



Phylogenetic relationships, taxonomic novelties, and combinations within *Stictidaceae* (*Ostropales*, *Lecanoromycetes*, *Ascomycota*): focus on *Absconditella*

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Received: 2 January 2023 / Revised: 12 April 2023 / Accepted: 16 April 2023
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Abstract

In this paper, we present new insights into the relationships of the lichen-forming genus *Absconditella*. The study is based on a phylogenetic analysis of DNA sequence data of three gene loci—two nuclear (the nuclear large subunit and internal transcribed spacer of rDNA) loci, and one mitochondrial (mitochondrial small subunit) locus, and an examination of species morphology. We show that *Absconditella* do not form a monophyletic lineage within *Stictidaceae*. One of the lineages, sister to *Xyloschistes*, *Ingvariella* and *Cryptodiscus* contains a recently described *Absconditella rubra*, and a species superficially similar to *A. lignicola* except it is smaller in all morphometric characteristics. Therefore, we introduce a new genus *Absconditonia* accommodating these two species. We confirm that the monotypic genus *Geisleria* belongs to *Absconditella*, and hence we combine *Geisleria* with the latter. Incorporation of public environmental ITS sequences into the analysis showed that both these genera—*Absconditella* and *Absconditonia*—may be more widespread than known only by specimen samples.

Keywords New species · Taxonomy · Overlooked taxa · Environmental sequences · Phylogeny

Introduction

Absconditella Vězda is a genus of fungi that belongs to *Stictidaceae* Fr. (*Ostropales*, *Lecanoromycetes*, *Ascomycota*), a family of saprotrophic, lichenized, including optionally lichenized, and lichenicolous species (e.g. Fries 1849; Baloch et al. 2013; Jaklitsch et al. 2016; Thiyagaraja et al. 2021). The family is a good example of the plasticity of nutritional modes among species which allows us to study fungal adaptations to their environment (Baloch et al. 2010; Kono et al. 2021; Thiyagaraja et al. 2021; Wedin et al. 2004). The family includes 33 genera (Thiyagaraja et al. 2021), with *Stictis* Pers. being the type. *Stictidaceae* is characterised by

having either minute, apothecioid (usually urceolate) or perithecioid ascomata, with thin-walled, non-amyloid asci with a small apical tholus and a ring structure containing 1–2 to multiple ascospores, and by having a hamathecium of either unbranched paraphyses or periphysoids lining the excipulum, and septate to muriform ascospores which in some genera disintegrate into part-spores (Jaklitsch et al. 2016). In lichenized species, the photobiont is usually chlorococcoid (Jaklitsch et al. 2016), but an association with *Trentepohlia* is described at least for one genus (Lücking et al. 2011). The lichenized genus *Absconditella* was erected by Vězda (1965) with *A. sphagnorum* Vězda and Poelt as a type species. The genus differs from other genera of *Stictidaceae* by having minute, urceolate ascomata (Fig. 1) with a non-amyloid hymenium without a dark pigment, simple and indistinctly septate paraphyses and asci with a distinct non-amyloid tholus. Since the description, 16 additional species have been placed to this genus. The species of *Absconditella* differ from each other mainly by the ascospores' shape and septation (e.g. van den Boom et al. 2015; Kantvilas 2005), but also by their excipular structure which is either composed of parallel conglutinated hyphae or is cellular (Coppins 2009). The genus is known from Europe (e.g. van den Boom et al. 2015, includes a key for European species), South America

Section Editor: Gerhard Rambold

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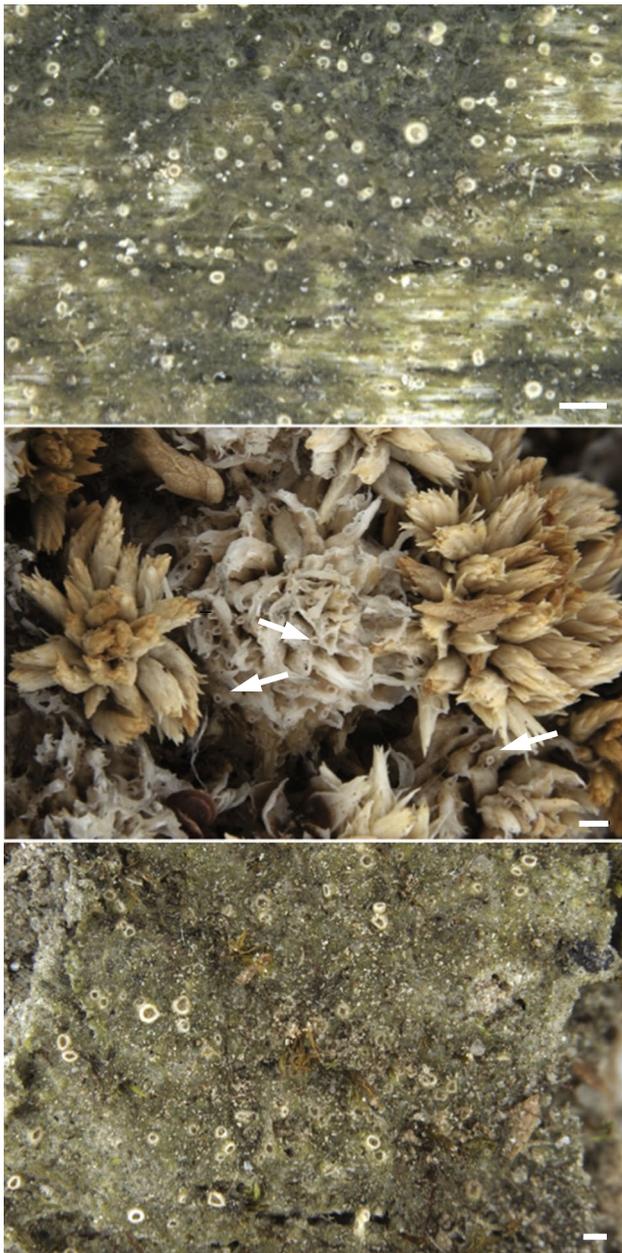


Fig. 1 Examples of *Absconditella* species. *Absconditella lignicola* on decaying wood (upper, Boom 56111); *A. sphagnorum* on turf mosses (middle, McCarthy 5b). *A. trivialis* on soil (lower, Boom 59653). Scale: 0.5 mm. Arrows indicate ascomata

(Cáceres and Aptroot 2016; Kalb and Aptroot 2018) and North America (Spribille et al. 2009), Australasia (Tasmania, Kantvilas 2005), Asia (South Korea, Kondratyuk et al. 2013) and even Antarctica (Søchting et al. 2004). Despite its global distribution, the species are sporadically collected mainly due to their minute ascomata, probably ephemeral nature and short-living substrate (decaying lignum, disturbed soil, turf mosses, termite nests) which is uncommon for most lichenized fungi (e.g. Czarnota and Kukwa 2008; Kantvilas

2005; Palice et al. 2006). Therefore, it is not surprising that sequence repositories (NCBI, UNITE) hold DNA sequences only from a limited number of *Absconditella* specimens.

The paper is based mainly on the second author's *Absconditella* collections from the Netherlands. Attempts to sequence, these specimens were successful in most cases allowing us to obtain new insights into this rarely collected lichenized genus.

Materials and methods

Taxon sampling and microscopy

The study is based on the material collected by the second author from the Netherlands and deposited in his private collection (hb v. d. Boom). Additional herbarium material for comparison was borrowed from TUF, GZU and UBC.

Specimens were initially examined under a dissecting microscope. Anatomical characteristics were studied using a light microscope on thin hand-cut sections of the ascomata mounted in water, in KOH (K) in c. 5% water solution or in Congo red in c. 0.5% water solution. Additional chemical reactions (ascus structure) were tested using Lugol's reagent (I) pre-treated with K. All microphotographs were taken either in water or in Congo red post-processed with K. Microscopical measurements all refer to material examined in water.

DNA extraction, PCR amplification and gene sequencing

DNA extraction, amplification and purification were carried out in the molecular lab of Mycology at the University of Tartu (TU). Total genomic DNA was extracted from ascomata of 19 specimens using High pure PCR Template Preparation Kit (Roche Applied Science®, Penzberg, Germany) and following the protocol provided by the manufacturer. We amplified three ribosomal loci. These include two nuclear (internal transcribed spacer (ITS), large subunit (LSU) and one mitochondrial (small subunit (mtSSU)) DNA regions. To amplify these loci, we used the following primer pairs respectively: ITS0F (Tedersoo et al. 2008) or ITS1 (White et al. 1990) or ITS1F (Gardes and Bruns 1993) and LA-W (Tedersoo et al. 2008) or ITS4 (White et al. 1990), LR0R and LR7 or LR5 (Vilgalys and Hester 1990), and mrSSU1 and mrSSU3R (Zoller et al. 1999). The PCR amplification was performed using a final volume of 25 µl, which was comprised of 0.8–3 µl of target-DNA, 5 µl 5× HOT FIRE-Pol Blend Master Mix (Solis BioDyne, Estonia), 0.5 µl of both primers (both in concentration 20 µM) and the rest of distilled water.

The PCR products were visualised on a 1% agarose gel stained with ethidium bromide, and for the purification of PCR products, 1 µL of FastAP and 0.5 µL of Exonuclease I (Thermo Scientific, Waltham, Massachusetts, USA) were added to each tube per 20 µL of the product.

Both strands were Sanger sequenced at MacroGen Inc. (Amsterdam, the Netherlands). The ITS region was sequenced with primer pair ITS4 and ITS5 (White et al. 1990), LSU with CTB6 (Garbelotto et al. 1997) and LR7, and mtSSU with same primers as amplified. Sequencher 4.10.1. (GeneCodes Corp.[®], Ann Arbor, Michigan, USA) was used to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) and UNITE (<https://unite.ut.ee/>; Nilsson et al. 2018; Kõljalg et al. 2020) databases to confirm the determination. Altogether, 19 specimens were used to create 40 new DNA sequences of them 16 ITS, 11 LSU and 13 mtSSU sequences (Table 1). The sequenced specimens are deposited in TUF and in the private collection of P. van den Boom (hb v. d. Boom); the extracted DNA samples are deposited in the DNA and Environmental Sample Collection of the Natural History Museum in Tartu University (TUE).

Phylogenetic analyses

To infer phylogenetic relationships, we compiled DNA alignments for each gene, using taxon sampling that encompassed as many of the segregate of *Stictidaceae* as possible.

For LSU and mtSSU, we downloaded from NCBI data repository sequences representing 22 genera of *Stictidaceae*, and *Baeomyces heteromorphus* Nyl. ex C. Bab. and Mitt. (*Baeomycetales*, *Lecanoromycetes*) and *Trapelia obtegens* (Th. Fr.) Hertel (*Trapeliales*, *Lecanoromycetes*) to root the trees (Supplementary 1, Table 1). Sequences were aligned with online version of MAFFT ver. 7 (Katoh et al. 2019; <https://mafft.cbrc.jp/alignment/server/index.html>) using default options. Minor manual adjustments were made with SeaView ver. 3.2. (Gouy et al. 2010). We excluded poorly aligned positions and divergent regions from LSU and mtSSU alignments by using web interface of Gblocks ver. 0.91b (Talavera and Castresana 2007; http://molevol.cmima.csic.es/castresana/Gblocks_server.html) allowing relaxed settings, i.e. allowing smaller final blocks and less strict flanking positions. The final mtSSU alignment contained 72 sequences and 569 nucleotide positions (43% from the original alignment) of them 105 variable and 94 informative; LSU 64 sequences and 524 positions (38%) of them 69 variable and 46 informative.

The ITS sequences were analysed separately. For that, we downloaded all ITS sequences (incl. those from environmental samples) annotated as *Stictidaceae* in the PlutoF database (Abarenkov et al. 2010; Supplementary 1, Table 2). The original file contained 783 sequences, but we excluded duplicate sequences from the same environmental sample, multiple sequences representing *Schizoxylon albescens* and sequences forming long branches in the first attempt to construct the tree. ITSx (Bengtsson-Palme et al. 2013) was used for extraction of neighbouring parts of conservative rDNA

Table 1 Information about voucher specimens (lab ID, voucher ID, taxon name and country of origin), and GenBank accession codes of the new DNA sequences. “—” means sequence not generated or not available for this specimen. The type specimens are marked in bold

Lab ID	Voucher ID	Taxon name	Country	ITS	LSU	mtSSU
AS398	TUF086935	<i>Absconditella delutula</i>	Argentina	OQ703739	OQ715329	OQ725024
AS282	Boom 56111	<i>Absconditella lignicola</i>	Netherlands	OQ703734	OQ715327	OQ725021
AS562	Boom 60065	<i>Absconditella lignicola</i>	Netherlands	OQ703741	OQ724819	OQ725026
AS189	Boom 55000	<i>Absconditella lignicola</i>	Netherlands	OQ703729	OQ715324	OQ725018
AS283	Boom 56108	<i>Absconditella lignicola</i>	Netherlands	OQ703732	OQ715325	—
AS284	Boom 56126	<i>Absconditella pauxilla</i>	Netherlands	—	—	OQ725019
AS188	Boom 54963	<i>Absconditella pauxilla</i>	Netherlands	OQ703730	OQ715323	OQ725017
AS354	Boom 57241	<i>Absconditella sphagnum</i>	Netherlands	OQ703737	—	OQ725022
AS136	Boom 52559	<i>Absconditella sphagnum</i>	Netherlands	OQ703727	OQ715321	—
AS186	Boom 54955	<i>Absconditella sphagnum</i>	Netherlands	OQ703728	—	—
AS388	McCarthy 5b	<i>Absconditella sphagnum</i>	Canada	OQ703738	OQ715328	OQ725023
AS187	Boom 53189	<i>Absconditella sphagnum</i>	Netherlands	OQ703731	OQ715322	OQ725016
AS281	Boom 56107	<i>Absconditella sphagnum</i>	Netherlands	OQ703733	OQ715326	OQ725020
AS560	Boom 59653	<i>Absconditella trivialis</i>	Netherlands	OQ703740	OQ715330	OQ725025
AS133	Boom 52517	<i>Absconditonia rubra</i>	Netherlands	KT454800	—	OQ725014
AS135	TUF074832	<i>Absconditonia rubra</i>	Netherlands	KT454799	—	OQ725015
AS563	Boom 59736	<i>Absconditonia sublignicola</i>	Netherlands	OQ703742	—	—
AS352	Boom 57276	<i>Absconditonia sublignicola</i>	Netherlands	OQ703735	—	—
AS353	TUF095005	<i>Absconditonia sublignicola</i>	Netherlands	OQ703736	—	—

regions in the ITS alignment. The final alignment contained 297 sequences, and 822 nucleotide positions (Supplementary 3; ITS alignment in fasta format).

For mtSSU and LSU datasets, phylogenetic relationships and the tree confidence were inferred using two different methodologies. Metropolis coupled Markov Chain Monte Carlo (MCMC; Bayesian) approach implemented in MrBayes ver. 3.2.7. (Ronquist et al. 2012) and maximum likelihood (ML) using RaxML ver. 8.2.12 (Stamatakis 2014). The best-fit nucleotide substitution model was selected amongst 56 candidate models using jModeltest ver. 2.1.10 (Darriba et al. 2012). According to the lowest value of AIC criterion, GTR + I + Γ model fitted for both datasets. As mtSSU and LSU trees were concordant in consensus tree topology, we concatenated the alignments (Supplementary 2: mtSSU + LSU concatenated alignment in fasta format). To construct 2-gene phylogeny, we used the following settings in Bayesian: two parallel simultaneous runs over 1 million generations starting with a random tree and employing four simultaneous chains; sampling frequency 1000. The analysis was run until average standard deviation of split frequencies across runs attained 0.01, and the average potential scale reduction factor (PSRF) for all models and parameters was close to 1. The first 25% of saved data was discarded as a burn-in; the consensus tree and posterior probabilities (PP) were calculated from the rest. In ML, the branch support was tested by rapid bootstrapping (BS) over 1000 pseudoreplicates, all other parameters were set as default. All analyses were performed in CIPRES Science Gateway ver. 3.3. (Miller et al. 2010). The ITS alignment was analysed by ML approach only using online version of IQTree (Trifinopoulos et al. 2016; <http://iqtree.cibiv.univie.ac.at>), applying GTR + I + G4 + F as nucleotide substitution model and testing branch support with ultrafast bootstrapping (Minh et al. 2013) using maximum 1000 iterations. In phylogenetic trees, we consider clades as significantly supported if the posterior probabilities (PP) are at least 0.95 and bootstrap values (BS) at least 0.75.

The phylogenetic trees were visualised and edited using FigTree ver. 1.4.4 (Rambaut et al. 2014), and post-processed with Adobe Illustrator CS3[®] ver. 13.0.0.

Results

Our two-locus and a single-locus ITS-based analysis of *Stictidaceae* sequences concord with previous studies on this family (e.g. Aptroot et al. 2014; Baloch et al. 2010; Thiyagaraja et al. 2021). The most striking result is the polyphyly of *Absconditella* within *Stictidaceae*. According to mtSSU + LSU (Fig. 2) and ITS analyses (Fig. 3), the recently described corticolous species—*Absconditella rubra* van den Boom, M. Brand and Suija (van den Boom

et al. 2015)—forms a distinct lineage sister to *Xyloschistes*, *Ingvariella* and *Cryptodiscus* (mtSSU + LSU: BS = 99, PP = 1; ITS: BS = 98). Some morphological–ecological deviations from the true *Absconditella* were already pointed out in van den Boom et al. (2015), and therefore we find transferring it to a genus of its own called *Absconditonia* is justified (see Taxonomy). The monotypic *Geisleria* is nested within *Absconditella* being a sister to *A. trivialis* (Fig. 2; BS = 100, PP = 1). Based on these results, we combined it with *Absconditella* (see Taxonomy).

In the ITS analysis, the sequences of *Absconditella lignicola* settled into two distinctive well-supported clades. One of the clades comprised most of the *Absconditella* sequences, including those from the type species—*A. sphagnorum* (Fig. 3). Another clade contained *A. rubra*, numerous environmental sequences all around the world, and the FJ904669 annotated in Baloch et al. (2009) as *A. lignicola*. We found that the *A. lignicola* specimens of these two clades are superficially very similar, but differ from each other by the size of ascomata, ascospores and asci (more details in Taxonomy). To clarify which of the two *A. lignicola* clades is the true *A. lignicola*, we examined isotype specimens of that species (GZU-000277841, GZU-000277842). We found that the measurements of the isotypes fit with those *A. lignicola* specimens that belong to the true *Absconditella* clade (Figs. 2 and 3). Next, we examined another *Absconditella* species—a recently described *A. amabilis* T. Sprib. (Spribille et al. 2009)—that by description resembles *A. lignicola*. The examination of a single specimen of *A. amabilis* (UBC L47003) revealed, however, several morpho-anatomical differences from our specimens, and therefore we found describing a new species reasonable (see Comments in Taxonomy section).

Both analyses also indicated that the name *Absconditella sphagnorum* may hide more than one species (Figs. 3 and 4), but at present, we do not have enough morpho-anatomical, ecological and distributional data to make any taxonomic conclusions.

The extended data set including both sequences of physical specimens and environmental sequences (Supplementary 3; Fig. 4) showed that both *Absconditella* and *Absconditonia* are widely and probably more commonly spread than previously thought (Fig. 3).

Taxonomy

Absconditella sychnogonioides (Nitschke) Suija and van den Boom, comb. nova

= *Geisleria sychnogonioides* Nitschke, Rabenhorst, Lich. Eur. Exs. Fasc. 21 no. 574 (1861), *non vidi*

Mycobank: MB848138

The name *Geisleria* was introduced by Nitschke (1861) in Rabenhorst, Lich. Eur. Exs. 21: 574 for a single species

Fig. 2 Two locus (nuclear large subunit (LSU) and mitochondrial small subunit (mtSSU) DNA region) consensus tree based on Bayesian approach, representing relationships within *Stictidaceae* (*Ostropales*, *Lecanoromycetes*), including genera *Absconditella* and *Absconditonia*. The branches with posterior probabilities (PPs) ≥ 0.95 and bootstrap values (BS) ≥ 75 are marked with a thicker line. Names in bold indicate sequences generated for this study

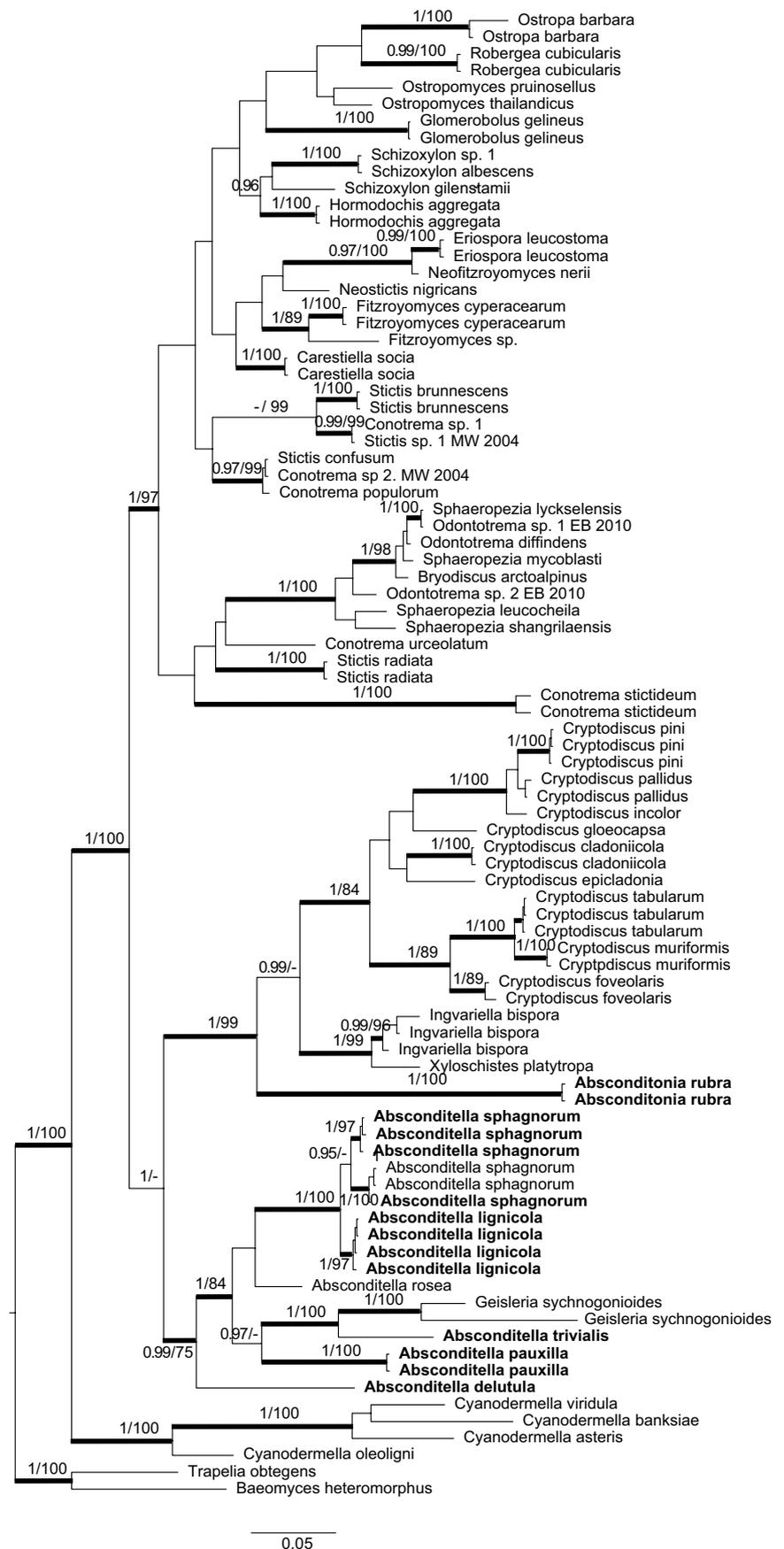


Fig. 3 Maximum likelihood (ML) phylogeny including sequences generated for this study (marked in bold) and UNITE+NCBI-derived rDNA ITS sequences annotated as *Stictidaceae*. The supported clades containing sequences from the same taxonomic unit are collapsed; environmental sequences are marked with (E); the thickness of the branch indicates the strength of the clade support (bootstrap values). *Absconditonia* and *Absconditella* are marked with grey box

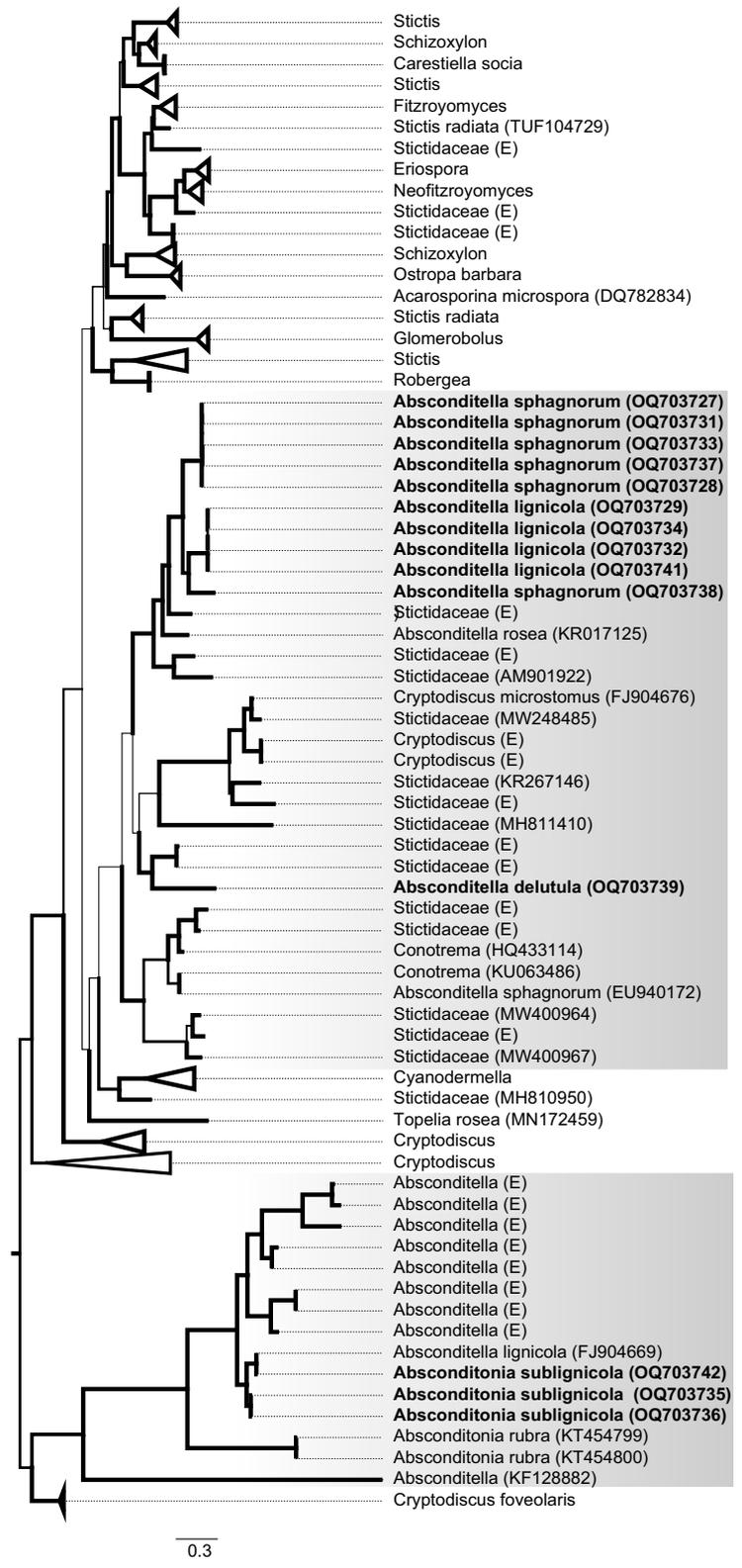
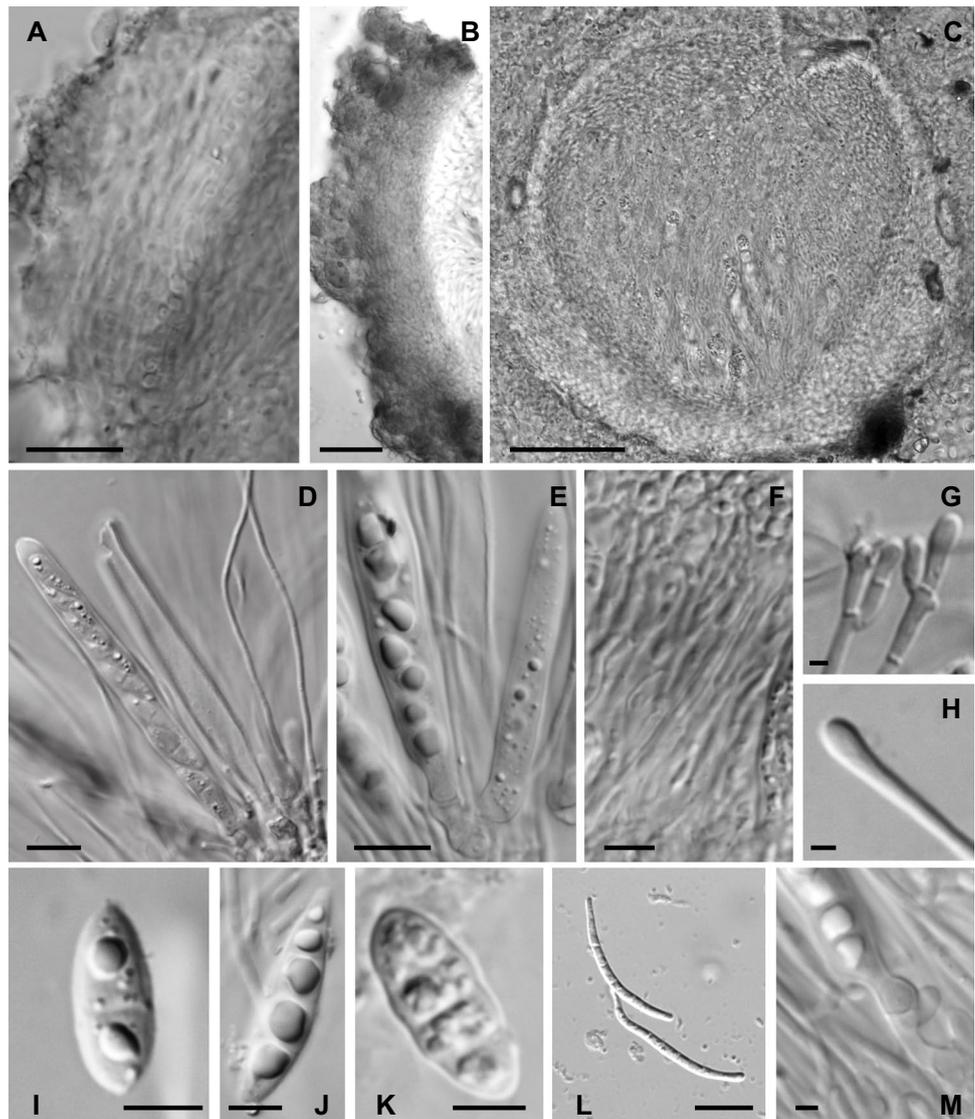


Fig. 4 Micromorphological characters of various *Absoconditella* (A.) and *Absoconditonia* (As.) species. **A** Exciple of *As. rubra* (holotype, TUF074832); **B** exciple of *A. delutula* (TUF086935); **C** cross-section of the ascomata of *A. lignicola* (isotype, GZU-000277841); **D** asci and paraphyses of *A. sphagnum* (McCarty 5b); **E** ascus of *A. trivialis* (Boom 59653); **F** paraphyses of *As. rubra* (TUF074832); **G** bifurcated tips of paraphyses of *A. delutula* (TUF086935); **H** widened tip of *A. sphagnum* (McCarty 5b); **I** ascospore of *A. sphagnum* (McCarty 5b); **J** ascospore of *A. trivialis* (Boom 59653); **K** ascospore of *A. lignicola* (GZU-000277841); **L** ascospores of *As. rubra* (TUF074832); **M** basis of the ascus of *A. trivialis* (Boom 59653). Scales: A, B, D = 10 μ m, C = 50 μ m, F, I, J, K, L = 5 μ m, G, H, M = 1 μ m



G. sychnogonioides Nitschke. Later, three more species were described, two of which—*G. alpina* Servit and *G. jamesii* Swinscow—were shown to belong to other genera; and *G. xylophila* Vězda is known only by the type (Vězda 1970; Roux and Serusiaux 2004). In their study, Aptroot et al. (2014) showed that the *Geisleria sychnogonioides* is a sister to *Absoconditella sphagnum*, forming a highly supported clade. Our phylogenetic analysis that includes more *Absoconditella* species coincides with their results, as *Geisleria* is nested within the *Absoconditella* clade being a sister to *A. trivialis* (Fig. 2), another species on unstable soil (Coppins 2009).

The ascomata of *Geisleria sychnogonioides* are perithecium-like with a narrow pore while in *Absoconditella* they are apothecioid (i.e. with widely opened hymenium). However, Aptroot et al. (2014) examined type material of *G. sychnogonioides* and revealed many structural similarities

in *Absoconditella* and *Geisleria* ascomata especially the fact that the ascomata of this species are typical apothecia but the disc is only narrowly exposed, leaving the impression of enclosed (angiocarpic) fruit-bodies. Indeed, in the study of some additional specimens, we can confirm this statement. The ascomata are c. 0.3 mm and are not pigmented, except that the upper part of the ascomata is sometimes pale brownish. Ascospores are 17–24 \times 7–9 μ m in size, and the paraphyses are very thin (< 1 μ m diam.), mostly simple, but some are also branched, and lax in water.

The need for nomenclatural re-arrangements—either synonymization of *Absoconditella* with *Geisleria* or proposal to conserve *Absoconditella* against *Geisleria*—was already pointed out in Aptroot et al. (2014), but, at that moment the combination of these two genera was not justified due to the small sampling size in their analysis. The larger sampling leaves no doubt that the nomenclatural act is necessary

following the principle of monophyletic taxa. In accordance to the principle of priority of taxonomic names (Art. 11.3; Turland et al. 2018), we should combine *Absconditella* with *Geisleria*. However, reasoned by the wider usage of the former name, we proposed to conserve the name *Absconditella* against *Geisleria* (Suija and van den Boom 2023).

Additionally examined species: *Absconditella sychnogonioides*: the Netherlands, Noord-Brabant, E of Helmond, 1.5 km S of Deurne centre, Leemskuilen, wasted grassland, along industrial area, terricolous, Grid ref. 52-41-14, 2 July 1991, van den Boom 11325 (hb v.d. Boom); S of Breda, Galdersche Heide, dry ditch along *Pinus* forest, terricolous, Grid ref. 50-23-12, 1 May 1993, van den Boom 14028 (hb v.d. Boom); SW of Son, Houtens, near bridge over channel, loamy ground nearby *Populus*, terricolous, Grid ref. 51-25-51, 12 March 2000, van den Boom 24017 (hb v.d. Boom).

Absconditonia Suija and van den Boom, gen. nova

Diagnosis: Similar to *Absconditella* but differs by the strongly branched-anastomosed paraphyses and excipulum containing radial hyphae.

Type species: *Absconditonia rubra* (van den Boom, M. Brand and Suija) Suija and van den Boom

Mycobank: MB848135

Notes: Based on the molecular phylogeny, *Absconditonia rubra* forms a lineage distinct from the core *Absconditella* group, and it is a sister to genera *Xyloschistes*, *Ingvariella* and *Cryptodiscus* (Fig. 2). Van den Boom et al. (2015) pointed out some morpho-anatomical and ecological characteristics that do not fit the traditional concept of *Absconditella*. Specifically, *A. rubra* has paraphyses that are distinctly septate and branched-anastomosed while in true *Absconditella* these are mostly simple, indistinctly septate, and occasionally branched only in the upper part. The excipulum of *A. rubra* is composed of radial hyphae while in true *Absconditella* these are either parallel or cellular. Also, the apothecial sections have some orange to reddish pigment as opposed to usually no pigment in true *Absconditella*. The only an exception *Absconditella* species having such pigment is *A. fossarum* Vězda and Pišút (Ceynowa-Giełdon 2003; Vězda and Pišút 1984), a rare terricolous species with strongly urceolate apothecia, up to 0.15 mm in diam., small asci of 7–8 µm wide and fusiform ascospores. However, ascospores are 3-septate and 12–14 × 4–4.5 µm in *A. fossarum* and 3–7 septate and 33–40 × 1.2–1.8 µm in *A. rubra*. There is no molecular data available for that species, but the excipulum (parallel hyphae) and paraphyses (simple to forked, without clear septation) structure corresponds to true *Absconditella* rather than the genus described here.

There is no information about asexual morph in *Absconditella*, but the pycnidia have been recorded for *A. rubra* (van

den Boom et al. 2015). In addition, typical *Absconditella* associates with algal films over short-living substrata like bryophytes, lignum, plant debris, or unstable soil, while *A. rubra* exceptionally inhabits long-living substratum such as the bark of living broad-leaved trees.

The analysis of ITS sequences adds one more *Absconditella*-like taxon to this clade. The taxon most resembles *Absconditella lignicola* but it deviates from the type by having smaller ascomata, different ascospores and paraphyses that are conglutinated.

Absconditonia rubra (van den Boom, M. Brand and Suija) Suija and van den Boom, comb. nova

Mycobank: MB848136

= *Absconditella rubra* van den Boom, M. Brand and Suija in Phytotaxa 238(3): 272 (2015)

Type: the Netherlands. Prov. Noord-Brabant, WNW of Son, between Sonniuswijk and Oud Meer, *Pinus* forest with some *Quercus robur*, *Q. rubra* and *Betula pendula* trees, grid. ref. 51-24-35, 20 July 2014, 15 m, P. & B. van den Boom 51882 (holotype: TUF074832 (previously TU74832); isotype: hb v.d. Boom; ITS barcoding sequence accession UDB024272, KT454799)

Distribution: *Absconditonia rubra* was first recorded from the Netherlands and Belgium (van den Boom et al. 2015) but is now reported also from Czech Republic (Malíček et al. 2018).

Absconditonia sublignicola van den Boom and Suija, sp. nov. (Fig. 5)

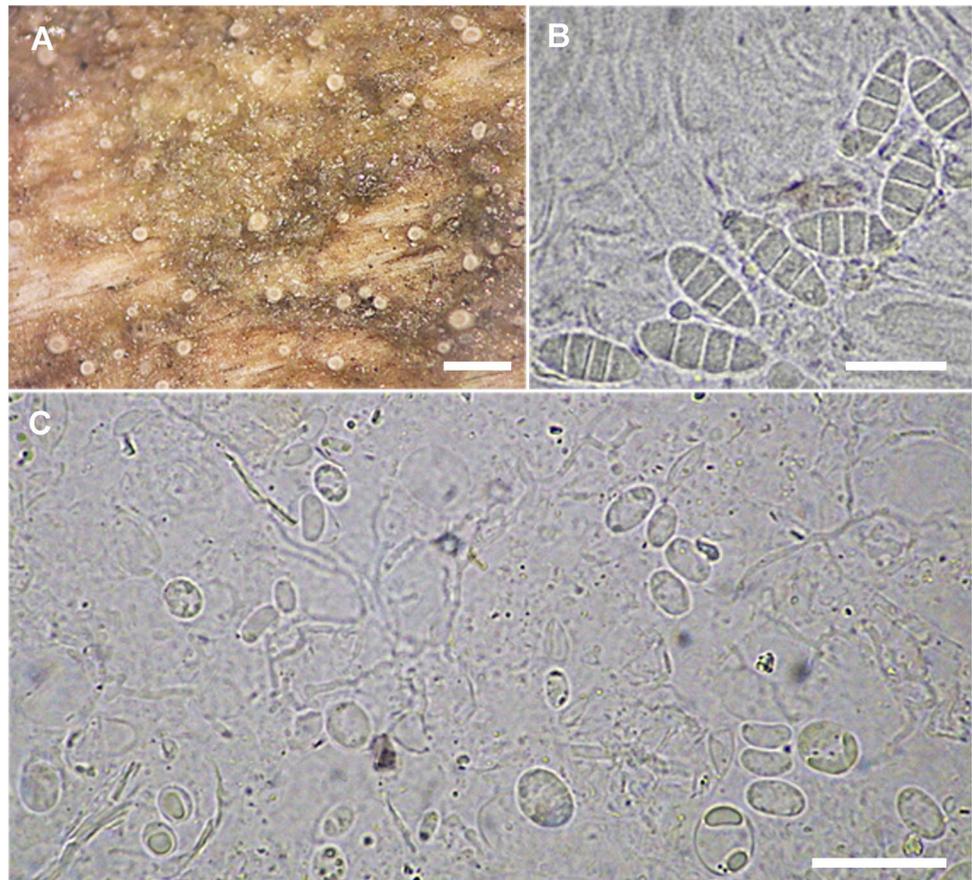
Mycobank: MB848137

Type: the Netherlands. Prov. Noord-Brabant, E of Oss, Berghem, N side of Groot Ganzeven (51°46'N 5°36'E), mixed forest with mainly *Pinus* and *Quercus*. Grid ref. 45-26-15. 5 October 2017, P. & B. van den Boom 57244 (holotype: TUF095005; isotype: hb v.d. Boom; ITS barcoding sequence accession UDB0819032; OQ703736)

Diagnosis: *Absconditonia sublignicola* is similar to *Absconditella lignicola* both having beige urceolate ascomata sunken into gel-like thallus and having 3-septate, ellipsoid ascospores with blunted ends. *Absconditonia sublignicola* differs by the cellular structure of the exciple (hyphae in parallel in *A. lignicola*), and by having smaller ascomata (0.05–0.15 mm vs. 0.1–0.2 mm) and ascospores (9–12 × 4–5 µm vs. 10–15 × 4.5–6.5 µm)

Description: Thallus lignicolous, inconspicuous, or visible as somewhat continuous pale greenish, patches, often with a gelatinous shiny algal film, containing mostly cells of *Coccomyxa*-like algae, cells 6–14 × 5–10 µm, but even chlorococcoid algae are sometimes present; hyphae thin, loose, attached among algal cells, but never clearly closely surrounded, without haustoria. Apothecia very

Fig. 5 **A, B, C** *Absconditonia sublignicola*, holotype (Boom 57244; TUF095005). **A** habitus; **B** ascospores; **C** paraphyses. Scale: A = 0.5 mm, B = 10 μ m, C = 30 μ m



inconspicuous, 0.05–0.15(–0.2) mm diameter, mostly up to 0.15 mm high, margin persistent, whitish, pale cream or waxy cream-like, disc plane to urceolate when mature, whitish, pale cream to pale brownish. Excipulum at the top 30–40 μ m wide, chondroid, inner part hyaline, at the top hyaline to very pale brown, containing radial hyphae, outer edge with irregular mostly concentric hyphae (lumina c. 2–3.5 μ m); excipulum below with c. 2–5 μ m, horizontal hyphae (*textura angularis*); subhymenium c. 20–25 μ m. Hymenium 40–55 μ m high; paraphyses, conglutinated, simple, only sometimes bifurcate in upper part, thin (0.5–1.5 μ m diam.); top not or slightly widened, up to 0.2 μ m, aseptate or with a few septa. Asci c. 38–50 \times 8–12 μ m, not amyloid, young asci thick-walled, at the top with thick, open tholus, when mature then thin-walled, 8-spored; ascospores ellipsoid cylindrical, thin-walled, smooth, mostly blunt-ended, not curved, perispore absent, 9–12 \times 4–5 μ m, 3-septate. Pycnidia not observed.

Chemistry: thallus and apothecia K–, C–, P–.

Etymology: The epithet “*sublignicola*” refers to the similarity with *Absconditella lignicola*.

Distribution and ecology: This new species is known from three localities in the south of the Netherlands, all of which were found growing on wood of a fallen tree, mainly

on a horizontal surface. The type was abundantly present with hundreds of apothecia. This locality is the only place in the study area where the very rare *Usnea wasmuthii* Räsänen was recently found on a *Quercus robur* tree, most probably influenced by a fault line in the landscape.

Notes: The species is morpho-anatomically most similar to *Absconditella lignicola*. Both species have gel-like thallus with sunken, urceolate beige ascomata and 4-celled, ellipsoid ascospores with blunted ends. In addition, both species grow on decorticated logs. The most obvious characteristics differentiating these two species are the size of ascospores and ascomata (both are smaller in *A. sublignicola*). Also, the disc in the new species is usually paler. The sequence FJ904669 annotated in Baloch et al. (2009) as *A. lignicola* most probably belongs to *A. sublignicola*. However, we did not have chance to examine the voucher specimen.

The species shares some similarities with *A. amabilis*, a species known only in North America (Spribille et al. 2009). We observed that the new species has ascomata sunken in a gel-like thallus, while the thallus of *A. amabilis* is sunken into the substratum, with scattered algae surrounding the ascomata, and ascospores of *A. amabilis* are a bit bigger and narrower 10–15 \times 3–4.5 μ m ($l/w = 2.4$ –4.2) vs. 9–12 \times 4–5 μ m ($l/w = 1.8$ –2.75) in *A. sublignicola*.

Only two accompanying lichens have been found, *Placynthiella dasaea* (Stirt.) Tønsberg and *Trapeliopsis granulosa* (Hoffm.) Lumbsch.

Additionally examined species: *Absconditonia sublignicola*. The Netherlands, Noord-Brabant, SSW of Lieshout, Molenheide, NE of Reigerlaan, trail in *Pinus Corylus Quercus* forest. Grid ref. 51-26-54, 18 December 2017, *P. & B. van den Boom 57276* (hb v.d. Boom; NCBI ID for ITS: OQ703735); N of Maarheeze, SE of Sterksel, trail in forest to Hoef aan de Pan (ENE side), mixed forest with mainly *Betula*, *Quercus rubra* and *Pinus*. Grid rif. 57-17-33, 20 April 2020, *P. & B. van den Boom 59736* (hb v.d. Boom; NCBI: OQ703742).

Absconditella lignicola Vězda and Pišút (isotypes). Bohemoslovakia. Slovakia, Tatra Magna: in valle rivi Hincov potek, alt. ca. 1300 m. Leg. *I. Pišút & A. Vězda*, 22 August 1983 (GZU-0000277841); Bohemoslovakia. Slovakia, Tatra Magna: in valle torrentes Hincov potok supra larum Štrbske pleso, alt. 1300 m. Ad truncum decorticatum Piceae excelsae cum algis ssp. *copiosis* et *Epigloea bactrospora*. Leg. *I. Pišút & A. Vězda*, 22 August 1983 (GZU-0000277842). The Netherlands, prov. Noord-Brabant, SE of Oostelbeers, Oostelbeersche Heide, NE of Rouwven, N of *Calluna* heathland, *Pinus* forest with a lot of fallen trunks. Grid ref. 51-32-55, 14 April 2016, *P. & B. van den Boom 55000* (hb v.d. Boom; NCBI ID: OQ703729); SSE of Oostelbeers, Oostelbeerse Heide, W of Rouwven, trail (SW/NE) in *Pinus* Forest with a few *Quercus* trees. Grid ref. 51-32-54, 16 March 2017, *P. & B. van den Boom 56108* (hb v.d. Boom; OQ703732); SSE of Oostelbeers, Oostelbeerse Heide, W of St. Martensberg, near t-junction, open place in *Pinus* forest with a few *Quercus* trees, former heathland. Grid ref. 51-32-54, 16 March 2017, *P. & B. van den Boom 56111* (hb v.d. Boom; OQ703734); SW of Heeze, SE of Heezerhut, SW of fen, open grassy place with stumps, fallen trunks, a few *Pinus* trees. Grid ref. 57-15-14, 20 November 2020, *P. & B. van den Boom 60065* (hb v.d. Boom; OQ703741); Estonia, Tartu County, Peipsiääre comm., Padakõrve village (58.57841°N 26.99173°E), mesotrophic boreal forest, on log of *Picea abies*, leg. & det. *P. Lõhmus*, 11 May 2006 (TUF047243); Ida-Viru County, Lügänuuse comm., Virunurme village (59.16423°N 27.02287°E), mesotrophic boreal forest, on log of *P. abies*, 19 October 2007, leg. & det. *P. Lõhmus* (TUF047333); Harju County, Harku comm., Sõrve village (59.42019°N 24.47919°E), on log, 5 August 2018, leg. & det. *A. Suija* (TUF051597.a).

Absconditella amabilis T. Sprib. Canada, British Columbia. Thompson Plateau. Opax/Mud Lake Sylviculture research forest (50°49'23"N 120°27'54"W). MLO 750, Stump 1. Alt. 1100 m, September 2007, leg. *C.R. Björk 15066*, det. *T. Spribille* (UBC L47003).

Absconditella fossarum Vězda and Pišút. The Netherlands, prov. Noord-Brabant, NW of Geldrop, S of 'Eindhovenens' channel, E of Hulster bridge, wasteland, on sandy soil. Grid ref. 51-46-31, 23 November 1997, leg. *P. & B. van den Boom 19766* (hb v.d. Boom), *ibid.* 7 December 1997, leg. *P. & B. van den Boom 19777* (hb v. d. Boom; hb Vězda). Belgium, prov. Luxembourg, W of St. Hubert, 2 km N of Smuid, trail in mixed forest, to Rocher de Marie Gobaille, on S exposed vertical soil (50°02.3'N 5°16.3'E), 4 May 2000, leg. *P. & B. van den Boom 24511* (hb v. d. Boom, hb Vězda).

Discussion

When Fries circumscribed *Stictidaceae* (Fries 1849: 372), it contained only two saprophytic genera (*Stictis* and *Cryptodiscus*). Today, 33 genera are included in this family (Thiyagaraja et al. 2021), and this study adds one more. In its current circumscription, there is no good synapomorphy to delimit *Stictidaceae* as a natural monophyletic unit because of considerable heterogeneity in morphology and anatomy (e.g. Jaklitsch et al. 2016) and lifestyles that may change during the life-cycle (Wedin et al. 2004). The new genus *Absconditonia* includes two lichenized species, the corticolous *A. rubra* and lignicolous *A. sublignicola*, but taking the sequence occurrence data into account, the genus may be even more diverse (Fig. 3). The placement of *Absconditonia rubra* in *Absconditella* was only provisional because at this moment sequences of only two *Absconditella* species were available in the DNA sequence repositories and the closest match was *A. lignicola* with 90% of sequence similarity (van den Boom et al. 2015). This study adds more DNA sequence data to public sequence repositories, allowing us to resolve some taxonomic uncertainties and creating new ones like the presence of *A. sphagnorum* complex (Figs. 2 and 3).

All *Absconditella* species are inconspicuous and easily overlooked (Coppins 2009; Kantvilas 2005; Vězda 1965; Vězda and Pišút 1984), and a few of them are known from the type locality only (e.g. Rossman 1980). Externally, all *Absconditella* species are very similar to each other. The exception is a species formerly known as *Geisleria sychonogonioides* combined here to preserve monophyly of *Absconditella*. The rest of the *Absconditella* species differ from each other mainly by the ascospores' shape and septation (e.g. van den Boom et al. 2015; Kantvilas 2005), excipular structure (Coppins 2009) and substrate preferences (Wirth et al. 2013). The new genus *Absconditonia* is morphologically and anatomically very similar to *Absconditella* but, based on DNA sequence data, it is only distantly related to it (Figs. 2 and 3). For example, the new species, *Absconditonia sublignicola* known in the Netherlands by three specimens from a rather small distribution area, and

in Sweden (GenBank acc. no. FJ904669) may be overlooked because of its overall similarity to *Absconditella lignicola*. As the morphological characteristics (structure of exciple and paraphyses, shape and size of ascospores) are heterogeneous within the genera (Figs. 4 and 5; Coppins 2009), we cannot do further taxonomic combinations here because we did not examine all types of *Absconditella* species, and the species descriptions and illustrations alone are insufficient to make a decision about this nomenclature. However, as when seen with the inclusion of environmental DNA data, both genera are more diverse than when considered only through specimen-derived data (Fig. 3).

Conclusions

Our phylogenetic results shed light on the circumscription of the lichenized genus *Absconditella* (*Stictidaceae*, *Ostropales*) by including *Geisleria synchogonioides* and excluding *Absconditella rubra*. The latter appeared to be only distantly related to *Absconditella* and therefore we described here a new genus: *Absconditonia*. For now, the genus includes two species, but inclusion of environmental sequences indicates a wider but hidden diversity of this genus.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-023-01889-2>.

Acknowledgements John W. McCarthy is thanked for collecting an *Absconditella sphagnorum* specimen from Canada. The curators of abovementioned collections are thanked for the loan of the specimens, and Rasmus Puusepp (University of Tartu) is thanked for lab work. Linguistic correction was made by Keelekord OÜ (Estonia).

Author contribution Conceptualization: (AS, PB), literature search and compilation: (AS, PB); writing abstract, introduction, conclusion, proof reading: (AS, PB). Preparation of data tables: (AS). Overall compilation and coordination: (AS). All authors have read and agreed to the published version of the manuscript.

Funding This work was supported by an Estonian Research Council grant (PRG1170), and by the European Regional Development Fund (Centre of Excellence EcolChange).

Data availability The voucher specimens are deposited in the registered collections and in the private collection of P. van den Boom and are available upon request. The data about the specimens, all new DNA sequences are deposited in the PlutoF data portal <https://plutof.ut.ee/> and in NCBI (<https://www.ncbi.nlm.nih.gov>), all taxonomic novelties are registered in MycoBank, and all supplementary files are available at <https://dx.doi.org/10.15156/BIO/2560638>

Declarations

Ethical approval Not applicable

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

References

- Abarenkov K, Tedersoo L, Nilsson RH et al (2010) PlutoF – a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evol Bioinform* 6:189–196. <https://doi.org/10.4137/EBO.S6271>
- Aptroot A, Parnmen S, Lücking R, Baloch E, Jungbluth P, Cáceres MES, Lumbsch HT (2014) Molecular phylogeny resolves a taxonomic misunderstanding and places *Geisleria* close to *Absconditella* s. str. (*Ostropales*: *Stictidaceae*). *Lichenologist* 46:115–128. <https://doi.org/10.1017/S0024282913000741>
- Baloch E, Gilenstam G, Wedin M (2009) Phylogeny and classification of *Cryptodiscus*, with taxonomic synopsis of the Swedish species. *Fungal Divers* 38:51–68
- Baloch E, Lücking R, Lumbsch HT, Wedin M (2010) Major clades and phylogenetic relationships between lichenized and non-lichenized lineages in *Ostropales* (*Ascomycota*: *Lecanoromycetes*). *Taxon* 59:1483–1494. <https://doi.org/10.2307/20774043>
- Baloch E, Lumbsch HT, Lücking R, Wedin M (2013) New combinations and names in *Gyalecta* for former *Belonia* and *Pachyphiale* (*Ascomycota*, *Ostropales*) species. *Lichenologist* 45:723–727. <https://doi.org/10.1017/S0024282913000492>
- Bengtsson-Palme J, Veldre V, Ryberg M et al (2013) ITSx: improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for use in environmental sequencing. *Methods Ecol Evol* 4:914–919. <https://doi.org/10.1111/2041-210X.12073>
- Cáceres MES, Aptroot A (2016) First inventory of lichens from the Brazilian Amazon in Amapá State. *Bryologist* 119:250–265. <https://doi.org/10.1639/0007-2745-119.3.250>
- Ceynowa-Giełdon M (2003) *Absconditella fossorum* and *A. sphagnorum* (Lichenes, *Stictidaceae*) in NW Poland. *Acta Mycol* 38:99–103. <https://doi.org/10.5586/am.2003.011>
- Coppins BJ (2009) *Absconditella* Vězda (1965) (2009). In: Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW, Wolseley PA (eds) *The Lichens of Great Britain and Ireland*. British Lichen Society, pp 123–124
- Czarnota P, Kukwa M (2008) Contribution to the knowledge of some poorly known lichens in Poland. I. The genus *Absconditella*. *Folia Cryptog Eston* 44: 1–7 <https://ojs.utlib.ee/index.php/FCE/article/view/13701>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9(8):772. <https://doi.org/10.1038/nmeth.2109>
- Fries EM (1849) *Summa vegetabilium Scandinaviae* [...]. Sectio posterior. Holmiae & Lipsiae, A. Bonnier, Uppsala
- Garbelotto MM, Lee HK, Slaughter G, Popenuck T, Cobb FW, Bruns TD (1997) Heterokaryosis is not required for virulence of *Heterobasidium annosum*. *Mycologia* 89:92–102. <https://doi.org/10.1080/00275514.1997.12026759>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224. <https://doi.org/10.1093/molbev/msp259>
- Jaklitsch W, Baral H-O, Lücking R, Lumbsch HT (2016) In: Frey W (ed) *Syllabus of plant families*, Volume 1/2: *Ascomycota*. Stuttgart: Gebrüder Borntraeger Verlag, 322 p.
- Kalb K, Aptroot A (2018) New lichen species from Brazil and Venezuela. *Bryologist* 121:55–66. <https://doi.org/10.1639/0007-2745-121.1.056>
- Kantvilas G (2005) Two ephemeral species of the lichen genus *Absconditella* (*Stictidaceae*) new to Tasmania. *Muelleria* 21:91–95. <https://doi.org/10.5962/p.291563>

- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform* 20(4):1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Köljalg U, Nilsson HR, Schigel D et al (2020) The taxon hypothesis paradigm—on the unambiguous detection and communication of taxa. *Microorganisms* 8(12):1910. <https://doi.org/10.3390/microorganisms8121910>
- Kondratyuk S, Lőkös L, Tchabanenko S et al (2013) New and noteworthy lichen-forming and lichenicolous fungi. *Acta Biol Hung* 55:275–349
- Kono M, Fernández-Brime S, Muggia L, Wedin M, Resl P, Terai Y (2021) Comparative genomic approaches towards the genetic basis of fungal lifestyles in *Stictidaceae*. In: International Association for Lichenology 9th Symposium (IAL 9) August 1 to 6, 2021 Brazil, IAL9 program and abstract book, p. 142
- Lücking R, Rivas Plata E, Mangold A et al (2011) Natural history of Nash's pore lichens, *Trinathotrema* (*Ascomycota*: *Ostropales*: *Stictidaceae*). *Biblioth Lichenol* 106:187–210
- Malíček J, Palice Z, Vondrák J (2018) Additions and corrections to the lichen biota of the Czech Republic. *Herzogia* 31(1):453–475. <https://doi.org/10.13158/hea.31.1.2018.453>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana. IEEE, New Orleans, LA, USA: 1–8
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* 30:1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Nilsson RH, Larsson K-H, Taylor AFS, et al (2018) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res* D259–D264. <https://doi.org/10.1093/nar/gky1022>
- Palice Z, Guttová A, Halda JP (2006) Lichens new for Slovakia collected in the National Park Muránska planina (W Carpathians). In: Lackovičová A, Guttová A, Lisická E, Lizoň P (eds) *Central European Lichens — Diversity and Threat*. Mycotaxon Ltd., Ithaca, and Institute of Botany, Slovak Academy of Sciences, Bratislava, pp. 179–192
- Rambaut A (2014) FigTree v. 1.4.2. <http://tree.bio.ed.ac.uk/software/figtree/>
- Ronquist F, Teslenko M, van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rossmann AY (1980) *Absoconditella duplicella* and *Cryptodiscus rutilus*: additions to the ostropalean fungi. *Mycotaxon* 10:365–368
- Roux C, Sérusiaux E (2004) Le genre *Strigula* (lichens) en Europe et en Macaronésie. *Biblioth Lichenol* 90:1–96
- Søchting U, Øvstedal DO, Sancho LG (2004) The lichens of Hurd Peninsula, Livingston Island, South Shetlands, Antarctica. *Biblioth Lichenol* 88:607–658
- Spribille T, Björk CR, Ekman S, Elix JA, Goward T, Printzen C, Tønnsberg T, Wheeler T (2009) Contributions to an epiphytic lichen flora of northwest North America: I. Eight new species from British Columbia inland rain forests. *Bryologist*. 112:109–137. <https://doi.org/10.1639/0007-2745-112.1.109>
- Stamatakis A (2014) RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* <https://doi.org/10.1093/bioinformatics/btu033> <http://bioinformatics.oxfordjournals.org/content/early/2014/01/21/bioinformatics.btu033.abstract>
- Suija A, van den Boom P (2023) (2953) Proposal to conserve the name *Absoconditella* against *Geisleria* (*Ascomycota*: *Lecanoromycetes*). *Taxon* (in press)
- Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol* 56:564–577. <https://doi.org/10.1080/10635150701472164>
- Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Köljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytol* 180:479–490. <https://doi.org/10.1111/j.1469-8137.2008.02561.x>
- Thiyagaraja V, Lücking R, Ertz D, Karunarathna SC, Wanasinghe DN, Lumyong S, Hyde KD (2021) The evolution of life modes in *Stictidaceae*, with three novel taxa. *J Fungi* (Basel) 7(2):105. <https://doi.org/10.3390/jof7020105>
- Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res* 44(W1):W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Turland NJ, Wiersema JH, Barrie FR et al (eds) (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books. <https://doi.org/10.12705/Code.2018>
- van den Boom PPG, Brand AM, Suija A (2015) A new species of *Absoconditella* from western and central Europe with a key to the European members. *Phytotaxa* 238:271–277. <https://doi.org/10.11646/phytotaxa.238.3.6>
- Vězda A (1965) Flechtensystematische Studien II. *Absoconditella*, eine neue Flechtengattung. *Preslia* 37:237–245
- Vězda A (1970) Neue und wenig bekannte Flechten in der Tschechoslowakei. I. *Folia Geobot Phytotax* 5(3–4):307–337
- Vězda A, Pišút I (1984) Zwei neue Arten der Flechtengattung *Absoconditella* (lichenisierte *Stictidaceae*, *Ostropales*) in der Tschechoslowakei. *Nova Hedwigia* 40:341–346
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172:4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Wedin M, Döring H, Gilenstam G (2004) Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the *Stictis-Conotrema* complex. *New Phytol* 164:459–465. <https://doi.org/10.1111/j.1469-8137.2004.01198.x>Citations:85
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR Protocols: a guide to methods and applications*. Academic Press Inc, San Diego, CA, USA, pp 315–322
- Wirth V, Hauck M, Schultz M (2013) *Die Flechten Deutschlands*, vol 1. Ulmer, Stuttgart, pp 1–672
- Zoller S, Scheidegger C, Sperisen C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* 31:511–516

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