Distribution of the Genus *Ceratocystiopsis* (Ascomycota, Ophiostomatales) on Conifers in Norway, With Description of Four New Species

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Abstract

The genus *Ceratocystiopsis* includes ascomycetes fungi belonging to the *Ophiostomatales* that are not well recognized in the world. Described species so far have been found mainly in association with bark beetles in the Northern Hemisphere. The aims of this study were therefore to survey of distribution of species of *Ceratocystiopsis* associated with bark beetles infesting *Picea abies* and *Pinus sylvestris* in Norway. Adults for 22 different bark beetle species were collected from 13 stands in Norway. During this study, we recovered 126 isolates showing affinities to *Ceratocystiopsis* representing six species, including two described and four undescribed taxa. The four undescribed taxa collected during this work were characterised based on their morphological characteristics and multi-gene phylogenies. Herein, we describe these new species as *Ceratocystiopsis chalcographii* sp. nov., *Ceratocystiopsis debeeria* sp. nov., *Ceratocystiopsis norroenii* sp. nov. and *Ceratocystiopsis troendelagii* sp. nov. *Ceratocystiopsis norroenii* and *C. rollhanseniana* were the most frequently isolated species although the latter species had a much wider vector range. This study expands our knowledge about the taxonomy and species diversity of *Ceratocystiopsis* and beetle-fungus relationships.

Introduction

Ceratocystiopsis was established by Upadhyay and Kendrick (1975) based on the morphological description of *Ophiostoma minutum* by Siemaszko (1939). Siemaszko (1939) based *O. minutum* on the characterization of perithecia found on the wood of *Picea abies* infested with *Ips typographus* in the Białowieża Forest (Poland), Siemaszko did not describe a conidial state for this fungus. The taxonomy of this group has a turbulent history, and species of *Ceratocystiopsis* (*Ascomycota*, *Ophiostomatales*, *Ophiostomataceae*) have been accommodated in various genera including *Ceratocystiopsis* (Upadhyay 1981; Marmolejo and Butin 1990; Hsiau and Harrington 1997; Zipfel et al. 2006; Strzałka et al. 2020; Nel et al. 2021), *Ceratocystis* (Griffin 1968; Olchowecki and Reid 1974), *Cornuvesica* (Viljoen et al. 2000), and *Ophiostoma* (Siemaszko 1939; Hausner et al. 1993, 2003; Kirschner and Oberwinkler 1999). Important historical developments in the taxonomy of *Ceratocystiopsis* include: 1) erecting the genus *Ceratocystiopsis* for species that have falcate ascospores with sheaths and short perithecial necks (Upadhyay and Kendrick (1975); 2) reducing *Ceratocystiopsis* to synonymy with *Ophiostoma*, based on partial SSU and LSU rDNA sequences (Hausner et al. 1993); 3) re-instating *Ceratocystiopsis* to accommodate taxa that have short ascomatal necks, produce falcate ascospores with sheaths and have *Hyalorhinocladiella* (occasionally sporothrix-like) anamorphs. The later was supported by molecular data that combined partial nuclear LSU and β -tubulin gene sequences (Zipfel et al. 2006). Currently, *Ceratocystiopsis* is one of nine more or less clearly defined genera in the *Ophiostomataceae* (De Beer and Wingfield 2013; De Beer et al. 2013a, 2013b, 2016; Hyde et al. 2020).

As currently circumscribed *Ceratocystiopsis* accommodates 20 recognized species (De Beer et al. 2013a; Strzałka et al. 2020; Nel et al. 2021; Chang et al. 2021), which are characterized by ascocarps that have short necks, and produce falcate ascospores surrounded by a hyaline sheath (De Beer and Wingfield 2013). According to De Beer and Wingfield (2013), *Ceratocystiopsis* species besides falcate ascospores (type E, F) may also produce cucullate ascospores (type G), in addition *Ceratocystiopsis* spp. typically have hyalorhinocladiella-like asexual morphs (De Beer and Wingfield 2013).

The genus *Ceratocystiopsis* has a wide geographic distribution, including records for Africa (Nel et al. 2021), Asia (e.g., Yamaoka et al. 1997; Wang et al. 2020; Chang et al. 2021), Europe (e.g., Hausner et al. 1993; Kirisits 2004; Jankowiak 2005; Linnakoski et al. 2012;) and North America (Olchowecki and Reid 1974; Bridges and Perry 1987; Marmolejo and Butin 1990; Hsiau and Harrington 1997; Hausner et al. 2003). They are abundant in the Northern Hemisphere, especially in conifer ecosystems in Europe and North America. The most frequently reported member of this genus is *Ceratocystiopsis minuta*. This species based on reports appears to be globally widespread, although Plattner et al. (2009) based on molecular phylogenetic analyses showed that strains from Europe and Asia assigned to *C. minuta* can be arranged into at least three well defined lineages (clades C, D, and E). In addition, “*C. minuta*-like” strains from North America failed to group within those lineages that accommodated *C. minuta* strains from Europe and Asia. So, *C. minuta s.l.* is a species complex that probably includes several cryptic species that have yet to be described. In the original description, type material was not designated for *C. minuta* (Siemaszko 1939). For this reason, Reid and Hausner (2010) proposed the epitypification of *Ceratocystiopsis minuta* based on UAMH 11218 (= a dried culture of UM1532 (=WIN(M)1532), originally isolated from *Picea abies* infested by *Ips typographus* in the Biebrzański National Park (Poland). With reference to the work of Plattner et al. (2009), UM1532 (=RJ705) belongs to clade D.

Ceratocystiopsis species occupy a relatively narrow range of habitats. The greatest number of species have been recorded on conifers in association with bark and wood boring beetles (e.g., Olchowecki and Reid 1974; Hsiau and Harrington 1997; Yamaoka et al. 1997; Kirisits 2004; Linnakoski et al. 2012; Nel et al. 2021). Other species have been described from hardwood trees (Olchowecki and Reid 1974; Li et al. 2018, Strzałka et al. 2020). Very little is known about the distribution of *Ceratocystiopsis* species in Norway. To date, only *C. minuta* and *C. rollhanseniana* have been described from Norway. The first of them has been isolated from *Picea abies* infested by *Ips typographus* (Solheim 1986, 1992, 1993) while the second species has been collected from beetle galleries in a standing tree of *Pinus sylvestris* (Hausner et al. 2003).

A survey of bark beetles and their associated fungi on conifers in Norway was conducted from October 2014 to September 2015. Among the fungi recovered from this study many isolates obtained from various species of bark beetles, and their galleries resembled species of *Ceratocystiopsis*. The aim of this study was to characterize the morphology, phylogenetic affinities, and taxonomy of *Ceratocystiopsis* isolates found in Norway and to resolve the phylogenetic relationships between these fungi and closely related known species within the genus *Ceratocystiopsis*. In addition, this study provides new insights on the abundance and distribution of *Ceratocystiopsis* species in Norway.

Materials And Methods

Fungal isolates

Between October 2014 and September 2015, 22 species of bark beetles were collected from several locations in Norway (Table 1, Fig. 1). The sampled bark beetles represented the following genera, *Cryphalus saltuarius* *Crypturgus cinerus*, *Crypturgus pusillus*, *Dryocoetes autographus*, *Dryocoetes hectographus*,

Hylastes brunneus, *Hylastes cunicularis*, *Hylurgops palliatus*, *Ips acuminatus*, *Ips sexdentatus*, *Orthotomicus laricis*, *Orthotomicus proximus*, *Orthotomicus suturalis*, *Pityogenes bidentatus*, *Pityogenes chalcographus*, *Pityogenes quadridens*, *Pityogenes saalasi*, *Pityophthorus micrographus*, *Polygraphus poligraphus*, *Polygraphus subopacus*, *Tomicus piniperda* and *Trypodendron lineatum*. The bark beetles were collected by hand from *Picea abies* and *Pinus sylvestris*. Bark beetle adults were excised from the bark and galleries with sterilized tweezers and stored individually in sterile 1.5 ml Eppendorf tubes for later fungal isolations. Each bark beetle was divided into three parts, elytra, head and the rest, before placing the parts in three separate Petri dishes with malt extract agar [MEA; 6.25 g malt Bacto™ agar powder (Beckton, Dickinson, Sparks, USA), 10 g agar (bactoagar powder from VWR International, Leuven, Belgium), 0.5 l deionized water] without any cycloheximide. Emerging cultures were purified by transferring small pieces of mycelium or spore masses from individual colonies to fresh MEA. Fungi were incubated at 20 °C, then subcultured onto 2% MEA and stored at 4 °C. After two weeks of incubation, the purified fungal cultures were grouped into morphotypes based on their morphological characteristics. Depending on the number of isolates belonging to the same morphotype, 2–19 isolates per morphotype were chosen for molecular identification (Table 2).

The cultures are maintained in the culture collection of Norwegian Institute of Bioeconomy (NIBIO), Norway. Ex-type isolates of new species described in this study were deposited in the collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, and in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Type specimens were deposited in the Mycological Herbarium (O), Natural History Museum, University of Oslo, Norway. The types of *C. neglecta* and *C. rollhanseniana* were sourced from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), and from the culture collection of the University of Manitoba (WIN) in Canada (Table 2). Taxonomic descriptions and nomenclatural data were registered in MycoBank (www.Mycobank.org) (Robert et al. 2013).

Microscopy and growth studies

Morphological characters were examined for selected isolates as well as for the herbarium specimens selected as types. Isolates were grown on 2% Malt Extract Agar (MEA) (20 g Bacto™ malt extract, 20 g agar Bacto™ agar powder (Becton Dickinson and Company, Franklin Lakes, USA) in 1 l deionized water). In attempts to induce the formation of ascomata, autoclaved twigs of host trees including the bark were placed at the centres of agar plates containing MEA. Fungal cultures were derived from single conidia. To promote the production of ascomata, single conidial isolates were crossed in all possible combinations, following the technique described by Grobbelaar et al. (2009). These cultures were incubated at 25 °C and monitored regularly for the appearance of fruiting structures.

Morphological features were examined by mounting fungal tissue in 80% lactic acid on glass slides, and fruiting structures were observed using a Nikon Eclipse 50/ microscope (Nikon® Corporation, Tokyo, Japan) with an Invenio 5S digital camera (DeltaPix®, Maalov, Denmark) to capture photographic images. Microscopy followed the technique described by Kamgan Nkuekam et al. (2011). Colour designations were based on the colour charts of Kornerup and Wanscher (1978).

For each taxonomically relevant structure, fifty measurements were made, when possible, using the Coolview 1.6.0 software (Precoptic®, Warsaw, Poland). Averages, ranges and standard deviations were calculated for the measurements, and these are presented in the format '(min–)(mean–SD)–(mean+SD)(–max)'.

Growth characteristics for the novel species were determined by analysing the radial growth for two isolates (two for each species). Agar disks (5 mm diam.) were cut from the actively growing margins of fungal colonies and these disks were placed at the centres of plates containing 2% MEA. Four replicate plates for each of the six putative new species were incubated at temperatures between 5, and 35 °C at 5 °C intervals. The radial growth (two measurements perpendicular to each other per plate) was determined 14 d after inoculation, and growth rates were calculated as mm/d.

PCR, sequencing, and phylogenetic analyses

Morphotypes were initially identified by sequencing the internal transcribed spacer 1 and 2 (ITS1–5.8S–ITS2), internal transcribed spacer 2 and large subunit (ITS2–28S) or β -tubulin (β T) gene region of representative isolates (Table 2). DNA extractions were performed as described by Aas et al. (2018). For more detailed analysis two loci were also amplified and sequenced and subjected to phylogenetic analyses: calmodulin (CAL), and the translation elongation factor 1- α (TEF1- α). The primers used for PCR and sequencing of the various loci were as follows: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for the ITS region; T10 (O'Donnell and Cigelnik 1997) or Bt2a together with Bt2b (Glass and Donaldson 1995) for β T; F-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) were used for TEF1- α ; CL1 and CL2a (O'Donnell et al. 2000) or CL3F and CL3R (De Beer et al. 2016) were used for CAL. Protocols for PCR amplification and DNA sequencing were as described previously (Aas et al. 2018).

All phylogenetic analyses were performed independently for each gene partition (Figs. 2–6). Resulting trees were visually compared for topological incongruence. Gene partitions showing no topological incongruences (i.e., ITS1–5.8S–ITS2–28S+ β T) were combined and presented as a concatenated construct (Fig. 7).

For phylogenetic analyses, sequence alignments were performed using the online version of MAFFT v7 (Kato and Standley 2013). The ITS1–5.8S–ITS2, ITS2–28S, β T, CAL, and TEF1- α datasets were aligned using the E-INS-i strategy with a 200PAM/ κ =2 scoring matrix, a gap opening penalty of 1.53 and an offset value of 0.00. The alignments were checked manually with BioEdit v.2.7.5 (Hall 1999). The resulting alignments and trees were deposited into TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S28950>).

Phylogenetic trees were inferred for each of the datasets using three different methods: Maximum likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI). For ML and BI analyses, the best-fit substitution models for each aligned dataset were established using the corrected Akaike Information Criterion (AICc) in jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). ML analyses were carried out with PhyML 3.0 (Guindon et al. 2010), utilizing the Montpellier online server (<http://www.atgc-montpellier.fr/phyml/>). The ML analysis was combined with bootstrapping (1000 bootstrap

pseudoreplicates) to assess node support values and the overall reliability of the tree topology. The best evolutionary substitution models were as follows: GTR+I+G for ITS1-5.8S-ITS2 (-lnL = 4817.94), ITS2-28S (-lnL = 1452.82) and CAL (-lnL = 3350.13); GTR+G for β T (-lnL = 4368.71) and ITS-LSU+ β T (-lnL = 9793.62); and HKY+I+G for TEF1- α (-lnL = 5374.80).

MP analyses were performed using PAUP* 4.0b10 (Swofford 2003). Gaps were treated as fifth state. Bootstrap analysis (1000 bootstrap replicates) was conducted to determine the levels of confidence for the nodes within the inferred tree topologies. Tree bisection and reconnection (TBR) was selected as the branch swapping option. The tree length (TL), Consistency Index (CI), Retention Index (RI), Homoplasy Index (HI) and Rescaled Consistency Index (RC) were recorded for each analysed dataset after the trees were generated.

BI analyses using Markov Chain Monte Carlo (MCMC) methods were carried out with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Four MCMC chains were run in parallel for 10 million generations applying the best-fit model for each dataset. Trees were sampled every 100th generations, resulting in 100,000 trees. Tracer v1.4.1 (Rambaut and Drummond 2007) was utilized to determine the burn-in value for each dataset. The remaining trees were utilized to generate a 50% majority rule consensus tree, which allowed for calculating posterior probability values for the nodes.

Results

Collections of bark beetles and fungi

Altogether 22 bark beetle species were collected from Norway during this study (Table 3). Among the bark beetles carrying *Ceratocystiopsis* species only *P. chalcographus* occurred on both pine and spruce. Five bark beetle species with *Ceratocystiopsis* species were found on pine, while four bark beetle species were encountered only on spruce. The isolations from bark beetles yielded a total of 126 isolates resembling *Ceratocystiopsis* spp.

In total, 126 *Ceratocystiopsis* spp. isolates were obtained from 459 sampled beetles (Table 3). Fifty isolates were collected from *I. acuminatus*, 21 isolates were collected from *O. proximus*, 15 isolates were collected from *P. chalcographus*, and 12 isolates were collected from *C. cinereus*. The additional twenty-eight isolates were collected from *H. palliatus*, *P. saalasi*, *Pol. subopacus*, *C. pusillus*, *P. bidentatus*, and *P. quadridens* (Table 3). Based on morphological observations the fungal isolates obtained from this study could be arranged into six taxa. Fifty-five isolates represented taxon 6, were isolated from six different bark beetles. This taxon was the most frequently encountered on *P. bidentatus* (72%) and *O. proximus* (70%). The frequency of occurrence of this taxon on *C. cinereus* was also relatively high (40%). Fifty isolates belonged to taxon 2. This taxon was found only in high frequency on *I. acuminatus* (54%). The remaining 16 isolates represented four *Ceratocystiopsis* taxa and were collected from 1 or 2 different beetle species (Table 3). The frequency of occurrence of these species was less than 11% (Table 3).

Phylogenetic Analyses

Alignments for the ITS1-5.8S-ITS2 data set contained 738 characters; for the ITS2-28S data 496 characters; for the CAL data 613 characters; for the TEF1- α data 785 characters; for the β T data 526 characters for the ITS1-5.8S-ITS2-28S+ β T data 1839 characters (including gaps). The exon/intron arrangement of the CAL data included intron 3, exon 4, intron 4, exon 5 intron 5, exon 6 and intron 6. The exon/intron arrangement of the TEF1- α data included intron 3, exon 4, intron 4 and exon 5. The aligned β T gene region consisted of exons 3 to 6, interrupted by introns 3, 4 and 5.

DNA sequence data were obtained for 45 isolates representing all six morphological groups represented among the 126 *Ceratocystiopsis* resembling fungal isolates recovered from 459 sample beetle specimens (Table 2). BLAST analyses of the ribosomal DNA sequences placed the examined isolates in the genus *Ceratocystiopsis*. Sequences from our 45 representative strains when combined with those of other members of the genus *Ceratocystiopsis* formed six independent well-supported terminal clades representing six phylogenetic species (Taxa 1–6) in the ITS1-5.8S-ITS2, ITS2-28S, β T, CAL and TEF1- α phylogenetic inferences (Figs. 2-6). Based on the phylogenetic analyses of the ITS1-5.8S-ITS2 and ITS2-28S data sets (Figs. 2-3), the isolates emerged as two known and four undescribed taxa of the genus *Ceratocystiopsis*.

Four isolates recovered from *P. abies* and *P. sylvestris* infested by *P. chalcographus* (i.e., taxon 1) in the ITS1-5.8S-ITS2 and ITS2-28S trees grouped in the “*C. minuta*” clade C as defined by Plattner et al. (2009), this clade includes European and Japanese strains of *Ceratocystiopsis minuta* s.l. In the β T tree, eight isolates of taxon 1 recovered in this study clustered into a distinct monophyletic clade next to a *Ceratocystiopsis* strain (CMW4352) from Austria and Japanese isolates of *Ceratocystiopsis* spp. (YCC330 and YCC329) (Fig. 4). Although only poorly supported this clade appears to share a common node with strains assigned to *C. minuta* s. str. (including UM1532) and *C. weihaiensis* (Fig. 4).

Nine isolates obtained from *P. sylvestris* infested by *I. acuminatus* (i.e., taxon 2) in the ITS1-5.8S-ITS2, ITS2-28S, β T, CAL, and TEF1- α trees (Figs. 2-6) form a well-supported lineage, distinct from all the other known species of *Ceratocystiopsis*. Six isolates representing taxon 3 and taxon 4 also form a well-supported lineages distinct from other known species of *Ceratocystiopsis* (Figs. 2-6). Taxon 3 appears to represent a phylogenetically distinct lineage, potentially sharing ancestry with *C. neglecta* (Figs. 2-4), however the nodes supporting monophyly are only poorly supported. Members of taxon 4 form a lineage adjacent to *C. minima* within the ITS2-28S sequences based phylogenetic tree (Fig. 3), however the other dataset do not support monophyly for these two species (Figs. 2, 4 and 7).

The analyses of the combined ITS1-5.8S-ITS2-28S+ β T datasets clearly separated Taxa 1 to 4 from the other known species of *Ceratocystiopsis* and also from each other (Fig. 7). Nineteen isolates of taxon 5 grouped with the ex-type isolate of *C. rollhanseniana* (UM113) and other isolates of this species based on ITS2-28S (Fig. 3). The β T, CAL and TEF1- α data also confirmed that those isolates represented *C. rollhanseniana* (Figs. 4-6). The ITS1-5.8S-ITS2, ITS2-28S, β T, CAL and TEF1- α datasets confirmed that four isolates of taxon 6 grouped with ex-type isolate of *C. neglecta* (Figs. 2-6).

Taxonomy

The morphological characterization and phylogenetic comparisons based on five genetic loci, showed that four herein described morphotypes (taxon 1, 2, 3 and 4) are distinct from each other and from other known taxa in the genus *Ceratocystiopsis*. Therefore, they are described here as new species:

Ceratocystiopsis chalcographii Jankowiak & H. Solheim, **sp. nov.**

MycoBank: 841844

(Fig. 8)

Etymology: The epithet *chalcographii* (Latin), refers to the species name of the bark beetle vector of this fungus, *Pityogenes chalcographus*.

Diagnosis: This species is closely related to *C. minuta* and *C. norroenii*. *Ceratocystiopsis chalcographii* has longer perithecial necks (36–124 µm) compared to *C. minuta* (20–45 µm) (Reid and Hausner 2010). Their ostiolar hyphae is also longer (7.7–16.4 vs. to 12 µm) just as the length of the ascospores (9.9–15 vs. 10–13 µm) (Reid and Hausner 2010). *Ceratocystiopsis chalcographii* has obovoid conidia, whereas *C. minuta* has oblong conidia (Reid and Hausner 2010). *Ceratocystiopsis chalcographii* has longer conidia (2.9–6 µm) compared to *C. minuta* (2–4 µm) (Reid and Hausner 2010). In addition, conidiophores of *C. chalcographii* are simple or slightly branched while *C. minuta* produces simple or verticillate to irregular branched conidiophores (Reid and Hausner 2010). *Ceratocystiopsis norroenii* also produces branched conidiophores, however this taxon could be distinguished from *C. minuta* by oblong and larger conidia (2.7–5 × 1.1–2.3 vs. 2–4 × 1.2 µm). In addition, *C. norroenii* does not produce a sexual morph.

Type: **Norway**, Nannestad, from *Pityogenes chalcographus* infesting *Picea abies*, 15 Oct. 2014, H. Solheim and M.E. Waalberg (O-F-259424 – holotype; CBS 147954 = CMW 57280 – ex-type culture).

Description: *Sexual morph* perithecial. *Perithecia* abundantly produced on media and sterilized spruce twigs, various in shape, bases black, globose or sub-prolate (46–)55–101(–125) µm diam. Necks black, straight or slightly curved, (36–)55–88(–124) µm long. Diameter of the necks (5.9–)8.2–13(–17) µm at the apex and (20.3–)23.9–32.3(39.7) µm at the base. *Ostiolar hyphae* present, hyaline, aseptate, straight, tapering extensively towards the apex, 5–8 in number, (7.7–)9.2–13(–16.4) µm long. Asci not observed. *Ascospores* one-celled, hyaline, including the sheath falcate in side view (9.9–)11.6–14(–15) × (1–)1.2–1.6(–1.8) µm, accumulated in a white-coloured mass at the tip of the neck.

Asexual morphs: hyalorhinocladia-like.

Hyalorhinocladia-like morph: *conidiophores* mononematous, micronematous or semi-macronematous, arising from vegetative hyphae, simple or slightly branched, upright, straight or undulate, producing conidiogenous cells at their apices, (1.7–)6.9–23.4(–40.4) × (0.7–)1–1.8(–2.2) µm. *Conidiogenous cells* integrated, hyaline, blastic, not denticulate. *Conidia* hyaline, aseptate, obovoid with the upper part swollen, apex round, tapering toward base, sometimes pyriform, no sheath, (2.9–)3.6–5(–6) × (0.9–)0.9–1.2(–1.9) µm, no sheath.

Cultures: *Colonies* with optimal growth at 30°C on 2% MEA with radial growth rate 1.11 (± 0.05) mm/d. Colonies white, margin smooth, flocculose, with white lumps of slimy conidia. Hyphae hyaline to pale grey in colour, reverse pale orange (Kornerup and Wanscher 1978), smooth, submerged in the medium and aerial mycelium abundant, not constricted at the septa, 1–2.6 (mean 1.7±0.4) µm diam., asexual morph very abundant.

Ecology: Isolated from *Pityogenes chalcographus* infesting *Picea abies* and *Pinus sylvestris*.

Habitat: *Picea abies* and *Pinus sylvestris* forests.

Distribution: Currently only known from Norway.

Notes: *Ceratocystiopsis chalcographii* and *C. norroenii* formed two distinct, well-supported clades within the *C. minuta* group, in which they were closely related to *C. minuta* (Figs. 2-4). They can be both differentiated from *C. minuta* by the morphology of sexual morph as well as morphology of the conidia and conidiophores.

Ceratocystiopsis chalcographii was rarely isolated from bark beetles infesting *P. abies* and *P. sylvestris* (Table 3). It was found only in association with *P. chalcographus* on Scots pine in Rendalen and on Norway spruce in Nannestad. This species is one of two *Ceratocystiopsis* species isolated from *P. chalcographus* (Taxa 1 and 3).

Additional specimen examined: **Norway:** Rendalen, from *Pityogenes chalcographus* infesting *Pinus sylvestris*, 29 Oct. 2014, H. Solheim, (O-F-259425, culture CBS 147955 = CMW 57282).

Ceratocystiopsis norroenii Jankowiak & H. Solheim, **sp. nov.**

MycoBank: 841845

(Fig. 9)

Etymology: The epithet *norroenii* (Latin), refers to the word Norrøn, which in Norse mythology means from north, 49 out of 50 specimens collected during this study are from the northernmost part of Norway, Troms and Finnmark.

Diagnosis: See comparisons between *C. norroenii* and *C. minuta* under *C. chalcographii*.

Type: **Norway**, Karasjok, from *Ips acuminatus* infesting *Pinus sylvestris*, 21 Jun. 2015, H. Solheim (O-F-259426 – holotype; CBS 147956 = CMW 57283 – ex-type culture).

Description: *Sexual morph:* not observed.

Asexual morphs: hyalorhinocladia-like.

Hyalorhinocladia-like morph: *conidiophores* micronematous or macronematous. The micronematous conidiophores, arising from vegetative hyphae, simple or branched (1 branches), hyaline, upright, straight or undulate, producing conidiogenous cells at their apices $(1.4\text{--})12.1\text{--}28.1\text{--}(33.3) \times (0.9\text{--})1.2\text{--}1.7\text{--}(2.1) \mu\text{m}$, often conidia formed singly, either directly on the side of vegetative hyphae or on short lateral branches. The macronematous conidiophores, hyaline, $(9.3\text{--})29.6\text{--}48.2\text{--}(64.9)$ long, and $(8.5\text{--})11.2\text{--}19.4\text{--}(15.8) \mu\text{m}$ wide at the apex, arising from a basal cell, $(4.3\text{--})6.2\text{--}11.7\text{--}(16) \times (1.4\text{--})1.7\text{--}2.6\text{--}(3.1) \mu\text{m}$. The basal cells branch irregularly or penicillate and form 1-2 branches producing conidiogenous cells at their apices, 3–4 per branch. *Conidiogenous cells* integrated, hyaline, blastic, sometimes denticulate. *Conidia* hyaline, smooth, unicellular, oblong, sometimes slightly obovoid $(2.7\text{--})3.2\text{--}4.1\text{--}(5.1) \times (1.1\text{--})1.2\text{--}1.6\text{--}(2.3) \mu\text{m}$, no sheath.

Cultures: Colonies with optimal growth at 25°C on 2% MEA with radial growth rate $1.11 (\pm 0.15)$ mm/d. Colonies orange white, margin smooth, with suede-like surface, but occasionally funiculose. Hyphae hyaline, reverse pale orange (Kornerup and Wanscher 1978), smooth, submerged in the medium and aerial mycelium rarely, not constricted at the septa, $0.5\text{--}1.8$ (mean 1 ± 0.3) μm diam., asexual morph very abundant.

Ecology: Isolated from *Ips acuminatus* and *Pityogenes quadridens* infesting *Pinus sylvestris*.

Habitat: *Pinus sylvestris* forests.

Distribution: Currently only known from Norway.

Notes: See comparisons between *C. norroenii* and *C. minuta* under *C. chalcographii*. *Ceratocystiopsis norroenii* was commonly isolated from *Ips acuminatus* and *Pityogenes quadridens* infesting Scots pine growing in the north part of Norway.

Additional specimen examined: **Norway:** Målselv, from *Ips acuminatus* infesting *Pinus sylvestris*, 21 Jun. 2015, H. Solheim (O-F-259427, culture CBS 147957 = CMW 57284).

Ceratocystiopsis debeeria Jankowiak & H. Solheim, **sp. nov.**

MycoBank: 841846

(Fig. 10).

Etymology: Named for Prof. Wilhelm de Beer who has contributed greatly to the fungal taxonomy of ophiostomatoid species.

Diagnosis: *Ceratocystiopsis debeeria* is the closest phylogenetic relative to *C. neglecta*. It can be differentiated from *C. neglecta* by the absence of perithecia and producing simple or very slightly irregularly branched conidiophores. *Ceratocystiopsis neglecta* produces simple or strongly penicillately branched conidiophores (Kirschner and Oberwinkler 1999). Morphologically, these two species also differ by the size of their conidia ranging from $3\text{--}4.5 \times 1\text{--}1.5 \mu\text{m}$ in *C. neglecta* (Kirschner and Oberwinkler 1999) vs. $2.7\text{--}5.2 \times 0.9\text{--}1.7 \mu\text{m}$ in *C. debeeria*.

Type: **Norway**, Flesberg, from *Pityogenes chalcographus* infesting *Picea abies*, 31. Oct. 2014, M.E. Waalberg and R.A. Lindseth (O-F-259428 – holotype; CBS 147958 = CMW 57288 – ex-type culture).

Description: *Sexual morph:* not observed.

Asexual morphs: hyalorhinocladia-like.

Hyalorhinocladia-like morph: *conidiophores* mononematous, micronematous, arising from vegetative hyphae, simple or slightly and irregularly branched, upright, straight or undulate, producing conidiogenous cells at their apices, $(7.8\text{--})10.6\text{--}25.8\text{--}(37.9) \times (0.8\text{--})1\text{--}1.4\text{--}(1.8) \mu\text{m}$. *Conidiogenous cells* integrated, hyaline, blastic, rarely denticulate. *Conidia* hyaline, aseptate, obovoid with the upper part swollen, apex round, tapering toward base, sometimes oblong, no sheath, $(2.7\text{--})3.1\text{--}4.2\text{--}(5.2) \times (0.9\text{--})1.1\text{--}1.4\text{--}(1.7) \mu\text{m}$.

Cultures: Colonies with optimal growth at 25°C on 2% MEA with radial growth rate $0.82 (\pm 0.18)$ mm/d. Colonies yellowish white, margin smooth, with suede-like surface. Hyphae hyaline to pale grey in colour, reverse pale orange (Kornerup and Wanscher 1978), smooth, submerged in the medium and aerial mycelium rarely, not constricted at the septa, $0.8\text{--}2.9$ (mean 1.6 ± 0.4) μm diam., asexual morph very abundant.

Ecology: Isolated from *Pityogenes chalcographus* infesting *Picea abies* and *Pinus sylvestris*.

Habitat: *Picea abies* and *Pinus sylvestris* forests.

Distribution: Currently only known from Norway.

Notes: In a phylogenetic perspective, *C. debeeria* is closely related to *C. neglecta* (Figs. 2-4). *Ceratocystiopsis debeeria* is characterized by the absence of perithecia, which are present in *C. neglecta* (Kirschner and Oberwinkler 1999). In addition, *C. debeeria* can be distinguished from *C. neglecta* by the size of

conidia.

Ceratocystiopsis debeeria was only isolated on a few occasions from *Pityogenes chalcographus* infesting *Picea abies* in Rendalen and *Pinus sylvestris* in Flesberg.

Additional specimen examined: Norway, Rendalen, from *Pityogenes chalcographus* infesting *Pinus sylvestris*, 29 Oct. 2014, H. Solheim (O-F-259429, culture CBS 147959 = CMW57290).

Ceratocystiopsis troendelagii Jankowiak & H. Solheim, **sp. nov.**

MycoBank: 841847

(Fig. 11)

Etymology: The epithet *troendelagii* (Latin) refers to the area in mid-Norway, Trøndelag, from which this fungus was collected.

Type: Norway, Lierne, from *Polygraphus subopacus* infesting *Picea abies*, 8 Aug. 2015, T. Kvamme and Å. Lindelöv (O-F-259430 – holotype; CBS 147962 = CMW57294 ex-type culture).

Diagnosis: The species is characterized by perithecia and a hyalorhinocla-diella-like morph. It can be differentiated from the closely related species *C. minima* by smaller perithecial base diameter 25–54 µm vs. 40–85 µm (Olchowecki and Reid 1974). Also *C. troendelagii* has shorter conidia (2.7–5.2 µm) compared to *C. minima* (2.5–8.0 µm) (Olchowecki and Reid 1974). In addition, the conidia of *C. minima* are clavate or oblong while *C. troendelagii* has mainly obovoid conidia.

Description: Sexual morph perithecial. *Perithecia* abundantly produced on media and sterilized spruce twigs, bases black, globose or sub-globose (25–)33–45(–54) µm diam. Necks black, straight or slightly curved, variable in length, (9–)11–29(–72) µm long. Diameter of the necks (4–)4.7–7.7(–10.7) µm at the apex and (12.5–)14.9–20.1(24.6) µm at the base. *Ostiolar hyphae* present, hyaline, aseptate, straight, tapering extensively towards the apex, 3–6 in number, (3.9–)7.2–10.5(–11.8) µm long. Asci not observed. *Ascospores* one-celled, hyaline, including the sheath falcate in side view (7–)8.8–11.8(–13.5) × (0.7–)0.8–1.1(–1.3) µm, accumulated in a white-coloured mass at the tip of the neck.

Asexual morphs: hyalorhinocla-diella-like.

Hyalorhinocla-diella-like morph: conidiophores micronematous or macronematous. The micronematous conidiophores, arising from vegetative hyphae, simple, hyaline, upright, straight or undulate, producing conidiogenous cells at their apices (5.9–)8.7–20.4(–30.4) × (0.8–)1.3–2.2(–2.7) µm. The macronematous conidiophores, hyaline, (17.8–)21.9–34.8(–41.2) long, and (4.4–)11.7–28.8(–37.7) µm wide at the apex, arising from a basal cell, (3.5–)5.1–10.9(–16.5) × (1.7–)2.2–3.3(–4.1) µm. The basal cells branch irregularly and form 2–3 branches producing conidiogenous cells at their apices, 2–4 per branch. *Conidiogenous cells* integrated, hyaline, blastic, sometimes denticulate. *Conidia* hyaline, smooth, unicellular, oblong or obovate (2–)2.6–3.5(–4.1) × (0.8–)1.1–1.8(–2.2) µm, no sheath.

Cultures: Colonies with optimal growth at 20°C on 2% MEA with radial growth rate 0.72 (± 0.05) mm/d. Colonies yellowish white, margin smooth, with suede-like surface, but occasionally funiculose. Hyphae hyaline, reverse pale orange (Kornerup and Wanscher 1978), smooth, submerged in the medium and aerial mycelium rarely, not constricted at the septa, 0.7–2.2 (mean 1.2±0.4) µm diam., asexual morph very abundant.

Ecology: Isolated from *Pityogenes saalasi* and *Polygraphus subopacus* infesting *Picea abies* and *Pinus sylvestris*

Habitat: *Picea abies* and *Pinus sylvestris* forests

Distribution: Currently only known from Norway.

Notes: *Ceratocystiopsis troendelagii* is phylogenetically close to *C. minima* but formed a distinct clade in both the ITS1-5.8S-ITS2 and ITS2-28S trees as well in the βT based tree (Figs. 2–4). It can be differentiated from *C. minima* by dimension of the perithecial base as well as morphology of conidia.

Ceratocystiopsis troendelagii was the rarest *Ceratocystiopsis* species isolated in this study. It was isolated twice in the same plot, once from *Pityogenes saalasi* and once from *Polygraphus subopacus*. Both bark beetles seem to have a northern distribution in Fennoscandia.

Additional specimen examined: Norway, Lierne, from *Pityogenes saalasi* infesting *Picea abies*, 8 Aug. 2015, T. Kvamme and Å. Lindelöv (O-F-259431, culture: CBS 147963 = CMW57295).

Discussion

In the present study, we collected 22 species of bark beetles from *Picea abies* and *Pinus sylvestris* located in Norway. From these beetles, we recovered 126 isolates that based on morphology showed affinities towards the genus *Ceratocystiopsis*. Analyses of molecular and morphological data indicated that four out of the six *Ceratocystiopsis* species recovered in this study were previously undescribed. Herein, we described these new species as *C. chalcographii*, *C. debeeria*, *C. norroenii* and *C. troendelagii*. Description of these new species brings the total number of recognized species in this genus to 24, of which seven occur in Norway. These include the six species recovered in this study as well as *C. minuta*, which was previously found in association with *I. typographus*

(Solheim 1986, 1992, 1993). In addition, *C. rollhanseniana* was described by Hausner et al. (2003) based on the Norwegian isolates collected by James Reid from *P. sylvestris*.

Ceratocystiopsis norroenii and *C. rollhanseniana* were the most frequently isolated *Ceratocystiopsis* species in this study. However, these species appear to have different distribution and insect associations. *Ceratocystiopsis norroenii* appears to be a consistent associate of *Ips acuminatus* on *P. sylvestris* while *C. rollhanseniana* has broader host/insect associations including different bark beetle species found on *P. abies* and *P. sylvestris*. Our phylogenetic analysis of the ITS2-28S sequences (Fig. 3) showed that some Polish isolates previously identified as *C. minuta* and collected from *P. abies* infested by *I. typographus* (CBS 116963, CBS 116796) actually represented *C. rollhanseniana*, showing its wider distribution in Europe.

Ceratocystiopsis norroenii belongs to the *C. minuta* s.l. grouping that includes several lineages from Europe and Japan (Plattner et al. 2009). According to Plattner et al. (2009) *C. minuta* contains different phylogenetic species, which are well-defined based on molecular and to some degree morphological criteria. Therefore, to stabilize the taxonomic position of *C. minuta* an epitype was designated for *C. minuta* based on the Polish strain RJ705=UAMH 11218 = WIN(M) 1532 by Reid and Hausner (2010). The genetic lineage of *C. norroenii* was not represented in Plattner et al.'s study (2009) suggesting that there are many *C. minuta*-like species that are morphologically similar. For example, *C. weihaiensis* a species from China was shown to be phylogenetically close to *C. minuta* (Chang et al. 2021).

In this study, besides *C. norroenii* we recovered eight other isolates that are part of the *C. minuta* s.l. species complex. These isolates were recovered from *P. chalcographus* on *P. abies* and represented a new species designated as *C. chalcographii*. The ITS1-5.8S-ITS2, ITS2-28S and β T sequences of this taxon were identical or very close to several isolates from Scotland, Austria and Japan, which were identified previously as *C. minuta*. Therefore, *C. chalcographii* probably has a wider geographical distribution in Europe and Asia. More studies are needed on the taxonomic/genetic diversity of the *C. minuta* s.l. complex to resolve the various cryptic species that might be part of this group. In this study, *P. chalcographus* was also associated with *C. debeeria*. However, this fungus was found only in association with this beetle species, suggesting that *C. debeeria* is a specific ectosymbiont of *P. chalcographii*.

Ceratocystiopsis troendelagii and *Ceratocystiopsis neglecta* were found only on a few occasions in association with conifer-infesting bark beetles in Norway. *Ceratocystiopsis troendelagii* seems to be a fungal associate of *P. saalasi* and *P. subopacus* while *C. neglecta* was found associated with *H. palliatus* and *P. subopacus*. *Ceratocystiopsis neglecta* so far was only known from studies on different bark- and ambrosia beetles infesting coniferous trees in Germany (Kirschner 2001; Kirschner and Oberwinkler 1999). This is the first report of *C. neglecta* as a fungal associate of *P. subopacus*.

All of the species described in this study are morphologically similar, having a hyalorhinocladia-like asexual states with hyaline conidia produced on non-denticulate conidiogenous cells. *Ceratocystiopsis norroenii* and *C. troendelagii* formed only simple conidiophores while *C. chalcographii* and *C. troendelagii* formed simple as well as conidiophores with irregular branching patterns resembling loose structures. Where ascomata were present, these tended to have globose or elongated bases with short necks terminating in ostiolar hyphae and falcate ascospores surrounded by hyaline sheaths. The newly described species demonstrated different optimal growth temperatures on 2% MEA. *Ceratocystiopsis norroenii* and *C. debeeria* grew optimally at 25 °C, *C. chalcographii* at 30 °C, and *C. troendelagii* at 20 °C. The four species described in this study can easily be distinguished from each other and from the other species of *Ceratocystiopsis* based on DNA sequence comparisons. Analyses of the ITS region sequence data were already sufficient to distinguish these species.

Most of the formally described species of *Ceratocystiopsis* are known only from conifers although *C. lunata* and *C. synnemata* were recently collected from hardwood-infesting beetles (Li et al. 2018; Strzalka et al. 2020; Nel 2021). The results of the present study confirm that in general there is a close affinity of *Ceratocystiopsis* species to conifers.

In this study, six species of *Ceratocystiopsis* were isolated from 22 species of bark beetles. Based on morphological characters and DNA sequence data, four new species of *Ceratocystiopsis* were described in addition this is the first report of *C. neglecta* for Norway. Although the relationships between fungi and bark beetles are relatively well recognized in Europe our survey led to the discovery and description of new species, suggesting that there are more novel fungal taxa to be discovered. The results of this study have substantially expanded our knowledge of *Ceratocystiopsis* species living in northern parts of Europe. Broadly, the results suggest that *Ceratocystiopsis* species are frequently occurring members of the *Ophiostomatales* in conifer ecosystems in Norway.

Abbreviations

BI: Bayesian Inference; β T: β -tubulin; CAL: Calmodulin; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CMW: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; ITS: The internal transcribed spacer; LSU: The large ribosomal subunit (28S); MEA: Malt extract agar; ML: Maximum likelihood; MP: Maximum Parsimony; NCBI: National Center for Biotechnology Information; NIBIO: Culture collection of Norwegian Institute of Bioeconomy, Norway; O: the Mycological Herbarium, Natural History Museum, University of Oslo, Norway; s.l.: *sensu lato*; s.str.: *sensu stricto*; TEF1- α : Translation elongation factor 1- α ; UAMH: The UAMH centre for global microfungi biodiversity, University of Toronto, Canada; WIN(M or UM): Culture Collection of the University of Manitoba (Winnipeg); Canada.

Declarations

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Authors' contribution

HS conceived the idea of this study, performed isolations and DNA sequencing, and edited the final version of the manuscript. RJ performed DNA sequencing, performed microscopy for the species descriptions and edited the description text, and wrote the manuscript. PB performed phylogenetic analysis. JM performed DNA sequencing, GH performed DNA sequencing and edited the final version of the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and/or is available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Bark beetle sampling sites

Picea abies (Pa), *Pinus sylvestris* (Ps)

Table 2 Isolates used in the present study

Sampling site	Latitude	Longitude	Municipality	County	Bark beetle species	Host
1	69.818212	25.157902	Porsanger	Finnmark	<i>P. quadridens</i>	<i>Ps</i>
2	69.551479	25.831215	Karasjok 4	Finnmark	<i>I. acuminatus</i> / <i>P. bidentatus</i>	<i>Ps</i>
3	69.465227	25.568562	Karasjok 6	Finnmark	<i>P. quadridens</i> / <i>P. bidentatus</i>	<i>Ps</i>
4	69.060888	18.995351	Målselv 5	Troms	<i>I. acuminatus</i>	<i>Ps</i>
5	69.001709	18.995351	Målselv 6	Troms	<i>I. acuminatus</i>	<i>Ps</i>
6	64.403248	13.548321	Lierne	Trøndelag	<i>P. saalasi</i> / <i>P. subopacus</i>	<i>Pa</i>
7	61.728590	11.344890	Rendalen	Innlandet	<i>P. chalcographus</i>	<i>Ps</i>
8	60.373333	12.461444	Grue	Innlandet	<i>P. quadridens</i>	<i>Ps</i>
9	60.237895	10.986056	Nannestad	Viken	<i>P. poligraphus</i> / <i>P. chalcographus</i>	<i>Pa</i>
10	59.668680	10.718710	Ås	Viken	<i>H. palliatus</i>	<i>Pa</i>
11	59.639736	10.654259	Frogn1	Viken	<i>C. pusillus</i> / <i>O. proximus</i>	<i>Ps</i>
12	59.634348	10.668942	Frogn 2	Viken	<i>C. cinereus</i> / <i>O. proximus</i>	<i>Ps</i>
13	59.743666	9.564444	Flesberg	Viken	<i>P. chalcographus</i>	<i>Ps</i>

Fungal species	Isolate no. ¹			Tree host	Insect vector	Site ²	GenBank accession	
	CBS	CMW	NIBO or WIN ^C				ITS	LSU
<i>Ceratocystiopsis chalcographii</i> sp. nov.	147954 ^T	57280	N2015-0121/2	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Nannestad	OL309930	OL30
Taxon 1		57281	N2015-0132/2/3	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Nannestad	OL309931	OL30
	147953	57279	N2015-0158/3/3	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	OL309932	OL30
	147955 ^A	57282	N2015-0167/2/1	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	OL309933	OL30
			N2015-0123/3/3	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Nannestad	-	-
			N2015-0134/2	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Nannestad	-	-
			N2015-0171/1/4	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	-	-
			N2015-0174/2/1	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	-	-
<i>Ceratocystiopsis norroenii</i> sp. nov.		57285	N2015-0494/1/2	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 5	OL309934	OL30
Taxon 2		57287	N2015-0528/2/3	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 6	OL309935	OL30
	147956 ^T	57283	N2015-0537/2/3	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Karasjok 6	OL309936	OL30
		57286	N2015-0545/2/4	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Karasjok 6	OL309937	OL30
	147957 ^A	57284	N2015-0526/2/3	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 6	OL309938	OL30
			N2015-0032/2/2	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Frogn 2	-	-
			N2015-0503/1/4/1	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 5	-	-
			N2015-0523/2/3	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 6	-	-
			N2015-0842/2/5	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Porsanger	-	-
<i>Ceratocystiopsis debeeria</i> sp. nov.	147959 ^A	57290	N2015-0165/1/3/1	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	OL309939	OL30
Taxon 3		57289	N2015-0176/3/3/1	<i>Pinus sylvestris</i>	<i>Pityogenes chalcographus</i>	Rendalen	OL309940	OL30
		57291	N2015-0177/1/2	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Flesberg	OL309941	OL30
	147958 ^T	57288	N2015-0179/5/2	<i>Picea abies</i>	<i>Pityogenes chalcographus</i>	Flesberg	OL309942	OL30
<i>Ceratocystiopsis troendelagii</i> sp. nov.	147962 ^T	57294	N2015-0879/2/5	<i>Picea abies</i>	<i>Polygraphus subopacus</i>	Lierne	OL309943	OL30
Taxon 4	147963 ^A	57295	N2015-0918/2/4	<i>Picea abies</i>	<i>Pityogenes saalasi</i>	Lierne	OL309944	OL30
<i>Ceratocystiopsis neglecta</i>	147960	57292	N2015-0445/3/4	<i>Picea abies</i>	<i>Hylurgops palliatus</i>	Ås	OL309945	OL30
Taxon 5			N2015-0888/3/1	<i>Picea abies</i>	<i>Polygraphus subopacus</i>	Lierne	OL309946	OL30
	147961	57293	N2015-0438/1/3	<i>Picea abies</i>	<i>Hylurgops palliatus</i>	Ås	-	-
			N2015-0439/1/3	<i>Picea abies</i>	<i>Hylurgops palliatus</i>	Ås	-	OL30
	100596 ^T			<i>Picea abies</i>	<i>Hylurgops palliatus</i>	Baden-Württemberg	MH862711	MH8

<i>Ceratocystiopsis rollhanseniana</i>	147950	57276	N2015-0837/2/2	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Porsanger	-	OL30
Taxon 6	147952	57278	N2015-0850/3/1	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	OL30
	147951	57277	N2015-0946/3/2	<i>Pinus sylvestris</i>	<i>Orthotomicus proximus</i>	Frogn 1	-	OL30
			N2015-0946/3/1	<i>Pinus sylvestris</i>	<i>Orthotomicus proximus</i>	Frogn 1	-	OL30
			N2015-1162/2/2/1	<i>Pinus sylvestris</i>	<i>Crypturgus pusillus coll.</i>	Frogn 1	-	OL30
			N2015-1169/1/2/1	<i>Pinus sylvestris</i>	<i>Crypturgus pusillus coll.</i>	Frogn 1	-	OL30
			N2015-0003/2/3	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Grue	-	-
			N2015-0859/3/2	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	-
			N2016-0836/3/4	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Porsanger	-	-
			N2015-0847/3/2	<i>Pinus sylvestris</i>	<i>Pityogenes quadridens</i>	Karasjok 6	-	-
			N2015-0493/3/2	<i>Pinus sylvestris</i>	<i>Ips acuminatus</i>	Målselv 5	-	-
			N2015-0846/2/4	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 6	-	-
			N2015-0849/1/1	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 6	-	-
			N2015-0853/1/1	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	-
			N2015-0855/3/2	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	-
			N2015-0857/2/1	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	-
			N2015-0857/3/1	<i>Pinus sylvestris</i>	<i>Pityogenes bidentatus</i>	Karasjok 4	-	-
			N2015-0938/2/3	<i>Pinus sylvestris</i>	<i>Orthotomicus proximus</i>	Frogn 1	-	-
			N2015-0947/1/2	<i>Pinus sylvestris</i>	<i>Orthotomicus proximus</i>	Frogn 1	-	OL30
			WIN110,UAMH9797	<i>Pinus sylvestris</i>	Bark beetle galleries	Kongsvinger	EU913719	EU91
			WIN113,UAMH9774 ^T	<i>Pinus sylvestris</i>	Bark beetle galleries	Tangen	EU913718	EU91
			WIN117	<i>Pinus sylvestris</i>	Bark beetle galleries	Kongsvinger	-	-

¹CBS Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CMW Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; NIBO (N) Culture collection of Norwegian Institute of Bioeconomy (NIBIO), Norway; UAMH: The UAMH centre for global microfungi biodiversity, University of Toronto, Toronto, Canada; WIN Culture Collection of the University of Manitoba (Winnipeg), Canada.

²All isolates were collected in Norway except *C. neglecta* CBS 100596 which was collected in Germany.

³ITS = internal transcribed spacer regions 1 and 2 of the nuclear ribosomal DNA operon, including the 5.8S region; LSU = internal transcribed spacer region 2 and the 28S large subunit of the nrDNA gene; β T = Beta-tubulin; CAL = Calmodulin; TEF1 α = Translation elongation factor 1-alpha; **Bold** type = GenBank accession numbers of sequences obtained in the present study.

T = ex-holotype isolates

A = additional specimen examined

Table 3 Number and frequencies (%)* of *Ceratocystiopsis* isolates and frequency of occurrence obtained from 22 bark beetle species during the course of this study.

		<i>Picea abies</i> (Pa)											
		<i>Pinus sylvestris</i> (Ps)											
Beetle species		1	2	3	4	5	6	7	8	9	10	11**	Total
Fungus species													
<i>C. chalcographii</i>	taxon 1					8(11%)							8
<i>C. norrenii</i>	taxon 2							49(54%)			1(11%)		50
<i>C. deberia</i>	taxon 3					7(9%)							7
<i>C. trendelagii</i>	taxon 4			1(3%)	1(3%)								2
<i>C. neglecta</i>	taxon 5		3(3%)		1(3%)								4
<i>C. rollhanseniana</i>	taxon 6	12(40%)					6(29%)	1(11%)	21(70%)	13(72%)	2(22%)		55

*The frequency of occurrence was calculated according to the following formula: $F = (NS/NTs) \times 100$, where F represents the frequency of occurrence (%) of the fungus, NS represents the number of bbtles from which a particular fungus was isolated, and NTs represents the total number of beetles.

Bark beetle species: 1 = *Crypturgus cinereus*; 2 = *Hylurgops palliatus*; 3 = *Pityogenes saalas*; 4 = *Polygraphus subopacus*; 5 = *Pityogenes chalcographus*; 6 = *Crypturgus pusillus*; 7 = *Ips acuminatus*; 8 = *Orthotomicus proximus*; 9 = *Pityogenes bidentatus*; 10 = *Pityogenes quadridens*.

** No *Ceratocystiopsis* species were detected in samples from *Cryphalus saltuarius*; *Dryocoetes autographus*; *Dryocoetes hectographus*; *Hylastes cunicularis*, *Hylastes brunneus*; *Ips sexdentatus*; *Orthotomicus laricis*; *Orthotomicus suturalis*; *Pityophthorus micrographus*; *Polygreaphus poligraphus*; *Tomicus piniperda*; *Trypodendrus lineatum*.

Figures

Figure 1

The sample sites in Norway where isolations from bark beetles yielded *Ceratocystiopsis* species.

Figure 2

Phylogram obtained from Maximum Likelihood (ML) analyses of the ITS1-5.8S-ITS2-28S data for the *Ceratocystopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values $< 75\%$. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *Ophiostoma quercus* represent the outgroup.

Figure 3

Phylogram obtained from Maximum Likelihood (ML) analyses of the ITS2-28S data for the *Ceratocystopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold

branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *O. quercus* represents the outgroup in analyses of ITS2-28S.

Figure 4

Phylogram obtained from Maximum Likelihood (ML) analyses of βT data for the *Ceratocystopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *O. quercus* represents the outgroup in analyses of βT .

Figure 5

Phylogram obtained from Maximum Likelihood (ML) analyses of TEF1- α data for the *Ceratocystiopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values $<75\%$. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *O. quercus* represents the outgroup in analyses of TEF1- α .

Figure 6

Phylogram obtained from Maximum Likelihood (ML) analyses of CAL data for the *Ceratocystiopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values $<75\%$. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *O. quercus* represents the outgroup in analyses of CAL.

Figure 7

Phylogram obtained from Maximum Likelihood (ML) analyses of the combined datasets of TS1-5.8S-ITS2-28S+ β T data for the *Ceratocystiopsis* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values $\geq 75\%$ for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. *Bootstrap values $< 75\%$. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Ophiostoma karelicum* and *O. quercus* represents the outgroup in analyses of the combined datasets of ITS1-5.8S-ITS2-28S+ β T.

Figure 8

Morphological characteristics of *Ceratocystiopsis chalcographii* sp. nov. (CBS 147954, Taxon 1). a-b. Perithecium; c. Ostiolar hyphae; d. Ascospores; e-f. Micronematous conidiophores; g. Slightly branched micronematous conidiophores; h. Conidia; i. Fourteen-day-old culture on MEA. Scale bars: a = 25 μm ; b = 50 μm ; c = 25 μm ; d = 10 μm ; e = 25 μm ; f-g = 10 μm .

Figure 9

Morphological characteristics of *Ceratocystiopsis norroenii* sp. nov. (CBS 147956, Taxon 2). a-c. Simple (a, b) and slightly (c) branched micronematous conidiophores; d-e. Conidia arising directly from hyphae; f-g. Branching macronematous conidiophores; h. conidia; i. Fourteen-day-old culture on MEA. Scale bars: a-d = 10 μ m, e = 25 μ m, f-h = 10 μ m.

Figure 10

Morphological characteristics of *Ceratocystiopsis debeeria* sp. nov. (CBS 147958, Taxon 3). a-d. Simple (a, b) and slightly (c, d) branched micronematous conidiophores; e. Conidia; f. Fourteen-day-old culture on MEA. Scale bars: a-e = 10 μ m.

Figure 11

Morphological characteristics of *Ceratocystiopsis troendelagii* sp. nov. (CBS 147962, Taxon 4). a-b. Perithecium; c. Ostiolar hyphae; d. Ascospores; e. Micronematous conidiophores; f-g. Branching macronematous conidiophores; h. Conidia; i. Fourteen-day-old culture on MEA. Scale bars: a-b = 25 μm ; c-f = 10 μm ; g = 25 μm ; h = 10 μm .