erinacea* only at LSU (0.1%). Although LSU provided a better resolved phylogeny tree, analyses of both of the loci resulted in similar tree topology both with the Bayesian approach and maximum parsimony (Online Resource 2). *O. erinacea* was placed unambiguously and with high statistical support as sister to *Octosporopsis nicolai*. *O. kelabitiana* was nested among other *Octospora* species. Analysis of concatenated alignment placed *O. kelabitiana* as a basal lineage of the *O. ithacaensis/humosa/wrightii* group (Fig. 1).

Taxonomy

Octosporopsis erinacea Egertová & Döbbeler, sp. nov.
Figures 2 and 3, Online Resource 2.
MycoBank: ***MB 822433.***

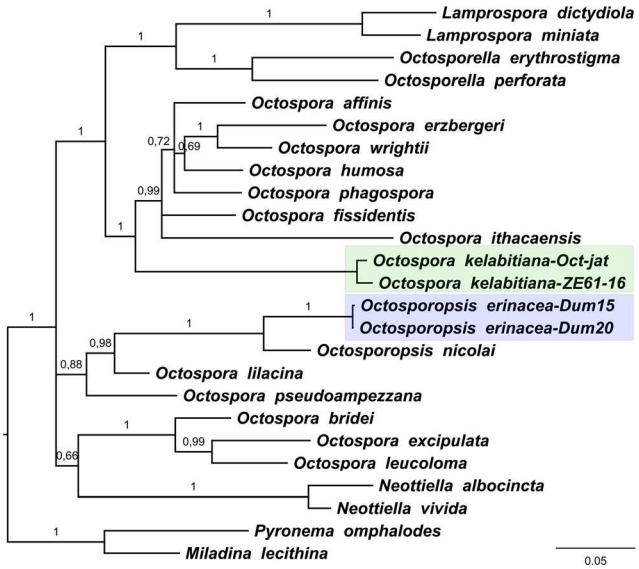

Fig. 1 Bayesian phylogeny inference based on concatenated alignment of LSU and EF1α sequences. Bayesian posterior probabilities are shown above branches; *Pyronema omphalodes* and *Miladina lecithina* serve as an outgroup; Bayesian and maximum parsimony trees based on analysis of each locus are shown in Online Resource 2

Etymology: From the Latin *erinaceus* (with bristles like a hedgehog); named after the conspicuous setae on the apothecia.

Holotype: Malaysia: Borneo, Sarawak, Kelabit Highlands, Pa′Lungan, upper Pa′Lungan river, 5.5 km N of the village, 3°51′41″N, 115°31′19″E, 1255 m asl., on *Dumortiera hirsuta* on a boulder in a riverbed, 20 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–20-01; isotype PRM 945774).

Diagnosis: Differs from all other species of bryophilous Pezizales by the following combination of characters: apothecia very small, pale yellow to almost colourless, without a rim, setose; excipulum with *textura angularis*; asci usually 8-spored; ascospores ellipsoid, delicately rough; superficial and internal, intracellular mycelium present, consisting of thick and thick-walled hyphae with appressoria and often mycelial haustoria; parasitic on thalli of *Dumortiera hirsuta*.

Description: Apothecia superficially on the dorsal thallus surface, exceptionally also on male receptacles, at first more or less barrel-shaped, later turbinate, light yellow to almost colourless, setose, 400–600 μm high and 450–550 μm wide, apically with a plane, rimless, concolorous disc, basally

Fig. 2 *Octosporopsis erinacea* (**a**. SAR KH2017–1–16-02; **b**, **c**. SAR KH2017–1–20-01, PRM 945774; **d**. SAR KH2017–1–20-01, PRM 945774) **a**, **b**, **c**. apothecia on thalli (a, b) and atheridia (c) of *Dumortiera hirsuta,* **d**. locality of the holotype. For more pictures see Online Resource 3

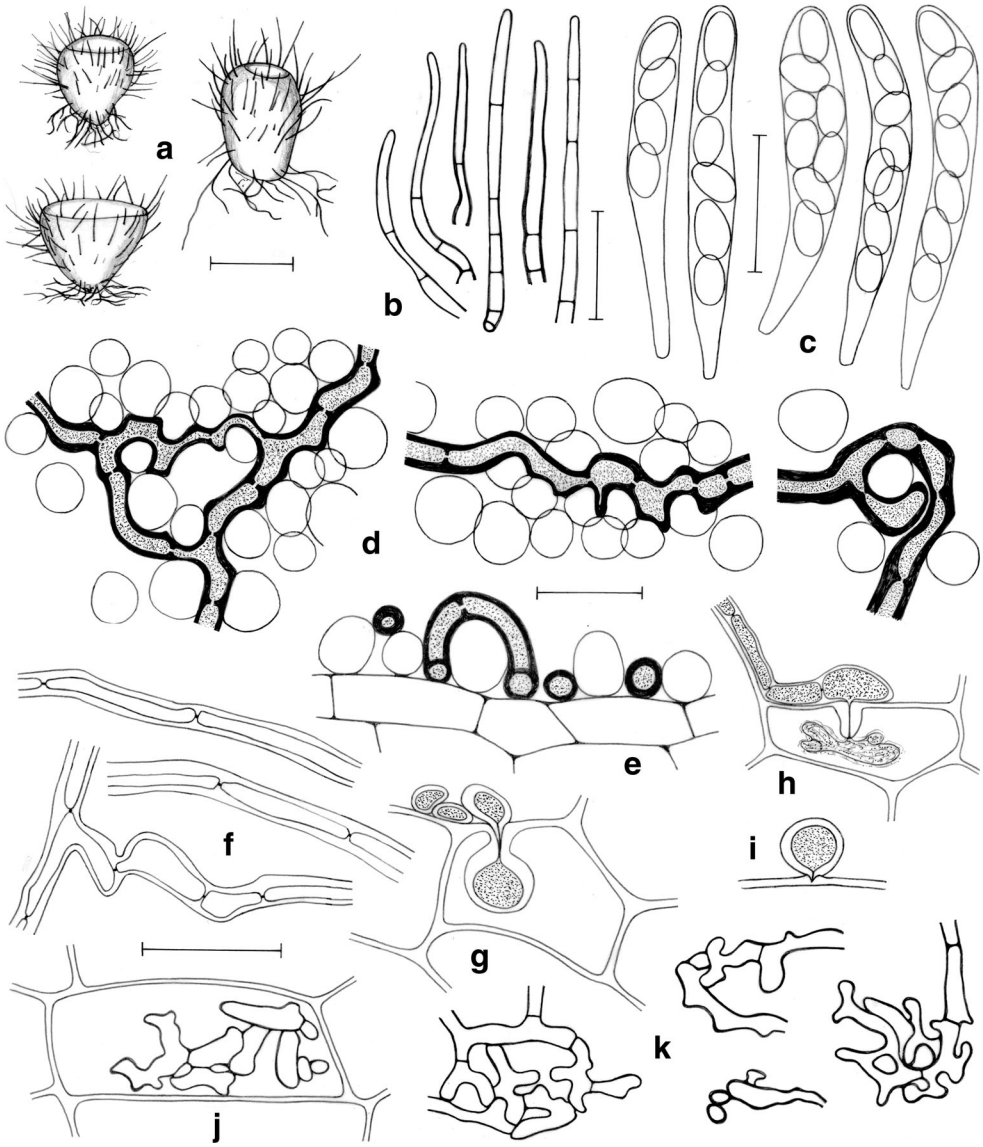

Fig. 3 *Octosporopsis erinacea* (**a**–**k**. PRM 945775). **a**. apothecia, **b**. setae, **c**. asci with 4, 6, 7 or 8 spores, **d**, **e**. hyphae (walls black, cytoplasm stippled) at the dorsal thallus side growing between papillate cells in surface view (**d**) and section (**e**), **f**. hyphae of the ventral thallus side (without papillate cells), **g**–**k**. hyphae within the cells of the host thallus as seen in sections (cytoplasm of hyphal cells stippled in **g**–**i**), **g**. two hyphae and an appressorium with haustorium encapsulated by the host cell wall, **h**. infection apparatus consisting of appressorium, haustorium and penetration peg surrounded by a lignituber-like swelling of the host cell wall, **i**. beginning penetration of a host cell wall, **j**, **k**. haustoria-like hyphae, infected cell indicated in **j.** Scale bars: **a** = 500 μm; **b** = 100 μm; **c** = 50 μm; **d**, **e** = 40 μm; **f**–**k** = 50 μm. Drawn by P. Döbbeler. For microphotographs see Online Resource 3

connected to the substrate by numerous anchoring hyphae that remain on detached ascomata. Setae numerous, mostly straight and stiff, generally oriented upwards, arising at all parts of the excipulum, projecting above the apothecial disc, slightly tapering towards the apex, blunt, up to 300(350) μm long, at the base 10–20(25) μm wide, at the apex 6–13 μm wide, wall 1–4 μm thick, lumina sometimes reduced, with one to four septa; even apothecial primordia with long setae. Excipulum seen externally with angular or slightly sinuose, (10)15–30(40) μm large cells. Paraphyses filiform, unbranched, straight, very pale, septate, 3–4 μm broad, apically slightly enlarged and 3–7 μm wide. Asci unitunicate, operculate, not amyloid, cylindrical to usually claviform (depending on spore position), with a spore-free foot, 120–160 × 14–24 μm, (4-, 5-, 6-, 7-) or 8-spored. Ascospores variable even in the same ascus, ellipsoid, colourless, (16)18–25(31) × 10–14(16) μm, epispore delicately rough, 1- or partly 2-seriate. Hyphae variable, abundant, on and within the host thallus, colourless, with ramifications, anastomoses and appressoria; external hyphae irregularly growing on both sides of the thallus surface, 6–11(15) μm wide; walls up to 5 μm thick, smooth; hyphae at the dorsal side growing between papillate cells often sinuose and with varying diameter due to available space. Appressoria comparatively scarce and inconspicuous, 1-celled, lateral ones on short stalks, terminal and also intercalary, elliptical (as seen from above), about 25–40 μm long and 14–20 μm wide, giving rise to internal, sometimes haustoria-like hyphae. No anamorph observed. Cell walls of excipulum, setae, ascospores, external and internal hyphae and appressoria cyanophilous, especially the epispore and the outermost layer of mycelial elements; cytoplasmic content of mature spores not reacting with CB.

Host: *Dumortiera hirsuta* (Sw.) Nees var. *nepalensis* (Taylor) Frye & L. Clark (Dumortieraceae, Marchantiales, Marchantiopsida).

Known geographical distribution: So far known only from the vicinity of Pa′Umor and Pa′Lungan villages in the Bario part of the Kelabit Highlands, Sarawak, Malaysia.

Additional specimens examined: Malaysia: Borneo, Sarawak, Pa′Lungan, Arur Bedalawid, 3.17 km N of the village, 3°50′21″N, 115°30′58″E, 1260 m asl., on *Dumortiera hirsuta* on a boulder in a riverbed, 4 Feb 2016, leg. Z. Egertová and M. Sochor (PRM 945775). Pa′Lungan, Arur Bedalawid, 3.12 km N of the village, 3°50′20″N, 115°31′1″E, 1230 m asl., on *Dumortiera hirsuta* on a boulder in a riverbed, 15 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–15-04, PRM 945776); ibid. 16 Jan 2017 (SAR KH2017–1–16-02).

Octospora kelabitiana Egertová & Döbbeler, sp. nov.
Figures 4, 5 and 6, Online Resource 2.
MycoBank: ***MB 822432.***

Etymology: Named after the Kelabit Highlands and their inhabitants, the Kelabit people.

Holotype: Malaysia: Borneo, Sarawak, Kelabit Highlands, Pa′Lungan, Pa′Lungan river, 3.85 km N of the village, 3°50′46″N, 115°31′23″E, 1170 m asl., on *Riccardia* sp. on a decaying trunk in a riverbed, 20 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–20-03; isotype PRM 945777).

Diagnosis: Differs from all other species of bryophilous Pezizales by following combination of characters: apothecia very small, often taller than wide, light flesh-coloured or orange, without a rim, setose; excipulum with *textura angularis*; asci 8-spored; ascospores ellipsoid, usually with two guttules, delicately rough; mycelium superficial, consisting of very thick hyphae with conspicuous warts and ridges, appressoria and intracellular haustoria; parasitic on thalli of *Riccardia* spp.

Description: Apothecia superficially on the dorsal thallus surface, barrel- or cup-shaped, tapering at the base, light flesh-coloured or orange, sometimes with a pink tint, setose, ca. 180–350 μm high, 160–400 μm wide, often taller than wide, apically with a plane or slightly concave, rimless, concolorous disc, basally connected to the substrate by numerous anchoring hyphae that remain on detached ascomata. Setae numerous, straight and stiff, mostly oriented upwards, arising from all parts of the excipulum, considerably projecting above the ascoma apex, gradually tapering towards the apex, blunt to subacute, with walls up to 4 μm thick, (35)60–150(190) μm long, basally 7–11(12.5) μm wide, with one to three septa,

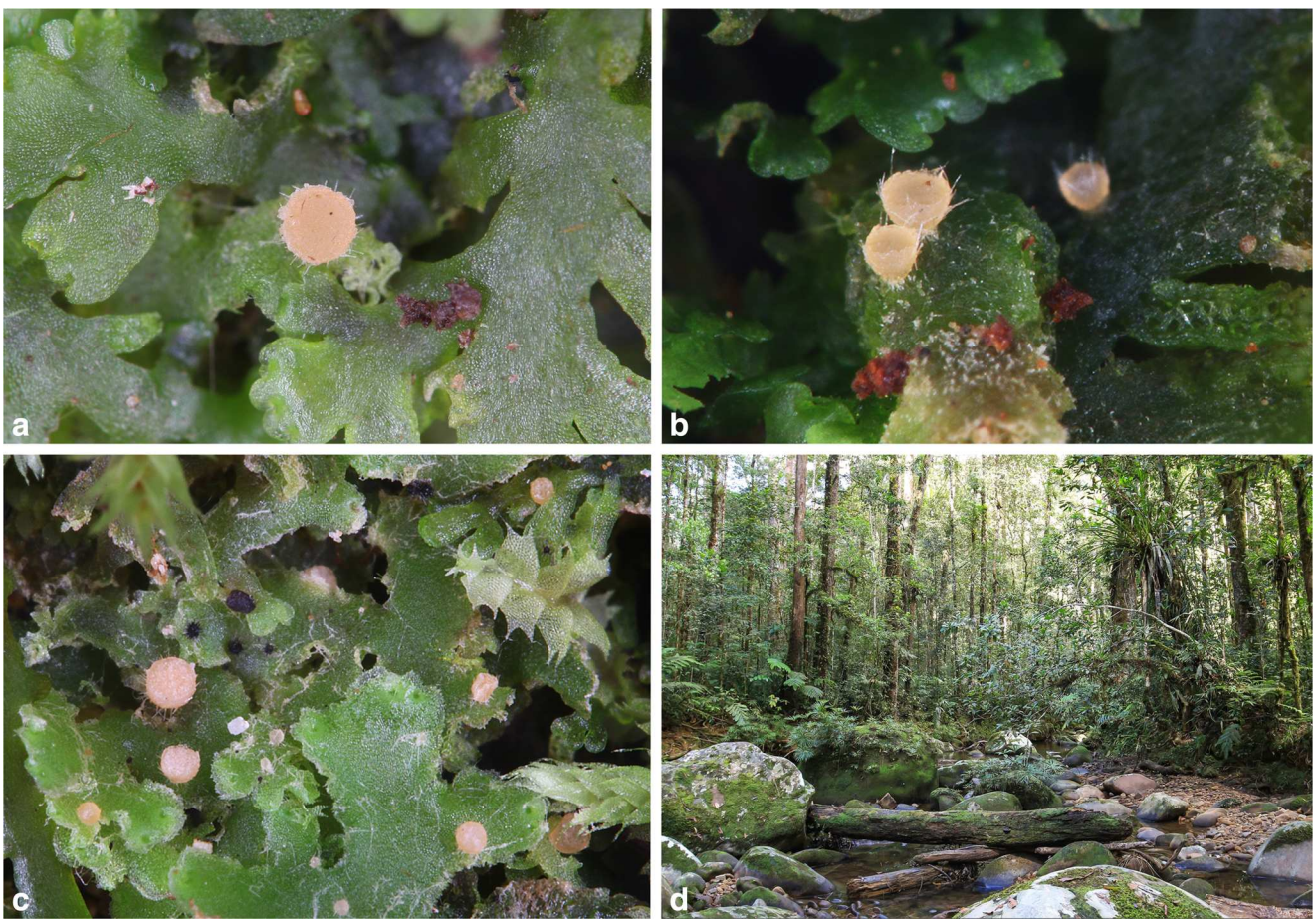

Fig. 4 *Octospora kelabitiana*. (**a**. SAR KH2017–1–14-01, PRM 945779; **b**. SAR KH2017–1–15-01, PRM 945782; **c**. SAR KH2017–1–15-03, PRM 945784; **d**. SAR KH2017–1–20-03, PRM 945777) **a**, **b**, **c**. apothecia on thalli of *Riccardia* spp. **d**. locality of the holotype. For further pictures see Online Resource 3

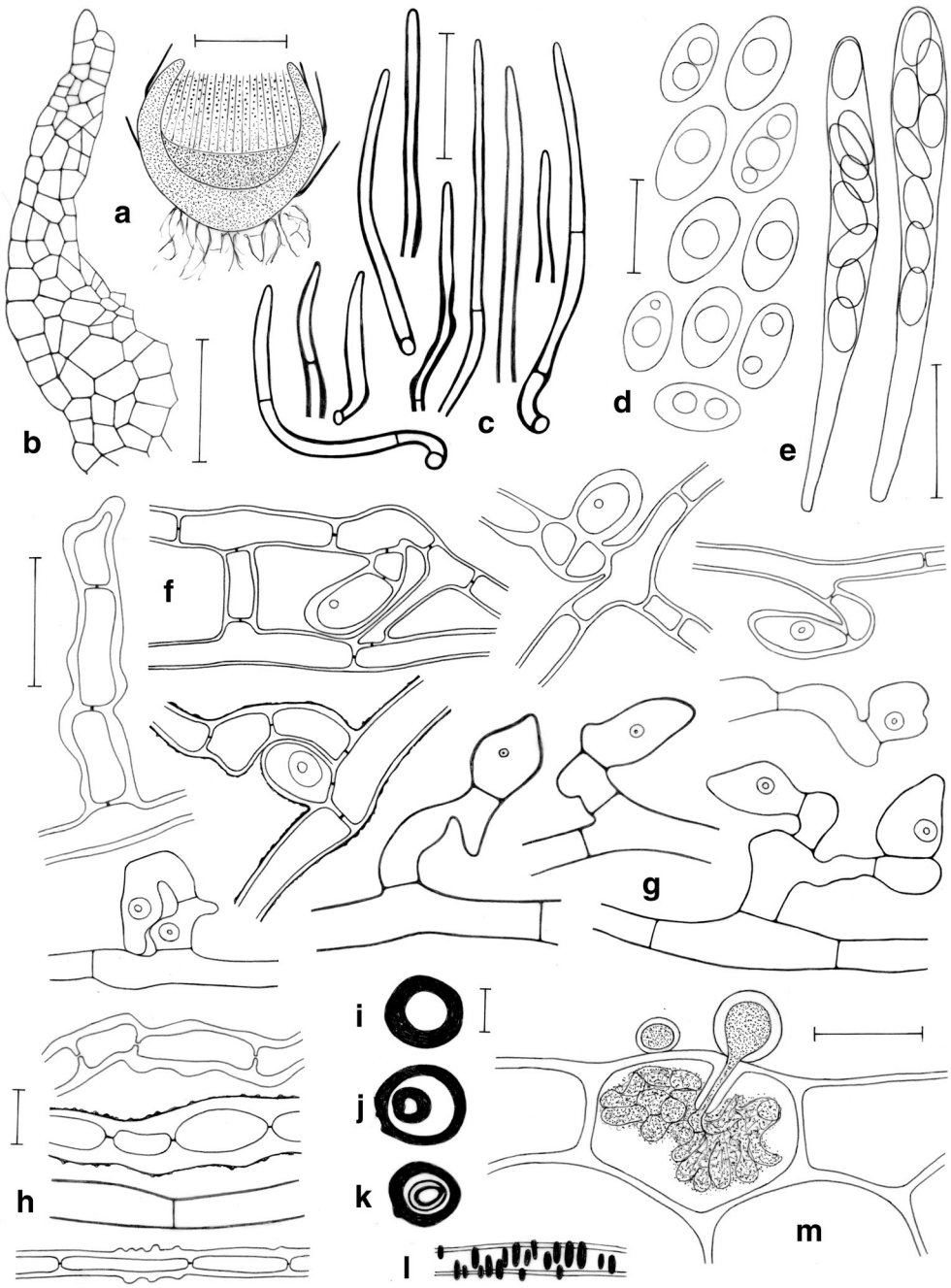

Fig. 5 *Octospora kelabitiana* (**a**–**m**. PRM 945781) **a**. apothecium in longitudinal medium section (schematically), **b**. lateral excipulum in longitudinal section, **c**. setae, **d**. ascospores (in H_2O), **e**. asci, **f**–**m**. hyphal features, **f**. superficial, thick-walled hyphae and appressoria with penetration pegs seen from above, **g**. thin-walled hyphae within thallus cells of the host, appressoria with penetration pegs, **h**. different hyphae, **i**–**k**. hyphae (walls black) in transverse sections, **i**. without internal hyphae, **j**. with one intrahyphal hypha, **k**. with two intrahyphal hyphae, **l**. hypha with conspicuous transversely elongated warts (black), **m**. infection apparatus consisting of appressorium, haustorium and penetration peg surrounded by a lignituber-like swelling of the host cell wall (cytoplasm stippled). Scale bars: **a** = 150 μm; **b** = 50 μm; **c** = 60 μm; **d** = 15 μm; **e** = 35 μm; **f**, **g** = 25 μm; **h**, **l** = 10 μm; **i**–**k** = 5 μm; **m** = 20 μm. Drawn by P. Döbbeler. For microphotographs see Online Resource 3

basally often sharply bent upwards. Excipulum from external view and in longitudinal section with *textura angularis*, cells about 7–23(28) μm; excipular wall laterally composed of few cell layers, about 8–15(23) μm thick; below the hymenium a zone of distinctly smaller, irregularly arranged cells. Paraphyses filiform, unbranched, apically slightly enlarged, 2–3.5 μm wide in the middle, 2.5–4 μm at the apex. Asci unitunicate, operculate, not amyloid, cylindrical to slightly claviform (depending on spore position), with a rather long spore-free foot, 98–150 × 10–16 μm, (4-, 7-)8-spored. Ascospores ellipsoid, colourless, in H_2O (13.5)14.5–17(18) × 7–8(9) μm, in CB (12.5)13–16(17) × (6.5)7–8(8.5) μm, containing (1)2(3) rather large guttules measuring up to 6.5 μm in diameter (when single), epispore delicately rough (or seemingly smooth), 1- or partly 2-seriate; cytoplasmic content of mature spores not reacting with CB. Hyphae variable, abundant, irregularly growing over the dorsal and ventral thallus surfaces, some hyphae occasionally also within the thallus, with ramifications and anastomoses, often thick-walled, (3)5–9(−11) μm wide (without warts); hyphae smooth, verruculose or more or less densely covered with conspicuous warts up to 2.5 μm high and 3.5 μm wide (in optical section); warts often elongating perpendicularly to the direction of the growing hyphae forming transverse ridges; hyphae within hyphae

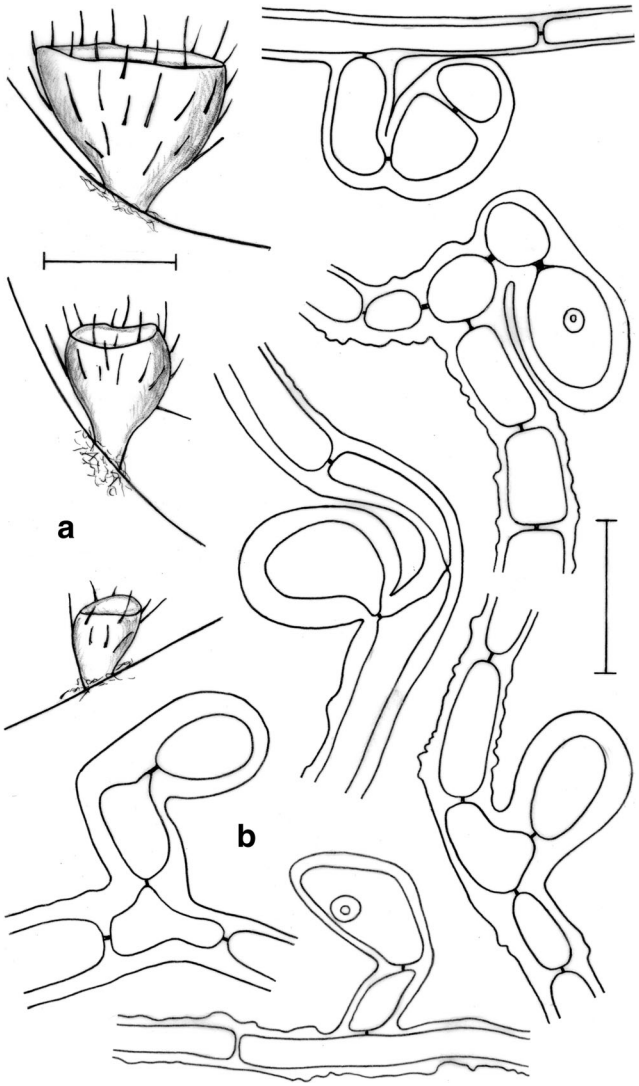

Fig. 6 *Octospora kelabitiana* (**a**–**b**. PRM 945780). **a**. mature apothecia, **b**. superficial hyphae with appressoria, two of them with penetration pegs seen from above. Scale bars: **a** = 300 μm; **b** = 20 μm. Drawn by P. Döbbeler. See Online Resource 3 for microphotographs

frequently present. Appressoria 1-(2-)celled, formed laterally at the hyphae, sessile or with a 1-celled stalk, stalk and/or appressorium often bent; appressoria in surface view elliptical, (14)16–23(26) μm long, (8)10–15(18) μm wide, about 12 μm high, walls up to 4 μm thick, smooth or verruculose; outgrowing or anastomosing appressoria result in an intercalary position; each appressorium connected to a haustorium by a perforation peg; pegs surrounded by lignituber-like tubes consisting of host cell wall material, up to 7(10) μm wide; perforations seen from above as circles. Haustoria intracellular, consisting of thick, thin-walled and intricate filaments rich in cytoplasmic content, sometimes occupying the whole infected cell, restricted to the invaded cell; sometimes a bladder-like cell is visible between the end of the perforation peg and the haustorial filaments; in comparison to the numerous appressoria relatively few haustoria observed. No anamorph observed. Cell walls of excipulum, setae, ascospores, hyphae and appressoria cyanophilous, especially the epispore and the outermost layer of mycelial elements including wart-like outgrowths.

Hosts: *Riccardia* spp. (Aneuraceae, Metzgeriales, Jungermanniopsida).

Known geographical distribution: So far known only from the vicinity of Pa′Umor and Pa′Lungan villages in the Bario part of the Kelabit Highlands, Sarawak, Malaysia.

Additional specimens examined:

Malaysia: Borneo, Sarawak, Pa′Umor, 4.15 km SE of the village, on Riccardia sp. on a decaying stem in a brook in a forest, 3°42′17″N, 115°31′45″E, 1145 m asl., 6 Feb 2016, leg. Z. Egertová and M. Sochor (PRM 945780); Pa′Umor, 2.7 km SE of the village, 3°42′59″N, 115°31′22″E, 1115 m asl., on *Riccardia* sp. on a decaying trunk near a river, 14 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–14-01, PRM 945779); Pa′Umor, eastern margin of the village, 3°44′4″N, 115°30′55″E, 1070 m asl., on Riccardia sp. on a decaying trunk in a forest, 10 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–10-01, PRM 945778); Pa′Lungan, 2.9 km NE of the village, on *Riccardia* sp. on a decaying stem in a brook in a forest, 3°49′46″N, 115°32′29″E, 1135 m asl., 3 Feb 2016, leg. Z. Egertová and M. Sochor (PRM 945781); Pa′Lungan, Arur Bedalawid, 2.95 km N of the village, 3°50′16″N, 115°31′7″E, 1190 m asl., on *Riccardia* sp. on a decaying trunk in a river, 15 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–15-01, PRM 945782); Pa′Lungan, Arur Bedalawid, 3 km N of the village, 3°50′17″N, 115°31′7″E, 1195 m asl., on *Riccardia* sp. on a decaying trunk in a river, 15 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–15-02, PRM 945783); Pa′ Lungan, Arur Bedalawid, 3.05 km N of the village, 3°50′18″ N, 115°31′2″E, 1225 m asl., on *Riccardia* sp. on a decaying trunk in a river, 15 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–15-03, PRM 945784); Pa′Lungan, Arur Bedalawid, 3.09 km N the village, 3°50′19″N, 115°31′1″E, 1230 m asl., on *Riccardia* sp. on a decaying trunk in a river, 16 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–16-01, PRM 945785); Pa′Lungan, Pa′Lungan river, 5 km N of the village, 3°51′23″N, 115°31′15″E, 1260 m asl., on *Riccardia* sp. on a decaying trunk in a river, 20 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–20-07, PRM 945787); Pa′Lungan, Upper Pa′Lungan river, 5.5 km N of the village, 3°51′40″N, 115°31′19″E, 1250 m asl., on Riccardia sp. on a boulder in a river, 20 Jan 2017, leg. Z. Egertová and M. Sochor (SAR KH2017–1–20-02, PRM 945786).

Discussion

Generic placement

The new species clearly belong to a well circumscribed group of bryophilous Pezizales, which includes the six genera

Octospora, *Lamprospora*, *Neottiella*, *Octosporopsis*, *Octosporella,* and *Filicupula*. Ecologically these fungi are characterised by parasitism on mosses and liverworts, morphologically – as far as analysed – by elaborate infection structures consisting of distinct appressoria and intracellular haustoria. However, the generic placement of both species was problematic due to the unusual combination of small apothecia with an exposed hymenium, presence of setae and infection of thallose liverworts. Another complicating fact is that some of the genera are polyphyletic and the taxonomic concept requires a thorough revision (Perry et al. 2007, Hansen et al. 2013, Z. Egertová unpubl. data, L. Janošík pers. comm.).

With the help of molecular data, we decided to classify the parasite of *Riccardia* as *Octospora* and that one of *Dumortiera* within *Octosporopsis. Octospora* is a polyphyletic genus, as preliminary molecular studies show (Perry et al. 2007, Hansen et al. 2013, Z. Egertová unpubl. data, L. Janošík pers. comm., see also Fig. 1). In its current concept it includes more than 50 species with discoid ascomata and ellipsoid, broadly ellipsoid, subglobose or fusiform ascospores, which can be smooth or ornamented with warts or ridges. Setae can be found in some species, e.g., *Octospora erzbergerii* Benkert, *O. hetieri* (Boud.) Dennis & Itzerott or *O. ithacaensis* (Rehm) K.B. Khare. Growth on liverworts is exceptional in the genus. Morphologically the most similar species to *O. kelabitiana* is *O. ithacaensis*, a parasite of *Marchantia polymorpha* L. Besides the growth on thallose liverworts, they share pale, rimless apothecia with more or less adjacent hyphae and setae. Ascospores are similar in shape and presence of two guttules, but in *O. ithacaensis* they are slightly larger (18–22 × 9–11 μm) and ornamented with distinct warts (e.g., Khare 1975; Benkert 1998c; Egertová et al. 2015). Gamundí (1973) described *Lamprospora cashiae* Gamundí from Tierra del Fuego as growing "between liverworts, especially *Riccardia* sp." Microscopically the species differs strongly from *O. kelabitiana*. In the original description spores are characterised as globose with diameter 14.3–17.6(19.2) μm, ornamented with 1–1.6 μm high warts. Benkert (1998d) found broadly ellipsoid spores with size 18–22 × 16–19 μm in the holotype and transferred the species to the genus *Octospora*.

The genus *Octosporopsis,* recently separated from *Kotlabaea* mainly by molecular data, was so far monotypic, including only *O. nicolai* (Maire) U. Lindem., M. Vega & T. Richt. Both it and *O. erinacea* inhabit thallose liverworts; *O. nicolai* grows on *Lunularia cruciata* (L.) Lindb. Both species also share thick-walled setae and ellipsoid, hyaline spores without large guttules. However, there are also certain differences between them. *O. nicolai* has apothecia reaching up to 7 mm in diameter, while those of *O. erinacea* are maximally 600 μm wide. In *O. nicolai,* they are at first obconical, then disc-shaped, with light yellow, pink or orange colour, while in *O. erinacea,* they are at first barrel-shaped, later turbinate, light yellow to almost colourless. Spores of *O. nicolai* are ca 1.5× larger, measuring (26)27–39(41) × (12)15–18.5(20.5) μm (Lindemann et al. 2014).

Classification of the two new species from Borneo to the genus *Lamprospora* was excluded. First, its species have globose, less often subglobose or broadly ellipsoid ascospores, which are variously ornamented (Benkert 1987). An exception would be *Humaria aurantiaca* Bres., recently combined in the genus *Lamprospora* based on molecular characters (Lindemann and Alvarado 2017). However, we consider this combination dubious, as the species has smooth ellipsoid spores, tetrasporic asci and an unproven relationship with bryophytes. The sequences obtained could be a result of contamination. Second, almost none of *Lamprospora* species has setae. The only exception known to us is an undescribed species from Croatia, growing on *Gymnostomum lanceolatum* M.J. Cano, Ros & J. Guerra (Z. Egertová unpubl. data). Species of *Lamprospora* parasitize mostly acrocarpous mosses, only *L. aneurae* Benkert grows on the liverwort *Aneura pinguis* (L.) Dumort. This species, so far known only from Germany, has globose to subglobose spores (12.5–15 × 12–13 μm) ornamented with 0.1–0.2 μm broad ridges which form a dense irregular reticulum (Benkert 1990).

Small size of fruitbodies, setae and growth on liverworts are typical for the genus *Octosporella*, but all its ten species deviate by having ovoid, ellipsoid or barrel-shaped ascomata which resemble perithecia (Döbbeler 1980b; Yao et al. 2006; Moyne et al. 2011).

The genus *Filicupula* was established to segregate *Octosporella suboperculata* (Döbbeler & P. James) Döbbeler from the genus *Octosporella*. *Filicupula suboperculata* (Döbbeler & P. James) Y.J. Yao & Spooner, so far still the only species in the genus, differs from both of the new species in the filamentous structure of the excipulum, the manner of ascus dehiscence and absence of setae (Döbbeler 1978; Yao and Spooner 1996).

Neottiella is a genus with an unstabilized concept; several species have been assigned to *Neottiella* and *Octospora*, depending on the author. The type species *Neottiella albocincta* (Berk. & M.A. Curtis) Sacc. and two other related species – *Neottiella rutilans* (Fr.) Dennis and *N. vivida* (Nyl.) Dennis – resemble the two new species in having distinct setae and typical apothecia. However, fruitbodies of *Neottiella* are much larger, they often reach over 10 mm. They differ also in parasitizing mosses from the family Polytrichaceae (Benkert 1994).

Ecology

Both species were found in similar ecological conditions. *Octospora kelabitiana* was collected in lower-montane primary rainforests far from villages in most cases, only once in a

man-influenced (probably secondary) forest at a margin of the Pa′Umor village, in altitude 1070–1260 m asl. It grew on *Riccardia* spp. that covered decaying trunks or, in a single case, a boulder. The host species always occurred in shady places in surroundings of brooks or rivers or directly in them. *O. kelabitiana* seems to be rather frequent in the Kelabit Highlands, as it was discovered in nine localities with a distance of at least 100 m from each other. It often fructified in large groups counting tens or even hundreds apothecia.

Octosporopsis erinacea occurred in the same type of forest as *Octospora kelabitiana,* in altitude 1230–1260 m asl. It grew on *Dumortiera hirsuta* covering boulders in rivers. The fungus can occur repeatedly (or continuously) in its localities, as it was noticed already in February 2016 in Arur Bedalawid and almost in the same place in January 2017. In the studied region, it is probably distinctly rarer than *Octospora kelabitiana*; it is so far known from three localities (and two of them are located only ca 100 m from each other). In the Arur Bedalawid waterfalls, *O. erinacea* and *O. kelabitiana* grew only a few meters apart.

The higher altitude connected with lower temperatures may be an important factor influencing the occurrence of bryoparasitic Pezizales in Borneo. While around twenty populations of five species were found in the Kelabit Highlands during our two excursions, none was recorded in the lowland areas of Borneo, to which similar efforts were dedicated.

Host bryophytes, infectious structures

Hyphae of *Octospora* and related genera within the host tissue are usually confined to haustoria within single host cells. The most commonly infected parts are rhizoids, leaves, outermost cells of stem and epidermal cells of thalli. Mycelia within the interior of thallose liverworts, which were observed in the two new species from Borneo, have so far been only sporadically recorded (e.g., in *Marchantia polymorpha* and *Riccia* sp., Döbbeler 1980a).

Octosporopsis erinacea and *Dumortiera hirsuta*

Dumortiera, the only genus in the family Dumortieraceae (Long 2006), is a new host taxon for octosporaceous fungi and generally for bryophilous ascomycetes. *Dumortiera hirsuta* s. lat. has a subcosmopolitan distribution from tropical to warm-temperate regions, where it is restricted to deeply shaded, continuously damp to wet sites in and beside rivers, streams and waterfalls (Schuster 1992). In Borneo, two varieties have been reported – the nominative one and *D. hirsuta* var. *nepalensis* (Chuah-Petiot 2011).

There is a striking imbalance between the thin-walled, chlorophyllose papillae of *Dumortiera hirsuta*, which are not protected by a dorsal epidermis with air pores as in other Marchantiales, and the thick and thick-walled hyphae of *Octosporopsis erinacea* growing between and over them. The robust hyphae and abundance of mycelia present on and inside the host tissue also contrast with the scattered, small and apparently depauperate apothecia. The fungus seems to invest most of its resources into the mycelium, which probably surpasses the ascomata in longevity. It probably guarantees the persistence of the highly adapted parasites under adverse conditions and may continuously follow the growth of their hosts.

The endobiotic hyphae of *O. erinacea* within the large thallus cells are principally similar to the epibiotic hyphae. However, they are somewhat thinner and less thick-walled. Intracellular appressoria also occur but crossing a cell wall by a usually delicate perforation does not necessarily need the presence of morphologically recognisable appressoria. Sometimes the haustorial character of hyphal segments is apparent due to short ramifications, relatively thin walls and dense cytoplasmic content (Fig. 3j, k). Lignituber-like structures 8.5–13 μm diam and perforating hyphae enclosed by capsules measuring up to 25 μm diam have been frequently observed (Fig. 3h). In these cases, host cell walls were yellowish or light brown discoloured as a manifestation of a defence reaction. Hyphae within the dorsal photosynthetic papillae have never been found.

Octospora kelabitiana and *Riccardia* spp.

The genus *Riccardia* is large with perhaps 90–100 species (Damsholt 2002). Seven taxa have been reported from Sarawak (Menzel 1988). *Riccardia* belongs to the family Aneuraceae, which exhibits an exceptionally wide range of interactions with symbiotic tulasnelloid fungi (Krause et al. 2011). Ascomycetes, however, seem not to be favoured associates of these liverworts. There are only a few records of species that form fruitbodies on Aneuraceae. Benkert (1990) reports *Lamprospora aneurae* Benkert on *Aneura pinguis* (L.) Dumort., Döbbeler (1978) two perithecial ascomycetes *Bryopelta variabilis* Döbbeler & Poelt and *Epibryon bryophilum* (Fuckel) Döbbeler on *Riccardia*. *Octospora cashiae* (Gamundí) Benkert, collected between liverworts of the genus *Riccardia*, was discussed above.

As in *O. erinacea*, one also sees a disproportion between sterile and fertile structures in *O. kelabitiana.* Hyphae have an uneven surface caused by warts or ridges, which seems to be a novel feature within *Octospora* s. lat. However, in many species information regarding mycelium characters is not yet available.

Acknowledgements We thank local Kelabit guides Rian John Pasan (Pa′Umor) and David Attu (Pa′Lungan). Petr Včelička and Marcel Vega are acknowledged for providing specimens of bryophilous fungi for the phylogenetic study, Jan Eckstein for determination of host bryophytes in some species used in the phylogenetic study, Lukáš Janošík for sharing his findings in *Octospora* s.l. and Chris Yeates for language correction. PD thanks Franz Höck who kindly helped with the layout of the

illustrations. ZE was supported by an internal grant from Palacký University (IGA_PrF_2017_001), MS by grant No. LO1204 (Sustainable development of research in the Centre of the Region Haná) from the National Program of Sustainability I, MEYS. The research was conducted under the permit No. NCCD.907.4.4(JLD.13)-337.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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4.1.2 *Octospora conidiophora* (Pyronemataceae) – a new species from South Africa and the first report of anamorph in bryophilous Pezizales

MycoKeys 54: 49–76 (2019)
doi: 10.3897/mycokeys.54.34571
http://mycokeys.pensoft.net

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Octospora conidiophora (Pyronemataceae) – a new species from South Africa and the first report of anamorph in bryophilous Pezizales

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Academic editor: *D. Haelewaters* | Received 15 March 2019 | Accepted 26 April 2019 | Published 10 June 2019

Citation: Sochorová Z, Döbbeler P, Sochor M, van Rooy J (2019) *Octospora conidiophora* (Pyronemataceae) – a new species from South Africa and the first report of anamorph in bryophilous Pezizales. MycoKeys 54: 49–76. https://doi.org/10.3897/mycokeys.54.34571

Abstract

Octospora conidiophora is described as a new species, based on collections from South Africa. It is characterised by apothecia with a distinct margin, smooth or finely warted ellipsoid ascospores, stiff, thick-walled hyaline hairs, warted mycelial hyphae and growth on pleurocarpous mosses *Trichosteleum perchlorosum* and *Sematophyllum brachycarpum* (Hypnales) on decaying wood in afromontane forests. It is the first species of bryophilous Pezizales in which an anamorph has been observed; it produces long, claviform, curved, hyaline and transversely septate conidia. Three other cryptic species of *Octospora* were detected using three molecular markers (LSU and SSU nrDNA and EF1α), but these could not be distinguished phenotypically. These are not described formally here and an informal species aggregate *O. conidiophora* agg. is established for them. The new species and finds of *Lamprospora campylopodis* growing on *Campylopus pyriformis* and *Neottiella albocincta* on *Atrichum androgynum* represent the first records of bryophilous Pezizales in South Africa.

Keywords

Afromontane forests, bryosymbionts, conidia, cryptic biodiversity, muscicolous parasites, *Sematophyllum*, *Trichosteleum*, South Africa

Introduction

The family Pyronemataceae is not only highly diverse in terms of morphology but also ecologically (Perry et al. 2007, Hansen et al. 2013). It includes six related genera that obligately grow on bryophytes – *Octospora* Hedw., *Lamprospora* De Not., *Neottiella* (Cooke) Sacc., *Octosporopsis* U.Lindem. & M.Vega, *Octosporella* Döbbeler and *Filicupula* Y.J.Yao & Spooner. These ascomycetes, known as bryoparasitic, bryophilous or bryosymbiotic Pezizales, form ca. 0.2–15 mm broad apothecia or perithecia-like apothecia (in *Octosporella*), coloured in shades of orange or red. They infect their hosts by elaborate infection structures consisting of superficial appressoria and intracellular haustoria (Döbbeler 1980). Together with their hosts, they can be found on various substrates like soil, burnt ground, rocks or bark and wood, both in natural and anthropogenic habitats in arctic to tropical regions (e.g. Benkert 1987; Schumacher 1993; Döbbeler 1997; Egertová et al. 2018).

Only rare reports of bryophilous Pezizales from the African continent are known: *Lamprospora maireana* Seaver, described on the basis of material from Algeria (Seaver 1914); *Octospora tetraspora* (Fuckel) Korf var. *aegyptiaca* J.Moravec from Egypt (Moravec 1972), later revised by D. Benkert as *O. leucoloma* Hedw. var. *tetraspora* Benkert, as indicated by his revision label; and *O. kilimanjarensis* J.Moravec, described from Tanzania (Moravec 1997) and later reported from Ethiopia together with a probably undescribed *Octospora* species (Lindemann 2013).

From southern Africa, thus far, no finds of these fungi have been reported and no vouchers are deposited in the South African National Collection of Fungi (PREM; Riana Jacobs-Venter pers. comm.). Surprisingly, during three weeks of our field excursions in KwaZulu-Natal and Mpumalanga, eastern South Africa, in February and March 2018, 39 populations of bryophilous Pezizales (*Octospora*, *Lamprospora* and *Neottiella*) were recorded. Only three of them could be assigned to described species, based on morphological characters, host association and DNA sequencing: *Lamprospora campylopodis* W.D.Buckley growing on *Campylopus pyriformis* (Schultz) Brid. (two collections) and *Neottiella albocincta* (Berk. & M.A.Curtis) Sacc. on *Atrichum androgynum* (Müll. Hal.) A.Jaeger (one collection). The remaining specimens were separated into six morphospecies. One of them, an undescribed *Octospora* species, growing on pleurocarpous mosses from the family Sematophyllaceae (Hypnales), turned out to be very common and remarkable in several aspects after detailed analysis. The aim of this contribution is to provide a description of this species, clarify its phylogenetic relationships and discuss associated taxonomical problems.

Methods

Sample collection and observation

Fungi were collected in February and March 2018 in South African Provinces KwaZulu-Natal and Mpumalanga. The description of *Octospora conidiophora* is based on 11 collections belonging to the most frequent genotype. Observations of apothecial fea-

tures were made on vital (marked by *) or rehydrated (†) material mostly in tap water, cresyl blue (CRB), lactophenol cotton blue (LPCB) or lactic acid cotton blue (LACB). Absence of amyloidity of asci was confirmed in Lugol´s solution. Infection structures were observed on rehydrated material. Parts of the host plants (leaves and rhizoids) close to an apothecium were separated, pulled apart, treated with LPCB and studied by light microscopy. The preparations were screened at 100× to 200× magnification for the presence of conidia. Infection structures and conidia usually occurred in the same mounts. Illustrations and measurements of hyphae, appressoria and haustoria, as well as conidia, were done in LPCB. The mosses were identified as hosts, based on the presence of appressoria on leaves or rhizoids. The host species were determined using standard techniques for bryophytes (Magill 1981). Collections are deposited in the Mycological department of the National Museum in Prague (PRM) and the herbarium of the Botanische Staatssammlung München (M).

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried apothecia by the CTAB method as outlined by Doyle and Doyle (1987). Up to three apothecia were homogenised by a pestle and incubated in 300 µl extraction buffer at 65 °C for one hour; the extract was subsequently purified in chloroform-isoamyl alcohol mixture, precipitated by isopropanol and finally dissolved in water and incubated with RNase for 30 min at 37 °C. DNA quality was checked on agarose gel. Molecular sequence data were generated for three loci: the 28S subunit of ribosomal DNA (LSU) was amplified with primers LR0R and LR6 (Vilgalys and Hester 1990), the 18S subunit of rDNA (SSU) with primers NS1 and NS6 (White et al. 1990) and translation elongation factor-1alpha (EF1α) with primers EF1-983F and EF1–1567R (Rehner and Buckley 2005). PCR was performed with Kapa polymerase (Kapa Biosystems, Wilmington, USA) following a standard protocol with 37 cycles and annealing temperature of 54 °C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced from both directions using the same primers by the Sanger method at Macrogen Europe, Amsterdam, The Netherlands.

Phylogenetic analysis

Newly generated sequences were assembled, edited and aligned in GENEIOUS 7.1.7. (Biomatters, New Zealand) using the MAFFT plugin, manually corrected and deposited in NCBI GenBank under accession numbers MK569288–MK569376. Datasets were compiled from these and previously published sequences (Table 1), aligned, trimmed in order not to contain too many missing data at the ends and concatenated in GENEIOUS 7.1.7. Bayesian Inference for concatenated data was computed in MRBAYES (ver. 3.2.4; Ronquist et al. 2012) with 2×10^7 generations, sampling every 1000th tree, in two independent runs, each with 4 chains, the first 50% (10^7) generations being ex-

Table 1. Specimens used for the phylogeny inference and their GenBank accession numbers. Newly generated sequences are MK569288–MK569376.

Taxon	Collection code	LSU	SSU	EF1α
Lamprospora campylopodis W.D.Buckley	48633	MF066054	MK569364	MK569289
Lamprospora dictydiola Boud.	ldic	MF754056	MK569365	MF754054
Lamprospora miniata De Not. var. *parvispora* Benkert	LMSk	MF066065	MK569366	MF754055
Lamprospora sylvatica Egertová & Eckstein	UA1	MG947604	MK569367	MK569290
Neottiella rutilans (Fr.) Dennis	46853	MK569313	MK569336	MK569288
Neottiella vivida (Nyl.) Dennis	NVZla	MF066068	MK569337	MF754051
Octospora affinis Benkert & L.G.Krieglst.	OAFZla	MF754075	MK569347	MF754045
Octospora conidiophora Sochorová & Döbbeler	ZE11/18	MK569315	MK569348	MK569291
Octospora conidiophora	ZE23/18	MK569324	MK569349	MK569294
Octospora conidiophora	ZE45/18	MK569316		MK569296
Octospora conidiophora	ZE46/18	MK569317	MK569350	MK569298
Octospora conidiophora	ZE48/18	MK569321	MK569351	MK569297
Octospora conidiophora	ZE57/18	MK569318	MK569352	MK569295
Octospora conidiophora	ZE62/18	MK569323	MK569354	MK569299
Octospora conidiophora	ZE63/18	MK569319	MK569355	MK569292
Octospora conidiophora	ZE71/18	MK569322	MK569356	MK569293
Octospora conidiophora	ZE75/18	MK569320	MK569357	MK569300
Octospora conidiophora	ZE77/18	MK569331	MK569353	MK569301
Octospora conidiophora agg. – lineage B	ZE37/18	MK569325	MK569358	MK569302
Octospora conidiophora agg. – lineage B	ZE38/18	MK569329	MK569359	MK569303
Octospora conidiophora agg. – lineage B	ZE51/18	MK569327	MK569362	MK569306
Octospora conidiophora agg. – lineage B	ZE52/18	MK569326	MK569360	MK569304
Octospora conidiophora agg. – lineage B	ZE53/18	MK569328	MK569361	MK569307
Octospora conidiophora agg. – lineage B	ZE65/18	MK569330	MK569363	MK569305
Octospora conidiophora agg. – lineage C	ZE44/18	MK569332	MK569373	MK569308
Octospora conidiophora agg. – lineage C	ZE56/18	MK569333	MK569374	MK569309
Octospora conidiophora agg. – lineage D	ZE69/18	MK569334	MK569375	MK569310
Octospora erzbergeri Benkert	ERZ	MF754068	MK569340	MF754042
Octospora excipulata (Clem.) Benkert	OExc	MF754062	MK569369	MF754047
Octospora fissidentis Benkert & Brouwer	Fis	MF754073	MK569341	MF754044
Octospora humosa (Fr.) Dennis	OHZla	MF754074	MK569343	MF754043
Octospora ithacaensis (Rehm) K.B.Khare	OLOi	MF754071	MK569346	MF754053
Octospora kelabitiana Egertová & Döbbeler	Oct-Jat	MF754065	MK569372	MF754048
Octospora kelabitiana	ZE61/16	MF754064	MK569376	MF754049
Octospora leucoloma Hedw.	Oleu	MF066067	MK569370	
Octospora orthotrichi (Cooke & Ellis) K.B.Khare & V.P.Tewari	HR8	MK569314	MK569342	MK569311
Octospora phagospora (Flageolet & Lorton) Dennis & Itzerott	PHG44	MF754072	MK569344	MF754046
Octospora pseudoampezzana (Svrček) Caillet & Moyne	OP1	MF754069	MK569339	MF754050
Octospora wrightii (Berk. & M.A.Curtis) J.Moravec	WRIG	MF754070	MK569345	
Octosporella perforata (Döbbeler) Döbbeler	PERF	MF754060	MK569368	MF754052
Octosporopsis erinacea Egertová & Döbbeler	DUM20/1	MF754057	MK569338	MF754041
Otidea leporina (Batsch) Fuckel	KGOL	MK569335	MK569371	MK569312

cluded as burn-in. The most suitable substitution model for each locus was determined in PARTITIONFINDER 2.1.1 (Lanfear et al. 2017) using the AIC corrected for small samples (AICc) and a greedy search. Single-locus phylogenies were computed with similar settings, but with 6×10^6 MCMC generations and the parameter temp. = 0.01.

Divergence times were estimated with BEAST 2.5.1 (Bouckaert et al. 2014) using the LSU and SSU data from our sample set (one sample per species or phylogenetic lineage) and six additional species: *Caloscypha fulgens* (Pers.) Boud., *Scutellinia scutellata* (L.) Lambotte, *Cheilymenia stercorea* (Pers.) Boud., *Aleuria aurantia* (Pers.) Fuckel, *Pyronema domesticum* (Sowerby) Sacc. and *Sarcoscypha coccinea* (Gray) Boud. (all sequences obtained from Beimforde et al. 2014; EF1α was not analysed by these authors and, therefore, not included in our molecular dating). Four calibration points were used for the analysis and the divergence times, together with their confidence intervals, were also taken from Beimforde et al. (2014), namely divergence *Cheilymenia-Scutellinia*, divergence *Aleuria*-(*Cheilymenia*+*Scutellinia*), split-off of *Sarcoscypha* and split-off of *Caloscypha*. Monophyly was forced for all of the points except the second one due to an unclear position of the *Octospora* clade. Analysis was run under GTR+I+G substitution model (as for MRBAYES), with relaxed clock log normal model and 10^8 MCMC generations, but the first 50% were excluded as burn-in. Priors included the Yule model with uniform birth rate and exponential gamma shape. Convergence and stationarity were analysed using TRACER v1.7.1 (Rambaut et al. 2018) and results were considered when effective sample size (ESS) ≥ 1000. Statistical uncertainty of divergence time estimates was assessed through the calculation of highest probability density (HPD) values.

Results

Phylogenetic and phenotypic analysis

After trimming, the total length of the concatenated alignment was 2702 bp (539 bp from EF1α, 1102 bp from LSU and 1061 bp from SSU, including gaps). Every studied locus provided sufficient polymorphism both amongst and within previously phenotypically delimited groups (Suppl. material 1: Table S1). Four distinct phylogenetic lineages were detected in the concatenated data, as well as in single-locus data within the group of specimens that were hosted by Sematophyllaceae (Fig. 1, Suppl. material 2: Fig. S1). Divergence between them was between 4 and 59 nucleotide differences at every locus (Suppl. material 1: Table S1). The four South African lineages formed a highly supported and distinct clade together with *O. kelabitiana* (Fig. 1). Molecular dating analysis estimated the basal split of bryophilous Pezizales to be 87–172 Ma old (95% confidence interval; mean = 149 Ma), the basal split of the South African accessions was estimated at 23–73 Ma (mean = 47 Ma; Fig. 2).

No significant differences in phenotypic traits were detected amongst the South African lineages using standard characters and methods. They shared the structure of excipulum, stiff, thick-walled hyaline hairs, ellipsoid hyaline ascospores which can be either smooth or ornamented with fine warts and which contain 1 or 2 guttules, warted mycelial hyphae, appressoria, haustoria and presence of anamorph. Although differences amongst individual collections were observed, phenotypic characters did not correspond to the molecular markers and many characters exhibited variability both amongst and within the four phylogenetic lineages (Table 2).

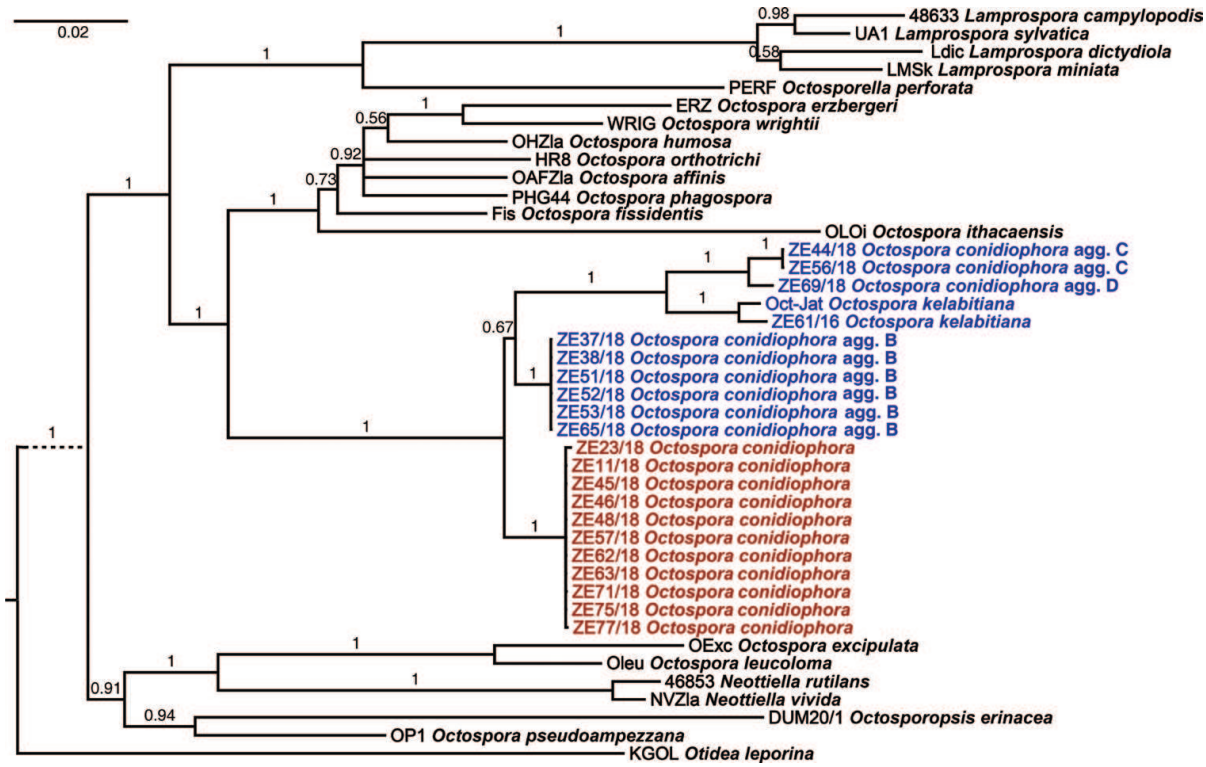

Figure 1. Bayesian phylogeny inference, based on concatenated alignment of EF1α, LSU and SSU sequences. Bayesian posterior probabilities are shown above branches; *Otidea leporina* serves as outgroup; trees based on analysis of each locus are shown in Suppl. material 2: Fig. S1.

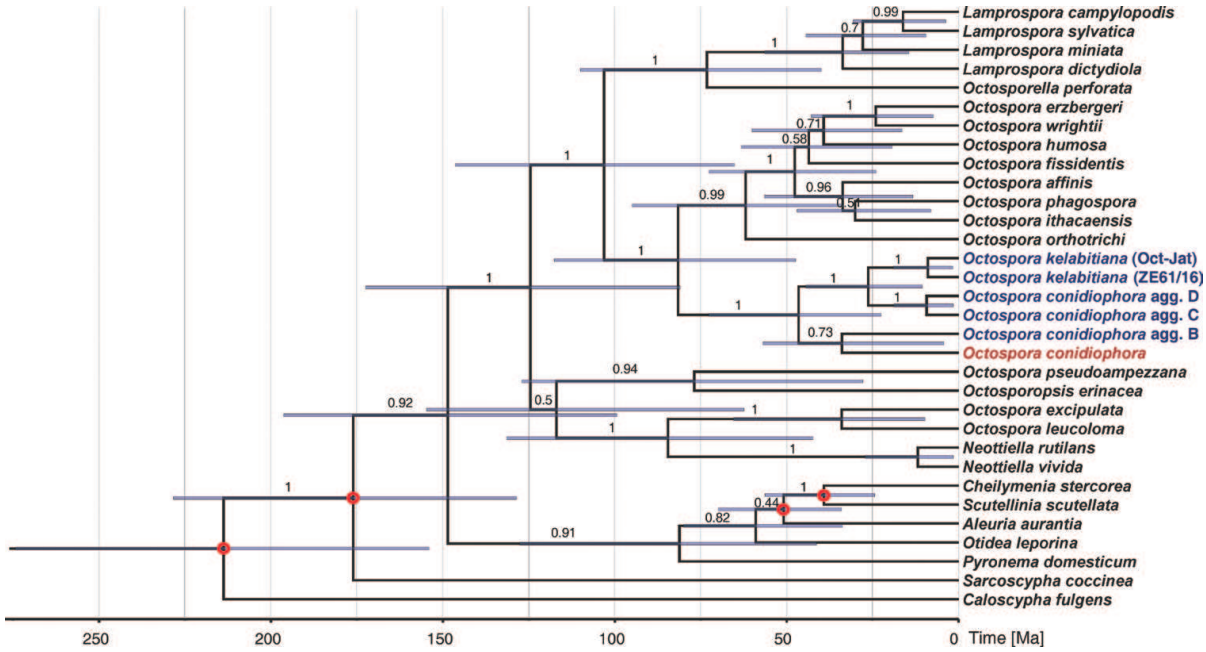

Figure 2. Maximum clade credibility tree with estimated divergence times, based on SSU and LSU data; calibration points are marked by red circles, posterior probabilities shown above branches, bars indicate the 95% highest posterior density (HPD) intervals.

Table 2. Variability of selected characters in *O. conidiophora* agg.

Voucher	Ornament of ascospores	(†) Size of ascospores [µm]	(†) Mean size of ascospores [µm]	(†) Q of ascospores	(†) Qm	Observation of conidia	Host
Lineage A - *Octospora conidiophora* s. str.							
ZE11/18	smooth	14.4–16.0 × 8.0–9.1	15.1 × 8.4	1.65–1.91	1.79	yes	*S. brachycarpum*
ZE23/18	smooth	14.0–16.3 × 7.5–9.0	14.9 × 8.0	1.66–2.03	1.84	yes	*T. perchlorosum*
ZE45/18	smooth	13.6–16.2 × 7.5–8.3	15.2 × 7.9	1.74–2.08	1.92	yes	*T. perchlorosum*
ZE46/18	smooth	15.0–17.0 × 8.0–9.9	15.9 × 8.7	1.63–2.06	1.82	yes	*T. perchlorosum*
ZE48/18	smooth	14.5–17.0 × 8.3–9.9	16.1 × 9.0	1.64–1.99	1.79	yes	*T. perchlorosum*
ZE57/18	smooth	14.9–16.2 × 7.9–9.0	15.5 × 8.2	1.69–1.99	1.87	yes	*T. perchlorosum*
ZE62/18	smooth	14.0–16.7 × 8.0–8.9	15.2 × 8.2	1.72–1.99	1.85	yes	*T. perchlorosum*
ZE63/18	smooth	14.0–17.0 × 8.0–9.2	15.4 × 8.7	1.67–1.89	1.77	yes	*T. perchlorosum*
ZE71/18	warted	13.0–15.0 × 8.0–9.9	14.1 × 8.9	1.39–1.73	1.59	no	*T. perchlorosum*
ZE75/18	smooth	14.0–16.1 × 7.8–9.1	15.1 × 8.4	1.65–1.94	1.79	yes	*T. perchlorosum*
ZE77/18	warted	13.5–15.4 × 8.7–10.5	14.4 × 9.4	1.40–1.67	1.53	yes	*T. perchlorosum*
all specimens		13.0–17.0 × 7.5–10.5	15.2 × 8.5	1.39–2.08	1.79		
Lineage B							
ZE37/18	warted	13.3–15.5 × 8.3–9.9	14.6 × 9.1	1.47–1.82	1.60	yes	*S. brachycarpum*
ZE38/18	warted	12.5–15.1 × 8.0–9.7	13.8 × 8.8	1.46–1.78	1.57	no	*T. perchlorosum*
ZE51/18	warted	13.5–15.7 × 8.4–10.2	14.5 × 9.4	1.45–1.65	1.54	yes	*T. perchlorosum*
ZE52/18	warted	13.5–16.0 × 8.0–9.5	14.7 × 8.8	1.47–1.83	1.66	yes	*T. perchlorosum*
ZE53/18	warted	14.0–15.3 × 8.0–10.1	14.7 × 9.3	1.47–1.85	1.56	no	*T. perchlorosum*
ZE65/18	warted	13.5–16.2 × 7.7–9.9	14.4 × 8.8	1.47–1.75	1.60	yes	*T. perchlorosum*
all specimens		12.5–16.2 × 7.7–10.2	14.5 × 9.1	1.45–1.85	1.59		
Lineage C							
ZE44/18	warted	13.5–15.9 × 7.2–8.1	14.6 × 7.8	1.71–2.01	1.87	yes	*S. brachycarpum*
ZE56/18	warted	13.1–15.4 × 7.0–8.2	14.1 × 7.6	1.66–2.11	1.84	yes	*S. brachycarpum*
both specimens		13.1–15.9 × 7.0–8.2	14.3 × 7.7	1.66–2.11	1.86		
Lineage D							
ZE69/18	smooth or lightly warted	13.5–18.0 × 7.9–9.9	15.5 × 8.6	1.61–2.02	1.80	yes	*S. brachycarpum*

Taxonomy

***Octospora conidiophora* Sochorová & Döbbeler, sp. nov.**
MycoBank no.: MB829095
Figs 3–9

Etymology. *Conidiophorus* (Gr./Lat.) refers to production of conidia.

Diagnosis. Differs from *Octospora kelabitiana* by larger apothecia with a distinct margin, infection of pleurocarpous mosses of the family Sematophyllaceae and frequent formation of a *Spermospora*-like anamorph.

TYPE: SOUTH AFRICA. KwaZulu-Natal Province: Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°7.72'S, 29°25.27'E, 1750 m alt., on *Trichosteleum perchlorosum* on decaying wood, 2 Mar. 2018, Z. Egertová (So-

Figure 3. *Octospora conidiophora*. **A–E** Apothecia in situ **F** Habitat **A, E** ZE63/18 **B** ZE57/18 **C, F** ZE11/18 **D** holotype ZE48/18.

chorová) and M. Sochor ZE48/18, holotype: PRM 951743, isotype: M; LSU GenBank accession number: MK569321, SSU GenBank accession number: MK569351, EF1α GenBank accession number: MK569297.

Description. *Apothecial features*: Apothecia in groups on plants of *Trichosteleum perchlorosum* or *Sematophyllum brachycarpum* or between them, 0.2–1.5 mm broad, up to 0.65 mm high, first subglobose with a small apical opening, later hemispherical, turbinate to disc-shaped, pinkish-orange, sessile, mostly with a well-developed margin, outer surface of excipulum with adpressed to shortly protruding hairs or hyphae.

Hairs *55–205 × 4–10.5 µm, scattered at flanks, hyaline, scarcely septate, obtuse, thick-walled, wall *0.5–3.5 µm thick. Excipulum at the base *230–330 µm thick,

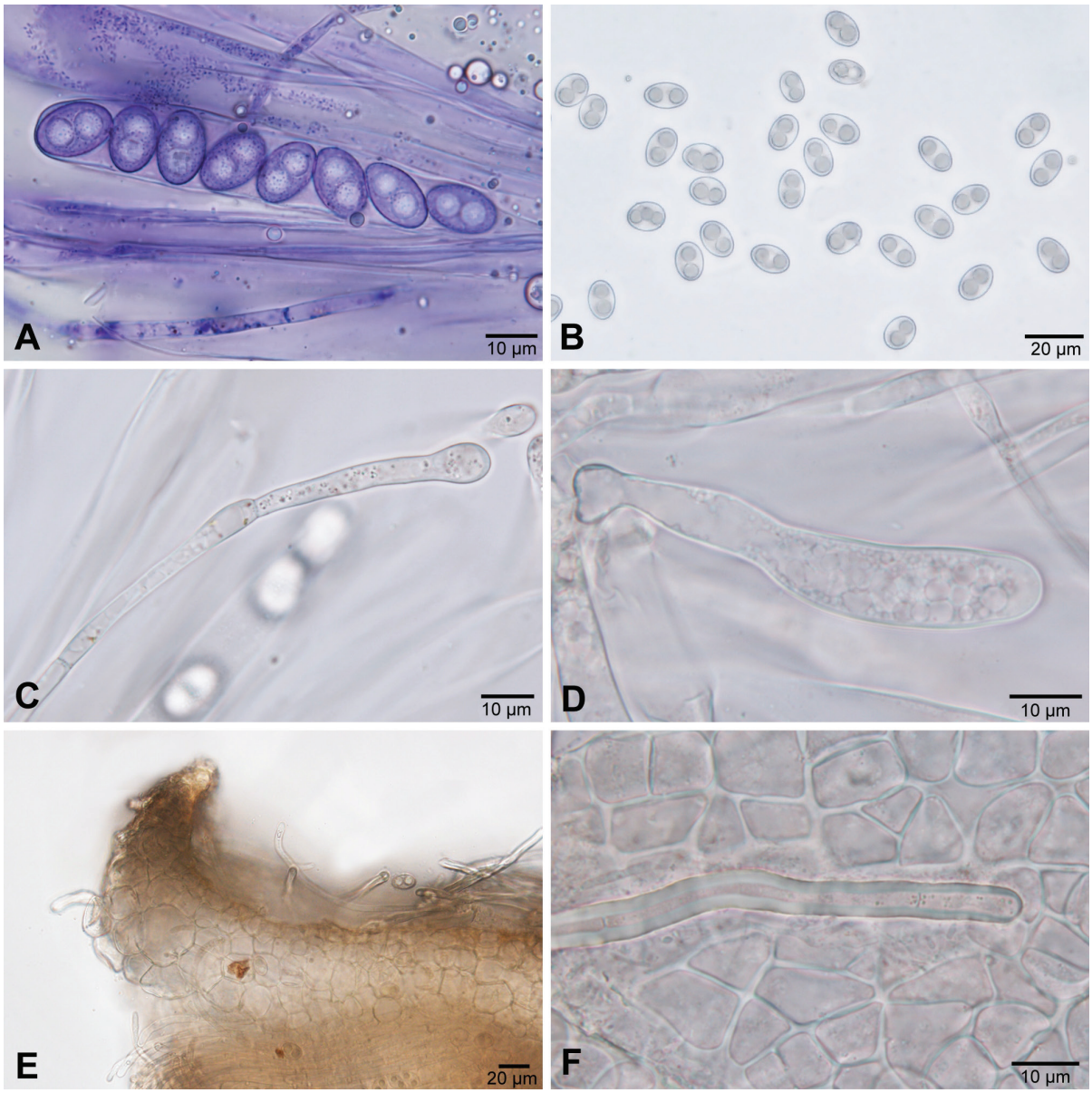

Figure 4. Microscopic characters of *Octospora conidiophora*. **A** Ascospores inside ascus stained with CRB **B** Free ascospores in tap water **C** Paraphyse in tap water **D** Young ascus in tap water **E** Margin of apothecium in tap water **F** Hair and excipular cells in tap water **A–F** ZE77/18.

laterally about 50 µm thick, composed of angular to subangular (triangular, trapezoid, rectangular), globose, subglobose or irregularly shaped cells, *6–43 × 5–42 µm, outermost cells thick-walled (neighbouring cells divided by up to *6 µm broad wall). Margin *60–280 µm broad, consisting of globose, subglobose, pyriform or trapezoid cells, *10–38 × 7–30 µm.

Subhymenium *40–75 µm wide, consisting of densely packed cylindrical cells *3–7 µm wide mixed with angular or irregularly shaped cells, *4.5–8 × 4.5–6 µm. Paraphyses filiform, straight or bent, unbranched, septate, uppermost one or two cells containing little very pale droplets (*0.5–2 µm in diameter), *2.1–3.5 µm broad (†1.5–2.3 µm), terminal cell *19–83 × 3–7 µm (†18–57 × 3–5.5 µm). Asci *146–197 × 12–15.5 µm (†135–192 × 9.5–12.5 µm), cylindrical, unitunicate, operculate, inamyloid, aris-

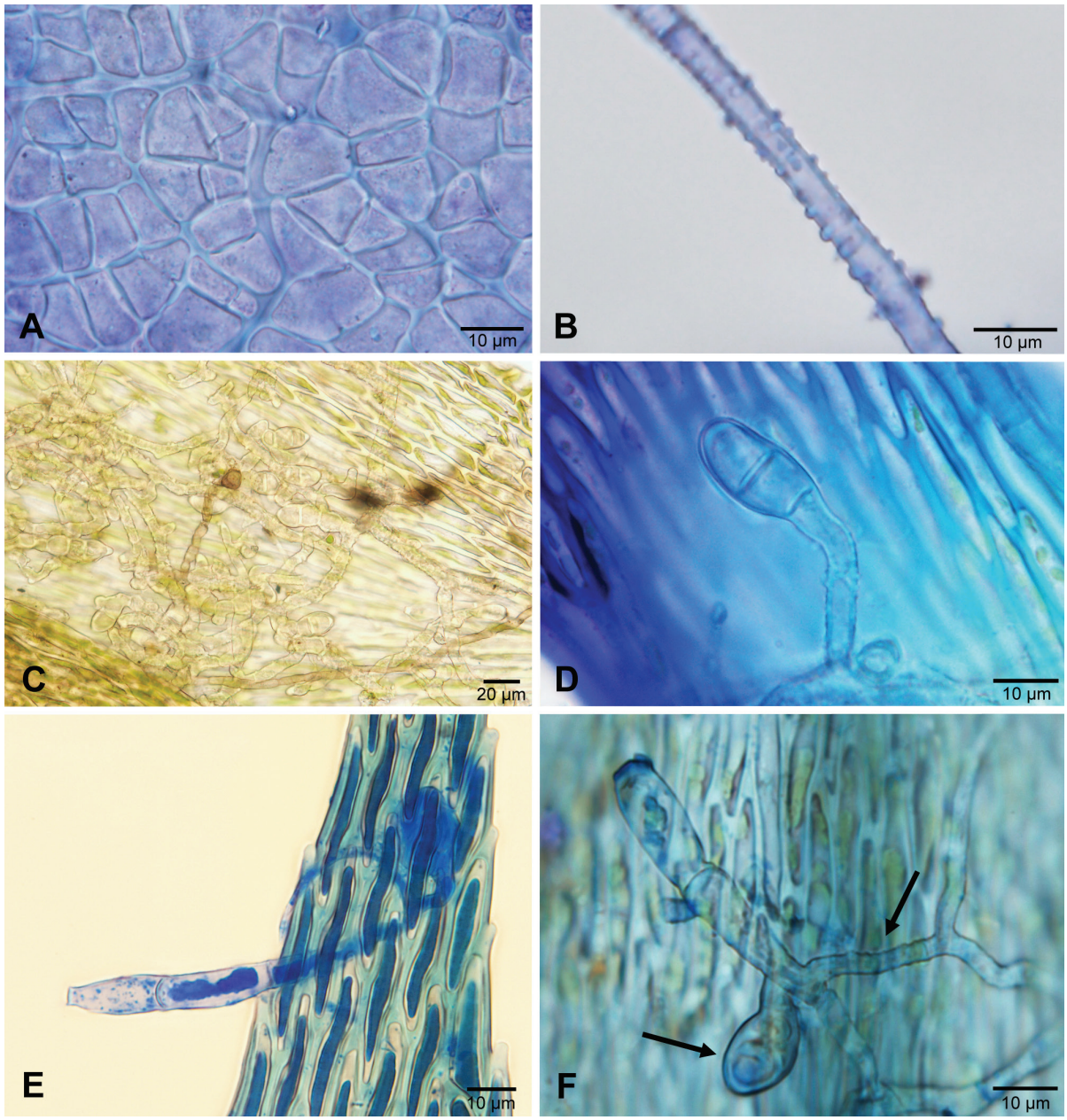

Figure 5. Microscopic characters of *Octospora conidiophora*. **A** Cells of the outermost layer of excipulum from an outside view stained with CRB **B** Mycelial hypha stained with CRB **C** Appressoria and hyphae on a leaf of *Sematophyllum brachycarpum* in tap water **D** Appressorium stained with LACB **E** Germinating conidium stained with LPCB **F** Germinated conidium produced a bifurcate warted hypha (right arrow), appressorium (left arrow) probably not connected to the conidium, in LACB **A, B, F** ZE77/18 **C, E** ZE11/18 **D** holotype ZE48/18.

ing from croziers, with 8 uniseriate ascospores. Ascospores *13–17.2 × 7–10.5 µm, mean 15.2 × 9 µm, Q = 1.34–1.99, Qm = 1.69 (†13–17 × 7.5–10.5 µm, mean 15.2 × 8.5 µm, Q = 1.39–2.08, Qm = 1.79), ellipsoid to narrowly ellipsoid, hyaline, containing one or two lipid guttules (up to *8 µm in diameter if one, *4–5.5 µm if two), smooth or ornamented with cyanophilous, very small, obtuse warts 0.1–0.3 µm broad; germinating with a single germ tube.

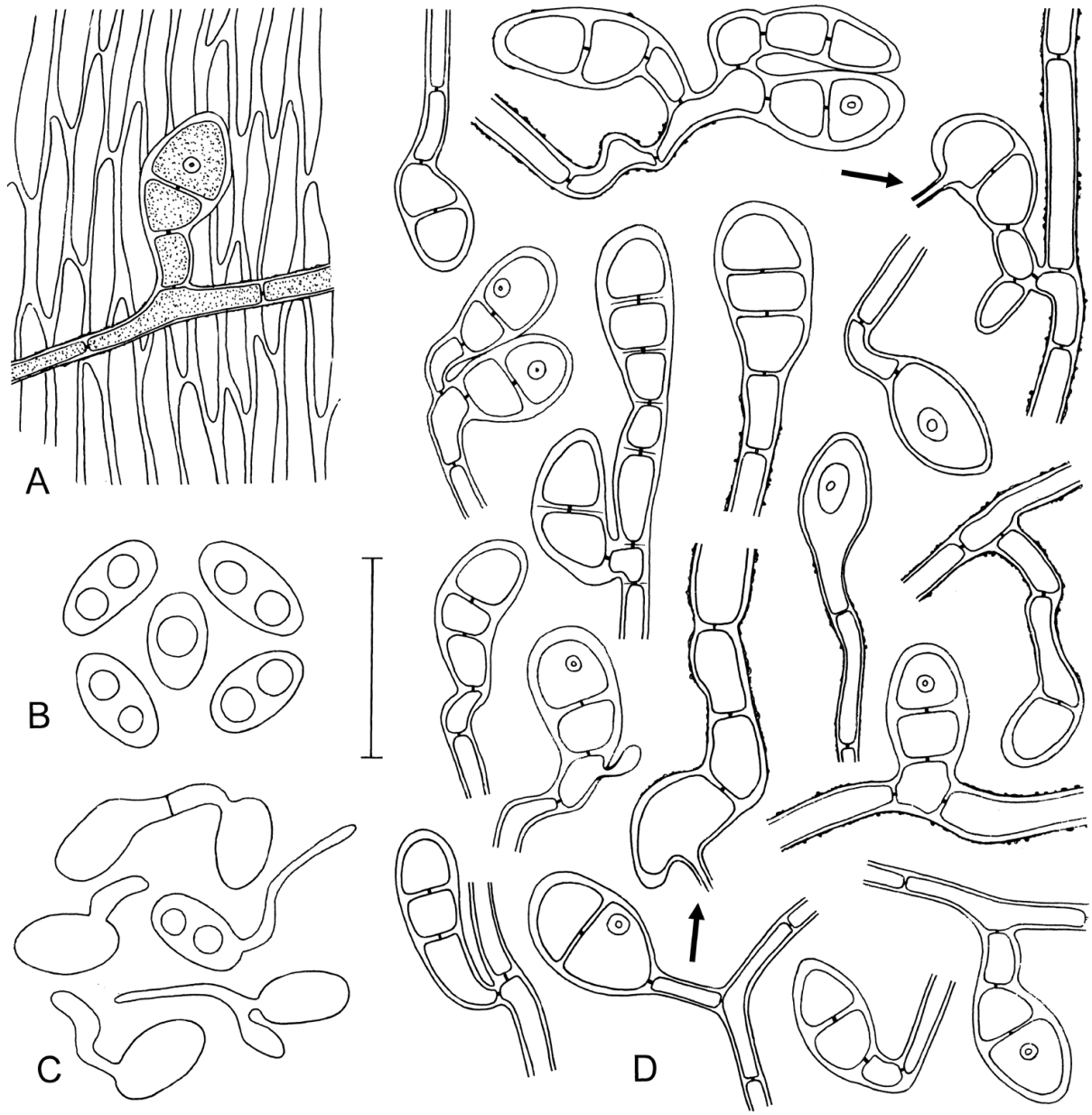

Figure 6. Microscopic characters of *Octospora conidiophora*. **A** Hypha with two-celled appresorium closely attached to the cells of the host leaf **B** Ascospores **C** Germinating ascospores found on leaves **D** Variation of appressoria mostly seen from above, infection pegs not always observed, appressoria seen in lateral view with infection pegs (indicated by arrows) **A, B, D** holotype ZE48/18 **C** ZE11/18. Scale bar: 30 µm. Illustrated by P.D.

Mycelial features (†): Hyphae restricted to the lowermost plant parts, irregularly growing on and between the leaf bases, stems and especially the rhizoids, hyaline, with ramifications and anastomoses, often thick-walled, (2–)3–6(–7) µm in diameter (excluding ornamentation); hyphal surface with minute to large protuberances, in optical section with numerous minute or larger, semi- or subglobose warts or spines, in surface view, these structures sometimes looking like ridges extended perpendicularly to the hyphal axis; largest warts up to 1.5(–2) µm high; hyphae growing within hyphae present; whole hyphal wall slightly cyanophilous, outermost rough part strongly cyanophilous.

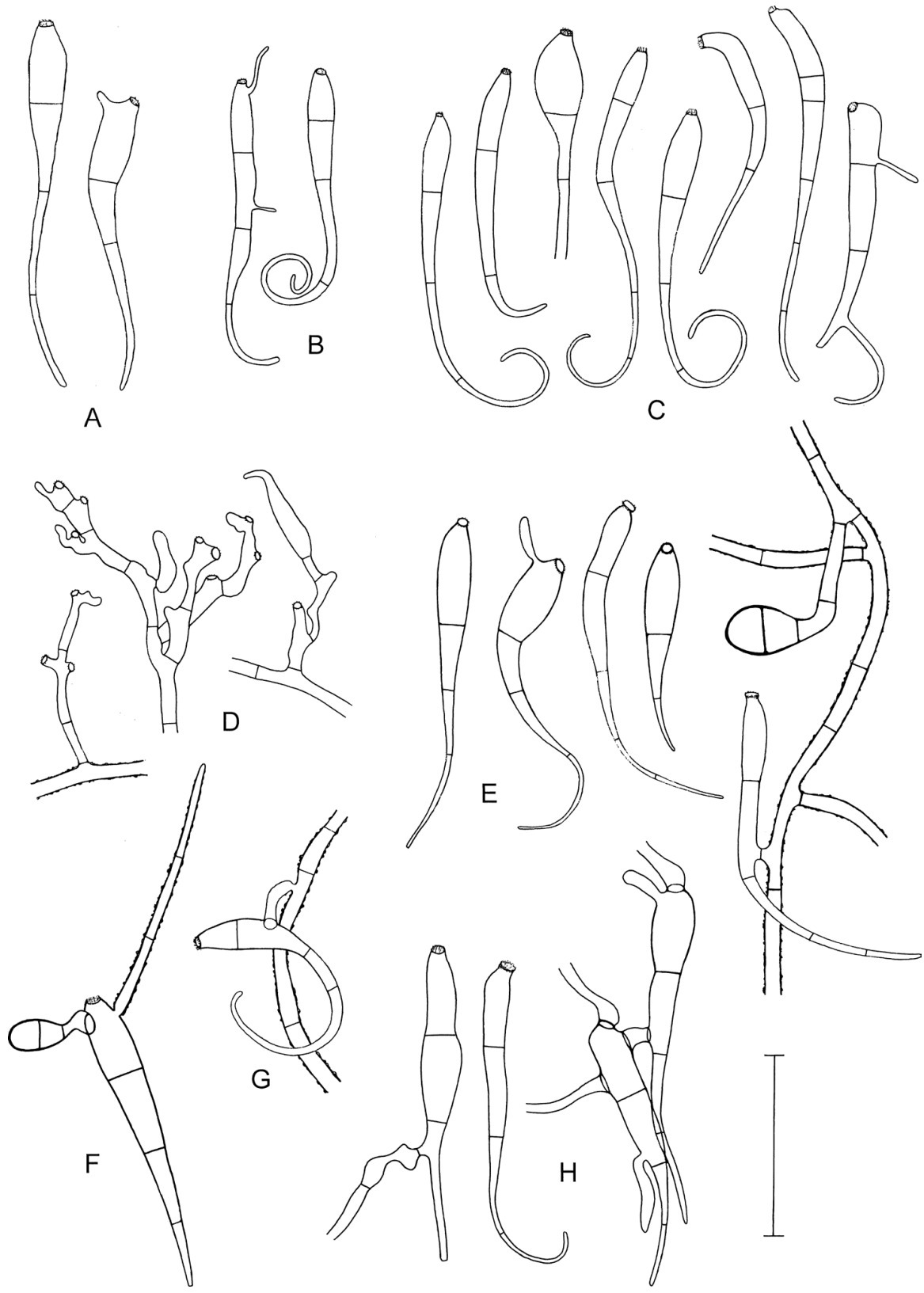

Figure 7. Microscopic characters of *Octospora conidiophora*. **A–C, E–H** Conidia, distal curved part apparently sometimes broken off, some conidia germinating **D** Conidogenous cells, on the right with a developing conidium **E** (on the right) Conidium anastomosing to mycelial hypha with two-celled appressorium **F** Conidium germinating by a hypha with a warty surface and a two-celled appressorium **G** Conidium with anastomosis to mycelial hypha **H** (on the right) Two germinating conidia with an anastomosis between them **A, F** ZE63/18 **B** ZE46/18 **C** ZE77/18 **D, E** holotype ZE48/18 **G** ZE57/18 **H** ZE11/18. Scale bar: 50 µm. Illustrated by P.D.

Appressoria variable, frequent (even more than 30 per leaf observed) and easy to detect, closely attached to both leaf sides or to rhizoids, colourless, 1-, 2- or 3-celled, from above elliptical, (14–)16–23(–26) µm long, (8–)11–16 µm wide, laterally seen slightly kidney-shaped, (7–)9–13(–16) µm high, with walls up to 2.5(–4) µm thick; surface rough but not warty, cyanophilous; appressorial cytoplasma strongly cyanophilous; appressoria mostly laterally formed on short stalks; stalks often gradually expanding toward the appressorium; perforation of the host cell wall by means of a delicate peg; peg often surrounded by a brown, straight or curved lignituber-like swelling measuring up to 10(–15) × 2–4(–6) µm; rhizoid wall at the perforation point slightly uplifted towards the appressorium; perforation point not always visible from above.

Haustoria within living leaf cells or rhizoidal cells, at first as a thick short filament, later becoming up to 55 µm long, orientated longitudinally in the rhizoid and developing ramifications (in wider rhizoids), rarely filling out the whole host cell; haustorial cytoplasm strongly cyanophilous.

Anamorph (†): Conidia variable in shape and size, claviform, hyaline, transversely septate, ca. (50–)70–115(–154) µm long (including the tail); proximal cell usually distinctly wider than the subproximal cell, rarely cells almost cylindrical, both cells measuring together (30–)35–48(–55) × (6–)7.5–12(–15) µm, subproximal cell continuously attenuating into a tail; tail typically curved to curled, 1- or 2-(3-) celled, (15–)30–60(–100) µm long and (1.5–)2(–2.5) µm in diameter at the distal end; proximal cell of the conidia with a conspicuous, circular, slightly protruding, delicately fringed scar, (3–)4(–4.5) µm in diameter, resulting from detachment from the conidiogenous cell; scar sometimes slightly laterally positioned; walls of conidia cyanophilous; the two proximal cells smooth, the tail sometimes warty (like the hyphae); germ tube one (to three) per conidium, arising from the scar or laterally from different regions of the conidia, including the tail cells.

Conidiogenous cells irregularly shaped, shorter and wider than sterile hyphal cells, rich in cytoplasmic content, usually with 1(–2) scars; shape and size of the scars like those at the conidia, also with a delicately fringed margin.

Hosts. *Trichosteleum perchlorosum*, *Sematophyllum brachycarpum* (Sematophyllaceae, Hypnales)

Distribution. South Africa, Mpumalanga and KwaZulu-Natal Provinces (Fig. 8).

Conservation status. *Octospora conidiophora* seems to be a common representative of the genus in South Africa, widespread and forming abundant populations. Its hosts are also common and widespread in the region (see below). Although the main habitat (afromontane forest) is naturally fragmented, it is often protected against human activities by nature reserves or national parks. Therefore, *O. conidiophora* does not fulfil the criteria for categories CR (critically endangered) to NT (near threatened) and we propose its evaluation as LC (least concern) for the present moment.

Additional specimens examined. South Africa. Mpumalanga Province: Ehlanzeni District Municipality, Graskop Gorge, 24°56.74'S, 30°50.8'E, 1355 m alt., on *Trichosteleum perchlorosum* on decaying wood, 6 Mar. 2018, Z. Egertová and M. Sochor ZE62/18 (PRM 951745); Ehlanzeni District Municipality, Graskop Gorge, 24°56.88'S, 30°50.75'E, 1435 m alt., on *Trichosteleum perchlorosum* on decaying wood,

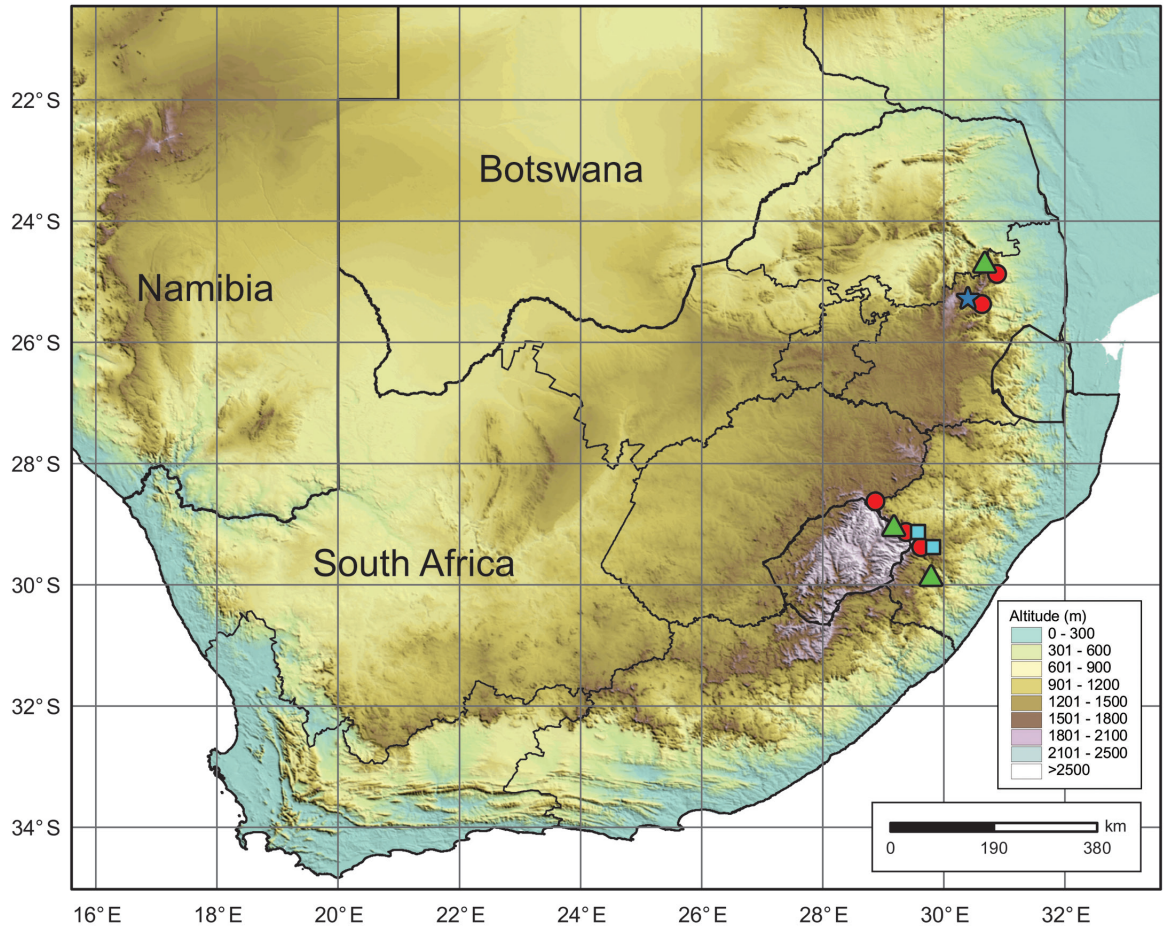

Figure 8. Geographical distribution of the four lineages of *Octospora conidiophora* agg. in South Africa. Red circle: lineage A (O. con*idiophora* s.str.); green triangle: lineage B; light blue square: lineage C, dark blue star: lineage D.

6 Mar. 2018, Z. Egertová and M. Sochor ZE63/18 (PRM 951746); Ehlanzeni District Municipality, Buffelskloof Nature Reserve, 25°15.98'S, 30°31.08'E, 1725 m alt., on *Trichosteleum perchlorosum* on decaying wood, 10 Mar. 2018, Z. Egertová and M. Sochor ZE75/18 (PRM 951748); Ehlanzeni District Municipality, Buffelskloof Nature Reserve, 25°16.37'S, 30°30.62'E, 1605 m alt., on *Trichosteleum perchlorosum* on decaying wood, 9 Mar. 2018, Z. Egertová and M. Sochor ZE71/18 (PRM 951747); Ehlanzeni District Municipality, Buffelskloof Nature Reserve, 25°16.53'S, 30°30.25'E, 1625 m alt., on *Trichosteleum perchlorosum* on decaying wood, 10 Mar. 2018, Z. Egertová and M. Sochor ZE77/18 (PRM 951749). KwaZulu-Natal Province: Uthukela District Municipality, Royal Natal National Park, 28°40.88'S, 28°55.73'E, 1760 m alt., on *Sematophyllum brachycarpum* on decaying stem, 19 Feb. 2018, Z. Egertová and M. Sochor ZE11/18 (PRM 951739); Uthukela District Municipality, Royal Natal National Park, 28°44.05'S, 28°54.85'E, 1800 m alt., on *Trichosteleum perchlorosum* on decaying stem, 20 Feb. 2018, Z. Egertová and M. Sochor ZE23/18 (PRM 951740). Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°8.95'S, 29°25.35'E, 1665 m alt., on *Trichosteleum perchlorosum* on decaying wood, 3 Mar. 2018, Z. Egertová and M. Sochor ZE57/18 (PRM 951744); Uthukela District Mu-

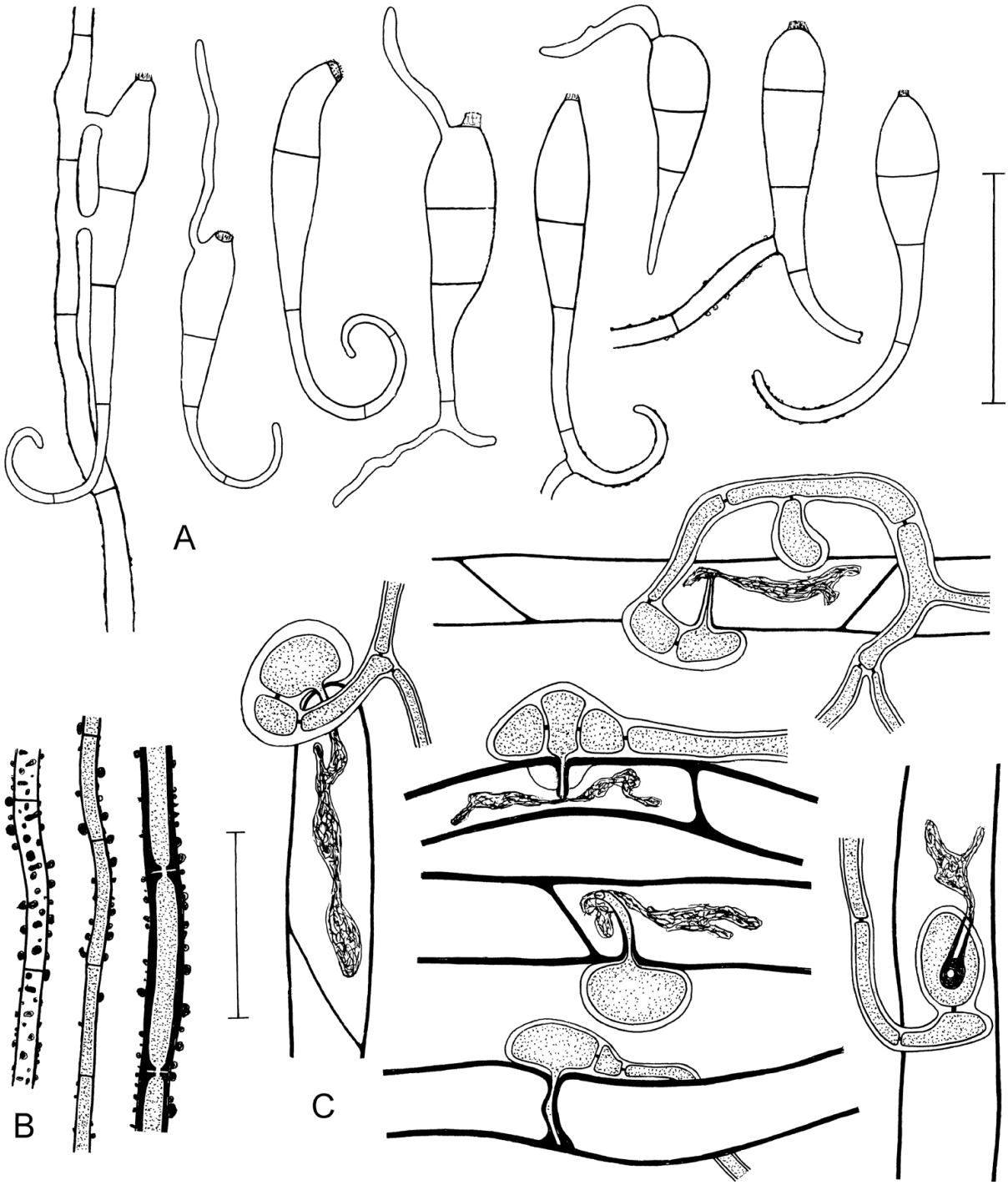

Figure 9. Microscopic characters of *Octospora conidiophora* agg. (lineage B). **A** Conidia, distal curved part apparently sometimes broken off, five conidia germinating by formation of usually a single hypha, conidium on the left connected to a hypha by two anastomoses **B** Strongly warted hyphae, the left one seen from above, the two others in optical section **C** Appresssoria infecting rhizoids in lateral view, the right one seen from above, infection pegs surrounded by lignituber-like tubes formed by the host cell wall, intracellular haustoria present apart from the lowermost infection where the peg is completely encapsulated by the host cell wall **A, B, C** ZE37/18. Scale bars: 50 μm (**A**); 30 μm (**B, C**). Illustrated by P.D.

nicipality, uKhahlamba Drakensberg Park, Giants Castle Nature Reserve, 29°16.93'S, 29°30.93'E, 1765 m alt., on *Trichosteleum perchlorosum* on decaying wood, 1 Mar. 2018, Z. Egertová and M. Sochor ZE46/18 (PRM 951742); Uthukela District Mu-

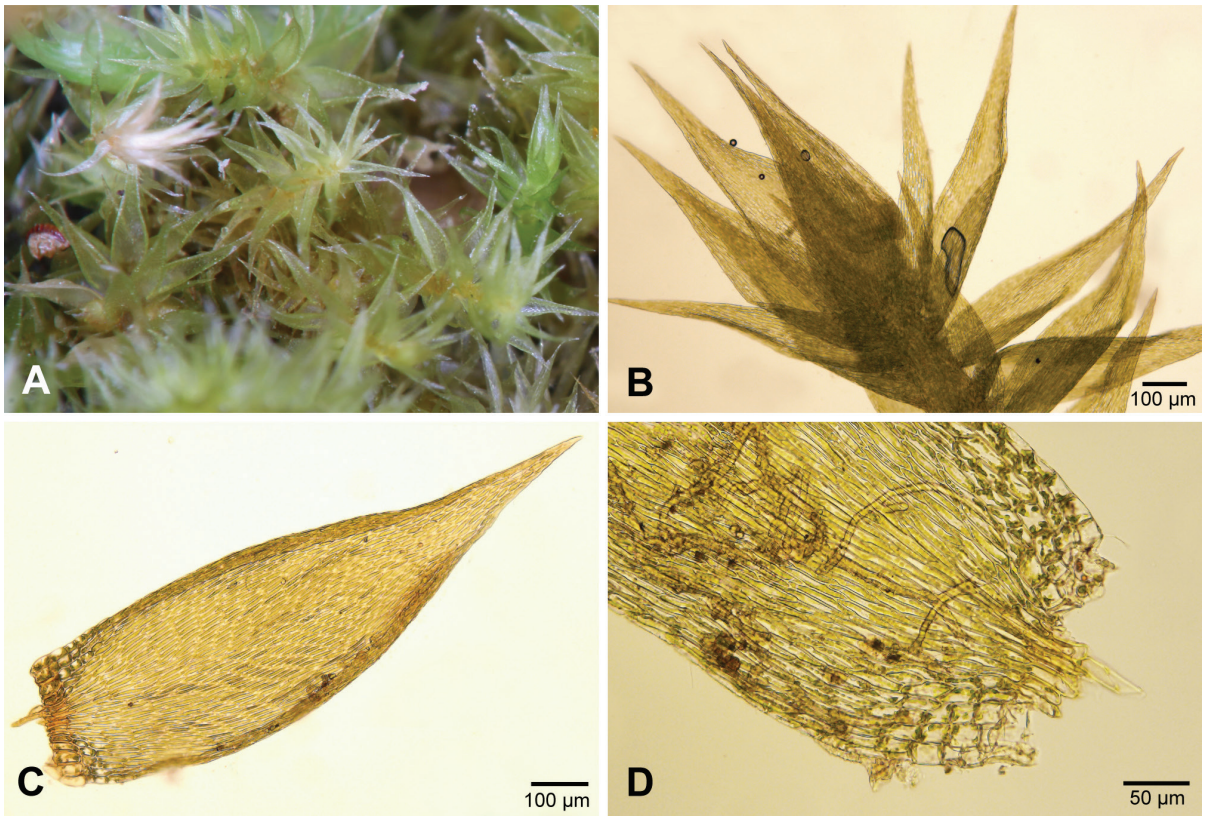

Figure 10. *Sematophyllum brachycarpum.* **A** Plants **B** Typical shoot with leaves **C** Leaf **D** Leaf base with alar cells.

nicipality, uKhahlamba Drakensberg Park, Giants Castle Nature Reserve, 29°16.98'S, 29°30.87'E, 1775 m alt., on *Trichosteleum perchlorosum* on decaying wood, 1 Mar. 2018, Z. Egertová and M. Sochor ZE45/18 (PRM 951741).

Data to other lineages. Lineage B: Mpumalanga Province: Ehlanzeni District Municipality, 3040 m WSW from the Graskop railway station, 24°56.28'S, 30°48.65'E, 1495 m alt., on *Trichosteleum perchlorosum* on decaying wood, 7 Mar. 2018, Z. Egertová and M. Sochor ZE65/18 (PRM 951735). KwaZulu-Natal Province: Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°7.62'S, 29°25.33'E, 1725 m alt., on *Trichosteleum perchlorosum* on decaying wood, 2 Mar. 2018, Z. Egertová and M. Sochor ZE51/18 (PRM 951732); Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°7.57'S, 29°25.38'E, 1715 m alt., on *Trichosteleum perchlorosum* on decaying wood, 2 Mar. 2018, Z. Egertová and M. Sochor ZE52/18 (PRM 951733); Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°7.98'S, 29°26.25'E, 1500 m alt., on *Trichosteleum perchlorosum* on decaying wood, 2 Mar. 2018, Z. Egertová and M. Sochor ZE53/18 (PRM 951734); Sisonke District Municipality, Marutswa Forest, 29°48.55'S, 29°47.28'E, 1465 m alt., on *Sematophyllum brachycarpum* on decaying stem, 24 Feb. 2018, Z. Egertová and M. Sochor ZE37/18 (PRM 951730); Sisonke District Municipality, Marutswa Forest, 29°48.6'S, 29°47.37'E, 1480 m alt., on *Trichosteleum perchlorosum* on decaying stem, 24 Feb. 2018, Z. Egertová and M. Sochor ZE38/18 (PRM 951731).

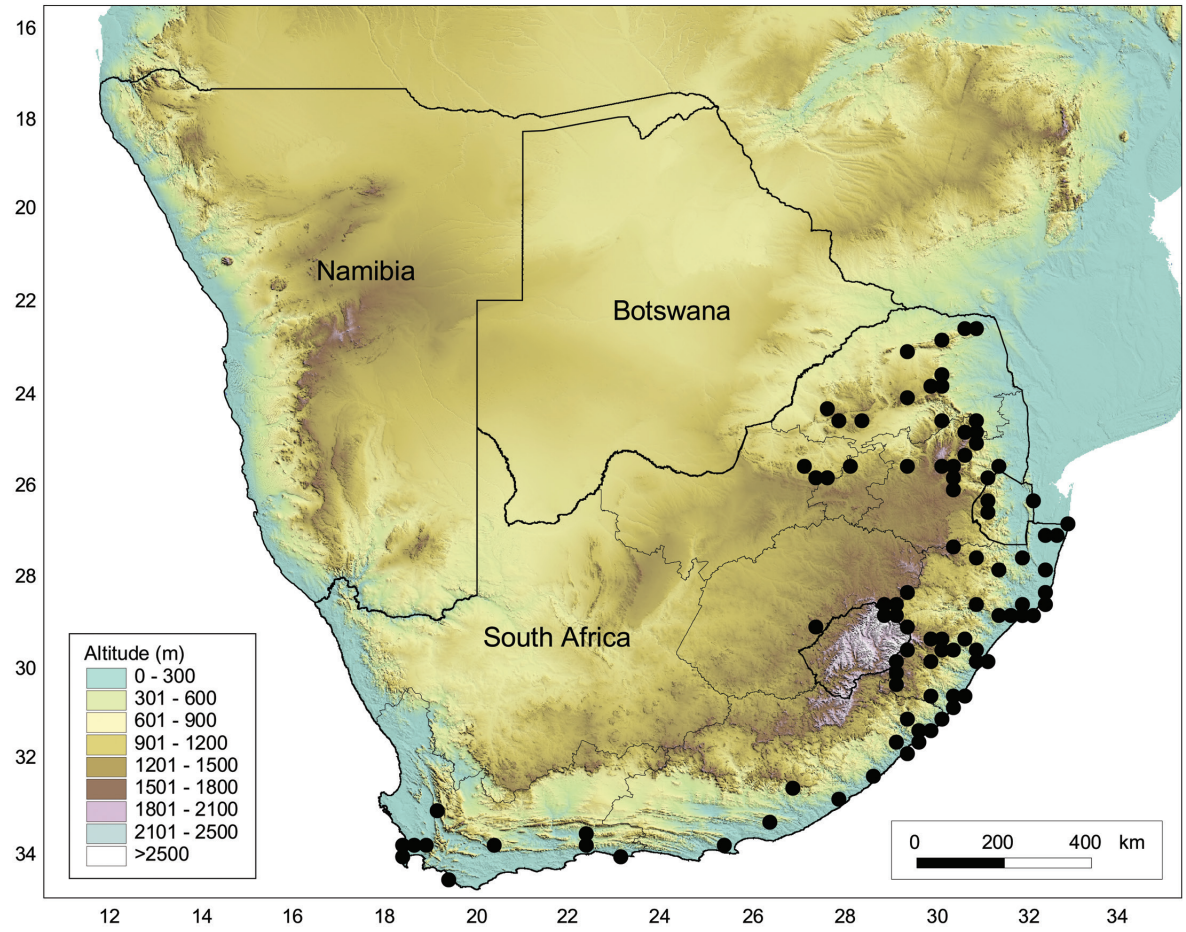

Figure 11. Geographical distribution of *Sematophyllum brachycarpum* in southern Africa based on records in BM, L, MO and PRE.

Lineage C: KwaZulu-Natal Province: Uthukela District Municipality, uKhahlamba Drakensberg Park, Giants Castle Nature Reserve, 29°17.02'S, 29°30.87'E, 1780 m alt., on *Sematophyllum brachycarpum* on decaying wood, 28 Feb. 2018, Z. Egertová and M. Sochor ZE44/18 (PRM 951736); Uthukela District Municipality, uKhahlamba Drakensberg Park, Injasuti, 29°8.33'S, 29°25.68'E, 1565 m alt., on *Sematophyllum brachycarpum* on decaying wood, 3 Mar. 2018, Z. Egertová and M. Sochor ZE56/18 (PRM 951737).

Lineage D: Mpumalanga Province: Ehlanzeni District Municipality, Buffelskloof Nature Reserve, 25°16.93'S, 30°30.45'E, 1470 m alt., on *Sematophyllum brachycarpum* on decaying wood, 9 Mar. 2018, Z. Egertová and M. Sochor ZE69/18 (PRM 951738).

Taxonomic affinities. The phylogenetically closest and phenotypically most similar species is *Octospora kelabitiana* described from Borneo, which shares most characters with the African species. It also has apothecia with stiff, thick-walled hyaline hairs, ellipsoid, hyaline ascospores of similar size like *O. conidiophora* († in H_2O (13.5)14.5–17(18) × 7–8(9) µm, in LPCB (12.5)13–16(17) × (6.5)7–8(8.5) µm), filiform, unbranched paraphyses, smooth appressoria of similar size and even the warted mycelial hyphae, which is a character unknown in any other species of bryophilous Pezizales (Egertová et

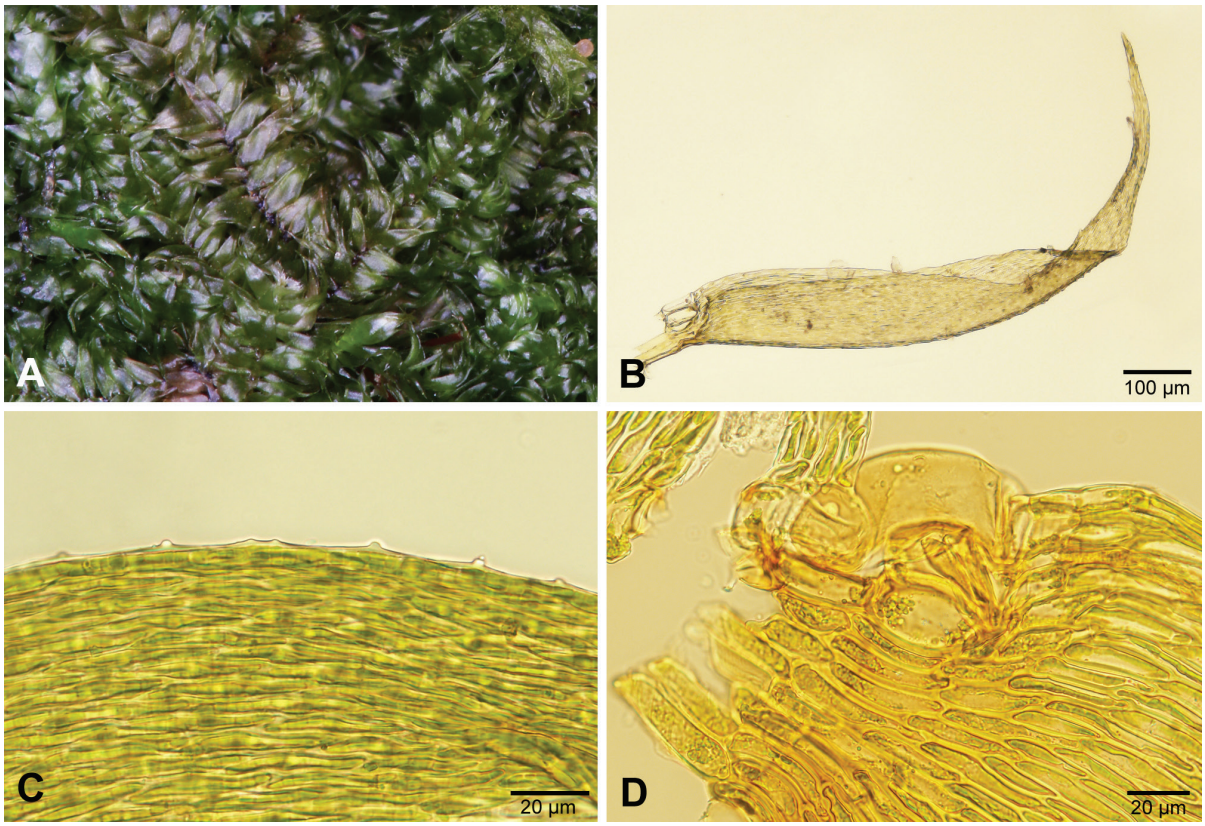

Figure 12. *Trichosteleum perchlorosum*. A. Plants. B. Leaf. C. Leaf papillae. D. Alar cells.

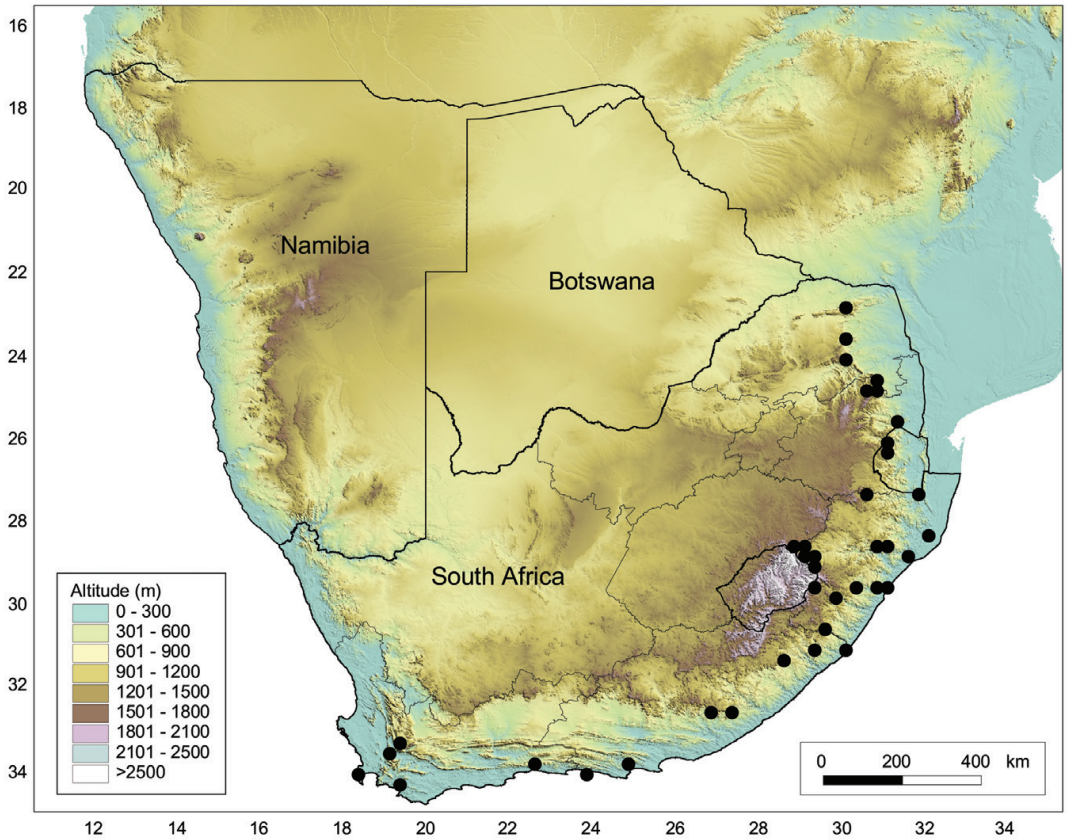

Figure 13. Geographical distribution of *Trichosteleum perchlorosum* in southern Africa based on records in BM, L, MO and PRE.

al. 2018). Nevertheless, it can be distinguished easily by growth on a completely different host – thallose liverworts from the genus *Riccardia* Gray. Furthermore, its apothecia are smaller, often taller than wide and lack a distinct margin. Its appressoria are usually one-celled, less often two-celled, while in *O. conidiophora*, two-celled appressoria are very common and even three-celled ones were found. Anamorph has not been detected in *O. kelabitiana*.

Discussion

According to the available literature and data from the main South African public fungarium (PREM), bryophilous Pezizales are completely unknown from southern Africa, despite the fact that this is a large and species-rich region, which hosts a very diverse bryoflora (Van Rooy and Phephu 2016). Our initial work revealed that this group of fungi is relatively common and probably also very diverse in southern Africa, despite the fact that the work was carried out in extraordinarily dry (and thus unsuitable) summer. Amongst others, four phenotypically similar, yet molecularly distinct lineages were discovered on two host species (lineages A and B on *Trichosteleum perchlorosum* and *Sematophyllum brachycarpum*, lineages C and D only on *S. brachycarpum*). This research brings novel insights into evolution and systematics of bryophilous ascomycetes and also raises important questions on taxonomic evaluation of these lineages. Therefore, we briefly discuss the taxonomy of cryptic taxa and suggest a suitable taxonomic solution for our collections. As *O. conidiophora* is the first species of bryophilous Pezizales with a detected anamorph, we also discuss this finding. Finally, diagnostic characters and data on distribution of the host mosses are provided as they may help expand the known distribution area of *O. conidiophora* in the future.

Taxonomic approach

The four lineages could not be distinguished phenotypically on the basis of characters that are normally studied in bryophilous Pezizales, although genetic differentiation was very high at all of the three studied loci (Suppl. material 1: Table S1). Such great genetic distances are usually observed amongst different species or even genera. The observed genetic distances, together with molecular dating, imply that the phenotypically more or less homogeneous morphotype actually represents a group of several cryptic species that have already become reproductively isolated in the Tertiary (Fig. 2). Similar cryptic diversity is probably quite common in fungi, including many genera of Pezizales, e.g. *Genea* Vittad. (Smith et al. 2006, Alvarado et al. 2016), *Geopyxis* (Pers.) Sacc. (Wang et al. 2016), *Helvella* L. (Nguyen et al. 2013, Skrede et al. 2017), *Terfezia* (Tul. & C.Tul.) Tul. & C.Tul. (Ferdman et al. 2009), *Trichophaea* Boud. (Van Vooren 2016) and *Tuber* P.Micheli ex F.H.Wigg. (Bonuso et al. 2010). In bryophilous Pezizales, intraspecific sequence variability was observed, e.g. in *Octosporopsis nicolai* (Maire) U.Lindem., M.Vega & T.Richt. (Lindemann et al. 2014) and *Octospora kela-*

bitiana (Egertová et al. 2018). Each of the species comprised two genetic lineages that, nevertheless, were relatively weakly diverged and were therefore not treated taxonomically. Besides the significant genetic distances amongst the South African populations, another fact speaks against the possibility that the four lineages could be treated as a single species; the whole clade includes *Octospora kelabitiana* (Fig. 1), a distinct species from Borneo infecting liverwort *Riccardia*. A widely defined species (i.e. including the four lineages but excluding *O. kelabitiana*) would therefore be paraphyletic.

The current approach of many authors to delimitation of species is based primarily or solely on DNA sequence data and sequence-based diagnoses have become almost a common practice in macromycetes (e.g. Buyck et al. 2016, Leacock et al. 2016, Taşkın et al. 2016, Wang et al. 2016, Korhonen et al. 2018). Some authors even aim to base descriptions of new species on environmental sequence data only (e.g. Hibbett et al. 2011). Although molecular phylogenetics is an excellent tool for evaluation of biodiversity, assignment of scientific binominal to molecularly defined species leads to several practical problems, mainly those related to limited accessibility of the methods for many field mycologists. Especially in developing countries, in which even standard optical microscopy can be barely affordable at the leading institutes, determination of species via DNA sequencing is still a matter for the distant future. This methodological obstacle may soon result (or has already resulted in some groups) in the split of traditional phenotype-based taxonomy and molecular taxonomy. Until recently, molecular taxonomy mostly worked with groups, such as molecular operational taxonomic unit (MOTU; Hibbett et al. 2011), phylogenetic species (O'Donnell et al. 2011), virtual taxon (Öpik et al. 2010) etc. and designated an alphanumeric code to them. Nevertheless, many of the molecular taxa are currently given traditional scientific names, often without studying related, validly described species that cannot be sequenced for various reasons. This process, although justified by the aim of cataloguing of global biodiversity, makes the resulting taxonomy impractical or even unusable for field mycologists (and sometimes also for molecular biologists). Another problem with descriptions of species, based on molecular data, is the fact that the borderline between intraspecific and interspecific molecular variation is often unclear (Thines et al. 2018), dependent on many evolutionary factors (e.g. Leliaert et al. 2014) and may become fuzzy after a more intensive and/or extensive sampling is performed, particularly if only one or few molecular markers are used. Nevertheless, this problem also exists with traditional taxonomy (e.g. Flynn and Miller 1990, Paal et al. 1998, Benkert 2001). One solution to the problems mentioned above is an integrative approach. This takes advantage of both multiple characters (morphology, DNA, ecology etc.) and results in robust, phylogeny-based taxonomy that is accessible to various users (e.g. Araújo et al. 2015, Skrede et al. 2017, Haelewaters et al. 2018).

After thorough consideration of the above-mentioned facts, we decided not to formally describe all of the four discovered cryptic species at the present moment. Instead, we prefer to establish two taxa: *O. conidiophora* (s.str.), which refers to the most common phylogenetic lineage A and the informal taxon *O. conidiophora* agg., which applies to all of the four South African cryptic species, but also to the morphologically distinct and host-specific Bornean *O. kelabitiana*. Although the name *O. kelabitiana* is older and should therefore be selected for the aggregate, we believe that the name *O. conidiophora* agg. better suits the pragmatic

purposes of this informal taxon. Our approach enables field mycologists to determine their specimens at least on the aggregate level and, at the same time, preserves a monophyletic taxonomical system. Detailed studies may reveal phenotypic differences between the South African lineages of *O. conidiophora* agg., which can then be formally described as species. Until then, we prefer to leave lineages B, C and D without a Latin binominal.

Anamorph

Conidia have been reported in several genera of Pezizales. The most frequent type of conidia are amerospores which are produced, e.g. in *Caloscypha* Boud. (Paden et al. 1978), *Desmazierella* Lib. (Hughes 1951), *Iodophanus* Korf (Korf 1958, sub *Ascophanus* Boud.), *Pachyphlodes* Zobel (Healy et al. 2015), *Peziza* Fr. (Berthet 1964a, Paden 1967, 1972), *Ruhlandiella* Henn. (Warcup and Talbot 1989, sub *Muciturbo* P.H.B.Talbot), *Thecotheus* Boud. (Conway 1975), *Urnula* Fr. (Davidson 1950), *Cookeina* Kuntze, *Phillipsia* Berk. (Paden 1975), *Pithya* Fuckel (Paden 1972), *Nanoscypha* Denison (Pfister 1973), *Sarcoscypha* (Fr.) Boud. (Harrington 1990), *Geopyxis* (Paden 1972), *Pyropyxis* Egger (Egger 1984, Filippova et al. 2016) and *Trichophaea* (Hennebert 1973). Staurospores can be found in *Miladina lecithina* (Cooke) Svrček (Descals and Webster 1978). The conidia of *O. conidiophora* can be classified as scolecospores or phragmospores and are therefore unique amongst Pezizales with known teleomorph. In their shape, they resemble the conidia of the anamorphic genus *Spermospora* R. Sprague (Ascomycota, Pezizomycotina), a parasite of grasses (Sprague 1948, Seifert et al. 2011).

Detached conidia were regularly found between the rhizoids and leaves in almost all collections of *Octospora conidiophora* agg. (with the exception of specimens ZE38/18, ZE53/18 and ZE71/18, probably due to limited material). The distal part of the conidia is sometimes short and straight. It is not clear whether this is an artefact caused by breaking off during preparation, although tail fragments have not been found. Germinating conidia are not rare. Longer germination tubes look like normal hyphae with the characteristic warty surface structure (Figs. 5F, 7F). Conidia germinating by a two-celled appressorium (Fig. 7F) or connected to a mycelial hypha by an anastomosis (Figs. 7E, G, 9A) have been repeatedly observed. Conidiogenous cells (Fig. 7D) are much more difficult to detect than conidia and have only been found in a few collections. The scars formed by detachment of conidia must not be confused with the ends of torn-off hyphae, which inevitably result during preparation. Fully developed conidia still connected to the conidiogeneous cells have not been found. Apparently, mature conidia easily detach from their conidiogenous cells. A developing, still attached conidium was observed once (Fig. 7D).

Octospora conidiophora agg. is the first case amongst bryophilous Pezizales in which an anamorph has been detected. The absence of records of anamorphic states in other species can be caused either by their real rarity or only by their difficulty in detection. The latter can have many reasons. First, bryophilous ascomycetes, in general, stand rather on the periphery of researchers´ interest (see Döbbeler 1997). Second, anamorphs are usually inconspicuous and therefore not easy to encounter. Even if an

anamorph is found, it can be difficult to link it with the corresponding teleomorph, because many fungal species commonly occur together. Moreover, anamorphs and teleomorphs are often formed in different environmental conditions (Kendrick 1979) and often at different times. And third, anamorphs are often studied in aseptic cultures and subsequently cultures are used for confirmation of their identity by molecular methods; unfortunately, cultivation of bryophilous Pezizales seems to be problematic (Berthet 1964b) and is not commonly attempted. Although an anamorph has not been confirmed by cultivation methods in *O. conidiophora* agg., the connection of anamorph and teleomorph is based on the evidence discussed above: conidia were repeatedly found amongst the moss plants near the teleomorph; germinating conidia have hyphae with the same ornamentation as observed in the mycelium bearing apothecia; conidiogenous cells occur on the mycelial hyphae; conidia anastomose with mycelial hyphae; the germlings form appressoria.

Hosts

***Sematophyllum brachycarpum* (Hampe) Broth.**

Syn: *Hypnum brachycarpum* Hampe

Sematophyllum brachycarpum can be distinguished from other species of *Sematophyllum* in southern Africa by the complanate, straight leaves with relatively large groups of alar cells (in 3–4 rows) that are not much inflated or coloured (Fig. 10, see also Câmara et al. 2019).

The species is by far the most common and widespread species of *Sematophyllum* in South Africa; *S. brachycarpum* is found in forests and wooded areas of the Limpopo, Mpumalanga, North West, Gauteng, Free State, KwaZulu-Natal, Eastern Cape and Western Cape Provinces (Fig. 11, see also Câmara et al. 2019). It occurs as an epiphyte or occasionally on soil or rocks, from sea level up to 1900 m alt. The species is widely distributed throughout the Afromontane Region, as defined by Van Rooy and Van Wyk (2010) and was found to belong to the Widespread Afromontane Subelement, a subdivision of the Afromontane Forest Element (Van Rooy and Van Wyk 2011). The Widespread Afromontane Subelement is centred in the Midlands of KwaZulu-Natal and the Drakensberg escarpment of Mpumalanga as well as in forests in the south-western Cape. The species has also been recorded from Lesotho, Swaziland, Mozambique, Zimbabwe, Zambia, Uganda and Kenya (O'Shea 2006).

***Trichosteleum perchlorosum* Broth. & Bryhn**

Trichosteleum perchlorosum is the only southern African species of Sematophyllaceae (sensu stricto) with papillose leaf cells. However, the papillae are sometimes difficult to see or may be absent on some leaves. The falcate leaves with enlarged, inflated

and coloured alar cells will also help to identify the species (Fig. 12, see also Câmara et al. 2019).

The species is endemic to the southern part of Africa and occurs as an epiphyte and also on decaying logs or rocks from sea level up to 3090 m high (Drakensberg of KwaZulu-Natal). It is most frequently collected in the KwaZulu-Natal Province of South Africa, but it is also known from Limpopo, Mpumalanga, Eastern Cape and Western Cape Provinces, as well as Swaziland (Fig. 13, see also Câmara et al. 2019). *Trichosteleum perchlorosum* is widespread throughout the Afromontane Region sensu Van Rooy and Van Wyk (2010), but unknown from Afromontane outliers in the Magaliesberg of Gauteng and the North West, the eastern Free State and the Waterberg of Limpopo. It was therefore included in the Tropical Afromontane Subelement (Van Rooy and Van Wyk 2011), which is centred in the Drakensberg escarpment of Mpumalanga and the Midlands of KwaZulu-Natal. This species was also reported from Zimbabwe (O'Shea 2006).

Acknowledgements

ZS and MS thank John Manning for financial support of the travel to South Africa. Riana Jacobs-Venter is acknowledged for providing information on the absence of bryophilous Pezizales in the South African National Collection of Fungi and Markéta Šandová and Miriam Brožíková (PRM) for information about *Octospora tetraspora* var. *aegyptiaca*. We are grateful to Hester Steyn and Elizma Fouche for editing the maps and the editor Danny Haelewaters and the reviewers, Nicolas Van Vooren and Donald H. Pfister, for comments to the manuscript. The work was partly supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0418. ZS was supported by an internal grant from Palacký University (IGA_PrF_2019_004). Research was conducted under permit no. OP 1264/2018.

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Supplementary material 1

Table S1. Distance matrices (nucleotide difference) for each locus of *Octospora conidiophora* agg. and several randomly selected taxa

Authors: Zuzana Sochorová, Peter Döbbeler, Michal Sochor, Jacques van Rooy

Data type: molecular data

Link: https://doi.org/10.3897/mycokeys.54.34571.suppl1

Supplementary material 2

Figure S1. Bayesian phylogeny inference based on single-locus analyses

Authors: Zuzana Sochorová, Peter Döbbeler, Michal Sochor, Jacques van Rooy

Data type: phylogenetic tree

Explanation note: Bayesian posterior probability are shown above branches.

Link: https://doi.org/10.3897/mycokeys.54.34571.suppl2

4.2 Studies in bryophilous ascomycetes in Europe

4.2.1 *Lamprospora sylvatica* (Pyronemataceae), a new bryophilous ascomycete on *Dicranum montanum*

Phytotaxa 357 (1): 017–029
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.357.1.2

Lamprospora sylvatica (Pyronemataceae), a new bryophilous ascomycete on *Dicranum montanum*

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Abstract

Lamprospora sylvatica is described as a new species based on finds from Ukraine, Slovakia, Germany and Norway. It is characterised by the combination of the following features: pinkish, orange to reddish-orange apothecia with a fimbriate margin, globose ascospores with more or less regular areolate ornamentation, infecting strong rhizoids of *Dicranum montanum* with an infectious structure consisting of a one-celled appressorium surrounded by a multi-layered cluster of thick-walled cells and haustorium within the rhizoids. The apothecia were always found on rotten wood, which is an unusual habitat for hosts of bryophilous Pezizales. The new species is compared to similar taxa morphologically and by means of DNA sequencing. In the phylogenetic analysis based on LSU and ITS regions, *L. sylvatica* forms a well-supported clade close to *L. feurichiana* (on *Ceratodon purpureus*), *L. kristiansenii* (also on *C. purpureus*) and *L. campylopodis* (on *Campylopus* spp.).

Keywords: Ascomycota, bryosymbiotic fungi, haustoria within rhizoids, Hainich National Park, Malá Fatra National Park

Introduction

The genus *Lamprospora* De Notaris (1864: 388; Pyronemataceae, Pezizales) belongs to a group of operculate ascomycetes that infects mosses or liverworts, and that also includes the related genera *Octospora* Hedwig (1789: 4), *Neottiella* (Cooke 1879: 261) Saccardo (1889: 190), *Octosporella* Döbbeler (1980: 827), *Filicupula* Y.J. Yao & Spooner (1996: 193) and *Octosporopsis* U. Lindemann & M. Vega (2014: 566). Members of this genus are characterised by disc-shaped, sessile, orange to red apothecia that are c. 0.5–4 mm wide and often have a fimbriate margin. Microscopically, they are distinguished particularly by inamyloid operculate asci and globose or subglobose, exceptionally broadly ellipsoid, ornamented ascospores. Ascospore ornamentation is remarkably diverse and very important for species identification. It can consist of warts, tubercles, ridges, bands or a combination thereof.

Fruit bodies occur mostly on soil, less often on stones or wood, together with the host bryophyte. Almost all *Lamprospora* species grow on acrocarpous mosses, with the exception of *L. aneurae* Benkert (1990: 631) that infects the liverwort *Aneura pinguis* (Linné 1753: 1136) Dumortier (1831: 86) and whose placement within the genus *Lamprospora* is debatable on the grounds of its ascospore shape and ornamentation that are quite unusual for this genus. Apart from ascospore ornamentation, infectious structures and host species identity are the most important distinguishing characteristics of *Lamprospora* species. Preliminary molecular data indicate a very high host specificity within the bryophilous Pezizales, which appears to be even more pronounced than previously assumed (Döbbeler 1980, Benkert 1987) and which could play a key role in addressing morphologically similar species complexes, such as *L. miniata* De Notaris (1864: 388; Z. Egertová unpubl. data, L. Janošík pers. comm). The genus as currently understood includes around 40 species.

In November 2014, fruit bodies of an undescribed *Lamprospora* were found on rotten wood with *Dicranum montanum* Hedwig (1801: 143), a species not previously known as a host for members of the bryophilous Pezizales, in Malá Fatra National Park in Slovakia. Less than one year later, the same species was found in Hainich National Park in

Germany. In 2017, a locality in Ukraine and another locality in Slovakia were added. Moreover, a herbarium specimen of the species, already collected in 1997, was sent to us from Norway by Roy Kristiansen.

The aim of the present article is to describe this taxonomic novelty as a new species and to compare it with other *Lamprospora* species with a similar ornamentation of ascospores.

Material and methods

Sample collection and observation

The description of macroscopic and microscopic characters is based on live material from Ukraine, Slovakia and Germany, and rehydrated material from Norway. Measurements of microscopic structures were made in tap water using standard light microscopy. Between ten and 70 measurements of ascospore size (from a spore print) and at least 10 measurements for other structures were carried out for each vital collection. Measurements of ascospores of all species always included ornamentation, unless stated otherwise. Ornamentation of ascospores was studied in cotton blue, and non-amyloidity checked in Lugol´s solution. Scanning electron micrographs (SEM) were taken from air-dried samples using a LEO-438 VP environmental scanning electron microscope. Bryophyte taxonomy refers to Hill *et al.* 2006 for mosses and Söderström *et al.* 2016 for liverworts.

DNA extraction, PCR amplification and sequencing

Lamprospora sylvatica and other *Lamprospora* species with similar ascospore ornamentation were included in molecular analysis (see Supplementary File 1). DNA was extracted from fresh or dried apothecia using the CTAB method (Doyle & Doyle 1987). Sequence data were generated for two loci; amplification was carried out for internal transcribed spacers (ITS) of ribosomal DNA (ITS1–5.8S rDNA–ITS2 region) with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and for the large subunit of ribosomal DNA (LSU) with primers NL1 (Maier *et al.* 2003) and LR6 (Vilgalys & Hester 1990). PCR was performed with Kapa polymerase (Kapa Biosystems), following a standard protocol with 37 cycles and annealing temperature of 56°C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25M NaCl in the precipitation mixture) and sequenced in both directions using the Sanger method (Macrogen Europe, The Netherlands).

Phylogenetic analysis

Sequences were edited and aligned in Geneious (ver. 7.1.7., Biomatters). Phylogeny was reconstructed using the Maximum Parsimony (MP) method and tested by means of bootstrapping, using 1000 pseudoreplicates in MEGA (ver. 6.06, Tamura *et al.* 2011). Bayesian phylogeny inference (BI) was computed in MrBayes (ver. 3.2.4, Ronquist *et al.* 2012) using the GTR+I+G substitution model, as determined by AICc in jModeltest (ver. 2.1.4, Darriba *et al.* 2012). The analysis was run for 6 millions generations in four independent runs, sampling every 1000th generation and excluding the first 50 % of generations as burn-in. The Basic Local Alignment Search Tool (BLAST; Zhang *et al.* 1990) was used for searching similar sequences in publicly available sequence databases (https://blast.ncbi.nlm.nih.gov).

Results

Taxonomy

Lamprospora sylvatica Egertová & Eckstein, *sp. nov.* (Figs 1–5)
Mycobank MB 824682

Diagnosis:—Differs from other species of the genus *Lamprospora* by the following combination of characters: pinkish, orange to reddish-orange apothecia with a fimbriate margin, globose ascospores with more or less regular areolate ornamentation, infection of *Dicranum montanum*, and infectious structures consisting of an appressorium surrounded by many thick-walled cells, located on strong rhizoids of the host, and a haustorium within the rhizoids.

FIGURE 1. *Lamprospora sylvatica* (a. PRM 946419, b. B Eckstein-43421, c. holotype, PRM 946415, d. PRM 946418). a. typical habitat—a moss covered rotten conifer log, b, c, d. apothecia between shoots of *Dicranum montanum*. Scale bar: b = 1 mm. Photos M. Sochor (a), J. Eckstein (b), Z. Egertová (c, d).

Etymology:—The specific epithet reflects the occurrence in forests.

Holotype:—UKRAINE. Zakarpattia Oblast, 3.3 km NW from Nimetska Mokra, 48°23’42”N, 23°48’27”E, 740 m asl., on *Dicranum montanum* covering a rotten stem of a conifer, 8 July 2017, *Z. Egertová and M. Sochor* (PRM 946415).

Description:—*Apothecia* (0.3–)0.5–1(–1.3) mm broad, first closed, later hemispherical to discoid, thick, sessile, with a narrow, fimbriate margin, hymenium pinkish, orange or reddish orange, rarely whitish, outer surface concolorous. *Asci* 240–370(420) × 20–27(29) µm, cylindrical, 8-spored, operculate, inamyloid, arising from croziers. *Ascospores* globose, (15.5–)16–18(–19) µm in diameter, hyaline, with a large spherical drop of 9–11.5 µm, uniseriate. Ornamentation areolate, consisting of ridges 0.4–1.6 µm wide and 0.8–1.4 µm high, forming a complete, ± regular reticulum of 5–7 meshes/diameter, meshes 1.5–4.5(8) µm broad. *Paraphyses* filiform, straight, mostly simple, rarely forked, pluriseptate, containing orange pigment, 2.5–4 µm broad, terminal cell 33–103 µm × 4–6.5 µm. *Subhymenium* consisting of thin-walled, variously shaped, hyaline cells 2.5–6 µm broad, merged with the medullary excipulum. *Medullary excipulum* about 150–220 µm thick (in the thickest part), made up of thin-walled, variously shaped cells - cylindric, pyriform, broadly ellipsoid, subglobose or indefinite. *Ectal excipulum* about 70–120 µm thick, consisting of thick-walled, globose, subglobose to subangular cells (*textura subglobulosa*) of 6–35 × 7–26 µm, the outermost cells often flattened, cell wall up to 5 µm thick. *Margo* of parallel hyphae of size 13–47 × 6–24 µm *(textura prismatica-porrecta*). *Infections* located on strong rhizoids of *Dicranum montanum*. *Infectious structures* consisting of spherical to irregular clusters of cells firmly attached to and often completely surrounding the rhizoid. Cells within the clusters more or less isodiametric, 10–20 µm wide with walls 2–5 µm thick. Appressoria situated at the centre of these clusters sitting directly on the rhizoid wall, very thick-walled, ± isodiametric or often slightly higher than wide, with 20–28 × 17–25 µm in diameter only slightly larger than surrounding cells. Infectious clusters up to 110 µm wide. Infection peg 4–5 µm wide, surrounded by a tube of rhizoid cell-wall material, forming intracellular haustoria.

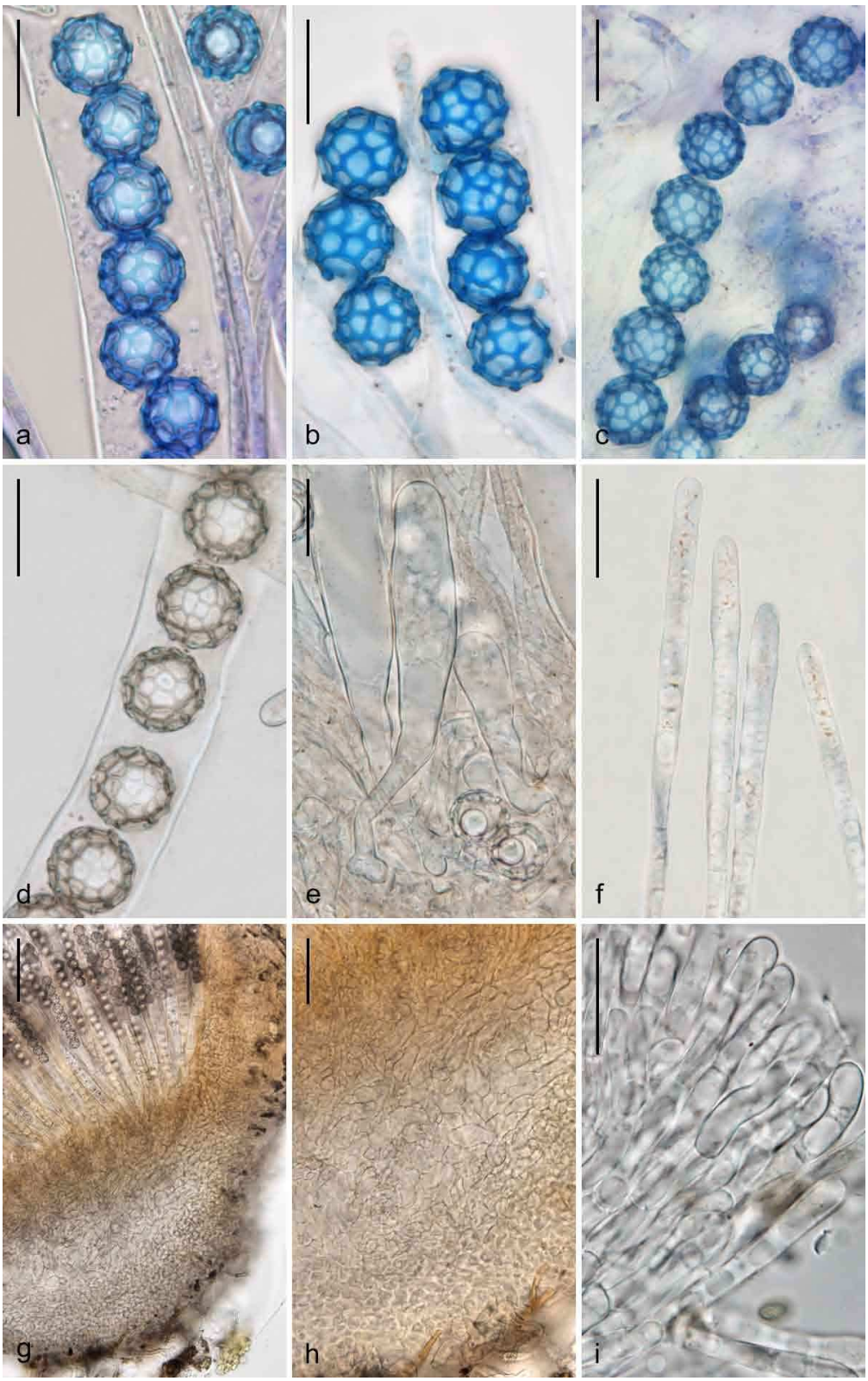

FIGURE 2. *Lamprospora sylvatica* (a–b, e–i. holotype, PRM 946415, c–d. PRM 946419) a–c. ascospores stained with cotton blue, d. ascospores in water, e. young asci, f. upper part of paraphyses, g. cross section of an apothecium showing hymenium, subhymenium, medullary and ectal excipulum, h. cross section showing subhymenium, medullary and ectal excipulum, i. marginal hyphae of an apothecium. Scale bars: a–f, i = 20 µm; g = 100 µm; h = 50 µm. Photos Z. Egertová.

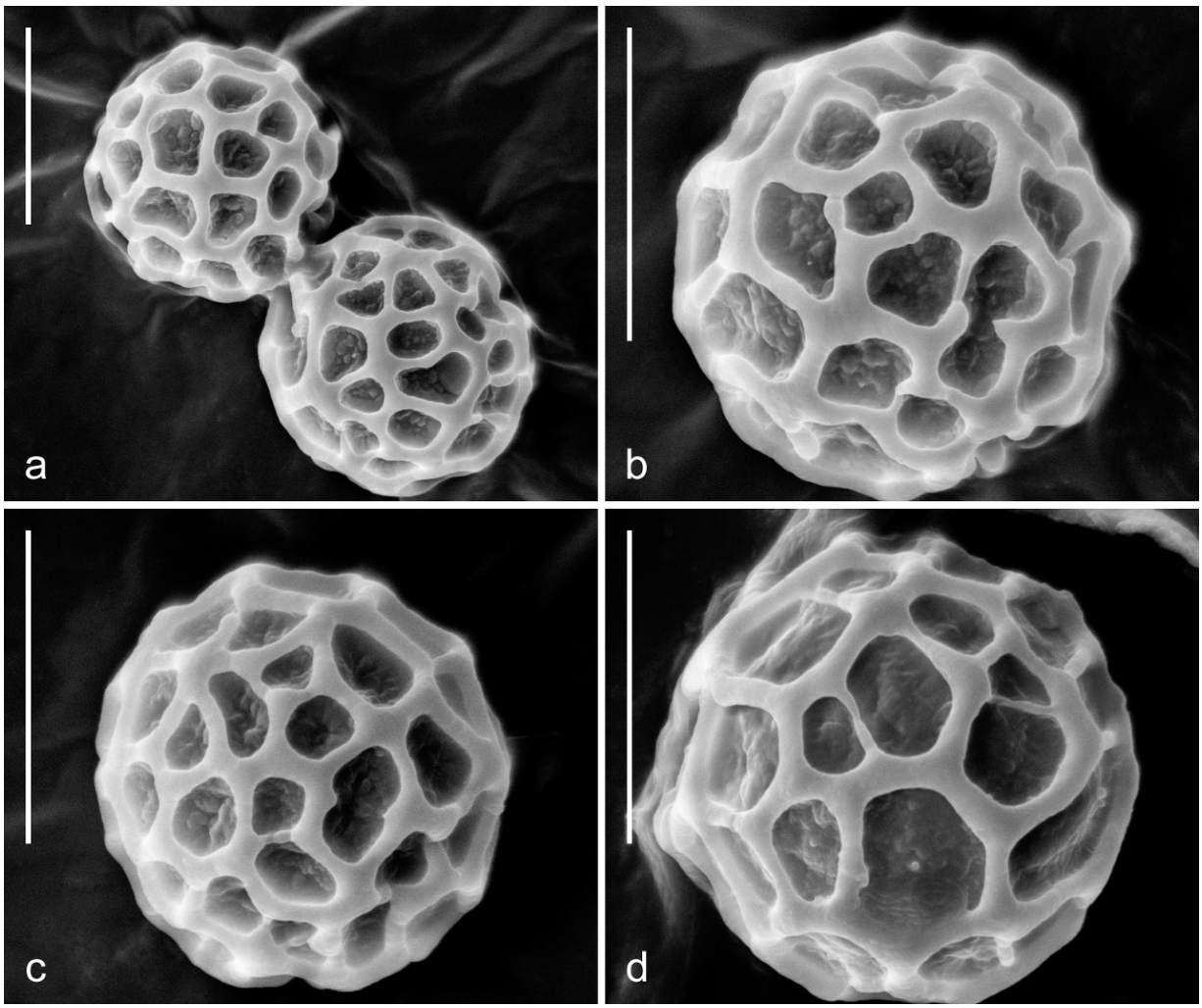

FIGURE 3. *Lamprospora sylvatica* (a–b. B Eckstein-43421, c, d. PRM 946416) SEM of ascospores. Scale bars: a–d = 10 µm. Photos J. Eckstein.

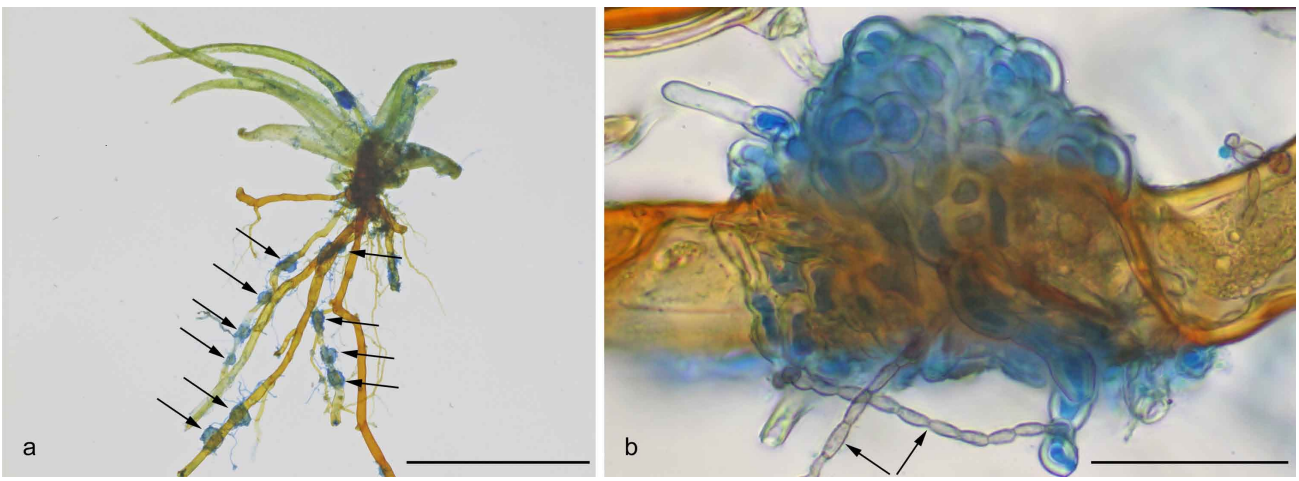

FIGURE 4. *Lamprospora sylvatica* (a–b. PRM 946416) a. shoot of *Dicranum montanum* with infections on rhizoids indicated by arrows, b. microscopic image of an infection, note the cluster of thick-walled cells, the brown short-celled hyphae at bottom (arrows) do not belong to *L. sylvatica*. Scale bars: a = 1 mm; b = 50 µm. Photos J. Eckstein.

Habitat and distribution:—In all cases the fungi grew in forests on strongly decayed logs covered by *Dicranum montanum*. Other accompanying bryophytes included *Buxbaumia viridis* (De Candolle 1815: 227) Mougeot & Nestler 1823: 724, *Cephaloziella* spec. (Spruce 1882: 62–63) Schiffner 1893: 98), *Herzogiella seligeri* (Bridel 1801a: 47–48) Iwatsuki (1970: 374) and *Hypnum cupressiforme* Hedwig (1801: 291). The species is so far known from locations in Ukraine, Slovakia, Germany and Norway.

Additional specimens examined: GERMANY. Thuringia, Hainich National Park, 51°04'06"N, 10°26'26"E, 420 m asl., on *Dicranum montanum* on strongly rotten conifer wood, 26 October 2015, *J. Eckstein* (B Eckstein-43421). NORWAY. Østfold, Aremark Bøen sæter, Tjøstøltjern, 150 m asl., on *Dicranum montanum* on wood in mixed forest, mostly *Picea*, 15 November 1997, *R. Kristiansen* (97.65). SLOVAKIA. Malá Fatra National Park, 110 m N of the Kopa Peak, 49°10'58"N, 18°55'46"E, 1093 m asl., on *Dicranum montanum* on a strongly rotten *Picea* stem, 17 November 2014, *Z. Egertová and M. Sochor* (PRM 946416); ibid. 1 November 2015 (PRM 946417); ibid. 4 November 2017 (PRM 946418); Malá Fatra National Park, 550 m SSW of the Kopa Peak, 49°10'37.3"N, 18°55'39.8"E, 895 m asl., on *Dicranum montanum* on a rotten conifer stem, 4 November 2017, *Z. Egertová and M. Sochor* (PRM 946419).

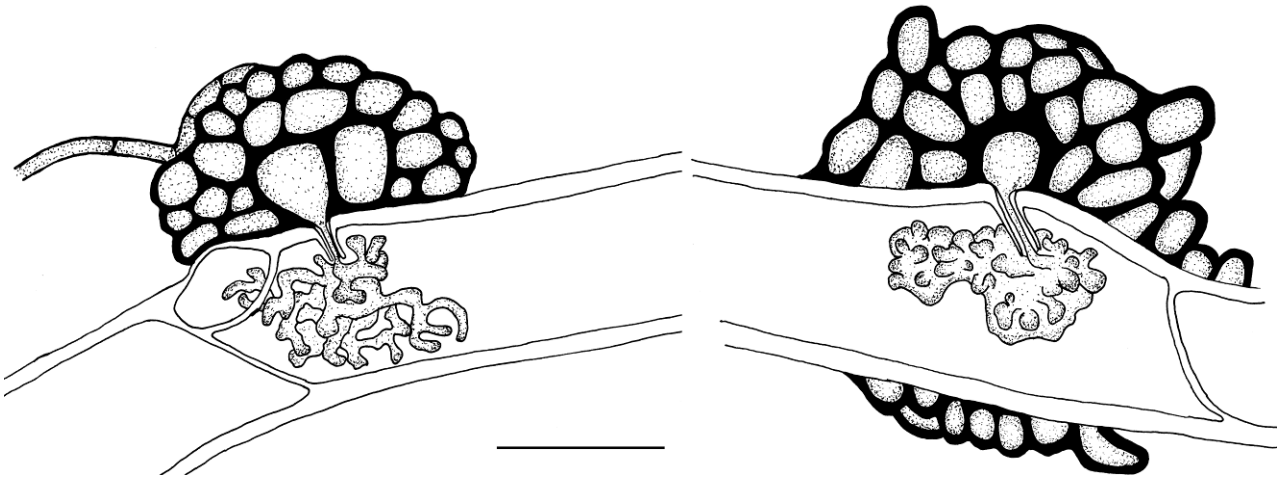

FIGURE 5. *Lamprospora sylvatica* (PRM 946416) drawings of two infectious structures in optical section. Scale bar = 50 µm. Illustrated by J. Eckstein.

Phylogenetic analyses: *Lamprospora sylvatica* was represented by five collections in the analysis. Of these, the Norwegian one was the oldest, and its DNA was very fragmented. As a result, an ITS sequence from this accession could not be obtained. However, the LSU region was identical in all five specimens, and ITS exhibited a single-nucleotide deletion in a poly-C microsatellite in the Slovak collections. The BLAST search resulted in one entry 100% identical to *L. sylvatica* in the LSU sequence—*Lamprospora* sp., accession number EU940123. Unfortunately, this sequence contains reading gaps precisely in the most variable regions, and therefore cannot be assigned to the new species with certainty. Furthermore, the specimen has been destroyed (S. Huhtinen pers. comm.) and is not available for study anymore.

Based on both markers (particularly LSU), it is clear that *L. sylvatica* is related to *L. feurichiana* (Kirschstein (1935: 205) Benkert (1976: 639) and *L. kristiansenii* Benkert (1990: 635), but differs significantly (similarity around 71 % in ITS and 96 % in LSU) from both species. Nevertheless, both BI and MP for LSU indicated its closer relationship with *L. campylopodis* W.D. Buckley (1923: 44; Fig. 6; similarity 59 % in ITS and 95 % in LSU), although with very low bootstrap support in MP (33 %; Supplementary File 2). ITS was hypervariable in the group, and its alignment was very ambiguous among distant species; phylogeny inference was therefore highly dependent on the model/method used and resulted mostly in low statistical support of many splits (Fig. 6 and Supplementary File 2). Nonetheless, ITS proved useful as a species-specific marker.

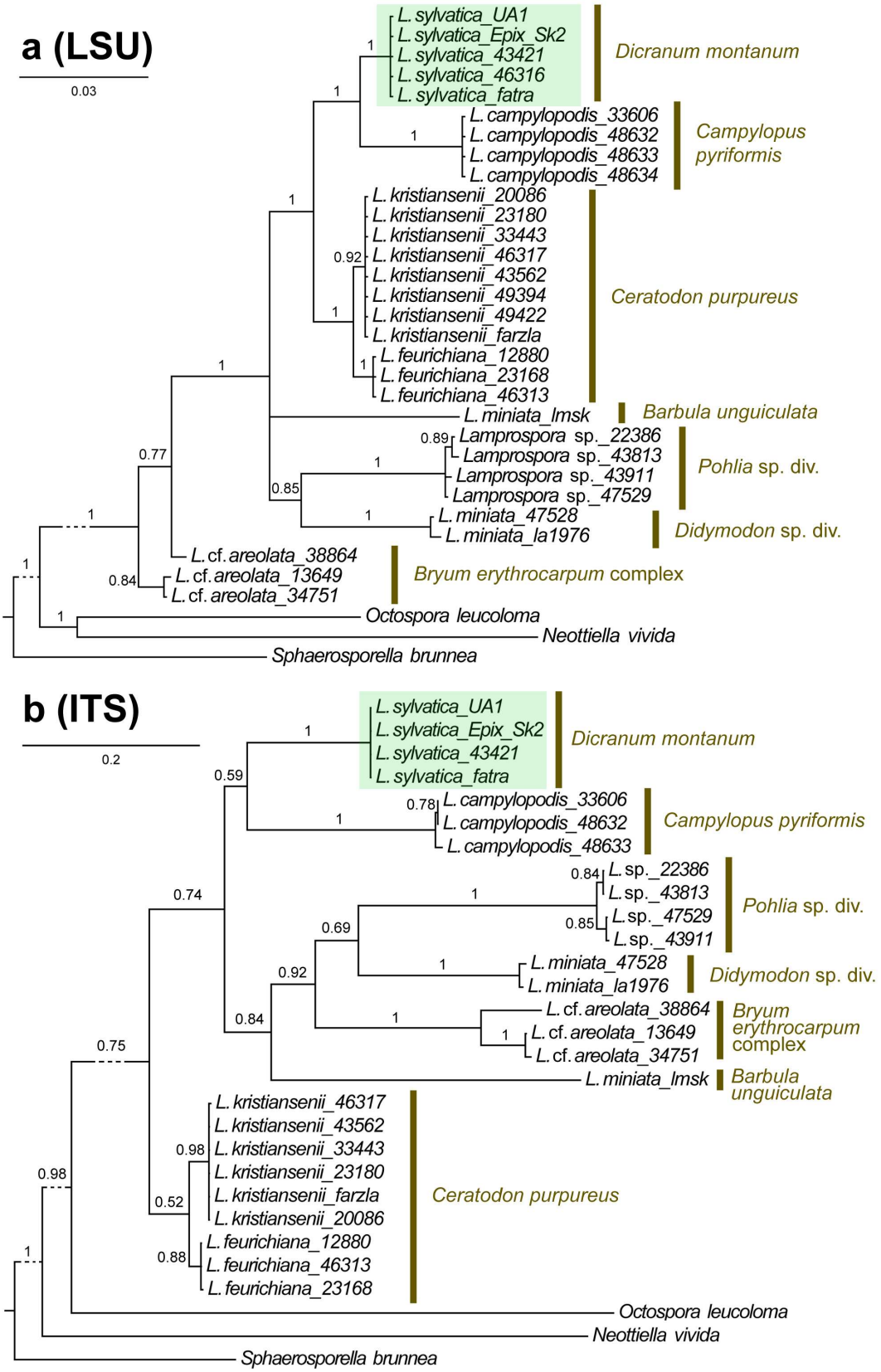

FIGURE 6. Bayesian inference phylogeny trees based on LSU and ITS loci; posterior probabilities shown above branches. Note that basal splits in the ITS tree may be influenced by its hypervariability in the group and ambiguous alignment. Collection numbers are given after underscores.

Discussion

The most important characteristics for determination of species in bryophilous Pezizales are ascospores (shape, size and ornamentation) and host bryophytes. Ascospores with an areolate reticulum can be found in many species of *Lamprospora*. It is a rather difficult genus and delimitation of several species has not been fully settled. In the following, a more detailed comparison with *L. sylvatica* is provided for those known species having globose ascospores ornamented with ridges less than 2 µm wide forming a complete, areolate reticulum with less than eight meshes/diameter, namely for *L. areolata* Seaver (1912: 48), *L. australis* (McLennan & Cookson 1923: 155) Rifai (1968: 182), *L. campylopodis*, *L. faroensis* Benkert (1987: 222), *L. feurichiana*, *L. kristiansenii*, *L. miniata* and *L. rugensis* Benkert (1987: 240). Collection data of all studied specimens from these species can be found in Supplementary File 1. Species-level differences between these species and *L. sylvatica* are as follows:

a) ***Lamprospora areolata*** was originally described from the USA (Seaver 1912) and later reported from France (Le Gal 1939) and Spain (De La Torre 1975, Rubio *et al.* 2002). Recent collections from Madeira and Germany (unpublished) at first glance appear to fit the description of *L. areolata*. However, identity of the European and American findings remains uncertain. The type specimen is characterised by globose ascospores 18–20 µm in diameter, ornamented by very high and narrow ridges (Seaver 1912, Benkert 1987, Wang & Kimbrough 1992). The recent collections from Germany and Madeira have larger ascospores, (19–)20–22(–23) µm in diameter (from spore prints), with ridges 2.4–3.2 µm high (Fig. 7a). Moreover, both De La Torre (1975) and Rubio *et al.* (2002) report for their collections pustules in the net's meshes—a feature neither described by Seaver (1912), Benkert (1987) or Wang & Kimbrough (1992), nor observed from the collections from Madeira and Germany. Confusingly, Seaver (1912) and Benkert (1987) emphasize that apothecia of *L. areolata* have no margin, whereas Wang & Kimbrough (1992) report and illustrate a ‚slightly raised margin' in the type specimen. Differences were observed in potential hosts as well. Seaver's specimens were associated with *Funaria* (Hedwig 1801: 172; Wang & Kimbrough 1992), although an infection was not demonstrated. The host in the recent European finds was *Bryum* sp. (Hedwig 1801: 178–187; unpublished). Whether any of these specimens are conspecific with the American species is difficult to decide with any certainty as the type is extremely sparse (Benkert 1987) and very old and therefore unavailable for sequencing. In any case, all these collections differ from *L. sylvatica* by the clearly higher than wide ridges of the ascospore ornamentation and with respect to host taxonomic identity.

b) ***Lamprospora australis***, known only from Australia, has ascospores very similar to those of *L. areolata*, 18–20 µm in diameter (McLennan & Cookson 1923, as *Lamprospora areolata* var. *australis*), and frequently with dotted ridges about 1 µm wide and 1.5–3 µm high, forming a reticulum with 3–4(–5) meshes per diameter (Rifai 1968, Benkert 1987, Wang & Kimbrough 1992). According to Benkert (1987), the host of *L. australis* is *Campylopus introflexus* (Hedwig 1801: 147) Bridel (1819: 72).

Therefore, *L. australis* (like *L. areolata*) is readily distinguished from *L. sylvatica* via its considerably higher ridges and via its host species.

c) ***Lamprospora campylopodis*** has ascospores that are (15–)16–19(–20) µm wide and often have dotted ridges 0.5–1.5 wide and high, forming an areolate ornamentation with (3–)4–6(–8) meshes per diameter, most of them 4–5 µm wide (Benkert 1987; Fig. 7b). In the original description of this species, *Campylopus fragilis* (Bridel 1801b: 296) Bruch & Schimper (1847: 164) was listed as host (Buckley 1923); however, the holotype appears to have been lost, and thus, a neotype had been chosen for which *C. pyriformis* (Schultz 1819: 73) Bridel (1826: 471) was described as host (Benkert 1987, p. 213). A specimen growing on *C. oerstedianus* (Müller 1851: 259) Mitten (1869: 81) has been reported from Greece (Benkert 2007). In addition to having a different host species, *L. campylopodis* also differs from the new species by having a reticulum with fewer and larger meshes, and by its slightly larger ascospores.

d) ***Lamprospora faroensis*** is a slightly enigmatic species. According to its original description, it has ascospores 15–17 µm in diameter (excl. orn.), that are ornamented by often dotted ridges 0.5–0.7 µm wide and ca 1 µm high, forming a reticulum with 5–8 meshes per diameter, and it infects rhizoids of *Ceratodon purpureus* (Hedwig 1801: 36) Bridel (1826: 480; Benkert 1987). To compare it with the new species, we studied the type (UPS F-016650) and found the ascospores 16–17.5 µm in diameter, ornamented by ridges 0.3–0.6 µm wide and high, forming a ± complete, irregular reticulum (miniata-type) with 6–9 meshes/diameter (Fig. 7c). The ridges were never dotted and rarely incomplete. The ascospore surface was covered by many small warts within the meshes of the reticulum. Importantly, we could prove that a *Bryum* species is the host, and not *C. purpureus*. Although *C. purpureus* is present in the holotype sample, no infection could be found. Therefore, *L. faroensis* differs from *L. sylvatica* by its finer ridges, and by a reticulum with on average more meshes/diameter, as well as by the species identity of its host.

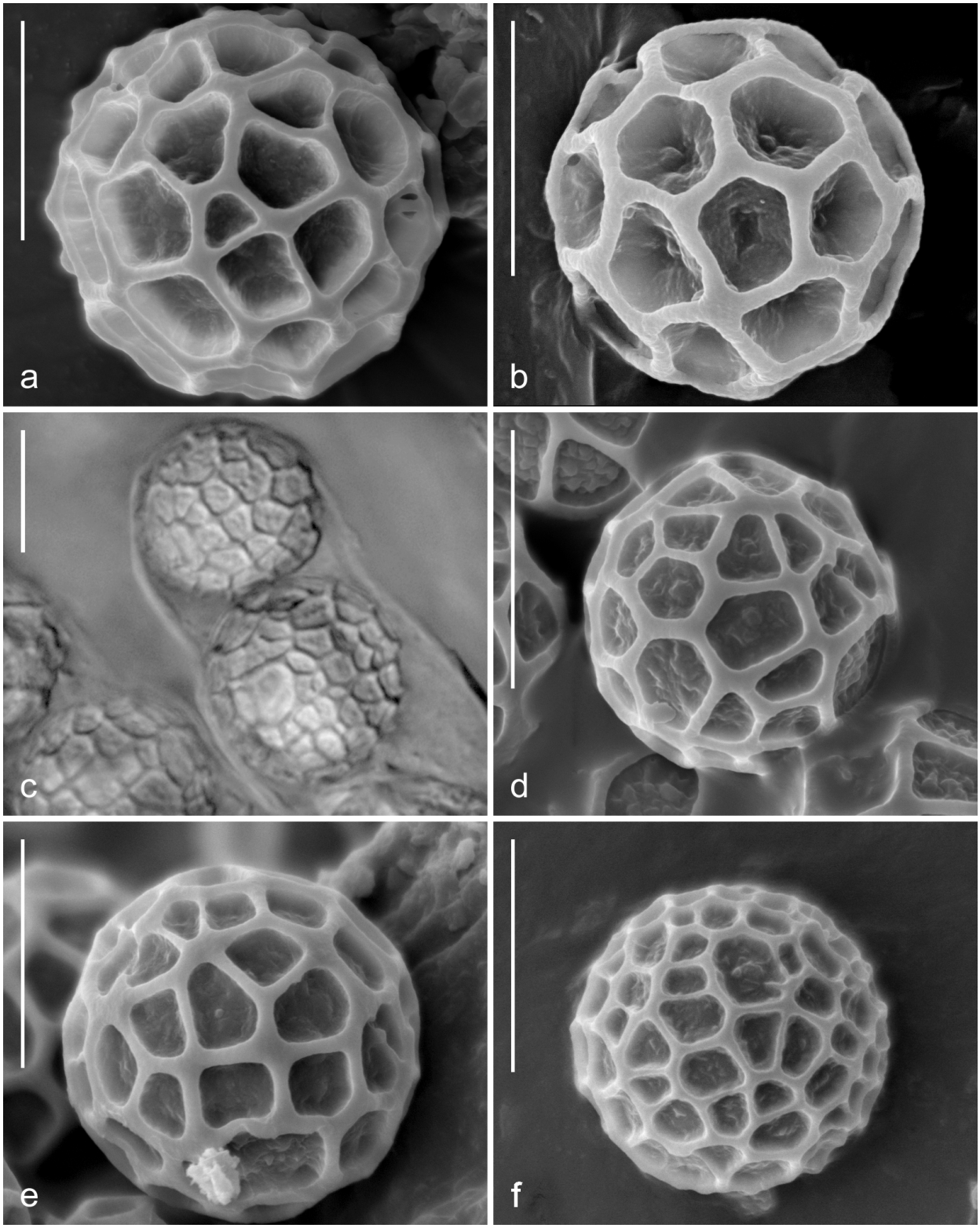

FIGURE 7. Ascospores similar to *Lamprospora sylvatica.* a. *L.* cf. *areolata* (col. no. 34751), SEM of ascospore, b. *L. campylopodis (*14246), SEM of ascospore, c. *L. faroensis* (holotype, UPS F-016650), light microscopic image of ascospores, d. *L. feurichiana* (46313), SEM of ascospore, e. *L. kristiansenii (*46317), SEM of ascospore, f. *L. miniata (*43741), SEM of ascospore. Scale bar = 10 µm. Photos J. Eckstein.

e) ***Lamprospora feurichiana*** is characterised by globose ascospores 14–16 µm in diameter, with ridges 0.6–1(–2) µm wide and 1(–1.5) µm high, forming a large-meshed reticulum with 3–5(–6) unequal meshes per diameter (Benkert 1987, based on a study of an isotype from DR). The holotype has probably been destroyed, and the isotype is accompanied by *Ceratodon purpureus* and *Bryum* sp. Later, Benkert (2009) also included samples accompanied by

Barbula Hedwig (1801: 115), *Bryum*, *Pohlia* Hedwig (1801: 171) and *Pottia* Ehrhart ex Fürnrohr (1829: 10), in his species concept of *L. feurichiana*. We studied the isotype in DR (leg. G. Feurich 27 October 1925) and found the host to be unambiguously *C. purpureus*. In the future, the concept of *L. feurichiana* should be restricted to collections on *C. purpureus*, as similar samples growing on other mosses most likely represent other species. Schumacher (1993, p. 328) regarded *L. feurichiana* as synonymous with *Barlaea retinosa* Velenovský (1934: 323), and listed collections accompanied by *Pohlia* and *Bryum*. These, in our opinion, also represent species other than *L. feurichiana*. Unfortunately, the original diagnosis of *Barlaea retinosa* (Velenovský 1934) is very brief and the holotype has practically been destroyed, consisting only of a fragment of a microscopic slide with remnants of one apothecium (Svrček 1976). Svrček´s later description of the holotype could be applied to more than one species and its true identity remains unclear. However, by restricting *L. feurichiana* to collections on *C. purpureus*, it becomes clear that the ascospores are almost undistinguishable from those of the new species; at the very best, the ascospores of *L. feurichiana* are slightly smaller than those of *L. sylvatica* (Fig. 7d). The only differences between the two species are the identities of their host species and their ecological habitat requirements, with *L. feurichiana* growing on open sandy soil, and *L. sylvatica* growing on rotten wood. DNA analysis does however clearly confirm our assumption that *L. feurichiana* and *L. sylvatica* are two different species (Fig. 6).

f) ***Lamprospora kristiansenii*** also has ascospores very similar to those of the new species (Fig. 7e), but differs with respect to the identity of its host, *Ceratodon purpureus*, and through its ecology, as it grows on open sandy soil. The ascospores of *L. kristiansenii* are at best slightly larger (16–19 µm) than those of *Lamprospora sylvatica* (16–18 µm). A sample of *L. kristiansenii* collected from the type locality was also available for our study. Not only does *L. kristiansenii* have ascospores very similar to those of the new species, but the type of infection is also very similar. DNA analysis (Fig. 6) confirms that existing differences in host and habitat between the species are mirrored by genetic differences. The question of whether *L. feurichiana* and *L. kristiansenii* are conspecific or not is particularly pertinent as they both share the same host and are characterised by morphologically very similar ascospores that differ only slightly in size (15–17 µm in *L. feurichiana* and 16–19 µm in *L. kristiansenii*). However, while both species are clearly closely related, our molecular data unequivocally indicate two distinct clusters, within the analysed samples of *L. feurichiana/kristiansenii*, which appear to indicate the existence of two separate species (Fig. 6). Further studies involving additional collections should help clarify this matter.

g) ***Lamprospora miniata*** is certain to represent a taxonomic aggregate. As no original material was preserved, a neotype was assigned by Benkert (2001, p. 50). This neotype was collected on *Pottia* and had ascospores 14–16 µm in diameter (excl. orn.), with a reticulum about 0.5 µm wide as well as high (Fig. 7f). Beside the nominative variety, two further ones have so far been described. *Lamprospora miniata* var. *parvispora* Benkert (2001: 51), growing on *Barbula* has slightly smaller ascospores that are 13–15 µm in diameter and ornamented with 0.3–0.5 µm wide ridges forming the reticulum (Benkert 2001). *Lamprospora miniata* var. *ratisbonensis* Benkert (2001: 53) grows on *Didymodon* Hedwig (1801: 104), and its ascospores are 14–16 µm wide with an ornamentation 0.5–1 µm wide and 0.5 µm high (Benkert 2001). Collections from other moss genera (e.g. *Encalypta* Hedwig (1801: 60), *Phascum* Hedwig (1801: 19) have also been reported, but according to preliminary molecular data analyses, these represent separate taxa (Z. Egertová pers. obs., L. Janošík pers. comm.). *L. miniata* on *Pottia* differs from the new species by its ascospores being characterised by smaller ridges and a denser reticulation (usually 7–10 meshes/diameter).

h) ***Lamprospora rugensis*** has globose ascospores (15–)16–18(–20) µm wide (excl. orn.), with undotted ridges 0.5–1 µm wide and high, forming an areolate reticulum with 5–9 meshes per diameter, and its host is a *Bryum* species (Benkert 1987). Later, Benkert (pers. comm.) also included samples on *Pohlia* in his concept of *L. rugensis*. Schumacher (1993) also identified several samples accompanied by *Pohlia drummondii* (Müller 1862: 328) Andrews (1935: 196) or *Pohlia* sp. as *L. rugensis*. Collections on *Pohlia* seem to be more frequent than those on *Bryum* (unpublished data, L. Janošík pers. comm.) and possibly represent a separate taxon. *L. rugensis* differs from the new species by its ascospores being ornamented by thinner and lower ridges, forming a reticulum with on average more meshes/diameter, and also by having a different host species.

Conclusion

Morphologically, the species closest to *L. sylvatica* are *L. feurichiana* and *L. kristiansenii*. All three species share an ascospore ornamentation consisting of relatively broad ridges forming a ± regular, complete reticulum. Also shared by all three species is the type of infection, with a one-celled appressorium surrounded by a multilayered cluster of thick-

walled cells. However, the molecular results confirm *L. sylvatica* as a distinct species. The results clearly demonstrate the importance of host specificity within the group of bryoparasitic Pezizales, which should not be underestimated. All current species of the group known from different hosts must be re-evaluated using both morphological and molecular methods, to potentially uncover previously hidden diversity within this group.

The occurrence on decaying wood is almost unique within the genus. Most species of *Lamprospora* grow on soil between shoots of the host moss or directly on their host. *L. sylvatica* shares its habitat, strongly rotten wood, only with *L. esterlechnerae* Benkert (2011: 152), a species known to infect *Dicranodontium denudatum* (Bridel 1806: 184) E. Britton (1913: 151) and that is so far only known from the type locality in Bavaria, Germany. Apart from its different host, *L. esterlechnerae* also differs from *L. sylvatica* through its ascospores that are ornamented with coarse, isolated, bluntly pyramidal warts up to 2.5 µm high (Benkert 2011).

The host species of *L. sylvatica*, *Dicranum montanum*, does not only grow on rotten wood, it also occurs frequently on the bark or roots of living trees, on silicate rock, or on bare soil. However, these habitats usually dry out quickly and may therefore be unfavourable for fungi. Further finds are thus expected to mainly take place in habitats with a stable moisture regime, i.e. mainly on rotten wood, although a possibility of occurrence in other micro-habitats, such as on permanently moist soil or rock, cannot be excluded.

Acknowledgements

We thank Markus Wagner (England) for the improvement of the English language and Lukáš Janošík (Slovakia) for sharing his findings in bryophilous Pezizales, especially regarding the concepts of *L. feurichiana* and *L. kristiansenii*. Roy Kristiansen (Norway), Günter Eckstein (Germany), Dieter Benkert (Germany), Peter Otto (Germany), Joschi Siembida (Germany) and Henk-Jan Van der Kolk (The Netherlands) are acknowledged for providing their collections of *Lamprospora* species with reticulate ornamentation. We also thank the curators of GOET, DR and UPS for the opportunity to study type specimens, and the staff of the Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology in Göttingen for allowing us to take SEM photographs using their equipment. Reviewers are acknowledged for providing valuable comments to the manuscript. The project was financed by the Hainich NP. ZE was supported by an internal grant from Palacký University (IGA PrF-2018-001), MS by grant No. LO1204 (Sustainable development of research in the Centre of the Region Haná) from the National Program of Sustainability I, MEYS. The specimens from Malá Fatra NP were collected in accordance with research permit OU-ZA-OSZP1-2017/003575-004/Ryb.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

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4.2.2 *Octospora doebbeleri*, a new bryophilous species on *Dicranoweisia cirrata*

DOI 10.12905/0380.sydowia73-2021-0233 Published online 26 February 2021

Octospora doebbeleri, a new bryophilous species on *Dicranoweisia cirrata*

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Sochorová Z., Eckstein J., Sedlářová M. & Sochor M. (2021) *Octospora doebbeleri*, a new bryophilous species on *Dicranoweisia cirrata*. – Sydowia 73: 233–244.

Octospora doebbeleri, a new bryophilous species infecting rhizoids of the acrocarpous moss *Dicranoweisia cirrata,* is described and illustrated based on collections from the Czech Republic. The delineation of the new species, based on the unique set of morphological characters, is also supported by phylogenetic analysis of LSU and SSU nrDNA and EF1α. Morphologically, *O. doebbeleri* is characterised by apothecia with a thin margin and ellipsoid, mostly binucleate ascospores ornamented with small isolated warts. Differences from other morphologically similar species of *Octospora* are discussed.

Keywords: bryosymbiotic fungi, Děvín a Ostrý national monument, nuclei, vital taxonomy.

Octospora Hedw. (Pezizales) is a species-rich genus of bryophilous ascomycetes, whose members infect a diverse range of bryophytes and form elaborate infection structures consisting of appressoria, penetration pegs and intracellular haustoria (Döbbeler 1980). *Octospora* spp. form sessile, discoid, saucer-shaped or turbinate apothecia of ca. 0.3–15 mm in diameter which are coloured in shades of orange, red or pink. The ascomata are usually hairless, except in a few species with hyaline hairs. At microscopic level, the genus *Octospora* is characterized mainly by operculate, inamyloid asci containing 8 or 4 ascospores, and by its content of carotenoid pigments, especially in paraphyses. Ascospores of different *Octospora* spp. vary widely in shape (subglobose, ovoid, broadly to narrowly ellipsoid, cylindrical, fusoid or lemon-shaped), size (ranging from 11–15 × 9–13.5 µm in *O. wrightii* (Berk. & M.A. Curtis) J. Moravec to 27–34 × 9–11 µm in *Octospora coccinea* var. *tetraspora* Benkert and 26–32 × 10–12 µm in *Octospora axillaris* var. *tetraspora* Benkert), content of lipid bodies, and perispore ornamentation (smooth, warted, spiny or reticulate; e.g. Caillet & Moyne 1987; Benkert 1995, 1998a, b; Janošík 2020; pers. obs.). Nevertheless, delimitation of the genus *Octospora* from the other genera of bryophilous Pezizales has not been fully clarified yet and varies between authors (e.g. Caillet & Moyne 1980; Wang & Kimbrough 1992; Benkert 1998c, 2009; Janošík 2020).

Octospora species are found worldwide (Gamundí 1973, Gamundí & Spinedi 1988, Benkert 1998a, Olech & Mleczko 2000, Egertová et al. 2017, Sochorová et al. 2019) in a range of habitats, including rocks, boulders and stone walls covered by bryophytes. Occurrence in this type of habitat, which is generally poor in non-lichenized fungi, is even reflected in the name of *Octospora musci-muralis* Graddon, but several other species of bryophilous Pezizales have also been recorded there.

At Christmas 2014, an interesting *Octospora* species growing on the acrocarpous moss *Dicranoweisia cirrata* (Hedw.) Lindb. ex Milde was found on sandstone rocks which used to form a natural bastion of the medieval castle Děvín in Northern Bohemia, Czech Republic. Subsequent microscopy and molecular studies confirmed that the find represented a previously undescribed species, which is introduced in this contribution as *Octospora doebbeleri,* sp. nov.

Materials and methods

Sample collection and observation

Octospora doebbeleri was found five times over the years 2014–2020 at the Děvín a Ostrý national

monument, Northern Bohemia, Czech Republic. Apothecia were collected together with the host. Description of macroscopic characters is based on fresh apothecia, microscopic characters were observed both on vital (annotated*) and rehydrated (annotated†) material. Microscopy was performed employing microscopes BX60, CX21 and CX23 (Olympus, Czech Group, Prague), with magnifications of 40×, 100×, 400× and 1000×. Apothecial characters were observed in tap water (H_2O), 3 % potassium hydroxide (KOH), Lugol's solution (IKI), Brilliant Cresyl Blue (CRB) and Lactic Acid Cotton Blue (LACB). Infection was investigated on rehydrated material in tap water and LACB. Nuclei were visualized in rehydrated apothecia upon staining with DAPI (10 µg/ml, 10 min, room temperature, dark) and fluorescence signal excited by a mercury lamp was combined with transmission light channel (50:50) (Olympus BX60 with attached DP73 camera). Unless stated otherwise, the features were measured in tap water, on photographs using the Piximètre 5.10 software (Henriot & Cheype 2020). Measurements of living ascospores relate to freshly ejected, fully mature, normally developed ascospores. Values of ascospore size are given as (minimum) mean ± standard deviation (maximum), Q = length/width ratio (n = sample size) and do not include ornamentation. Vouchers are deposited in the herbarium of the Mycological Department of the National Museum in Prague (PRM), Czech Republic.

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried apothecia using the CTAB method as outlined by Doyle & Doyle (1987). Apothecia were homogenised using a pestle, incubated in 300 µl of extraction buffer at 65 °C for one hour, and the extract was subsequently purified in chloroform–isoamyl alcohol mixture, precipitated by isopropanol and finally dissolved in water and incubated with RNase for 30 min at 37 °C. DNA quality was checked on 1.5 % agarose gel. Molecular sequence data were generated for three loci: the 28S subunit of ribosomal DNA (LSU) was amplified with primers LR0R and LR6 (Vilgalys & Hester 1990), the 18S subunit of rDNA (SSU) with primers NS1 and NS6 (White et al. 1990) and translation elongation factor-1alpha (EF1α) with primers EF1-983F and EF1–1567R (Rehner & Buckley 2005). PCR was performed with Kapa polymerase (Kapa Biosystems, Wilmington, USA) following a standard protocol with 37 cycles and annealing temperature

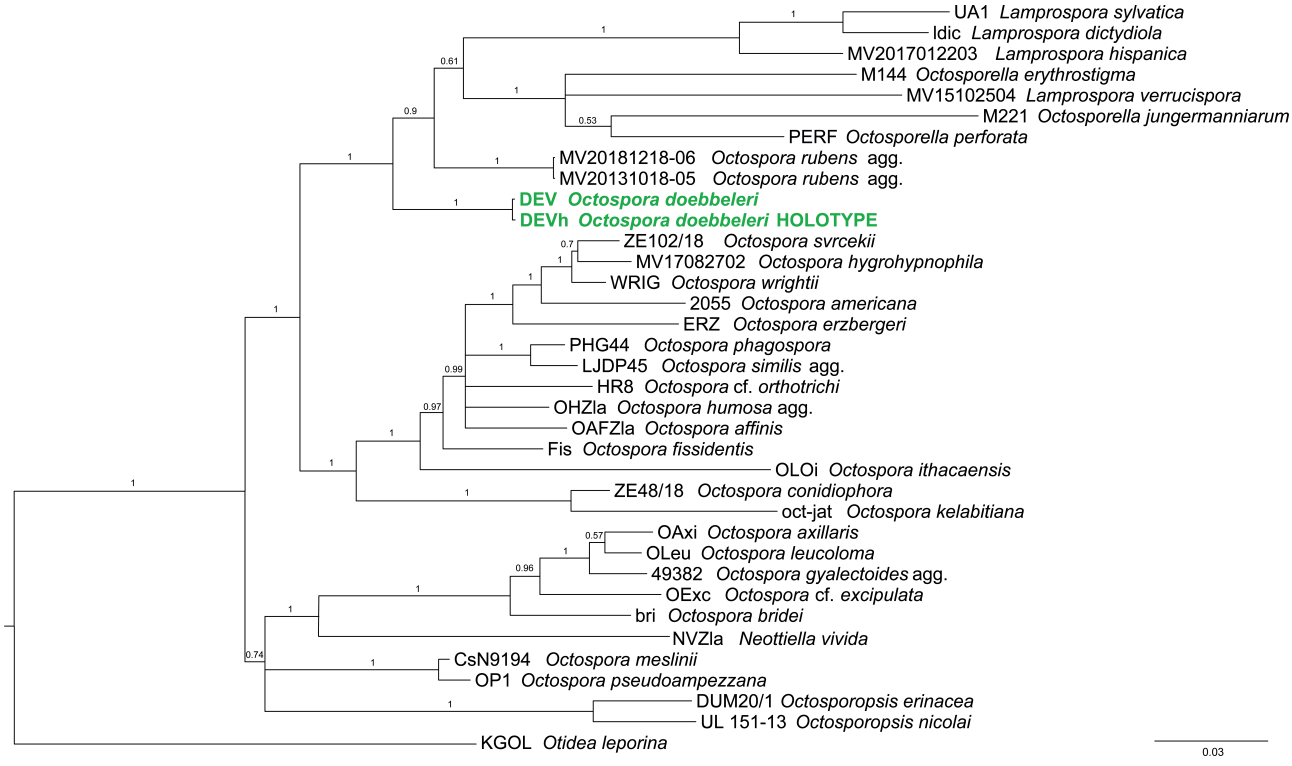

Fig. 1. Bayesian phylogeny inference, based on concatenated alignment of EF1α, LSU and SSU sequences. Bayesian posterior probabilities are shown above branches; *Otidea leporina* is used as an outgroup.

Fig. 2. *Octospora doebbeleri* (holotype PRM 954007). **a–d.** Apothecia of *Octospora doebbeleri* between shoots of *Dicranoweisia cirrata*; **e, f.** The type locality - bastion on the Děvín hill. Photos Z. Sochorová.

of 54 °C. The PCR products were purified by precipitation with polyethylene glycol (10 % PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced from both directions using the same primer pairs by the Sanger method at Macrogen Europe, Amsterdam, The Netherlands.

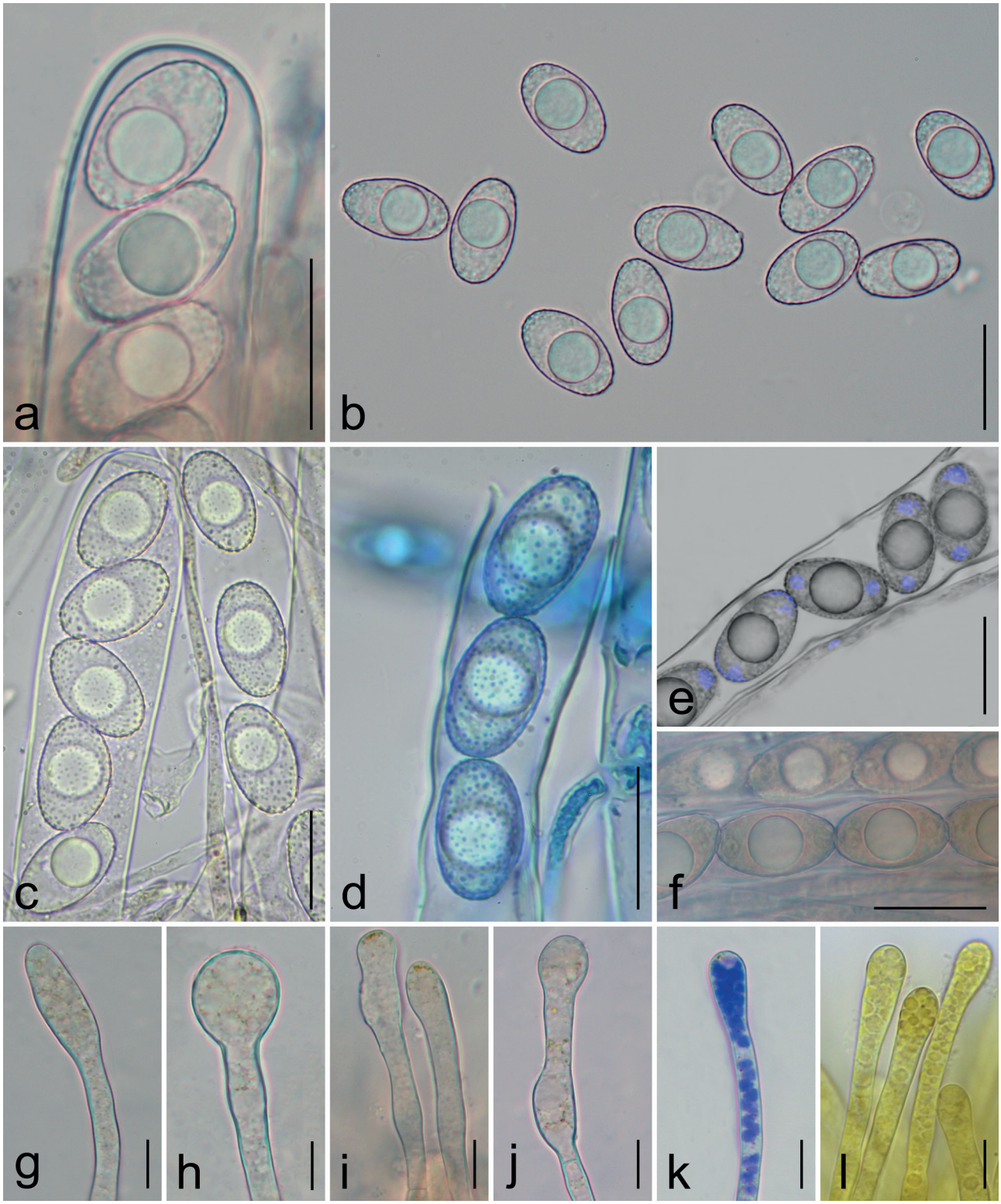

Fig. 3. *Octospora doebbeleri*, microscopic characters. (a, b, e–l holotype PRM 954007; c, d PRM 954008) **a.** Ascus apex in water; **b, c.** Ascospores in water; **d.** Ascospores stained with LACB; **e.** Ascospores in DAPI showing the two nuclei; **f.** Ascospores in IKI; **g–j.** Apical cells of paraphyses in tap water; **k.** Apical cell of a paraphysis in CRB; **l.** Apical cells of paraphyses in IKI. Scales a–f. Ascospores in IKI = 20 µm, g–l = 5 µm. Photos a, b, f–l Z. Sochorová, c, d – J. Eckstein, e – M. Sedlářová.

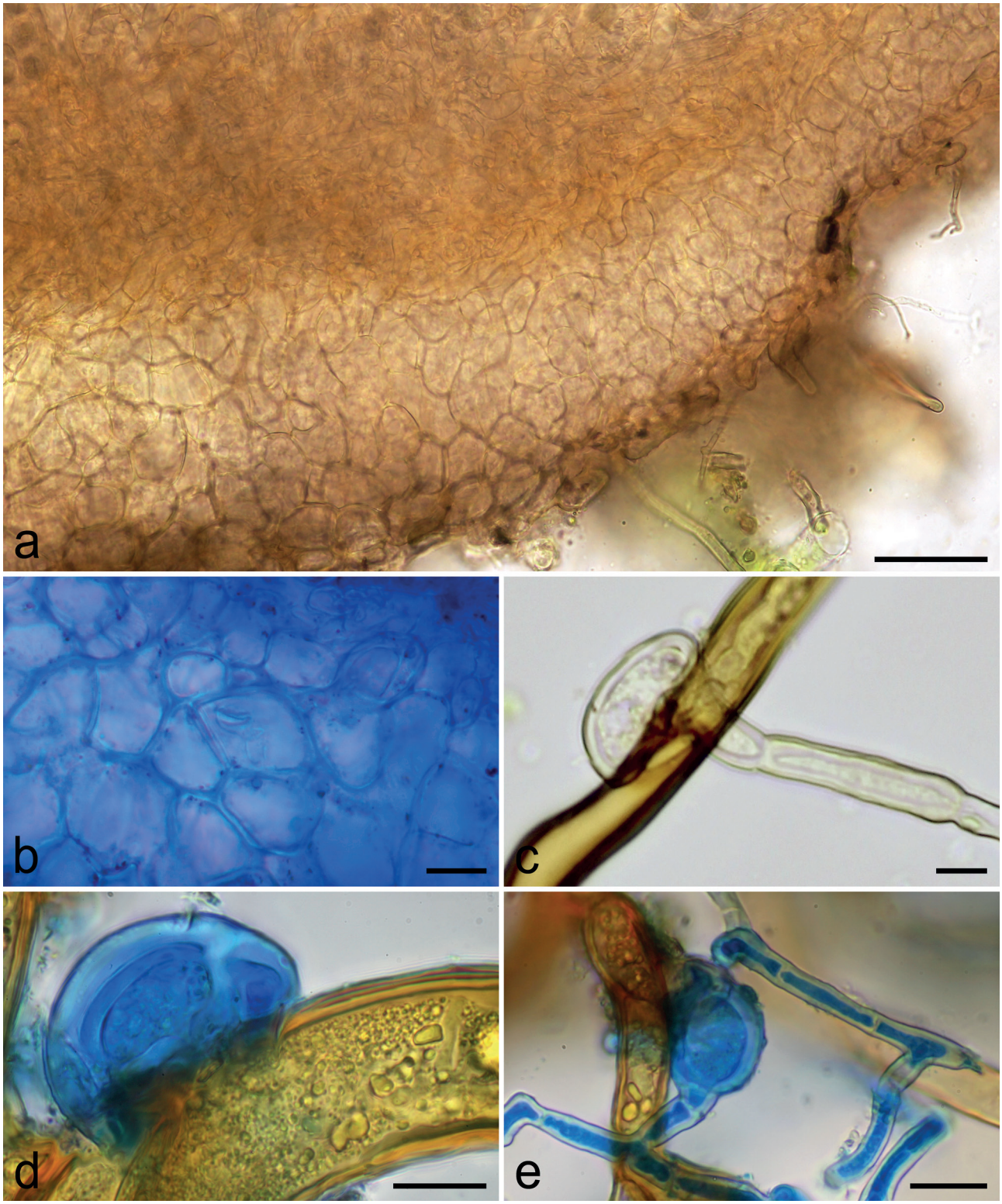

Fig. 4. *Octospora doebbeleri* (a–c holotype PRM 954007; d, e PRM 954008). **a.** Cross section of a vital apothecium showing subhymenium, medullary and ectal excipulum in water; **b.** Ectal excipulum in LACB; **c–e.** Appressoria attached to rhizoids of *Dicranoweisia cirrata* in water (c) and stained with LACB (d, e). Scales a = 50 µm, b–d = 10 µm, e = 20 µm. Photos a–c Z. Sochorová, d, e – J. Eckstein.

Phylogenetic analysis

Specimens used in the phylogenetic analysis are listed in Tab. 1. Sequences of *Octospora similis* agg. were provided by Lukáš Janošík, Charles University in Prague. Sequences were edited and aligned using the Geneious software (ver. 7.1.7., Biomatters). Phylogeny was reconstructed using the Maximum Parsimony (MP) method and tested by bootstrapping, using 1000 pseudoreplicates in MEGA (ver. 6.06, Tamura et al. 2011). Bayesian phylogeny inference (BI) was computed in MrBayes (ver. 3.2.4, Ronquist et al. 2012) using the GTR+I+G substitution model, as determined by AICc in PartitionFinder 2.1.1 (Lanfear et al. 2017). The analysis was run for 20 million generations in four independent runs, sampling every 1000th generation and excluding the first 50 % of generations as burn-in. The Basic Local Alignment Search Tool (BLAST; Zhang et al. 1990) was used for searching similar sequences in publicly available sequence databases (https://blast.ncbi.nlm.nih.gov).

Results

Phylogeny

Octospora doebbeleri forms a distinct and highly supported clade together with *O. rubens* agg. (smooth-spored species infecting *Ceratodon purpureus*) and several species currently included in the genera *Lamprospora* and *Octosporella* (Fig. 1). Due to a somewhat conflicting phylogenetic signal from the SSU locus, basal topology within the clade is rather weakly supported in the analysis of concatenated data (but see LSU and EF1α in Supplementary material 1). Despite that, *O. doebbeleri* appears to be the basal lineage of this diverse and species-rich clade.

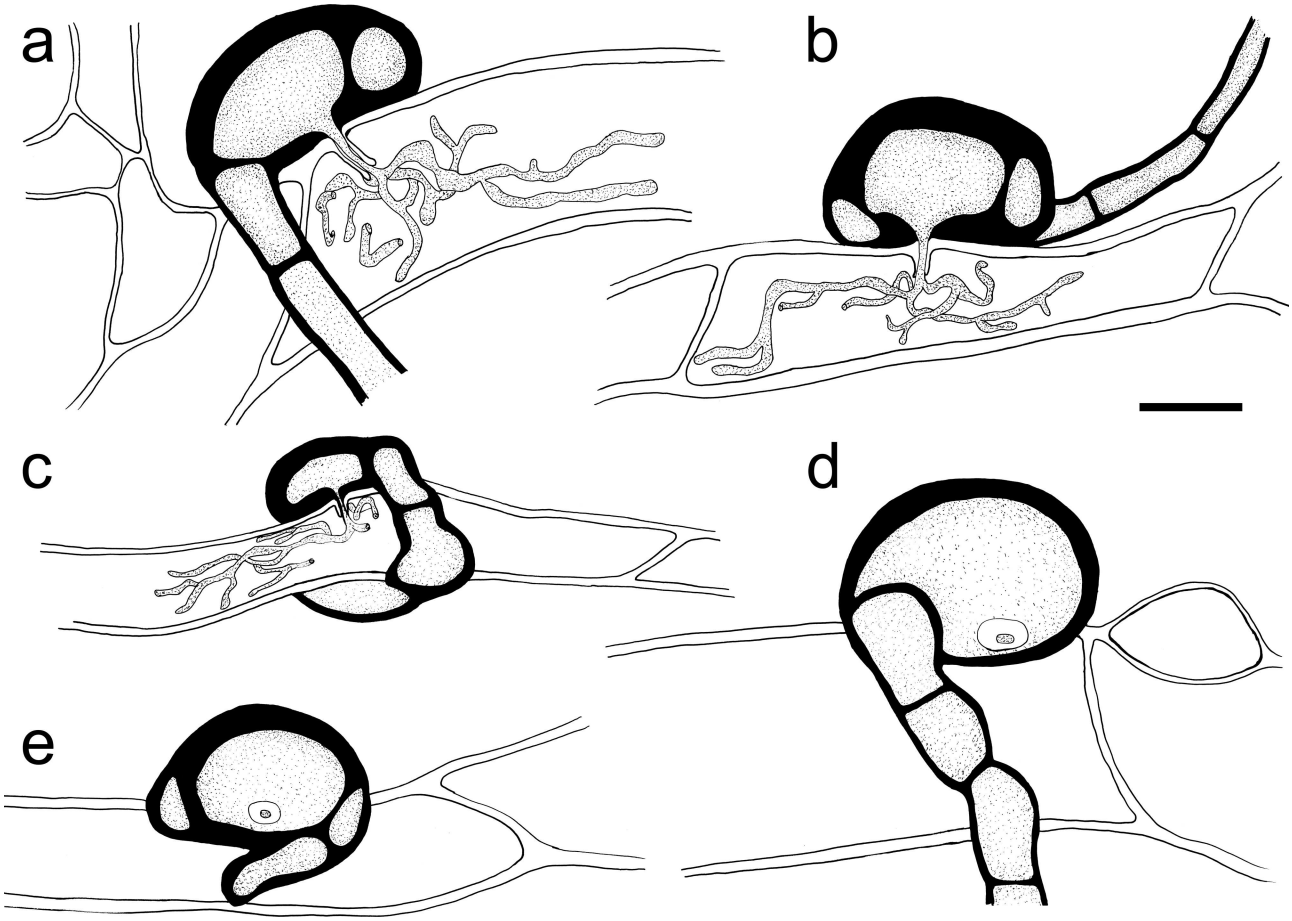

Fig. 5. *Octospora doebbeleri* (PRM 954008, fungal cells dotted). **a–c.** Appressoria (seen laterally) attached to rhizoids of *Dicranoweisia cirrata*, also showing infection pegs and intracellular haustoria; **d–e.** Appressoria seen from above, haustorium not indicated. Scale a–e = 20 µm. Illustration J. Eckstein.

Taxonomy

Octospora doebbeleri Sochorová & Eckstein, **sp. nov.** – Figs. 2–5.
MycoBank no.: MB 836340

Etymology. – Named after Dr. Peter Döbbeler for his merits in research of bryophilous fungi.

Diagnosis. – Differs from known species of *Octospora* by the following combination of characters: apothecia with a thin margin, ellipsoid to narrowly ellipsoid, binucleate ascospores ornamented with isolated warts, and infections on rhizoids of *Dicranoweisia cirrata*.

Holotypus. – CZECH REPUBLIC. Hamr na Jezeře (Liberec region, Česká Lípa district), Děvín a Ostrý NM, on *Dicranoweisia cirrata* on sandstone rocks, 50° 41' 37" N, 14° 51' 19" E, 390 m a. s. l., 25 December 2019, leg. Zuzana Sochorová & Michal Sochor, herb. PRM 954007.

Description. – Apothecia first spherical, later discoid, rounded from the top view, up to 2.3 mm in diameter and 0.9 mm high, sessile, pinkish, reddish or reddish orange; margin thin, toothed, hairless; growing in groups between the host moss. – Asci *318–460 × 20–28.5 µm, †240–370 × 15–20 µm, 8-spored, operculate, inamyloid, unitunicate, cylindrical, with a hemispherical to slightly truncate apex, often with a very long thinning base, arising from perforated croziers, young asci rich in glycogen especially in the basal part (turning rusty in IKI), protruding above paraphyses for up to 72 µm, *pars sporifera* *105–128 µm when all 8 ascospores fully developed. Sometimes a few spores inside the asci aborted. – Ascospores *(18.9)21.2±1.06(24.2) × (10.6)12.3±0.57(13.6) µm, *Q = (1.47)1.73±0.1(2) (n = 415), †(16.8)19.4±1.23(23.6) × (9.6)11.3±0.65(13.1) µm, †Q = (1.43)1.72±0.16(2.42) (n=100), ellipsoid to narrowly ellipsoid, sometimes with one side more flattened, hyaline, thin-walled, containing one lipid body *8.3–11.1 µm in diam. or less often two lipid bodies (*6.3–8.5 µm in diam.), uniseriate or subbiseriate in living asci, binucleate, nuclei contrasting in IKI and 3 % KOH, in IKI *2.8–4.8 µm in diam. Ornamentation formed by isolated warts 0.3–1.2 µm broad and 0.2–0.8 µm high, obtuse, cyanophilous, dissolving in 3 % KOH. – Paraphyses *2.9–4 µm wide in lower part, †2.2–3 µm, usually straight, less often apically bent, multiseptate, often forked, sometimes with short lateral projections, containing carotenoid granular pigment (turning green in IKI, not changing in 3 % KOH and CRB); apical cell *40–109 µm long, 3.5–11.3 µm wide in the broadest part, †36–97 × 3.3–9 µm, very variable in shape – gradually widening, capitate, irregular or not inflated. – Subhymenium *25–40 µm thick, composed of *textura angularis-intricata*, cells *3.2–15 µm wide, containing carotenoid granular pigment turning green in IKI. – Medulla *60–95 µm thick at flanks, composed of *textura intricata*, cells *4–15 µm wide, containing carotenoid granular pigment which turns green in IKI. – Ectal excipulum sharply differentiated from medulla, *70–200 µm at flanks, composed of *textura subangularis*, cells hyaline, angular, subglobose, pyriform, shortly cylindrical or irregularly shaped, *11–55 × 10–26 µm, wall thickness increasing towards the outer surface, in the outermost cells up to *4.5 µm thick. – Margin consisting of two layers: inner layer *20–80 µm wide, extending from the medullary excipulum, very similar to paraphyses. Outer layer, extending from the ectal excipulum, *80–130 µm wide, end cells *26–70 × 4.5–17 µm, clavate, cylindric, pyriform, bowling pin shaped or irregular, containing carotenoid granular pigment, thick-walled, wall up to *3 µm thick, thickest at the apex. – Anchoring hyphae growing from the outermost cells of the ectal excipulum *4.5–10 µm wide, †4.5–8.5 µm, hyaline to subhyaline, branching, anastomosing, hyaline, thick-walled, wall up to 3 µm thick, lumen in LACB strongly cyanophilous.

Infection. – Hyphae †5–11 µm wide, thick-walled. – Infection structures consisting of appressoria, infection pegs and haustoria. – Appressoria †20–50 µm long, †12–30 µm high and †18–40 µm wide, closely attached to the infected rhizoid cells, convex, broadly elliptical in outline seen from above, mostly free or partly covered by hyphae, consisting of one large cell often laterally with one or two additional smaller cells, distal wall to 7 µm thick. – Infection peg one per appressorium, surrounded by a lignituber-like tube consisting of host cell material. – Haustoria †(1)2–3(4) µm wide, intracellular, thin-walled, ramified, with undulating cell walls, septa missing or not discernible, not growing through cross walls of the rhizoids.

Host plant. – Occurring on the rhizoids of *Dicranoweisia cirrata*.

Distribution. – Known so far only from the type locality on the Děvín hill, Northern Bohemia, Czech Republic.

Other specimens examined. – From the type locality, 26 December 2014 (PRM 954008), 28 February 2015 (PRM 954009), 30 December 2016 (PRM 954010), 14 March 2020 (PRM 954011).

Discussion

Octospora doebbeleri is currently the only species of bryophilous Pezizales known to infect *Dicranoweisia cirrata*. This moss grows epiphytic or epixylic on broad-leaved trees, on rocks or on soil,

Tab. 1. Specimens used for the phylogeny inference and their GenBank accession numbers. Newly obtained sequences in bold.

species	Identification code in GenBank	Herbarium code	Country, collection date	Host	GenBank accession numbers		
					LSU	SSU	EF1α
Lamprospora dictydiola	ldic	PRM 945794	Czech Republic, 12 February 2014	*Tortula muralis*	MF754056	MK569365	MF754054
Lamprospora hispanica	MV2017012203	B 70 0100986	Spain, 22 January 2017	*Aloina ambigua*	MN394599	MW242827	MN366468
Lamprospora sylvatica	UA1	PRM 946415 (holotype)	Ukraine, 8 July 2017	*Dicranum montanum*	MG947604	MK569367	MK569290
Lamprospora verrucispora	MV15102504	HBG 1412 (holotype)	Germany, 25 October 2015	*Campylopus pyriformis*	MN994551	MN994527	
Neottiella vivida	NVZla	PRM 945797	Czech Republic, 22 October 2016	*Polytrichum piliferum*	MF066068	MK569337	MF754051
Octospora affinis	OAFZla	PRM 945798	Czech Republic, 22 October 2016	*Orthotrichum affine*	MF754075	MK569347	MF754045
Octospora americana	2055	S F43718 (holotype)	USA, 18 February 1981	*Forsstroemia trichomitria*	MN967346	MN994516	MT078729
Octospora axillaris	OAxi	PRM 954016	Czech Republic, 8 November 2016	*Phascum cuspidatum*	**MW242829**	**MW242828**	**MW430761**
Octospora bridei	bri	PRM 935151	Czech Republic, 18 October 2015	*Ephemerum minutissimum*	MF754061	MT001890	
Octospora conidiophora	ZE48/18	PRM 951743 (holotype)	South Africa, 2 March 2018	*Trichosteleum perchlorosum*	MK569321	MK569351	MK569297
Octospora doebbeleri	DEVh	PRM 954007 (holotype)	Czech Republic, 25 December 2019	*Dicranoweisia cirrata*	**MW152148**	**MW152156**	**MW159137**
Octospora doebbeleri	DEV	PRM 954010	Czech Republic, 30 Dec 2016	*Dicranoweisia cirrata*	**MT813511**	**MW152157**	
Octospora erzbergeri	ERZ	PRM 945799	Czech Republic, 10 December 2016	*Pseudoleskeella nervosa*	MF754068	MK569340	MF754042
Octospora cf. *excipulata*	OEXc	PRM 945800	Czech Republic, 16 Nov 2015	*Funaria hygrometrica*	MF754062	MK569369	MF754047
Octospora fissidentis	Fis	PRM 945801	Czech Republic, 13 November 2016	*Fissidens bryoides*	MF754073	MK569341	MF754044
Octospora gyalectoides agg.	49382	B 70 0100075	Germany, 22 November 2016	*Pottia lanceolata*	MT001891	MT001889	MN990995

species	Identification code in GenBank	Herbarium code	Country, collection date	Host	GenBank accession numbers		
					LSU	SSU	EF1α
Octospora humosa agg.	OHZla	PRM 945802	Czech Republic, 22 October 2016	*Polytrichum piliferum*	MF754074	MK569343	MF754043
Octospora hygrohypnophila	MV17082702	PRM 953064	France, 27 August 2017	*Hygrohypnum luridum*	MN994543	MN994520	MN990988
Octospora ithacaensis	OLOi	PRM 945803	Czech Republic, 6 May 2016	*Marchantia polymorpha*	MF754071	MK569346	MF754053
Octospora kelabitiana	oct-jat	PRM 945781	Malaysia, 3 February 2016	*Riccardia* sp.	MF754065	MK569372	MF754048
Octospora leucoloma	OLeu	PRM 945804	Czech Republic, 21 October 2016	*Bryum argenteum*	MF754063	MK569370	
Octospora meslinii	CsN9194	PRM 954637	Hungary, 7 January 2018	*Grimmia pulvinata*	**MW152147**	**MW152158**	**MW159139**
Octospora cf. *orthotrichi*	HR8	CNF 2/10561	Croatia, 2 January 2018	*Orthotrichum diaphanum*	MK569314	MK569342	MK569311
Octospora phagospora	PHG44	PRM 945805	Germany, 24 October 2015	unknown	MF754072	MK569344	MF754046
Octospora pseudoampezzana	OP1	PRM 935156	Czech Republic, 5 March 2016	*Schistidium crassipilum*	MF754069	MK569339	MF754050
Octospora rubens agg.	MV20181218-06	PRM 954641	Spain, 18 December 2018	*Ceratodon purpureus*	**MW221931**	**MW206790**	**MW219144**
Octospora rubens agg.	MV20131018-05	without voucher	Germany, 18 October 2013	*Ceratodon purpureus*	**MW221930**	**MW206791**	**MW219145**
Octospora similis agg.	LJDP45	PRC 4667	Slovakia, 26 November 2019	*Bryum* cf. *rubens*	**MT766281**	**MT766280**	**MT759840**
Octospora svrcekii	ZE102/18	PRM 951720	Croatia, 20 May 2018	*Cratoneuron filicinum*	MN967348	MN994518	MN974532
Octospora wrightii	WRIG	PRM 945807	Czech Republic, 22 April 2017	*Amblystegium serpens*	MF754070	MK569345	MT078728
Octosporella erythrostigma	M144	TUR 178060	Denmark, 14 May 2005	*Frullania dilatata*	EU940108	EU940035	
Octosporella jungermanniarum	M221	TUR 178050	Switzerland, 29 August 2005	*Plagiochila asplenioides s.* l.	EU940133	EU940060	
Octosporella perforata	PERF	PRM 945808	Czech Republic, 10 December 2016	*Porella platyphylla*	MF754060	MK569368	MF754052
Octosporopsis erinacea	DUM20/1	PRM 945774 (isotype)	Malaysia, 20 January 2017	*Dumortiera hirsuta*	MF754057	MK569338	MF754041
Octosporopsis nicolai	UL 151-13	pers. herb. U. Lindemann	Germany, 2013	*Lunularia cruciata*	KF771033		KF771042
Otidea leporina	KGOL	CNF 2/9962	Kyrgyzstan, 15 July 2016	–	MK569335	MK569371	MK569312

and is distributed in Europe, Macaronesia, North Africa, Middle East, Caucasus, Polynesia and North America (Sharp et al. 1994, Smith 2004).

Further noteworthy characteristics are the binucleate ascospores within 8-spored asci. According to Perry et al. (2007), species of the family Pyronemataceae form uninucleate ascospores. Jaklitsch et al. (2016) similarly characterise the *Octospora* lineage, comprising all the bryophilous genera of Pezizales and a few others, as having uninucleate ascospores in 8-spored asci (see also e.g. Berthet 1964, Dissing & Sivertsen 1983, Billekens 1992, Kullman 2002 or Sochorová et al. 2020), or binucleate ascospores in 4-spored asci (see also e.g. Senn-Irlett 1988, Weber 1992 or Benkert 1998b). A few species of the genus *Octosporella* are known to produce tetranucleate ascospores (Corner 1929, Janošík 2020). Binucleate ascospores in 8-spored asci have been reported in *Octosporopsis nicolai* (Maire) U. Lindem., M. Vega & T. Richt. by Lindemann et al. (2014) (but tetranucleate by Janošík 2020). Out of 52 species of bryophilous Pezizales analysed by Janošík (2020), only five had binucleate ascospores in octosporic asci, none of them classified to the genus *Octospora*.

Comparison with morphologically similar species

Ascospore ornamentation consisting of isolated warts is very common in the genus *Octospora*. In the following, *O. doebbeleri* is compared to species having 8-spored asci, ellipsoid ascospores with a similar size and ornamentation, lacking long hairs and infecting acrocarpous mosses. The comparison is a bit complicated in some species, as the Q value is often missing in the older literature or it is not clear whether the measurements relate to vital or dead ascospores and whether they include ornamentation.

Octospora fissidentis Benkert & Brouwer has smaller, ellipsoidal to subfusoid ascospores, measuring 17–21 × 9–10.5(11) µm (Q = 1.8–2), which contain one or less often two lipid bodies, and are ornamented with isolated warts of 0.5–1 µm in diameter. Its host is *Fissidens bryoides* Hedw. (Benkert & Brouwer 2004). It is known from European localities (Benkert & Brouwer 2004; pers. obs.).

Octospora heterosculpturata T. Schumach. has shorter, (broadly) ellipsoid ascospores 16.5–19 × 12.6–14.2 µm (Q not known), ornamented with isolated or confluent warts 0.4–1.2 µm broad and 0.2–0.5 µm high. It has been collected on manured soil and dung of reindeers between mosses *Splachnum vasculosum* Hedw. and *Pohlia* sp. in Norway (Schumacher 1992).

Octospora meslinii forms ascospores size of which can overlap with those of *O. doebbeleri*, i.e. *18.3–21.9 × 11.7–13.5 µm, *Q = 1.46–1.71, ornamented with warts ca. 0.25–0.4 µm broad (C. Németh, pers. com.). It differs from *O. doebbeleri* by blackening apothecia and infecting *Grimmia pulvinata* (Hedw.) Sm., inducing galls on its rhizoids (Itzerott & Döbbeler 1982). It occurs in Europe (Le Gal 1939, Itzerott 1981, Caillet & Moyne 1987).

Octospora orthotrichi (Cooke & Ellis) K.B. Khare & V.P. Tewari is a somewhat mysterious taxon. In Europe, specimens from *Orthotrichum diaphanum* Schrad. ex Brid. are being named *O. orthotrichi* (Benkert 1998a, Egertová et al. 2015, Vega 2017, Janošík 2020). However, the host moss in the type collection from the USA was a species of *Orthotrichum* lacking a hyaline hair (therefore, not *O. diaphanum*; Benkert 1998a). Taking in mind the high host specificity of *Octospora* spp., it is possible that the European collections represent a species different from that in American collections. The ascospores in American collections measured 16–21 × 11–14 µm (Benkert 1998a). In material from Europe, ascospores having a size of *16.5–19 × 11–13.5 µm (*Q = 1.3–1.65 in Egertová et al. 2015, while *Qav = 1.74 in Janošík 2020), i.e. smaller than in *O. doebbeleri*, were reported. The ornamentation consists of warts 0.2–0.8 µm broad. Unlike in *O. doebbeleri*, the ascospores are uninucleate (Janošík 2020), often asymmetrical and their arrangement inside the asci can be biseriate (Egertová et al. 2015).

Octospora pseudoampezzana (Svrček) Caillet & Moyne has ellipsoid ascospores, which are distinctly broader than those of *O. doebbeleri,* 19–26 × 14–17 µm (Q = 1.2–1.6), contain a single lipid body and are ornamented with isolated warts 0.3–1 µm broad. Like *O. meslinii*, *O. pseudoampezzana* has blackening apothecia, especially at the margin (Svrček 1969, Caillet & Moyne 1987, Rubio et al. 2000, pers. obs.). It further differs from *O. doebbeleri* in uninucleate ascospores (Janošík 2020). It infects *Schistidium* sp. and induces galls on its rhizoids (Eckstein & Eckstein 2009). It is known from Europe (Svrček 1969, Caillet & Moyne 1987, Rubio et al. 2000, Eckstein & Eckstein 2009).

Octospora similis (Kirschst.) Benkert has smaller, ellipsoid ascospores measuring (15)16–18(20) × (10)11–13(14.5) µm (*Qav = 1.55), ornamented with warts 0.5–1(1.5) µm broad and containing mostly a single lipid body (Benkert 1996, Janošík 2020). The ascospores are, in contrast to *O. doebbeleri*, uninucleate (Janošík 2020). Mosses from the genus *Bryum*

Hedw. have been reported as hosts (Benkert 1996, Vega 2017, Janošík 2020), with induction of galls proven by Itzerott (1983). It has been reported from Europe (e.g. Caillet & Moyne 1987, Benkert 1996, Vega 2017) and the USA (Benkert 1996). It is possible that *O. similis* is conspecific with *O. ciervensis* Gamundí & Spinedi, described from the Antarctic Peninsula (Gamundí & Spinedi 1988, Benkert 1998a).

Acknowledgements

We thank Lukáš Janošík for sharing his sequences of *Octospora similis* agg., Marcel Vega for providing specimens of *Octospora rubens* agg., Csaba Németh for information about *Octospora meslinii*, Seppo Huhtinen for information about *Octosporella erythrostigma* and *O. jungermanniarum*, Vojtěch Taraška for enabling to use the microscope of the Regional Museum in Olomouc, Markus Wagner and Caroline Hobart for the improvement of the English language. M. Sochor was supported by the Ministry of Agriculture of the Czech Republic (grant no. RO0418).

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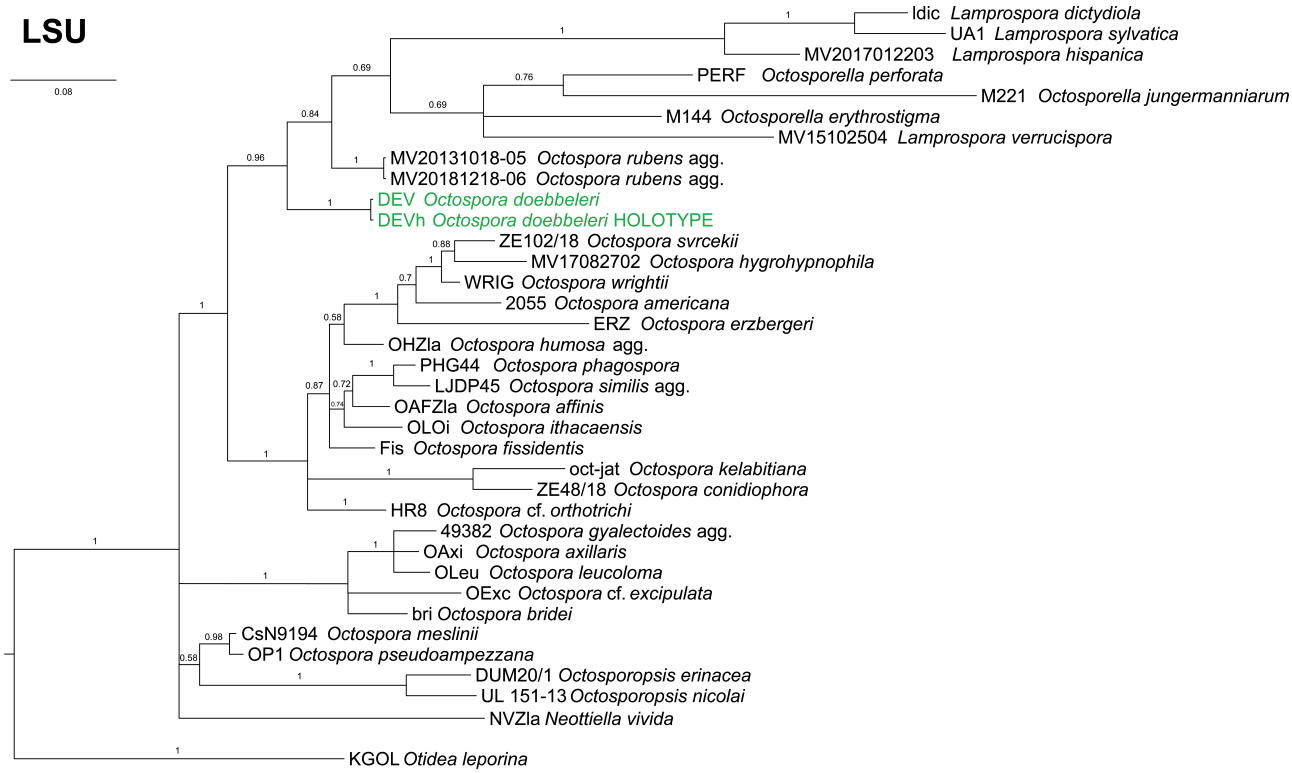
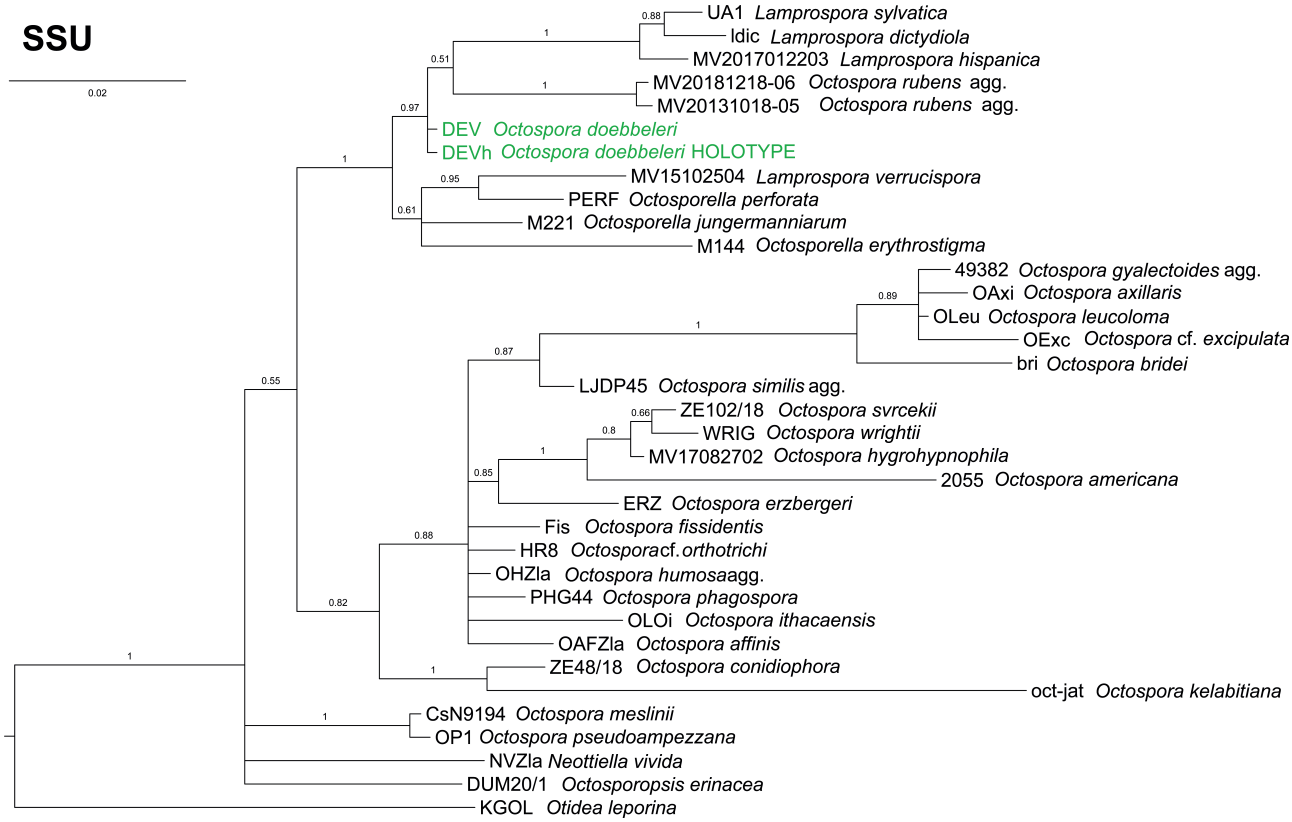
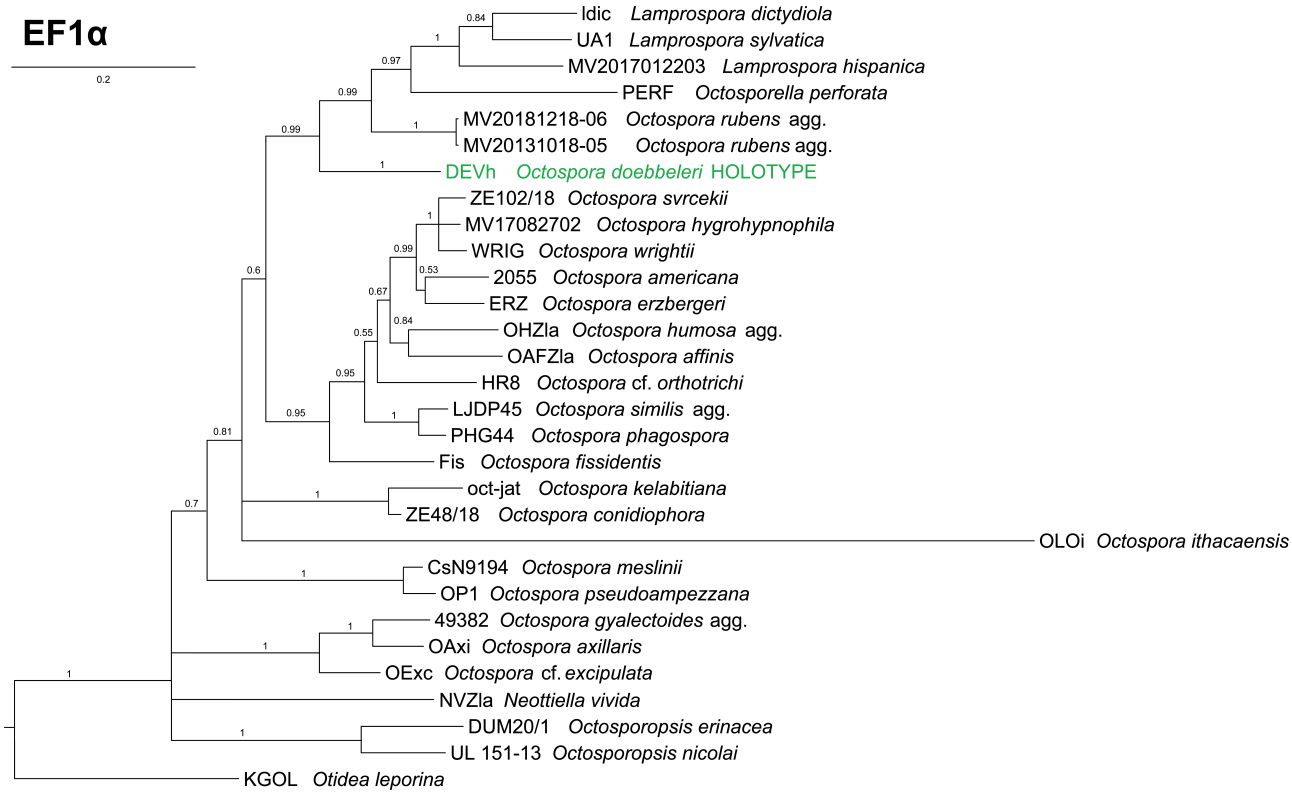
(Manuscript accepted 25 January 2021; Corresponding Editor: I. Krisai-Greilhuber)

Supplementary material 1. Bayesian phylogeny inferences based on single-locus analyses of the LSU, SSU and EF1α.






EF1α
0.2
Idic Lamprospora dictydiola
UA1 Lamprospora sylvatica
MV2017012203 Lamprospora hispanica
PERF Octosporella perforata
MV20181218-06 Octospora rubens agg.
MV20131018-05 Octospora rubens agg.
DEVh Octospora doebbeleri HOLOTYPE
ZE102/18 Octospora svrcekii
MV17082702 Octospora hygrohypnophila
WRIG Octospora wrightii
2055 Octospora americana
ERZ Octospora erzbergeri
OHZla Octospora humosa agg.
OAFZla Octospora affinis
HR8 Octospora cf. orthotrichi
LJDP45 Octospora similis agg.
PHG44 Octospora phagospora
Fis Octospora fissidentis
oct-jat Octospora kelabitiana
ZE48/18 Octospora conidiophora
OLOi Octospora ithacaensis
CsN9194 Octospora meslinii
OP1 Octospora pseudoampezzana
49382 Octospora gyalectoides agg.
OAxi Octospora axillaris
OExc Octospora cf. excipulata
NVZla Neottiella vivida
DUM20/1 Octosporopsis erinacea
UL 151-13 Octosporopsis nicolai
KGOL Otidea leporina

4.2.3 *Lamprospora aberrans* sp. nov. (Pezizales) – the first species of *Lamprospora* with hairy apothecia

Lamprospora aberrans sp. nov. (Pezizales) – the first species of *Lamprospora* with hairy apothecia

Zuzana Sochorová, Marcel Vega, Jorge Hernanz, Jan Eckstein & Michal Sochor

Abstract: Sochorová, Z., Vega, M., Hernanz, J., Eckstein, J. & Sochor, M. 2023. *Lamprospora aberrans* sp. nov. (Pezizales) – the first species of *Lamprospora* with hairy apothecia. – Herzogia **36**: 206–221.

Lamprospora aberrans is described as a new species based on collections from Spain (Community of Madrid and the island of Mallorca) and Croatia (Paklenica NP). It is unique due to the combination of globose ascospores ornamented with bent ridges, as well as stiff, thick-walled hyaline hairs – the latter feature unknown in the genus *Lamprospora* so far. It is the first species of bryophilous Pezizales known to infect mosses from the genus *Gymnostomum* (Pottiaceae, Pottiales). In the Bayesian analysis of LSU, SSU and EF1-α loci it clustered in a well-supported clade with *L. cailletii*, *L. tuberculatella* agg., *L. benkertii* and *L. paechnatzii*. The role of the hairs in the taxonomy of bryophilous Pezizales is discussed.

Zusammenfassung: Sochorová, Z., Vega, M., Hernanz, J., Eckstein, J. & Sochor, M. 2023. *Lamprospora aberrans* sp. nov. (Pezizales) – die erste Art der Gattung *Lamprospora* mit behaarten Apothecien. – Herzogia **36**: 206–221.

Lamprospora aberrans wird als neue Art mit Aufsammlungen aus Spanien (Stadt Madrid und Mallorca) und Kroatien (Nationalpark Paklenica) beschrieben. Die Art ist durch die folgende Merkmalskombination einmalig: globose Ascosporen mit einem Ornament aus gebogenen Leisten sowie das Vorkommen von starren, dickwandigen, hyalinen Haaren – das letzte Merkmal ist in der Gattung *Lamprospora* bisher unbekannt. Es handelt sich um die erste Art bryophiler Pezizales, die Moose aus der Gattung *Gymnostomum* (Pottiaceae, Pottiales) befällt. Eine Bayes-Analyse der LSU, SSU und EF1-α Sequenzen zeigt *L. aberrans* in einer statistisch gut begründeten Gruppe zusammen mit *L. cailletii*, *L. tuberculatella* agg., *L. benkertii* und *L. paechnatzii*. Der Wert von Haaren in der Taxonomie bryophiler Pezizales wird diskutiert.

Key words: bryophilous ascomycetes, Paklenica NP, phylogeny, setae, vital taxonomy.

Introduction

The family Pyronemataceae includes a monophyletic group of obligately bryophilous fungi classified to the genera *Octospora* Hedw., *Lamprospora* De Not., *Neottiella* (Cooke) Sacc., *Octosporopsis* U.Lindem. & M.Vega, *Octosporella* Döbbeler and *Filicupula* Y.J.Yao & Spooner. Due to the species richness in this group, high morphological similarity between individual species, many undescribed taxa, „old“ species with no type material available and sometimes unclear species boundaries, these fungi are often difficult to identify.

In general, the most important characters for identification are the morphology of the ascospores and the link to a certain host bryophyte species or genus (rarely to related genera). Nevertheless, the fungi possess additional characters valuable for determination. One of the most conspicuous ones, which may be detected by the naked eye, is hairs. Thick-walled, hyaline hairs (sometimes called setae; e.g. Döbbeler 2011, Döbbeler et al. 2018, Egertová et al. 2018a) occur in several

species of bryophilous Pezizales. They are present in all species classified in recent works to the genera *Octosporella* (Döbbeler & Menjívar 1992, Yao et al. 2006, Döbbeler 2011, Döbbeler et al. 2018, Döbbeler & Davison 2021, Janošík et al. 2022), *Neottiella* (Benkert 1995, Yuan et al. 2020), both species of *Octosporopsis* (Lindemann et al. 2014, Egertová et al. 2018a), and can also be found in some members of the large genus *Octospora* (Dennis & Itzerott 1973, Benkert 2006, Németh 2017, Sochorová et al. 2019, Vega & Moyne 2019). The only genera of bryophilous Pezizales in which no hairy species were known are *Filicupula* (Yao & Spooner 1996a, Döbbeler & Davison 2021) and *Lamprospora* (e.g. Benkert 1987, Egertová et al. 2018b, Vega et al. 2019, Vega et al. 2021a).

Therefore, it was a surprise when, in January 2016, a species of bryophilous Pezizales was found in the Paklenica NP (Croatia) with hairy apothecia and globose ornamented ascospores – the latter a typical character of the genus *Lamprospora* (Benkert 1987). Since then, the same species has also been discovered in Spain. In all instances, it was found alongside a moss in the genus *Gymnostomum.* It is introduced in this paper as *Lamprospora aberrans* sp. nov.

Material and methods

Macroscopic features were described based on fresh apothecia, microscopic characters were studied both on vital (annotated *) and rehydrated (†) material. Microscopy was performed using a microscope CX21 (Olympus, Czech Group, Prague, Czech Republic), with magnifications of 40×, 100×, 400× and 1000×. The microscopic elements were observed in tap water (H_2O), 5% potassium hydroxide (KOH), Lugol's solution (IKI), Brilliant Cresyl Blue (CRB) and Lactic Acid Cotton Blue (LACB). Infection was studied on rehydrated material in tap water and LACB. Unless stated otherwise, the features were measured in tap water, on photographs using the Piximètre 5.10 software (Henriot & Cheype 2020). Measurements of living, freshly ejected ascospores include fully mature, randomly selected and normally developed ascospores. Values of ascospores´ size are given as minimal measured value – arithmetic mean – maximal measured value, Q = length/width ratio (n = sample size) and include ornamentation. Vouchers are deposited in the herbarium of the University of Alcalá (AH), the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem (B), the Croatian National Fungarium (CNF) and the herbarium of the Mycological department of the National Museum in Prague (PRM).

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried apothecia using the CTAB method as outlined by Doyle & Doyle (1987). Apothecia were homogenised using a pestle, incubated in 300 µL of extraction buffer at 65 °C for one hour, and the extract was subsequently purified in chloroform–isoamyl alcohol mixture, precipitated by isopropanol and finally dissolved in water and incubated with RNase for 30 min at 37 °C. DNA quality was checked using agarose gel electrophoresis. Molecular sequence data were generated for three loci: the 28S subunit of ribosomal DNA (LSU) was amplified with primers LR0R and LR6 (Vilgalys & Hester 1990), the 18S subunit of rDNA (SSU) with primers NS1 and NS6 (White et al. 1990) and translation elongation factor-1alpha (EF1α) with primers EF1–983F and EF1–1567R (Rehner & Buckley 2005). PCR was performed with EliZyme FAST Taq mix (Elisabeth Pharmacon, Brno, Czech Republic) following a standard protocol with 37 cycles and annealing temperature of 54 °C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 m NaCl in the precipitation mixture) and sequenced from both directions using the same primer pairs by the Sanger method at Macrogen Europe, Amsterdam, the Netherlands.

Table 1. List of sequences used in the phylogenetic analysis. Newly generated sequences in bold.

Species	Herbarium code	Geographic origin, collector	Host	LSU	SSU	EF1-α	Reference
Filicupula suboperculata	GG12	Great Britain, G. Greiff	*Frullania tamarisci*	**OQ077718**	**OQ077717**		
Lamprospora aberrans	AH-56305 (holotype)	Spain, J. Hernanz	*Gymnostomum viridulum*	**OQ023285**	**OQ023282**	**OQ023971**	
L. aberrans	CNF 2/9852	Croatia, Z. Egertová	*Gymnostomum calcareum*	**OQ023283**	**OQ023280**		
L. aberrans	B 70 0108109	Spain, M. Vega, J. L. Siquier & J. C. Salom	*Gymnostomum viridulum*	**OQ023284**	**OQ023281**	**OQ023972**	
L. aneurae	B 70 0005997 (holotype)	Germany, D. Benkert	*Aneura pinguis*	MZ343191	MZ343180	MZ336038	Eckstein et al. 2021
L. angularis	AH-44756 (holotype)	Spain, M. Vega	*Campylopus pilifer*	MZ190473	MZ190475	MZ189736	Vega et al. 2021b
L. arvensis	PRC 4964	Germany, J. Siembida	*Ceratodon purpureus*	ON087134	ON087208	ON093896	Janošík et al. 2023
L. benkertii	PRC 4601	Slovakia, L. Janošík	*Trichostomum crispulum*	ON087070	ON087158	ON093858	Janošík et al. 2023
L. bulbiformis	B 70 0100012 (holotype)	Portugal, M. Vega	*Fissidens viridulus*	MT792684	MT792707	MT783993	Vega et al. 2021a
L. cailletii	PRM 951726	Slovakia, Z. Egertová, M. Sochor	*Tortella tortuosa*	MN394605			Eckstein et al. 2022
L. campylopodis	HBG-024817	Germany, M. Vega	*Campylopus pyriformis*	MF066054	MK569364	MK569289	Egertová et al. 2018b, Sochorová et al. 2019
L. carbonicola	PRC 4118	Czech Republic, L. Janošík	*Funaria hygrometrica*	MH818440	ON087212	ON093899	Vega et al. 2019, Janošík et al. 2023
L. densireticulata	HBG-024587 (paratype)	Germany, M. Vega & T. Richter	*Aloina ambigua*	MH818449			Vega et al. 2019
L. dicranellae	PRC 4619	Austria, L. Janošík	*Ditrichum heteromallum*	MT792686	MT792709	MT783995	Vega et al. 2021a
L. dictydiola	PRM 945794	Czech Republic, Z. Egertová	*Tortula muralis*	MF754056	MK569365	MF754054	Egertová et al. 2018a, Sochorová et al. 2019
L. ditrichi	PRC 4620	Czech Republic, L. Janošík	*Flexitrichum flexicaule*	MT792687	MT792710	MT783996	Vega et al. 2021a
L. ecksteinii	JE 66514	Germany, J. Eckstein & G. Eckstein	*Microbryum curvicollum*	ON087152	ON087222		Janošík et al. 2023
L. esterlechnerae	PRC 4621	Germany, L. Janošík & M. Vega	*Dicranodontium denudatum*	MT792689			Vega et al. 2021a
L. feurichiana	PRC 4980	Spain, L. Janošík	*Ceratodon purpureus*	ON087153	ON087223	ON093905	Janošík et al. 2023
L. gibbosa	B 70 0100017 (holotype)	France, M. Vega	*Fissidens crassipes*	MT792691	MT792712	MT783997	Vega et al. 2021a
L. hispanica	B 70 0100998	Spain, M. Vega	*Aloina aloides*	MN394607	MT792713	MT783998	Eckstein et al. 2022, Vega et al. 2021a

Species	Herbarium code	Geographic origin, collector	Host	LSU	SSU	EF1-α	Reference
L. kristiansenii	PRC 4968	Czech Republic, L. Janošík	*Ceratodon purpureus*	ON087138	ON087213	ON093900	Janošík et al. 2023
L. leptodictya	ZT Myc 61079	Switzerland, E. Stöckli	*Aongstroemia longipes*	MN394610	MT792714		Eckstein et al. 2022, Vega et al. 2021a
L. lubicensis	PRC 4622	Czech Republic, L. Janošík & Z. Egertová	*Hennediella heimii*	MT792693	MT792715	MT783999	Vega et al. 2021a
L. lutziana	MA-Fungi 90544	Spain, M. Vega, R. Martínez-Gil & J. De La Cruz	*Philonotis fontana*	MN434188	MT792716	MT784000	Martínez-Gil et al. 2019, Vega et al. 2021a
L. maireana	PRC 4927	Cyprus, M. Vega	*Fossombronia caespitiformis*	ON087073	ON087161		Janošík et al. 2023
L. miniata var. *parvispora*	PRM 945795	Slovakia, Z. Egertová	*Barbula unguiculata*	MF066065	MK569366	MF754055	Egertová et al. 2018a, 2018b, Sochorová et al. 2019
L. miniata var. *ratisbonensis*	PRM 946421	Croatia, Z. Egertová	*Didymodon luridus*	MF066064			Egertová et al. 2018b
L. norvegica	HBG-024743	Switzerland, M. Vega & B. Senn-Irlet	*Ditrichum pusillum*	MT792694	MT792717	MT784001	Vega et al. 2021a
L. paechnatzii	B 70 0100018	Germany, M. Vega, T. Richter	*Ptychostomum pseudotriquetrum* var. *bimum*	MN394613			Eckstein et al. 2022
L. pseudoarvensis	PRC 4965	Czech Republic, L. Janošík	*Pleuridium acuminatum*	ON087135	ON087210	ON093897	Janošík et al. 2023
L. rehmii	S F317032 (epitype)	Spain, R. Martínez-Gil	*Pleuridium acuminatum*	MH087070	MT792719		Vega et al. 2018, 2021a
L. retispora	PRC 4931	Czech Republic, O. Koukol	*Syntrichia ruralis*	ON087077	ON087165	ON093861	Janošík et al. 2023
L. seaveri	PRC 4581	Montenegro, L. Janošík	*Ceratodon purpureus*	MT792695	ON087209		Janošík et al. 2023, Vega et al. 2021a
L. sylvatica	PRM 946415 (holotype)	Ukraine, Z. Egertová & M. Sochor	*Dicranum montanum*	MG947604	MK569367	MK569290	Egertová et al. 2018b, Sochorová et al. 2019
L. thelespora	MA-Fungi 90701 (holotype)	Spain, R. Martínez-Gil	*Cheilothela chloropus*	MT792701	MT792724	MT784006	Vegaet al. 2021a
L. tortulae-ruralis	PRM 956465	Czech Republic, Z. Egertová	*Syntrichia ruralis*	ON087079	ON087166	ON093862	Janošík et al. 2023

Species	Herbarium code	Geographic origin, collector	Host	LSU	SSU	EF1-α	Reference
L. tuberculata agg.	PRC 4624	Slovakia, L. Janošík	*Pleuridium subulatum*	MT792703	MT792726	MT784008	Vega et al. 2021a
L. tuberculatella agg.	PRC 4625	Slovakia, L. Janošík	cf. *Trichostomum crispulum*	MT792705	MT792728	MT784009	Vega et al. 2021a
L. verrucispora	HBG-1412 (holotype)	Germany, M. Vega	*Campylopus pyriformis*	MN994551	MN994527	MN990993	Sochorová et al. 2020
Neottiella albocincta	PRC 4935	Germany, M. Vega	*Atrichum undulatum*	ON087103	ON087181	ON093872	Janošík et al. 2023
N. gigaspora	HKAS 104669 (holotype)	China, M. Zeng	unknown	MK589293		MK577716	Yuan et al. 2020
N. ricciae		Germany, J. Eckstein	*Riccia beyrichiana*	**OQ077715**	**OQ077716**		
N. rutilans	B 70 0100473	Poland, J. Eckstein	*Oligotrichum hercynicum*	MK569313	MK569336	MK569288	Sochorová et al. 2019
N. vivida	PRM 945797	Czech Republic, Z. Egertová	*Polytrichum piliferum*	MF066068	MK569337	MF754051	Egertová et al. 2018a, 2018b, Sochorová et al. 2019
Octospora affinis	PRM 945798	Czech Republic, A. Polhorský, L. Janošík & Z. Egertová	*Lewinskya affinis*	MF754075	MK569347	MF754045	Egertová et al. 2018a, Sochorová et al. 2019
O. americana	S F43718 (holotype)	USA, G. Thor	*Forsstroemia trichomitria*	MN967346	MN994516	MT078729	Sochorová et al. 2020
O. bridei	PRM 935151	Czech Republic, Z. Egertová	*Ephemerum serratum*	MF754061	MT001890		Egertová et al. 2018a, Sochorová et al. 2020
O. conidiophora	PRM 951743 (holotype)	South Africa, Z. Egertová & M. Sochor	*Trichosteleum perchlorosum*	MK569321	MK569351	MK569297	Sochorová et al. 2019
O. doebbeleri	PRM 954007 (holotype)	Czech Republic, Z. Sochorová & M. Sochor	*Dicranoweisia cirrata*	MW152148	MW152156	MW159137	Sochorová et al. 2021
O. erzbergeri	PRM 945799	Czech Republic, Z. Egertová	*Pseudoleskeella nervosa*	MF754068	MK569340	MF754042	Egertová et al. 2018a, Sochorová et al. 2019
O. cf. *excipulata*	PRM 945800	Czech Republic, Z. Egertová	*Funaria hygrometrica*	MF754062	MK569369	MF754047	Egertová et al. 2018a, Sochorová et al. 2019
O. gyalectoides agg.	B 70 0100075	Germany, J. Eckstein	*Tortula lindbergii*	MT001891	MT001889	MN990995	Sochorová et al. 2020
O. hetieri	B 70 0108147	Germany, J. Eckstein	*Funaria hygrometrica*	ON087091	ON087174	ON093867	Janošík et al. 2023
O. humosa agg.	PRM 945802	Czech Republic, Z. Egertová	*Polytrichum piliferum*	MF754074	MK569343	MF754043	Egertová et al. 2018a, Sochorová et al. 2019
O. ithacaensis	PRM 945803	Czech Republic, Z. Egertová	*Marchantia polymorpha*	MF754071	MK569346		Egertová et al. 2018a, Sochorová et al. 2019

Species	Herbarium code	Geographic origin, collector	Host	LSU	SSU	EF1-α	Reference
O. kelabitiana	PRM 945781	Malaysia, Z. Egertová & M. Sochor	*Riccardia* sp.	MF754065	MK569372	MF754048	Egertová et al. 2018a, Sochorová et al. 2019
O. kilimanjarensis	U.L. 294	Ethiopia, U. Lindemann	*Pogonatum* sp.	**OQ220948**			
O. leucoloma	PRC 4952	Czech Republic, L. Janošík	*Bryum argenteum*	ON087120	ON087195	ON093885	Janošík et al. 2023
O. oscarii	PRM 955619	Czech Republic, Z. Sochorová	*Pseudotaxiphyllum elegans*	MZ343189	MZ343179	MZ336037	Eckstein et al. 2021
O. phagospora	PRM 945805	Germany, M. Vega	*Pohlia lutescens*	MF754072	MK569344	MF754046	Egertová et al. 2018a, Sochorová et al. 2019
O. pseudoampezzana	PRM 935156	Czech Republic, Z. Egertová & M. Sochor	*Schistidium crassipilum*	MF754069	MK569339	MF754050	Egertová et al. 2018a, Sochorová et al. 2019
O. svrcekii	PRM 951720	Croatia, Z. Egertová, N. Matočec & I. Kušan	*Cratoneuron filicinum*	MN967348	MN994518	MN974532	Sochorová et al. 2020
O. wrightii	PRM 945807	Czech Republic, Z. Egertová	*Amblystegium serpens*	MF754070	MK569345	MT078728	Egertová et al. 2018a, Sochorová et al. 2019, 2020
Octosporella australis	AD-C61399 (holotype)	Australia, L. Janošík	*Lethocolea pansa*	OM991664	OM991697	ON012511	Janošík et al. 2022
O. erythrostigma	PRC 4919	Austria, L. Janošík	*Frullania dilatata*	OM991674	OM991704		Janošík et al. 2022
O. jungermanniarum	TUR 178050	Switzerland, P. Döbbeler	*Plagiochila asplenioides*	EU940133	EU940060		Stenroos et al. 2010
O. microtricha	PRM 956007	Spain, M. Vega	*Frullania polysticta*	ON087100	ON087178		Janošík et al. 2023
O. ornithocephala	PRC 4918	France, J. P. Priou	*Radula complanata*	OM991673	OM991703	ON012515	Janošík et al. 2022
O. perforata	PRM 945808	Czech Republic, Z. Egertová	*Porella platyphylla*	MF754060	MK569368	MF754052	Egertová et al. 2018a, Sochorová et al. 2019
Octosporopsis erinacea	PRM945774 (isotype)	Malaysia, Z. Egertová & M. Sochor	*Dumortiera hirsuta*	MF754057	MK569338	MF754041	Egertová et al. 2018a, Sochorová et al. 2019
O. nicolai	UL151–13	Germany, M. Vega	*Lunularia cruciata*	KF771033		KF771042	Lindemann et al. 2014
Otidea concinna	KH.09.183 (S) (epitype)	Sweden, K. Hansen & I. Olariaga		NG060279	NG064990	KM823275	Hansen & Olariaga 2015, Schoch et al. 2012

Phylogenetic analysis

Specimens used in the phylogenetic analysis are listed in Tab. 1. Sequences were edited and aligned in Geneious software (ver. 7.1.7., Biomatters, New Zealand) using the MAFFT plugin. Bayesian phylogeny inference (BI) was computed in MrBayes (ver. 3.2.4, Ronquist et al. 2012) using the GTR+I+G substitution model, as determined by AICc in PartitionFinder 2.1.1 (Lanfear et al. 2017). The analysis was run for 20 million generations in four independent runs, sampling every 1000th generation and excluding the first 50 % of generations as burn-in. The Basic Local Alignment Search Tool (BLAST; Zhang et al. 1990) was used for searching similar sequences in publicly available sequence databases (https://blast.ncbi.nlm.nih.gov).

Results

Phylogenetic analysis

LSU and SSU sequences were obtained from three specimens of *Lamprospora aberrans* and EF1-α from two (AH-56305 and B 70 0108109). In LSU and EF1-α, the sequenced samples were fully identical and in SSU only 1-bp indel was present. In our Bayesian analysis, *L. aberrans* clustered in a well-supported clade with *L. cailletii* Benkert, *L. tuberculatella* agg., *L. benkertii* Eckstein, M.Vega, Sochorová & Janošík and *L. paechnatzii* Benkert (Fig. 1).

Taxonomy

Lamprospora aberrans Sochorová, M.Vega, J.Hernanz & Eckstein, **sp. nov.** (Figs 2–5) [MycoBank no. MB 847133]

Etymology: aberrans = aberrant, for being unique among known species of *Lamprospora* due to the hairy apothecia

Diagnosis: Differs from all known species of the genus *Lamprospora* by the presence of stiff, long, thick-walled hairs at the apothecial margin and receptacular surface.

Holotype: **Spain**, Alcalá de Henares (Community of Madrid), Parque de los Cerros, 40º27'30.7"N/3º20'21.7"W, 650 m a.s.l., on *Gymnostomum viridulum* on a bank in a dark ravine in a forest of *Pinus halepensis*, 10 Apr 2021, leg. J. Hernanz, herb. AH-56305 (isotype: PRM 958539).

Hosts: *Gymnostomum calcareum*, *G. viridulum;* accompanying mosses: *Aloina aloides*, *Dicranella howei*, *Didymodon insulanus*, *D. sicculus*, *Eucladium verticillatum*, *Trichostomum* sp.

Description: **Apothecia** growing solitary or in groups on soil among the host moss, globose when young, later barrel-shaped or cup-shaped, sessile, up to 950 µm in diameter and 570 µm high, vividly orange; hymenium sometimes slightly roughened by protruding asci; margin and receptaculum richly beset by stiff, hyaline hairs; in section orange except the part occupied by ectal excipulum which is subhyaline.

Asci *(200–)250–350 × 20–28 µm, †180–250 × 14–18.5 µm, cylindrical, thinning toward the base, unitunicate, operculate, inamyloid, with a hemispherical apex, 8-spored, arising from croziers; young asci containing glycogen, especially in the basal part; protruding above paraphyses for up to 80 µm; pars sporifera *97–112.5 µm when all eight ascospores are fully developed, †92–108 µm.

Ascospores globose, in H_2O *12.9–14.4–15.3(–16) µm in diam. (n=150), in LACB †11.3–13.5–15.2 µm (n=150), hyaline, thin-walled, uninucleate, containing a single large lipid body *9–10.5(–11) µm in diam.; in asci uniseriate or less often subbiseriate; De Bary bubbles formed in some ascospores after heating in LACB. Ornamentation consisting of bent ridges, *0.2–1(–1.6) µm wide, 0.3–1(–1.2) µm high, often anastomosing, ending either blunt or pointed at their meeting points, cyanophilous, completely dissolving in 5% KOH.

Paraphyses mostly straight, simple or forked, septate, with a high content of carotenoid pigment (turning green in IKI), without vacuolar bodies (VBs), sometimes anastomosing or having short lateral

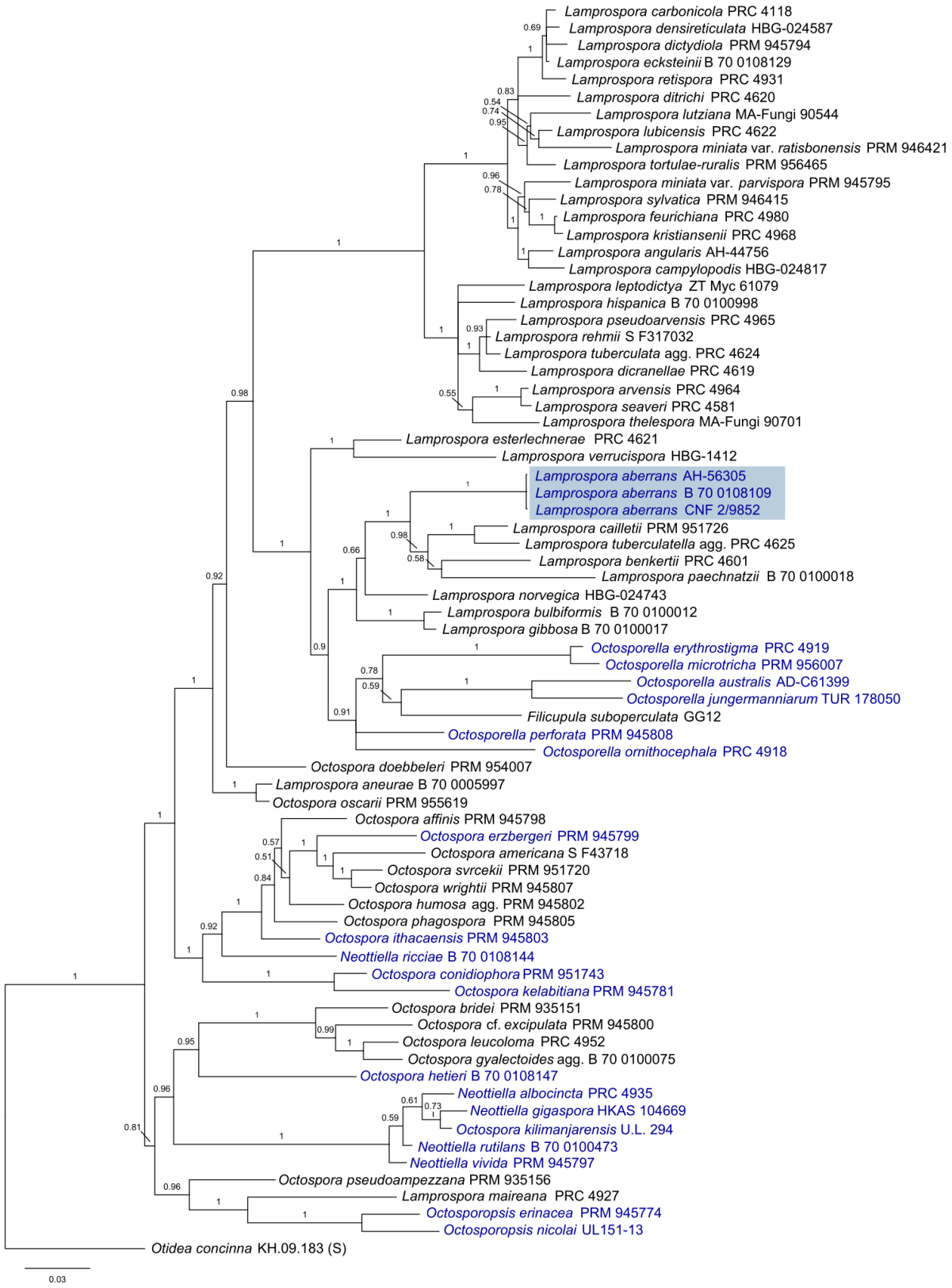


Fig. 1. Fifty percent majority rule Bayesian phylogram obtained from the concatenated LSU, SSU and EF1-α sequences showing the phylogenetic position of *Lamprospora aberrans*. Species having stiff, thick-walled hairs are highlighted in blue. *Otidea concinna* serves as outgroup. Posterior probabilities are shown above branches.

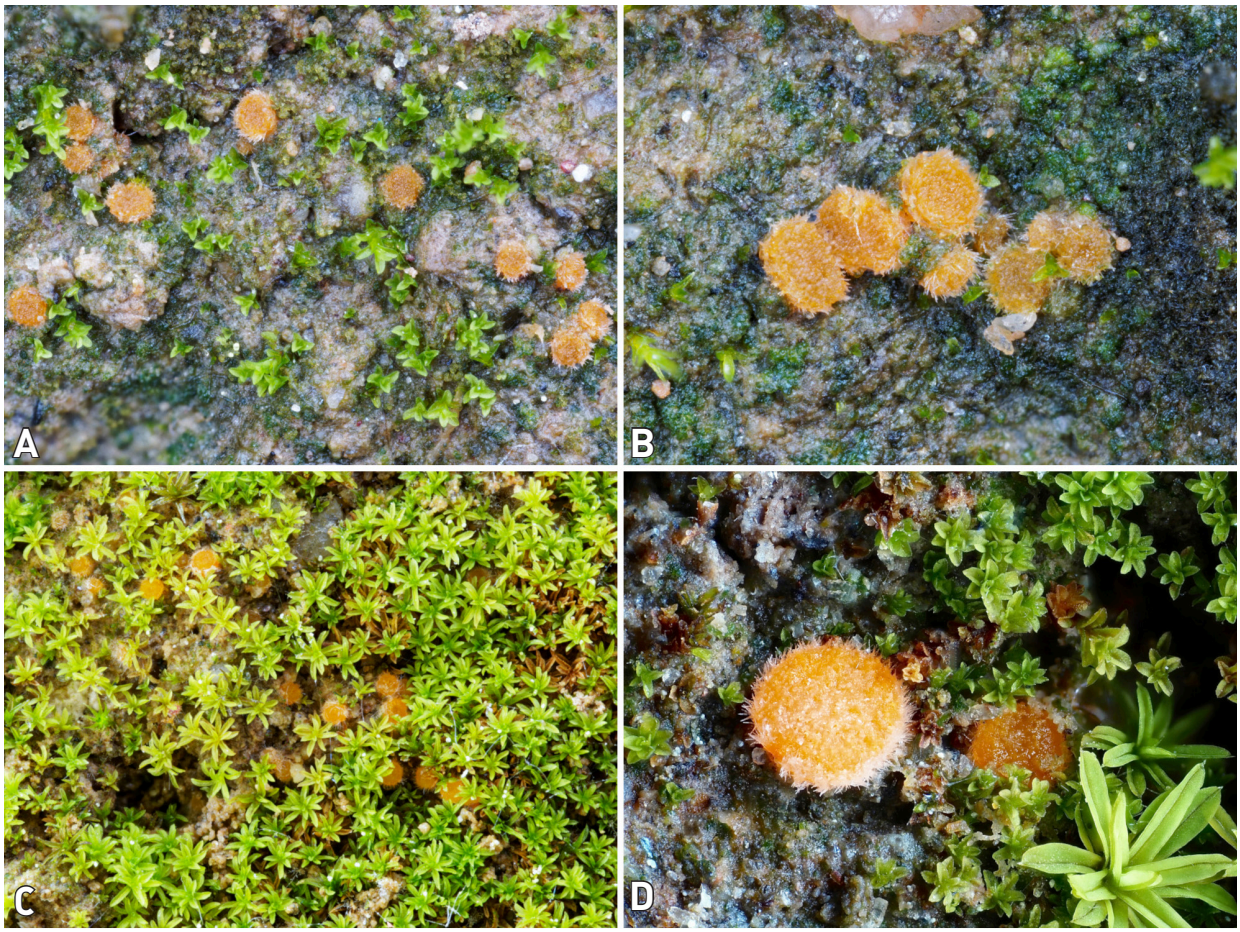


Fig. 2. *Lamprospora aberrans* – macroscopic characters (A–D holotype AH-56305). **A–D**–Apothecia between shoots of *Gymnostomum viridulum*. Photo 2D by Sandra Bernhardt.

projections; apical cell usually cylindrical, less often slightly clavate, capitate or spathulate, *31–100 × 3–8 µm, †30–75 × 2–5.3 µm, lower cells *3–5 µm wide, †1.5–3.5 µm.

Medulla sharply delimited from ectal excipulum, merging with subhymenium, *30 µm (in the middle flank) to 180 µm thick (in the thickest part), formed by textura intricata, cells cylindrical, thin-walled, containing carotenoid pigment (turning green in IKI), *3–10 µm wide.

Ectal excipulum *45–125 µm thick at the base, *25–70 µm at flanks, formed by textura angularis-prismatica, cells angular, subglobose, pyriform, cylindrical or irregular, thick-walled (wall up to 3.2 µm thick), subhyaline, rich in glycogen (rusty in IKI), *(8–)11–25(–33) × 6–16 µm.

Margin formed by textura prismatica-porrecta, cells containing carotenoid pigment, terminal cells cylindrical, clavate or with an irregular apex, sometimes anastomosing, *(13–)34–75 × 6–20 µm, lower cells cylindrical, *15–60 × 6.5–13(–17) µm.

Hairs arising from the outermost cells of margin and the ectal excipulum, hyaline, sparsely septate, simple or less often bifurcate, at the margin oriented upwards, more randomly oriented in lower parts of the apothecium; *(40–)90–215 × 8–16 µm (in the thickest part), very thick-walled, the opposite walls often merging and the lumina therefore absent; apices pointed or blunt; exceeding the level of hymenium for up to 100 µm.

Anchoring hyphae hyaline, thick-walled, septate, branching, *4–7 µm wide.

Infection

Hyphae 3–6 µm wide, thick-walled, forming complex infection structures on rhizoids consisting of appressoria, infection pegs and haustoria. Appressoria of two kinds probably represent developmental

Fig. 3. *Lamprospora aberrans* – habitat (A–B holotype AH-56305). **A–B**–The ravine in the vicinity of Alcalá de Henares.

stages: first 1–3-septate, 15–28 µm long and 10–15 µm wide and high, uncovered; later or on strong rhizoids appressoria larger, up to 25 µm wide and high and completely covered by a layer of connate hyphae, without discernable septa. Infection peg 2–3 µm wide, surrounded by a short tube of rhizoid cell-wall material. Haustoria (1–)2–3(–4) µm wide, intracellular, thin-walled, ramified, contorted, septa missing or difficult to see, not growing through the rhizoid cross walls. No growth modifications relating to the infection were observed.

Other specimens examined: Croatia, Paklenica NP, Mala Paklenica, ca 44°17'9.8"N/15°30'11"E, 90 m a.s.l., on *Gymnostomum calcareum* on a rock above the dried out river, 2 Jan 2016, leg. Z. Egertová (CNF 2/9852); Velika Paklenica canyon, ca 44°17'55"N/15°27'54"E, 60 m a.s.l., on *Gymnostomum calcareum* on a rock above the road, 25 Mar 2016, leg. Z. Egertová (CNF 2/9871). **Spain**, Esporles (Mallorca), Área recreativa de Son Tries, 39°39'44.1"N/2°34'30.8"E, 290 m a.s.l., on *Gymnostomum viridulum* on the bank of a road, 24 Nov 2018, leg. M. Vega, J. L. Siquier & J. C. Salom (B 70 0108109); Alcalá de Henares (Community of Madrid), Parque de los Cerros, 40°27'28.3"N/3°20'21.2"W, 670 m a.s.l., on *Gymnostomum viridulum*, 19 Nov 2022, leg. J. Hernanz (PRM 958328).

Discussion

The combination of hairy apothecia, globose ascospores ornamented with bent ridges and growth on *Gymnostomum* spp. (Pottiaceae, Pottiales) makes the species unique and almost impossible to misidentify.

Mosses of the genus *Gymnostomum* have not been recorded as host for any other species of bryophilous Pezizales to date. *Gymnostomum viridulum* forms dense bright green tufts less than 5 mm high on calcareous outcrops and on calcareous or gypsum soil mostly in sheltered situations. In Europe, it has a Mediterranean-Atlantic distribution extending north to

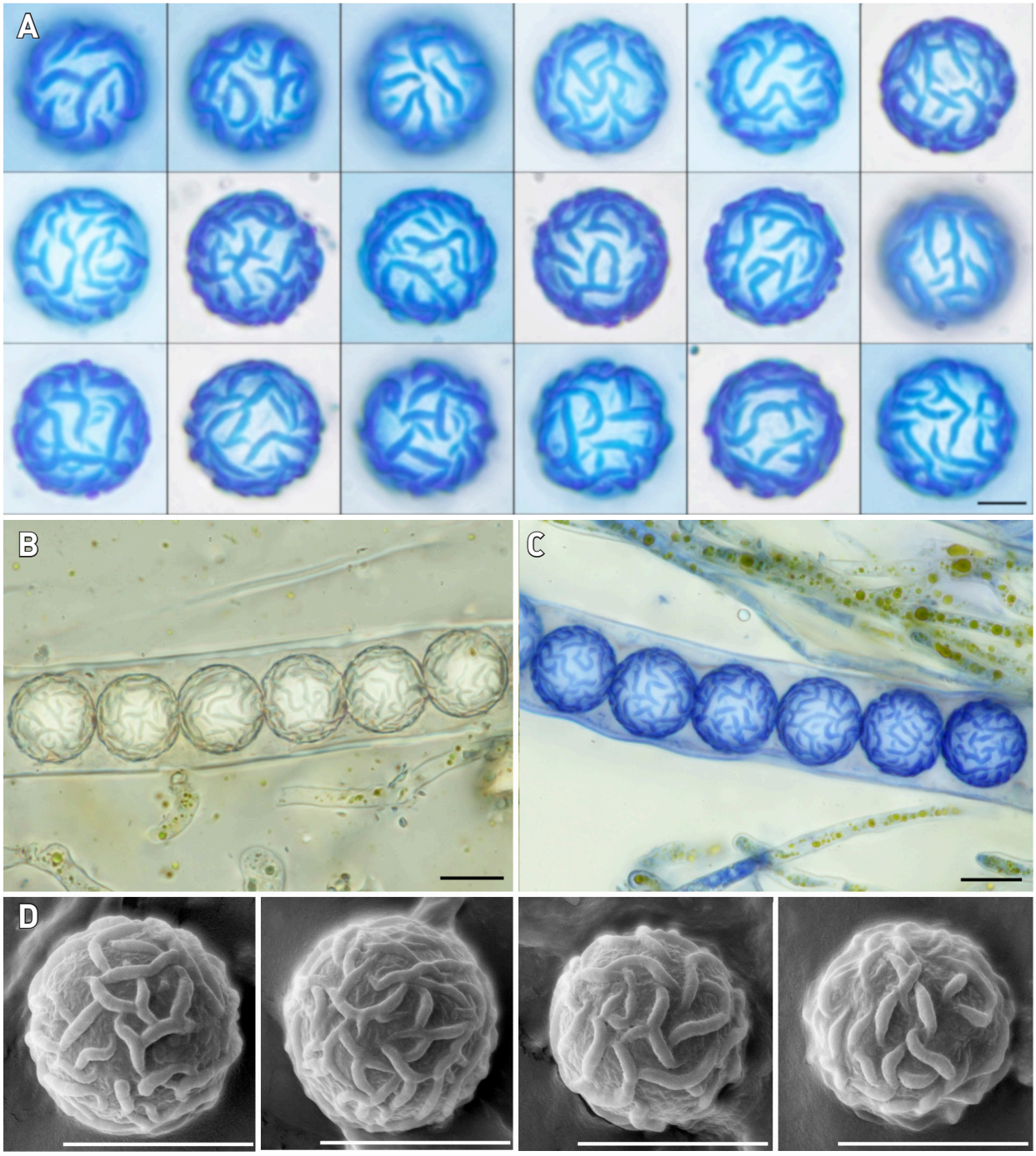


Fig. 4. *Lamprospora aberrans* – ascospores (A–C holotype AH-56305, D CNF 2/9852). **A** – free ascospores stained in LACB. **B** – ascospores inside ascus in water. **C** – ascospores inside ascus stained in LACB. **D** – SEM figures of ascospores. Scale bars: A = 5 µm, B–G = 10 µm.

Czech Republic, Germany, Belgium and the British Isles. Outside Europe it is known from Macaronesia, Southwest Asia, North and East Africa as well as western North America (Blockeel et al. 2014). *Gymnostomum calcareum* also forms dense bright green tufts but up to 20 mm height. It grows on soft calcareous rocks and rock crevices in damp and sheltered situations. The distribution for *G. calcareum* is Eurosiberian Southern-temperate with European occurrences mainly in the southern, central and western parts. Outside Europe it is widely distributed in both hemispheres but detailed distribution is obscured by taxonomic difficulties (Blockeel et al. 2014).

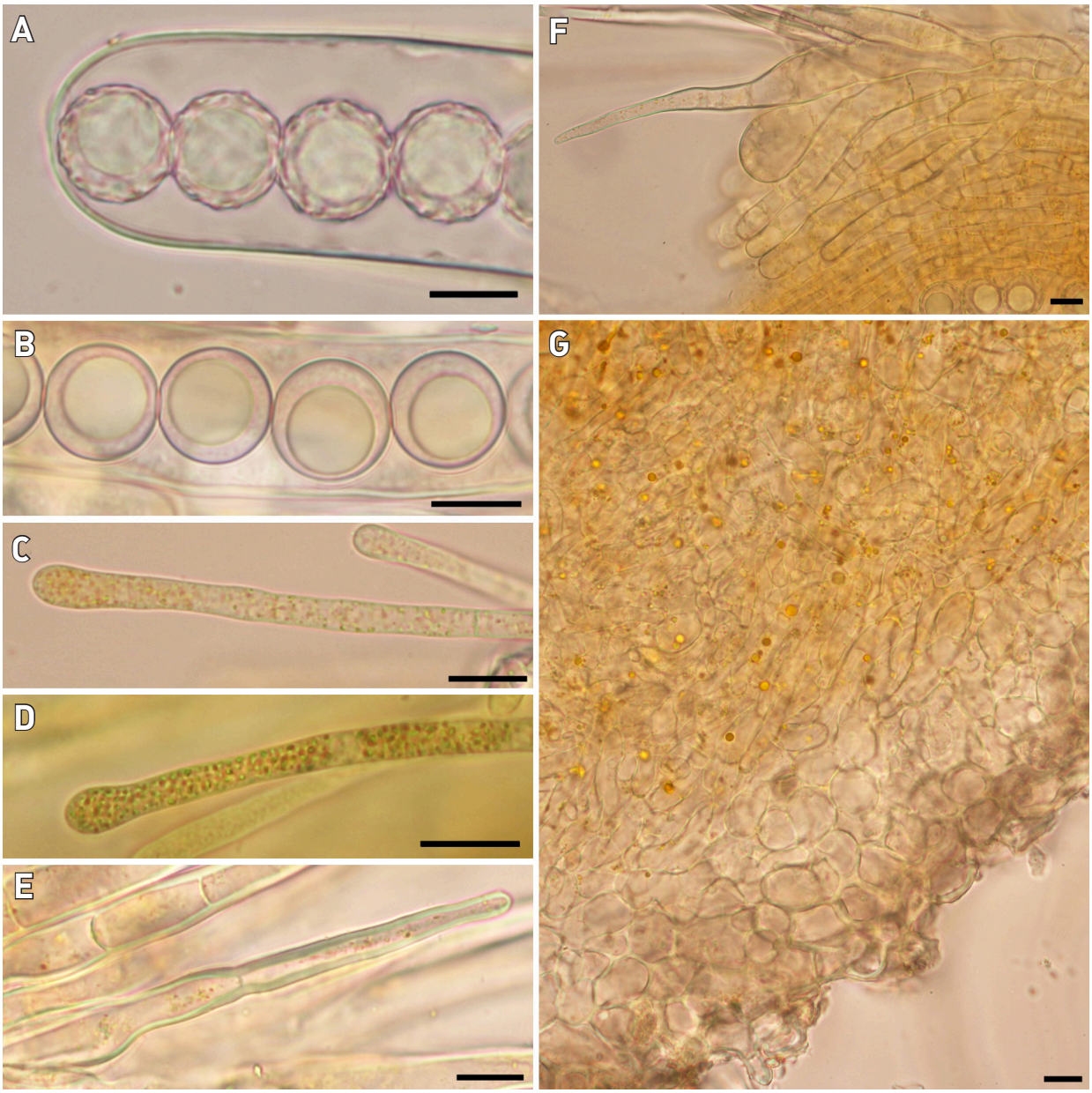


Fig. 5. *Lamprospora aberrans* – microscopic characters (A–C, E–G PRM 958328, D holotype AH-56305). **A** – Ascospores inside a fully turgescent ascus in tap water. **B** – Ascospores in 5% KOH. **C** – Paraphyses in water. **D** – Paraphysis in IKI. **E** – Hair in tap water. **F** – Margin of apothecium in tap water. **G** – Detail of section of the apothecium in tap water. Scale bars in all figures = 10 µm.

Role of hairs in the taxonomy of bryophilous Pezizales

Three of the genera belonging to bryophilous Pezizales were described a very long time ago: *Octospora* (Hedwig 1789), *Lamprospora* (De Notaris 1864) and *Neottiella* (Saccardo 1889), while the other three were established much more recently: *Octosporella* (Döbbeler 1980), *Filicupula* (Yao & Spooner 1996a) and *Octosporopsis* (Lindemann et al. 2014).

While it is rather easy to recognize that a certain species belongs to the group of bryophilous Pezizales, it is often problematic to classify it to a genus. In particular, the concepts of the old genera vary greatly between different authors. In the past, several superficially similar species had been lumped into these genera, many of which turned out to be non-bryophilous and not

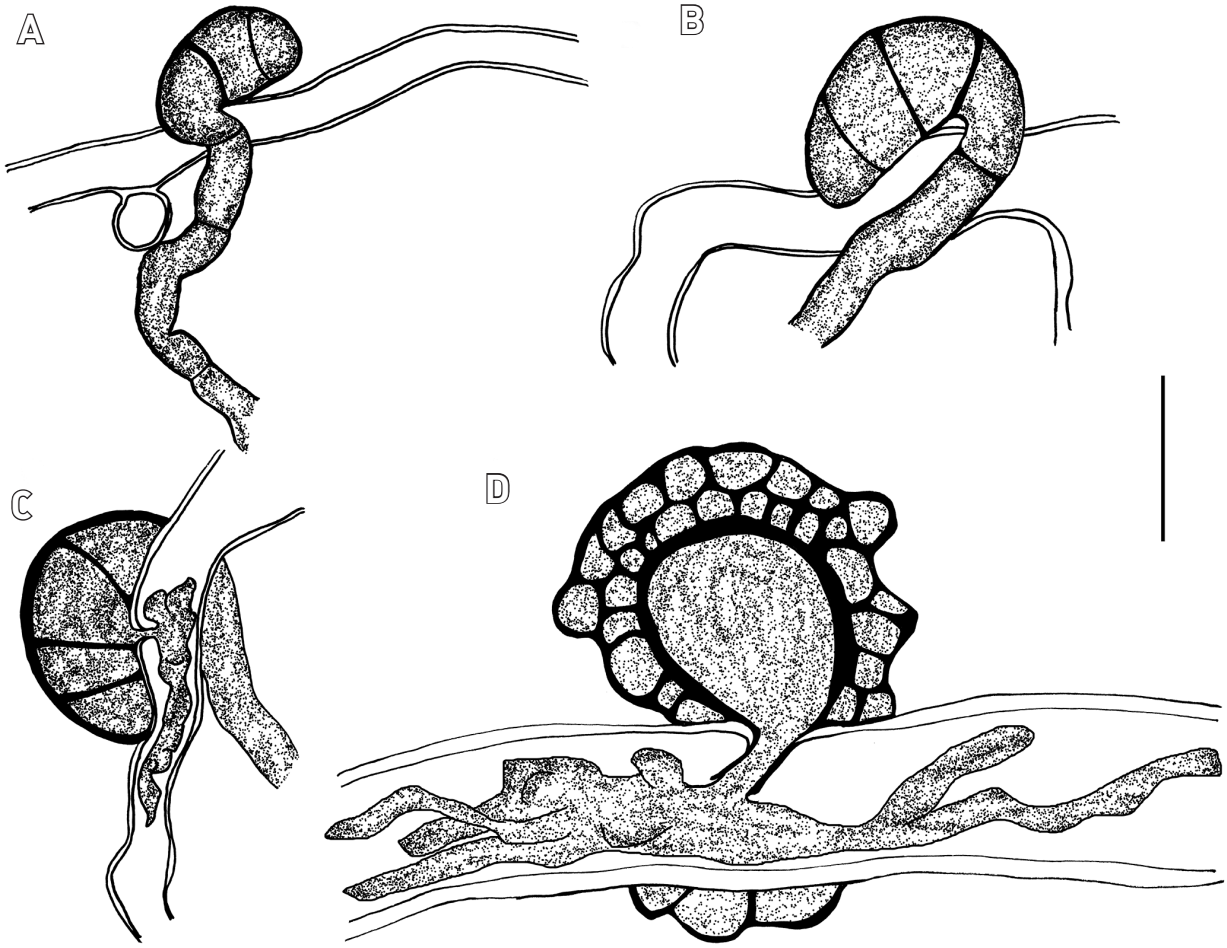


Fig. 6. *Lamprospora aberrans* – infection, fungal cells dotted (A–D holotype AH-56305). **A–B –** appressoria attached to rhizoids, surface view, **C** – appressorium with haustorium, optical section. **D –** appressorium covered with accompanying hyphae as well as haustorium inside rhizoid, optical section. Scale bar = 20 µm. Illustrated by Jan Eckstein.

very closely related. In more recent works on these fungi, the main systematic issues can be found in setting the boundaries of the genera *Neottiella* and *Lamprospora* against *Octospora*.

The presence of stiff, thick-walled hairs has been used as an important argument for distinguishing *Neottiella* as a separate genus (Dennis 1981, Benkert 1998). On the other hand, several authors prefered a broader concept of *Octospora* – some species previously assigned to *Neottiella* were recombined to *Octospora* by Dennis & Itzerott (1973), which was accepted also e.g. by Yao & Spooner (1996b); Caillet & Moyne (1987) established a section *Neottiellae* in *Octospora* for the hairy species, which was followed by Moravec (1997). In more modern works, *Neottiella* is usually accepted as a separate genus for species with stiff hairs and infecting Polytrichaceae (Eckstein et al. 2014, Vega 2017, Eckstein et al. 2020, Janošík 2020). Molecular data have shown that the type species of *Neottiella – N. albocincta* (Berk. & M.A.Curtis) Sacc., forms a monophyletic lineage with two European species infecting Polytrichaceae – *N. rutilans* (Fr.) Dennis and *N. vivida* (Nyl.) Dennis (Janošík 2020), as well as with *Octospora kilimanjarensis* J.Moravec from Africa (Moravec 1997) and *N. gigaspora* M.Zeng, Q.Zhao & K.D.Hyde, recently described from China (Yuan et al. 2020). The host of *N. gigaspora* is not known; it has been described as "saprobic on soil", but the host apparently is a species of *Atrichum*, based on the photos in Yuan et al. (2020). Hairy species sometimes classified to *Neottiella*, but not linked to Polytrichaceae – *Octospora hetieri* (Boud.) Dennis

& Itzerott (syn. *Neottiella hetieri* Boud.), *Octospora ithacaensis* (Rehm) K.B.Khare (syn. *Neottiella ithacaensis* (Rehm) Schweers) and *Neottiella ricciae* (P.Crouan & H.Crouan) Korf & W.Y.Zhuang do not cluster to the group and should not be treated as members of *Neottiella*. The position of *N. megapolitana* Benkert & T.Richter is uncertain, as no molecular data are available for this species and its host is unknown (no species of Polytrichaceae is given as an accompanying bryophyte in Benkert 1998). *Octospora erzbergeri* Benkert, which also has hairy apothecia, belongs to the group around *Octospora wrightii* (Berk. & M.A.Curtis) J.Moravec, the so-called section *Wrightoideae* (Sochorová et al. 2020). Species of *Octospora conidiophora* agg. and *O. kelabitiana* Egertová & Döbbeler are also unrelated to the *N. albocincta* lineage (Egertová et al. 2018a, Sochorová et al. 2019).

The second case in which hairs have been emphasized, was the delimitation of *Filicupula* from *Octosporella* (Yao & Spooner 1996a). Beside the absence of hairs, *Filicupula* differs from *Octosporella* in having typical discoid apothecia and a filamentous construction of the excipulum. Our Bayesian analysis included the type species of the genus *Filicupula – F. suboperculata* (Döbbeler & P.James) Y.J.Yao & Spooner, which clustered in a clade with *Octosporella erythrostigma* (Mont.) Döbbeler, *O. microtricha* Döbbeler, Negrín & M.Vega, *O. australis* Janošík & Döbbeler, *O. jungermanniarum* (P.Crouan & H.Crouan) Döbbeler, *O. perforata* (Döbbeler) Döbbeler and *O. ornithocephala* Döbbeler, although with low support. Phylogenetic analyses including more genes will be needed to convincingly clarify the boundaries of the genera.

It can be concluded that hairs either have developed independently several times or represent a plesiomorphic character which is not expressed in most of the contemporary species of bryophilous Pezizales.

Acknowledgements

We thank the authorities of Paklenica NP for permission to conduct the research in the area of National Park, George Greiff for the improvement of the English language and valuable comments to the manuscript, Uwe Lindemann and Pablo Alvarado for providing the sequence of *Octospora kilimanjarensis* and Sandra Bernhardt for a photo of apothecia (Fig. 2D). Zuzana Sochorová was supported by IGA UP PrF-2023–001 and Michal Sochor by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0423.

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Manuscript accepted: 2 May 2023.

Communicated by: Frank Müller

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4.2.4 *Bryorutstroemia* (Rutstroemiaceae, Helotiales), a new genus to accommodate the neglected sclerotiniaceous bryoparasitic discomycete *Helotium fulvum* Boud.

life

Article

Bryorutstroemia (*Rutstroemiaceae*, *Helotiales*), a New Genus to Accommodate the Neglected Sclerotiniaceous Bryoparasitic Discomycete *Helotium fulvum*

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Abstract: The new genus *Bryorutstroemia* is established for the red-brown, stipitate, bryoparasitic discomycete *Helotium fulvum* Boud. Combined phylogenetic analysis of ITS and LSU rDNA and *EF1α* revealed that *Bryorutstroemia fulva* belongs to the sclerotiniaceous clade, which comprises the paraphyletic families *Rutstroemiaceae* and *Sclerotiniaceae*. *Bryorutstroemia* formed with *Clarireedia* a supported clade (*Rutstroemiaceae* s.l.), though with high distance. *Bryorutstroemia* closely resembles other *Rutstroemiaceae* in having uninucleate ascospores with high lipid content and an ectal excipulum of textura porrecta, but is unique because of its bryophilous lifestyle and is extraordinary with its thick-walled inamyloid ascus apex. Although *B. fulva* was described in 1897, very few records came to our notice. The present study summarizes the known distribution of the species, including 25 personal collections from the years 2001–2022. *Bryorutstroemia fulva* was most often encountered on *Dicranella heteromalla*, and rarely on other members of *Dicranales* or *Grimmiales*, while inducing necrobiosis of the leaves. A detailed description based on mainly fresh apothecia is provided together with a rich photographic documentation. Six new combinations are proposed based on our phylogenetic results and unpublished personal morphological studies: *Clarireedia asphodeli*, *C. calopus*, *C. gladioli*, *C. henningsiana*, *C. maritima*, and *C. narcissi*.

Keywords: *Bucklandiella*; *Clarireedia*; *Dicranella*; *Dicranum*; elongation factor-1alpha (*EF1α*); fungal diversity; nuITS+LSU rDNA; sandstone; vital taxonomy

Citation: Baral, H.-O.; Sochorová, Z.; Sochor, M. *Bryorutstroemia* (*Rutstroemiaceae*, *Helotiales*), a New Genus to Accommodate the Neglected Sclerotiniaceous Bryoparasitic Discomycete *Helotium fulvum*. *Life* **2023**, *13*, 1041. https://doi.org/10.3390/life13041041

Academic Editor: Ang Ren

Received: 30 January 2023
Revised: 21 March 2023
Accepted: 7 April 2023
Published: 18 April 2023

1. Introduction

Helotium fulvum was described by Boudier in 1897 based on his collection from Forêt de Carnelle north of Paris (France) [1]. The apothecia grew on sandy soil among *Phascum*, *Dicranella*, and other small mosses. They consistently arose from leaf axils (leaf bases) at the tip of stems of what was obviously a *Dicranella*. Reliable reports of the species in the literature are very sparse up to now and include collections from Great Britain [2] and Belgium [3]. During an ascomycete foray in Luxembourg in April 2001 [4], the first author collected and documented a single apothecium on *Dicranella*, which remained undetermined for many years. Because of its very short stipe, large, ellipsoid, multiguttulate ascospores, and large, inamyloid asci, an affinity with the genus *Mniaecia* Boud. was considered, despite its reddish-brown colour. Further fresh collections from Sweden (Småland), France (Bretagne), Germany (Sachsen), Czech Republic (regions of Ústí nad Labem, Liberec, Hradec Králové, Vysočina, Olomouc, Moravian-Silesian and Zlín), Poland (Silesia), and Hungary (near Budapest) all deviated from our first record in possessing comparatively long stipes. Apart from the brown, stipitate apothecia, a ± gelatinized ectal excipulum of textura (prismatica-)porrecta with ochre-brown, partially encrusted cortical

cells pointed to a relationship with the genus *Rutstroemia* P. Karst. The aim of our work was to clarify the phylogenetic position of *Helotium fulvum*, summarize its known distribution, and provide a detailed description based on recent collections.

2. Materials and Methods

2.1. Sampling and Observation

Macro- and microscopic characters were studied from fresh apothecia, predominantly from living (*) elements following the standards of vital taxonomy [5], in comparison also with samples from dead (†) elements. Apothecia were rehydrated after different intervals for testing their drought tolerance. Tap water (H_2O) was used as a mounting medium. Colour reactions were tested with IKI and KOH. The latter was also applied for testing pigment solubility, resistance of oil drops (LBs), and iodine reactions under KOH-pre-treatment. CX21 (Olympus, Czech Group, Prague, Czech Republic) and Zeiss Standard 14 microscopes, with magnifications of 40×, 100×, 400×, and 1000×, were used in our study. Measurements were conducted in tap water, either directly or on photographs using the Piximètre 5.10 software [6] or by calculating from scales prepared using a Zeiss calibration slide.

Collections are deposited in the herbaria of PRA (Z. Palice), PRM (Z. Sochorová), and UPS (R. Isaksson), and in the private herbaria of H.O. Baral (H.B.), M. Lüderitz (M.L.), C. Németh (C.N.), and J.P. Priou (J.P.P.).

The following abbreviations are used: H_2O = tap water, KOH = potassium hydroxide (~5%), LBs = lipid bodies (oil drops), VBs = refractive KOH-soluble vacuolar bodies, IKI = ~1% iodine (I_2) in 3% KI (potassium iodide), MLZ = Melzer's reagent, OCI = lipid content, PVA = polyvinyl acetate, idem = the same, ibid. = from the same geographical region, l.c. = reference cited, doc. vid. = documentation seen, non vid. = no documentation seen. Values in {} indicate the number of collections, thereby numbers after a slash refer to uncertain hosts.

2.2. DNA Extraction, PCR Amplification and Sequencing

DNA was extracted from dried apothecia using the CTAB method described by Doyle et Doyle [7]. Apothecia were homogenised using a pestle and incubated in 300 µL of extraction buffer at 65 °C for one hour. The extract was subsequently purified in chloroform-isoamyl alcohol mixture (24:1), precipitated by isopropanol, washed in 70% ethanol, dried and finally dissolved in water and incubated with RNase for 30 min at 37 °C. DNA quality was checked using agarose gel electrophoresis. Three genomic regions including the internal transcribed spacers (ITS = ITS1-5.8S-ITS2 region) and the 28S subunit (LSU) of ribosomal DNA (rDNA) plus the translation elongation factor-1alpha (*EF1α*) were amplified and sequenced with the primers ITS1F [8] / ITS4 [9], LR0R/LR6 [10], and EF1-983F/EF1-1567R [11], respectively. PCR was performed with EliZyme FAST Taq MIX Red (Elisabeth Pharmacon, Brno, Czech Republic) following a standard protocol with 37 cycles and annealing temperature of 54 °C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced from both directions using the same primer pairs by the Sanger method at Macrogen Europe, Amsterdam, the Netherlands.

2.3. Phylogenetic Analysis

Specimens used in the phylogenetic analysis are listed in Table 1. Newly generated sequences were edited using the Geneious software (ver. 7.1.7., Biomatters, Auckland, New Zealand). Alignment was achieved with MAFFT plugin and subsequently manually checked. Phylogeny was reconstructed using the Maximum Likelihood (ML) method with the substitution model GTR+G+I tested by bootstrapping, using 1000 pseudoreplicates in MEGA (ver. 6.06) [12]. Bayesian phylogeny inference (BI) was computed in MrBayes (ver. 3.2.4) [13] using the GTR+I+G (for ITS), SYM+I+G (LSU) and GTR+G (*EF1α*) substitution model, as determined by AICc in PartitionFinder 2.1.1 [14]. Besides the combined trees,

single gene trees were calculated. The analysis was run for 15 million generations in four independent runs, sampling every 1000th generation and excluding the first 50% of generations as burn-in, and temperature parameter was set to 0.05 for better chain mixing. The Basic Local Alignment Search Tool (BLAST) [15] was used for searching similar sequences in publicly available sequence databases [16].

Table 1. Sequences included in phylogenetic analysis (T = type). Newly generated sequences in bold. - = gene region missing, ? = data missing, # = as *Rutstroemia cuniculi*.

Species	Collection Number	Country	Host	ITS	LSU	*EF1α*
Bicornispora seditiosa	AH 44702 T	Spain	*Acer monspessulanum*	KF499362	KF499362	MW001933
Bryorutstroemia fulva	**C.N. 103**	**Hungary**	***Dicranum scoparium***	**OP035831**	**OP035831**	**OP058106**
Bryorutstroemia fulva	**Z.S. 2/2021**	**Czech Republic**	***Dicranella heteromalla***	**OP035812**	**-**	**-**
Bryorutstroemia fulva	**Z.S. 7/2021**	**Czech Republic**	***Dicranella heteromalla***	**OP035830**	**OP035830**	**OP058105**
Bryorutstroemia fulva	**Z.S. 9/2021**	**Czech Republic**	***Dicranella heteromalla***	**OP035829**	**OP035829**	**OP058104**
Bryorutstroemia fulva	**Z.S. 19/2021**	**Czech Republic**	***Dicranella heteromalla***	**OP035828**	**OP035828**	**OP058103**
"Cenangium" acuum	KL 243	Germany	*Pinus sylvestris*	LT158439	KX090822	KX090674
Chlorociboria glauca	KL 238	France	*Salix* sp.	LT158438	KX090821	KX090673
Ciboria amentacea	**HR 98838**	**Czech Republic**	***Alnus* sp.**	**OP901951**	**OP897698**	**OP958788**
Ciboria americana	**HR 102055**	**Czech Republic**	**indet. gall**	**OP901952**	**OP897699**	**OP958784**
Ciboria betulae	1145.P	Norway	*Betula* sp.	Z81427	Z81403	-
Ciboria conformata	**HR B008890**	**Czech Republic**	***Alnus glutinosa***	**OP902277**	**OP897705**	**OP958790**
Ciboria coryli	**HR B008735**	**Czech Republic**	***Corylus avellana***	**OP902275**	**OP897703**	**OQ023970**
Ciboria viridifusca	**HR B006315**	**Czech Republic**	***Alnus glutinosa***	**OP901954**	**OP897702**	**OP958783**
Clarireedia asphodeli	F142282	Spain	*Asphodelus fistulosus*	KJ941085	KJ941065	-
Clarireedia bennettii	CBS 309.37	unknown	indet. *Poaceae*	MF964321	-	-
Clarireedia calopus	CBS 854.97	Netherlands	indet. *Poaceae*	KF545314	AB926155	-
Clarireedia calopus #	CBS 465.73	Great Britain	rabbit dung	KF588375	MH878367	-
Clarireedia gladioli	CBS 265.28 T	unkown	*Gladiolus* sp.	MH855008	MH866477	-
Clarireedia henningsiana	**HR B013053**	**Czech Republic**	***Scirpus sylvaticus***	**OP901955**	**OP897706**	**OP958787**
Clarireedia homoeocarpa	CBS 310.37	Great Britain	*Festuca* sp.	MF964322	MH867420	-
Clarireedia maritima	H.B. 6860	Spain	*Ammophila arenaria*	KF588372	KJ941063	-
Clarireedia narcissi	CBS 339.33	Netherlands	*Narcissus* sp.	MH855451	MH866916	-
Clarireedia paspali	XC5	China	*Paspalum vaginatum*	MH392087	-	MH444193
Clarireedia sp.	BVV	USA	*Bromus tectorum*	MT850272	MG937748	NJPS01000062
Dumontinia tuberosa	TU109263	Estonia	*Anemone nemorosa*	LT158412	KX090843	KX090697
Encoelia furfuracea	KL 107	Estonia	*Corylus avellana*	LT158416	KX090798	KX090653
Hymenoscyphus scutula	G.M. 2014-12-25.2	Luxembourg	indet. herb	MK674606	MK674606	-
Lambertella corni-maris	CLX4075	USA	*Malus* sp.	KC958562	KC964858	-
Lambertella palmeri	AH 7655	Spain	*Quercus rotundifolia*	KF499365	KF499365	-
Lambertella pyrolae	TNS-F 40132 T	Japan	*Pyrola incarnata*	AB926081	AB926164	-
Lambertella subrenispora	CBS 811.85	Japan	*Aster ageratoides*	MH861915	DQ470978	DQ471101
Lanzia allantospora	CBS 124334	New Zealand	*Agathis australis*	AB926099	AB926154	-
Martininia panamaensis	CBS 207.47	Panama	indet. log	MH856219	MH867749	-
Monilinia fructicola	2014/FC48	Hungary	*Prunus persica*	LT615175	LT615175	-
Monilinia oxycocci	1087.P	Norway	*Vaccinium oxycoccos*	Z73789	Z73754	-
Piceomphale bulgarioides	**HR B004019**	**Czech Republic**	***Picea abies***	**OP901953**	**OP897701**	**OP958786**
Pycnopeziza sejournei	KL 267	France	*Hedera helix*	LT158443	KX090827	KX090679
Rutstroemia bolaris	1526.P	Norway	*Betula pubescens*	Z80894	Z81419	-
Rutstroemia elatina	**HR B000521**	**Czech Republic**	***Abies* sp.**	**OP902274**	**OP897700**	**OP958785**
Rutstroemia firma	KL 290	Estonia	indet. angiosperm	LT158448	KX090830	KX090682
Rutstroemia longipes	TNS: F-40097	Japan	*Daphniphyllum macropodum*	AB926073	AB926142	-
Rutstroemia luteovirescens	**HR B008840**	**Czech Republic**	***Acer platanoides***	**OP902276**	**OP897704**	**OP958789**
Rutstroemia tiliacea	KL 160	Germany	*Tilia* sp.	LT158423	KX090808	KX090661
Schroeteria decaisneana	A.U. 2273	Germany	*Veronica hederifolia*	MZ048345	MZ048345	-
Schroeteria delastrina	V.K. P1652-26	Germany	*Veronica arvensis*	MW915645	MW915645	-
Sclerencoelia fraxinicola	KL 156	Germany	*Fraxinus excelsior*	LT158420	KX090805	KX090659
Scleromitrula shiraiana	Hirayama062001	?	?	AY789408	AY789407	-
Sclerotinia sclerotiorum	1980 UF-70	USA	bean pods	CP017820	CP017820	-
Torrendiella setulata	H.B. 9775	Canada	*Acer spicatum*	KF588367	KJ941052	-

Maximum Likelihood (ML) phylogenetic analysis was performed in MEGA6 with the settings 'use all sites, nearest-neighbour-interchange, weak branch swap filter'. Distance

analyses were performed with MEGA6 using the settings 'p-distances, transitions + transversions, uniform rates, pairwise deletion'.

3. Results

3.1. Taxonomy

Bryorutstroemia Sochorová and Baral, gen. nov.—MycoBank MB 847031

Diagnosis: Differs from *Rutstroemia* and *Clarireedia* by its inamyloid asci, bryoparasitic habitat, and genetic profile.

Etymology: named after the bryicolous habitat and the similarity with the genus *Rutstroemia*.

Type: *Bryorutstroemia fulva* (Boud.) Sochorová, Baral and Priou

Bryorutstroemia fulva (Boud.) Sochorová, Baral and Priou, comb. nov.—MycoBank MB 847033

Figures 1–8

Basionym: *Helotium fulvum* Boud., Bull. Soc. mycol. Fr. 13(1): 16 (1897)

≡ *Hymenoscyphus fulvus* (Boud.) Hengstm., in Arnolds et al., Overzicht paddest. Nederl.: 654 (1985)

Etymology: after the red-brown apothecial colour caused by brown wall deposits on paraphyses and cortical hyphae of ectal excipulum.

Holotype: France, Île-de-France, Val d'Oise, Paris, Forêt de Carnelle, on *Dicranella* cf. *heteromalla*, II.1896, É. Boudier (doc. vid.).

Apothecia (0.4–)0.5–1(–1.5) mm diam. when fresh {16}, receptacle 0.25–0.33 mm thick at lower flanks, 0.2–0.26 mm thick at margin {3}, singly or rarely in fascicles of two to four fused at the base, non-gelatinous; disc rounded in upper view, flat, eventually slightly convex, light to mostly bright to deep reddish- to purplish-brown (carmine-brown), also ochre-brown to dark brown, non-translucent, margin distinct, not protruding, even, exterior concolorous, flesh pale brown; mostly with a distinct **stipe** (0.1–)0.5–1.5(–1.8) × (0.12–)0.15–0.3(–0.55) mm {12}, cylindrical or widened above or sometimes below, pale to deep red-brown, basal (1/10–)1/4–1/3 of stipe blackish-brown {15}, base inserted in leaf axils at tip of stem, seemingly superficial. **Asci** *(150–)170–220(–233) × (17–)18–24(–27) µm {8}, †(100–)110–155(–165) × (12–)13–17(–18) µm {4}, cylindric-clavate, eight-spored, spores *obliquely biseriate, pars sporifera *(50–)60–70(–87) µm long if all eight ascospores fully developed, living mature asci protruding 20–50 µm beyond paraphyses; **apex** */†obtuse or slightly to strongly conical, dome immature †(4–)5–7(–10) µm thick (*2–2.5 µm), mature †3–7 µm (*1–1.2 µm) {9}, IKI– {22}, MLZ– {5}, when KOH-pretreated IKI–/MLZ– {1}, dome hemispherically protruding into ascoplasm, without apical chamber, lateral ascus wall †0.5–1 µm thick, subapically †1.2–1.5 µm; **base** with medium to long stalk, arising from simple septa {21} with basal downward-oriented protuberance {11}, sometimes bifurcate by one branch forming the protuberance {4}. **Ascospores** *(14–)16–25(–27.5) × (6–)7–10(–11) µm {13} [*Q = 2.27–2.76–4.2 (n = 50), *Me = 23.1 × 8.4 µm, Z.S. 2/2021; *Q = 2.16–2.57 (n = 20), C.N. 103], †(14.5–)16–22(–24.7) × (5.5–)6–8(–9) µm {3} [†Q = 2.1–2.6–3(–3.8) (n = 50), †Me = 18.6 × 7.2 µm, Z.S. 2/2021], ellipsoid, also cylindric-ellipsoid or ellipsoid- to fusoid-clavate, homopolar, straight, ends obtuse, smooth; containing numerous **LBs** of (0.5–)0.8–2(–2.5) µm diam. (multiguttulate) {22}, LBs in young spores much smaller and more numerous, OCI 4.5–5 {20}, leaving an area occupied by the single nucleus, when freshly ejected sometimes surrounded by a sheath that separates from the spore wall {7} (Figure 7: 1e,3,6); overmature spores one-septate {12}, hyaline, rarely germinating with one hypha at the pole or more laterally. **Paraphyses** cylindrical-filiform throughout, sometimes slightly clavate above, rarely slightly capitate, spathulate, or narrowly obtusely-sublanceolate, straight to slightly flexuous, sometimes curved under a wide arc, hyaline, terminal cell *21–53 {4} × (1.7–)2–3(–3.4) µm {6}, †(14–)19–45(–50) {3} × (1.5–)2–2.5(–3) µm {4}, without VBs {15}, sometimes with groups of LBs {1}, embedded above in (very) pale fox-brownish, smooth, gel-like exudate, lower cells *16–30 × 1.7–3.1 µm {2}, †1.8–2 µm wide {1}; sparsely to frequently branched in middle part. **Subhymenium** hyaline, *17–33 µm thick, non-gelatinized, cells angular, subglobose or irregular, *5–11 × 3–8 µm. **Medullary excipu-**

lum with pale brass-ochre to brownish intercellular exudate, non-gelatinized, *90–150 µm thick in centre, *60–120 µm at lower flanks, in receptacle of dense textura intricata with tendency to an upward orientation, cells *8–24 × 2.5–5.5(–11) µm {2}, thin-walled, sharply delimited from ectal excipulum by a thin, parallel, pale brown layer of t. porrecta; in stipe of vertically oriented hyaline to pale brown t. porrecta, cells cylindrical, *(13–)20–60(–75) × (3.5–)5–6(–11) µm {2}. **Ectal excipulum in receptacle** of hyaline to pale ochre-brown, thin-walled, *not or slightly (†medium to strongly) gelatinized, textura (prismatica-)porrecta from base to margin, oriented at a (0–)10–30(–50)° angle to the surface (often very irregularly, Figure 5: 3b), *(30–)40–55 µm thick at lower flanks, cells *(10–)15–33(–48) × 3.5–7(–9) µm {2}, †19–28 × 3.5–6 µm {1}; *20–40 µm thick near margin, smaller-celled, bright reddish-brown, marginal cortical cells *12–16 × 3–3.5 µm {1}, ± flexuous, forming hair-like elements; cortical cells of similar size, with pale to bright ochre- to red-brown, thin, smooth {2} or granular to ridge-like encrustation {6} (Figure 6: 1a,2a), in surface view straight to sometimes ± undulating, often with short, scattered lateral protrusions, *5–14 × 3.5–5 µm {3}; **in stipe** of not to slightly gelified t. porrecta oriented parallel to the surface, formed by cylindrical, often anastomosing or branching, thin- to thick-walled (*0.2–0.7 µm) cells *12–40 × 2.7–7(–9.5) µm {1}; cortical cells as on receptacle. **Tissues** without crystals, without IKI reaction, excipular pigment in KOH not changing colour, not dissolved {3}. **Anchoring hyphae** sparse, brown, forming chains of †8–12 × 5–6.5 µm large cells, walls †~0.5–0.8 µm thick {1}. **Anamorph** unknown.

Habitat: on leaf axils of living or mainly dead individuals of *Bucklandiella heterosticha* {1}, *Dicranella cerviculata* {1}, *D. heteromalla* {35/2}, *Dicranella* sp. {1}, *Dicranum scoparium* {2}, causing yellowish discolouration of the host, mosses growing on rocks or equally often on sandy to loamy or humous soil. **Associated** (± remotely): *Mniaecia* cf. *gemmata* {3}, *M. jungermanniae* {4}. **Drought tolerance**: only a few ascospores survived when dry apothecia were examined after 10 days up to 2 $^{1}/_{3}$ months. **Altitude**: 10–530(–835) m above sea level. **Phenology**: X–VII(VIII–IX) (throughout the year, especially in winter and spring). **Geology**: Bretagne: acidic quartzite, sandstone, argillaceous siltite, shale-like schist (Ordovician, Brioverian); Luxembourg: Lower Lias (sandstone); Czechia and Poland: acidic sandstone, alluvial sediments, gneiss, migmatite, granulite.

Specimens included: **Sweden**: **Småland**, **Jönköpings län**, 4 km WNW of Sävsjö, 3.5 km SSW of Bringetofta, 0.5 km SSE of Rickelstorp, 245 m, *Bucklandiella heterosticha* on silicate stonewall, 13.XII.2020, R. Isaksson (UPS F-990878).—1.3 km SSE of Rickelstorp, 235 m, on *Dicranum scoparium* on silicate stonewall, 29.XII.2020, R. Isaksson (doc. vid.).—**Great Britain**: **Scotland**, **East Lothian**, SSE of Haddington, Gifford, ~120 m, on *Dicranella heteromalla*, 18.X.1964, D.M. Henderson (E, non vid.).—idem, 25.X.1965.—idem, X.1968.—idem, 10.X.1969.—**Southwest England**, **West Gloucestershire**, 30 km N of Bristol, Rodmore Grove, 140 m, host not stated, 1.IX.1991, A. Yelland (non vid.) [17].—**Netherlands**: **Groningen**, 2.5 km S of Vlagtwedde, 1 km NW of Weende, Liefstinghsbroek, 10 m, on *D. heteromalla*, 2.II.2022, J. Boers (unpreserved, doc. vid.).—**Belgium**: **Vlaanderen**, **Antwerpen**, 11.5 km NE of Antwerpen, 4 km NE of Schoten, La Garenne, 12 m, on *D. cerviculata*, 24.II.1992, J. Slembrouck and H. De Meulder (H.B. 4632).—**France**: **Bretagne**, **Côtes-d'Armor**, 4.5 km WNW of Mur-de-Bretagne/Guerléda, 1 km SW of Caurel, Lac de Guerlédan, 133 m, on *D. heteromalla*, 7.III.2005, J.P. Priou (J.P.P. 15051).—**Ille-et-Vilaine**, 4.5 km E of La Gacilly, 1.2 km SE of Sixt-sur-Aff, Dessous Le Guerche, D255, 66 m, on *D. heteromalla*, 3.III.2006, J.P. Priou (J.P.P. 26054, H.B. 8083).—**Morbihan**, 3 km SW of La Gacilly, 2.8 km NW of Glénac, route de La Forêt Neuve, 80 m, on *D. heteromalla*, 6.IV.2004, J.P. Priou (J.P.P. 24120).—3.7 km S of Montfort-sur-Meu, 2 km WSW of Talensac, 110 m, on *D. heteromalla*, 19.III.2021, J.P. Priou (J.P.P. 2021050, non vid.)—**Île-de-France**, **Val d'Oise**, ~29 km N of Paris, Forêt de Carnelle, ~200 m, on *D.* cf. *heteromalla*, II.1896, É. Boudier (holotype, doc. vid.).—**Luxembourg**: **Gutland**, Petite Suisse, 11.5 km WNW of Echternach, 2.2 km W of Beaufort, Esselbur, Elteschmuer S of Tinnes, 405 m, on *D.* cf. *heteromalla*, 25.IV.2001, H.O. Baral (H.B. 6917 [PVA-slide]).—**Germany**: **Niedersachsen**, 5 km ESE of Ratzeburg, ~2.8 km WSW of Mustin, SW of Garrensee, NW of Garrenseeholz, on *D. heteromalla*, 9.III.1995, M. Lüderitz (M.L., non

vid.) [18].—**Sachsen**, 8.5 km SSW of Zittau, 0.8 km S of Kurort Oybin, 465 m, on *D. heteromalla* on a sandstone rock, 15.V.2021, Z. Sochorová (ex Z.S. 44/2021, PRM 956027).—**Bayern**, **Oberbayern**, near Ingolstadt, ~400 m, on *D. heteromalla*, 31.VIII.1979, J. Poelt (Plantae Graecensis 255, PDD 60714, non vid.)— **Poland: Lower Silesian Voivodeship**, 16 km SE of Wałbrzych, 1.6 km SE of Walim, Owl Mountains landscape park, 775 m, on *D. heteromalla* on soil, 17.IV.2022, Z. Sochorová (ex Z.S. 1/2022, PRM 957650).—**Czech Republic**: **Ústí nad Labem region**, **Děčín district**, 5 km N of Jetřichovice, České Švýcarsko National Park, Křinice valley, ENE of Jankův kopec, 348 m, on *D. heteromalla* on soil, 10.XI. 2021, Z. Palice, I. Marková and P. Uhlík (ex Z.P. 32329, PRA, vid.).—**Liberec region, Česká Lípa district**, 6.5 km NNE of Česká Lípa, 1.3 km NW of Svojkov, 1 km SSW of Sloup v Čechách, group of rocks above the road no. 268, 350 m, on *D. heteromalla* on a sandstone rock, 1.I.2021, Z. Sochorová (ex Z.S. 2/2021, PRM 956016, sq.: ITS OP035812).—idem, 28.II.2021 (ex Z.S. 11/2021, PRM 956020).—10 km ENE Mimoň, 2.8 km SE of Hamr na Jezeře, Divadlo Nature Monument, 375 m, on *D. heteromalla* on a sandstone rock, 27.II.2021, Z. Sochorová (ex Z.S. 8/2021, PRM 956018).—3.2 km S of Hamr na Jezeře, 2.7 km NE Svébořice, Stohánek Nature Monument, 350 m, on *D. heteromalla* on a sandstone rock, 27.II.2021, Z. Sochorová (ex Z.S. 9/2021, PRM 956019, sq.: ITS + LSU OP035829, *EF1α* OP058104).—**Liberec district**, 21 km WNW of Liberec, 4 km N of Jablonné v Podještědí, 1.2 km SW of Petrovice, 410 m, on *D. heteromalla* on soil, 4.III.2021, Z. Sochorová (ex Z.S. 17/2021, PRM 956023).—2.1 km NE of Jablonné v Podještědí, 345 m, on *D. heteromalla*, 4.VII.2021, Z. Sochorová (ex Z.S. 61/2021, PRM 956032).—idem, 15.XI.2021 (ex Z.S. 153/2021, PRM 956457).—1.8 km NE of Jablonné v Podještědí, at St. Zdislava's spring, 335 m, on *D. heteromalla*, 4.VII.2021, Z. Sochorová (ex Z.S. 62/2021, PRM 956033).—2 km ENE of Jablonné v Podještědí, 1 km S Lvová, 365 m, on *D. heteromalla* on a sandstone rock, 1.III.2021, Z. Sochorová (ex Z.S. 15/2021, PRM 956021).—4.5 km E of Jablonné v Podještědí, 0.5 km N of Janovice v Podještědí, 260 m NNW of cemetery, 370 m, on *D. heteromalla* on a sandstone rock, 3.III.2021, Z. Sochorová (ex Z.S. 16/2021, PRM 956022).—9 km SW of Liberec, 0.8 km SE of Rozstání pod Ještědem, Horka forest park, 460 m, on *D. heteromalla*, 3.VII.2021, Z. Sochorová (ex Z.S. 60/2021, PRM 956031).—3 km SW of Česká Lípa, Peklo National Nature Monument, 270 m, on *D. heteromalla*, 16.XI.2021, Z. Sochorová (ex Z.S. 158/2021, PRM 956458).—**Jablonec nad Nisou district**, 2.3 km WNW of Koberovy, 1 km NW of Besedice, 445 m, on *D. heteromalla* on soil, on sandstone bedrock, 26.II.2021, Z. Sochorová (ex Z.S. 7/2021, PRM 956017, sq.: ITS + LSU OP035830, *EF1α* OP058105).—**Hradec Králové region, Náchod district**, Broumovské stěny National Nature Reserve, 6 km SSW of Broumov, 1.6 km ENE of Slavný, 650 m, on *D. heteromalla* on soil on sandstone bedrock, 18.IV.2022, Z. Sochorová (ex Z.S. 2/2022, PRM 957651).—idem, 0.9 km ENE of Slavný, Zaječí rokle, 605 m (ex Z.S. 4/2022, PRM 957652).—**Vysočina region**, **Havlíčkův Brod district**, Údolí Doubravy Nature Reserve, 3.5 km ESE of Chotěboř, 820 m WNW of Bílek railway station, 545 m, on *D. heteromalla* on soil over migmatite to orthogneiss, 21.V.2021, Z. Sochorová (ex Z.S. 48/2021, PRM 956029).—ibid., 600 m WNW of Bílek railway station, 545 m, on *D. heteromalla* on soil, 21.V.2021, Z. Sochorová (ex Z.S. 47/2021, PRM 956028).—**Olomouc region**, **Olomouc district**, Dolany u Olomouce, W of Nové Sady, 305 m, on *D. heteromalla* on soil, 28.III.2021, Z. Sochorová (ex Z.S. 18/2021, PRM 956024).—ibid., S of Nové Sady, 340 m, on *D. heteromalla* on soil-stony bedrock, 28.III.2021, Z. Sochorová (ex Z.S. 19/2021, PRM 956025, sq.: ITS + LSU OP035828, *EF1α* OP058103).—**Šumperk district**, 4.6 km NW of Staré Město, 1 km NNW of the church in Stříbrnice, 835 m, on *D. heteromalla* on soil, 30.V.2021, Z. Sochorová (ex Z.S. 58/2021, PRM 956030).—**Moravian-Silesian region**, **Opava district**, 2 km NW of Těškovice, 360 m, on *D. heteromalla* on soil, 4.IV.2021, Z. Sochorová (ex Z.S. 23/2021, PRM 956026).—**Zlín region**, **Zlín district**, 5 km SE of Bystřice, 2 km SSE of Hostýn, 690 m, on *D. heteromalla* on soil, 28.X.2022, Z. Sochorová (ex Z.S. 136/2022, PRM 958329).—**Hungary**: **Pest county**, **Budakeszi district**, 12 km WNW of Budapest, 3 km W of Budakeszi, 320 m, on *Dicranum scoparium* on soil, 8.XII.2020, C. Németh (C.N. 103, sq.: ITS + LSU OP035831, *EF1α* OP058106).

3.2. Phylogeny

ITS sequences were obtained from five collections of *B. fulva*, while LSU and *EF1α* were obtained from four (Table 1). The S1506-intron is absent in all of them, according to the used ITS1F primer. The five sequences are fully identical in the overlapping parts. In BLASTn searches in GenBank, *B. fulva* had the highest ITS similarity to members of the *Clarireedia* clade: *Clarireedia narcissi* (90%), *C. monteithiana* and *C. jacksonii* (89.5%), *C. asphodeli*, *C. calopus*, *C. henningsiana*, *C. homoeocarpa*, *C. maritima*, and *C. paspali* (88–89%). Also in the LSU D1–D2 domain the highest similarity (95%) was to members of *Clarireedia* but also to *Piceomphale*, followed by other rutstroemiaceous taxa, including *Rutstroemia firma* (92.5–93.5%).

Helotium fulvum was only once recombined into another genus when Hengstmengel [19] suggested a relationship with the genus *Hymenoscyphus.* Our phylogenetic analysis of nuITS+LSU rDNA + *EF1α* (Figure 9 and Figure S4), in which we used *Hymenoscyphus scutula* (Pers.) W. Phillips (isolate G.M. 2014-12-25.2, ITS + LSU: MK674606) as outgroup, indicated a high distance between *B. fulva* and that species. Instead, *B. fulva* nested in the strongly supported sclerotiniaceous lineage as circumscribed by Baral [20] p. 173, a group which currently includes two families, *Rutstroemiaceae* and *Sclerotiniaceae*. Two further families in our dataset, *Cenangiaceae* and *Chlorociboriaceae*, clustered outside the sclerotiniaceous lineage.

In our Bayesian analysis, the paraphyletic family *Rutstroemiaceae* appears in three different clades (Figures 9 and 10). One clade (*Rutstroemiaceae* s.str.) comprises species growing on wood and bark but also on the leaves of trees; it includes two strongly supported subclades, one containing the type species of *Rutstroemia*, *R. firma*, and four other *Rutstroemia* spp., but also *Torrendiella setulata,* the other containing *Lambertella subrenispora* and *Lanzia allantospora*.

A different, strongly supported clade comprises species growing on monocots and also on dung. It represents the recently established genus *Clarireedia* L.A. Beirn et al. [21], with the type species *C. homoeocarpa* (F.T. Benn.) L.A. Beirn et al. (≡ *Sclerotinia homoeocarpa* F.T. Benn.), and includes species currently assigned to *Rutstroemia* but also *Ciboria*, *Sclerotinia*, and *Stromatinia*. Within *Clarireedia*, *C. paspali* clustered in our ITS+LSU analysis closest to *B. fulva* despite its comparatively high ITS distance (Figure 10), perhaps because the specimen lacks LSU, whereas in our ITS+LSU+*EF1α* analysis it clustered supported with other *Clarireedia* spp. (Figure 9).

The following new combinations are proposed to harmonize the nomenclature of species on monocots which cluster in the supported *Clarireedia* clade. The listed taxonomic synonyms are to be taken as tentative and require type studies for clarification. *C. henningsiana* (= *R. paludosa*) is here understood as a species on *Cyperaceae* and *Juncaceae* characterized by simple-septate asci, whereas *C. calopus* (= *C. bennettii*) and *C. maritima* represent species on *Poaceae* characterized by asci arising from croziers, *C. maritima* also by asci with inamyloid, moderately thick-walled apex (pers. obs.). We tentatively regarded *R. cuniculi* as a synonym of *C. calopus* because available ITS sequences in GenBank differed from those of *C. calopus* by only one nucleotide.

Clarireedia asphodeli (Duvernoy and Maire) Baral and Sochorová, comb. nov.—MycoBank MB 847034

Basionym: *Ciboria asphodeli* Duvernoy and Maire, in Maire, Bull. trimest. Soc. mycol. Fr. 44: 54 (1928)

≡ *Rutstroemia asphodeli* (Duvernoy and Maire) R. Galán and Matočec, in Galán et al., Mycologia 107(4): 799 (2015)

Clarireedia gladioli (Drayton) Baral and Sochorová, comb. nov.—MycoBank MB 847035

Basionym: *Sclerotinia gladioli* Drayton, Phytopathology 24: 397 (1934)

≡ *Stromatinia gladioli* (Drayton) Whetzel, Mycologia 37(6): 674 (1945)

Clarireedia henningsiana (Plöttn.) Baral and Sochorová, comb. nov.—MycoBank MB 847036

Basionym: *Ciboria henningsiana* Plöttn., in Maire, Verh. bot. Ver. Prov. Brandenb. 41: X (1899)

= *Rutstroemia paludosa* (E.K. Cash and R.W. Davidson) J.W. Groves and M.E. Elliott, Can. J. Bot. 39: 225 (1961)

= *Ciboria blanda* Svrček, Česká Mykol. 12(4): 225 (1958)

Clarireedia maritima (Roberge ex Desm.) Baral and Sochorová, comb. nov.—MycoBank MB 847037

Basionym: *Peziza maritima* Roberge ex Desm., Ann. Sci. Nat., Bot., sér. 3 3: 366 (1845)

≡ *Rutstroemia maritima* (Roberge ex Desm.) Dennis, Persoonia 3(1): 52 (1964)

Clarireedia narcissi (Drayton and J.W. Groves) Baral and Sochorová, comb. nov.—MycoBank MB 847038

Basionym: *Stromatinia narcissi* Drayton and J.W. Groves, Mycologia 44(1): 126 (1952)

Clarireedia calopus (Fr.) Baral and Sochorová, comb. nov.—MycoBank MB 847039

Basionym: *Peziza calopus* Fr., Observ. mycol. (Havniae) 2: 307 (1818)

≡ *Rutstroemia calopus* (Fr.) Rehm, Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.3(lief. 39): 768 (1893) [1896]

= *Clarireedia bennettii* C. Salgado, L.A. Beirn, B.B. Clarke and J.A. Crouch, in Salgado-Salazar et al., Fungal Biology 122(8): 769 (2018)

= *Rutstroemia cuniculi* (Boud.) M.E. Elliott, Can. J. Bot. 45(4): 521 (1967)

A third clade is formed by the type species of *Lambertella*, *L. corni-maris*, and two more *Lambertella* spp., but also includes *Bicornispora seditiosa*, and with less support *Rutstroemia longipes* and *Martininia panamaensis*. A further, strongly supported clade represents the family *Sclerotiniaceae*, which includes in the present analysis members of *Ciboria*, *Dumontinia*, *Monilinia*, *Pycnopeziza*, *Schroeteria*, *Sclerencoelia*, and *Sclerotinia*, with partly high distances among the species.

Four species of the sclerotiniaceous lineage clustered outside the four above-mentioned clades (Figures 9 and 10): *Bryorutstroemia fulva* formed with *Clarireedia* a strongly supported clade, though with high distance. *Scleromitrula shiraiana* is morphologically similar to *Ciboria* but it clustered unsupported in Figure 9 but formed a moderately supported sister clade to *Rutstroemia* s.str. in Figure 10. As in other published analyses [22], *Piceomphale bulgarioiodes* clustered with "*Cenangium*" *acuum* distant from all other sclerotiniaceous taxa, despite its morphological similarity with *Ciboria* and an ascus structure of the *Sclerotinia*-type. The two species form the "*Piceomphale*-clade", which is difficult to assign to a family, but may be better recognized in the sclerotiniaceous lineage than in *Cenangiaceae* to which *Encoelia furfuracea* belongs [22].

A phylogenetic tree generated with MEGA6 (ML, GTR+G+I, 1000 replicates, Figure S4), based on the very same dataset as in Figure 9, gave a similar tree topology though with only weak support for *Rutstroemia* s.str. and moderate support for *Lambertella* s.str. Again, *B. fulva* clustered sister to *Clarireedia*, though with only moderate support and by forming with *C. paspali* an unsupported clade. Contrary to the Bayesian analysis, *Martininia panamaensis* clustered strongly supported with *Lambertella* in the ML ITS+LSU analysis of Baral et al. [23] but unresolved in Figure S4, and *Scleromitrula shiraiana* clustered unresolved in both Baral et al. [23] and in Figure S4.

4. Discussion

4.1. Morphological Remarks

Bryorutstroemia fulva is characterized by deep reddish-brown, stipitate or rarely subsessile apothecia, a gelatinized ectal excipulum of textura porrecta covered by ochre-brown cortical hyphae with short outgrowths, inamyloid asci arising from simple septa, and large, multiguttulate, ellipsoid ascospores. Especially the latter varied among the collections, particularly in width, some being predominantly narrowly ellipsoid, the others more broadly ellipsoid. The living paraphyses usually looked empty and colourless by lacking vacuolar bodies (VBs), but sometimes they contained groups of lipid bodies (LBs). The pale to bright

ochre-brown cortical hyphae of the receptacle and stipe often had an encrusted surface but were occasionally smooth.

In order to summarize the most important differences between *Bryorutstroemia* and related genera, the following key is provided. It needs to be taken as provisional, as the taxonomy of *Rutstroemiaceae* is still insufficiently solved and nomenclatural changes in the circumscription of the family and its members can be expected.

Provisional key to the recognized genera of *Rutstroemiaceae* s.l.

1. Asci (†) with prominent, inamyloid apical wall thickening; ascospores permanently hyaline; growing on bryophytes..... ***Bryorutstroemia***
1. Ascus apex (†) with amyloid apical ring of the *Sclerotinia*-type, rarely faintly amyloid or inamyloid, but then only moderately thick-walled; growing on phanerogams..... 2
2. On monocotyledons.....***Clarireedia***
2. On dicotyledons or gymnosperms..... 3
3. Apothecia externally with prominent, septate, thick-walled setae..... ***Torrendiella***
3. Apothecia without setae..... 4
4. Ascospores permanently hyaline.....***Rutstroemia*** (including *Dencoeliopsis*), ***Lanzia***
4. Ascospores turning brown with age, either within the living asci or when overmature..... ***Bicornispora***, ***Lambertella***, ***Martininia***

4.2. Phylogenetic Remarks

Based solely on cultural isolates, Salgado-Salazar et al. described three new species in the new genus *Clarireedia* in 2018 [21] and Hu et al. added a fourth species, *C. paspali* Jian Hu and Lamour in 2019 [24]. Because teleomorphs were absent in their samples, the authors overlooked close relationships of their *Clarireedia* spp. with old taxa recognized in *Rutstroemia*. For instance, their wide concept of *Clarireedia bennettii* C. Salgado et al. encompasses ITS sequences which fully match GenBank uploads under the names *R. calopus* (Fr.) Rehm, *R. henningsiana* (Plöttn.) Dennis, and *R. paludosa* (E.K. Cash and R.W. Davidson) J.W. Groves and M.E. Elliott, here classified as *Clarireedia calopus* and *C. henningsiana* (Figures 9 and 10).

The type clade of *Clarireedia homoeocarpa* is closely related to *R. maritima* (Roberge ex Desm.) Dennis and *R. asphodeli* (Duvernoy and Maire) R. Galán and Matočec, here classified as *Clarireedia maritima* and *C. asphodeli*, whereas the remaining three species (*Clarireedia jacksonii* C. Salgado et al., *C. monteithiana* C. Salgado et al., *C. paspali*) represent a distinct group of genotypes which includes strains that are misnamed as *Sclerotinia homoeocarpa* in GenBank (based on our ML analysis of ITS rDNA, not shown).

Delimitation of the families *Sclerotiniaceae* and *Rutstroemiaceae* within the sclerotiniaceous lineage is still not clear in all respects. In the morphology-based classification defined by ascospores with a low vs. high lipid content coupled with globose vs. prismatic excipular cells, respectively, both families are paraphyletic (Figures 9 and 10). Hereafter, *Sclerotinia*, *Martininia*, and *Piceomphale* share characters with the core clade of *Sclerotiniaceae*, while *Lambertella* and *Clarireedia* share characters with the core clade of *Rutstroemiaceae*. Additionally, *Bryorutstroemia* shares characters with *Rutstroemiaceae*, for which it could represent an ancestor on a phylogenetically old host, although the tree topology of Figure 9 suggests an evolution from mainly woody plants to monocots and mosses. The current concept that characterizes *Rutstroemiaceae* by a stroma and *Sclerotiniaceae* by sclerotia [25] largely coincides with the morphology-based concept, but both concepts include some problematic genera.

The difficulty of conducting phylogenetic analysis on sclerotiniaceous fungi based on rDNA data alone became obvious when trying to resolve the position of *Schroeteria* [23]. Multigene analyses probably better resolve phylogenetic affinities in this group. However, in a preliminary analysis of the *EF1α* gene with MEGA6 (TN+G, not shown), which comprised members of *Helotiales* (mainly sclerotiniaceous taxa), *Pezizales*, *Phacidiales*, *Rhytismatales*, *Dothideomycetes*, *Eurotiomycetes*, and *Sordariomycetes*, *B. fulva* clustered

with *Sordariomycetes*, though with a high distance. *B. fulva* formed a clade with *Clarireedia* only when non-helotialean sequences were excluded from the analysis (Figure S3). Despite this curious result, BLAST search (megablast) for *EF1α* (strain Z.S. 19/2021) yielded *Rutstroemia firma* as the second most similar species (85.3%, query cover 61%), with the highest similarity of 88% (query cover 51%) to *Spathularia* (*Rhytismatales*) and 83.5–83.6% (query cover 68%) to *Sordaria* (*Sordariomycetes*) and *Lasiobolidium* (*Pezizales*). BLASTn search, however, yielded *R. firma* on top with 84.4% similarity (80% query cover). The *EF1α* sequences obtained from *B. fulva* strongly deviate at various positions from any other group of *Ascomycota*, which impedes a reasonable conclusion about its phylogenetic relationships. *EF1α* sequences obtained from four collections of *B. fulva* in this study were about the same length of 500 nucleotides and fully identical (except for nine ambiguities in C.N. 103), thus confirming the reliability of the result.

4.3. Ecological Remarks

Bryorutstroemia fulva is a necrotrophic parasite, causing bleaching of the host tissues. These striking substrate discolourations help one to spot the apothecia in the field (Figure 3), similarly as in several other species of bryophilous *Helotiales*, such as *Belonioscyphella hypnorum* [26], *Bryoscyphus dicrani* (pers. obs.), *B. hyalotectus* [27], and *Roseodiscus subcarneus* [28]. In the present study, *B. fulva* has been collected on mosses of the family *Dicranaceae* (*Dicranales*), mostly *D. heteromalla*. Only a single collection from Sweden grew on a moss from a different family and order, *Bucklandiella heterosticha* (≡ *Racomitrium heterostichum*, *Grimmiaceae*, *Grimmiales*). Suitable localities are shaded surfaces of acidic bedrock, very often in planted spruce forests. In several collections *B. fulva* grew more or less remotely associated with *Mniaecia jungermanniae*, a common hepaticolous ascomycete with deep blue apothecia (observed in collections J.P.P. 24120, Z.S. 23/2021, 44/2021, 48/2021), and *M.* cf. *gemmata* with whitish apothecia (J.P.P. 15051, Z.S. 18/2021, 23/2021).

We have encountered *B. fulva* mainly during the colder season, i.e., from November to May, but three of our records were from July. Collections from August by J. Poelt [29], September by A. Yelland [17], and October by D.M. Henderson [29] also exist. Although only a few records have been published, *B. fulva* seems to be common in colline to montane regions with acidic bedrock, which was exemplified in the present study for Czechia (24 collections during 2021–2022). The presently known distribution (Figure 11) is certainly incomplete. However, as the most frequent host *D. heteromalla* prefers acidic pH and grows most often on acidic forest soil or less often on sandy soil or directly on silicate boulders [30], the fungus might be rarer in areas with neutral to basic soil.

In France (Bretagne), the Netherlands, Luxembourg, Germany, Poland (Silesia), and Czechia the host was always *Dicranella*, which mainly grew on acidic sandstone (Figure 2: 1a), but also on sandy or loamy soil over sandstone, slate (Ordovician shale), silt, orthogneiss, migmatite, or granulite, etc. Sometimes the moss grew on soil on an uprooted fallen tree. The vegetation was preferably an acidic pure coniferous forest (predominantly *Picea* but also *Pinus*), also mixed with *Betula* or *Fagus,* etc. In Divadlo, the main vegetation was a *Vaccinio myrtilli-Pinetum sylvestris*, in Stohánek (Figure 3: 1) a *Vaccinio vitis-idaeae-Quercetum* with *Pinus sylvestris* and *Quercus petraea*, less often *Q. robur*, with admixture of *Betula pendula*, *Sorbus aucuparia*, and *Frangula alnus*, but also *Dicrano-Pinion* with the dominant *P. sylvestris* admixed with *Quercus petraea*, *Betula pendula*, *Frangula alnus* or *Sorbus aria*. Collections were often from the margins of forest pathways and also in ditches at the edges of roads. At the French sites the host moss occurred in close association with *Diplophyllum albicans*, *Calypogeia*, and *Cephalozia,* etc. Especially when growing on rock, the plant community in which *Bryorutstroemia fulva* parasitizes *Dicranella* may be classified as *Dicranellion heteromallae* [31]. In Sweden *B. fulva* grew on *Bucklandiella* (Figure 8: 2a) or *Dicranum* covering silicate stonewalls, and at the Hungarian site it grew in cushions of *D. scoparium* occurring scattered on open soil in an acidophilous *Quercus petraea* forest (Figure 8: 1a).

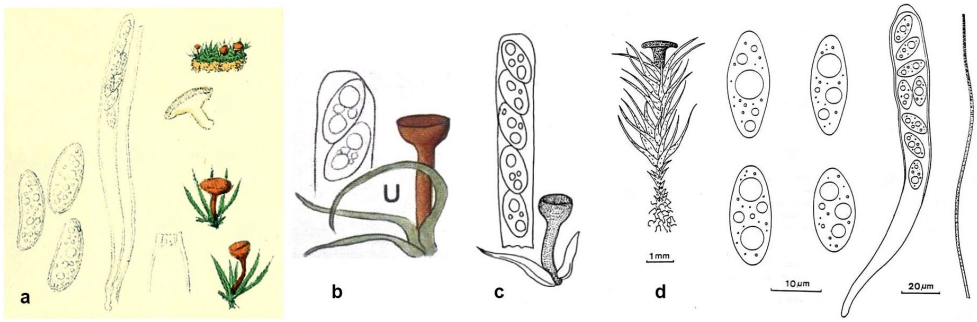

Figure 1. *Bryorutstroemia fulva* as illustrated under the name *Helotium fulvum* by (**a**) Boudier (1897 [1] pl. III fig. III), (**b**) Dennis (1978 [2] pl. XVIII U), (**c**) Ellis et Ellis (1988 [32] fig. 5), and (**d**) De Meulder (1992 [3] p. 80).

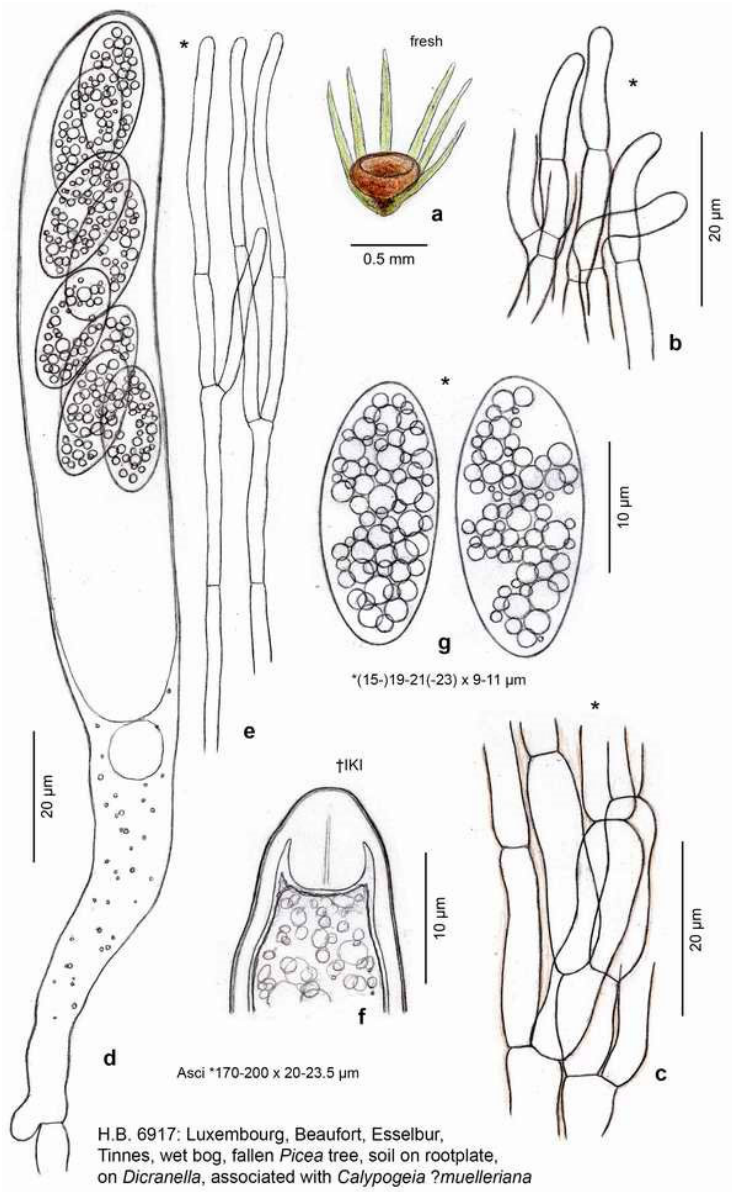

Figure 2. *Bryorutstroemia fulva* on *Dicranella* cf. *heteromalla*. (**a**) fresh apothecium formed in leaf axils at tip of plant; (**b**) hair-like marginal elements; (**c**) ectal excipular cells in surface view; (**d**) mature ascus; (**e**) upper part of paraphyses; (**f**) apex of immature ascus with prominent wall thickening expanding into the ascoplasm; (**g**) mature ascospores containing numerous LBs. Living state, except for f (in IKI).—Del. H.O. Baral.

Figure 3. *Bryorutstroemia fulva* on *Dicranella* growing on sandstone in northern Bohemia. (**1a**,**2a**) collection sites; (**1b**,**2b**,**c**,**3**,**4a**,**b**) apothecia in leaf axils of bleached leaves.—(**1**) Stohánek (Z.S. 9/2021), (**2**) Sloup v Čechách (Z.S. 11/2021), (**3**) Petrovice (Z.S. 17/2021), (**4**) Sloup v Čechách (Z.S. 2/2021). Phot. Z. Sochorová.

Figure 4. *Bryorutstroemia fulva* in leaf axils of *Dicranella* (from northern Bohemia, Bretagne, and Luxembourg) or *Dicranum* (from Hungary). (**1a–c**,**2**,**5**,**6a**,**b**) apothecia in reflected light; (**3**,**4a**,**b**,**6c**) apothecia in transmitted light (**4b** showing protruding mature asci); (**4c**) median section of apothecium; (**7**) lower part of leaf with brown fungal tissue. (**1–5**,**6a**,**b**) fresh apothecia, (**6c**) rehydrated apothecium; (**4a–c**,**6c**) in water, (**7**) in PVA.—(**1–4**) phot. Z. Sochorová: (**1**) Sloup v Čechách (Z.S. 11/2021), (**2**) Janovice v Podještědí (Z.S. 16/2021), (**3**) Jablonné v Podještědí (Z.S. 15/2021), (**4**) Sloup v Čechách (Z.S. 2/2021); (**5**) phot. C. Németh: Budakeszi (C.N. 103), (**6a**,**b**) phot. J.P. Priou, (**6c**) H.O. Baral: Sixt-sur-Aff (J.P.P. 26054, H.B. 8083), (**7**) phot. H.O. Baral: Beaufort (H.B. 6917).

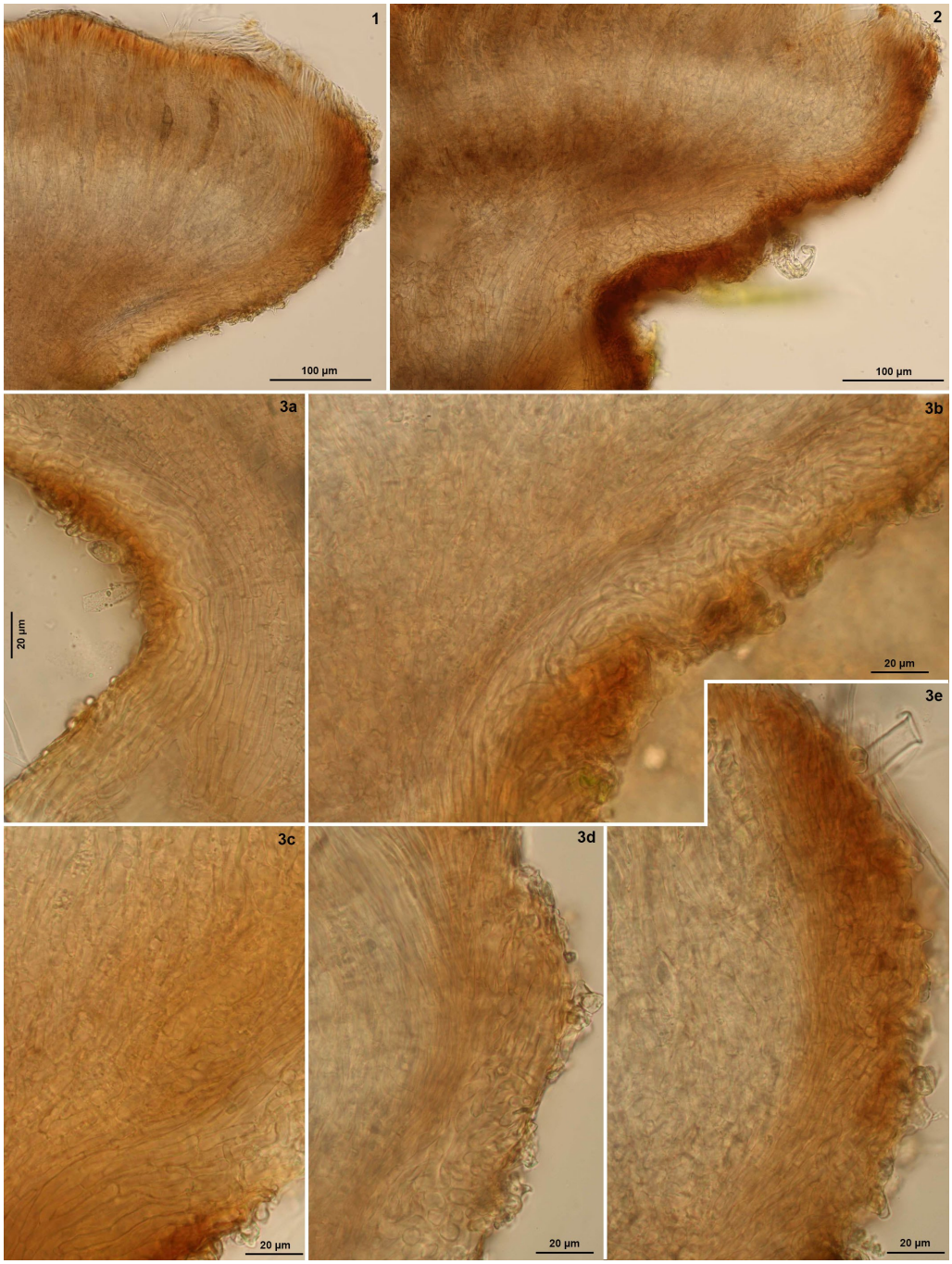

Figure 5. *Bryorutstroemia fulva* on *Dicranella* (from northern Bohemia). Median section of apothecia: (**1**,**2**) receptacle, (**3a**,**b**) upper stipe and lower flanks, (**3c**) lower flanks, (**3d**,**e**) margin. Living state.—(**1**) Divadlo (Z.S. 8/2021); (**2**) Besedice (Z.S. 7/2021); (**3**) Sloup v Čechách (Z.S. 2/2021). Phot. Z. Sochorová.

Figure 6. *Bryorutstroemia fulva* on *Dicranella* (from northern Bohemia). Apothecial stipe in surface view (**1a**,**c**,**2a**) and median section (**1b**,**2b**). Living state.—(**1**) Sloup v Čechách (Z.S. 2/2021); (**2**) Divadlo (Z.S. 8/2021). Phot. Z. Sochorová.

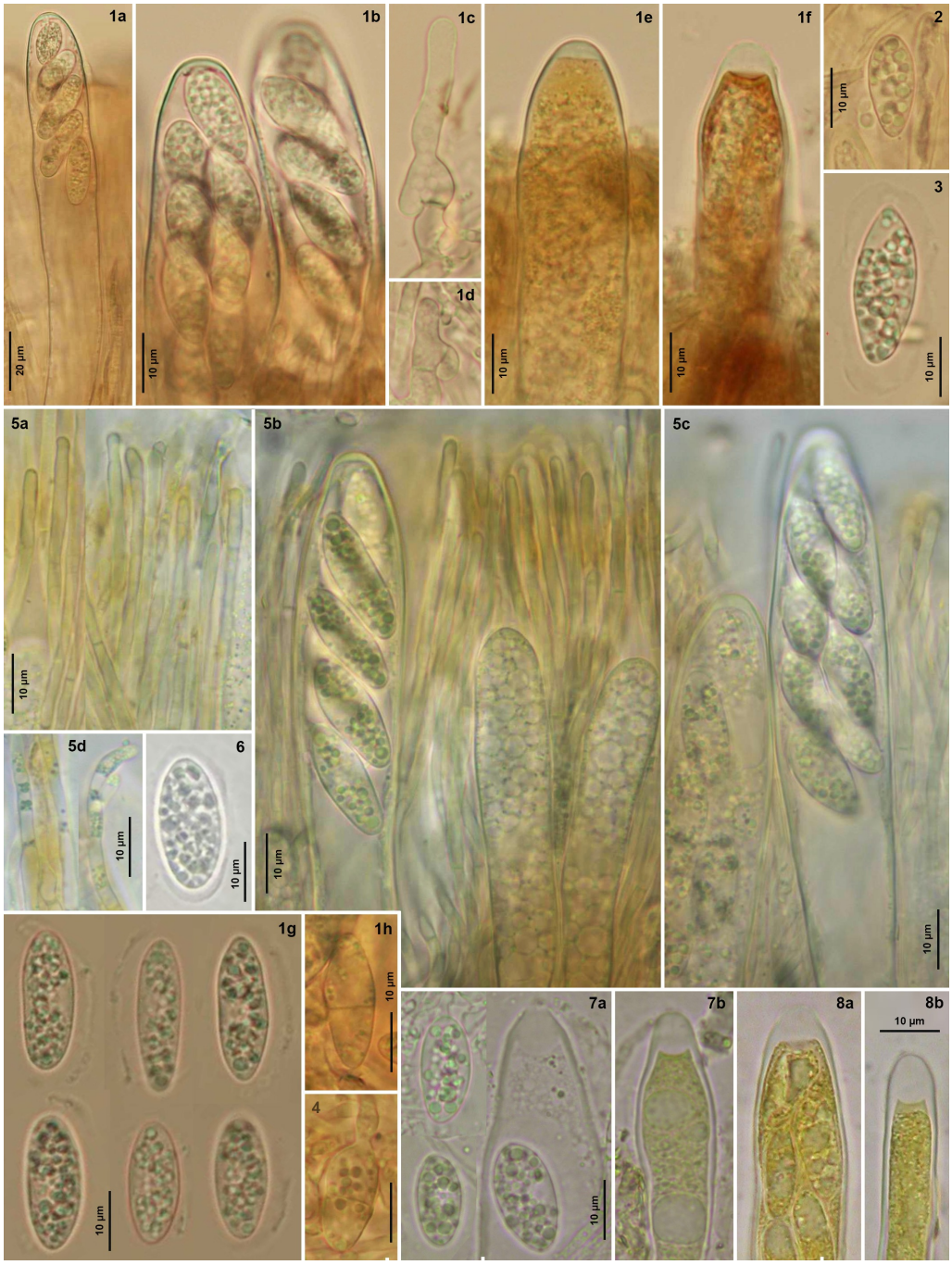

Figure 7. *Bryorutstroemia fulva*. (**1a**,**b**,**5b**, **c**,**7a**,**8a**) mature asci; (**1c**,**d**,**5c left**,**7b**,**8b**) immature asci; (**5b right**) young asci; (**5a**,**b**,**d**) paraphyses; (**1e**,**2**,**3**,**6**,**7a**) mature ascospores; (**1f**,**4**) overmature ascospores. Living state, except for (**7a**) asci, in H_2O, (**7b**,**8**) in IKI.—(**1**–**4**) from northern Bohemia, on *Dicranella* (phot. Z. Sochorová), (**1**) Z.S. 2/2021, (**2**) Z.S. 15/2021, (**3**) Z.S. 16/2021, (**4**) Z.S. 17/2021; (**5**) Hungary, on *Dicranum* (phot. C. Németh, C.N. 103); (**6**) Sweden, on *Bucklandiella* (phot. R. Isaksson, UPS F-990878); (**7**,**8**) France, on *Dicranella*, (**7**) phot. J.P. Priou, J.P.P. 15051, (**8**) H.O. Baral, H.B. 8083.

Figure 8. *Bryorutstroemia fulva*. (**1a**) collection site in *Quercus petraea* forest, (**1b–e**) apothecia in cushions of *Dicranum scoparium* (Hungary, phot. C. Németh, C.N. 103); (**2a**,**b**) *Bucklandiella heterosticha* on silicate stonewall (Sweden, phot. R. Isaksson, UPS F-990878).

4.4. Literature Reports

Boudier [1] described the apothecia of *H. fulvum* with a diameter and height of 0.5–1.5 mm, asci 150–200 × 17–18 µm, paraphyses apically slightly widened to 3–4 µm, eguttulate, and ascospores oblong-ellipsoid, subinaequilateral, rarely somewhat curved, *16–21 × 7–10 µm, multiguttulate (see Figure 1a). He illustrated bright reddish-brown apothecia but described them as brown to yellow-brown ("brunneo-fulvum") or fawn-brownish ("fauve brunâtre"), with hymenium and stipe base the most deeply coloured. He apparently did not test the asci with iodine and did not observe overmature spores as he stated that the spores were never septate. When taking the ascus width in Boudier's drawing as 17 µm, ascus length becomes 250 µm, spores in the asci 18–21 × 7–7.5 µm, and paraphysis width about 4 µm. Evaluation of the scales based on the 225× and 820× magnifications yield values of *295 × 18.5 µm for the ascus, *21–22 × 7–8.5 µm for the free ascospores, and 4 µm for the paraphysis, suggesting some scale and length/width error in Boudier's drawing regarding ascus length (Figure 1a).

British records of *H. fulvum* from leaf axils of *Dicranella heteromalla* were figured by Dennis [2] (as '*D. heteromera*') and Ellis et Ellis [32], but no collection data were given (see Figure 1b,c). The database of the British Mycological Society [17] indicates two collections, one from Gloucestershire made in 1991 and one without data. Dennis mentioned the negative ascus iodine reaction and considered an affinity with *Rutstroemia*, but also referred to a "small group of similar species parasitic on bryophytes, for which a separate genus may perhaps be needed" (Dennis probably meant the later erected genus *Bryoscyphus* Spooner). The almost identical measurements by Dennis and Ellis et Ellis (l.c., asci 150–180 × 13–16 µm, ascospores 16–21 × 6–9 µm) concur well with the present data. The ascospores were illustrated with two large and some smaller LBs, probably because the material was studied in a rehydrated state.

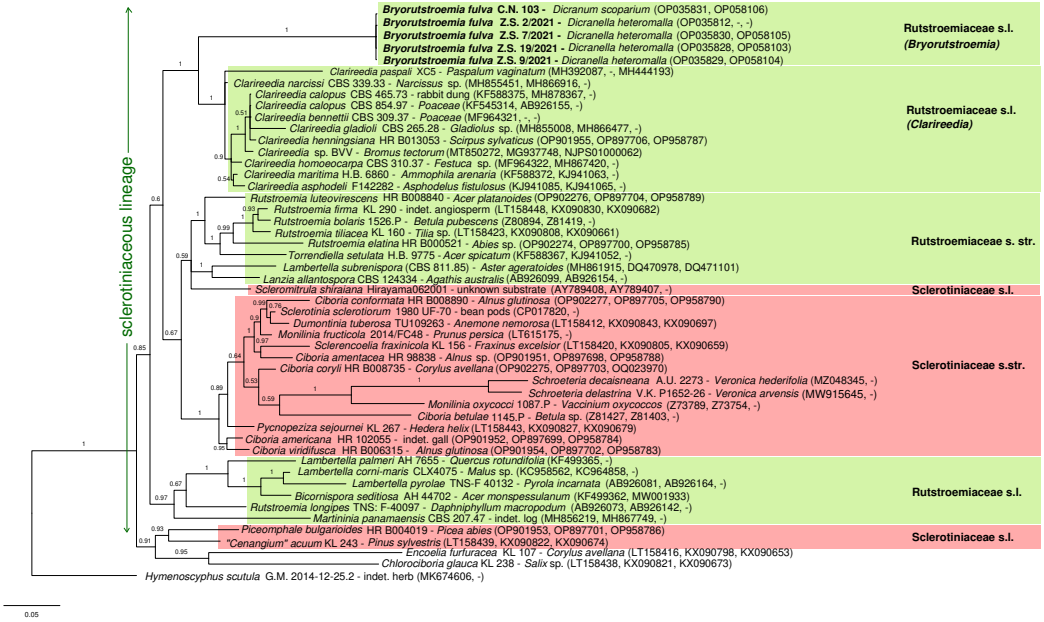

Figure 9. Bayesian analysis of the sclerotiniaceous lineage which comprises *Rutstroemiaceae* s.l. and *Sclerotiniaceae* s.l., based on ITS1-5.8S-ITS2 and LSU D1–D3 rDNA and *EF1α*. The chosen outgroup comprises members of *Cenangiaceae*, *Chlorociboriaceae*, and *Helotiaceae*.

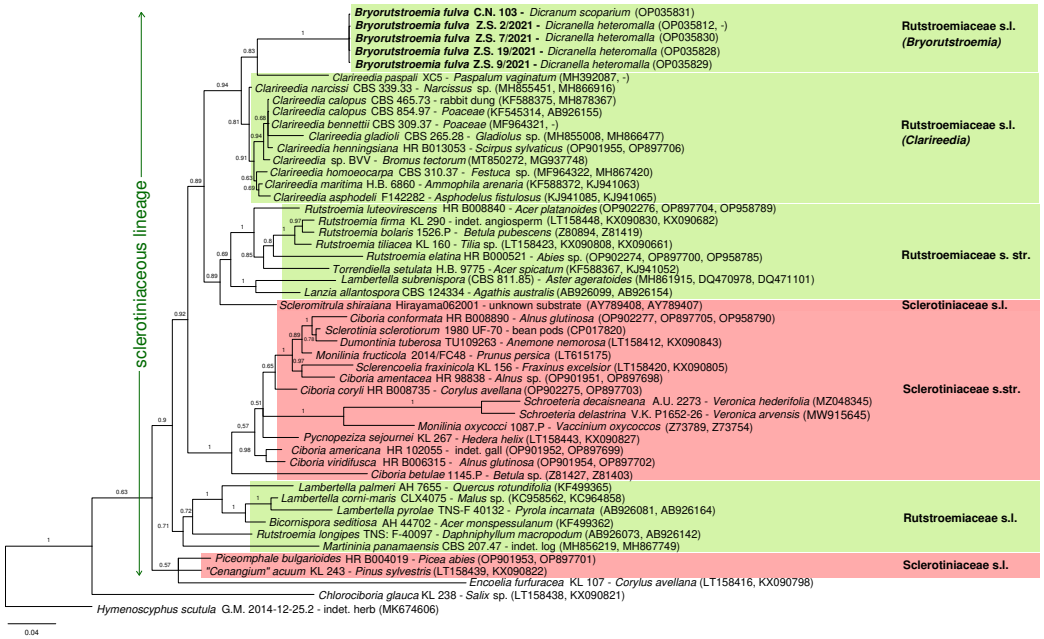

Figure 10. Bayesian analysis of the sclerotiniaceous lineage based on ITS1-5.8S-ITS2 and LSU D1–D3 rDNA.

Much earlier, Dennis ([33] p. 58) compared *H. fulvum*, based on Boudier's description, with a collection on *Lycopodium* from Norway which he identified as *Poculopsis ogrensis* Kirschst. This species he combined as *Allophylaria ogrensis* (Kirschst.) Dennis, although the inner ectal excipulum was drawn with thin-walled cells and the texture described as very soft. Contrary to *H. fulvum*, the apothecia were yellow when fresh but turned dark brown on drying, and the much shorter asci had an amyloid ring. At that time, Dennis did not know *H. fulvum* by personal study. For *A. ogrensis*, he saw some similarities with the *Sclerotiniaceae* (as *Ciborioideae*), but the absence of a substratal blackening or a sclerotium excluded such a relationship.

Figure 11. Known distribution of *Bryorutstroemia fulva* in Europe (white dots referring to the collections cited under "Specimens included").

De Meulder [3] described and illustrated a personal collection of *H. fulvum* on *Dicranella cerviculata* collected in 1992 in Belgium (Figure 1d). Ten days after this collection was made, the first author received a part of the dried specimen from the collector. Despite the short time span, no living elements could be found. The obtained measurements differed from De Meulder's data by much narrower paraphyses (†2–2.2 vs. †3–4 µm), slightly shorter asci (†130–158 × 14–18 vs. †137.5–175 × 12.5–18 µm), and distinctly narrower ascospores (†15–20 × 6–7.5 vs. *15.5–22.75 × 7.5–8.7 µm). De Meulder might have studied a rehydrated apothecium with still living spores, judging from the larger size, and also from the included partly large LBs which were likely formed by the fusion of smaller ones during rehydration. Paraphysis width is hardly over 1.5 µm when evaluated from De Meulder's drawing, hence his given width of 3–4 µm should be an error or a mere copy of Boudier's data, whereas values around †1.5–2.5 µm would have been closer to what was here observed in the other collections.

4.5. Misinterpretations

Under the name *Helotium fulvum*, Velenovský ([34] p. 209) gave an unillustrated record on *Hylocomium splendens*, *H. squarrosum* (≡ *Rhytidiadelphus squarrosus*), and *Hypnum cupressiforme*. When revising the cited collection, Svrček ([35] p. 149, pl. 19 fig. 7) found only *Hylocomium splendens* inside the voucher, with apothecia on the leaves, and concluded that it is a species very different from *H. fulvum*, for which he could not give a name. The ascospores were much more slender (†17–19 × 4–4.5 µm), with two large guttules, and the inamyloid asci much smaller (†90–100 × 6–10 µm, Velenovský: †100–120 × 5–8 µm) compared to Boudier's *H. fulvum*, with a strongly inflated foot (crozier?). The apothecia were 1–1.5 mm diam., blackish-brown, sessile or short-stalked. Svrček's description suggests a species of *Hymenoscyphus* s.l. Another specimen found in Velenovský's herbarium under the name *H. fulvum* was on *Rhytidiadelphus squarrosus*, and Svrček (l.c.) identified it as *Hymenoscyphus rhytidiadelphi* (≡ *Bryoscyphus rhytidiadelphi*).

Bryorutstroemia fulva may be confused with *Bryoscyphus dicrani* because of a similar ascospore size and shape and inamyloid asci. However, confusion is only possible when comparing herbarium specimens in which *B. dicrani* may attain a reddish-brownish colour due to secondary pigmentation of the multiguttulate contents of paraphyses and excipular cells. In the living state, *B. dicrani* has white apothecia, binucleate ascospores with a lower lipid content (OCI 2–3), and multiguttulate paraphyses and cortical excipular cells due to strongly refractive vacuolar bodies (VBs). A further difference lies in the asci which are also inamyloid but arise from croziers.

Hengstmengel [19] studied a collection on *Brachythecium rutabulum* from the Netherlands (Drenthe, Rolde, Deurzerbroek). We have seen no documentation of this collection, but we consider the possibility that it might be a misidentification, judging from the deviating host.

4.6. Other Bryicolous Species of the Sclerotiniaceous Lineage

Bryorutstroemia fulva is exceptional within the sclerotiniaceous lineage by its ecological restriction to acrocarpous mosses. Only a very small number of other bryicolous discomycetes with a clear affinity to the sclerotiniaceous lineage are known up to now. One of them is *Sclerotinia atrostipitata* Svrček from Czechia, which was described as emerging from a 2 mm large subglobose sclerotium among rhizoids of *Ceratodon purpureus*, with globose excipular cells, amyloid asci, and comparatively small, ellipsoid-ovoid, eguttulate ascospores [36]. Svrček's remark of an attachment of the sclerotium to the moss rhizoids might be an argument for a real connection to the moss, but interactions at the cellular level have not been assessed. The North American *Sclerotinia incondita* (Ellis) Sacc. mentioned by Svrček likewise grew among mosses, but its description which includes four-spored asci is too brief to permit any conclusion.

Supplementary Materials: The following supplementary Bayesian analyses are available online at https://www.mdpi.com/article/10.3390/life13041041/s1; Figure S1: Bayesian analysis of ITS1-5.8S-ITS2 region; Figure S2: Bayesian analysis of D1–D2 domain of LSU rDNA; Figure S3: Bayesian analysis of *EF1α*; Figure S4: combined Maximum Likelihood analysis of dataset of Figure 9. For further data see legend to Figure 9 (as outgroup for S3 we selected *Chlorociboria glauca*).

Author Contributions: H.-O.B.: fieldwork, microscopy, writing the manuscript, illustrating the material; Z.S.: fieldwork, microscopy, writing the manuscript, photographic documentation; M.S.: sequencing, phylogenetic analysis. All authors have read and agreed to the published version of the manuscript.

Funding: Zuzana Sochorová was funded by IGA UP PrF-2023-001 and Michal Sochor by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0423.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Names of the new species and combinations were formally registered in MycoBank. Newly generated sequences were deposited in GenBank.

Acknowledgments: We thank Csaba Németh for providing data and photos of his collection from Hungary and for identification of the host moss in a part of the collections, Jannes Boers, Robin Isaksson, Zdeněk Palice, and Jean-Paul Priou for providing data on their collections, and Hubert De Meulder (†) for sending his Belgian specimen to the first author in 1992. Bernard Declercq kindly provided De Meulder's paper and an excerpt of Arnolds et al. Tereza Tejklová, the curator of HR, is thanked for providing specimens of the sclerotiniaceous clade for sequencing. Chris Yeates and Georges Greiff are thanked for linguistic suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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4.2.5 Further important finds of bryophilous ascomycetes in the Czech Republic

Several interesting species have been collected by the author during the last decade. Some of them have already been published before starting the Ph.D. studies in September 2016 (Egertová 2015, Egertová et al. 2015, 2016b). In this chapter, the most interesting newer or unpublished finds are presented (Fig. 6).

Helotiales
Pezoloma marchantiae (Sommerf.) Benkert
South Bohemian region: NE of Kladenské Rovné, ca 48°48'2"N, 14°13'34.5"E, 700 m a. s. l., on *Marchantia polymorpha* on an old burnt place, 28 September 2020, leg. et det. Z. Sochorová (herb. CB)

Moravian-Silesian region: Karlov pod Pradědem, Moravice valley, 50°2'4.3"N, 17°16'12"E, 835 m a. s. l., on *Marchantia polymorpha* on a path, 20 August 2017, leg. et det. Z. Egertová (PRM 955988)

Olomouc region: Jeseníky Protected Landscape Area, Ostružná – at the Čerňava cable car station, 50°10'52.2"N, 17°4'47.6"E, 1065 m a. s. l., on *Marchantia polymorpha* on wet soil, 8 August 2020, leg. et det. Z. Sochorová (PRM 954620); Bílá Lhota – arboretum, 49°42'35.6"N, 16°58'32"E, 295 m a. s. l., on *Marchantia polymorpha* on soil, 12 August 2017, leg. et det. Z. Egertová (PRM 955989)

South Moravian region: bottom of the Macocha abyss, on *Marchantia polymorpha*, 15 July 2021, leg. et det. Z. Sochorová (PRM 957649)

Zlín region: Záhlinické rybníky nature park, 49°17'12.7"N, 17°26'42.7"E, 195 m a. s. l., on *Marchantia polymorpha* on soil, 14 December 2014, leg. et det. Z. Egertová (PRM 955987); Buchlovice castle park, 49°4'56"N, 17°20'21"E, 255 m a. s. l., on *Marchantia polymorpha* on a crushed brick pathway, 25 October 2014, leg. et det. Z. Egertová (PRM 955990)

Pezizales
Lamprospora annulata Seaver
Zlín region: Kroměříž – the Chateau garden, 49°18'17"N, 17°23'41"E, 196 m a. s. l., on *Ephemerum minutissimum*, in a grassland, 27 November 2015 (PRM 935149)

Lamprospora lubicensis Benkert
South Moravian region: Sedlec, Slanisko u Nesytu NNR, 175 m a. s. l., on *Hennediella heimii* on soil, 2 April 2017, leg. et det. Z. Egertová et L. Janošík (PRM 956015)

Lamprospora moynei Benkert
Olomouc region: Plané loučky NR – northern part, west of the Mlýnský brook, 49°37'33.6"N, 17°13'57.5"E, 215 m a. s. l., on *Ephemerum* sp. on soil in a meadow, 18 July 2022, leg. et det. Z. Sochorová (PRM 957695)

Octospora bridei Caillet & Moyne
South Bohemian region: Chvalšiny – park of the Červený dvůr psychiatric hospital, 48°50'41"N, 14°13'48"E, 550 m a. s. l., on *Ephemerum* sp. in a grassland, 29 September 2020, leg. et det. Z. Sochorová (herb. CB)

Olomouc region: Plané loučky NR – northern part, west of the Mlýnský brook, 49°37'33.6"N, 17°13'57.5"E, 215 m a. s. l., on *Ephemerum* sp. on soil in a meadow, 18 July 2022, leg. et det. Z. Sochorová (PRM 957696)

Zlín region: Kroměříž – the Chateau garden, 49°18'17"N, 17°23'41"E, 196 m a. s. l., on *Ephemerum minutissimum* in a grassland, 18 October 2015 (PRM 935151); ibid. 6 November 2015 (PRM 955970); 20 November 2015 (PRM 958334, PRM 956479); 27 November 2015 (PRM 956480); 28 November 2015 (PRM 956484); 19 December 2015 (PRM 955969)

Octospora erzbergeri Benkert
Olomouc region: Špraněk NNR, 49°40'2"N, 16°54'16"E, 440 m a. s. l., on *Pseudoleskeella nervosa* on a boulder, 13 September 2014, leg. et. det. Z. Egertová (PRM 955971)

Octospora ithacaensis (Rehm) K. B. Khare
Olomouc region: Olomouc – Sudova street, 49°34'48"N, 17°16'35"E, 210 m a. s. l., on thalli of *Marchantia polymorpha*, 6 May 2016, leg. et det. Z. Egertová (PRM 945803)

Octospora oscarii Eckstein, Sochorová & Janošík
Olomouc region: Dolany u Olomouce, 49°39'10.2"N, 17°20'38.8"E, 310 m a. s. l., on *Pseudotaxiphyllum elegans* on soil in a mixed forest, 6 February 2021, leg. et det. Z. Sochorová (PRM 955619); ibid. 23 February 2021 (PRM 955620)

Octospora pseudoampezzana (Svrček) Caillet & Moyne
Moravian-Silesian region: Štramberk, 49°35'16"N, 18°7'11"E, 440 m a. s. l., on *Schistidium crassipilum* on a boulder, 5 March 2016, leg. et det. Z. Egertová (PRM 945806)

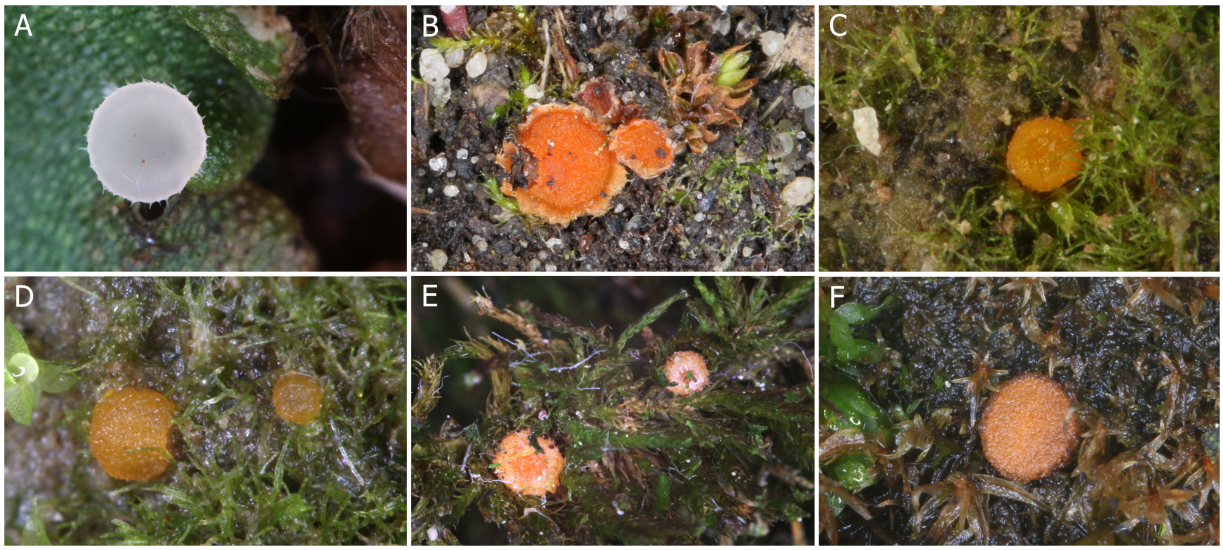

Fig. 6: Finds of rarely reported species in the Czech Republic. A) *Pezoloma marchantiae* (PRM 955989), B) *Lamprospora lubicensis* (PRM 956015), C) *Lamprospora moynei* (PRM 957695), D) *Octospora bridei* (herb. CB), E) *Octospora erzbergeri* (PRM 955971), F) *Octospora pseudoampezzana* (PRM 945806)

4.3 *Octospora svrcekii* and phylogeny of the section *Wrightoideae*

4.3.1 Amended description of the rarely reported bryophilous ascomycete *Octospora svrcekii* (Pyronemataceae) with notes on the phylogeny of the section *Wrightoideae*

Phytotaxa 475 (1): 001–017
https://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.475.1.1

Amended description of the rarely reported bryophilous ascomycete *Octospora svrcekii* (Pyronemataceae) with notes on the phylogeny of the section *Wrightoideae*

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Abstract

The bryophilous ascomycete *Octospora svrcekii*, belonging to the section *Wrightoideae*, has so far been reported from only three localities in the world. New collections from Albania, Austria, Croatia, France, Slovakia and Spain have enabled a better understanding of its variability, ecology, distribution and phylogenetic relationships with other taxa within the section *Wrightoideae*. *Octospora svrcekii* was always found associated with *Cratoneuron filicinum* growing in constantly humid habitats (brooks, rivers or waterfalls), on calcareous bedrock. A species description based on both living and dead material is provided and compared with previous observations. A phylogenetic analysis of the section *Wrightoideae*, performed using the EF1α, SSU rDNA and LSU rDNA loci, revealed that *Octospora svrcekii* forms a monophyletic group with *O. wrightii*, *O. erzbergeri, O. hygrohypnophila* and *O. americana,* all of which are characterised by subglobose to broadly ellipsoid ascospores ornamented with isolated warts, and infect mosses in the order Hypnales, inducing galls on their rhizoids. Based on the molecular analysis, *O. orthotrichi* and *O. affinis,* formerly also considered as members of the section *Wrightoideae*, do not belong to the group.

Keywords: Bryophilous fungi, fungal systematics, galls, pleurocarpous mosses, vital taxonomy

Introduction

Octospora svrcekii Benkert (1998a: 26), a member of the Pyronemataceae, Pezizales, is a bryophilous ascomycete growing on the pleurocarpous moss *Cratoneuron filicinum* (Hedwig 1801: 285) Spruce (1867: 21) in the Amblystegiaceae, Hypnales. It was first collected in Zádielska dolina in the Slovak Karst in 1961, by Kubička (1972), but identified as *Lamprospora lutziana* Boudier (1917: 15). This collection was later re-studied by Benkert, who identified it as a new species and named it after the well-known Czech mycologist Mirko Svrček (Benkert 1998a).

He assigned it to the section *Wrightoideae* Benkert (1998a: 18), which was established by him in the same work. The section was defined by the following criteria: 1) parasitism of mosses in the orders Hypnales and Neckerales, 2) formation of stipitate galls on rhizoids of the hosts and 3) ellipsoid or broadly ellipsoid ascospores ornamented by isolated warts. Besides *O. svrcekii*, five more species were included in the section – the type species *Octospora wrightii* (Berk. & M.A. Curtis in Berkeley & Broome 1865: 444) J. Moravec (1969: 227)*, O. americana* Benkert (1998a: 20), *O. texensis* Benkert (1998a: 28)*, O. hygrohypnophila* Dissing & Sivertsen (1983: 415) and *O. orthotrichi* (Cooke & Ellis 1877: 7) K.B. Khare & V.P. Tewari (1978: 2118). Later, *O. erzbergeri* Benkert (2006: 1) and *O. affinis* Benkert & L.G. Krieglsteiner (2006: 54) were added to *Wrightoideae*, although *O. affinis* does not induce gall formation and its inclusion is therefore in conflict with the diagnosis of the section.

Besides the type locality, *O. svrcekii* has so far only been reported from Savoy in France (Capoen 2018) and Sarajevo County in Bosnia and Herzegovina (Jukić *et al*. 2020). Between 2014 and 2020, the species was recollected from its type locality and recorded from ten more localities in Albania, Austria, Croatia, France, Slovakia and Spain. In this study, *Octospora svrcekii* was sequenced for the first time, including the collection from the type locality in Slovakia, as well as those from Albania, Croatia, France and Spain. The holotype collection was not included in the analysis because the species protologue fully corresponded to our observations and could not be studied in the living state for additional data. The new collections enabled a detailed study of both living and dead material and the resulting observations broadened the original description. Molecular analysis was performed to verify the positioning of species assigned to the section *Wrightoideae*. The aim of this contribution is to present the new observations and clarify phylogenetic relationships within the section.

Material and methods

Specimen collection and observation

Octospora svrcekii was collected in six European countries from 2014–2020. The fungi were always collected together with the accompanying bryophytes to study the infection and identify the host. This description is based mainly on the Croatian collection (PRM 951720) and a voucher collected on type locality in Slovakia (PRC 4125). Macroscopic features were described from living fruit bodies. Microscopic characters were studied in living (*) cells and tissues following the methods of vital taxonomy (Baral 1992) and its systemic additions (Kušan 2015) as well as rehydrated material (†), using light microscopes at magnifications up to 1600×. The structures were observed in tap water (H_2O), 5% potassium hydroxide (KOH), Lugol's solution (IKI), Brilliant Cresyl Blue (CRB), Lactic Acid Cotton Blue (LACB) and acetocarmine (AC). Measurements of living, freshly ejected ascospores were made on 100 fully mature normally developed and randomly selected ascospores to an accuracy of 0.1 µm and are given without ornamentation. Measurements were made directly with an ocular micrometer scale or on photographs with the Piximètre 5.10 programme (Henriot & Cheype 2020). Length, width, and length/width ratio (Q value) of ascospores are given as: (min.) stat. min.—*arith. mean*—stat. max. (max.) where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic mean minus two times standard deviation), arith. mean = arithmetic mean, "stat. max." = statistical maximum (arithmetic mean plus two times standard deviation), "max." = maximum (highest measured value). In cases where stat. min./stat. max. are outside the measured range or equal to min/max., min. and max. are written without a bracket. Ascospore shapes follow the standard terminology proposed by Kušan *et al*. (2014). Vouchers are deposited in public herbaria and fungaria CNF, PRC, PRM, VIT and WU.

DNA extraction, PCR amplification and sequencing

The analysis included all species assigned to the section *Wrightoideae* with the exception of *Octospora texensis* which was not available for our analysis, and chosen representatives of the genera *Octospora* Hedwig (1789: 4), *Lamprospora* De Notaris (1864: 388) and *Octosporopsis* U. Lindemann & M. Vega in Lindemann *et al*. (2014: 566) outside the section. Specimens used in the analysis and their GenBank accession numbers are listed in Tab. 1, with newly obtained sequences in bold letters. DNA was extracted from fresh, dried, or CTAB-stored apothecia using the *Quick*-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Orange, USA) or the CTAB method (Doyle & Doyle 1987). Sequence data were generated for three loci: the large subunit of ribosomal DNA (LSU) was amplified with primers NL1 and NL4 (O'Donnell 1993) or LR6 (Vilgalys & Hester 1990); 18S subunit of rDNA (SSU) with primers NS1 and NS6 (White *et*

al. 1990); and translation elongation factor-1alpha (EF1α) with primers EF1-983F and EF1-1567R (Rehner & Buckley 2005). PCR was performed with Kapa polymerase (Kapa Biosystems), following standard protocol, with 37 cycles and an annealing temperature of 54°C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25M NaCl in the precipitation mixture) or using the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taipei, Taiwan), and sequenced in both directions using the Sanger method (Macrogen Europe, The Netherlands, and Sequencing laboratory of the Faculty of Science, Charles University, Prague, Czech Republic).

Obtained sequences, together with those of other *Octospora, Octosporopsis* and *Lamprospora*, as well as *Otidea leporina* (Batsch 1783: 117) Fuckel (1870: 329), serving as an outgroup, were manually checked, trimmed, edited and aligned in Geneious 8 (Biomatters) using the MAFFT plugin. *Otidea leporina* was selected as an outgroup because of its basal phylogenetic position within the family Pyronemataceae. Bayesian phylogeny reconstruction was conducted in MrBayes 3.2.4 (Ronquist *et al*. 2012), with two independent runs of six million generations and four chains, sampling every 1000th generation; the first 50 % of samples were discarded as burn-in. The most suitable substitution model for each locus was determined in PartitionFinder 2.1.1 (Lanfear *et al*. 2017), using the AIC corrected for small samples (AICc) and a greedy search, which selected the general time reversible substitution model (GTR + G + I) as the best fitting.

TABLE 1. List of collections used in the phylogenetic study together with their GenBank accession numbers.

Species	Identification code in GenBank	Herbarium code	Country, collection date	Host	GenBank accession numbers		
					LSU	SSU	EF1α
Lamprospora sylvatica	UA1	PRM 946415 (holotype)	Ukraine, 8 Jul 2017	*Dicranum montanum*	MG947604	MG947607	MK569290
Lamprospora verrucispora	MV14020201	PRM 953061	Netherlands, 2 Feb 2014	*Campylopus pyriformis*	**MN994549**		
Lamprospora verrucispora	MV15100303	PRM 953062	Germany, 3 Oct 2015	*Campylopus pyriformis*	**MN994550**	**MN994526**	**MN990992**
Lamprospora verrucispora	MV15102504	HBG 1412 (holotype)	Germany, 25 Oct 2015	*Campylopus pyriformis*	**MN994551**	**MN994527**	**MN990993**
Octospora affinis	OAfZla	PRM 945798	Czech Republic, 22 Oct 2016	*Orthotrichum affine*	MF754075	MK569347	MF754045
Octospora affinis	L915	PRC 4610	Austria, 1 May 2015	*Orthotrichum affine*	**MN994530**		
Octospora affinis	L1115	PRC 4609	Austria, 2 May 2015	*Orthotrichum affine*	**MN994531**		
Octospora affinis	LJ14003	PRC 4611	Germany, 14 Feb 2015	*Orthotrichum affine*	**MN994532**		
Octospora affinis	LJ16015	PRC 4613	Czech Republic, 5 Jun 2016	*Orthotrichum affine*	**MN994533**		
Octospora americana	2055	S F43718 (holotype)	USA, 18 Feb 1981	*Forsstroemia trichomitria*	**MN967346**	**MN994516**	**MT078729**
Octospora bridei	bri	PRM 935151	Czech Republic, 18 Oct 2015	*Ephemerum minutissimum*	MF754061	**MT001890**	
Octospora conidiophora	ZE48/18	PRM 951743 (holotype)	South Africa, 2 Mar 2018	*Trichosteleum perchlorosum*	MK569321	MK569351	MK569297
Octospora erzbergeri	ERZ	PRM 945799	Czech Republic, 10 Dec 2016	*Pseudoleskeella nervosa*	MF754068	MK569340	MF754042
Octospora erzbergeri	L1615	PRC 4603	Slovakia, 28 Dec 2015	*Pseudoleskeella nervosa*	**MN994547**		
Octospora erzbergeri	LJ16016	PRC 4615	Slovakia, 28 May 2016	*Pseudoleskeella nervosa*	**MN994548**		
Octospora cf. *excipulata*	OExc	PRM 945800	Czech Republic, 16 Nov 2015	*Funaria hygrometrica*	MF754062	MK569369	MF754047

.....continued on the next page

TABLE 1. (Continued)

Species	Identification code in GenBank	Herbarium code	Country, collection date	Host	GenBank accession numbers		
					LSU	SSU	EF1α
Octospora gyalectoides agg.	49382	B 70 0100075	Germany, 22 Nov 2016	*Pottia lanceolata*	**MT001891**	**MT001889**	**MN990995**
Octospora humosa agg.	OHZla	PRM 945802	Czech Republic, 22 Oct 2016	*Polytrichum piliferum*	MF754074	MK569343	MF754043
Octospora hygrohypnophila	LJ18039	PRC 4604	Austria, 19 Jul 2018	*Hygrohypnum luridum*	**MN994540**		
Octospora hygrohypnophila	LJ18044	PRC 4616	Austria, 19 Jul 2018	*Hygrohypnum luridum*	**MN994541**	**MN994519**	**MN990986**
Octospora hygrohypnophila	MV17082702	PRM 953064	France, 27 Aug 2017	*Hygrohypnum luridum*	**MN994543**	**MN994520**	**MN990988**
Octospora hygrohypnophila	MV17083004	PRM 953065	France, 30 Aug 2017	*Hygrohypnum luridum*	**MN994544**	**MN994521**	
Octospora hygrohypnophila	MV17082601	PRM 953063	France, 26 Aug 2017	*Hygrohypnum luridum*	**MN994542**	**MN994522**	**MN990987**
Octospora hygrohypnophila	KH.03.30 (FH)	KH.03.30 (FH)	Norway, 2003	*Hygrohypnum luridum*	DQ220379	DQ646539	KC109258
Octospora ithacaensis	OLOi	PRM 945803	Czech Republic, 6 May 2016	*Marchantia polymorpha*	MF754071	MK569346	MF754053
Octospora kelabitiana	oct-jat	PRM 945781	Malaysia, 3 Feb 2016	*Riccardia* sp.	MF754065	MK569372	MF754048
Octospora leucoloma	Oleu	PRM 945804	Czech Republic, 21 Oct 2016	*Bryum argenteum*	MF754063	MK569370	
Octospora cf. *orthotrichi*	HR8	CNF 2/10561	Croatia, 2 Jan 2018	*Orthotrichum diaphanum*	**MK569314**	**MK569342**	**MK569311**
Octospora cf. *orthotrichi*	L916	PRC 4612	Czech Republic, 27 Nov 2016	*Orthotrichum diaphanum*	**MN994545**		
Octospora cf. *orthotrichi*	LJ16038	PRC 4614	Czech Republic, 8 Oct 2016	*Orthotrichum diaphanum*	**MN994546**		
Octospora pannosa	JE51883	B 70 0100137	Serbia, 10 Jan 2018	*Brachytheciastrum velutinum*	**MN994528**		
Octospora pannosa	PRC 8124	PRC 8124 (isotype)	Germany, 28 Dec 2017	*Brachytheciastrum velutinum*	**MN994529**		
Octospora phagospora	PHG44	PRM 945805	Germany, 24 Oct 2015	unknown	MF754072	MK569344	MF754046
Octospora pseudoampezzana	OP1	PRM 935156	Czech Republic, 5 Mar 2016	*Schistidium crassipilum*	MF754069	MK569339	MF754050
Octospora svrcekii	ZE102/18	PRM 951720	Croatia, 20 May 2018	*Cratoneuron filicinum*	**MN967348**	**MN994518**	**MN974532**
Octospora svrcekii	PRC 4125	PRC 4125	Slovakia, 28 Dec 2015	*Cratoneuron filicinum*	**MN994537**	**MN994525**	**MN990991**
Octospora svrcekii	MV17082502	PRM 953066	France, 25 Aug 2017	*Cratoneuron filicinum*	**MN994536**	**MN994523**	**MN990989**
Octospora svrcekii	MV18062203	PRM 953067	Spain, 22 Jun 2018	*Cratoneuron filicinum*	**MN994538**	**MN994524**	**MN990990**
Octospora svrcekii	51959	PRM 954236	Albania, 8 Jul 2014	*Cratoneuron filicinum*	**MN967347**	**MT065902**	**MN974531**
Octospora svrcekii	MV18062301	PRM 953068	Spain, 23 Jun 2018	*Cratoneuron filicinum*	**MN994539**		
Octospora wrightii	WRIG	PRM 945807	Czech Republic, 22 Apr 2017	*Amblystegium serpens*	MF754070	MK569345	**MT078728**
Octospora wrightii	LJ15010	PRC 4617	Czech Republic, 24 Jan 2015	*Amblystegium serpens*	**MN994534**	**MN994517**	**MN990994**
Octospora wrightii	LJ16047	PRC 4618	Czech Republic, 21 Sep 2016	*Amblystegium serpens*	**MN994535**		
Octosporopsis erinacea	DUM20/1	PRM 945774 (isotype)	Malaysia, 20 Jan 2017	*Dumortiera hirsuta*	MF754057	MK569338	MF754041
Otidea leporina	KGOL	CNF 2/9962	Kyrgyzstan, 15 Jul 2016		MK569335	MK569371	MK569312

Results

Phylogenetic analysis

The analysed species with broadly ellipsoid or subglobose ascospores, ornamented with isolated warts, and forming galls on rhizoids of mosses in the order Hypnales, i. e. *O. wrightii, O. americana, O. erzbergeri, O. hygrohypnophila* and *O. svrcekii,* form a well-supported monophyletic lineage (Fig. 1; trees based on analysis of each locus shown in Suppl. Fig. S1). However, *O. orthotrichi* and *O. affinis,* infecting mosses in the genus *Orthotrichum* Hedwig (1801: 162–163), order Orthotrichales, also formerly considered members of the section *Wrightoideae*, do not belong to the group. Species infecting mosses in the order Hypnales, but not inducing formation of galls, or having a different type of ornamentation (*O. conidiophora* Sochorová & Döbbeler in Sochorová *et al*. 2019: 55*,* and *O. pannosa* T. Richter, M. Vega & D. Savić in Vega *et al*. 2018: 1001) also have different positions in the tree.

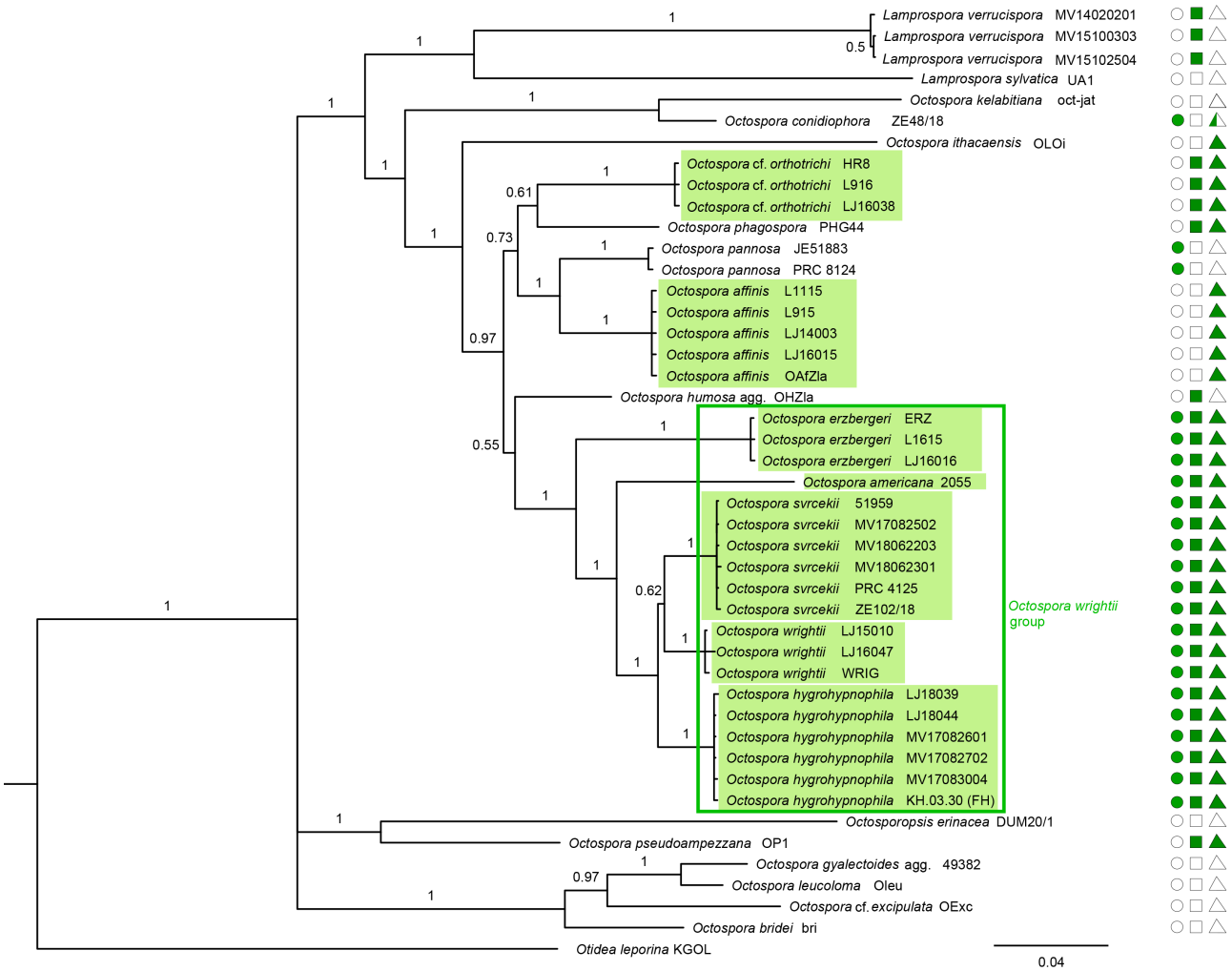

FIGURE 1. Bayesian phylogeny inference, based on concatenated alignment of EF1α, LSU and SSU sequences. Bayesian posterior probabilities are shown above branches; *Otidea leporina* serves as an outgroup. Species assigned to the section *Wrightoideae* are highlighted in light green, the monophyletic *Octospora wrightii* lineage is enclosed in a green frame. The symbols represent diagnostic characters for the section *Wrightoideae*: circle = growth on mosses in the order Hypnales, square = induction of galls on rhizoids of the host moss, triangle = ellipsoid or broadly ellipsoid ascospores ornamented with isolated warts (here, we include also subglobose ascospores, as these were called broadly ellipsoid by the author of the section, D. Benkert). Full symbol means presence of the character, empty symbol its absence, and halved symbol variability in trait presence

Taxonomic treatment

Octospora svrcekii (Figs 2–5)

Type:—SLOVAKIA. Slovenský kras, Turňa nad Bodvou surroundings, Zádielska dolina valley, in a mossy place, 6 July 1961, *J. Kubička* (PRM 616314)

Description:—*Apothecia* up to 1430 µm broad and 620 µm high, first spherical, later turbinate or saucer–shaped, finally discoid, orange, often with a narrow dentate margin, hymenium roughened due to protruding asci, sessile on plants of *Cratoneuron filicinum*.

FIGURE 2. *Octospora svrcekii* (a, b. PRC 4125, c, d. PRM 951720). a. typical habitat—rocks on the banks of the Blatný potok stream, Slovakia, b–d. apothecia on shoots of *Cratoneuron filicinum*. Scale bars = 1 mm. Photos a, b. L. Janošík, c, d. Z. Sochorová.

Hymenium *260–340 µm thick. *Asci* cylindrical, *243–322 × 22–28 µm, †190–232 × 18–19 µm, containing 8 ascospores arranged in a single row, *pars sporifera* *105–120 µm when all eight ascospores are fully developed; unitunicate, arising from croziers, in IKI inamyloid; operculate region not distinctly differentiated, subapical ring present; young asci containing glycogen nearly throughout the extrasporal protoplasm, but in maturity only in the basal part. *Ascospores* subglobose, in H_2O *(14)14.6–*16.1*–17.7(18) × (13)13.7–*15.1*–16.6(17) µm, Q=(1.01)1.02–*1.07*–1.11(1.13) (n=100); in H_2O †(12)12.3–*14.7*–17 × 11.5–*13.6*–15.5 µm, Q=1.00–*1.09*–1.19(1.31) (n=100); in heated CB †(12)12.7–*14.4*–16.1(16.7) × (10.9)11.6–*13.2*–14.7(15.1) µm, Q=1.00–*1.09*–1.20(1.28) (n=180); hyaline, with

a large lipid body 8–11 µm broad, uninucleate. Nucleus both in acetocarmine and IKI strongly contrasted, but not stained. Spore wall elastic, De Bary bubbles not formed after 24 h treatment in LACB, perispore slightly cyanophilic, not loosened. Ornamentation consisting of more or less regularly distributed warts, *0.3–1.4 µm broad and 0.2–1.5 µm high, rounded from the top view, mostly obtuse, less often slightly conical or truncate from the side view, sometimes two warts confluent, in IKI distinctly yellow, in heated LACB sky blue, in 5% KOH slightly dissolving. *Paraphyses* cylindric-obtuse to cylindric-clavate, straight, sometimes forked, septate, without exudations, apical cells *44–107(158) × (5)6.3–10.5 µm, †43–58 × 4–6.4 µm, in lower part *3.5–4.8 µm wide, †2.5–4 µm wide. * Paraphyses contain carotenoid granular pigment, deposited in freely floating vacuoles, that turns herbage green to leaf green in IKI, * apical cells very rich in moderately refractive VBs *1.2–7.3 µm in diam., in CRB turning turquoise, in IKI rusty orange; †paraphyses devoid of VBs, in heated LACB cytoplasm pale greyish, walls cyanophobic, carotenoids unchanged. *Subhymenium* *30–50 µm thick, composed of dense *textura intricata-epidermoidea*, cells short, thin-walled, hyaline, *4–10.7 µm wide, with a cyanophilic cytoplasm. *Medulla* *200–230 µm thick in the thickest part, formed by vertically oriented *textura epidermoidea-prismatica*, cells *30–102 × 7.5–19 µm, hyaline, cylindrical, sometimes bent, forked or anastomosing, extremely rich in glycogen (turning rusty-orange in IKI), cytoplasm pale blue in heated LACB, walls cyanophobic, cytoplasm additionally with two types of corpuscles: (1) vacuolar ones, slightly refractive in H_2O, greyish-blue in CRB; (2) possibly resinaceous ones, of a medium refractivity in H_2O, dark purplish in CRB. *Margin* *60–100 µm wide, composed of *textura porrecta*, cells *21–92 × 6–17 µm, †27–41 × 5.5–8 µm, terminal cells *36–57 × 6.5–8.2 µm, †23–55 × 5.5–8 µm, surface covered with a layer of exudations; *containing orange granular pigment, terminal cell's cytoplasm also with VBs, 2–3.5 µm in diam., turning turquoise in CRB, reaction with IKI similar like in paraphyses but in somewhat lesser degree, †devoid of VBs, carotenoid pigment in vacuoles detached from walls, walls hyaline to pale greenish. *Outer cortical layer* *30–79 µm thick, composed of *textura epidermoidea-intricata*, cells *24–45 × 5.8–10 µm, thick-walled, walls gelatinous, LACB-, pale greenish in CRB, cytoplasm stained blue in LACB, with dark purplish bodies in CRB.

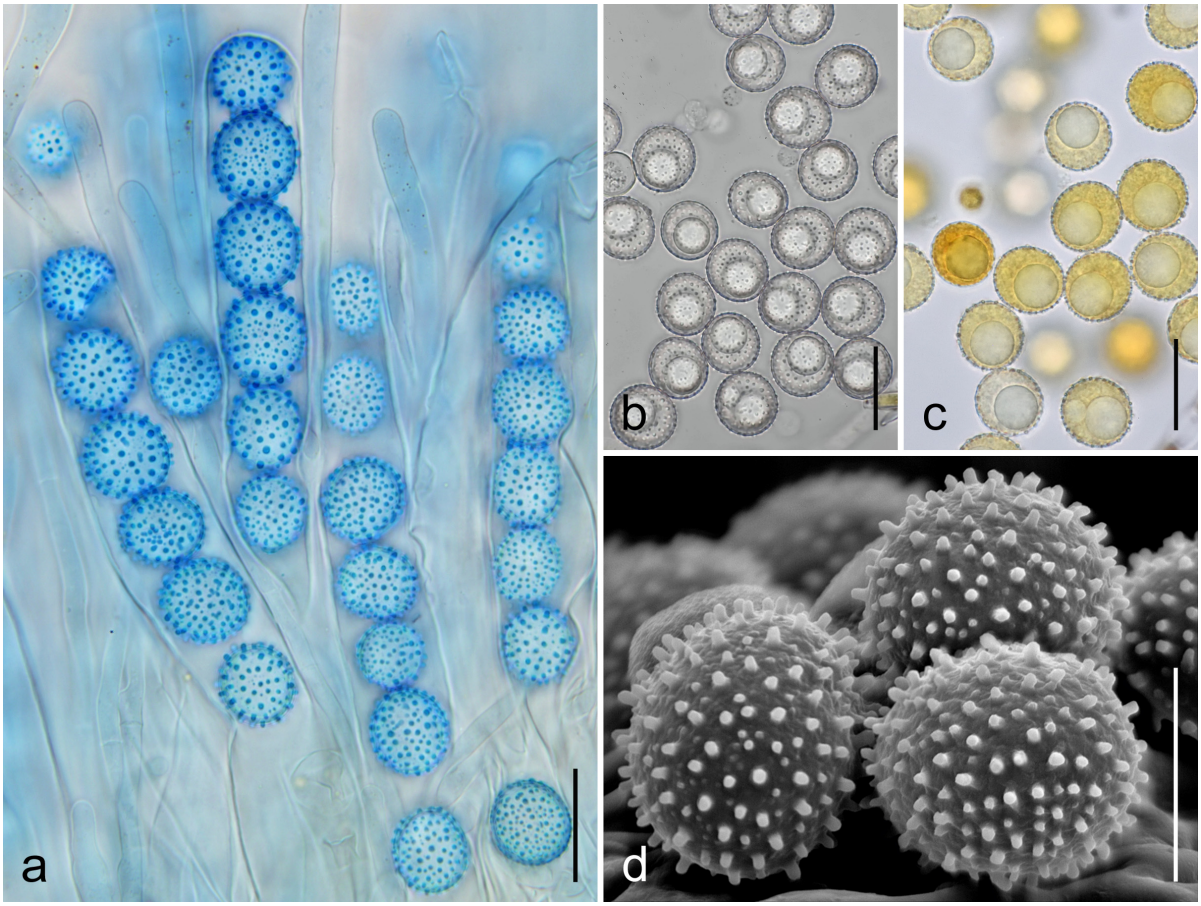

FIGURE 3. *Octospora svrcekii* (a. PRC 4125, b, c. PRM 951720, d. PRM 954236) a. ascospores stained with LACB, b. ascospores in water, c. ascospores in IKI, d. SEM image of ascospores. Scale bars: a–c = 20 µm; d = 10 µm. Photos a. L. Janošík, b,c. Z. Sochorová, d. J. Eckstein.

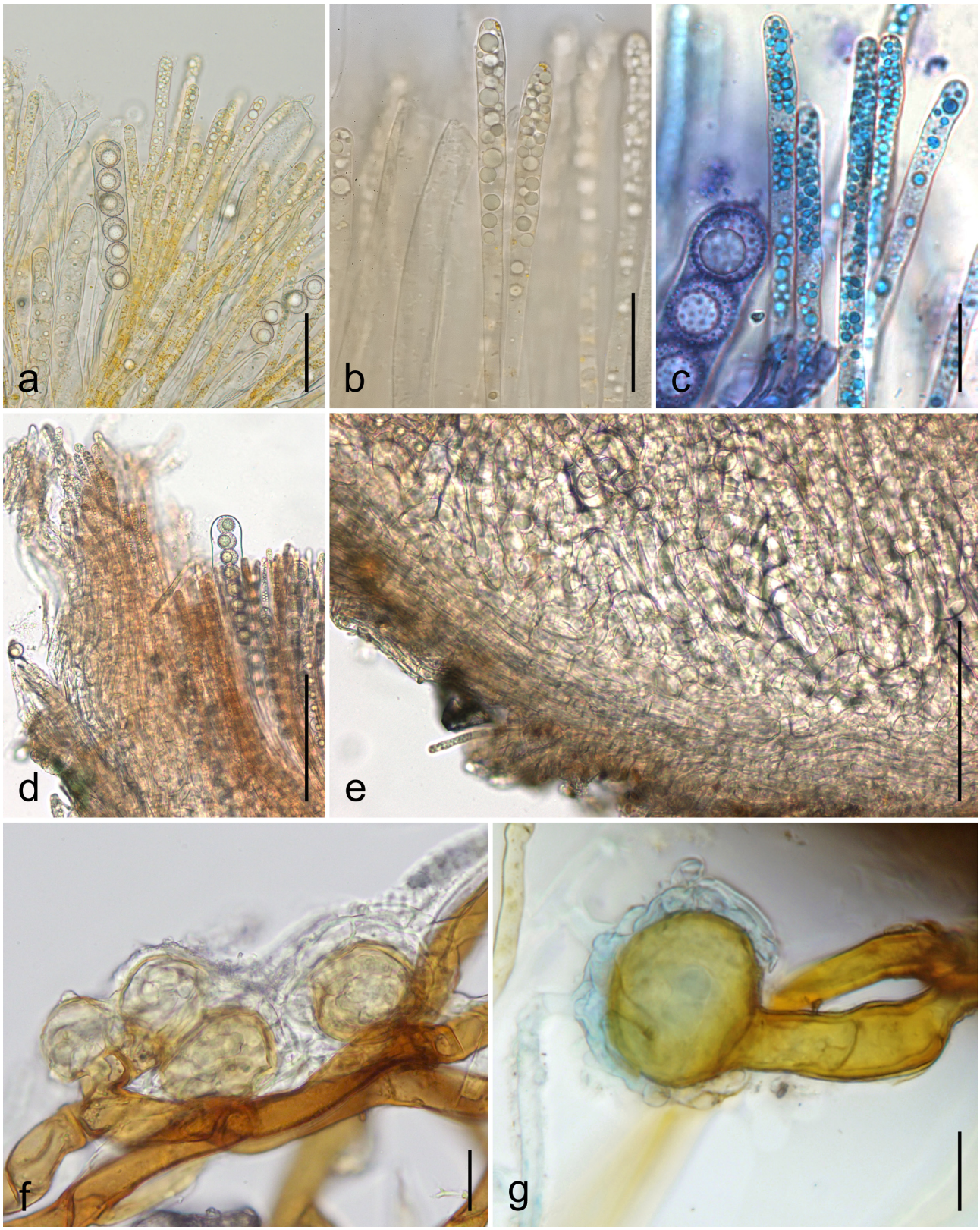

FIGURE 4. *Octospora svrcekii* (a, g. PRC 4125, b–e. PRM 951720, f. PRM 954236) a. hymenium in water showing paraphyses and asci, b. paraphyses in water, c. paraphyses stained with CRB, d. cross section of an apothecium showing hymenium and margin, e. cross section of an apothecium showing medullary and ectal excipulum, f–g. infection of rhizoid of *Cratoneuron filicinum*, f. in KOH, g. hyphae stained with LACB. Scale bars: a, d–e = 100 µm; b–c, f–g = 20 µm. Photos a, g. L. Janošík, b–e. Z. Sochorová, f. J. Eckstein.

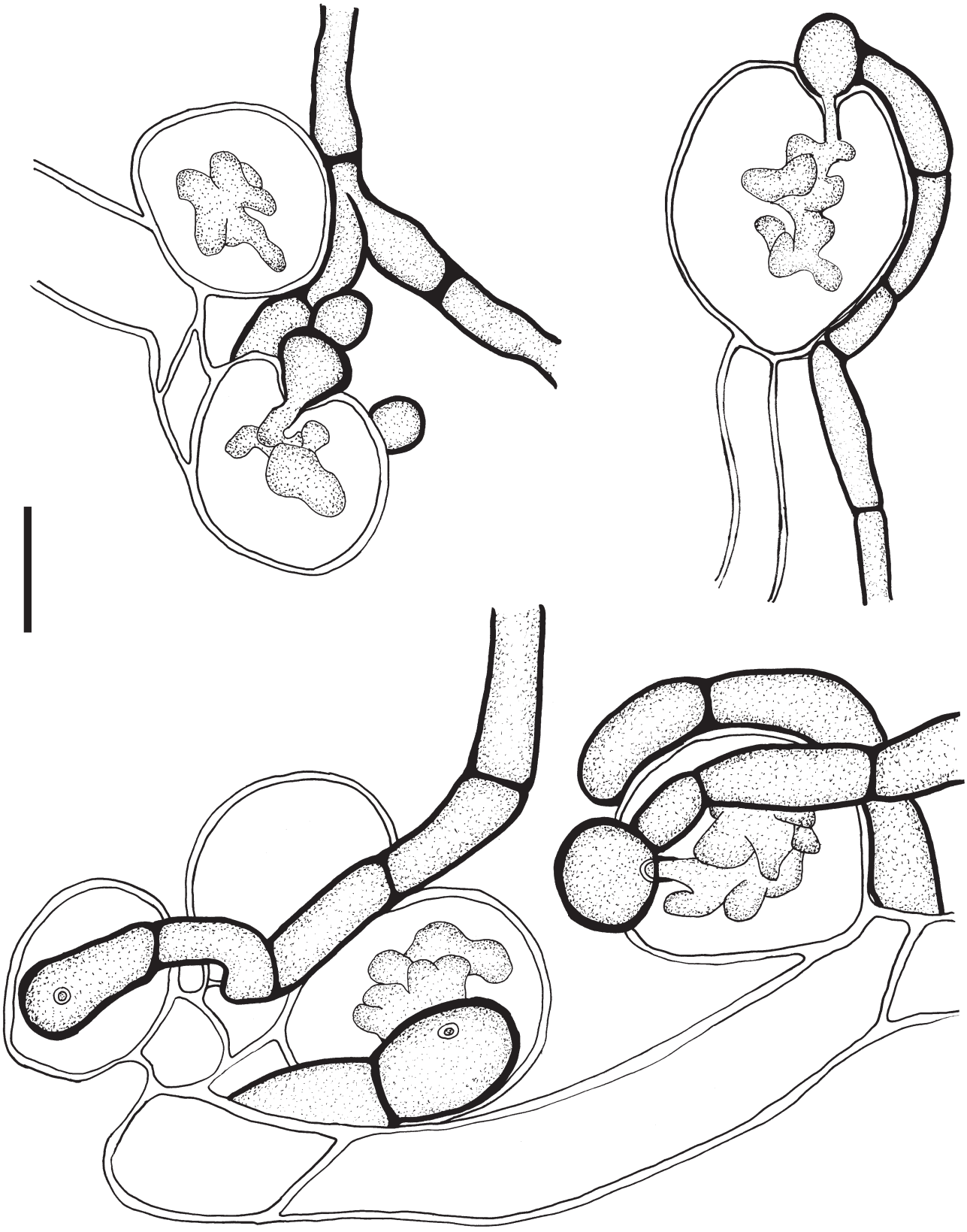

FIGURE 5. Infection structures of *Octospora svrcekii*: galls on rhizoids of *Cratoneuron filicinum* together with attached appressoria, infection pegs and intracellular haustoria, hyphae dotted (PRM 954236). Scale bar = 20 µm. Illustration J. Eckstein.

Infection (Fig. 4 f, g and Fig. 5):—*O. svrcekii* infects the rhizoids of the pleurocarpous moss *Cratoneuron filicinum*. The infection causes the terminal cell of a rhizoid to swell and form a more or less spherical gall. The linear growth of infected rhizoids is stopped, resulting in a terminal position of the gall; galls are also formed terminally on ramifications of the rhizoid. Often, rhizoid initials are infected resulting in clusters of brown galls sitting directly on the underside of the moss stems. The one-celled galls are 30–50 (60) µm in diam. and have brown walls not differing in colour from the unaffected rhizoid parts. At first the galls are only partly covered by thick-walled hyphae 5–8 µm wide, which are later glued together forming a complete cover around the gall. One of the attached hyphal cells surrounding the gall is the appressorium, which at 10–15 µm is only slightly wider than adjacent cells. Often, a depression is formed in the rhizoid-gall in the place where the appressorium is attached. From the appressoria an infection peg, 1–2 µm wide, penetrates the rhizoid wall. On the outside, the penetration point is surrounded by a ring-like ridge 3–4 µm in diam. formed by the rhizoid wall. Inside, the infection peg is surrounded by a tube of host cell wall material. Growing from the infection peg the fungus develops a thin-walled, strongly ramified and curled haustorium which does not grow through the cross walls of the rhizoids. No septa have been observed within the haustoria. The infection does not weaken the host discernibly.

Ecology and distribution:—In all cases, *O. svrcekii* was found on *Cratoneuron filicinum* on calcareous bedrock in very humid habitats – on boulders in brooks or rivers, or in the spray zone of waterfalls. It is known from Albania, Austria, Bosnia and Herzegovina, Croatia, France, Slovakia and Spain.

Specimens examined:—ALBANIA. Tropojë District: Rragam, Prokletije Mts., Valbona, 1420 m a.s.l., 42°24’38’’N, 19°49’30’’E, on *C. filicinum,* 8 July 2014, leg. P. Erzberger, det. J. Eckstein (PRM 954236).

—AUSTRIA. Neunkirchen District: NW of Sonnleiten, 775 m a.s.l., 47°47’44.1”N, 15°51’19.6”E, on *C. filicinum* on a boulder in the Sebastianbach brook, 21 July 2019, leg. Z. Sochorová and M. Sochor, det. Z. Sochorová (WU 42408!); NW of Sonnleiten, 820 m a.s.l., 47°47’50.2”N, 15°51’7.2”E, on *C. filicinum* on a boulder in the Sebastianbach brook, 21 July 2019, leg. Z. Sochorová and M. Sochor, det. Z. Sochorová (WU 42405!); Liezen District: Ardning, Ennstaler Alpen – Ardningalm, 47°36’46.1”N, 14°21’39.2”E, 1040 m a.s.l, on *C. filicinum* on rocks in a small stream, 3 November 2019, leg. L. Janošík and K. Daňková, det. L. Janošík (PRC 4600!).

—CROATIA. Zagreb County: Žumberak - Samoborsko gorje Nature Park, Brisalo waterfall in Slapnica valley, 320 m a.s.l., 45°44’15.1”N, 15°29’34.0”E, on *C. filicinum* in waterfall spray zone and on boulders in a brook, 20 May 2018, leg. N. Matočec, I. Kušan and Z. Egertová, det. Z. Egertová (PRM 951719!, 951720!), duplex (CNF 2/10628!)

—FRANCE. Savoy: Beaufort, Ruisseau du Dorinet near the Camping Municipal Domelin, 720 m a.s.l., 45°43’21.6”N, 6°33’51.8”E, on *C. filicinum* on rocks on the banks of the Ruisseau du Dorinet stream, 25 August 2017, leg. et det. M. Vega (PRM 953066!).

—SLOVAKIA. Košice District: Zádiel, Slovenský kras – Zádielska tiesňava, 370 m a.s.l., 48°37’44.4”N, 20°49’49.7”E, on *C. filicinum* on rocks on the banks of the stream Blatný potok, 29 December 2015, leg. et det. L. Janošík (PRC 4125!); Liptovský Mikuláš District: Hybe, Podtatranská kotlina – Hybická tiesňava, 49°5’23.3”N, 19°53’54.3”E, 820 m a.s.l, on *C. filicinum* on boulders on the banks of the Hybica stream, 21 August 2019, leg. et det. L. Janošík and P. Mlčoch (PRC 4602!).

—SPAIN. Basque Country: Santa Cruz de Campezo, near Oteo, Arroyo Rosaria, 625 m a.s.l., 42°42’15.7”N, 2°21’21.9”W, on *C. filicinum* on the banks of the Rosaria stream, 22 June 2018, leg. et det. M. Vega and R. Martínez-Gil (PRM 953067!); La Rioja: Almarza de Cameros, Arroyo de Tómalos, 990 m a.s.l., 42°15’7.8”N, 2°35’1”W, on *C. filicinum* on rocks on the banks of the Tómalos stream, 23 June 2018, leg. et det. M. Vega and R. Martínez-Gil (PRM 953068!, VIT-Micoteca 9738!). Torrecilla en Cameros, Arroyo de Tómalos, 900 m a.s.l., 42°14’48.8”N, 2°36’48.5”W, on *C. filicinum* on wet boulders, 29 February 2020, leg. et det. R. Martínez-Gil (VIT-Micoteca 9739!).

Discussion

Comparison of *O. svrcekii* microscopic features with older descriptions

So far, only two descriptions of *O. svrcekii* have been published – by Kubička (1972, as *Lamprospora lutziana*) and by Benkert (1998a). Colour photographs were presented by Jukić *et al*. (2020). A comparison of the main features is shown in Tab. 2.

Differences in ascospores, ascal and paraphyses sizes are probably caused by observations of vital vs. herbarium material and mounting media (cf. Baral 1992), although it is not stated in the older descriptions whether the measurements of ascospores include ornamentation. Kubička (1972) reported numerous unequally sized “oil droplets” in the upper

part of the paraphyses. However, the turquoise-blue reaction obtained after staining with CRB *in statu vivo* excludes a lipid origin for these droplets; it is typical for vacuolar bodies (see Baral 1992). Benkert noticed hyaline exudations covering the paraphyses. In our specimens, such exudations were observed only outside the terminal cells of the margin.

TABLE 2. Comparison of features of *O. svrcekii* - our own observations versus previously published data.

Character	Kubička 1972	Benkert 1998a	Our observation
colour of hymenium	brown-orange	brown-orange	orange
diam. of apothecia	1–3 mm	1–3 mm	to 1.4 mm
asci	200–240 × 18–24 µm	ca 200–250 × 17–20 (24) µm	*H_2O 243–322 × 22–28 µm †H_2O 190–232 × 18–19 µm
ascospores	13–15 µm	14–16 × 13–15 µm	*H_2O *(14)14.6–*16.1*–17.7(18) × (13)13.7–*15.1*–16.6(17) µm †H_2O (12)12.3–*14.7*–17 × 11.5–*13.6*–15.5 µm CB (12)12.7–*14.4*–16.1(16.7) × (10.9)11.6–*13.2*–14.7(15.1) µm
shape	globose, very rarely broadly ellipsoid	subglobose to broadly ellipsoid	globose to subglobose
Q	not given	1.07	*H_2O (1.01)1.02–*1.07*–1.11(1.13) †H_2O 1.00–*1.09*–1.19(1.31) †heated CB 1.00–*1.09*–1.20(1.28)
warts	not given	ca 0.5–1 µm high and broad	*0.3–1.4 µm broad, 0.25–1.5 µm high
ascospore guttule diam.	8–9 µm	8–10 µm	*8–11 µm
paraphyses width	5–6 µm	apically 5–7 µm	*3.5–4.8 µm wide, apically (5)6.3–10.5 µm †2.5–4 µm wide, apically 4–6.4 µm

Section *Wrightoideae*

Interestingly, the section *Wrightoideae* is the only section currently recognized within the genus *Octospora*. Moravec (1997) used the term "sectio *Neottiellae*", which is nowadays usually treated as a separate genus, *Neottiella* (Cooke 1879: 261) Saccardo (1889: 190) (cf. Benkert 1998b, Eckstein *et al*. 2014, Vega 2017). Kubička (1972) introduced the section *Ovalisporae* for species in the genus *Lamprospora* (which in his concept also included some species recently assigned to the genus *Octospora*) with broadly ellipsoid to subglobose ascospores. It was typified by *Octospora wrightii* (sub *Lamprospora wrightii*) - the same species later chosen by Benkert for the sect. *Wrightoideae*. However, the concept of sect. *Ovalisporae* is different from the one for the sect. *Wrightoideae*. Being defined by the shape of ascospores only, *Ovalisporae* could include very different and unrelated species, e.g. also those with reticulate ornamentation of the ascospores like *L. dictydiola* Boudier (1907: 68), *L. carbonicola* Boudier (1907: 68), or *L. retispora* (Itzerott & Thate 1974: 506) T. Schumacher (1986: 61), and would not form a monophyletic group. Therefore, we consider the section *Ovalisporae* obsolete and of no value in current *Octospora* taxonomy.

In the following, the diagnostic characters of the section *Wrightoideae* are discussed. Since none of these traits alone is sufficient to effectively define the section, only species exhibiting all three characters can qualify for placement in the section, such that monophyly is preserved.

1) Parasitism on mosses in the order Hypnales (and originally also Neckerales)

The species forming a monophyletic group around *O. wrightii* are all linked to mosses in the order Hypnales. This also applies to *O. americana*, with its hosts *Forsstroemia trichomitria* (Hedwig 1801: 82–83) Lindberg (1863: 605) and *Cryphaea glomerata* Schimp. ex Sull. in Gray (1856: 656). These mosses were earlier placed in the Neckerales by some authors (Frey *et al*. 1995), but later phylogenetic analyses have placed them in the order Hypnales (Olsson *et al*. 2009). Considering the high degree of host specificity among bryophilous Pezizales, the two hosts identified in samples of *O. americana* suggest two closely related, unresolved taxa. Our sample set included the holotype specimen on *Forsstroemia trichomitria*, but unfortunately no collection from *C. glomerata* was available for comparison.

The two bryophilous species *O. affinis* and *O. orthotrichi* were also included in section *Wrightoideae* by Benkert (1998a) and Benkert & Krieglsteiner (2006), but this placement is not supported by our phylogenetic analysis. They grow on species of the moss genus *Orthotrichum*, which was formerly placed in the Neckerales but today is placed in the Orthotrichales (Goffinet *et al*. 1998, Goffinet *et al*. 2008). Recently, two more species on Hypnales were described: *Octospora pannosa* on *Brachytheciastrum velutinum* Hedwig (1801: 272) Ignatov & Huttunen (2002: 260) (Vega *et al.* 2018) and *O. conidiophora* on *Trichosteleum perchlorosum* Brotherus & Bryhn in Bryhn (1911: 24) and *Sematophyllum brachycarpum* (Hampe 1844: pl. 11) Brotherus in Engler & Prantl (1925: 431) (Sochorová *et al*. 2019). These do not induce galls and do not cluster in the lineage with *O. wrightii*.

2) Formation of rhizoid galls

This feature was reported in several species of *Octospora* outside the section (including smooth-spored species) and even in two species of *Lamprospora* (for details see Vega *et al*. 2016).

3) Ellipsoid, broadly ellipsoid or subglobose ascospores ornamented by isolated warts

In the diagnosis of section *Wrightoideae*, ellipsoid and broadly ellipsoid ascospores are mentioned, while in the rest of the paper subglobose ascospores are considered as well (Benkert 1998a). Such a combination of ascospores´ shapes and the ornamentation type is common in bryophilous Pezizales. Apart from species in the *Wrightoideae* it also occurs in *Octospora* species inducing galls on acrocarpous mosses (e.g. *Octospora pseudoampezzana* Svrček (1969: 83) Caillet and Moyne (1987: 180) or not inducing galls but linked to acrocarpous mosses (e.g. *O. similis* (Kirschstein in Jaap 1922: 9) Benkert (1996: 51), or even liverworts (*O. ithacaensis* (Rehm 1904: 35) K.B. Khare 1975: 111). It can also be found in *Neottiella vivida* (Nylander 1865: 467) Dennis (1960: 28) or *L. ecksteinii* Benkert (2009: 52), which infect acrocarpous mosses but are not known to induce galls.

The species in the *O. wrightii* lineage have a Q-value of ascospores between 1.01 and 1.28 (our data and Benkert 1998a) and could therefore be judged as globose, subglobose or broadly ellipsoid, according to the classification of Kušan *et al*. (2014).

Distinguishing *O. svrcekii* from similar species

Octospora svrcekii could most likely be confused with other species in the *O. wrightii* lineage (Figs 1, 6). *Octospora americana* has smaller ascospores (12)13–15 × (10)11–13 µm (Q = 1.17) ornamented with conical warts. The host in the holotype specimen was *Forsstroemia trichomitria,* with other records (possibly representing a different taxon) on *Cryphaea glomerata.* It is only known from a few localities in North America (Benkert 1998a). *Octospora erzbergeri* has slightly smaller ascospores with a higher Q value: 13–15(15.5) × 11–13(14) µm (Q = 1.17). Furthermore, it is conspicuous due to long hyaline hairs at the margins of the apothecia. It infects *Pseudoleskeella nervosa* (Bridel 1806: 132) Nyholm (1969: 776) and can be found on the bark of trees or on calcareous rocks (Benkert 2006, Németh 2017). *Octospora hygrohypnophila* inhabits similar environments to *O. svrcekii*, but has slightly smaller ascospores: 13–15(16) × (10)10.5–12(12.5) µm with a higher Q value (about 1.24) and its host is *Hygrohypnum luridum* (Hedwig 1801: 291) Jennings (1913: 287) (Benkert 1998a). *Octospora texensis*, known from North America, has ascospores 16–18 × (12.5)13–14(15) µm µm (Q = 1.28) (Benkert 1998a). Its host is *Schwetschkeopsis fabronia* (Schwägrichen 1830: 294) Brotherus in Engler & Prantl (1907: 878). *Octospora wrightii* differs in having smaller ascospores (11)12–14(15) × 11–13(13.5) µm (Q = 1.08) and infects mosses in the genus *Amblystegium* Schimper in Bruch *et al*. (1853: 45). It is usually found on stones or bark (Benkert 1998a). *Lamprospora lutziana* (original identification of the type specimen of *O. svrcekii*) occurs in similar habitats to *O. svrcekii,* but has globose ascospores 15.2–18.6 µm in diameter, infects the leaves and stems of *Philonotis fontana* (Hedwig 1801: 195) Bridel (1827: 18) and does not induce galls (Martínez-Gil *et al*. 2019).

Conclusion

Octospora svrcekii is currently known from 13 localities, making its known range ca. 1950 km (from the most western locality in Torrecilla en Cameros, Spain, to the most eastern locality in Zádielska dolina, Slovakia). It always occurs in

constantly wet places with its host *Cratoneuron filicinum*. Both phenotypically and phylogenetically, it clearly belongs to the section *Wrightoideae*.

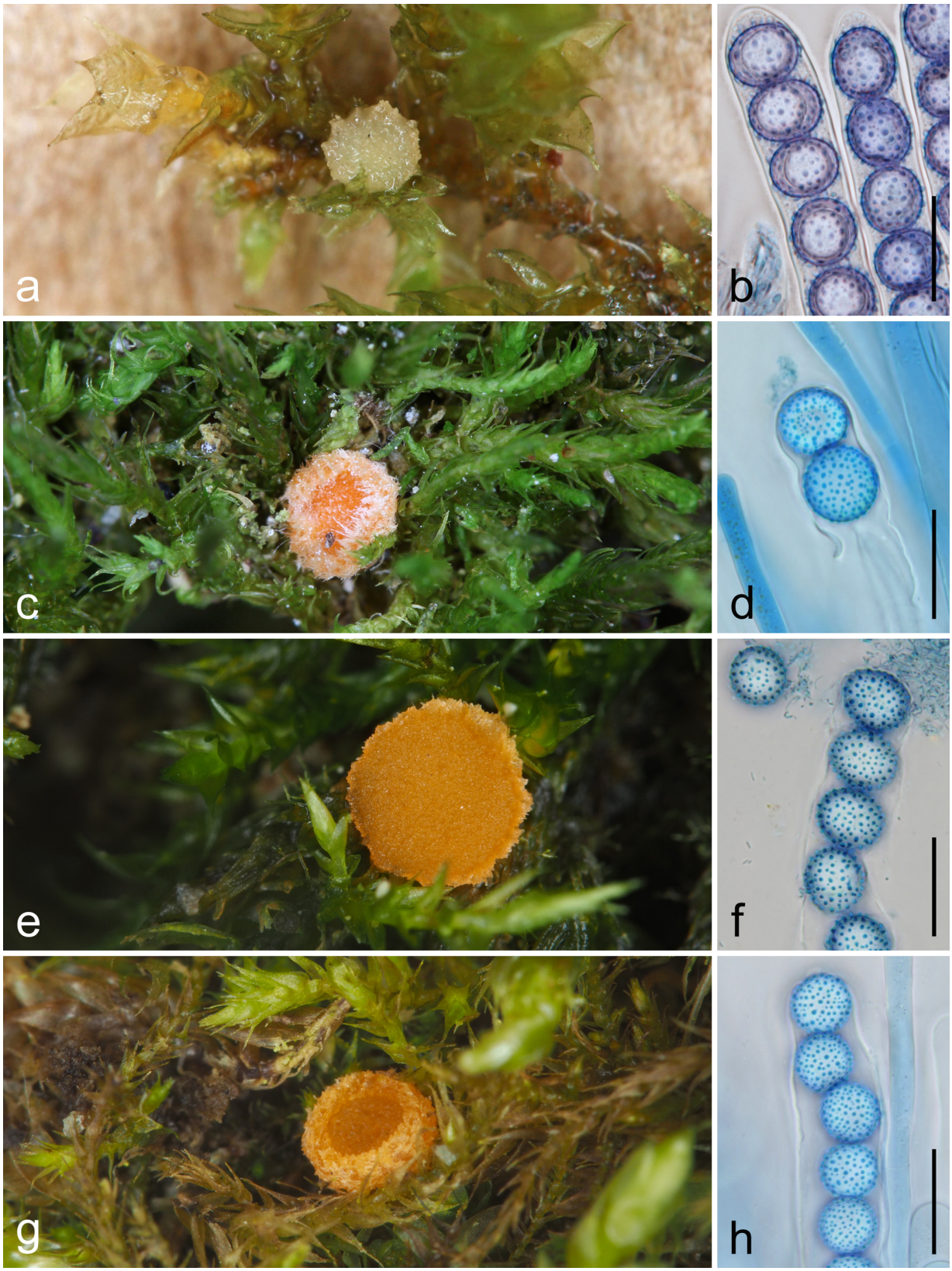

FIGURE 6. Other members of the section *Wrightoideae* a–b. *Octospora americana* (S F43718, holotype), a. apothecium on shoot of *Forsstroemia trichomitria*, b. ascospores stained with cotton blue, c–d. *O. erzbergeri*, c. apothecium on shoots of *Pseudoleskeella nervosa* (PRM 945799), d. ascospores stained with cotton blue (PRC 4603), e–f. *O. hygrohypnophila*, e. apothecium on shoot of *Hygrohypnum luridum* (PRC 4605), f. ascospores stained with cotton blue (PRC 4604), g–h. *O. wrightii*, g. apothecium on shoots of *Amblystegium serpens* (PRC 4607), h. ascospores stained with cotton blue (PRC 4606). Scale bars = 20 µm. Photos a–c. Z. Sochorová, d–h. L. Janošík.

None of the diagnostic criteria alone are exclusive to this section. Species of the monophyletic lineage around *O. wrightii* can be characterised by the combination of 1) the growth on mosses in the order Hypnales (not Neckerales), 2) formation of rhizoid galls and 3) globose, subglobose or broadly ellipsoid ascospores ornamented with isolated warts. Taxa infecting mosses from orders other than the Hypnales, or not inducing the formation of galls (including *O. orthotrichi* and *O. affinis*), do not belong to the group. Therefore, we suggest that the section *Wrightoideae* should be restricted to *O. wrightii*, *O. erzbergeri*, *O. hygrohypnophila*, *O. svrcekii*, *O. americana* and probably also *O. texensis* which fulfils all the above-mentioned criteria.

Acknowledgments

We thank Arne Anderberg (Swedish Museum of Natural History) for the loan of the holotype specimen of *Octospora americana*, Csaba Németh for providing the specimen of *Octospora svrcekii* from Albania and Nedim Jukić for information on the Bosnian & Herzegovinian record of *Octospora svrcekii*. The work was partly supported by the Ministry of Agriculture of the Czech Republic (institutional support MZE-RO0418) and also by the Croatian Science Foundation under the project ForFungiDNA (IP-2018-01-1736). Z. Sochorová and M. Sedlářová were supported by an internal grant from Palacký University (IGA PrF-2020-003), L. Janošík by Institutional Support for Science and Research of the Ministry of Education, Youth and Sports of the Czech Republic. The authors are grateful to Lee Knight for making improvements to the English text.

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5. Discussion

During the study of bryophilous ascomycetes, esp. Pezizales, large amount of data (records, collections, descriptions, photographs and sequences) was gathered, primarily to fullfil the aims of the Ph.D. thesis but also exceeding its scope. The following discussion is divided according to the main goals and results presented within the thesis.

5.1 Bryophilous Pezizales in Borneo and South Africa

(this chapter is based on Egertová et al. 2018b and Sochorová et al. 2019)

During the excursions to the Kelabit Highlands (Borneo, Sarawak, Malaysia) in 2016 and 2017, two remarkable hepaticolous species were collected. These were described as *Octosporopsis erinacea* and *Octospora kelabitiana*.

Octosporopsis erinacea is a unique species due to the following combination of characters: very small apothecia (400–600 µm high and 450–550 µm wide) which are pale yellow to almost hyaline, lacking a prominent margin and covered by thick-walled hyaline hairs; ectal excipulum composed of textura angularis, usually 8-spored asci and ellipsoid, delicately rough ascospores sized (16)18–25(31) × 10–14(16) µm. It grows on the dorsal thallus surface of *Dumortiera hirsuta*, exceptionally also on male receptacles, and it was always found on boulders in riverbeds (Egertová et al. 2018b).

Octosporopsis is the newest genus of bryophilous Pezizales so far, established in 2014 for *Octosporopsis nicolai* (Maire) U. Lindem., M. Vega & T. Richt., growing on *Lunularia cruciata* (Lindemann et al. 2014). *Octosporopsis erinacea* is only the second known member of the genus. The two species share the host preferences for thallose liverworts, presence of thick-walled hairs and ellipsoid, hyaline ascospores without large lipid bodies. Nevertheless, they can be easily distinguished; apothecia of *O. nicolai* reach up to 7 mm in diameter, are first obconical and then disc-shaped, light yellow, pink or orange. Its ascospores are ca 1.5× larger than in *O. erinacea*, sized (26)27–39(41) × (12)15–18.5(20.5) µm (Lindemann et al. 2014). *Octosporopsis nicolai* is known from Europe, while *O. erinacea* from Asia; beside Borneo, it was reported also from Japan (Hosono et al. 2021).

Octospora kelabitiana is macroscopically characterised by very small apothecia (180–350 µm high, 160–400 µm wide) which are often taller than wide, barrel- or cup-shaped, light pinkish or orange, without a prominent margin and covered by thick-walled hyaline hairs. Characteristic microscopic features are 8-spored asci, ellipsoid, delicately rough ascospores containing mostly two lipid bodies and measuring (13.5)14.5–17(18) × 7–8(9) µm (in water), ectal excipulum composed of textura angularis and superficial mycelium bearing conspicuous warts and ridges – a feature unknown in any other species of bryophilous Pezizales at the time when it was described. It grows on thalli of *Riccardia* spp., most often on decaying trunks in streams or in their surroundings, less also on boulders (Egertová et al. 2018b).

In February and March 2018, an excursion to South Africa (provinces KwaZulu-Natal and Mpumalanga) was carried out. During three weeks, 39 collections of bryophilous ascomycetes belonging to genera *Octospora*, *Lamprospora* and *Neottiella* were gathered, although no records had been published from the country previously. Only three collections were possible to assign to already described species – two collections were identified as

Lamprospora campylopodis (on *Campylopus pyriformis*) and one as *Neottiella albocincta* (Berk. & M. A. Curtis) Sacc. (on *Atrichum androgynum*). The remaining specimens were separated into six morphospecies. The most frequent one was an undescribed *Octospora* species growing on pleurocarpous mosses *Sematophyllum brachycarpum* and *Trichosteleum perchlorosum* (Sematophyllaceae, Hypnales). It shared many features with *Octospora kelabitiana* – apothecia with stiff, thick-walled hyaline hairs, ellipsoid, hyaline ascospores of similar size (*13–17.2 × 7–10.5 µm, †13–17 × 7.5–10.5 µm), filiform, unbranched paraphyses, and even the warted mycelium. Nevertheless, it could be distinguished easily by the growth on a completely different host. Furthermore, its apothecia were larger (0.2–1.5 mm broad, up to 0.65 mm high) and often had a prominent margin. Its appressoria were very often two-celled, sometimes even three-celled, while *O. kelabitiana* usually had one-celled appressoria.

Molecular analysis revealed that the morphospecies included four cryptic taxa and that they were very closely related to *O. kelabitiana*. As no distinct morphologic differences were found between the four cryptospecies, we decided to describe the most common lineage (11 collections) as *Octospora conidiophora* (s. str.) and for the three remaining ones to establish an informal taxon *O. conidiophora* agg., which comprises also *O. kelabitiana* (Sochorová et al. 2019).

Interestingly, *Spermospora*-like anamorph was detected in most of the collections of *Octospora conidiophora* agg., thanks to which the taxon became the first one among bryophilous Pezizales with discovered anamorph. To date, there is reported one more species with anamorph – *Octospora bicarpa* producing ameroconidia (Döbbeler et al. 2021a).

It is certain that both Borneo and South Africa, as well as other remote regions, hide many undescribed species of bryophilous ascomycetes. Some of them (e.g. a very tiny pezizalean species occurring in the phyllosphere and infecting the liverwort *Caudalejeunea recurvistipula*, perhaps representing a new genus, or *Octospora* on *Lindbergia pseudoleskeoides*) have been discovered by the author, but need to be recollected in a better shape. Unfortunately, the research in these regions is very limited due to the lack of specialists, often challenging terrain and bureaucratic obstructions.

5.2 Bryophilous ascomycetes in Europe

(this chapter is based on Egertová et al. 2018a, Sochorová et al. 2021, 2023 and Baral et al. 2023, and on chapter 4.2.5)

Taking into account the number of scientists interested in mycology and bryology, Europe is certainly the best inspected continent concerning bryophilous ascomycetes. Despite this fact, new species are still being discovered in European countries even in the most recent years (e.g. Döbbeler et al. 2018b, Eckstein et al. 2021, 2022, Vega et al. 2016, 2017, 2018, 2019, 2021a, 2021b). During Christmas 2014, an interesting *Octospora* species infecting the acrocarpous moss *Dicranoweisia cirrata* was found for the first time in the vicinity of the ruin of castle Děvín in the surroundings of Hamr na Jezeře, Czech Republic. It was recollected at the same locality in years 2015, 2016, 2019 and 2020 (Sochorová et al. 2021) and 2021 (unpublished record, PRM 956464) and described as *Octospora doebbeleri*, in honour of Peter Döbbeler. Beside infecting the moss *Dicranoweisia cirrata,* it is characterised by pinkish, reddish or reddish orange apothecia with a thin margin and 8-spored asci with ellipsoid, mostly binucleate ascospores ornamented with small isolated warts (Sochorová et al. 2021).

Especially the binucleate ascospores within octosporic asci represent an interesting character. Bryophilous Pezizales, similarly to other species of the family Pyronemataceae, usually produce uninucleate ascospores (Berthet 1964, Dissing & Sivertsen 1983, Billekens 1992, Jaklitsch et al. 2016, Kullman 2002, Perry et al. 2007 or Sochorová et al. 2020). Binucleate ascospores within 4-spored asci are known in some *Octospora* spp. (Senn-Irlett 1988, Weber 1992, Benkert 1998a, Janošík 2020), tetranucleate ascospores have been reported in a few species of the genus *Octosporella* (Corner 1929, Janošík 2020). Binucleate ascospores in 8-spored asci have been observed in *Octosporopsis nicolai* Lindemann et al. (2014), but tetranucleate ascospores in the same species by Janošík (2020). Janošík (2020), who analysed the number of nuclei in ascospores of 52 species of bryophilous Pezizales, found binucleate ascospores in octosporic asci only in five species, none of them belonging to the genus *Octospora*.

The second species new to science, *Lamprospora sylvatica,* was described based on specimens from Ukraine, Slovakia, Germany and Norway. It is characterised by the combination of the following features: pinkish, orange to reddish-orange apothecia with a fimbriate margin, globose ascospores with more or less regular areolate ornamentation, infecting strong rhizoids of *Dicranum montanum* with an infectious structure consisting of a one-celled appressorium surrounded by a multi-layered cluster of thick-walled cells and haustorium within the rhizoids. The apothecia were always found on rotten wood, which is an unusual habitat for hosts of bryophilous Pezizales (Egertová et al. 2018a).

Another new species described from Europe is *Lamprospora aberrans*, described based on collections from Spain and Croatia (Sochorová et al. 2023). It is a unique species due to the combination of hairy apothecia, globose ascospores ornamented with bent ridges and infection on rhizoids of *Gymnostomum* spp. While hairs are known in members of the genera *Octosporella*, *Octosporopsis*, *Octospora* and *Neottiella*, these have not been documented in *Lamprospora* before. Therefore, *L. aberrans* is the first species of the genus with this noticeable character. Furthermore, *Gymnostomum* became known as a new host for bryophilous Pezizales.

On the 1st January 2021, an inoperculate bryophilous ascomycete *Helotium fulvum* was found by the author for the first time in the vicinity of Sloup in North Bohemia. Subsequent searching in similar localities (especially sandstone areas in North Bohemia) brought 23 other collections of the species from Czechia, although the species was reported in literature only twice after being described by Boudier (1897). A similar case was *Mniaecia jungermanniae*, collected in 66 localities in the Liberec region within two years (Egertová et al. 2016b). Both these cases demonstrate how severely overlooked bryophilous ascomycetes have been.

Clarification of the phylogenetic position of *Helotium fulvum* was rather complicated. It resembled the family Rutstroemiaeceae in having uninucleate ascospores with high lipid content and an ectal excipulum of textura porrecta, but deviated in its bryophilous lifestyle and the thick-walled inamyloid ascus apex. Combined phylogenetic analysis of ITS and LSU rDNA and EF1α confirmed that the species belongs to the sclerotiniaceous clade, which comprises the paraphyletic families Rutstroemiaceae and Sclerotiniaceae. *Helotium fulvum* formed with *Clarireedia* a supported clade (Rutstroemiaceae s.l.), though with high distance. We have decided to establish the new genus *Bryorutstroemia* to accommodate the species. Nevertheless, the phylogeny of the sclerotiniaceous clade is still not fully clear and will require including more species and analysing additional loci.

Additionally, the research on bryophilous ascomycetes in the Czech Republic brought findings of several rare species. To the most surprising collections belong *Lamprospora moynei* and *Octospora bridei* found during hot summer 2022 in the Plané Loučky NR, which is one of the mycologically best explored localities in the Olomouc region (Sochorová & Kříž 2022). In February 2021, an undescribed species on *Pseudotaxiphyllum elegans* was collected in the vicinity of Dolany u Olomouce; this was subsequently described as *Octospora oscarii* Eckstein, Sochorová & Janošík (Eckstein et al. 2021). An important find is *Octospora pseudoampezzana*, which has been listed in the Red list of fungi (macromycetes) of the Czech Republic in the category ?EX (probably extinct). Another locality in the Czech Republic has been discovered by Lukáš Janošík (Janošík 2020). Therefore, it needs to be reclassified in the new version of the Red list, which is currently under preparation. Other rare species of bryophilous Pezizales (e.g. *Lamprospora esterlechnerae*, *L. lubicensis*, *L. moynei*, *L. pseudoarvensis*, *Neottiella ricciae*, *Octospora bridei*, *O. doebbeleri*, *O. oscarii* and *O. pannosa*) have been nominated by Zuzana Sochorová, Lukáš Janošík and Jan Běták to complete the Red list.

5.3 *Octospora svrcekii* and phylogeny of the section *Wrightoideae*

(this chapter is based on Sochorová et al. 2020)

Octospora svrcekii was described by Benkert (1998b) based on a collection from Slovakia and later published only from two other localities in the world (Capoen 2018, Jukić et al. 2020). In our paper, *O. svrcekii* was described in detail following the principles of vital taxonomy and reported from new localities in Albania, Austria, Croatia, France, Slovakia and Spain (Sochorová et al. 2020). Additionally, the species was collected in Armenia in summer 2023 (Tavush province, NP Dilijan, 1.1 km N of Haghartsin Monastery, at the Hidden Waterfall, 40°48'42.5"N, 44°53'15.8"E, on *Cratoneuron filicinum* on travertine in a broad-leaved forest, 1510 m a. s. l., 9 July 2023, leg. et det. Z. Sochorová, PRM 959596). The Armenian locality is now the most eastern known locality of the species, located ca 2080 km far from Zádielská dolina, which was the most eastern before. Thus, it can be assumed that distribution of the species, as well as other bryophilous ascomycetes, is larger than current records indicate.

Another part of the work was dedicated to the phylogeny of the section *Wrightoideae*, which was established by Benkert (1998b) and defined by the following criteria: 1) parasitism of mosses in the orders Hypnales and Neckerales, 2) formation of stipitate galls on rhizoids of the hosts and 3) ellipsoid or broadly ellipsoid ascospores ornamented by isolated warts. The section was named after the type species *Octospora wrightii* and further included *O. americana* Benkert, *O. hygrohypnophila*, *O. orthotrichi* (Cooke & Ellis) K. B. Khare & V. P. Tewari, *O. svrcekii* and *O. texensis* Benkert. Two more species, *O. erzbergeri* (Benkert 2006) and *O. affinis* Benkert & L. G. Krieglsteiner (Benkert & Krieglsteiner 2006), were added to *Wrightoideae* later, although *O. affinis* does not induce galls and therefore it does not fullfil all the three diagnostic criteria.

Our phylogenetic analysis, which included all species of *Wrightoideae* but *O. texensis*, has shown that a monophyletic group is formed by *O. wrightii*, *O. americana, O. erzbergeri, O. hygrohypnophila* and *O. svrcekii*, but does not include *O. affinis* and *O. orthotrichi* which are linked to *Orthotrichum* spp. (earlier classified in the order Neckerales, recently in Orthotrichales).

In 2023, *O. tucumanensis* Catania & G. M. Suárez was described from Argentina, also clustering to the section *Wrightoideae* and forming a sister lineage to *O. americana* (Suárez et al. 2023). The newly described species induces galls on rhizoids of *Dimerodontium balansae* (Fabroniaceae, Hypnales) and has subglobose warted ascospores.

6. Conclusion and future perspectives

In 1997, Peter Döbbeler stated that 'our knowledge of the bryophilous ascomycetes is in an almost Linnean situation, especially outside Central Europe' (Döbbeler 1997). Though many interesting discoveries have been made since that time (new species, new localities, interactions of fungi with their host bryophytes), bryophilous ascomycetes remain a rather marginal research topic. This thesis represents a modest contribution to the knowledge on this fascinating, but often overlooked group of fungi. Publications included in the thesis are based on collections from the Czech Republic and other European countries, as well as from remote regions like Borneo and South Africa. Three species of the genus *Octospora*, two of *Lamprospora* and one of *Octosporopsis* were described as new to science. Moreover, the first anamorph among bryophilous Pezizales was detected, namely in *Octospora conidiophora* agg. from South Africa. *Octospora svrcekii*, previously a rarely reported species, was collected from new localities in Europe and described in detail based on vital material. The new genus *Bryorutstroemia* was established for the enigmatic species *Helotium fulvum*. Several rare species were found in the Czech Republic, including many which were previously not reported from the country, along with *Octospora pseudoampezzana* which was listed as probably extinct in the Red list of macromycetes of the Czech Republic.

Although bryophilous ascomycetes have been studied for more than two centuries, there are still many aspects in the need of further study. First of all it is discovery of the global species richness and unveiling their distribution in both recently inspected as well as new regions. Several countries still lack any data on the occurrence of fungi associated to bryophytes but their presence in the ecosystems is more than probable. My experience from both South Africa and Borneo illustrates that such underexplored regions can be very rich in undescribed species. Even in Europe, specialists are aware of dozens of them, but in many cases they need to be recollected in sufficient quantity and quality, and described in detail, ideally following the principles of vital taxonomy. Many species still await to be discovered.

Molecular data indicate that the genus *Octospora* is paraphyletic and could be split into several smaller genera. A multigene phylogeny will be needed to reliably elucidate the generic boundaries. Another task following primary descriptions of species and their host range is disentangling species complexes, e.g. *Lamprospora miniata* agg. or *Octospora gyalectoides* agg., employing integrative taxonomy. This will require also studies on type specimens, including verification of their host bryophytes.

Another interesting topic is disclosure of the interaction between ascomycetes and the host bryophytes. This is challenging especially in biotrophic parasites which need living tissues for their growth, as cultivation of host bryophytes as well as tiny fungi in the laboratory is rather complicated. Similarly to interactions of higher plants with fungi we can expect that environmental factors and other interacting microorganisms, esp. bacteria, might influence balance between a bryophyte and an ascomycete. Techniques of molecular biology, esp. new generation sequencing, could help to gather more information to interpret raising questions.

It is obvious that there is a lot of work to do in bryophilous ascomycetes. I hope this Ph.D. thesis will encourage other mycologists and bryologists to study these beautiful and interesting organisms.

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