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Botulisporaceae (Cephalothecales, Sordariomycetes), a new family for *Botulispora fagicola*, gen. et sp. nov., from branches of *Fagus sylvatica* in Austria

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An ascomycete repeatedly collected in Austria on the inner bark of branches of *Fagus sylvatica* is described as a new species, accommodated in the new genus *Botulispora*. Multi-locus (partial SSU-ITS1-5.8S-ITS2-LSU nrDNA, *RPB1, RPB2*) phylogenetic and morphological analyses place this genus in a sister clade to the family Cephalothecaceae in the order Cephalothecales, thus the new family Botulisporaceae is proposed. The previously monofamilial Cephalothecales included genera with cleistothecioid ascomata with a cephalothecioid (i.e. polygonally splitting) peridium and small, simple, evanescent asci. The inclusion of *Botulispora fagicola* extends the morphological circumscription of the order to include fungi with perithecioid (i.e. ostiolate) ascomata with a non-cephalothecioid peridium and non-evanescent asci.

Keywords: Ascomycota, beech, cephalothecioid fungi, molecular phylogeny, taxonomy. – 1 new family, 1 new genus, 1 new species.

The order Cephalothecales was first introduced by Hubka & Réblová (2019) within the Sordariomycetes. The same order was proposed at a later point by Hyde et al. (2020b), which is therefore to be regarded as an isonym based on Article 6.3, Note 2 of the Code (Turland et al. 2018). The Cephalothecales contain a single family, the Cephalothecaceae (Hubka & Réblová 2019, Hyde et al. 2020b), which was originally described by Höhnel (1917) for the following seven species: *Argynna polyhedron* (Schwein.) Morgan, *Cephalotheca pulcherrima* Höhn., *C. sulfurea* Fuckel, *Testudina terrestris* Bizz. (= *Marchaliella zopfielloides* E. Bommer & M. Rousseau), *Zopfia rhizophila* Rabenh., *Zopfiella curvata* (Fuckel) G. Winter and *Z. tabulata* (Fuckel) G. Winter. From today's point of view, Höhnel's family concept is extremely heterogenous as some of the species included by him in the Cephalothecaceae are placed far apart phylogenetically. For example, the genus *Cephalotheca*, typified by *C. sulfurea* (Fuckel 1872, Clements & Shear 1931), belongs to the Sordariomycetes (Perdomo et al. 2013, Hyde et al. 2020b), whereas the genus *Zopfia*, typified by *Z. rhizophila* (Rabenhorst, Fungi europ. exsicc., Cent. 13: no 33, 1874), is a member of the Dothideomycetes (Kruys et al. 2006, Schoch et al. 2009, Jayasiri et al. 2019). Thus, it is not surprising that in modern literature the family Cephalothecaceae is circumscribed very differently to Höhnel's concept. The family currently comprises five genera, i.e. *Albertiniella*, *Cephalotheca*, *Cryptendoxyla*, *Phialemonium* and *Victoriomyces* (Perdomo et al. 2013, Davolos et al. 2019, Hyde et al. 2020b).

Over the course of several years, multiple collections of an unknown ascomycete were gathered on the inner bark of dead branches of *Fagus sylvatica* in Austria. Since an initial attempt to identify this species based on morphological characteristics failed, it was isolated in pure culture, and multilocus phylogenetic analyses as well as detailed morphological studies were conducted, revealing its isolated phylogenetic position within the order Cephalothecales.

Materials and methods

Morphological observations

Due to their small size, ascomata were usually not spotted in the field. Instead, pieces of inner bark from dead branches of *Fagus sylvatica* were collected and studied under a stereo microscope. The ascomata were measured in rehydrated state using

a Keyence VHX digital microscope, and photographs of the ascomata were taken with the same microscope. Micro-morphological studies were carried out with an Olympus B51 microscope and the Olympus cellSens software, as well as a Zeiss Axio Imager.A1 compound microscope (Oberkochen, Germany) equipped with a Zeiss Axiocam 506 color digital camera. Measurements and photos were taken in tap water. The holotype collection is deposited in the herbarium of the Universalmuseum Joanneum (GJO) under the accession number GJO 0137210, and the ex-holotype culture is deposited at the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands.

Culture preparation, DNA extraction, PCR and sequencing

Isolates were prepared from ascospores as described in Jaklitsch (2009) and grown on 2 % corn meal agar plus 2 % w/v dextrose (CMD). Growth of liquid culture and extraction of genomic DNA was performed as reported previously (Voglmayr & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QIAgen GmbH, Hilden, Germany).

The following loci were amplified and sequenced: the complete internal transcribed spacer region (ITS1–5.8S–ITS2) and a ca. 0.9 kb fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA), amplified and sequenced as a single fragment with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); a ca. 1.7 kb fragment of the small subunit nuclear ribosomal DNA (nSSU rDNA) with primers SL1 (Landvik et al. 1997) and NS24mod (Voglmayr & Jaklitsch 2011); a ca. 1.2 kb fragment of the RNA polymerase II subunit 1 (*RPB1*) gene with primers RPB1-Af (Stiller & Hall 1997) and RPB1-6R1asc (Hofstetter et al. 2007); and a ca. 1.2 kb fragment of the RNA polymerase II subunit 2 (*RPB2*) gene with primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) with the same primers as in PCR; in addition, the primers ITS4 (White et al. 1990), LR2R-A (Voglmayr et al. 2012) and LR3 (Vilgalys & Hester 1990) were used as internal primers for the complete ITS-LSU region and nSSU1088 (Kauff & Lutzoni 2002) for the SSU region. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems).

Data analyses

The newly generated sequences were aligned to a representative multigene matrix of Boliniaceae, Cephalothecaceae, Chaeotosphaeriaceae, Coniochaetaceae and Cordanaceae, with Vermiculariopsiellaceae as outgroup according to Réblová et al. (2021). The GenBank accession numbers of sequences used in the phylogenetic analyses are given in Table 1.

Sequence alignments were produced with the server version of MAFFT v. 7.526 (http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2019), checked and refined using BioEdit v. 7.2.6 (Hall 1999). The SSU-ITS-LSU rDNA, *RPB1* and *RPB2* matrices were combined for subsequent phylogenetic analyses. After exclusion of ambiguously aligned regions and long gaps, the final combined data matrix contained 5423 characters (1644 nucleotides of SSU, 657 nucleotides of ITS, 844 nucleotides of LSU, 1207 nucleotides of *RPB1* and 1071 nucleotides of *RPB2*).

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI v. 2.0 (Edler et al. 2021), using the ML + rapid bootstrap setting and the GTR-GAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the different gene regions. For evaluation and discussion of bootstrap support, values below 70 % were considered low, between 70 and 90 % medium/moderate, above 90 % high and 100 % maximum.

Maximum parsimony (MP) bootstrap analyses were performed with PAUP v. 4.0a169 (Swofford 2002), with 1000 bootstrap replicates using five rounds of heuristic search replicates with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect, COLLAPSE command set to MINBRLEN) during each bootstrap replicate. All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to minbrlen.

Results

Molecular phylogeny

The combined multilocus matrix used for phylogenetic analyses comprised 5423 characters, of which 1489 were parsimony informative (83 from SSU, 251 from ITS, 179 from LSU, 429 from *RPB1* and 547 from *RPB2*). The best ML tree (-lnL = 36226.4864) obtained by RAxML is shown in

Tab. 1. Details of strains and sequences analysed in this study, including taxon name, strain identifiers, GenBank accession numbers and references.

Taxon	Strain	GenBank accession numbers					References
		SSU	ITS	LSU	*RPB1*	*RPB2*	
Albertiniella polyporicola	CBS 457.88	AF096170	LT633939	AF096185	LT634060	LT634061	SSU, LSU: Suh & Blackwell (1999), ITS, *RPB1*, *RPB2*: Hubka, unpublished
Apiorhynchostoma curreyi	UAMH 11088	NG_065685	NR_120207	NG_042715	–	KY931926	SSU, *RPB2*: Réblová et al. (2018), ITS: Schoch et al. (2014), LSU: Untereiner et al. (2013)
Botulispora fagicola	**WSF**	**PQ824977**	**PQ824977**	**PQ824977**	**PQ826828**	**PQ826829**	**Present study**
Botulispora fagicola	**WSF1**	**–**	**PQ824978**	**PQ824978**	**–**	**PQ826830**	**Present study**
Camarops ustulinoides	AFTOL-ID 72	DQ470989	–	DQ470941	DQ471121	DQ470882	Spatafora et al. (2006)
Cephalotheca foveolata	UAMH11631	–	KC408422	KC408398	–	KC408404	Mankowski et al., unpublished
Cephalotheca sulfurea	CBS 135.34	AF096173	LT633933	LT633933	LT634024	LT634025	SSU: Suh & Blackwell (1999), ITS, LSU, *RPB1*, *RPB2*: Hubka, unpublished
Chaetosphaeria aquatica	MFLUCC 18-1341	MK834748	–	NG_068639	MN156524	–	Luo et al. (2019)
Chaetosphaeria innumera	SMH2748	–	AY906956	AY017375	–	–	Huhndorf & Fernández (2005)
Coniochaeta acaciae	MFLUCC 17-2298	–	MG062735	MG062737	–	MG194423	Samarakoon et al. (2018)
Coniochaeta africana	CBS 120868	NG_062776	NR_137725	NG_066150	–	–	Damm et al. (2010)
Coniochaeta baysunika	MFLUCC 17-0830	NG_063676	MG828880	MG828996	–	–	Wanasinghe et al. (2018)
Coniochaeta boothii	CBS 381.74	AJ875181	NR_159776	AJ875226	–	–	SSU, LSU: García et al. (2006), ITS: Vu et al. (2019)
Coniochaeta coluteae	MFLUCC 17-2299	–	MG137251	MG137252	MG194424	–	Samarakoon et al. (2018)
Coniochaeta discoidea	CBS 158.80	AJ875179	NR_159779	NG_064120	–	–	SSU: García et al. (2006), ITS, LSU: Vu et al. (2019)
Coniochaeta endophytica	AEA 9094	–	EF420005	EF420069	MK693167	MZ241219	ITS, LSU, *RPB1*: Harrington et al. (2019), *RPB2*: Arnold et al. (2021)
Coniochaeta fodinicola	CBS 136963	KF857171	JQ904603	KF857172	–	–	Vázquez-Campos et al. (2014)
Coniochaeta hoffmannii	CBS 245.38	AJ496245	NR_167688	AF353599	–	–	SSU: Prillinger et al. (2002), ITS: Leonhardt et al. (2018), LSU: Weber et al. (2002)
Coniochaeta ligniaria	CBS 424.65	–	MH858650	AF353584	–	–	ITS: Vu et al. (2019), LSU: Weber et al. (2002)
Coniochaeta lignicola	CBS 267.33	AJ496246	NR_111520	NG_067344	MK693175	–	SSU: Prillinger et al. (2002), ITS: Schoch et al. (2014), LSU: Vu et al. (2019), *RPB1*: Harrington et al. (2019)
Coniochaeta mutabilis	CBS 157.44	AJ496247	NR_111519	NG_042382	–	–	SSU: Prillinger et al. (2002), ITS: Schoch et al. (2014), LSU: Weber et al. (2002)
Coniochaeta navarrae	CBS 141016	–	KU762326	KU762326	–	KU762329	Friebes et al. (2016)
Coniochaeta ostrea	CBS 507.70	NG_065536	NR_159772	NG_064080	DQ471151	DQ470909	SSU, *RPB1*, *RPB2*: Spatafora et al. (2006), ITS, LSU: Vu et al. (2019)
Coniochaeta prunicola	CBS 120875	NG_062777	NR_137037	NG_066151	MK693170	–	SSU, ITS, LSU: Damm et al. (2010), *RPB1*: Harrington et al. 2019
Coniochaeta rosae	MFLUCC 17-0810	MG829110	NR_157509	NG_066204	–	–	Wanasinghe et al. (2018)

Taxon	Strain	GenBank accession numbers					References
		SSU	ITS	LSU	*RPB1*	*RPB2*	
Coniochaeta savoryi	CBS 725.74	AJ875180	MH860890	MH872627	–	–	SSU: García et al. (2006), ITS, LSU: Vu et al. (2019)
Coniochaeta taeniospora	CBS 141014	–	KU762324	KU762324	–	KU762327	Friebes et al. (2016)
Coniochaeta vineae	KUMCC 17-0322	NG_068419	NR_168225	MN473512	–	MN480811	Hyde et al. (2020a)
Cordana aquatica	MFLUCC 16-0954	–	NR_168791	MK835799	MN156529	–	Luo et al. (2019)
Cordana bisbyi	CBS 213.65	–	KF733464	KF746880	–	–	Hernández-Restrepo et al. (2014)
Cordana ellipsoidea	IMI 183415	–	HE672155	HE672166	–	–	Hernández-Restrepo et al. (2014)
Cordana inaequalis	CBS 508.83	–	HE672146	HE672157	–	–	Hernández-Restrepo et al. (2014)
Cordana lignicola	MFLUCC 17-1332	MK834761	NR_168790	MK835797	MN156527	–	Luo et al. (2019)
Cordana mercadoana	CBS 131866	–	HE672154	HE672165	–	–	Hernández-Restrepo et al. (2014)
Cordana pauciseptata	IMI 232041a	–	HE672148	HE672159	–	–	Hernández-Restrepo et al. (2014)
Cordana solitaria	CBS 214.86	–	HE672150	HE672161	–	–	Hernández-Restrepo et al. (2014)
Cordana terrestris	CBS 401.52	–	KF733463	MH868631	–	–	ITS: Hernández-Restrepo et al. (2014), LSU: Vu et al. (2019)
Cordana verruculosa	CBS 127868	–	HE672151	HE672162	–	–	Hernández-Restrepo et al. (2014)
Cryptendoxyla consimilis	CBS 508.70	LT633942	NR_159848	NG_058720	LT634047	LT634048	SSU, *RPB1*, *RPB2*: Hubka, unpublished, ITS: Vu et al. (2019), LSU: Suh et al. (2006)
Endoxyla mallochii	UAMH 11087	–	NR_111790	NG_042717	–	–	ITS: Schoch et al. (2014), LSU: Untereiner et al. (2013)
Endoxyla operculata	UAMH 11085	KY931895	JX460987	JX460992	–	KY931927	SSU, *RPB2*: Réblová et al. (2018), ITS, LSU: Untereiner et al. (2013)
Neurospora tetraspora	CBS 178.33	DQ471032	AY681178	NG_068996	DQ471178	DQ470932	SSU, LSU, *RPB1*, *RPB2*: Spatafora et al. (2006), ITS: Cai et al. (2006)
Phialemonium atrogriseum	CBS 604.67	NG_065691	LT633906	MH870783	LT633958	LT633959	SSU, ITS, *RPB1*, *RPB2*: Hubka, unpublished, LSU: Vu et al. (2019)
Phialemonium obovatum	CBS 279.76	NG_065692	NR_165935	NG_057631	LT634005	LT634006	SSU, *RPB1*, *RPB2*: Hubka, unpublished, ITS, LSU: Suh et al. (2006)
Sordaria fimicola	CBS 723.96	–	MH862606	MH874231	–	DQ368647	ITS, LSU: Vu et al. (2019), *RPB2*: Tang et al. (2007)
Vermiculariopsiella dichapetali	CBS 137977	–	NR_168149	KJ869186	–	–	Crous et al. (2014)
Vermiculariopsiella immersa	CBS 140223	–	KY853476	KY853540	–	ON399363	ITS, LSU: Hernández-Restrepo et al. (2017), *RPB2*: Hernández-Restrepo et al. (2022)
Vermiculariopsis eucalypti	CBS 141281	–	NR_154637	NG_066169	–	–	Crous et al. (2016)
Victoriomyces antarcticus	FBL 165	NG_065553	NR_163275	EF611340	–	KY290262	Davolos et al. (2019)

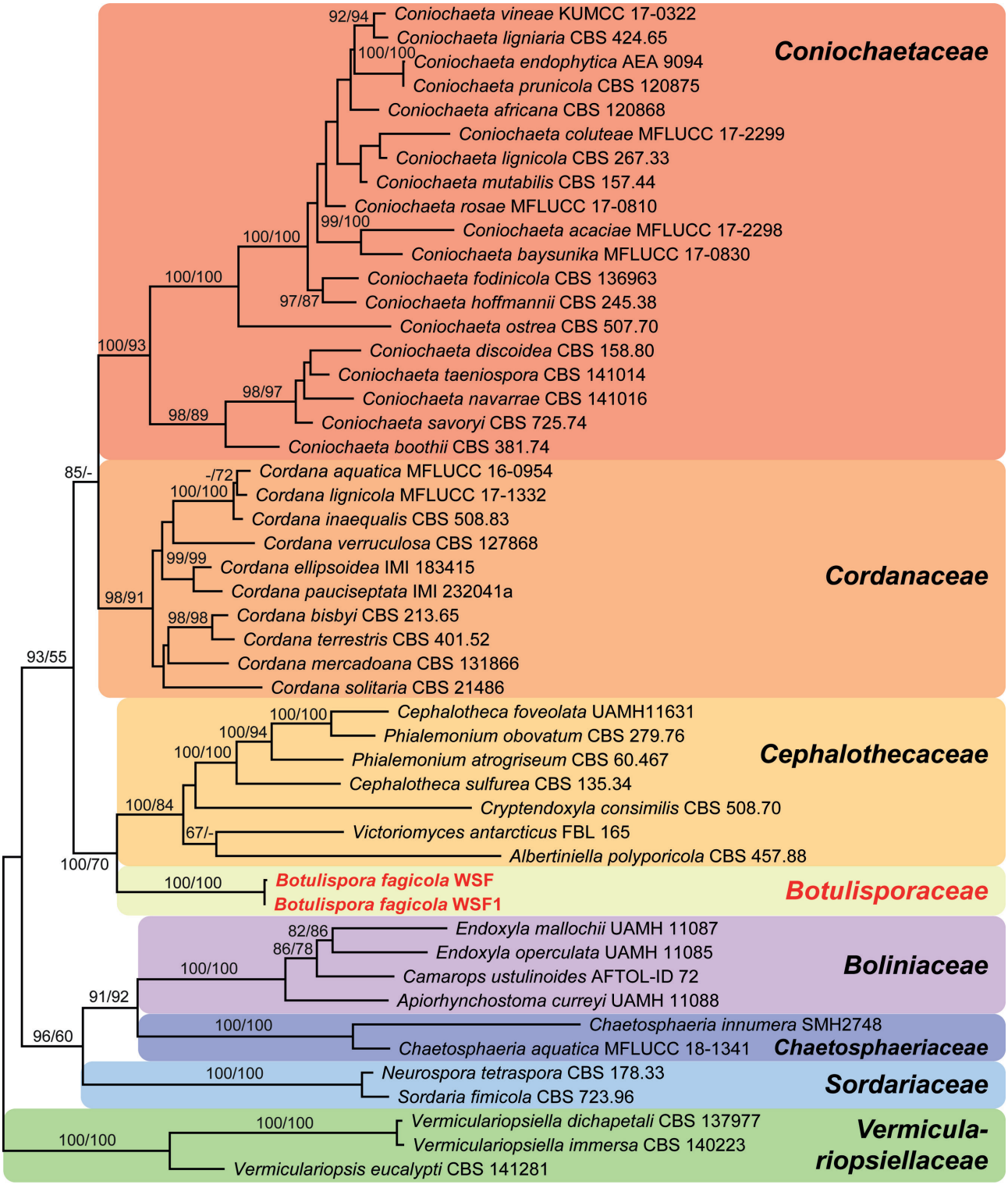

Fig. 1. ML phylogram (-lnL = 36226.4864) revealed by RAxML from an analysis of the combined SSU-ITS-LSU-*RPB1*-*RPB2* matrix of selected Sordariomycetes, showing the phylogenetic position of *Botulispora*. Strain/culture numbers are given following the taxon names. ML and MP bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches.

Fig. 1. Overall tree topology is similar to previous analyses, e.g. of Davolos et al. (2019) and Réblová et al. (2021). Almost all families received high to maximum bootstrap support in both ML and MP analyses; only Cephalothecaceae received maximum support in ML analyses, while support was medium (84 %) in MP analyses. Sister group relationship of the new genus *Botulispora* to Cephalothecaceae received maximum ML and medium MP support (Fig. 1).

Taxonomy

Botulisporaceae Friebes & Voglmayr, **fam. nov.**
MycoBank no.: MB857178

Type genus. – *Botulispora* Friebes & Voglmayr.

Description. – Ascomata non-stromatic, perithecioid, ostiolate. – Peridium soft, towards the outside comprised of dark brown cells, non-cephalothecioid. – Hyphal cover of the peridium generally present. – Interascal filaments paraphysoid. – Asci (4)8-spored, thin-walled, non-evanescent, without a clearly visible apical apparatus. – Ascospores smooth, light brown, allantoid.

Etymology. – Named after the type genus *Botulispora*.

Botulispora Friebes & Voglmayr, **gen. nov.**
MycoBank no.: MB857179

Type species. – *Botulispora fagicola* Friebes & Voglmayr.

Description. – Ascomata solitary to gregarious, non-stromatic, perithecioid, ostiolate, slightly immersed to superficial, often covered with a light brown to greenish brown hyphal felt, in fresh or rehydrated state ± globose or, more commonly, pyriform, laterally collapsing when dry. – Peridium soft, towards the outside comprised of dark brown cells, non-cephalothecioid, towards the outside often transitioning into short, elongate, dark brown hyphal outgrowths. – Hyphal cover of the peridium generally present, often very dense, most pronounced in the lower half of the ascoma, consisting of light brown, thick-walled, partly septate, wavy-sinuous to curly hyphae with obtuse ends. – Interascal filaments paraphysoid, thread-like, hyaline; ostiolar canal lined by short periphyses. – Asci (4)8-spored, thin-walled, non-evanescent, without a clearly visible apical apparatus, stipitate, IKI –. – Ascospores smooth, light brown, allantoid, filled with smaller and larger lipid drops, when overmature 1–3-septate.

Etymology. – From lat. botulus, sausage; refers to the allantoid ascospores of the type species *Botulispora fagicola*.

Botulispora fagicola Friebes & Voglmayr, **sp. nov.** – Figs. 2–3.
MycoBank no.: MB857180

Holotypus. – AUSTRIA, Steiermark, Voitsberg, Köflach, Zigöllerkogel, N 47.077792°, E 15.077134°, 576 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 24 February 2018, *leg.* G. Friebes (GJO 0137210), ex-holotype culture WSF = CBS 145629. GenBank SSU-ITS-LSU: PQ824977; *RPB1*: PQ826828; *RPB2*: PQ826829.

Description. – Ascomata solitary to gregarious, non-stromatic, perithecioid, with an apical, sometimes slightly eccentric ostiole, slightly immersed to superficial, surface finely rough, often covered with a light brown to greenish brown hyphal felt, especially in the lower half, the hyphae spreading over the adjacent host surface, in fresh or rehydrated state ± globose or, more commonly, pyriform, laterally collapsing when dry and then forming an apical, crest-like fold, quickly swelling up again when rehydrated, 150–310 × 120–280 µm. – Peridium soft, 15–35 µm thick, towards the outside comprised of dark brown, flat, irregularly shaped cells of 5–12 × 1.5–3.2 µm in diameter, cells lighter coloured and less flattened towards the inside, not changing colour in 3% KOH, non-cephalothecioid (i.e. not peeling off polygonally), towards the outside often transitioning into short, elongate, dark brown hyphal outgrowths. – Hyphal cover of the peridium generally present, often very dense, most pronounced in the lower half of the ascoma, consisting of light brown, thick-walled, partly septate, wavy-sinuous to curly hyphae with obtuse ends, 1.5–3.1 µm in diameter. – Interascal filaments paraphysoid, thread-like, hyaline, septate, 1.2–3.5 µm wide, ostiolar canal about 10–20 µm wide in section, lined by short periphyses. – Asci (4)8-spored, ± cylindrical, ascospores obliquely uniseriate or biseriate at least in the middle part, with a 12–23 µm long stipe, thin-walled when mature, without a clearly visible apical apparatus, IKI –, 83–105 × 8–12 µm. – Ascospores smooth, light brown, allantoid, filled with smaller and larger lipid drops, when overmature 1–3-septate, germinating with hyaline hyphae, (16.5)17.7–21.2(23.7) × (4.0)4.2–5.4(6.5) µm, Q: (3.2–)3.7–4.5(–5.1) (n = 61). Asexual morph not observed.

Culture characteristics. – On CMD growing very slowly, reaching ca. 3.3 mm diam after 10 wk, first pale ochraceous, becoming crimson brown in the centre, secreting a bright yellow pig-

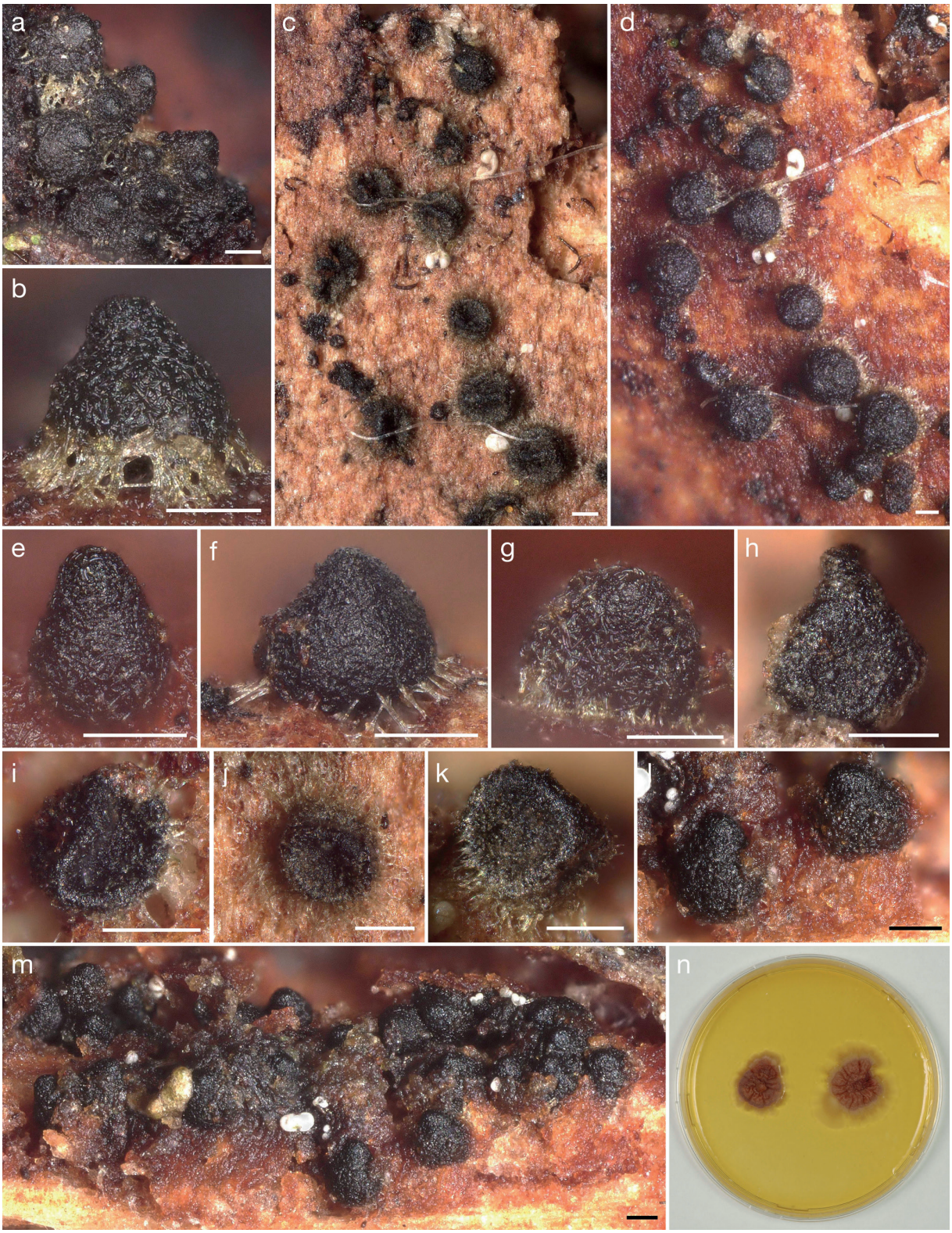

Fig. 2. *Botulispora fagicola*: **a–m.** Ascomata on bast (inner bark) (**c, i–k.** dried, collapsed ascomata; **d.** same ascomata as in **c**, but rehydrated). **n.** Culture on CMD after 3 months. **a, b, e–g.** GJO 0137213. **c, d, h–k.** GJO 0137212. **l, m.** GJO 0137210 (holotype). Bars: 100 µm.

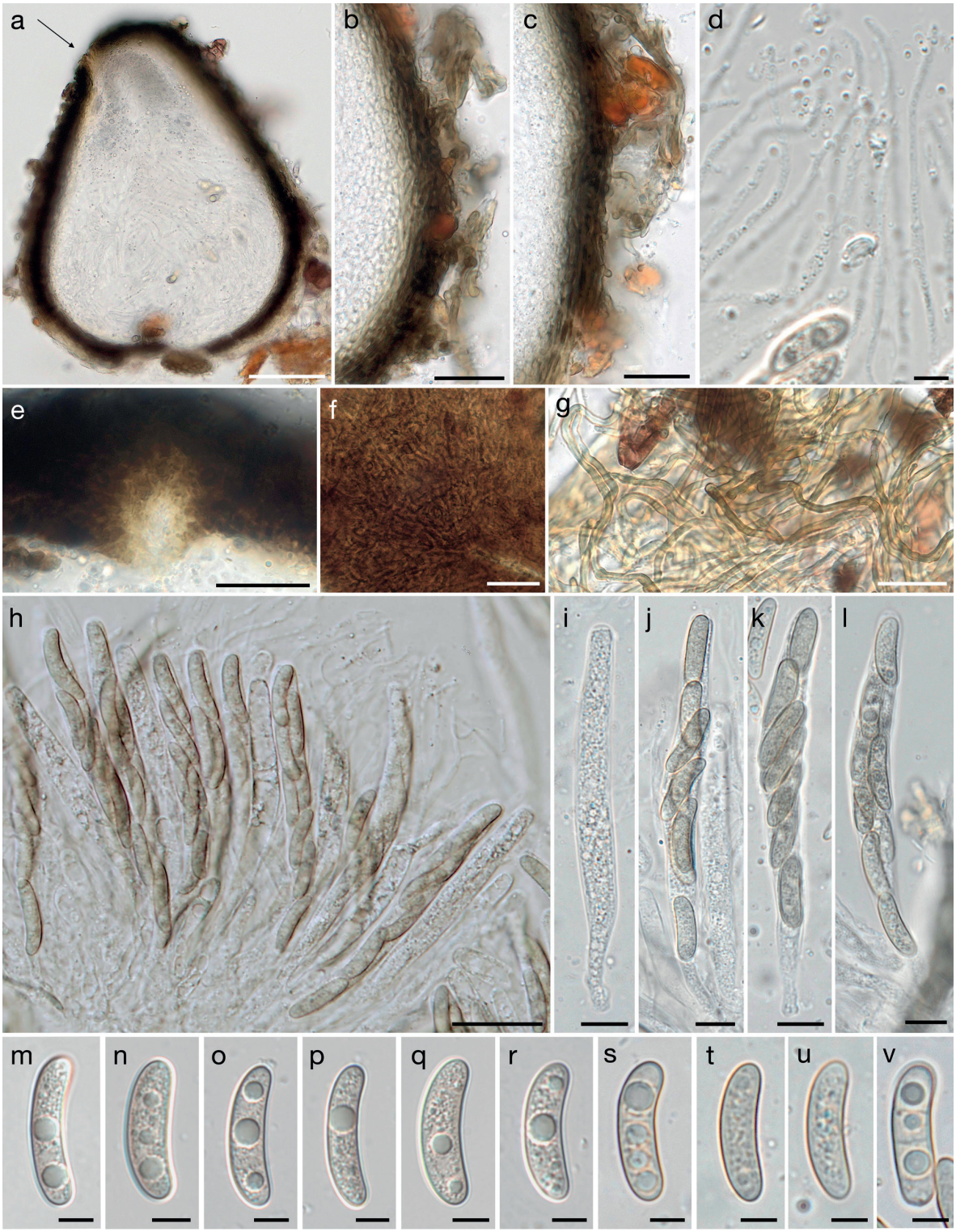

Fig. 3. *Botulispora fagicola*, microscopic characteristics: **a.** Section of an ascoma; arrow indicating the ostiole. **b, c.** Section of the peridium. **d.** Paraphyses. **e.** Ostiole seen from above**. f.** Peridial cells. **g.** Hyphae covering the ascomatal walls**. h.** Hymenium with asci, ascospores and paraphyses. **i.** Immature ascus. **j–l**. Mature asci. **m–v**. Ascospores (overmature, transversely septate ascospore in **v**). a–c, e–g, i–l, s–v. GJO 0137213. d, h, m–r. GJO 0137210 (holotype). Bars: a = 50 µm; b, c, e–g = 20 µm; d, m–v = 5 µm; h = 25 µm; i–l = 10 µm.

ment in the agar medium; surface waxy, initially smooth, in the centre becoming radially wrinkled to cerebriform with age, aerial mycelium sparse to absent; reverse ochraceous, becoming reddish to crimson brown in the centre.

Etymology. – Refers to its host (*Fagus sylvatica*).

Habitat. – On bast (inner bark) of dead branches of *Fagus sylvatica*. Mostly observed on recently fallen branches, although twice also on branches still attached to the tree, 1.5–2 m above the ground. Soc. *Asteromassaria macrospora* (Desm.) Höhn., *Asterosporium asterospermum* (Pers.) S. Hughes, *Merismodes confusa* (Bres.) D.A. Reid, *Orbilia* sp.

Distribution. – Known from multiple localities in Austria (Styria and Upper Austria) at an alt. of 408 m to 933 m, observed from February to July.

Additional material examined. – AUSTRIA. Oberösterreich, Eferding, Stroheim, Aschachtal, Kropfleiten, N 48.38144°, E 13.91153°, 408 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 23 June 2024, *leg.* G. Friebes (GJO 0137211). Oberösterreich, Schärding, Kopfing, between Königsedt and Paulsdorf, N 48.45622°, E 13.69311°, 615 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 25 July 2024, leg. H. Voglmayr (WU-MYC 0053574; living culture WSF1; GenBank ITS-LSU: PQ824978; *RPB2*: PQ826830). Steiermark, Deutschlandsberg, Wies, N and NE slopes of Buchenberg, European nature reserve "Schwarze und Weiße Sulm", N 46.71561°, E 15.18705°, 510 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 2 March 2024, *leg.* G. Friebes (GJO 0137212); Steiermark, Weiz, Gutenberg-Stenzengreith, Gutenberg, Raabklamm, N 47.21391°, E 15.56906°, 498 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 9 March 2024, *leg.* G. Friebes (GJO 0137213); Steiermark, Bruck-Mürzzuschlag, Turnau, Dürrsee approx. 2 km SE Seewiesen, N 47.60680°, E 15.28412°, 933 m a.s.l., on inner bark of a fallen branch of *Fagus sylvatica*, 17 March 2024, *leg.* G. Friebes (GJO 0137214); Steiermark, Leibnitz, Großklein, Oberfahrenbach, Kroasnbach-Graben, N 46.71548°, E 15.47058°, 421 m a.s.l., on inner bark of a still-attached branch of *Fagus sylvatica* 1.5–2 m above the ground, 23 March 2024, *leg.* G. Friebes (GJO 0137215); Steiermark, Weiz, Gutenberg-Stenzengreith, Raabklamm, near Grillbichl, N 47.22641°, E 15.55653°, 520 m a.s.l., on inner bark of a still-attached branch of *Fagus sylvatica* 2 m above the ground, 29 March 2024, *leg.* G. Friebes (GJO 0137216).

Discussion

Botulispora fagicola has been observed on branches of *Fagus sylvatica* over the course of several years, during winter, spring and summer. The authors were able to observe it repeatedly in different parts of Austria, indicating that it is at least locally common, yet easily over-looked. Initial morphological examination did not provide any hints regarding the potential relationship of this species below the level of Sordariomycetes. Therefore, a phylogenetic analysis based on an SSU-ITS-LSU rDNA-*RPB1*-*RPB2* multilocus matrix was carried out, which revealed a sister group relationship of *B. fagicola* to the family Cephalothecaeae (Fig. 1).

The Cephalothecaceae are currently the only family within the order Cephalothecales, subclass Sordariomycetidae, class Sordariomycetes (Hubka & Réblová 2019, Hyde et al. 2020b). The family comprises five genera, i.e. *Albertiniella*, *Cephalotheca*, *Cryptendoxyla*, *Phialemonium* and *Victoriomyces* (Perdomo et al. 2013, Davolos et al. 2019, Hyde et al. 2020b). Of these, only *Albertiniella*, *Cephalotheca* and *Cryptendoxyla* are known to produce sexual morphs, which are morphologically homogenous in that they produce cleistothecioid, non-ostiolate ascomata with a cephalothecioid (i.e. polygonally splitting) peridium as well as small, prototunicate, evanescent asci (e.g. Fuckel 1872, Malloch & Cain 1970, Takashi et al. 2006, Akulov 2013, Koukol 2016). *Botulispora fagicola* differs strongly from these in ostiolate ascomata without a cephalothecioid peridium and non-evanescent asci, thus extending the morphological circumscription of the Cephalothecales considerably. Based on these significant morphological differences as well as its isolated basal position within the Cephalothecales, the new family Botulisporaceae is proposed to accommodate *B. fagicola*. One of the few morphological characteristics shared by *B. fagicola* with some species of the Cephalothecaceae are the ascomata frequently densely covered with hyaline to light-brown hyphae. A similar hyphal covering is found in species of *Cephalotheca* (e.g. Chesters 1935, Yaguchi et al. 2006).

Botulispora fagicola is characterised by its distinct ecology. It was observed exclusively on the inner bark of dead branches of *Fagus sylvatica*. The branches consistently showed little decay, indicating that they only recently fell to the ground. Indeed, *B. fagicola* was never observed on branches that were covered to a larger extent by corticioid or other saprotrophic fungi that develop on deadwood on the ground. Based on the little decay shown by branches with *B. fagicola*, the authors had been suspecting for a while that this species might develop on still-attached rather than fallen branches. This was ultimately confirmed by the observed accompanying fungi which are typical for still-attached branches, e.g. *Asteromassaria macrospora*, *Asterosporium asterospermum*, *Merismodes confusa* and *Orbilia* sp., and especially by the collections GJO 0137215 and 0137216, which, after targeted search, were found on branches still attached to the

tree. The growth on dry, little-decayed corticated branches still attached to the tree in combination with the small, inconspicuous ascomata may be a reason why the species has been missed to date. Further observations from other parts of Europe are needed to reveal whether *B. fagicola* follows its host *Fagus sylvatica* throughout the continent or whether it is restricted to a smaller geographical area.

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