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A phylogenetic assessment of a fungicolous lineage in *Coniocybomycetes*: *Chaenotricha*, a new genus of *Trichaptum*-inhabiting species

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Abstract: The globally distributed genus *Trichaptum* is one of the most species-rich among polypores in terms of hosting other fungi. Among *Trichaptum*-associates, there is a group of mazaediate lichenized fungi (*Coniocybomycetes*, *Ascomycota*) that previously had an uncertain phylogenetic position. DNA sequences – mitochondrial small subunit (mtSSU), nuclear large subunit rDNA (nuLSU), and internal transcribed spacer (ITS) – were obtained from 29 specimens collected from Europe and North America. Maximum likelihood and Bayesian inference analyses of these three gene loci were used to infer phylogenetic position and relationships among lineages. Statistical tests were used to find which phenotypical characteristics distinguish species. The molecular sequence data provide evidence that the fungicolous specimens form a distinct lineage within *Coniocybomycetes* sister to the combined clade of *Chaenotheca s. lat.* and *Sclerophora.* Considering its phylogenetic placement and strict specialization, we describe a new genus – *Chaenotricha*. This fungicolous lineage contains three species based on molecular characteristics. Morphological characters mostly overlap except for spore size and stalk length of apothecia. We provide a new combination, *Chaenotricha obscura,* for the only previously described species for which we designate an epitype, and introduce a new species – *Chaenotricha cilians*. The third lineage remains undescribed because of a small sample size, which did not allow us to clearly delineate species boundaries.

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INTRODUCTION

The surface of polypore sporocarps can be a substratum for many organisms such as epiphytic algae (Zavada & Simoes 2001, Stonyeva *et al*. 2015, Vondrák *et al*. 2023), non-lichenized (*e.g*., Hutchison 1987, Sun *et al*. 2019, Maurice *et al*. 2021) and lichenized fungi (Hawksworth *et al.* 2014), forming so-called epimycotic or fungicolous communities (Stonyeva *et al*. 2015, Maurice *et al*. 2021). For example, a globally distributed genus of poroid white-rotting fungi, *Trichaptum* (*Hymenochaetales*, *Agaricomycetes*, *Basidiomycota*; Larsson *et al*. 2006), may host more than 20 green algal (*Chlorophyta*) species (Mukhin *et al*. 2018) and is one of the richest among polypores in terms of associated fungicolous fungi (Maurice *et al*. 2021). Among these, there are at least three lichenized *Chaenotheca* species, *C. gracillima* (Spribille *et al*. 2010), *C. trichialis* (Selva 2014), and *C. obscura* (= *C. balsamconensis*; Merrill 1909, Allen & McMullin 2015, Selva & McMullin 2020), reported as growing on *Trichaptum* sporocarps.

The genus *Chaenotheca*, together with the genus *Sclerophora*, belongs to the early diverging lichenized lineage *Coniocybomycetes*, species of which are characterized by having stalked and mazaediate ascomata (Prieto *et al*. 2013), and their closest relatives belong to *Lichinomycetes* (Prieto *et al*. 2013, Díaz-Escandón *et al*. 2022). The oldest report of a *Chaenotheca* species growing on *Trichaptum* sporocarps was made by Fries (1865), who mentioned a *Trichaptum*-inhabiting variant called *Chaenotheca brunneola β cilians* due to its similarities to eyelashes. Unfortunately, the original material of this taxon is lost (L. Tibell & A. Thell, pers. comm.). The second oldest evidence comes from North America, where Merrill (1909) described *Calicium obscurum* (= *Chaenotheca obscura*) growing on *Trichaptum.* In 2015, Allen & McMullin (2015) described *C. balsamconensis* from North America, but after examining Merrill's type material, Selva & McMullin (2020) concluded that this species is conspecific with *C. obscura* and synonymized accordingly. At the same time, a study by Suija *et al*. (2016) tested if morphologically similar, but ecologically distinct (wood inhabiting *vs*. fungal sporocarp-inhabiting) specimens belong to the same species – *Chaenotheca brunneola*. A single-gene (full-length ITS) analysis in that study showed that all *Trichaptum*-dwelling specimens, including the type of *C. balsamconensis* from North America, form a distinct lineage sister to the rest of *Chaenotheca* and *Sclerophora* species. Moreover, there was

a clear distinction in nucleotide sequences between European and North American specimens, suggesting that these two may represent different species.

In the current study, we aimed to clarify the taxonomic position of these fungi within *Coniocybomycetes*. We analyzed slow- and fast-evolving ribosomal DNA markers as well as their morphological characteristics. We also sampled widely to better understand the distribution of these *Trichaptum*-inhabiting fungi.

MATERIAL AND METHODS

Taxon sampling and morphological examination

We examined chaenotheca-like lichenized fungi growing on *Trichaptum* sporocarps. The specimens are deposited in BILAS, CANL, DAU, M, NY, UPS, TRH and TUF (fungarium acronyms follow Index Herbariorum; https://sweetgum.nybg.org/science/ih/). In total, we studied the morphology and anatomy of 29 specimens collected from Europe and North America (Table 1), including an isotype of *Chaenotheca obscura* from the exsiccate series *Merrill, Lich. Exs. Ser. II. 92*. (M0205375 and CANL) and the holotype of *C. balsamconensis* (NY02359896).

We selected 28 morpho-anatomical characters to describe the specimens (Supplementary Table S1). For each specimen, we recorded the thallus type (immersed or episubstratal), shape (granular or farinaceous) and presence of thallus cortex; if thallus was visible, we also tested thallus color reactions with standard spot tests with reagents following protocols described by Brodo *et al*. (2001): potassium hydroxide *ca*. 10 % solution (K), para-phenylenediamine ethanol solution (Pd) and commercial bleach containing sodium hypochlorite (C). The abbreviation KC refers to a color reaction after applying K and then C immediately afterwards to the same location. We examined up to five apothecia per specimen (the number examined depended on the availability/abundance of apothecia). We also recorded the location of the apothecia on the *Trichaptum* fruitbodies and stalk pigment reaction in K. For each apothecium, we described 13 characteristics: presence of pruina on the stalk, stalk color (dull or shining black), capitulum shape (spherical or obconical), development of excipulum (well or weakly), color of mazaedium (dark brown or black) and its structure (powdery or granular), height and width of the stalk and capitulum, shape of the asci (cylindrical or clavate) and its length (without stipe), shape of the ascospores (spherical, slightly elliptical or both), ascospore surface (smooth, with fissures or both) and diameter of ascospores. We calculated the average length-width ratio of the stalk based on up to five apothecia per specimen. Average size of ascospores and asci were calculated using 10 ascospores and four asci (if possible) for each apothecium to a maximum of 20 asci and 50 ascospores per specimen. Variable values were given as (min–)mean ±SD(–max) where SD is the standard deviation, and min and max are minimum and maximum values respectively.

We examined the anatomical characters of ascomata and ascospores in squash preparations under a Leica DM1000 LED compound light microscope. Our measurements were made in tap water with a precision of 1 µm using 10× objectives for apothecia and 100× (water) objectives for asci and ascospores. We produced scanning electron micrographs from air-dried material mounted on stubs coated with a thin layer of gold and observed using a FEI INSPECT Scanning Electron Microscope (The National Museum of Natural Sciences [MNCN, CSIC], Madrid). Our character selection and terminology of morphological and anatomical characteristics follows Schmidt (1970), Tibell (1980, 1999) and Allen & McMullin (2015).

Using a Mann-Whitney U Test, we assessed the difference in morphological and anatomical variables (except two variables with extremely low sample size) between two taxon groups with Statistica® v. 6.0 software (StatSoft 1984–2001).

DNA extraction, amplification, and sequencing

We extracted total DNA from 29 specimens growing on the sporocarps of *Trichaptum abietinum, T. fuscoviolaceum* and *T. biforme* and from several *Chaenotheca* and *Sclerophora* specimens (Table 1). For DNA extraction, we removed four to five ascomata per specimen from the substratum and placed them into a 1.5 mL test tube. We used a High Pure PCR Template Preparation Kit (Roche Applied Science®) and followed the protocol provided by the manufacturer.

We amplified two nuclear (full-length internal transcribed spacer [ITS] and partial large subunit [nuLSU]), and one mitochondrial (small subunit (mtSSU)) ribosomal DNA regions. To amplify these loci, we used the following primer pairs: ITS0F / ITS4, ITS0F / LA-W or ITS1 / LA-W (White *et al*. 1990, Tedersoo *et al*. 2008) for ITS, LR0R / LR7 or LR0R / LR5 (Vilgalys & Hester 1990) for nuLSU, and mrSSU1 / mrSSU3r (Zoller *et al*. 1999) for mtSSU. The PCR mix (25 µL) consisted of 5 µL 5× HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µL of both primers, 3–8 µL of target-DNA and the rest of distilled water. We visualized the PCR products on a 1 % agarose gel stained with ethidium bromide. For the purification of PCR products, we added 1 µL of FastAP and 0.5 µL of Exonuclease I (Thermo Scientific, Waltham, MA, USA) to each tube and the tubes were incubated at 37 °C for 45 min, and the enzymes were deactivated by heating at 85 °C for 15 min. We sequenced both complementary strands of ITS using primer pairs ITS4 and ITS5 (White *et al.* 1990), and nuLSU with CTB6 (Garbelotto *et al*. 1997) and LR7, and mtSSU with the same primers as amplified. We performed DNA extraction, amplification, and purification in the molecular lab of mycology at the University of Tartu (TU, Estonia) and we Sanger sequenced the amplicons by Macrogen Inc. (Amsterdam, the Netherlands).

We used Sequencher v. 4.10.1. (GeneCodes Corp.®, Ann Arbor, MI, USA) or CodonCode Aligner v. 8.0.2 (CodonCode Corporation®, Centerville, MA, USA) to check, assemble, and manually edit the sequence fragments. To avoid misidentifications, we compared the consensus sequences with those available in the nucleotide database of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) using the 'megablast' algorithm (Altschul *et al*. 1990). We deposited the newly generated DNA sequences in NCBI and UNITE (Abarenkov *et al.* 2010) data repositories. The sequenced voucher specimens are in DAU, TUF, NY, TRH, UPS, and the extracted DNA samples in the DNA and Environmental Sample Collection of the Natural History Museum in Tartu University (TUE). We also provide UNITE Species Hypotheses (SH; Kõljalg *et al.* 2013) at a distance value of 1.5 %, and a reference sequence for each recognized taxon.

Table 1. Information about voucher specimens (taxon name, voucher ID, country of origin, lab code, and morpho ID), and NCBI accession codes of the new and downloaded DNA sequences (full-length ITS, nuLSU and mtSSU) used for reconstruction of phylogeny presented on Figs 1 and 2. "—" means sequence not generated or not available for this specimen. The type specimens are in **bold.**

Taxon name	Voucher ID	Country	Lab code	Morpho ID	ITS	LSU	mtSSU
Chaenotheca biesboschii	A.v.d. Pluijm 3244 (UPS)	Netherlands			MK514539	—	—
	A.v.d. Pluijm 3244 (UPS)	Netherlands			MK376459	—	—
Chaenotheca brachypoda	Tibell 22193 (UPS)	Sweden			AF297963	—	—
	Tibell 17062 (UPS)	Sweden			AF297962	—	—
Chaenotheca brunneola	Tibell 22202 (UPS)	Sweden			AF297964	—	—
	TUF076414	Estonia	CB22		KX348121	—	—
	TUF076421	Estonia	CB14		KX348125	—	—
Chaenotheca chlorella	Tibell 16867 (UPS)	Sweden			—	AY804191	—
	Tibell 22372 (UPS)	Estonia			AF445356	—	—
	Tibell 22187 (UPS)	Sweden			AF297966	—	—
Chaenotheca chrysocephala	Tibell 21799 (UPS)	Sweden			AF298121	—	—
	PRA-Vondrak26008	Austria			OQ717362	—	—
Chaenotheca cinerea	TUF039194	Estonia	BF18		KX348119	—	—
	Tibell 22374 (UPS)	Estonia			AF421201	—	—
	Jonsson & Nordin (UPS)	Sweden			AF298122	—	—
Chaenotheca deludens	Tibell 16575	New Zealand			AF408678	—	—
Chaenotheca ferruginea	TUF089549	Estonia	CH463		OR661708	OR661698	—
	Tibell 22276 (UPS)	Sweden			AF298123	—	—
Chaenotheca furfuracea	Wedin 6366 (UPS)	Unspecified			—	JX000087	JX000121
	TUF091901	Estonia	AS899		OR661703	—	—
	Tibell 22364	Sweden			AF445357	—	—
Chaenotheca gracilenta	Wedin 7022 (S)	Unspecified			JX000100	JX000084	JX000119
	TUF030149	Estonia	BF30		KX348118	—	—
Chaenotheca gracillima	TUF091585	Estonia	CH289		OR661701	—	OR661671
	Tibell 16725 (UPS)	New Zealand			AF408682	—	—
	Tibell 17614 (UPS)	Argentina			AF408679	—	—
Chaenotheca hispidula	TUF051093	Latvia	CH361		OR661707	—	—
	Tibell 21900 (UPS)	India			AF298128	—	—
Chaenotheca hygrophila	TNS:YO9596	Japan			LC669601	—	—
	Thor 15612	Japan			AF298129	—	—
Chaenotheca laevigata	Tibell 21998b (UPS)	India			AF298131	—	—
	Tibell 22176 (UPS)	Sweden			AF298130	—	—
Chaenotheca nitidula	Tibell 21490 (UPS)	USA			AF492388	—	—
	Koffman 170 (UPS)	Canada			AF492387	—	—
Chaenotheca phaeocephala	Tibell 22291 (UPS)	Sweden			AF446045	—	—
	Tibell 21819 (UPS)	Sweden			AF445360	—	—
Chaenotheca sp.	Tibell 22113 (UPS)	India			AF298135	—	—
Chaenotheca sphaerocephala	Tibell 21939 (UPS)	India			AF298134	—	—
Chaenotheca stemonea	Tibell 22191 (UPS)	Sweden			AF408683	—	—
	WSL:BC-087-3	Switzerland			KX133006	—	—
Chaenotheca subroscida	Tibell 22150 (UPS)	Sweden			AF298136	—	—
	TUF049310	Estonia	CH760		OR661702	—	—
Chaenotheca trichialis	Prieto 3028 (S)	Unspecified			JX000102	JX000085	JX000120
	Tibell 22384 (UPS)	Sweden			AF421207	—	—
	KR-0051902	Unspecified			MW325680	—	—

Table 1. (Continued).

Taxon name	Voucher ID	Country	Lab code	Morpho ID	ITS	LSU	mtSSU
Chaenotheca xyloxena	Selva 7753 (UMFK)	Canada			AF421213	—	—
	Tibell 22329 (UPS)	Sweden			AF421212	—	—
Chaenotricha cilians	TUF095043	Norway	AS972		OR661716	—	OR661678
	UPS-L-941561	Sweden	CH419		OR661713	OR661686	OR661659
	TUF091610	Estonia	CH99		—	OR661699	—
	TUF089479	Canada	CH484	1	—	—	OR661670
	TUF050023	Estonia	AS699	2	OR661715	OR661694	OR661676
	TUF076412	Estonia	CH168	3	KX348131	—	—
	TUF076423	Estonia	CH98	4	KX348120	OR661693	OR661672
	TUF091611	Estonia	CH290	5	—	—	OR661669
	TUF091612	**Estonia**	**CH288**	**6**	**OR661721**	**OR661692**	**OR661665**
	DAU0602050	Latvia	CH310	7	OR661717	—	OR661666
	DAU0602051	Latvia	CH311	8	—	—	OR661667
	DAU0602052	Latvia	CH312	9	—	—	OR661668
	TUF089401	Latvia	CH481	10	—	OR661689	OR661662
	TUF090000	Latvia	CH480	11	OR661712	OR661688	OR661661
	TRH-L-18707	Norway	CH435	12	OR661709	OR661687	OR661660
	TRH-L-18708	Norway	CH434	13	OR661711	OR661690	OR661663
	TUF050022	Norway	AS698	14	OR661714	OR661695	OR661675
	TUF095044	Norway	AS973	15	OR661719	—	OR661677
	BILAS	Russia	CH436	16	—	OR661691	OR661664
	UPS-L-867275	Sweden	CH418	17	OR661710	—	—
	UPS-L-872283	Sweden	CH420	18	OR661718	—	—
	TUF076413	Estonia	CB1		KX348130	—	—
	TUF076417	Estonia	CB3		KX348129	—	—
	TUF076416	Estonia	CB5		KX348128	—	—
	TUF076420	Estonia	CB15		KX348124	—	—
	TUF076419	Estonia	CB19		KX348123	—	—
	TUF076422	Estonia	CB21		KX348122	—	—
Chaenotricha obscura	TUF089391	Canada	CH488	24	OR661720	OR661684	OR661657
	NY02359896	**USA**	**CH174**	**25**	**KX348132**	**OR661679**	**OR661652**
	NY02439109 (epitype)	**USA**	**CH175**	**26**	**KX348133**	**OR661680**	**OR661653**
	CANL20337 (lectotype)	**USA**		**27**	—	—	—
Chaenotricha sp.	TUF089393	Canada	CH490	19	—	OR661681	OR661654
	TUF089480	Canada	CH485	20	—	OR661682	OR661655
	TUF089481	Canada	CH486	21	—	OR661696	OR661674
	TUF089547	Estonia	CH461	22	—	OR661683	OR661656
	TUF089548	Estonia	CH462	23	—	OR661685	OR661658
Lempholemma polyanthes	Zoladeski & Lutzoni 11294-L1(2/2) (CANL	Unspecified			—	AF356691	AY584709
Peltula auriculata	B. Büdel 24902	Venezuela			MF766344	MF766385	MF766303
	Herb. B. Büdel 24901	Venezuela			—	DQ832330	DQ922953
Peltula rodriguesii	B. Büdel 15901	Namibia			MF766373	—	—
Sclerophora amabilis	PRA-Vondrak24780	Czechia			OQ718083	—	—
	PRA-Vondrak24776	Czechia			OQ718082	—	—
Sclerophora farinacea	TUF086803	Estonia	SC406		OR661706	OR661700	—
	TUF055034	Estonia	BF32		OR661705	—	—

Table 1. (Continued).

Taxon name	Voucher ID	Country	Lab code	Morpho ID	ITS	LSU	mtSSU
Sclerophora pallida	EDNA09-01585	United Kingdom			FR799288	—	—
	EDNA09-01513	United Kingdom			FR799287	—	—
Sclerophora peronella	TUF051090	Estonia	SC362		OR661704	OR661697	OR661673
	TUF038050	Estonia	BF16		KX348134	—	—

Phylogenetic analysis

We successfully generated 72 new sequences (18 ITS, 24 nuLSU and 30 mtSSU; Table 1). Sequence blasting in NCBI indicated that our specimens belong to *Coniocybomycetes*. We therefore downloaded sequences of *Chaenotheca* and *Sclerophora* from NCBI and UNITE data repositories (Table 1) to assess the phylogenetic position of these *Trichaptum*-habiting specimens. We included the closest relatives to *Coniocybomycetes*, species of *Lichinomycetes* – *Peltula auriculata* and *P. rodriguesii* to root the ITS-based phylogeny, and *P. auriculata* and *Lempholemma polyanthes* to root the three-locus phylogeny. Approximately 50 *Chaenotheca* species and seven *Sclerophora* species are described (Index Fungorum; https://www.indexfungorum.org; accessed 6 Sep. 2023; and salient literature), of them DNA sequences are available for 21 and four species respectively in publicly accessible repositories.

We aligned sequences with the online version of MAFFT v. 7 (Katoh *et al*. 2019; https://mafft.cbrc.jp/alignment/server/) using default options and then manually adjusted them in Seaview v. 3.2 (Gouy *et al*. 2010) or AliView v. 1.27 (Larsson 2014). We refined the nuLSU and mtSSU alignments, *i.e*., eliminated poorly aligned positions and divergent regions by using Gblocks v. 0.91b (Talavera & Castresana 2007; http://www.phylogeny.fr/). In Gblocks, we used relaxed settings by allowing gap positions within the final blocks and less strict flanking positions. From the ITS alignment, we used ITSx (Bengtsson-Palme *et al*. 2013) in the PlutoF workbench (https://plutof.ut.ee) for extraction of neighboring conservative rDNA regions.

We reconstructed single-gene phylogenies with Maximum Likelihood (ML) using IQ-TREE v. 2 (Trifinopoulos *et al*. 2016; http://iqtree.cibiv.univie.ac.at) to detect possible conflicts among individual genes. We selected GTR+I+G4+F as the nucleotide substitution model and tested branch support with ultrafast bootstrapping (Minh *et al*. 2013) by applying 1 000 iterations. No incongruences were found, so we concatenated the mtSSU, nuLSU and ITS alignments. In the concatenated alignment, we considered gaps as missing characters. Next, we analyzed these two datasets, *i.e*., the concatenated mtSSU+nuLSU+ITS (37 specimens; 2 229 characters, of which 714 bp are mtSSU, 816 bp nuLSU, and 669 bp ITS) and ITS (69 sequences: 737 characters, of which 117 were parsimony informative) datasets. We used both datasets to 1) assess the phylogenetic position of the fungicolous lineage in relation to *Sclerophora* and *Chaenotheca s. lat.*; and 2) evaluate the species limits within the fungicolous group. We deposited the alignments in the TreeBASE repository (study ID S30840).

Next, based on the concatenated alignment, we inferred the phylogenetic relationships and the tree confidence by using two different methodologies: the Metropolis coupled Markov Chain Monte Carlo (MCMC) approach implemented in MrBayes v. 3.2.1. (Ronquist *et al*. 2012) and Maximum Likelihood (ML) in RAxML (Stamatakis 2006), using RAxML-NG v. 1.0.0 software (Kozlov *et al*. 2019; https://raxml-ng.vital-it.ch/). We calculated the best-fit nucleotide substitution model using PartitionFinder v. 2.1.1 (Lanfear *et al*. 2012). The best-fit models according to the lowest value of the Akaike Information Criterion (AICc) were TrN+I+G for nuLSU and GTR+I+G for mtSSU and ITS. The settings for MCMC were as follows: two parallel, simultaneous runs with four incrementally heated chains starting with a random tree; *ngen* = 1 M generations, *samplefreq* and *diagnfreq* = 500, *printfreq* = 2 000. We ran the analysis until the standard deviation of split frequencies (SDSF) was below 0.01 and the potential scale reduction factor (PSRF) was close to 1 indicating convergence of the chains. We discarded the first 25 % as 'burn-in' and a consensus tree and posterior probabilities (PP) were calculated from the remaining tree distribution. We calculated Maximum Likelihood (ML) using a GTR+F0+G nucleotide substitution model, bootstrap support of the ML topology was obtained using *bootstrapping* with 1 000 pseudo-replicates (bootstrap cut-off was 0.01). In the phylogenetic trees, we considered clades supported when posterior probabilities (PP) were ≥ 0.95 and bootstrap values (BS) ≥ 70 %. Our consensus trees were visualized using FigTree v. 1.4.4 (Rambaut 2014) and annotated with Adobe Illustrator v. 13.0.0 CS3®.

RESULTS AND DISCUSSION

The analysis of two datasets (concatenated mtSSU+nuLSU+ITS and ITS only) indicate that the *Trichaptum*-dwelling specimens form a distinct, highly supported lineage sister to the *Chaenotheca-Sclerophora* clade (mtSSU+nuLSU+ITS: BS = 100 %, PP = 1; ITS: BS = 99 %) and in all combinations the *Sclerophora* clade is nested within *Chaenotheca s. lat.* (Figs 1, 2). In both analyses, the closest relatives are species in the *Chaenotheca brunneola* group (*i.e.*, *C. brunneola, C. deludens, C. ferruginea, C. hygrophila*, *C. sphaerocephala* and *C. stemonea*), but this relationship is supported only in the ITS-based phylogeny (Figs 1, 2). Following the smaller genus concepts of Tibell *et al*. (2019) based on an ITS-phylogeny and morphology, and instead of incorporating all species of *Coniocybomycetes* under the single name *Chaenotheca*, we describe a new genus – *Chaenotricha* – to accommodate all *Trichaptum*-specialists (see the Taxonomy section). This allows us to retain *Sclerophora* as a separate taxonomic unit without combining it with *Chaenotheca*.

The Bayesian and ML trees were topologically concordant and revealed three lineages within *Chaenotricha* (Fig. 2), instead of the previously recognized two (Suija *et al*. 2016). One of the groups includes the holotype of *C. balsamconensis* (= *C.*

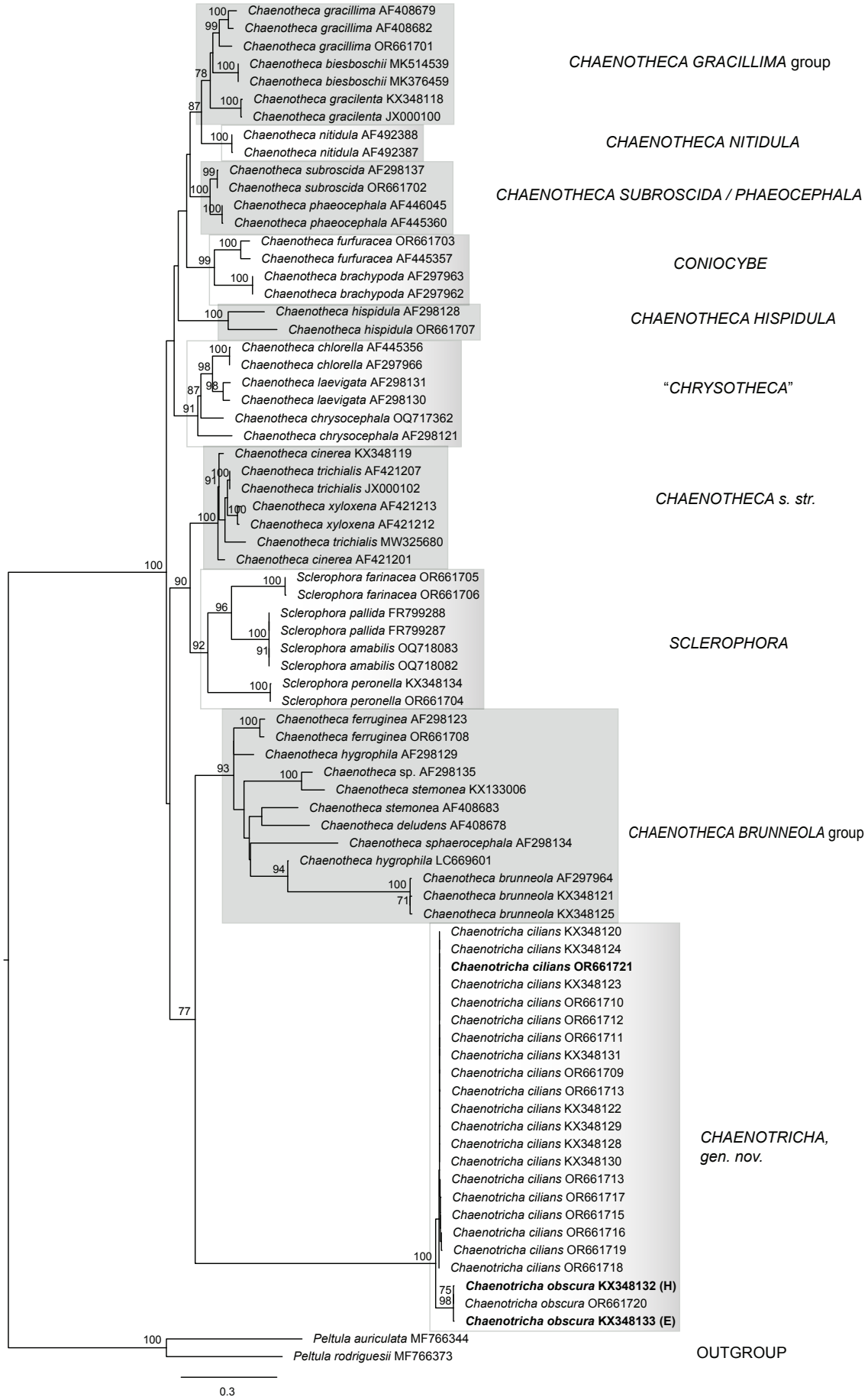

Fig. 1. Maximum likelihood (ML) phylogeny based on rDNA ITS sequences generated for this study and derived from NCBI and UNITE database (Table 1) and showing the position of *Chaenotricha* in relation to other "taxa" within *Coniocybomycetes*. The names of the clades follow Tibell *et al.* (2019); bootstrap support values (BS) ≥ 70 % are above branches. Letters "H" and "E" in brackets indicate the holotype of *Chaenotheca balsamconensis* (= *Chaenotricha obscura*) and epitype of *Chaeotricha obscura*, respectively.

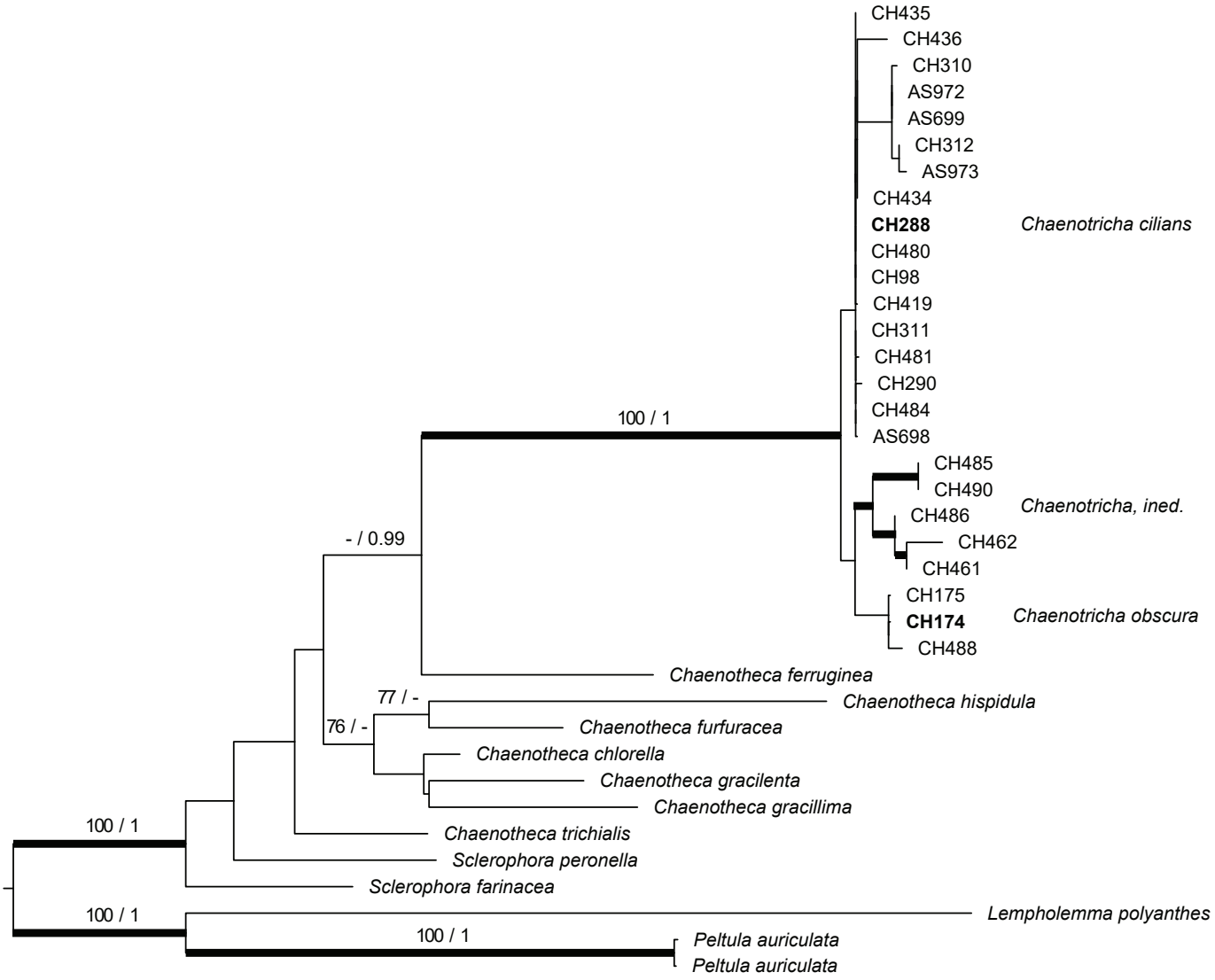

Fig. 2. Three-locus phylogeny (mtSSU+nuLSU+ITS) based on the Maximum Likelihood consensus tree showing the position of *Chaenotricha* within *Coniocybomycetes* and three clades within it corresponding to *Chaenotricha* species. The branches with posterior probabilities (PPs) ≥ 0.95 and bootstrap values (BS) ≥ 70 % are considered as supported and indicated with a thicker line. Support values are above branches. Species of *Lichinomycetes* form an outgroup. Type specimens are in **bold**. CH174 - holotype of *Chaenotheca balsamconensis*; CH175 - epitype of *Chaenotricha obscura*.

obscura, combined with *Chaenotricha* here), and two other specimens from USA and Canada. The second group includes 18 specimens from Europe (Estonia, Latvia, Norway, Sweden, European Russia), and one from Canada, and the third group is from Canada and Estonia (five specimens). The specimens in the second group have smaller ascospores and shorter stalk lengths compared to *C. obscura* (Fig. 3, Table 2). Also, the pairwise comparison of ITS sequences revealed that there are 23 parsimony informative characters (4.6 % of 504 bp, n = 25) separating these two groups as distinct species (Table 3). The third group is also well-supported molecularly, but we failed to obtain ITS sequences from any of these specimens. However, the sequences of slow-evolving genes were divergent enough not to incorporate these specimens under the names *C. cilians* or *C. obscura* (Fig. 2, Table 3). We found that the morphological characteristics of these specimens are somewhat intermediate between *C. obscura* and *C. cilians* (Fig. 3, Table 2), but because of the small sample size, we are only describing the two species that are morphologically distinct for now.

Taxonomy

Chaenotricha Suija, McMullin & P. Lõhmus, ***gen. nov.*** MycoBank MB 850355. Figs 4–7; fig. 1 in Allen & McMullin (2015).

Etymology: The name combines two genus names – *Chaenotheca*, a genus in which the type species was originally settled, and *Trichaptum*, a genus on which the lichenized species grow.

Type species: *Chaenotricha obscura* (G. Merr.) Suija, McMullin & P. Lõhmus

Typus: *Calicium (Allodium) obscurum* Merrill. Merrill, Lichenes Exsiccati prepared by G.K. Merrill no. 92. **USA**, Rockland, Maine, on dead fungus, 5 Sep. 1909, *G.K. Merrill* (**lectotype** designated here CANL 20337!, *vidi*, MBT 10016450; isotype M0205375!, *vidi*).

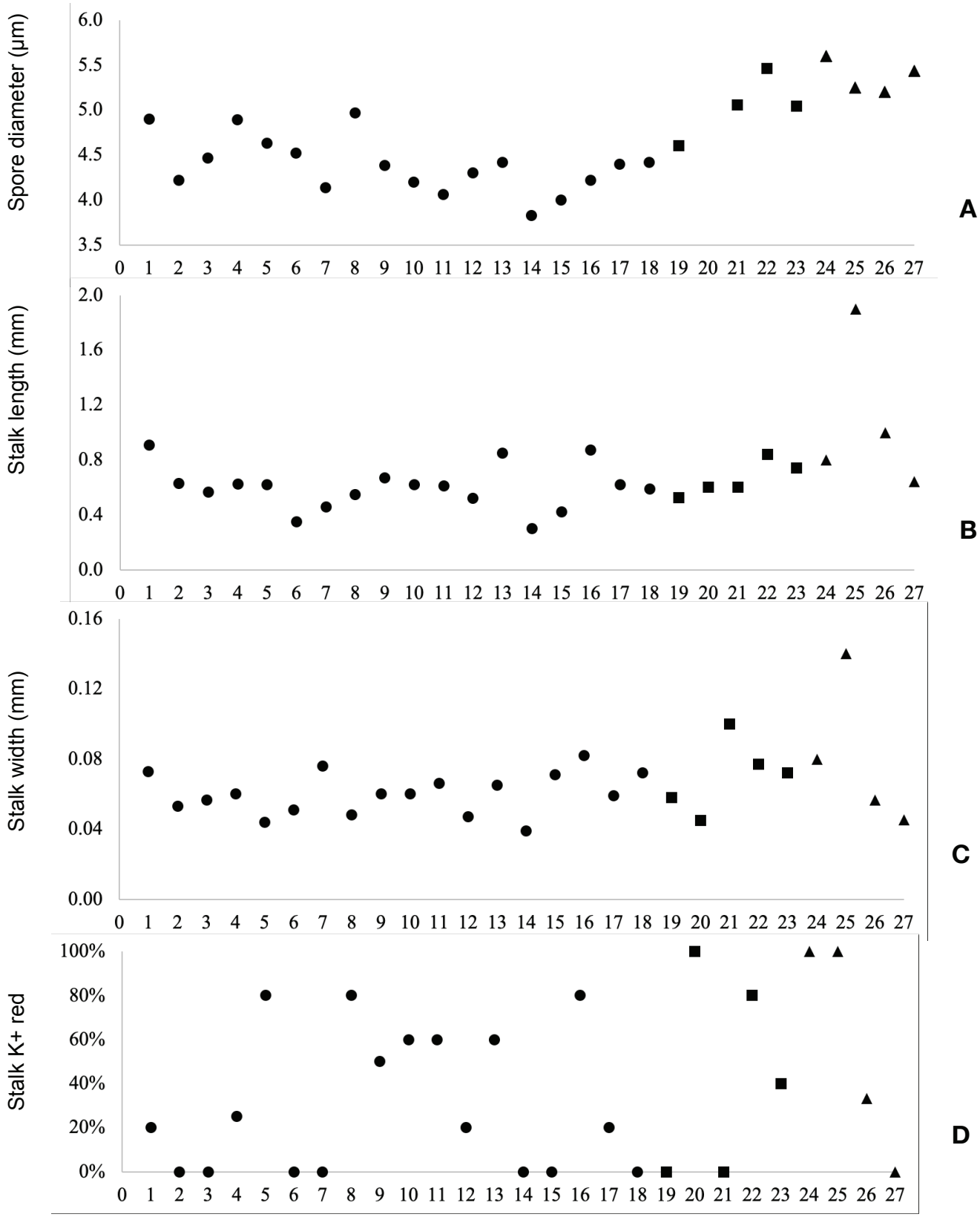

Fig. 3. Average values of anatomical (**A**) and morphological (**B**, **C**) characters and the proportion of apothecia with stalks that have a K+ red reaction (**D**) of the studied specimens of *Chaenotricha cilians* (●), *Chaenotricha* sp. (■) and *C. obscura* (▲). Specimen ID numbers correspond to vouchers in Table 1. Five apothecia were surveyed per specimen, except for ID #4 (four apothecia), #3, #26–27 (three), and #20, #24, #25 (one). Type specimen of *C. cilians* is ID #6. Spore data are missing for #20.

Table 2. Morphological and anatomical apothecial characters for three *Chaenotricha* species. Sample size (N) represents the number of specimens studied (for each specimen, up to five apothecia were studied; also see corresponding illustrations in Fig. 3).

Character	*Chaenotricha cilians*					*Chaenotricha* sp.					*Chaenotricha obscura*				
	N	min	**Mean**	max	SD	N	min	**Mean**	max	SD	N	min	**Mean**	max	SD
Stalk with K+ red (%)	18	0	**31**	80	32	5	0	**44**	100	46	4	0	**58**	100	50
Stalk+capitulum length (mm)	18	0.4	**0.7**	1.0	0.2	5	0.6	**0.8**	1.0	0.2	4	0.8	**1.3**	2.3	0.7
Stalk length (mm)	18	0.3	**0.6**	0.9	0.2	5	0.5	**0.7**	0.8	0.1	4	0.6	**1.1**	1.9	0.6
Stalk width (mm)	18	0.04	**0.06**	0.08	0.01	5	0.05	**0.07**	0.10	0.02	4	0.05	**0.08**	0.14	0.04
Stalk length to width ratio	18	6	**10**	14	2	5	6	**10**	13	3	4	10	**14**	18	3
Capitulum length (mm)	18	0.03	**0.08**	0.12	0.03	4	0.10	**0.12**	0.13	0.01	4	0.05	**0.19**	0.40	0.15
Capitulum width (mm)	18	0.1	**0.2**	0.3	0.0	4	0.2	**0.3**	0.3	0.0	4	0.1	**0.3**	0.6	0.2
Mazaedium length (mm)	18	0.03	**0.08**	0.16	0.04	4	0.08	**0.14**	0.22	0.06	2	0.05	**0.07**	0.08	0.02
Ascus length (µm)	17	16	**18**	20	1	4	18	**20**	21	1	2	16	**23**	30	10
Ascospore diameter (µm)	18	3.8	**4.4**	5.0	0.3	4	4.6	**5.0**	5.5	0.4	4	5.2	**5.4**	5.6	0.2

Table 3. An overview of the single-gene alignments (full-length ITS, nuLSU, and mtSSU) for the *Trichaptum*-specialized specimens characterized by the number of sequences in the alignment, number of base pairs in the alignment (length), number and percentage of variable nucleotide positions and parsimony informative nucleotide positions.

Locus	No. of sequences	Length (bp)	Variable (%)	Informative (%)
ITS	25	504	25 (5.0)	23 (4.6)
nuLSU	18	1 242	38 (3.1)	18 (1.5)
mtSSU	25	818	7 (1.3)	5 (0.9)

Diagnosis: Species in this genus grow exclusively on sporocarps of *Trichaptum*, which distinguishes it from the rest of *Coniocybomycetes*. It differs from the species in the *Chaenotheca brunneola* group by having ascospores without fissures and cracks.

Description: *Thallus* immersed or inconspicuous, forming loose associations with unicellular green algae on the surface of *Trichaptum* sporocarps or infrequently episubstratal forming ecorticate, granular aggregations of hyphae and algae. *Ascomata* stalked, stalk dark brown to black, mostly shiny, straight to somewhat curved, consisting of periclinally arranged brown hyphae, surface uneven, stalk K– or K+ red (color bleeds from the stalk). *Capitulum* spherical to obconical, epruinose. Mazaedium powdery. True excipulum and hypothecium well-developed, brown to dark brown, formed as a continuation of the stalk, with similar hyphal structure. *Hamathecium* consists of asci dissolving at the early stage of development, and paraphyses. *Asci* cylindrical, raising singly and directly from the ascogenous hyphae, no croziers, consisting of eight uniseriately arranged ascospores, stalked. *Paraphyses* hyaline, straight, not swollen at tips, without septa. *Ascospores* aseptate, at the early stage of development hyaline, brown when mature, smooth, spherical to irregularly spherical. *Asexual morph* not observed.

Fig. 4. Isotype of *Chaenotricha obscura* from Merrill, Lich. Exs. Ser. I 92 (CANL). Scale bar = 0.5 mm.

Chaenotricha obscura (G. Merr.) Suija, McMullin & P. Lõhmus, ***comb. nov.*** MycoBank MB 850356.
Basionym: *Calicium obscurum* G. Merr., *Bryologist* **12**: 107. 1909.
Synonyms: *Chaenotheca obscura* (G. Merr.) Nádv., *Stud. Bot. Čechoslov*. **5**: 124. 1942.
Chaenotheca balsamconensis J.L. Allen & McMullin, *Bryologist* **118**: 55. 2015.
Epitype: **USA**, Michigan, Chippewa county, Hiawatha National Forest, FS3343 1.5 mi E of jct w/ MI-123, 1.9 mi NE of Trout Lake, 4.3 mi NW of Old Dick (45.21444°N, 84.889722°W), bog dominated by *Pinus banksiana* with additional hardwoods (*Acer*, *Betula*, *Populus*, *Salix*) and conifers (*Abies*, *Larix*, *Picea*), on *T. abietinum* on dead *Pinus banksiana*, *leg. J.C. Lendemer*, #45283-A (**epitype** designated here NY02439109, *vidi*, MBT 10016475).

Species hypothesis: SH1265129.09FU.

Reference sequence from the epitype: ITS (GenBank KX348133), other available gene sequences nuLSU (GenBank OR661680), mtSSU (GenBank OR661653).

Materials examined: **Canada,** Lunenburg County, *ca*. 1 km N of Crouse's Settlement, *ca*. 1 km NE of Crouse's Settlement Road, 8 m E of Old Wood's Road (44.3561°N, 64.4050°W), coastal mature mixed-wood forest, on *T. abietinum*, on a dead *Abies balsamea*, *leg*. *F. Anderson*, *det*. *T. R. McMullin* (TUF089391). **USA**, North Carolina, Yancey County: Mount Mitchell State Park, Balsam Cone summit and vicinity, *ca*. 2 mi N of Mount Mitchell, *ca*. 3 mi W of US80 (35.7894°N, 82.2559°W), spruce (*Picea*)-fir (*Abies*) forest with *Betula, Rhododendron*, and *Sorbus* on top of narrow ridge with scattered, large rock outcrops, on *T. abietinum*, on dead *Abies*, *leg*. *J.L. Allen & J.C. Lendemer*, J. Allen #4108 (**holotype** of *C. balsamconensis* NY02359896).

Chaenotricha cilians Suija, McMullin & P. Lõhmus, ***sp. nov.*** MycoBank MB 850357. Figs 5–7, and fig. 1 in Suija *et al*. (2016).

Etymology: The epithet *cilians* was used by Theodor Magnus Fries (1832–1913) for a variant of *Chaenotheca brunneola* inhabiting *Trichaptum* sporocarps. The name was adopted by him due to the resemblance to eyelashes.

Diagnosis: This species differs from *Chaenotricha obscura* by having shorter stalks on average 0.6 mm *vs*. 1.1 mm, and slightly smaller ascospores, on average 4.5 µm *vs*. 5.5 µm. The species is similar to *Chaenotheca brunneola* (lignicolous) except inhabiting fruitbodies of *Trichaptum* spp., having exclusively cylindrical asci with uniseriately arranged smooth ascospores and stalks K– or with a K+ red reaction (bleeds from the stalk).

Typus: **Estonia**, Tartu Co., Kardla village (58.4204°N 26.5604°E), *Aegopodium* boreo-nemoral forest site type, 66-yr-old Norway spruce dominated forest, on *Trichaptum* on a *Picea abies* snag, 2 Jun. 2017, *leg. P. Lõhmus*, Kardla ID27 (**holotype** TUF091612).

Species hypothesis: SH1265130.09FU.

Reference sequence: ITS (UNITE: UDB0801842; GenBank OR661721), other gene sequences nuLSU (GenBank OR661692), mtSSU (GenBank OR661665).

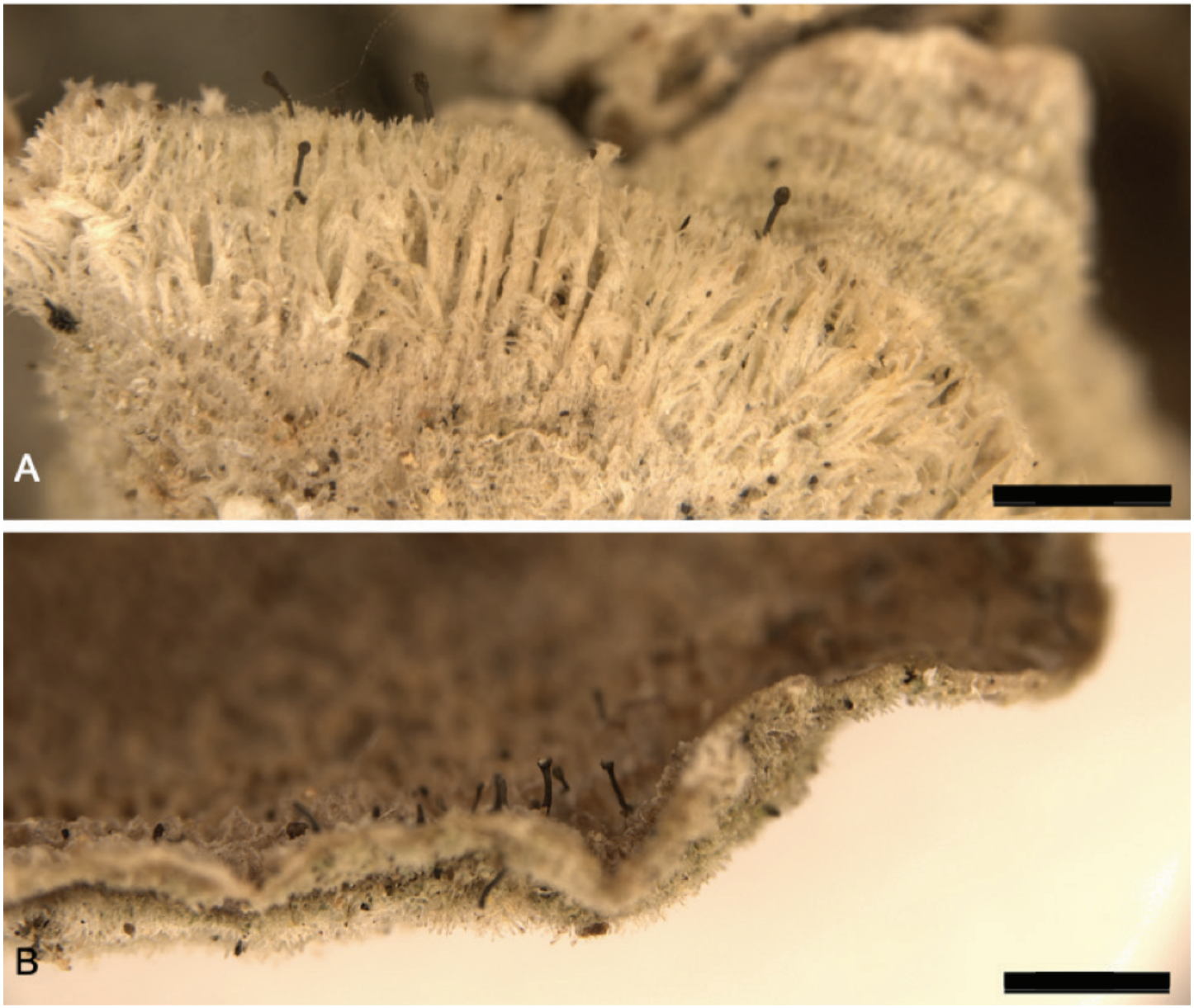

Fig. 5. *Chaenotricha cilians sp. nov*. **A.** Ascomata of *C. cilians* on the upper side of the *Trichaptum* fruitbodies (TUF091612, holotype). **B.** Ascomata on the hymenophore layer of the *Trichaptum* fruitbodies (TUF050023). Scale bars = 20 mm.

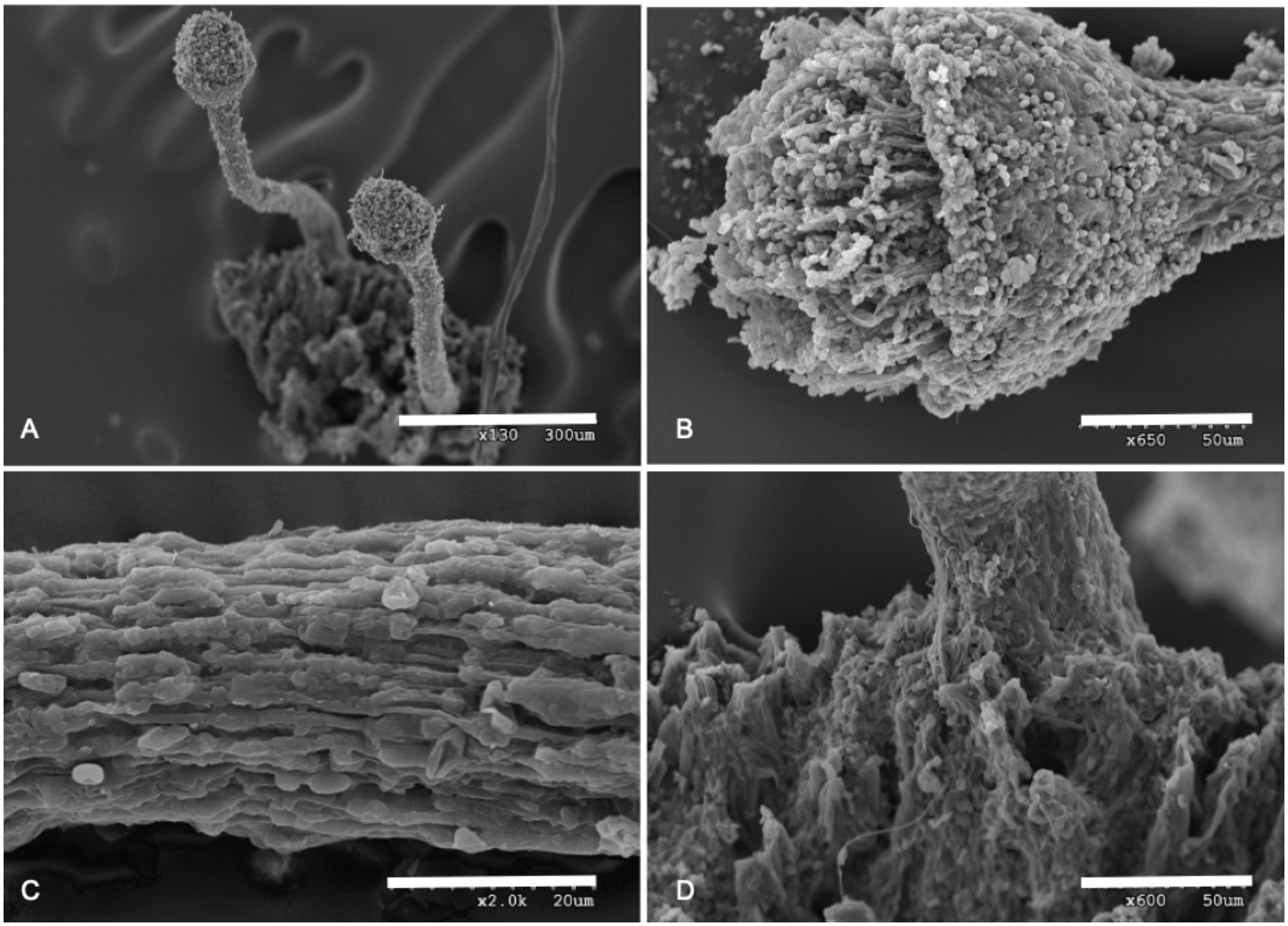

Fig. 6. Scanning electron microscopy of *Chaenotricha cilians* (specimen TRH-L-18708). **A.** Ascomata (note the obovate shape of the mazaedium). **B.** Exciple surrounding the hamathecium. **C.** Periclinally arranged hyphae of the stalk. **D.** Base of ascomatal stalk. Scale bars: A = 300 µm; B, D = 50 µm; C = 20 µm.

Description: *Thallus* inconspicuous, mycobiont hyphae loosely connected with cells of trebouxioid algae on the sporocarp surface. *Ascomata* developed on the upper surface and at the edge of the fungal sporocarp. *Stalk* epruinose, dark brown, K–, (0.35–)0.4 ± 0.07(–0.5) mm in length × (0.045–)0.05 ± 0.01(–0.06) mm in width, length to width ratio (6–)7 ± 1(–8). *Capitulum* spherical to obconical, (0.10–)0.13 ± 0.01(–0.14) mm in diameter (n = 5). *Excipulum* well developed, mazaedium dark brown, powdery. *Asci* cylindrical, born singly on a stalk, (15–)19.3 ± 4.4(–30) µm in length (n = 20); some measured asci (n = 9) had a stipe, (5–)5.6 ± 1.7(–10) µm long. *Ascospores* arranged within the ascus uniseriately, hyaline when young, brown, smooth, spherical to irregularly spherical, (3–)4.5 ± 0.6(–5) µm diam (n = 50). *Asexual morph* not observed.

Ecology and Distribution: *Chaenotricha cilians* grows on the sporocarps of three *Trichaptum* species, *T. abietinum, T. fuscoviolaceum* and *T. biforme.* So far, the distribution includes European countries (Denmark, Estonia, Latvia, Lithuania, Norway, Russia, Sweden) but there is also one record from Canada. *Chaeotricha cilians* is reported from hemiboreal and boreal forests and bog areas, on *Trichaptum* sporocarps inhabiting standing dead trunks, mainly of Norway spruce and Scots pine and rarely on birch.

Notes: Eighteen specimens were examined (see Table 1), and on a few occasions ascomata were produced on the hymenophore surface (Fig 4B), and in those cases, we did not find associations with algae using a compound light microscope. Moreover, we did not locate living or dead algal cells in the area around the ascomata. Life-style switching is common among fungi, and optional and weak lichenization has been demonstrated for several groups of ascomycetes (*e.g.*, Wedin *et al.* 2006, Pérez-Ortega *et al*. 2016). Our results suggest that *C. cilians* may be an example of optional lichenization. Genome screening, metatranscriptomics, and other techniques may provide further information about the relationships of this tri-partite association.

The intensity of the K+ red pigment reaction of the stalks and the degree of shininess can vary within and among specimens (Fig. 3D, Table 2). The length of apothecia (and other anatomical and morphological characters) may vary slightly among specimens (Fig. 3, Table 2); for example, the type specimen has 0.2 mm shorter stalks than the average length measured for 18 specimens (Table 2). The species differs significantly from *Chaenotricha obscura* by its 1 µm smaller spores, respectively (Table 2; Mann-Whitney U test, U = 7.00, p = 0.013) and in the length of the stalk, which is significantly shorter than that of *C. obscura* (on average 0.5 mm, Table 2; U = 0, p = 0.002), However,

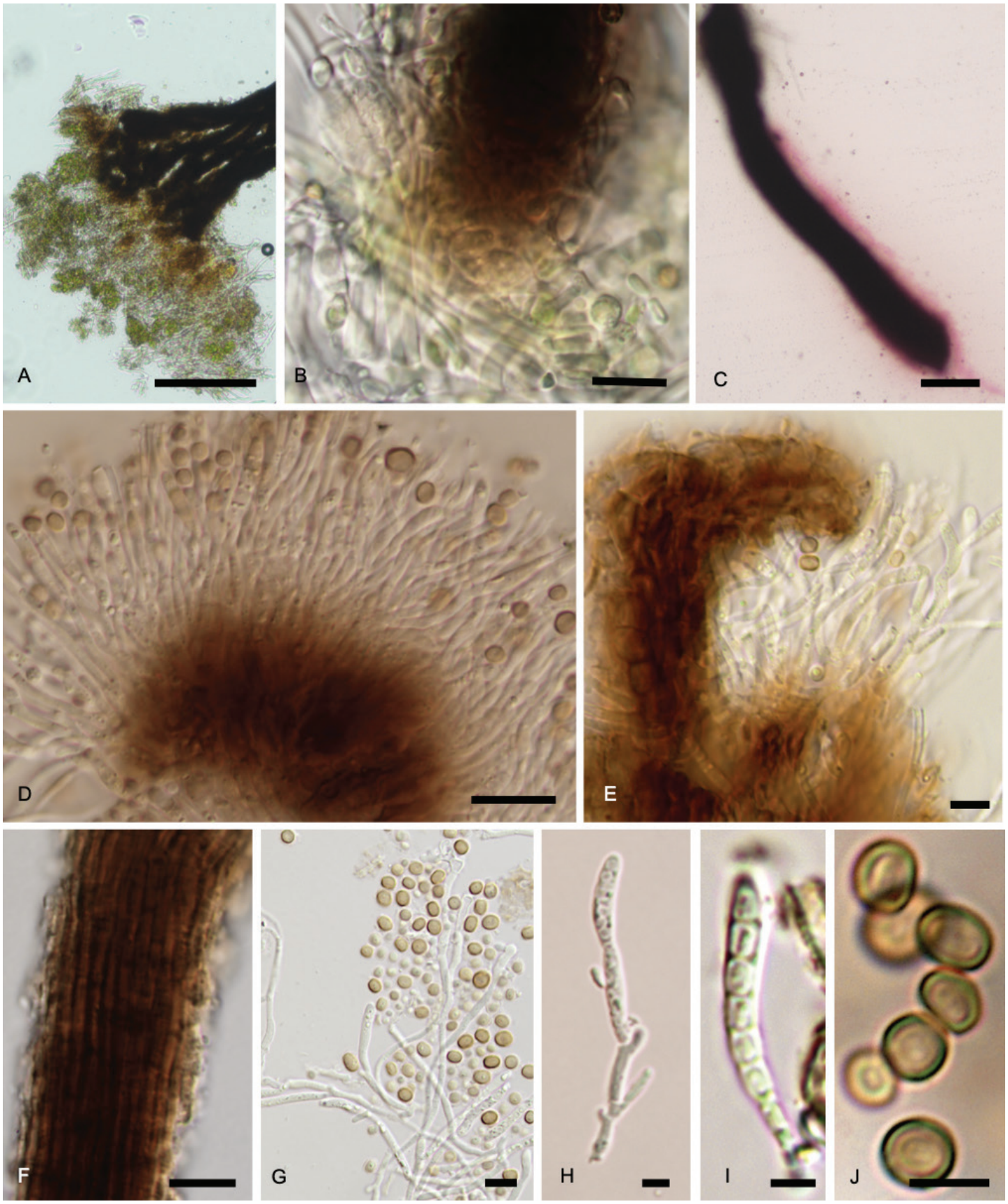

Fig. 7. Micromorphological characters of *Chaenotricha cilians* (TUF050023). **A, B**. Base of the stalk. **C.** K+ bleeding red reaction of the apothecium. **D.** Hypothecium and hamathecium consisting of asci at different developmental stages and paraphyses, and single liberated ascospores. **E.** Exciple. **F.** Stalk structure (note periclinally arranged hyphae and uneven surface of the stalk). **G.** Developing asci, paraphyses and ascospores **H.** Ascus (immature) developing singly from the ascogenous cells. **I.** Immature ascus with developing ascospores. **J.** Mature ascospores. Scale bars: A, C = 50 µm; B, F = 20 µm; D, E, G = 10 µm; H–J = 5 µm.

because of very unequal (and small) sample sizes of species groups, the results of statistical tests should be interpreted with caution.

Materials examined (selected): **Denmark**, North Denmark Region, Thisted Municipality, Nationalpark Thy (56.9769°N, 8.4274°E), on *Trichaptum* sp. on *Pinus contorta*, 14 Oct. 2023, *leg. P. Lõhmus & A. Suija* (TUF095157); *ibid*., Thagaard plantation (56.9817°N, 8.4283°N), on *T. abietinum* on standing corticated *Pinus cf. mugo*, 14 Oct. 2023, *leg. N. Johansson* (TUF095158). **Estonia**, Pärnu county, Saarde community (58.1224°N, 25.0914°E), on *T. abietinum* on a *Picea abies* snag, 18 Aug. 2014, *leg. P. Lõhmus* (TUF076419; TUF076420); Surju comm. (58.3099°N, 24.9826°E), on *T. abietinum* on a *P. sylvestris* snag, 26 May 2014, *leg. P. Lõhmus* (TUF076423); Tartu co., Puhja comm. (58.3337°N, 26.2631°E), on *T. abietinum* on snag of *P. sylvestris*, 10 Jun. 2014, *leg. P. Lõhmus* (TUF076422); Tartu comm., Valmaotsa village, Selli-Sillaotsa hiking trail, Alam-Pedja Nature Reserve, (58.4411°N, 26.2662°E), on *Trichaptum* sp. on a snag of *Betula*, 7 Aug. 2022, *leg. A. Suija & M. Suija* (TUF050023). **Latvia**, Alūksne Municipality, Liepna Parish, young boggy birch forest (57.4110°N 27.4843°E), on *T. abietinum* on *P. sylvestris*, unknown collection date, *leg. R. Moisejevs* (DAU0602050); *ibid*. (DAU0602051); Jēkabpils Municipality, Sauka Parish (56.3187°N 25.3954°E), on *T. abietinum* on *P. sylvestris*, unknown collection date, *leg. R. Moisejevs* (DAU0602052); Drabeši parish (57.2638°N, 25.1108°E), spruce forest, on *Trichaptum* sp. on natural spruce stump, 2020, *leg. P. Degtjarenko & R. Moisejevs* (TUF090000). **Lithuania**, Trakai district, Plomenai bog. close to Sibirka village (54.6425°N, 24.9004°E), on sporocarps of *Trichaptum* sp. growing on *Pinus*, 6 Feb. 2022, *leg. M. Ryla* (TUF095099, ex BILAS 11108). **Norway,** Steinkjer Municipality, W of Strukstadmyra (63.9873°N, 11.5801°E), boreal rainforest, on *Trichaptum* on dead spruce (*P. abies*), 9 Aug. 2018, *leg. A. Frisch* (TRH-L-18707); Vefsn Municipality, Langmoen, NW of Fustvatnet (65.912°N, 13.28372°E), on *Trichaptum* sp. on a spruce snag, 26 Jun. 2018, *leg. A. Frisch* (TRH-L-18706); Grong Municipality, Solemsmoen naturreservat, Kvernbekken (64.5760°N, 12.5557°E), boreal rainforest, on *T. abietinum* on *P. abies*, 16 Aug. 2019, *leg. H. Holien* (TRH-L-18708); Nordre Follo, Ås (59.6715°N,10.8846°E), on *T. fuscoviolaceum* on dead *P. sylvestris* in *Sphagnum* bog, 28 Jan. 2023, *leg. A. K. Ruud* (TUF050022); Fredrikstad Municipality, Askedalstangen (59.1367°N,11.0780°E), on *T. fuscoviolaceum* on pine, 5 Nov. 2022, *leg. A.G. Helle* (TUF095043); Moss, Vardasen nature reserve (59.3529°N,10.6764°E), on *T. fuscoviolaceum* on pine, 29 Jan. 2023, *leg. A.G. Helle & M. Angard* (TUF095044). **Russia,** Krasnoznamensky District, SE to Krasnoznamensk, forest "Michurinsky", near Kaban'e bog" (54.8892°N, 22.5622°E), old-growth pine forest with *Sphagnum* spp. and *Carex* sp., with mosses and *Vaccinium myrtillis* on hummocks, with young birches and spruce undergrowth, with upturned trees and big log, on *T. biforme* on trunk of *Betula* sp., 27 Sep. 2019, *leg. I. Stepanchikova & D. Himelbrant* (BILAS). **Sweden,** Uppsala, Kvarnbo (59.8410°N, 17.5668°E), on *T. fuscoviolaceum*, 21 Apr. 2019, *leg. R. Elleby* (UPS-L-941561); Åmål Municipality, Edelskog par., Baljasen Nature Reserve, *ca*. 750 m NW of the folk museum Petersborg (59.0812°N, 12.4657°E), on *T. abietinum* on the trunk of *P. abies*, 14 Apr. 2017, *leg. M. Westberg & C. Kannesten* (UPS-L-867275); Uppsala, Hammarparken, (59.8424°N, 17.5981°E), on *T. fuscoviolaceum*, 16 Mar. 2019, *leg. H. Lernefalk & B. Kühn* (UPS-L-941560); Uppland, Vänge par., Fiby urskog (59.8899°N, 17.3525°E), on *T. abietinum* on *P. abies*, 7 Apr. 2016, *leg. J. C. Zamora, M. Svensson, S. Ekman, M. Westberg & G. von Hirschheydt* (UPS-L-872283).

***Chaenotricha* sp.**

Five specimens that we examined form a well-supported clade in the three-marker phylogenetic tree (Fig. 2). These specimens have an intermediate set of morphological characteristics between the other two *Chaenotricha* species. They are similar to *C. cilians* in stalk length, and other morpho-anatomical characters (Fig. 3, Table 2), but they differ by having larger (average 5 µm) ascospores similar to those to *C. obscura* (for additional results see the Notes of *C. cilians*).

Distribution: Five localities in North America (Canada, USA) and Europe (Estonia) are known.

Specimens examined: **Canada**, Lunenburg County, *ca*. 1 km N of Crouse's Settlement, *ca*. 1 km NE of Crouse's Settlement Road, 3 m E of Old Wood's Road (44.3555°N, 64.4045°W), coastal mature mixed-wood forest, on *T. abietinum*, on an *Abies balsamea* snag, 30 Aug. 2020, *leg. R.T. McMullin* (TUF089393); Ontario, Thunder Bay District, Sibley Peninsula, Sleeping Giant Provincial Park, between park cabin 5 and the Marie Louise Lake Campground, (48.4584°N, 88.7368°W), 16 Oct. 2018, *leg. R.T. McMullin* (TUF089481). **Estonia**, Saaremaa, Lussu village (58.4575°N, 22.4370°E), on *T. fuscoviolaceum* on *P. sylvestris*, 12 Nov. 2019, *leg. M. Nõmm* (TUF089547); Järise village (58.4920°N, 22.3916°E), on *T. fuscoviolaceum*, on log of *P. sylvestris*, 31 Oct. 2019, *leg. M. Nõmm* (TUF089548). **USA**, Haywood County, Great Smoky Mountains National Park, McKee Branch Trail, 0.48 km (linear) SE of junction with Caldwell Fork Trail (35.5952°N, 83.0991°W), mature mixed-wood forest, deciduous dominated in protected river valley, on *T. abietinum*, 27 Oct. 2017, *leg. R.R. McMullin* (TUF089480).

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Conflict of interest: The authors declare that there is no conflict of interest.

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Table S1. List of evaluated morphological characters.