DOI 10.12905/0380.sydowia74-2021-0163 Published online 11 November 2021

Belonium coroniforme Rehm (Helotiales), a highly specialized muscicolous ascomycete on Orthotrichaceae and *Leucodon*

Peter Döbbeler[1], Arnold Büschlen[2], Jan Eckstein[3,*] & Andrin Gross[4]

[1] Ludwig-Maximilians-Universität München, Systematische Botanik und Mykologie, Menzinger Str. 67, 80638 München, Germany
[2] Lötschenmattstraße 10, 8912 Obfelden, Switzerland
[3] Arnoldiweg 20, 37083 Göttingen, Germany
[4] Swiss Federal Research Institute WSL, SwissFungi, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

* e-mail: jan.eckstein@octospora.de

Döbbeler P., Büschlen A., Eckstein J. & Gross A. (2021): *Belonium coroniforme* Rehm (Helotiales), a highly specialized muscicolous ascomycete on Orthotrichaceae and *Leucodon*. – Sydowia 74: 163–174.

The ascomycete *Belonium coroniforme* Rehm (Helotiales) forms both orange apothecia and apothecia-like conidiomata (sporodochia) on corticolous moss species of the genera *Lewinskya*, *Orthotrichum* and *Ulota* (Orthotrichales), as well as *Leucodon* (Hypnales). Both the apothecia and the sporodochia are restricted to the female shoot apices (perichaetia), producing a compact mycelial wedge within the host stems. The fungal morphs can occur together or separately on shoot apices of the same or different plants. Infection prevents the normal development of sporophytes, which means that capsules with mature spores are not produced, thus reducing the host's fertility. In contrast, except for the infected shoot apices, the gametophytic plants retain their green healthy appearance and continue to grow by subapical innovation. *Belonium coroniforme* is sporadically documented in Europe and also occurs in Nepal and Tunisia. Apart from having a hyphomycetous anamorph, it is characterized by a clear preference for orthotrichalean mosses and by occupying a distinct microniche. This high specialization has never been observed in muscicolous apothecial ascomycetes before. Sequence data from eight different collections taken from five different host species did not reveal any variation within the ITS1, ITS2 and LSU region of the rDNA, with inconclusive generic placement. Isolation attempts of the fungus from infected moss parts and ascospore germination experiments were not successful on standard malt-extract agar medium, strongly suggesting an obligate biotrophic lifestyle. The aim of this article is to present a comprehensive description of *B. coroniforme* and its host-parasite relationship as well as to provide initial genetic resources for the species.

Keywords: Bryophilous fungi, conidiomata, microniche, reduction of fertility, sex specificity.

In 1907 the German physician and mycologist Heinrich Rehm published a new helotialean ascomycete under the name *Belonium coroniforme* (Rehm 1907). Rehm based his description on a specimen collected by C. Laubinger near Bad Gastein in Styria (now in Salzburg, Austria). The fungus formed vividly coloured apothecia on shoot apices of an *Orthotrichum* sp. (sensu lato). The description is concise but gives the essential species-specific characteristics. Unusual is the information about the substrate, given that bryophytes were not yet regarded as common hosts for fungi at that time. The type collection remained the only known material of *B. coroniforme* for nearly a century. However, in 2000 an ascomycete matching Rehm's description was discovered on bark-inhabiting bryophytes in a riparian forest near Tiefencastel (Grisons, Switzerland). These collections, together with several samples from the same and other locations, were identified as being conspecific with the type material. After Rehm's description, the first new records were published only recently, based on specimens from Switzerland and France (Gross et al. 2021, Van Voorен et al. 2021), and knowledge about the geographic range of *B. coroniforme* is only beginning to emerge. More detailed studies of *B. coroniforme* have revealed that it represents an exceptional bryophilous fungus, exhibiting several features that were unknown previously. This paper presents a comprehensive description of *B. coroniforme*, with emphasis on the parasite-host relationship, and includes data from DNA analyses and cultivation trials.

Materials and methods

Sample collection and observation

The description of macroscopic and microscopic characters of the ascomycete was based on living

and dead material. Herbarium material of the bryophyte host was re-hydrated because dry fruit-bodies are typically hidden below appressed host leaves. Measurements of ascospores and conidia were made in tap water, and measurements of other microscopic characteristics (especially hyphae) were made in lactophenol cotton blue (CB), unless otherwise stated. More than 50 measurements were carried out to determine spore size and at least 10 measurements for other structures. The amyloid reaction was observed in Lugol's solution or in Melzer's reagent without or after pre-treatment with potassium hydroxide solution (KOH). Cross sections were made using a hand-and-table microtome with samples embedded in elder pith. The observed morphs (ascomata and/or conidiomata) are indicated for most records cited below, but only based on one or a few microscopic preparations. The bryophyte nomenclature follows Hodgetts et al. (2020).

DNA extraction, PCR amplification and sequencing

1) Method for specimens deposited in ZT:

Up to five apothecia were removed from fresh or dried *B. coroniforme* specimens and transferred to a 2 ml Eppendorf tube. Before DNA extraction, samples were lyophilized overnight using a Christ 1–8 LDplus lyophilizer. Subsequently, a steel bead of 4 mm diam. was added to each sample and the samples were ground for 2 min in a mixer mill Retsch MM300 (Verder Scientific, Haan, Germany) at 30 Hz. DNA was extracted using the chemicals and the extraction protocol of LGC Genomics (Berlin, Germany) developed for the automated DNA extraction module KingFisher 96/Flex (ThermoScientific, Waltham, USA) with the following modifications: a 20 instead of 6 µl sbeadex® magnetic bead particle suspension was used, and elution was accomplished using 100 instead of 30 µl elution buffer. Sequence data were generated for three loci: the internal transcribed spacer 1 and 2 region of the ribosomal DNA were amplified with the primer pair ITS1f and ITS4 (White et al. 1990, Gardes & Bruns 1993), the 28S subunit of ribosomal DNA (LSU) was amplified with LR0R and LR6 (Rehner & Samuels 1995, Vilgalys & Hester 1990). PCR amplification was performed using the JumpStart™ RedTaq® ReadyMix™ (Sigma-Aldrich, St. Louis, USA) following a standard protocol with a total volume of 10 µl per reaction, 2 µl of template DNA, 35 PCR cycles, and an annealing temperature of 55 °C. PCR products were visualized by gel electrophoresis, and successful amplifications were purified and sequenced in both directions by Microsynth (Balgach, Switzerland).

2) Method for specimens deposited in JE and PRM:

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 1 h, 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. The 28S subunit of ribosomal DNA (LSU) was amplified with primers LR0R and LR6 (Rehner & Samuels 1995, Vilgalys & Hester 1990). 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 LSU primer pairs (see above) by the Sanger method at Macrogen Europe, Amsterdam, The Netherlands. Sequences were edited and aligned in Geneious Prime (Biomatters Ltd., Auckland, New Zealand) and submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). GenBank accession numbers are given in Tab. 1.

Ascospore germination

Two fresh apothecia of *B. coroniforme* were detached from the moss and attached to the underside of the lid of a Petri dish containing malt-extract Agar [MEA, 20 g/l malt extract broth (Carl Roth, Karlsruhe, Germany), 16 g/l agar (Carl Roth) and streptomycin (50 mg/l, Carl Roth)]. Apothecia were attached to the lid such that ascospores were discharged directly onto the agar surface. Successful sporulation was verified using a stereo microscope after incubation overnight. As soon as spores were observed on the agar medium, apothecia were removed from the lid of the petri dishes and the plates were incubated at room temperature in the dark and checked regularly for germinating ascospores.

Isolation of endophytic fungi

For the isolation trials, single fresh apothecia-bearing moss branches were separated from the moss cushion of *L. speciosa* (collection ZT-Myc 64689) and the surface was sterilized as follows: 1 min soaking in 70 % EtOH, 3 min in Javel water

Tab. 1. *Belonium coroniforme*. Specimens used for DNA analysis.

Seqencing voucher	Place of record	Host	Genbank accession no.		Herbarium
			ITS	LSU	
KN-338	Switzerland, Flims, above Battaleins	*Orthotrichum pallens*	MW718702		ZT-Myc 64691
KN-619	Switzerland, Murgtal	*Lewinskya speciosa*	MW718703	MW718696	ZT-Myc 64689
KN-622	Switzerland, Laax	*Orthotrichum* sp.	MW718704		ZT-Myc 64692
KN-623	Switzerland, Ulrichen, Obergoms	*Orthotrichum pallens*	MW718705		ZT-Myc 64694
PRM 954622	Slovakia, Malá Fatra	*Orthotrichum pallens*		MW718697	PRM 954622
H.-J. Zündorf 26025a	Georgia, Greater Caucasus	*Lewinskya striata*		MW718698	JE
H.-J. Zündorf 27264a	Georgia, Greater Caucasus	*Orthotrichum alpestre*		MW718699	JE
H.-J. Zündorf 28816a	Georgia, Greater Caucasus	*Orthotrichum pallens*		MW718700	JE

containing 24 g/l sodium hypochlorite, and again 30 sec in 70 % EtOH. Afterwards, moss pieces were dried using a fresh paper towel and processed. The moss branch was cut aseptically approximately 1–2 mm below the apothecium or the asymptomatic apex of the branch and transferred to fresh MEA plates (prepared as described above). Two agar plates were prepared, each containing six individual moss pieces bearing an apothecium. An analogous isolation series was prepared using adjacent asymptomatic moss pieces from the same moss sample, adding up to 12 pieces per series or 24 pieces in total. Samples were incubated at room temperature in the dark and checked daily. Mycelia growing out of individual moss pieces were immediately transferred to fresh MEA plates. After approximately two weeks, samples were grouped into morphotypes according to the macroscopic culture morphology. From each morphotype, a culture was selected, DNA extracted, and the ITS region sequenced, using the same methods as described above. nBLAST analyses were used to identify the sequenced morphotypes.

Results

Taxonomy

Belonium coroniforme Rehm, Annales Mycologici 5: 534 (1907), (Dermateaceae, Helotiales, Leotiomycetes) – Figs. 1–3.

Description. – Apothecia at the shoot apices within perichaetia, disc-like with a more or less distinct stalk, arising from stromatic tissue within the stems, disc circular or irregularly sinuate, flat or uneven, often bordered by a small rim or some scales, glabrous, reddish to light or yellow orange or almost colourless in the living wet state, sometimes with a pink tint, horny and amber-like in the dry state, 500–1000(1200) µm in diam.; apothecia single or more commonly 2–6 growing closely together forming a confluent structure up to 2000 µm in diam.. – Excipulum, when viewed from the outside, with longitudinally oriented regular and compact hyphae ca 1.5–2.5 µm wide (textura porrecta). – Paraphyses abundant, filiform, septate, simple or rarely with a few basal ramifications, (1)1.5–2(2.5) µm wide, apically not widened or rarely slightly claviform or even subglobose and up to 4 µm wide; distance between septa (10)12–16(20) µm; easily breaking into pieces and then confusable with conidia, which are also fragile (however, conidia are unbranched and somewhat wider, and the individual cells are shorter). Septa in paraphyses, as well as in ascospores and conidia, are often difficult to observe. – Asci unitunicate, inoperculate, subcylindrical to evenly claviform, in H_2O (110)125–155(170) × (12)13–16 µm (Rehm 1907: 100–120 × 10 µm), foot attenuated, arising from ascogenous hyphae, croziers hardly recognizable; asci normally eight-spored; discharged asci with an apical hole, not collapsing. – Ascospores variable, subcylindrical to slightly claviform, slightly coloured by many yellowish droplets, with (5)8–12(15) transverse septa, distance between septa (3)4–6(7) µm, not or rarely slightly constricted at the septa, end

cells rounded, epispore smooth, in H_2O (32)40–65(80) × (4)4.5–5.5(6.5) µm (Rehm 1907: 25–30 × 4.5 µm, with 3–7–9 transverse septa), in CB (27)35–60 × (3.5)4–5.5(6) µm, arranged in 2 or 3 rows filling the whole ascus lumen, thicker spore half oriented to the ascus apex; ascospores germinate with 1–1.5 µm thick germ hyphae at one or both end cells, less often germ hyphae emerging also from the other ascospore cells. – Hyphae colourless, first intercellular within the stems, later forming a strong compact wedge in the widened host stems below the female gametangia, hyphal tissue replacing the host cells, except for the outermost cortical layer which is finally disrupted by fruit-body formation; individual hyphal cells small, irregular, mostly longitudinally oriented, (1)1.5–3 µm wide; no hyphae within the gametangia or slime cells observed, leaf cells also without hyphae, but superficial hyphae present on both sides of the basal parts of the perichaetial leaves. – Conidiomata (not mentioned by Rehm 1907) as sporodochia, at the same position as the ascomata, arising from stromatic tissue within the

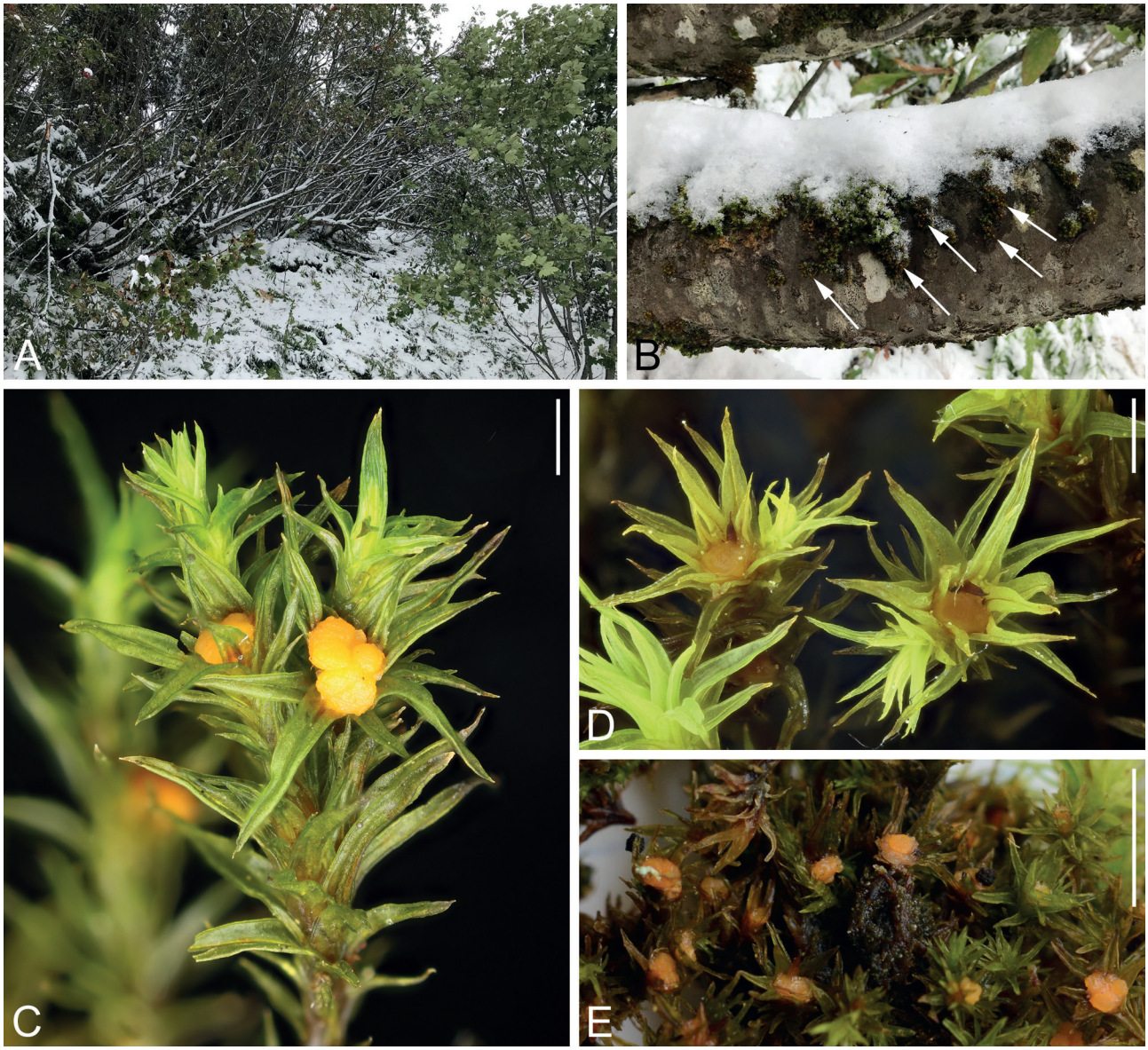

Fig. 1. *Belonium coroniforme* macroscopic features (A–C, E ZT-Myc 64688, D Zündorf 26025a). **A.** Habitat overview; **B.** Tufts of infected *Orthotrichum pallens* (arrows) on a horizontal *Sorbus aucuparia* stem; **C.** Apothecia on *Lewinskya speciosa*; **D.** Conidiomata on *Lewinskya striata*; **E.** Apothecia on *O. pallens*. Scale bars C–E = 1 mm.

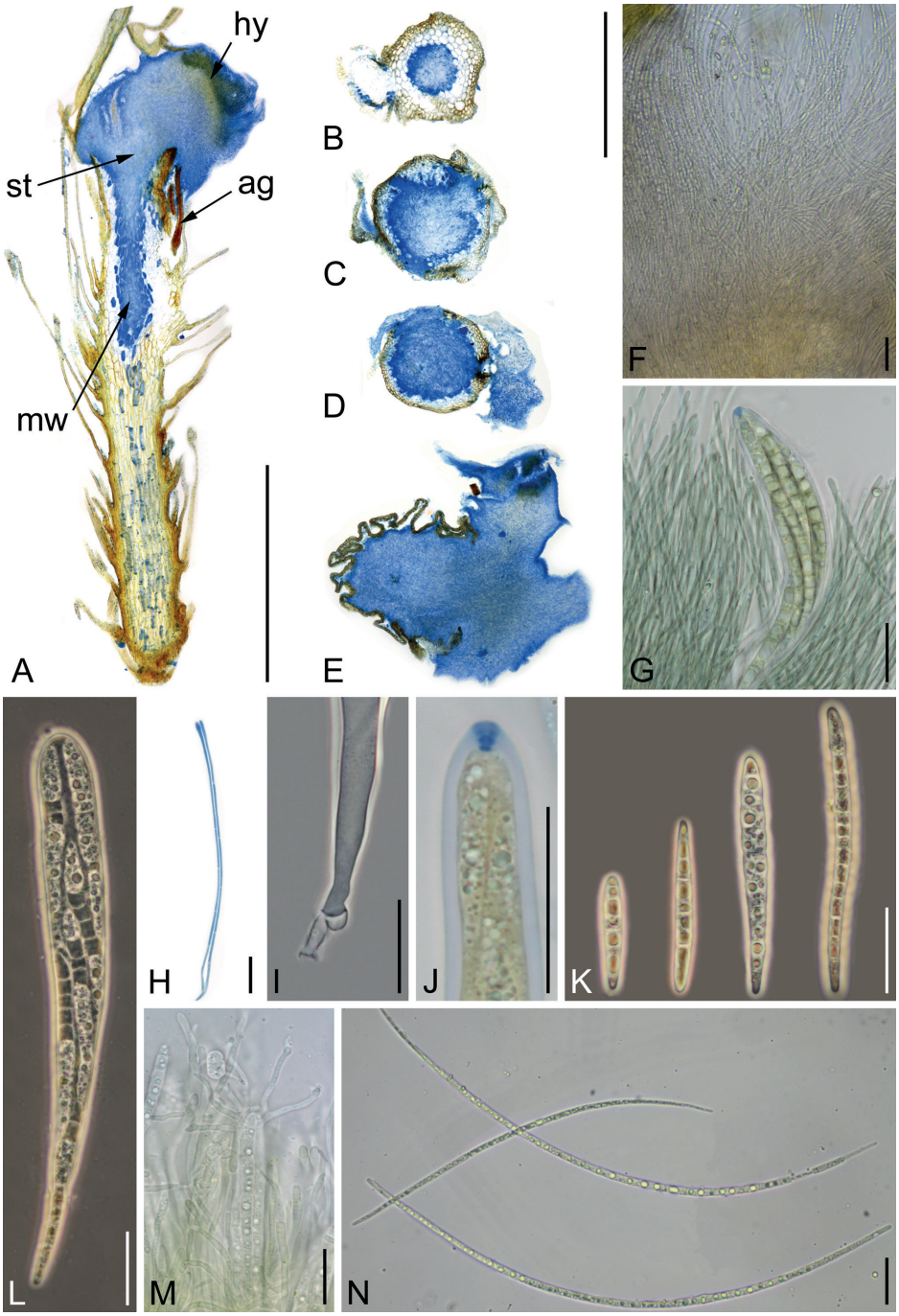

Fig. 2. *Belonium coroniforme* microscopic features (A–E, H–I, K–L ZT-Myc 64696; F, N Zündorf 26025a; G, J ZT-Myc 64693; M ZT-Myc 64690). **A.** Vertical section of ascoma on perichaetium of *Orthotrichum pallens* stained with CB, showing hymenium (hy) and stromatic tissue (st) extending as a mycelial wedge (mw) into the widened longitudinally-sectioned host stem and an archegonium (ag); **B–E.** Transverse sections cut at different levels through the infected stem and stained with CB. Central stromatic wedge surrounded by a thick layer of stem tissue (B), sections cut at higher levels (closer to fruit-bodies), with hyphal tissue strongly expanded and partly breaking through the stem cortex and cushion-like hyphal tissue outside the stem beginning to develop (C–D), near hymenium showing closely attached calyptra on the left side (E); **F.** Conidial layer in vertical section in water; **G.** Hymenium after treatment with KOH and Lugol's solution showing paraphyses and ascus; **H.** Two paraphyses stained with CB; **I.** Ascus base in phase contrast; **J.** Upper part of ascus after treatment with KOH and Lugol's solution; **K.** Ascospores in phase contrast; **L.** Ascus with ascospores in phase contrast; **M.** Ascus and paraphyses in water showing ascospores with germinating hyphae radiating from the ascus tip; **N.** Conidia in water. Scale bars A = 1 mm, B–E = 500 µm, F–N = 20 µm.

stems, sometimes stalked, apically disc-like, plane or convex, cup-like when overmature, light orange to flesh-coloured to almost colourless in the living wet state, herbarium material cream-coloured, gelatinous to mucilaginous, (350)500–850(1100) × 350–460(800) µm; single or two (three) growing closely together within one perichaetium; layer of conidia formation 150–180 µm thick in section, a textura intricata with hyphae 2–3 µm wide underneath. – Conidiophores filamentous, up to 3 µm wide, with lateral or dichotomous ramifications. – Conidiogenous cells not or slightly wider than the conidiophores, about 6–14 µm long. – Conidia filiform, light orange when viewed in large quantities in transmitted light, individual conidia almost colourless, straight or usually slightly bent, sometimes S-shaped, transversely septate, in H_2O (100)125–225(250) × (1.5)2–3 µm, tapering continuously towards both ends; end cells uniformly rounded or truncated at the proximal end; distance between septa (4)6–15(23) µm (best observable in dead, optically empty conidia). – Hyphae as in the ascomata.

Colour of both morphs due to numerous small, light coloured droplets of varying size within excipular cells, paraphyses, asci and ascospores, as well as conidiogenous cells and conidia. – Chemical reactions: Ascus apex in Lugol's solution with a pale reddish to delicate purple, sometimes indistinct ring about 3.5 µm in diam. (Rehm 1907: J–); ring in Lugol's solution and Melzer's reagent after pre-treatment with KOH blue; all structures of apothecia (including hymenia) and conidiomata (including conidial layer) in Lugol's solution slightly reddish brown, sometimes with a bluish front; dirty bluish in both reagents after pre-treatment with KOH. The hemiamyloid reaction is due to a very faint colouration of the cell walls. No cyanophilous reaction of hyphal cell walls with CB in any of the morphs observed. – General observations: Mature ascomata lose their individual boundaries when they grow together in the attenuated perichaetia. The hymenia contain many undischarged ascospores that germinate both inside and outside the asci. Germ hyphae coming out of the ascus apex have been repeatedly observed.

Host plants. – Orthotrichales, Orthotrichaceae: *Lewinskya affinis* (Schrad. ex Brid.) F. Lara, Garilleti & Goffinet, (syn. *Orthotrichum affine* Schrad. ex Brid.); *L. speciosa* (Nees) F. Lara, Garilleti & Goffinet, (syn. *O. speciosum* Nees); *L. striata* (Hedw.) F. Lara, Garilleti & Goffinet, (syn. *O. striatum* Hedw.); *Orthotrichum alpestre* Bruch & Schimp.; *O. pallens* Bruch ex Brid.; *O. patens* Bruch ex Brid.; *O. stramineum* Hornsch. ex Brid.; *Ulota bruchii* Hornsch. ex Brid.; *U. coarctata* (P. Beauv.) Hammar; *U. crispula* Bruch; *U. macrospora* Baur & Warnst. – Hypnales, Leucodontaceae: *Leucodon sciuroides* (Hedw.) Schwägr., (syn. *L. sciuroides* var. *morensis* (Schwägr.) De Not.)

Distribution. – Austria, France, Georgia, Nepal, Slovakia, Switzerland, Tunisia.

Genetic analyses and cultivation trials

ITS sequences were successfully generated from four specimens originating from three different host species (Table 1). All the sequences were identical. The best hit in an NCBI nucleotide BLAST (nBLAST) search (on 7 Jan 2021) against type sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed a rather weak similarity to *Geomyces auratus* Traeen (GenBank accession no. NR_111872), with 91.0 % sequence identity. *Pallidophorina paarla* (Damm & Crous) S. Bien & Damm (NR_155151) and *Tympanis inflata* S. Bien, C. Kraus & Damm (NR_165225) were the second and third best hits, with 86.7 % and 86.0 % sequence identity, respectively. No hits with higher sequence identities regarding *G. auratus* were obtained with nBLAST searches against the full nucleotide collection database (ITS genotyping of a 1968 herbarium specimen from Tunisia on *Leucodon sciuroides* failed).

Likewise, no variation was found within the LSU sequence of five samples originating from three different geographic regions and four different host species. nBLAST searches against the full nucleotide collection database revealed a *Leotiomycetes* sp. (MH470214.1), with 97.1 % sequence identity, as the best hit. *Belonium excelsior* (P. Karst.) Boud. (on 7 Jan 2021) is the only other *Belonium* species with sequences in GenBank. Its ITS sequence (MH856965) only shows 75.5 % sequence identity to *B. coroniforme*, whereas its LSU sequence (MH868487) shows 80.7 % sequence identity.

The ascospores from two different apothecia failed to germinate on malt-extract agar.

Cultivation attempts for endophytic fungi yielded a total of eleven isolates, six from the first series of apothecia-bearing branches and five from asymptomatic branches. The eleven isolates were grouped into seven different morphotypes. One morphotype was represented by four different isolates, two from series one and two from series two. One morphotype was represented by two different isolates while the others were only represented by a single isolate. Six morphotypes were successfully sequenced, while

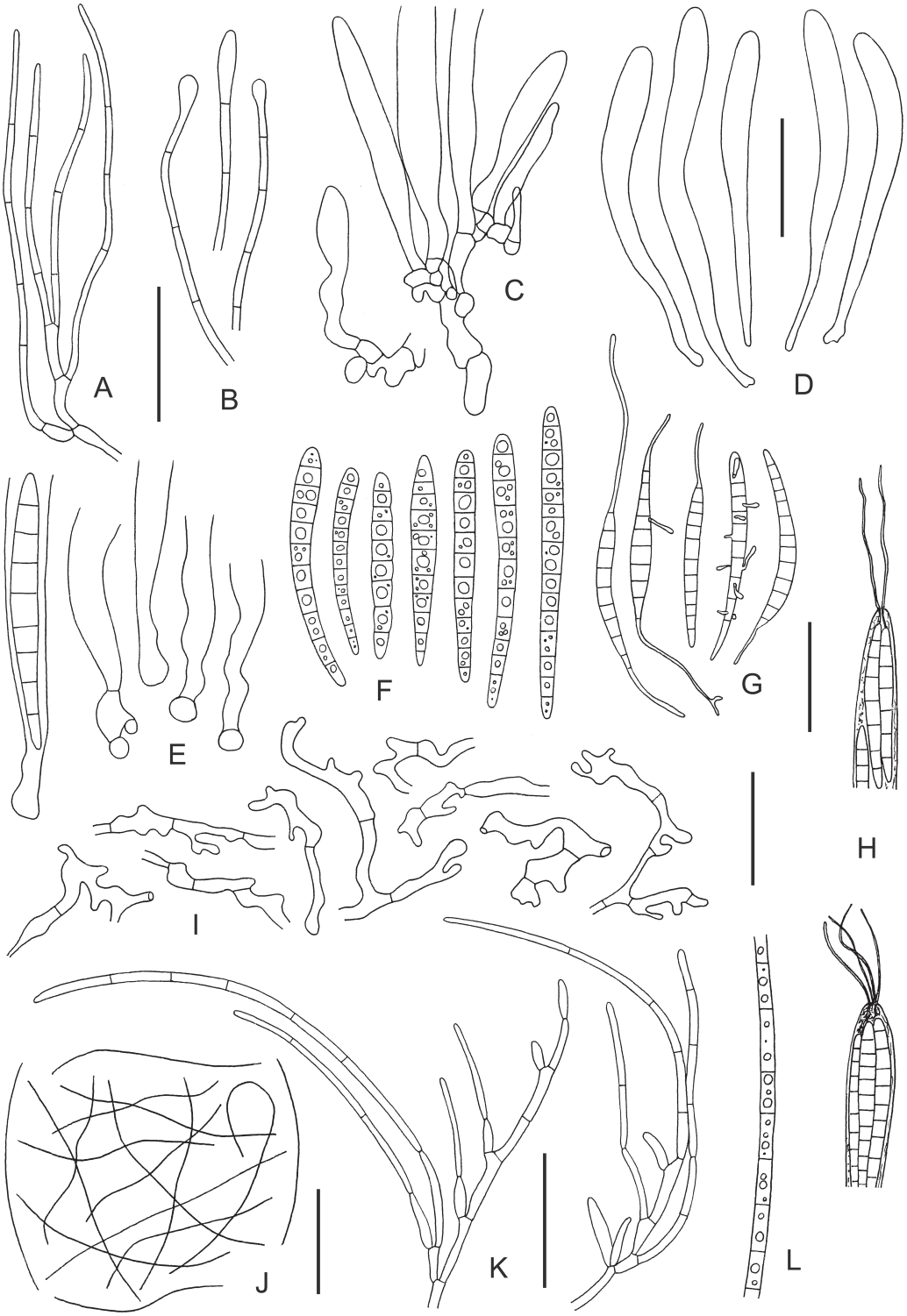

Fig. 3. *Belonium coroniforme* (A Döbbeler 7853; B–E, L Döbbeler 7857; F ZT-Myc 64688 #1,5; G, H ZT-Myc 64688 #1,8; I Döbbeler 9223; J ZT-Myc 64688 #1,1; K Döbbeler 7711). **A.** Paraphyses; **B.** Untypical end cells of paraphyses; **C.** Ascogenous hyphae with asci; **D.** Almost mature asci in outline; **E.** Basal parts of asci, left with an ascospore; **F.** Ascospores; **G.** Germinating ascospores; **H.** Apical part of asci with germinating ascospores; **I.** Isolated hyphae from the mycelial wedge; **J.** Conidia; **K.** Conidiophores with conidiogenous cells and developing conidia; **L.** Middle part of a conidium; (A–E and G–K in CB; F, L in water). Scale bars A–C, E = 25 µm, D =50 µm, F, I, L = 15 µm, G, H = 30 µm, J = 70 µm, K = 25 µm. Drawings by P. Döbbeler.

the sequencing of one morphotype (only isolated once) failed. *Belonium coroniforme* was not among the isolates since none of the sequences matched the *B. coroniforme* sequence. The sequences were deposited in GenBank, and nBLAST analyses were conducted to identify the species as following (accession numbers of newly generated sequences in parentheses): *Hypoxylon* cf. *fragiforme*, 618 of 619 bp identical to best hit MT644893 (MW907964); *H.* cf. *rubiginosum*, 609 of 609 bp identical to best hit MT214998 (MW907965); two times *Biscogniauxia* cf. *nummularia*, 608 of 609 bp identical to best hit MH860015 (MW907966, MW907969); *Coniochaeta* cf. *velutina,* 566 of 566 identical to best hit MN341294 (MW907967); *Nemania* cf. *serpens*, 575 of 578 bp identical to best hit MG098299 (MW907968).

Material examined. – ***Belonium coroniforme*** Rehm, C = conidiomata, A = ascomata: AUSTRIA. Salzburg [formerly Styria]: near Bad Gastein [Wildbad Gastein], on *Lewinskya striata*. (C, A), July 1905, C. Laubinger [Kassel, comm. Dr. F. Quelle] (holotype S F12182; isotype JE, both examined by J. Eckstein).

GEORGIA. Greater Caucasus [all specimens only with conidiomata]: SE Shantili near Georgsminda, 42.631 °N, 45.123 °E, 1700 m a.s.l., on *L. striata* on bark of *Betula* sp., 1 August 2007, H.-J. Zündorf 23913a (JE); Mtskheta-Mtianeti: 3 km SW Khone, 42.566 °N, 45.219 °E, 2000 m a.s.l., on *O. pallens* on bark of *Sorbus aucuparia*, 11 September 2009, H.-J. Zündorf 25434b (JE); SE Khone, 42.564 °N, 45.237 °E, 1900 m a.s.l., on *O. alpestre* on bark of *Betula* sp., 28 July 2012, H.-J. Zündorf 27264a (JE; see Table 1); Rache-Lechkhumi and Kvemo Svaneti: E Ghebi, 42.767 °N, 43.535 °E, 1450 m a.s.l., on *L. striata* on bark of *Acer* sp., 5 June 2014, H.-J. Zündorf 28911a (JE); SE Mravaldzali, 42.508 °N, 43.347 °E, 1800 m a.s.l., on *O. pallens* on bark of *Acer* sp., 4 June 2014, H.-J. Zündorf 28816a (JE; see Table 1): Kakheti: 6 km SE Dartlo, 42.404 °N, 45.624 °E, 2300 m a.s.l., on *L. striata* on bark of *Betula* sp., 5 September 2009, H.-J. Zündorf 25277a (JE); Alazani SW Omalo, 42.361 °N, 45.622 °E, 1700 m a.s.l., on *L. striata* on bark of *Sorbus* sp., 2 July 2010, H.-J. Zündorf 26025a (JE; see Table 1); Samtskhe-Javakheti: Goderzi-pass E Beshumi, 41.633 °N, 42.554 °E, 1850 m a.s.l., on *O. pallens* on bark of *Fagus orientalis*, 16 June 2014, H.-J. Zündorf 29332c (JE).

NEPAL. Foothills of the Himalayas [Vorhimalaya], Okhaldunga, *Abies-Rhododendron*-forest near Thodung, about 3000 m a.s.l., on cf. *L. speciosa* on *Berberis* sp. (C), 7 September 1962, J. Poelt F 31 (M).

SLOVAKIA. Žilina region: Malá Fatra NP, 150 m WNW from the tourist signpost "Sedlo pod Suchým", 49.168 °N, 18.954 °E, 1260 m a.s.l., on *O. pallens* on *Acer* sp. (C), 4 Nov 2017, Z. Egertová & M. Sochor, det. Z. Egertová (PRM 954622; see Tab. 1).

SWITZERLAND. St. Gallen: Quarten municipality S of Walensee, Murgtal, "Mornen" on the right-hand side of the path in the direction to the Murgsee, 47.052 °N, 9.197 °E, 1380 m a.s.l., on *L. affinis* #1,1 (C, A); on *L. speciosa* #1,2 (sequencing voucher KN-619, see Table 1); on *L. striata* #1,3 (C, A); on *O. pallens* #1,4 (A); on *O. patens* #1,5 (C, A); on *O. stramineum* #1,6; on *Ulota bruchii* #1,7 (A); on *U. coarctata* #1,8; on *Sorbus aucuparia*, 19 October 2020 (ZT-Myc 64688) and at the same location on *U. crispula*, 18 September 2020, A. Büschlen (ZT-Myc 64690). Grisons: Albulatal E of Tiefencastel, floodplain forest between the golf course and the Albula river, immediately E of Alvaneu Bad (in the direction of Filisur), 950 m a.s.l., on *L. speciosa* (C), 15 September 2000, P. Döbbeler 7915; ibidem, on *L. speciosa* (C, A), 19 September 2001, P. Döbbeler 7853; on *L. speciosa*, *O. pallens* (C, A), P. Döbbeler 7857; on *L. affinis*, *L. speciosa* (C, A), 19 September 2002, P. Döbbeler 7711; Albulatal E of Tiefencastel, floodplain and spruce forest near Zinols, between Alvaneu Bad and Filisur, on the southern side of the Albula river (about 2 km WSW of Filisur), 980–1000 m a.s.l., on *O. pallens* (C), 1 September 2005, P. Döbbeler 9192, 9223 (all vouchers in M); Flims, above Battaleins, 46.8585 °N, 9.2586 °E, 1650 m a.s.l., on *O. pallens*, 6 September 2020, A. Büschlen (ZT-Myc 64691; sequencing voucher KN-338, see Tab. 1); Laax, between the Nagans reservoir and the mountain station Nagans, 46.8555 °N, 9.2359 °E, 1960 m a.s.l., on *Orthotrichum* sp. on a limestone boulder (C), 6 July 2018, T. Kiebacher (ZT-Myc 64692; sequencing voucher KN-622, see Tab. 1). Bern: Adelboden, Stigelschwand, "Allebach", 46.4979 °N, 7.5230 °E, 1490 m a.s.l., on *O. pallens* and *L. speciosa* on *Salix* sp., 19 October 2016, A. Büschlen (ZT-Myc 64693). Wallis: Obergoms, Ulrichen, bank of the river Rotten (Rhone) near the bridge at "Herreeije", 46.5003 °N, 8.3001 °E, 1340 m a.s.l., on *O. pallens* on *Salix* sp., 25 June 2018, A. Büschlen (ZT-Myc 64694; sequencing voucher KN-623, see Table 1). Uri: Unterschächen, in the forest "Ruosalperwald" close to the "Gwalpetenbach", 46.91334 °N, 8.84798 °E, 1153 m a.s.l., on *Ulota macrospora*, 18 August 2020, A. Büschlen (ZT-Myc 64695); Isenthal, below "Steinhüttli" near the hiking trail, 46.87005 °N, 8.50986 °E, 1380 m a.s.l., on *O. pallens* on *Acer pseudoplatanus*, 17 June 2020, A. Büschlen (ZT-Myc 64696).

TUNISIA. Monts de la Medjerda: Immediately N Ain Draham, cork oak forest, 700 m a.s.l., on *Leucodon sciuroides* [sub *L. sciuroides* ssp. *morensis*] (C), 11 April 1968, H. Hertel 8341 (M).

Discussion

Useful distinguishing traits between the two fruit-body types (morphs) of *Belonium coroniforme* include a generally darker coloration of the apothecia and the presence of a delicate rim of scales formed by protruding hyphal tissue on the excipular margin of the apothecia. On the other hand, conidiomata tend to look slimy when wet. This slimy appearance reminds of *Octospora* apothecia undergoing bacterial decomposition.

Several genera of blastic Hyphomycetes have colourless, filiform or sigmoid, transversely septate conidia like *B. coroniforme*, but deviate in one or more features. For example, there are similarities with *Anguillosporella* U. Braun, which forms sporodochia but has unbranched conidiophores. *Anguillospora* Ingold is recognized as the anamorph of several helotialean genera, but does not form conidiomata and also deviates by having unbranched conidiophores. *Pseudoanguillospora* S.H. Iqbal produces sparingly branched conidiophores without conidiomata (Seifert et al. 2011).

Both morphs belong doubtlessly to the same species *Belonium coroniforme,* although they often occur spatially separated. Ascomata occurring adjacent to conidiomata in the same stand have been repeatedly observed. The morphs share the same host species, occupy the same highly specialized microniche, and originate from a hyphal wedge within the stem of the host gametophyte. A further argument for the affiliation of both morphs to the same holomorph is their common, unusual iodine reaction.

Apothecia and conidiomata strongly prefer perichaetia situated at the uppermost position of the main or lateral host shoots. Perichaetia bear the female gametangia, i.e., archegonia which have brown, up to 400 µm long necks. Sometimes a neck protrudes out of a fruit-body. Ascomata and sporodochia grow over the archegonia, hiding the host gametangia within the fungal tissue. Not only are the archegonia then in a state of arrested development, but often a single sporophyte suffering the same developmental fate can be found arising from within or adjacent to the fungal fruit-bodies. These sporophytes can remain very small, still covered by the calyptra, or may develop a little further with the calyptra dehisced. Mature moss spores do not develop.

The restricted space between the crowded, spirally arranged perichaetial leaf bases affects the shape and size of the fruit-bodies. They are often deformed by mutual pressure and tend to grow together laterally, forming a compound structure with a central depression or small hole. The leaf-bases are sometimes partly or completely enclosed by hyphal tissue. Several times apothecia were observed bursting out of the stem below archegonia. Fertile hyphal tissue may even come out of a fissure of the sporophyte seta or immature capsule. Once, small pulvinate conidiomata were found at the surface of a somewhat mummified but elevated, open capsule containing undispersed spores. The timing and intensity of fungal colonization seems to be an important factor in determining the varying aspects of mature infections.

The proven host species of *Belonium* are autoecious (*Lewinskya*, *Orthotrichum* and *Ulota*) or dioecious (*Leucodon*). In *Lewinskya*, *Orthotrichum* and *Ulota* the female and male organs (perichaetia and perigonia) occur on the same plant but in separate inflorescences. The bud-like perigonia are situated laterally at the shoot apices and contain antheridia with paraphyses surrounded by some modified leaves. It is remarkable that perigonia-borne fruit-bodies have been found only a few times even though infected female structures were nearby.

The *Belonium coroniforme* lifestyle – destruction of the sporophyte but causing only insignificant harm to the gametophyte – is reported for the first time in muscicolous ascomycetes. Furthermore, *B. coroniforme* is one of very few discomycetes on bryophytes observed to form compact conidiomata. The only recently described *Octospora bicarpa* Döbbeler, Büschlen & Eckstein develops sporodochia-like conidiomata which produce one-celled conidia (Döbbeler et al. 2021).

The host species of *Belonium coroniforme*

The known hosts of *Belonium coroniforme* belong to eleven species of the genera *Lewinskya*, *Orthotrichum* and *Ulota* (Orthotrichaceae, subfamily Orthotrichoideae), as well as *Leucodon sciuroides* (Leucodontaceae). It is likely that the complete host spectrum has yet to be determined. The most recent Swiss collections from the Murgtal near Quarten (St. Gallen) are particularly illustrative. Invariably, all eight species of *Lewinskya*, *Orthotrichum* and *Ulota* that were found growing together on the same phorophyte exhibited mature *B. coroniforme* fruit-bodies – an impressive example of a natural infection experiment.

The Orthotrichoideae comprise about 315 corticolous or saxicolous species (Frey & Stech 2009). The host genera have a cosmopolitan distribution, being most diverse in temperate and mediterranean climate zones (Lewinsky 1993, Lara et al. 2016). All mosses so far reported as infected dwell on bark, the typical substrate for Orthotrichaceae and *Leucodon sciuroides*, with the single exception of one record on an undetermined saxicolous species of *Orthotrichum* (ZT-Myc 64692).

Microniche and lifestyle

Belonium coroniforme does not occur at any other shoot apices of the host plants, only at the perichaetia. This is a novel targeted microniche for muscicolous ascomycetes. The vividly orange apothecia crowning the contrasting green shoots of the host probably inspired Rehm (1907) to use the epithet 'coroniforme'. The perichaetial leaves surrounding mature fruit-bodies initially remain green, providing a pleasant contrast to the orange fruit-bodies, but later die off. The necrotic zone does not expand further, but remains restricted to the infected apices. These do not continue to grow by terminal innovation, a behaviour also seen in unparasitized plants after sporophyte formation. The shoots ramify by subapical innovation. The only disadvantage of colonization by *B. coroniforme*

seems to be the prevention of sporophyte development, i.e., reduction of sexual propagation by spores. However, this effect may be significant. It was often noted that, apart from the main stem, lateral shoots also bore fruit-bodies instead of mature sporophytes. An interesting but currently unsolved question is whether healthy host tissue is directly invaded by mycelia of the primary infections or by dispersed ascospores or conidia. The infection events are presumably correlated with the phenology of the sexual cycle of the host mosses.

Colonization of apical shoot regions in mosses has been reported in several ascomycete species. For example, the helotialean *Bryoscyphus aestivalis* Gamundí & Spinedi always develops apothecia at the apices of living shoots of *Bryum dichotomum* Hedw. (Gamundí & Spinedi 1988). Ascomata of several species of the pyrenocarpous genus *Bryostroma* Döbbeler only occur at the uppermost stem region. Like *Belonium coroniforme*, *Bryostroma racomitrii* Döbbeler on *Racomitrium lanuginosum* (Hedw.) Brid. develops an intramatrical mycelial wedge (Döbbeler 1978, 2002). However, gametangia, or their remains, occurring together with developing ascomata have never been observed in hosts of *Bryostroma*. *Bryostroma* infects sterile shoots, whereas *Belonium coroniforme* targets shoots with archegonia.

Discussion of sequencing and cultivation results

The absent variability of ITS and LSU sequences of several specimens originating from different host species indicates that there are no cryptic species present, pointing to a host spectrum of *Belonium coroniforme* restricted to *Orthotrichum* s.l. Unfortunately, ITS genotyping of the only specimen on *Leucodon sciuroides* failed. Therefore, it remains unclear if the collection on the atypical host species is identical to the other samples on Orthotrichaceae. Fresh collections of *B. coroniforme* on *L. sciuroides* are needed to clarify its status. NCBI nBLAST analyses and GenBank searches indicated that no other *B. coroniforme* sequences had previously been deposited in GenBank. In addition, ITS sequences did not reveal any closely related species in GenBank. The only other *Belonium* species sequenced so far proved to be not closely related. The nBLAST analyses revealed only weak similarities to other species and a rather large spectrum of different genera among the best hits. Thus, it was not possible to genetically determine whether *B. coroniforme* is currently placed within the correct genus. However, morphological and ecological data indicate a unique set of characteristics that may justify the description of a separate genus.

Isolation trials for endophytic fungi resulted in the isolation of wood-degrading species, while the target fungus *Belonium coroniforme* could not be identified. All the isolated fungi are common and often found on dead wood in central European forests. The isolation of these fungi from moss might be surprising, but this occurrence is commonly observed in studies about endophytic moss fungi (e.g., Kauserud et al. 2008). Together with the failed ascospore germination experiment, this result indicates that *B. coroniforme* does not easily grow on artificial media, a typical characteristic of highly adapted obligate parasites.

Discomycetes reported on *Orthotrichum*

At least three species of the pezizalean genus *Octospora* Hedw. with vividly coloured apothecia also occur as obligate, biotrophic parasites on *Orthotrichum* sp. div. and may be confused with *B. coroniforme*. However, they have operculate, inamyloid asci, one-celled, ellipsoidal ascospores and elaborate infection structures consisting of large, superficial appressoria and intracellular haustoria.

Octospora orthotrichi (Cooke & Ellis) K.B. Khare & V.P. Tewari growing on *Orthotrichum* sp. was described from New Jersey (USA) by Cooke & Ellis (1877). Today, the fungus is better known from Europe, where it is regionally common. It seems to be restricted to *Orthotrichum diaphanum* Brid., where it infects the rhizoids by haustoria inducing spherical galls (Senn-Irlet 1988, Benkert 1998, Sochorová et al. 2020).

A further species of *Octospora*, *O. affinis* Benkert & L.G. Krieglst. grows on *Lewinskya affinis*. This species also attacks the rhizoids but does not cause galls (Benkert & Krieglsteiner 2006, Krieglsteiner 2006, Chaillet & Moyne 2013, Egertová et al. 2015, Sochorová et al. 2020). The introduction of the new species *O. affinis* was complemented by an ecological and distributional study based on more than 100 records, mainly from southern Germany. It is remarkable that Krieglsteiner (2006) does not mention the presence of *Belonium coroniforme* in any of his *Octospora* collections and observations at the collection sites, even though the host spectra at least partly overlap. Conversely, we did not succeed in detecting *O. affinis* within stands of *B. coroniforme*. An additional species of *Octospora* found on the shoots of an orthotrichaceous moss was recently discovered and newly described as *O. bicarpa* (Döbbeler et al. 2021).

The reddish or pinkish apothecia of *Belonioscyphella hypnorum* (Syd. & P. Syd.) Höhn. (Helotiales) also resemble *Belonium coroniforme*. This parasite is known to occur on various acrocarpous and pleurocarpous mosses and liverworts, causing yellowish necrotic areas (Racovitza 1960, Döbbeler 1986). Egertová et al. (2016) recorded this species on a number of bryophytes, including *Orthotrichum* sp. in Moravia. *Belonioscyphella hypnorum* can be distinguished from *Belonium coroniforme* based on two biological features: its apothecia are not restricted to the shoot apices of the host plants, and it is a necrotrophic parasite. This is combined with *B. hypnorum*'s ability to indiscriminately colonize a wide spectrum of unrelated hosts situated close together.

Belonium coroniforme belongs to the most highly specialized ascomycetes known to colonize bryophytes. Despite its comparatively large, vividly coloured ascomata, this species was almost forgotten for more than 110 years. Even in Central Europe, numerous new fungal taxa on mosses and liverworts remain to be detected, and already named species may hold surprising discoveries.

Acknowledgements

The first author (P. D.) had the privilege to participate in several excursions organized by Adrian Leuchtmann for students of ETH Zürich. Fieldwork resulted in several records of the species described here. Zuzana Sochorová (Egertová), Thomas Kiebacher, Michal Sochor, and especially Hans-Joachim Zündorf kindly allowed us to cite their collections. We thank Melissa Dawes for help with editing the manuscript and Michal Sochor for providing part of the LSU sequences. Two anonymous reviewers substantially improved the manuscript by critical remarks and corrections.

References

Benkert D. (1998) Beiträge zur Kenntnis bryophiler Pezizales-Arten. 6. Wrightoideae, eine neue Sektion der Gattung *Octospora*. *Zeitschrift für Mykologie* **64**: 17–40.

Benkert D., Krieglsteiner L. (2006) *Octospora affinis* (Ascomycetes, Pezizales), eine neue, offenbar nicht seltene bryoparasitische Art auf *Orthotrichum affine*. *Zeitschrift für Mykologie* **72**: 53–58.

Chaillet P., Moyne G. (2013) Une espèce à rechercher: *Octospora affinis* Benkert & L.G. Krieglsteiner (Pezizales), nouvelle pour la Franche-Comte. *Ascomycete.org* **5**: 105–107.

Cooke M.C., Ellis J.B. (1877) New Jersey fungi [cont.]. *Grevillea* **6**: 1–18.

Döbbeler P. (1978) Moosbewohnende Ascomyceten I. Die pyrenocarpen, den Gametophyten besiedelnden Arten. *Mitteilungen der Botanischen Staatssammlung München* **14**: 1–360.

Döbbeler P. (1986) *Belonioscyphella hypnorum* (Helotiales, Ascomycetes), ein nekrotropher Parasit auf Laubmoosen. *Berichte der Bayerischen Botanischen Gesellschaft* **57**: 153–158.

Döbbeler P. (2002) Microniches occupied by bryophilous ascomycetes. *Nova Hedwigia* **75**: 275–306.

Döbbeler P., Büschlen A., Eckstein J. (2021) *Octospora bicarpa* sp. nov. (Pezizales), the first species of the bryophilous genus *Octospora* Hedw. with ameroconidia. *Ascomycete.org* **13**: 85–91.

Doyle J.J., Doyle J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

Egertová Z., Eckstein J., Vega M. (2015) *Lamprospora tuberculata*, *Octospora ithacaensis*, *O. orthotrichi* and *O. affinis* – four bryoparasitic ascomycetes new to the Czech Republic. *Czech Mycology* **67**: 119–133.

Egertová Z., Hairaud M., Sochor M. (2016) *Belonioscyphella hypnorum* (Helotiales), a rarely reported bryoparasitic ascomycete new for the Czech Republic. *Ascomycete.org* **8**: 91–95.

Frey W., Stech M. (2009) Marchantiophyta, Bryophyta, Anthocerotophyta. In: *Syllabus of plant families*. *A. Englers Syllabus der Pflanzenfamilien*, 13th edn., Part 3 *Bryophytes and seedless vascular plants*. Gebr. Borntraeger Verlagsbuchhandlung, Stuttgart: 9–263.

Gamundí I.J., Spinedi H.A. (1988) Ascomycetes from Antarctica. New species and interesting collections from Danco Coast, Antarctic Peninsula. *Mycotaxon* **33**: 467–482.

Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Gross A., Blaser S., Senn-Irlet B.J. (2021) *SwissFungi: National data- and information center for the fungi of Switzerland* [database]. Version 2. Birmensdorf, Swiss Federal Institute WSL; https://www.wsl.ch/map_fungi (accessed 2021 Jan 03).

Hodgetts N.G., Söderström L., Blockeel T.L., Caspari S., Ignatov M.S., Konstantinova N.A., Lockhart N., Papp B., Schröck C., Sim-Sim M., Bell D., Bell N.E., Blom H.H., Bruggeman-Nannenga M.A., Brugués M., Enroth J., Flatberg K.I., Garilleti R., Hedenäs L., Holyoak D.T., Hugonnot V., Kariyawasam I., Köckinger H., Kučera J., Lara F., Porley R.D. (2020) An annotated checklist of bryophytes of Europe, Macaronesia and Cyprus. *Journal of Bryology* **42**: 1–116.

Kauserud H., Mathiesen C., Ohlson M. (2008) High diversity of fungi associated with living parts of boreal forest bryophytes. *Botany* **86**: 1326–1333.

Krieglsteiner L. (2006) Ökologie und Verbreitung von *Octospora affinis*, erste Erkenntnisse. *Zeitschrift für Mykologie* **72**: 59–66.

Lara F., Garilleti R., Goffinet B., Draper I., Medina R., Vigalondo B., Mazimpaka V. (2016) *Lewinskya*, a new genus to accommodate the phaneroporous and monoicous taxa of *Orthotrichum* (Bryophyta, Orthotrichaceae). *Cryptogamie, Bryologie* **37**: 361–382.

Lewinsky J. (1993) A synopsis of the genus *Orthotrichum* Hedw. (Musci, Orthotrichaceae). *Bryobrothera* **2**: 1–59.

Racovitza A. (1960) Contribuţii la cunoaşterea ciupercilor briofile din R. P. Romînă. *Comunicările Academiei Republicii Populare Romîne* **10**: 1111–1115.

Rehm H. (1907) Ascomycetes novi. *Annales Mycologici* **5**: 516–546.

Rehner S., Samuels G. (1995) Molecular systematics of the Hypocreales: A teleomorph gene phylogeny and the status of their anamorphs. *Canadian Journal of Botany* **73**: 816–823.

Seifert K.A., Morgan-Jones G., Gams W., Kendrick B. (2011) *The genera of Hyphomycetes*. CBS Biodiversity Series 9. CBS-KNAW Fungal Biodiversity Centre, Utrecht.

Senn-Irlet B. (1988) Zum Nachweis der bryoparasitischen Lebensweise von *Octospora orthotricha* und *Octospora tetraspora* (Pezizales, Ascomycetes). *Mycologia Helvetica* **3**: 173–181.

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

Van Vooren N., Mombert A., Stöckli E., Pellicier P., Moyne G., Clowez P. (2021) Ascomycètes non lichénisés nouveaux pour la France. Notules no. 1. *Ascomycete.org* **13**: 54–58.

Vilgalys R., Hester M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

White T.J., Bruns T., Lee S., Taylor J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications*. (eds. Innis M.A., Gelfand D.H., Sninsky J.J., White T.J.). Academic Press, New York: 315–322.

(Manuscript accepted 28 June 2021; Corresponding Editor: I. Krisai-Greilhuber)