

***Caesiodiscus populicola* gen. et sp. nov.**
(Leotiomyces, Helotiales), a remarkable new corticolous
ascomycete from Norway

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bemerkesesverdig ny barklevende sekkspore-
sopp fra Norge

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KEYWORDS

Caesiodiscus, Helotiales, new species, phylo-
geny, taxonomy

NØKKELOORD

Caesiodiscus, fylogeni, Helotiales, ny art,
taksonomi

SAMMENDRAG

Den nye sekksporesoppen *Caesiodiscus popu-
licola* Holien og Suija blir her beskrevet
basert på morfologiske, anatomiske og mole-
kylære karakterer. Artens systematiske plas-
sering innenfor orden Helotiales er uklar, men
analyser av nuLSU-sekvenser tyder på at de
nærmeste slektningene er *Connersia rilstonei*
og *Pleuroascus nicholsonii*. Den nye arten
har distinkte karakterer både habituellt og
mikroskopisk. Den vokser på stammer av

løvtrær med rik bark og det viktigste vertstreet
er osp. Arten er så langt bare kjent fra Norge
hvor den hovedsakelig er funnet langs kysten.

ABSTRACT

A new ascomycete genus and species *Caesio-
discus populicola* Holien and Suija is herein
described based on morphological, anatomical
and molecular characteristics. Its systematic
position within the Helotiales is unclear, but
analysis of nuLSU sequences indicated that
the closest relatives are *Connersia rilstonei*
and *Pleuroascus nicholsonii*. The species is
distinct both macroscopically and microscopi-
cally and is corticolous on deciduous trees
with a high bark-pH. The most common
phorophyte is *Populus tremula*. It is at present
only known from Norway, mainly along the
west coast.

INTRODUCTION

During the last decade of fieldwork along the
west coast of Norway we have come across a
distinct, corticolous ascomycete. The species
has pruinose, bluish grey to greyish apothecial
disc, distinct greyish white excipulum, 4-spored
asci and eumuriform ascospores, and it grows
mainly on old trunks of living *Populus tremula*
L. With such distinct combined microscopic,
macroscopic and ecological characters, we
optimistically searched for its name.

Surprisingly, we were not able to find neither a name nor a genus, not even a family, for this species. We first thought the species could belong in the family Tribliidiaceae (Leotiomycetes), which was recently revised (Karakehian et al. 2019), but preliminary studies indicated that this was probably not true. In the present study, we address the taxonomic affinity of this new species using morphological, anatomical and molecular data.

MATERIALS AND METHODS

Material

This study is based on material collected by the first author. In addition, we have received collections from several other persons. The material is deposited in the herbarium in Trondheim (TRH) with duplicates to O, TUF and UPS.

Microscopy

We examined dried herbarium specimens by standard techniques, using Leica MZ8 and Leica S4E stereomicroscopes, and Zeiss Axio A1 and Leica DM750 light microscopes. For examination of details of the ascomata we used razor-blade-cut sections mounted in tap water, in 10 % aqueous solution of potassium hydroxide (KOH, K), c. 50% nitric acid (HNO₃, N), Cresyl Blue (CRB) and in Lugol's iodine solution (I). For observation of amyloid reactions of the ascus apical apparatus, Lugol's

solution both with (denoted as K/I) and without pretreating with KOH were used.

Measurements were made in water and the sizes are presented as (minimum-)average (-maximum) value.

DNA extraction, PCR amplification and DNA sequencing

DNA extraction, amplification and purification were carried out at Tartu University (TU). Genomic DNA was extracted from ascomata of five specimens (Table 1) using High pure PCR Template Preparation Kit (Roche Applied Science®, Penzberg, Germany) following the protocol provided by the manufacturer. We amplified four ribosomal loci. These include three nuclear (internal transcribed spacer (nuITS), large subunit (nuLSU), and small subunit (nuSSU)) and one mitochondrial (small subunit (mtSSU)) DNA regions. To amplify these four loci, we used the following primer pairs, respectively: ITS0F-LA-W (Tedersoo et al. 2008), LROR-LR7 (Vilgalys and Hester 1990), SSU1-SSU3R (Pärtel et al. 2017), and mrSSU1-mrSSU3R (Zoller et al. 1999). Sample preparation, polymerase chain reaction (PCR) amplification and DNA purification are described in detail in Suija et al. (2017). Both strands were Sanger sequenced at MacroGen Inc. (Amsterdam, the Netherlands). The nuITS sequences were sequenced with primer pair ITS4 and ITS5 (White et al. 1990),

Table 1. Voucher information and GenBank accession codes corresponding to sequences generated for this study. Sequences from type specimen are marked in bold.

Laboratory code	Fungarium	Collector and collection number	nuITS	nuLSU	nuSSU	mtSSU
AS364	TRH-F-17978	Holien 17/18	MW298844	MW298855	-	MW298850
AS443	TUF-087471	Holien 15910	MW298848	MW298857	-	MW298854
TR391	TUF-091126	Olsen OO-L-19.02	MW298845	MW298856	-	MW298851
TR392	TRH-F-17225	Jordal s.n.	MW298846	-	-	MW298852
TR393	TRH-F-17224	Jordal s.n.	MW298847	-	MW298849	MW298853

Table 2. GenBank accession codes of nuLSU sequences used for building phylogeny. The taxonomy of species follows Jaklitsch et al. (2016) and Johnston et al. (2019).

GenBank acc. code	Species name	Taxonomy
MK226457	<i>Alatospora acuminata</i>	Leotiales, incertae sedis
AB481317	<i>Albotricha acutipila</i>	Lachnaceae, Helotiales
JN086746	<i>Arachnopeziza obtusipila</i>	Arachnopezizeaceae, Helotiales
MK226456	<i>Articulospora tetraccladia</i>	Discinella-Pezoloma lineage, Helotiales
JN086709	<i>Ascocoryne cylichnium</i>	Gelatinodiscaceae, Helotiales
JN086695	<i>Brunnipila fuscescens</i>	Lachnaceae, Helotiales
EU940107	<i>Bryoscyphus dicrani</i>	Helotiaceae, Helotiales
KJ663870	<i>Bulgaria inquinans</i>	Phacidaceae, Phacidiales
KR094163	<i>Calycellinopsis xishuangbanna</i>	Helotiales, incertae sedis
KX090811	<i>Cenangiopsis quercicola</i>	Cenangiaceae, Helotiales
KX090822	<i>Cenangium acuum</i>	Cenangiaceae, Helotiales
AY487083	<i>Chaetomella oblonga</i>	Chaetomellaceae, Helotiales
AY544669	<i>Chlorociboria cf. aeruginacens</i>	Chlorociboriaceae, Helotiales
MH729335	<i>Chlorosplenium chlora</i>	Mollisiaceae, Helotiales
JN086732	<i>Cistella albidolutea</i>	Stamnaria lineage/Han Clade 9
KX090815	<i>Claussenomyces prasinulus</i>	Tympanidaceae
AY544657	<i>Coccomyces dentatus</i>	Rhytismataceae
GQ154611	<i>Collophorina paarla</i>	Tympanidaceae
MK314598	<i>Collophorina rubra</i>	Tympanidaceae
FJ176866	<i>Connersia rilstonei</i>	Helotiales, incertae sedis
AY544680	<i>Crinula caliciiformis</i>	Leotiomycetes, incertae sedis
MH985296	<i>Cryptohymenium pycnidiophorum</i>	Helotiales sclerotinioid clade
DQ470944	<i>Cudoniella clavus</i>	Helotiaceae, Helotiales
EU107207	<i>Cyttaria darwinii</i>	Cyttariaceae
EU107223	<i>Cyttaria hariotii</i>	Cyttariaceae
DQ247801	<i>Dermea acerina</i>	Dermateaceae, Helotiales
AB570319	<i>Dictyocatenuata alba</i>	Ostropomycetidae
AB570320	<i>Dictyocatenuata alba</i>	Ostropomycetidae
KX090834	<i>Diplocarpa bloxamii</i>	Cordieritidaceae, Helotiales
EU940116	<i>Discinella schimperi</i>	Discinella-Pezoloma lineage, Helotiales
KX090798	<i>Encoelia furfuracea</i>	Cordieritidaceae, Helotiales
KX090809	<i>Encoelia heteromera</i>	Cordieritidaceae, Helotiales
EU940128	<i>Epiglia gloeocapsae</i>	Mniaeciaceae
KC834021	<i>Filospora fistucella</i>	Helotiales, incertae sedis
MH485386	<i>Gelatinopsis fungicola</i>	Helicogoniaceae, Phacidiales

GenBank acc. code	Species name	Taxonomy
KC834029	<i>Gyoerffyyella rotula</i>	Discinella-Pezoloma lineage, Helotiales
JN086755	<i>Hamatocanthoscypha laricionis</i>	Pezizellaceae, Helotiales
MH870940	<i>Haplographium delicatum</i>	Hyaloscyphaceae, Helotiales
JN086748	<i>Hyalopeziza pygmaea</i>	Hyaloscyphaceae, Helotiales
JN086705	<i>Hymenoscyphus caudatus</i>	Helotiaceae, Helotiales
NG66420	<i>Hymenoscyphus ohakue</i>	Helotiaceae, Helotiales
HM140528	<i>Hypoderma rubi</i>	Rhytismataceae
HQ609479	<i>Infundichalara microchona</i>	Pezizellaceae, Helotiales
KX090833	<i>Ionomidotis olivascens</i>	Cordieritidaceae, Helotiales
MH748087	<i>Lachnellula subtilissima</i>	Lachnaceae, Helotiales
JN086698	<i>Lachnum abnorme</i>	Lachnaceae, Helotiales
DQ470978	<i>Lambertella subrenispora</i>	Rutstroemiaceae, Helotiales
AB481319	<i>Lasiobelonium lonicerae</i>	Lachnaceae, Helotiales
DQ267627	<i>Lemonniera aquatica</i>	Discinella-Pezoloma lineage, Helotiales
AY544644	<i>Leotia lubrica</i>	Leotiaceae, Leotiales
FJ176884	<i>Leuconeurospora pulcherrima</i>	Pseudeurotiaceae, Phacidiales
KX090842	<i>Llimoniella terricola</i>	Cordieritidaceae, Helotiales
DQ470957	<i>Loramycetes macrosporus</i>	Loramycetaceae, Helotiales
MK599207	<i>Marthamycetes dracophylla</i>	Marthamycetaceae, Marthamycetales
DQ470954	<i>Meria laricis</i>	Cenangiaceae, Helotiales
KX090817	<i>Microglossum olivaceum</i>	Leotiaceae, Leotiales
DQ470981	<i>Microglossum rufum</i>	Leotiaceae, Leotiales
JN086721	<i>Microscypha ellisii</i>	Pezizellaceae, Helotiales
JN086706	<i>Microscypha sp</i>	Pezizellaceae, Helotiales
EU940115	<i>Mniaeciaceae nivea</i>	Tympanidaceae, Phacidiales
AB926162	<i>Moellerodiscus pinicola</i>	Rutstroemiaceae, Helotiales
DQ470942	<i>Mollisia cinerea</i>	Mollisiaceae, Helotiales
JN086707	<i>Mollisia uncinata</i>	Mollisiaceae, Helotiales
KC834033	<i>Mycofalcella calcarata</i>	Helotiaceae, Helotiales
AY541491	<i>Myxotrichum deflexum</i>	Myxotrichaceae, Helotiales
FJ176867	<i>Naemacyclus fimbriatus</i>	Marthamycetaceae, Marthamycetales
FJ176865	<i>Neobulgaria pura</i>	Gelatinodiscaceae, Helotiales
JN086683	<i>Olla millepunctata</i>	Pezizellaceae, Helotiales
DQ470967	<i>Pezicula carpinea</i>	Dermateaceae, Helotiales
DQ470976	<i>Phacidium lacerum</i>	Phacidaceae, Phacidiales
AB926130	<i>Phaeohelotium epiphyllum</i>	Helotiaceae, Helotiales
JN086727	<i>Phialina lachnabrachyoides</i>	Pezizellaceae, Helotiales
MH869755	<i>Phialocephala dimorphospora</i>	Mollisiaceae, Helotiales

GenBank acc. code	Species name	Taxonomy
KR859069	<i>Phlyctema vagabunda</i>	Dermateaceae, Helotiales
MH870939	<i>Pilidium acerinum</i>	Chaetomellaceae, Helotiales
MH872404	<i>Pleuroascus nicholsonii</i>	Helotiaceae, Helotiales
AB926136	<i>Poculum pseudosydowia</i>	Rutstroemiaceae, Helotiales
JN086753	<i>Polydesmia pruinosa</i>	Helotiales sclerotinioid clade
MG719709	<i>Polyphilus frankenii</i>	Stamnaria lineage/Han Clade 9
DQ470949	<i>Potebniomyces pyri</i>	Phacidiaceae, Phacidiales
HM140560	<i>Propolis versicolor</i>	Marthamycetaceae, Marthamycetales
DQ470988	<i>Pseudeurotium zonatum</i>	Pseudeurotiaceae, Phacidiales
KR859074	<i>Pseudofabraea citrica</i>	Dermateaceae, Helotiales
JN086699	<i>Psilachnum staphyleae</i>	Stamnaria lineage/Han Clade 9
AF356696	<i>Rhytisma acerinum</i>	Rhytismataceae
JN086717	<i>Rodwayella citrinula</i>	Pezizellaceae, Helotiales
AB628056	<i>Roesleria subterranea</i>	Helotiaceae, Helotiales
KT972712	<i>Roseodiscus formosus</i>	Helotiales, incertae sedis
KX090797	<i>Rutstroemia bulgarioides</i>	Rutstroemiaceae, Helotiales
DQ470963	<i>Rutstroemia firma</i>	Rutstroemiaceae, Helotiales
MH748088	<i>Sclerotinia pseudotuberosa</i>	Sclerotiniaceae, Helotiales
MH872858	<i>Sordaria fimicola</i>	Sordariomycetes (outgroup)
FJ176895	<i>Thelebolus ellipsoideus</i>	Thelebolaceae
JQ256441	<i>Thuemenidium atropurpureum</i>	Leotiaceae, Leotiales
MN540636	<i>Triblidium caliciiforme</i>	Triblidiaceae
JN086701	<i>Trichopeziza sulphurea</i>	Lachnaceae, Helotiales
AB481307	<i>Trichopezizella otani</i>	Lachnaceae, Helotiales
GQ477333	<i>Tricladium splendens</i>	Helotiaceae, Helotiales
EU940114	<i>Trizodia acrobia</i>	Mniaeciaceae
KX090835	<i>Trochila laurocerasi</i>	Cenangiaceae, Helotiales
DQ470983	<i>Tryblidiopsis pinastri</i>	Rhytismataceae
JN086682	<i>Urceolella crispula</i>	Stamnaria lineage/Han Clade 9
KC834036	<i>Varicosporium delicatum</i>	Discinella-Pezoloma lineage, Helotiales
AB546954	<i>Venturiocistella japonica</i>	Helotiales - Han Clade 4
FJ176874	<i>Vibrissea truncorum</i>	Vibrisseaceae, Helotiales
KX090824	<i>Xeropilidium dennisii</i>	Chaetomellaceae, Helotiales
AY544648	<i>Xylaria hypoxylon</i>	Sordariomycetes (outgroup)

nuLSU with CTB6 (Garbelotto et al. 1997) and LR7, and mtSSU and nuSSU with same primers as amplified. Sequencher 4.10.1. (GeneCodes Corp®, Ann Arbor, Michigan, USA) was used to check, assemble and manu-

The consensus sequences were compared with those publicly available in National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) and

Table 3. The results of *blastn* comparison of different genes (accessed October 2020). Per id % = identity percent. The taxonomy follows *Index Fungorum*.

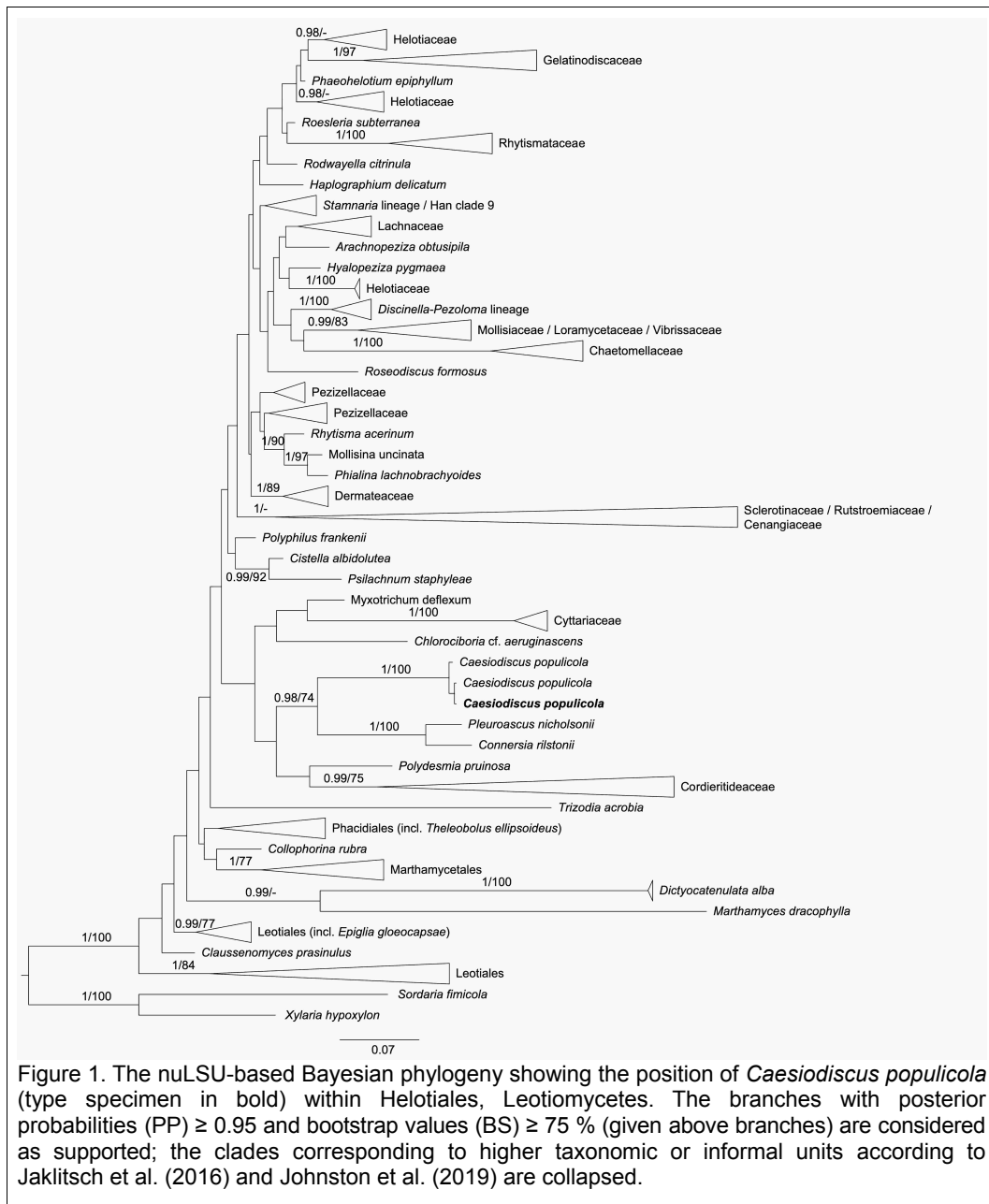
Gene	Closest match	Per id %	Taxonomy	Family / Order	Reference
nuITS	GU055726	86.88–88.32	Uncultured Helotiales	Incertae sedis, Helotiales	Klaubauf et al. 2010
nuLSU	AY541491	94.37–94.49	<i>Myxotrichum deflexum</i>	Amorphothecaceae, Erysiphales	Lumbsch et al. 2005
nuSSU	AB570321	91.88	<i>Dictyocatenulata alba</i>	Incertae sedis, Ostropomycetidae	An et al. 2012
mtSSU	FJ713604	91.66–91.93	<i>Cudoniella clavus</i>	Helotiaceae, Helotiales	Schoch and Sung, unpubl.

UNITE (<https://unite.ut.ee>) databases. The newly generated DNA sequences are available in NCBI (Table 1) and UNITE databases.

The phylogenetic analyses

We compiled DNA alignments for each gene, using taxon sampling that encompassed as many of the segregate of Leotiomycetes as possible (Table 2). The DNA sequences were aligned with the on-line version of Mafft ver. 7 (Katoh et al. 2019) using default options and corrected manually with SeaView ver. 4.6 (Gouy et al. 2010). These single gene alignments were analysed using the one-click mode in NGPhylogeny (Lemoine et al. 2019). After that we selected nuLSU dataset (110 sequences, including three new sequences; see Table 1 and 2) to analyse it further because the nuLSU dataset was the most comprehensive. The ambiguous regions were identified and eliminated with on-line version of Gblocks ver. 0.91b (Talavera and Castresana 2000), adjusting relaxed filtering parameters (smaller final blocks; gap positions within the final blocks). The resulting new alignment consisted of 768 nucleotide positions (121 variable, 96 informative) i.e., 23% of the original alignment. The best-fit nucleotide substitution model according to AIC criterion – GTR + I + G – was calculated with jModeltest ver. 2.1.6. (Darriba et al. 2012). The alignment was analysed using Markov Chain Monte Carlo (MCMC) and Maximum Likelihood (ML)

approaches. The Bayesian analysis was performed with MrBayes ver. 3.2.1. (Ronquist et al. 2012) using the following settings: two parallel simultaneous runs over 8 million generations starting with a random tree and employing four simultaneous chains; sampling after 1000 steps. The average standard deviation of split frequencies across runs reached 0.01, and the potential scale reduction factor (PSRF) for all models and parameters was close to 1. The first 25 % of saved data was discarded as ‘burnin’; the consensus tree and posterior probabilities (PP) were calculated from the rest. The ML analysis was run with RAXML ver. 8.2.12 and inferred assuming GTR + G as the nucleotide substitution model. Branch support was calculated by rapid bootstrapping over 1000 pseudoreplicates. All analyses were implemented at the CIPRES Science Gateway ver. 3.3 (Miller et al. 2010). The clades with posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 0.75 have been regarded as significantly supported. The phylogenetic tree was visualized and edited using FigTree ver. 1.4.2 (Rambaut 2014) and Adobe Illustrator CS3® was used for artwork.



RESULTS

Phylogenetic analyses

Based on *blastn* (Altschul et al. 1990) search in NCBI and UNITE nucleotide databases, no identical or similar sequences were found, and the percentage of identity of different genes ranged from 87.37% (nuITS) to 94.49% (nuLSU) (Table 3). Moreover, the closest matches of different genes pointed to different taxonomic groups in Leotiomycetes, except nuSSU which closest match belonged to Ostropomycetidae, Lecanoromycetes (Table 3). The initial analyses of single-gene alignments using one-click option (data not shown) gave ambiguous results, mainly due to differences in sequence data coverage. This made the exact phylogenetic arrangement of the species impossible, but still allowed placement among Leotiomycetes. In the analysis of the most comprehensive nuLSU dataset, the sister clade of the specimens was formed by *Pleuroascus nicholsonii* Massee and E.S. Salmon and *Connersia rilstonei* (C. Booth) Malloch (Fig. 1; PP=0.98, BS=74), both Helotiales, Helotiaceae (Johnston et al. 2019).

Taxonomy

Caesiodiscus Holien and Suija gen. nov.

MycoBank: MB838686

Diagnosis: Non-lichenized. Ascomata apothecial, marginate, erumpent; asci cylindrical, inoperculate, without croziers, but with long leg separated by septum, with distinct, hemiamyloid ascus apical apparatus; paraphyses filiform, distantly septate, mostly unbranched, apically not thickened; ascospores arranged uniseriately in asci, hyaline, inamyloid, eumuriform.

Typus generis: *Caesiodiscus populicola* Holien and Suija

Caesiodiscus populicola Holien and Suija sp. nov.

MycoBank: MB838687

Diagnosis: *Caesiodiscus populicola* is charac-

terized by the bluish grey to greyish (lead-grey) ascomata covered with greyish white pruina, up to 1.5 mm in diameter, the pale swollen margin and the more or less roundish shape, with 4-spored (more rarely 6–8-spored)

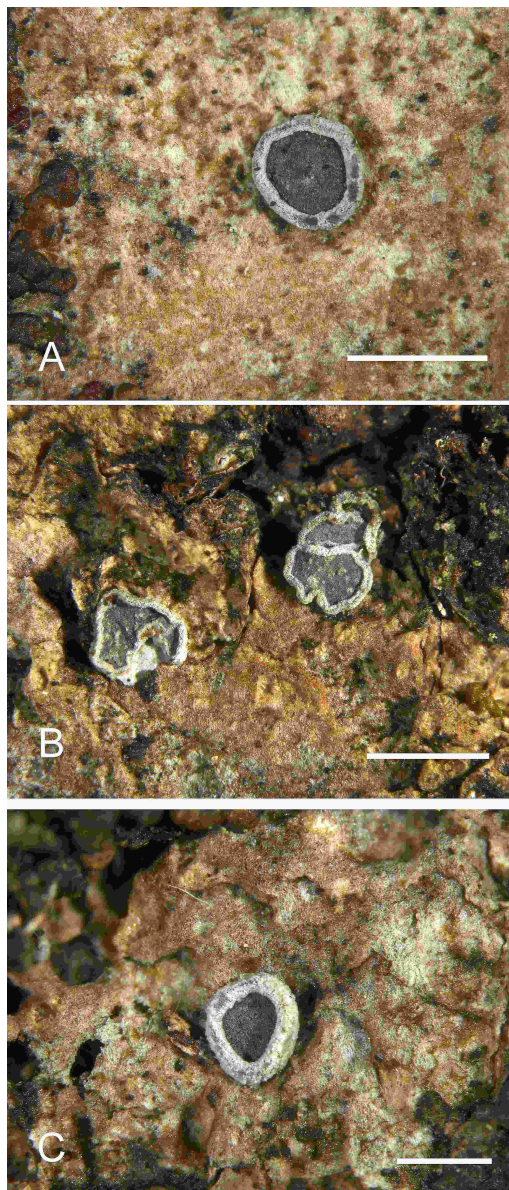


Figure 2. *Caesiodiscus populicola*, the holotype (TRH-F-17665). Scales: A-B= 2 mm; C= 1 mm. Photo: A. Frisch.



Figure 3. *Caesiodiscus populicola* with associate green algal film (TRH-F-17688). Photo: K. Mandal.

asci, eumuriform ascospores and the corticolous habit on deciduous trees with calcium rich bark.

Type: NORWAY: Nord-Trøndelag, Flatanger, between Hylla and Innervika, W side of Utvordveien, 64.55456°N, 11.07011°E, alt. ca 30 m, on trunk of *Populus tremula* in boreal rainforest, 2019-10-24, Holien 15910 (TRH-F-17665 Holotype; O, TUF-087471 and UPS Isotypes)

NCBI accession nos. from isotype (TUF-087471): MW298848 (nuITS; DNA barcode/reference sequence), MW298857 (nuLSU), MW298854 (mtSSU)

Ascomata apothecia, solitary, sometimes gregarious, two or more apothecia in clusters, sessile, narrowed at base, (0.7-)1.0(-1.5) mm (n=18) in diameter (Figs. 2 and 3), outline of the apothecia roundish to somewhat irregular in shape, disc plane to concave, bluish grey (Fig. 4A-C), pruinose, giving the frosty impression; apothecia with distinct, swollen and

almost white margin, (0.1-)0.2(-0.3) mm (n=18) in diameter; in young ascomata the margin is slightly turned inwards. All microscopical structures hyaline, except the outermost layers which are greyish, K- or K+ slightly olive green, N+ violet (reaction disappears quickly), with granular pigments, granules insoluble in K-, but stain in CRB; no crystals. **Hymenium** hyaline, c. 150 µm thick, I-, K-, K/I-, the uppermost part greyish to dark greenish; subhymenium hyaline to slightly beige, c. 30 µm, composed of elongated interwoven cells (*textura intricata* type). **Ectal exciple** distinct, hyaline in central part, of *textura intricata* type, greyish in outer part, composed of angular, irregular cells (*textura angularis* type); excipular hairs and setae missing, however, the tips of hyphae extend from the exciple (well-visible after treatment with K), (Fig. 4I) the tips of hyphae somewhat coiled. **Medullary exciple** (hypothecium) multilayered, hyaline in upper part, *textura intricata* type, c. 50 µm thick, the layer below *textura angularis* type, hyaline in upper part,

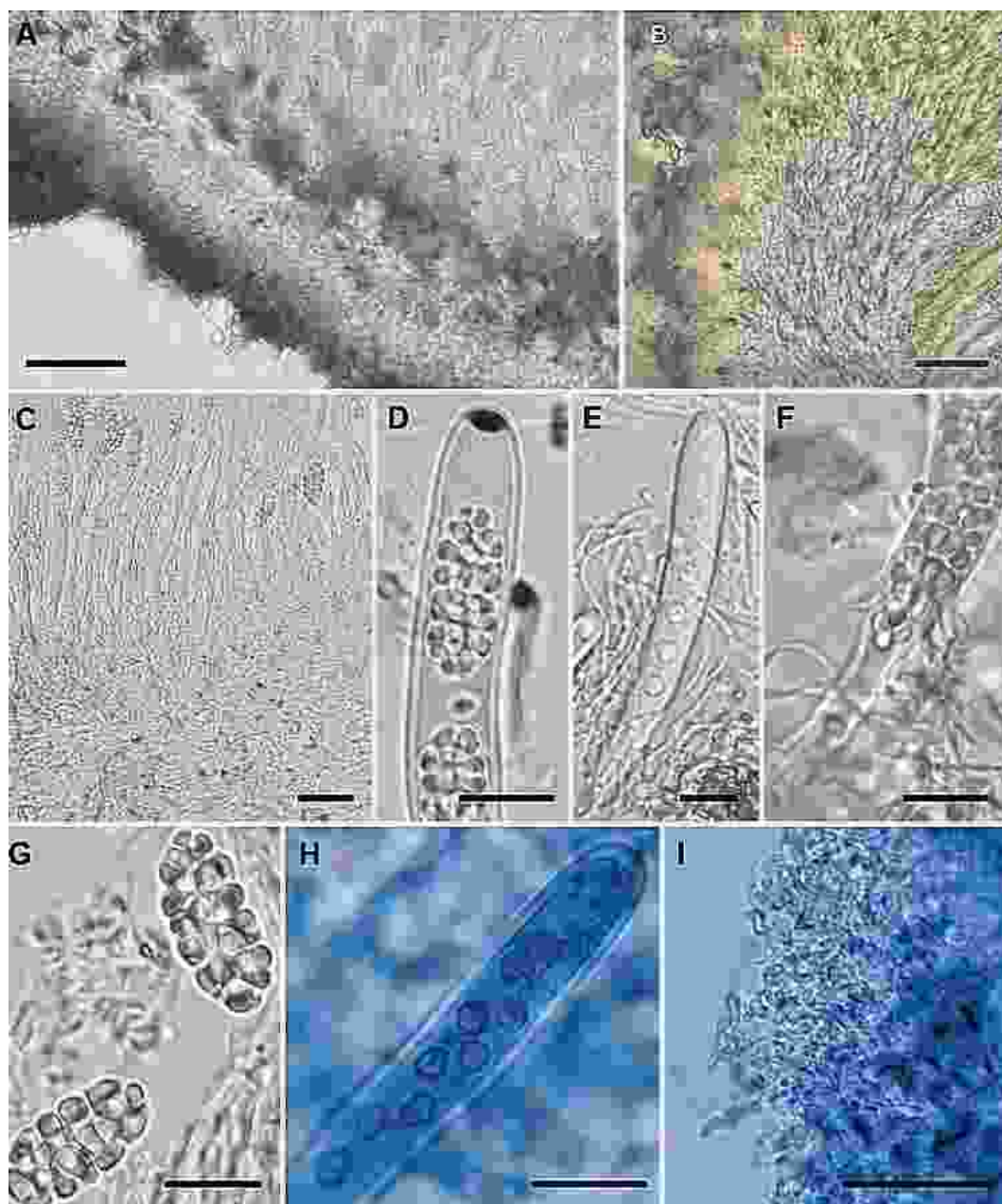


Figure 4. *Caesiodiscus populicola* (TUF-087471, isotype). A, section through ascomata, multilayered medullary exciple and lower part of hymenium (in water); B, section of ectal exciple (in water); C, lower part of the hymenium with asci and paraphyses, and upper part of medullary exciple (in K); D, mature ascus with mature ascospores (in K/I); E, young ascus with 1-celled ascospores (in K); F, base of the ascus (in K/I); G, ascospores (in K/I); H, young ascus with eight 1-celled ascospores (in CRB); I, tips of hyphae extending from the exciple (in CRB). Scales: A= 50 μ m; B, C=20 μ m; D, E, F, G, H=10 μ m; I= 5 μ m. Photos: A. Suija.

the outermost part greyish, c. 50 µm wide. **Asci** inoperculate, mostly 4-spored, more rarely 6- to 8-spored; the young asci contain eight 1-celled ascospores; asci narrow cylindrical (Fig. 4E, H) (125-)135(-150) × (10-)11(-13) µm, narrowed towards the base forming a long leg (Fig. 4F), c. 15–20 µm long and c. 5 µm wide, separated by septum; without croziers; asci with apical thickening c. 5 µm wide, ascus apical ring structure detectable already in water, hemiamyloid, I+ red, IKI+ blue (Fig. 4D); ascus wall c. 1 µm thick, wall surface CRB+ light blue in dead state. **Ascospores** uniseriate, hyaline, eumuriform, with 20–28 cells on surface view, broadly ellipsoid, with rounded ends, inamyloid, (20-)25(-30) × (9-)11(-13) µm (n=50) (Fig. 4G), with thick, c. 1 µm wide wall, wall surface CRB+ light blue in dead state; young spores 1-celled, globose to ellipsoid, the first spore division in transverse direction (Fig. 4E, H). **Paraphyses** narrow, filiform, distantly septate, unbranched or occasionally forked, apically not thickened, c. 1 µm wide, slightly exceeding asci. **Anamorph** not observed. **Photobiont** usually absent, but present in one specimen as a thin green algal film (Fig 3).

Etymology: *Caesiodiscus* from latin *caesius* = bluish, and *populicola* = growing on *Populus*.

Substrate and habitat: The species grows on deciduous trees with calcium-rich bark, mainly on old trunks of *Populus tremula*, more rarely on trunks of *Fraxinus excelsior* and dead stems of *Sambucus racemosa*. The epiphytic community on the host trees is dominated by small bryophytes. On the type locality the most common bryophyte on the host trees was *Frullania fragillifolia* (Taylor) Gottsche et al. with additional *Barbilophozia barbata* (Schmidel ex Schreb.) Loeske, *Lophozia longidens* (Lindb.) Macoun, *Sanionia uncinata* (Hedw.) Loeske and *Ulota phyllantha* Brid. Other associated species include the corticolous

ascomycete *Lasiobelonium corticale* (Pers.: Fr.) Raitv. and small amounts of the cyanolichens *Pannaria rubiginosa* (Ach.) Bory and *Parmeliella triptophylla* (Ach.) Müll. Arg. *Caesiodiscus populicola* was not found on trunks rich in foliose lichens. Other associate species found in the other localities include the liverwort *Radula complanata* (L.) Dumort., the fungi *Amphisphaerella dispersella* (Nyl.) O.E. Erikss. and *Stictis radiata* (L.) Pers. and the lichens *Megalania grossa* (Pers. Ex Nyl.) Hafellner, *Normandina acroglypta* (Norman) Aptroot and *Pectenium plumbea* (Lightf.) P.M. Jørg. et al.

The habitat in the type locality (Fig. 5) can be characterized as a rather open boreal rainforest with *Picea abies* and deciduous trees i.e. *Betula pubescens*, *Populus tremula*, *Salix caprea* and *Sorbus aucuparia*. On *Sorbus* close to the host trees interesting redlisted rainforest lichens i.e. *Pseudocyphellaria citrina* (Gyeln.) Lücking et al. and *Rinodina disjuncta* Sheard and Tønsberg were present.

Distribution: So far, the species has been recorded only from Norway where it has been found in humid coastal areas from Agder in the south to Hamarøy in Nordland county. An additional record from Akershus in the Oslofjord region is locally humid and can be characterized as suboceanic. Its altitudinal range is from just above sea level to about 280 m in the south. The known localities are situated within the south and middle boreal vegetation zones (Moen 1999).

Additional specimens examined: NORWAY: Akershus, Rælingen, Mørkåstjerna N, 59.85978°N, 11.05262°E, on *Populus tremula*, alt. ca. 260 m, 2014-05-10, Klepsland JK14-S004 (TRH-F-17671). Vest-Agder, Søgne, Øygardsbekken, 58.11110°N, 7.65085°E, on *Fraxinus excelsior*, alt. ca. 90 m, 2008-06-23, Klepsland JK08-S016 (TRH-F-17669). Møre og Romsdal, Aure, Vik,



Figure 5. The type locality for *Caesiodiscus populicola* in Flatanger. The host tree, *Populus tremula*, along with *Betula pubescens*, *Picea abies* and *Sorbus aucuparia*. 24th October 2019. Photo: H. Holien.

63.1297°N, 8.6127°E, on *Populus tremula*, alt. 67 m, 2018-11-18, Jordal s.n. (TRH-F-17224); Aure, Vik, 63.1926°N, 8.6124°E, on *Populus tremula*, alt. 61 m, 2018-11-18, Jordal s.n. (TRH-F-17225); Molde, Vikvatnet SE, Vass-skaret, 62.70120°N, 7.49900°E, on *Populus tremula*, alt. ca. 200 m, 2020-01-26, Olsen OOL-20.01 (TRH-F-17582); Molde, Høystakkliaasen, 62.8207°N, 7.7042°E, on *Populus tremula*, alt. ca. 200 m, 2020-10-12, Lorentzen s.n. (TRH-F-17703); Skodje, Liafjellet, 62.4832°N, 6.7361°E, on *Populus tremula*, alt. ca. 140 m, 2019-05-21, Olsen OOL-19.02 (TRH-F-17238 and 17239, TUF-091126); Surnadal, Hjellnes, 62.8395°N, 8.6593°E, on *Populus tremula*, alt. ca. 90 m, 2020-10-13, Lorentzen s.n. (TRH-F-17702); Volda, Bjørkedalsvatnet, 62.0257°N, 6.0417°E, on *Populus tremula*, alt. ca. 30 m, 2019-10-29, Olsen OOL-19.09 (TRH-F-17668). Sør-Trøndelag, Ørland (Bjugn), Fagerenget, 63.87250°N, 9.88810°E, on *Sambucus racemosa*, alt. ca. 20 m, 2020-10-09, Mandal s.n. (TRH-F-17688). Nord-Trøndelag, Flatanger,

between Hylla and Innervika, W side of Utvordveien, 65.55454°N, 11.07019°E, on *Populus tremula*, alt. ca. 30 m, 2018-08-08, Holien 17/18 (TRH-F-17239); Flatanger, between Hylla and Innervika, W side of Utvordveien, 65.55444°N, 11.07036°E, on *Populus tremula*, alt. ca. 30 m, 2019-10-24, Holien 15909 (TRH-F-17666). Nordland, Rødøy, Esvikelva, 66.70025°N, 13.39650°E, on *Populus tremula*, alt. ca. 30 m, 2013-06-07, Klepsland JK13-S005 (TRH-F-17670); Hamarøy, Dragskryssset nord, Kvannvatnet, 68.0556°N, 15.9538°E, on *Populus tremula*, alt. ca. 30 m, 2016-07-19, Gaarder 6808 (TRH-F-17667); Hamarøy, Buktaelva øvre, 67.9952°N, 15.9056°E, on *Populus tremula*, alt. ca. 65 m, 2020-07-15, Lorentzen s.n. (TRH-F-17631).

DISCUSSION

In this study, we wanted to determine the identity of some distinct specimens found along the Norwegian west coast. These specimens had the remarkable combination of bluish grey ascomata with 4-spored asci and eumuriform ascospores.

Eumuriform ascospores are uncommon within Leotiomycetes. The only family in which such type of ascospores are common is the Tribliaceae (Jaklitsch et al. 2016) that in the recent phylogeny by Karakehian et al. (2019) was proven to belong to Rhytismatales. Searching for similar DNA sequences in databases and rough analyses did not confirm the affiliation of *Caesiodiscus* neither with this family nor order. Another taxon having muriform spores is *Mellitiosporium* (Jaklitsch et al. 2016), that is currently placed in Marthamyetaceae, Marthamyetales (Jaklitsch et al. 2016, Johnston et al. 2019). However, DNA sequences of this genus are not available in public databases. *Mellitiosporium* differs from *Caesiodiscus* by having erumpent ascomata and non-amyloid asci (Senn-Irlet 2014, Jaklitsch et al. 2016, Johnston et al. 2019). *Connersia* and *Pleuroascus*, that showed up to be the closest genera according to nuLSU sequence analysis, have reduced, cleistothecial ascomata and single-celled hyaline ascospores within globose, evanescent asci (Malloch 1974, Plishka et al. 2008, Johnston et al. 2019). Thus, the affiliation of *Caesiodiscus* within Helotiales, Leotiomycetes remains unclear. This can probably, at least partly, be attributed to the fact that Leotiomycetes remains a poorly studied taxonomic group within the Ascomycota, as a high proportion of unsettled taxa are lacking molecular data and recent collections (Jaklitsch et al. 2016, Johnston et al. 2019). On the other hand, many helotialean taxa are known by environmental DNA sequences only (e.g. Johnston et al. 2019) without association of reliable taxon names.

In one specimen of *C. populicola* (TRH-F-17688), see Fig. 3, we observed a thin green algal film close to the apothecia. We believe that the species is non-lichenized, but it may be an example of a saprotrophic species that can be facultatively weakly lichenized. This is in need of further study.

Our observations indicate that the fruitbodies of *Caesiodiscus populicola* most often develop in late autumn. They are often browsed by herbivores (possibly molluscs) that remove the hymenium, as seen in nearly all collections so far.

Caesiodiscus populicola seems to have a strong preference for large, living trees of *Populus tremula* which is generally considered a tree species with calcium-rich bark, consequently with a high bark-pH (DuRietz 1945, Barkman 1958). However, the variation in bark-pH is considerable in *Populus*, mostly reflecting soil chemistry (Gustafsson and Eriksson 1995). The other known phorophytes, *Fraxinus excelsior* and *Sambucus racemosa* have normally even higher bark-pH and the latter could be classified as extremely calcium rich with a bark-pH about 7 (Poncet et al. 2015).

Old trees of *Populus tremula* have the highest diversity of red-listed species among the boreal deciduous trees in Norway (Bendiksen et al. 2008). The amount of old *Populus* is declining in Norway due to clear-cut logging and transformation of deciduous forest to spruce plantations (Bendiksen et al. 2008, Lorentzen 2020), but probably even more due to browsing by herbivores, red deer and moose in particular (Speed et al. 2020).

Among relevant red-listed species with a preference for *Populus* in Norway, the lichen *Staurolemma omphalarioides* (Anzi) P.M. Jørg. and Henssen and the fungus *Caliciopsis calicioides* (Ellis and Everh.) Fitzp. are the most interesting (see e.g. Holien and Jørgensen 2010, Holien 2011, Bendiksen et al. 2014, Jordal et al. 2014). It seems that *Caesiodiscus*

populicola shares the same habitat as the two species mentioned above and even the same host trees in several localities from Møre and Romsdal to Nordland (Lorentzen 2020). *Caesiodiscus populicola* should therefore be considered for the Norwegian red list of species.

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