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Ophiostoma denticiliatum sp. nov. and other *Ophiostoma* species associated with the birch bark beetle in southern Norway

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Key words

Betula
Ophiostoma
Scolytus
symbiosis

Abstract Ophiostomatoid fungi were isolated from *Scolytus ratzeburgi* infesting *Betula pendula* and *B. pubescens* in Norway. Fungi were identified based on morphology, DNA sequence comparison for two gene regions and phylogenetic analyses. The most abundant fungus was *Ophiostoma karelicum*, suggesting a specific relationship between the fungus, the vector insect and the host tree. Our results suggest that *O. karelicum* occurs across the geographic range of *S. ratzeburgi* and its close relatedness to the Dutch elm disease fungi suggests that it could be important if introduced into other parts of the world. Other fungi, only occasionally isolated from *S. ratzeburgi*, were identified as *O. quercus* and a novel taxon, described here as *O. denticiliatum* sp. nov.

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INTRODUCTION

The birch bark beetle, *Scolytus ratzeburgi* (*Coleoptera*: *Curculionidae*, *Scolytinae*), is the only *Scolytus* species known to infest birch. It is a very common bark beetle found on silver birch (*Betula pendula*) and downy birch (*Betula pubescens*) in Scandinavia and other parts of Europe (Postner 1974). *Scolytus ratzeburgi* typically infests weakened or dying trees, but can also attack apparently healthy trees (Saalas 1949). In a recent study, the occurrence of ophiostomatoid fungi in association with *S. ratzeburgi* was reported from the Karelia region of Finland and Russia (Linnakoski et al. 2008). A new *Ophiostoma* species, *O. karelicum*, together with *O. quercus* and a third taxon close to *O. catonianum,* were identified. *Ophiostoma karelicum* was the dominant fungus and consistently isolated from every beetle and gallery collected.

The pathogenicity of *O. karelicum* to birch and its role in the ecology of *S. ratzeburgi* remains unclear. The fact that the fungus is closely related to the Dutch elm disease fungi, *Ophiostoma ulmi*, *O. novo-ulmi* subsp. *novo-ulmi* and subsp. *americana* (Burges et al. 1979, Hubbes 1999, Brasier & Kirk 2001), is a matter of concern (Linnakoski et al. 2008). Another unanswered question is whether *O. karelicum* is also associated with the beetle in other geographical areas where this insect occurs. The aim of this study was thus to collect and identify ophiostomatoid fungi associated with *S. ratzeburgi* infesting birch in Norway.

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MATERIALS AND METHODS

Collection of bark beetles

Sampling was conducted during the flight period (5–7 July) of *Scolytus ratzeburgi* during the summer of 2007 in Akerhus and Østfold counties, Norway. Adult bark beetles and their galleries were collected from piles of birch logs at three different sites. These included a sawmill yard in Spydeberg (Svenneby), and in forests at Vestby (near Haug, Garder) and Hobøl (near Kattås). At the time of sampling, adult beetles were found on birch trunk surfaces and under the bark where gallery construction was active. A total of 34 adults and 33 galleries were collected and stored individually at 5 °C until further study.

Fungi were isolated from the bark beetles as well as their galleries. Methods of isolation were the same as those described by Linnakoski et al. (2008). Representative isolates were deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands and the CMW-collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Isolates of the new taxon are also maintained in the VTT Culture Collection, VTT Technical Research Centre of Finland, Espoo with herbarium specimens deposited in Kuopio Museum of Natural History (KUO), Kuopio, Finland.

DNA extraction and PCR

Fungal isolates were grown on malt extract agar (MEA; 20 g Difco Bacto™ malt extract from Becton, Dickinson & Company, Sparks, USA, 20 g Difco Bacto™ agar and 1 L Milli-Q water). DNA was extracted using PrepMan Ultra Sample preparation reagent (Applied Biosystems, Foster City, CA, USA) as described by Linnakoski et al. (2008). For the ribosomal DNA operon, the internal transcribed spacers (ITS) 1 and 2, including the 5.8S gene, were amplified using primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The β-tubulin gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). Primer Bt2b was replaced in some cases with primer T10 (O'Donnel & Cigelnik 1997).

Gene fragments were amplified in 25 µL reaction mixture containing 0.25 µL of Super-Therm DNA Polymerase mixture (250 U) (Hoffmann-La Roche, Nutley, USA), 2.5 µL of reaction

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Table 1 Fungal isolates obtained from *Scolytus ratzeburgi* infesting birch and used in this study.

Species identity	Isolate no.[1]			Geographical origin	GenBank accession no.	
	CBS	CMW	VTT		ITS	β-tubulin
Ophiostoma canum-like		29490		Hobøl, Norway	FJ804493	
	124499	29495		Hobøl, Norway		FJ804505
O. denticiliatum sp. nov.	124497[a,b]	29493[a,b]	D-091316	Hobøl, Norway	FJ804490	FJ804502
	124498[a,b]	29494[a,b]	D-091317	Hobøl, Norway	FJ804491	FJ804503
			D-091318[a,b]	Hobøl, Norway	FJ804492	FJ804504
O. flexuosum-like	124500	29496		Hobøl, Norway	FJ804494	FJ804506
O. karelicum	124494	29484		Spydeberg, Norway	FJ804482	FJ804495
	124495	29485		Vestby, Norway	FJ804483	FJ804496
		29486		Hobøl, Norway	FJ804484	FJ804497
		29487		Spydeberg, Norway	FJ804485	FJ804498
		29488		Vestby, Norway	FJ804486	FJ804499
		29489		Hobøl, Norway	FJ804487	FJ804500
O. multiannulatum-like		29492		Spydeberg, Norway	FJ804489	
O. quercus	124496	29491		Hobøl, Norway	FJ804488	FJ804501

[1] CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; VTT: Culture Collection of the Technical Research Centre of Finland, Espoo, Finland. All isolates were collected by R. Linnakoski.

[a] Isolates used in growth studies.

[b] Isolates crossed with each other to obtain perithecia for morphological descriptions.

buffer (10×) and 2.5 µL of $MgCl_2$ (25 mM) (supplied with the enzyme), 2.5 µL of dNTP's and 0.25 µL of each primer (10 mM). PCR reactions were performed using an Eppendorf Mastercycler® Personal (Perkin-Elmer, Hamburg, Germany). The PCR conditions were: an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of 30 s at 95 °C, 30 s at 54 °C and 1 min at 72 °C, and a final chain elongation at 72 °C for 8 min. Amplified products were sequenced with the BigDye v3.1 Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on the ABI Prism 377 Autosequencer (Applied Biosystems, Foster City, CA, USA).

Sequence analyses

Raw sequences were aligned and consensus sequences determined manually using Geneious Pro v4.0.4 for MacIntosh (Biomatters, Auckland, New Zealand). BLAST searches were conducted for preliminary identifications, after which datasets were compiled that included published GenBank sequences. Since the sequence data for intron 5 of the β-tubulin gene varies greatly between different species groups in *Ophiostoma*, the β-tubulin dataset was treated in subsets in order to obtain improved alignments for species definition. Alignments were done online within MAFFT v6 (Katoh et al. 2002), using the E-INS-I method with a gap opening penalty of 1.53 and an offset value of 0.00. All sequences obtained in this study were deposited in GenBank (Table 1).

Neighbour-joining (NJ) analyses (with the Kimura-2 parameter active) and maximum parsimony (MP) were conducted using MEGA v4 (Kumar et al. 2008). Bootstrap values were calculated for 1 000 replicates (Felsenstein 1985).

Bayesian analyses were carried out with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Four independent Markov chains were run for 6 million generations. Substitution models were determined for each dataset using the Akaike Information Criterion (AIC) in MrModeltest v2.3 (http://www.abc.se/~nylander/). Trees were sampled every 100 generations resulting in 60 000 trees, discarding the burn-in of the chain, as calculated for the respective datasets. The remaining trees for both runs were used to construct a majority rule consensus tree for each dataset.

Morphological studies

DNA sequence analyses suggested that some of the isolates from *S. ratzeburgi* represent an undescribed species of *Ophiostoma*, but only anamorph structures had been found in the galleries and in culture. In order to ascertain whether mating of strains would result in the teleomorph of this species, single conidial isolates were crossed in all possible combinations. Each culture was also crossed against itself as a control. These sexual compatibility tests were conducted on three different media including water agar (WA; 15 g Difco Bacto™ agar and 1 L Milli-Q water), MEA and oatmeal agar (OA; 15 g oatmeal, 15 g Difco Bacto™ agar and 1 L Milli-Q water) with sterilised birch twigs placed on the surface of the medium. Cultures were incubated at 20 °C and inspected regularly for fruiting structures.

For the descriptions, anamorph and teleomorph (where present) fruiting structures were mounted in 85 % lactic acid on glass slides and observed using a Nikon Eclipse 50i phase contrast microscope (Nikon Corporation, Tokyo, Japan). A Nikon DS-Fi1 camera system (Nikon Corporation, Tokyo, Japan) was used to photograph images. Fifty measurements were made of each of the taxonomically informative anamorph and teleomorph structures. Averages, ranges and standard deviations of the measurements were computed and measurements are presented in the format (minimum–) mean minus standard deviation – mean plus standard deviation (–maximum).

Three isolates (Table 1) of the unknown *Ophiostoma* species were chosen to determine the optimal temperature for growth in culture. Agar plugs (5 mm diam) bearing mycelium were

Table 2 *Ophiostoma* species isolated from *Scolytus ratzeburgi* beetles and galleries in Norway.

Species	Site 1: Spydeberg		Site 2: Vestby		Site 3: Hobøl		Total
	Beetle	Gallery	Beetle	Gallery	Beetle	Gallery	
Ophiostoma karelicum	17	21	10	13	11	15	87
O. quercus	0	0	0	0	5	0	5
O. denticiliatum sp. nov.	0	0	0	0	3	0	3
O. canum-like	0	0	0	0	0	2	2
O. flexuosum-like	0	1	0	0	0	0	1
O. multiannulatum-like	0	1	0	0	0	0	1

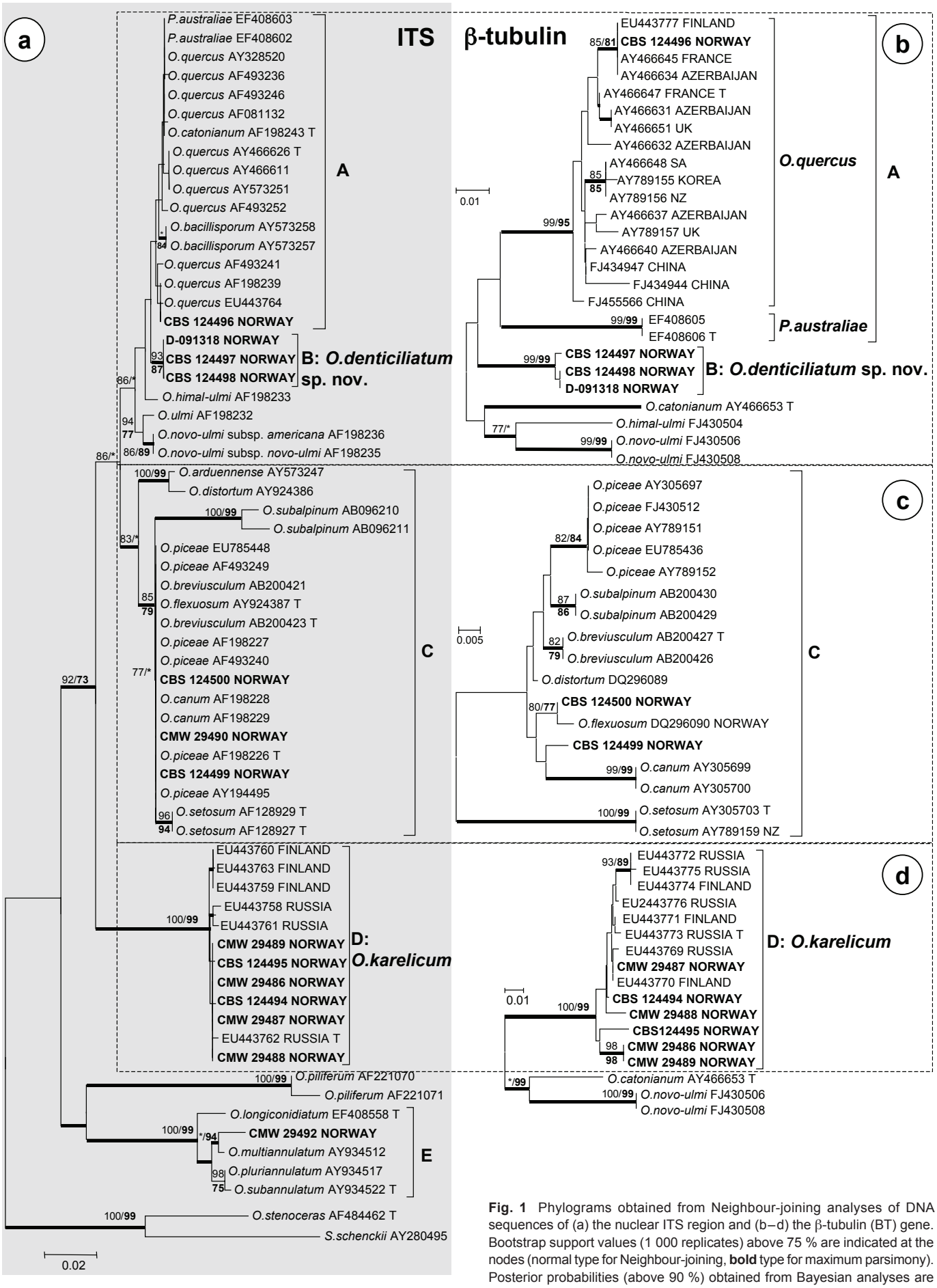


Fig. 1 Phylograms obtained from Neighbour-joining analyses of DNA sequences of (a) the nuclear ITS region and (b–d) the β-tubulin (BT) gene. Bootstrap support values (1 000 replicates) above 75 % are indicated at the nodes (normal type for Neighbour-joining, **bold** type for maximum parsimony). Posterior probabilities (above 90 %) obtained from Bayesian analyses are indicated by **bold** lines at the relevant branching points. * = bootstrap values lower than 75 %. Isolate numbers of sequences obtained in this study are printed in **bold** type. — Scale bar = total nucleotide difference between taxa.

transferred from the actively growing margins of one wk old cultures and placed at the centres of 9 cm Petri dishes containing 2 % MEA. The growth rates of isolates were determined at 5, 10, 15, 20, 25, 30 and 35 °C (± 0.5 °C) in the dark. Five replicate plates were used for each temperature interval and colony diameters (three colony diameter measurements per plate) were determined 8 d after inoculation. The results were calculated as mm/day (± standard deviation).

For scanning electron microscopy, small agar disks were cut from the sporulating colonies and fixed overnight in 2.5 % glutaraldehyde in a 0.15 M phosphate buffer (pH 7.4). After three buffer rinses, the specimens were post-fixed in 1 % osmium tetroxide for 60 min, dehydrated in an ethanol series, and critical point dried using CPD 010 (Balzers Union AG, Balzers, Liechtenstein). The specimens were coated with a 30 nm thick copper layer using an Emitech K675X sputter coater (Emitech, Ashford, UK). A LEO 1550 scanning electron microscope (Beamtech Nordiska AB, Gnesta, Sweden) operating at 5 kV was used to examine the specimens.

RESULTS

Fungal isolations

A total of 99 isolates were obtained from bark beetles and their galleries (Table 2). All isolates formed typical *Pesotum* and *Sporothrix* anamorphs in culture. No ascomata were observed. Six different morphological groups were recognized among the isolates. One of the taxa (87 isolates) was isolated from every gallery and every beetle sampled in the study. The other taxa were present in low numbers and were not consistently isolated from the samples.

DNA sequence analyses

Aligned DNA sequence from the ITS regions yielded 715 characters including gaps (Fig. 1a), while alignments of the three β-tubulin data subsets consisted of 339, 332 and 329 characters, respectively, including gaps (Fig. 1b–d). All the trees presented were obtained from NJ analyses (Fig. 1). The Consistency Indices (CI) obtained from heuristic searches in MP analyses for the four datasets were 0.757, 0.819, 0.886 and 0.933, respectively, while the Retention Indices were 0.926, 0.889, 0.925 and 0.941, respectively. The Bayesian analyses for ITS and β-tubulin gene regions produced trees with topologies similar to those of the NJ and MP analyses. The best fitting substitution model selected for the Bayesian analyses was GTR+I+G for all the datasets.

Comparisons of ITS sequences of the isolates from this study with sequences from GenBank, showed that *S. ratzeburgi* fungi resided in five groups which we designated A to E (Fig. 1a). Of these, groups A and C were not resolved in the ITS tree (Fig 1a), thus β-tubulin was used to distinguish between the species representing them (Fig. 1b, c). The groups representing *O. karelicum* (B and D) and an apparently undescribed species were well-resolved in the ITS tree (Fig. 1a) and in these cases β-tubulin sequence data were used to confirm their identities (Fig. 1b, d). For the isolate in group E, the ITS sequence data showed that it was closely related to, but distinct from, *O. multiannulatum* (Fig. 1a). No published β-tubulin sequences are available for species in this group and it could not be resolved further.

Analyses of the sequence data for the β-tubulin gene region revealed more variation among the so-called *O. piceae*-complex species (group C) than was present in the ITS tree. The isolates clustering in this group (Fig. 1c) were closely related to, but distinct from, *O. flexuosum* and *O. canum*.

Sexual compatibility tests

Pairing of single conidial cultures of the undescribed *Ophiostoma* species resulted in several successful crosses where ascomata with viable ascospores were produced. None of the single spore cultures formed ascomata when mated with themselves, indicating that the species is heterothallic.

Growth in culture

Isolates of the undescribed *Ophiostoma* species reached an average diam of 28 (± 4) mm within 8 d on MEA at 25 °C, which was also the optimal temperature for growth. The mean radial growth rate of isolates was 3.5 (± 0.5) mm/d at 25 °C. Very little growth occurred at 5 and 30 °C.

Taxonomy

Ophiostoma denticiliatum Linnakoski, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB509666; Fig. 2, 3

Anamorphs. *Pesotum* sp.
Synanamorph. Sporothrix sp.

Bases atrae, globosae vel subglobosae (88–)132–181(–196) µm diametro, inornatae vel sparse pilis brunneis hypharum ornatae. Hyphae ostiolares (7–)9–13(–16) hyalinae divergentes (17–)25–42(–56) µm longae, basi 1–1.5 µm latae, apice denticulata conidia formanti; denticulis acutis 0.5–1.5 µm longis, primo crescentibus simul ac generatione hypharum. Ascosporae hyalinae, unicellulares, lateraliter visae allantoides 3.5–4.5(–5.5) × 1–1.5 µm, e fronte visae ellipsoideae (3–)3.5–4.5(–5) × 1–1.5 µm, ab extremo visae globosae. Anamorpha *Pesotum* macronema dominans. Anamorpha *Sporothriciformis* adest.

Etymology. The epithet *denticiliatum* refers to the denticulate apices of the ostiolar hyphae.

Ascomata developing after 3 wk when cultures of opposite mating type are paired on agar with birch twigs; superficial or partly embedded in agar (Fig. 2). *Bases* dark, globose to subglobose, (88–)132–181(–196) µm diam; unornamented or sparsely ornamented with brown hyphal hairs. *Ascomatal necks* smooth, dark at base, becoming paler towards apex, straight or slightly curved, (394–)667–986(–1286) µm long excluding ostiolar hyphae, (22–)26–34(–43) µm wide at base, (7–)8.5–10.5(–12) µm wide at the apex (Fig. 2a). *Ostiolar hyphae* (7–)9–13(–16) in number, hyaline, divergent, (17–)25–42(–56) µm long, 1–1.5 µm wide at the base, with denticulate apices producing conidia; denticles pointed, 0.5–1.5 µm long, appearing soon after the appearance of ostiolar hyphae (Fig. 2b, c). *Asci* not observed. *Ascospores* hyaline, 1-celled, allantoid in side view, 3.5–4.5(–5.5) × 1–1.5 µm, ellipsoidal in frontal view, (3–)3.5–4.5(–5) × 1–1.5 µm, globose in end view (Fig. 2d). *Pesotum* macronematal anamorph predominant (Fig. 3). *Synnemata* simple, dark brown at the base, becoming paler toward the apex, (346–)421–542(–647) µm long including conidiogenous apparatus, (42–)75–126(–159) µm wide at base; *conidiogenous cells* (14–)16–21(–26) × 1–1.5 µm; *conidia* hyaline, 1-celled, smooth, oblong, clavate or obovoid (2.5–)3–4.5(–5.5) × 1–1.5 µm. Synnematal anamorph usually common on different agar media with birch twigs. Attached to substrate by brown rhizoid-like hyphae (Fig. 3a–d). *Sporothrix* synanamorph present. *Conidiogenous cells* micronematous, mononematous, hyaline, (10–)13–22(–30) × 1–1.5(–2) µm, apical part consisting of swollen clusters bearing pointed denticles, 0.5–1(–1.5) µm; *conidia* hyaline, 1-celled, smooth, oblong, clavate or obovoid (3–)4–5(–6) × 1–1.5 µm (Fig. 3e, f).

Culture characteristics — Colonies at first hyaline, later becoming moderately to dark brown at the centre. Mycelium superficial on the agar, white to pale greyish aerial mycelium present. *Pesotum* anamorph dominant in cultures. Optimal temperature for growth 25 °C, growth reduced at 5 and 30 °C. The mean radial growth rate 3.5 (± 0.5) mm/d.

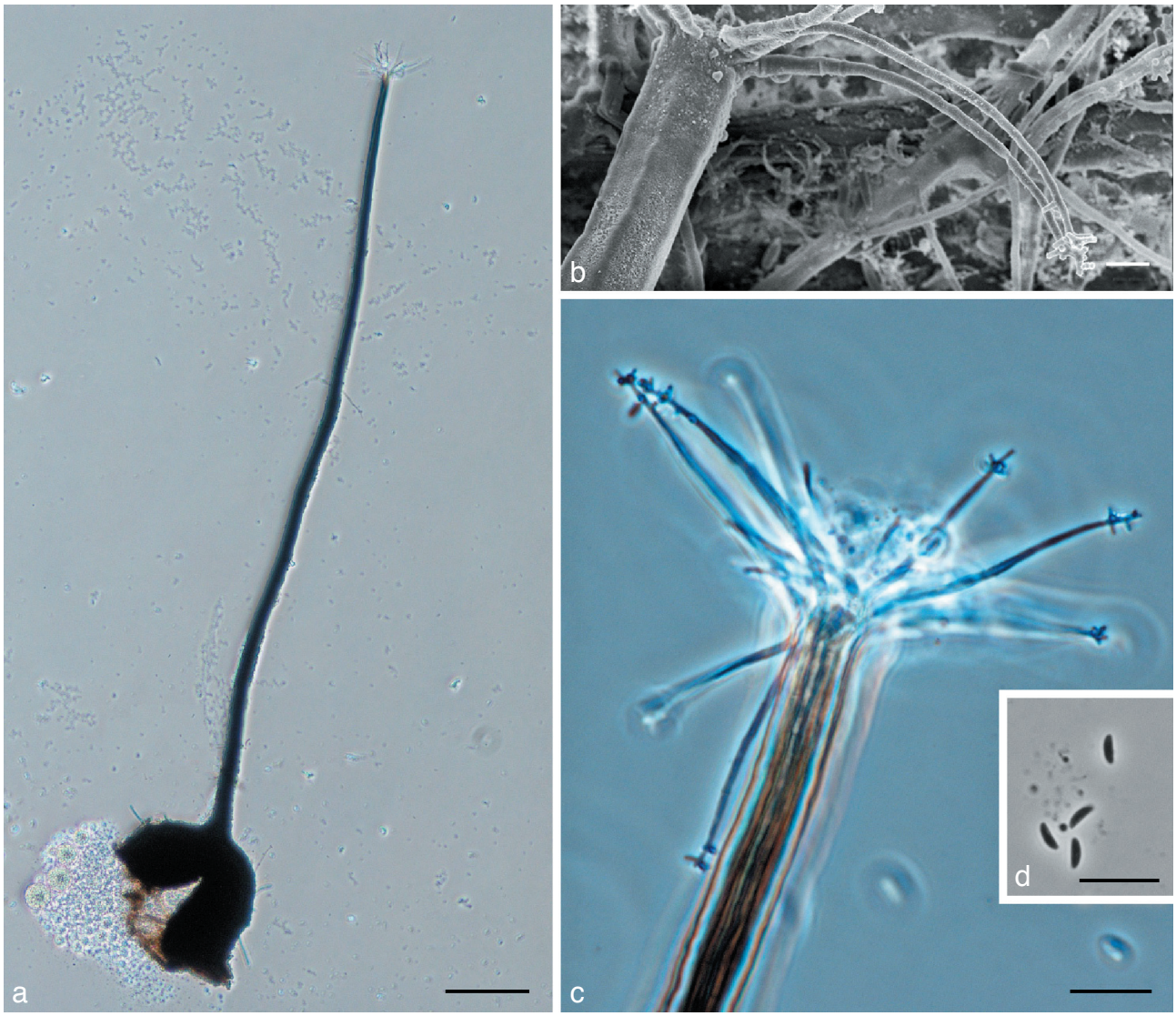


Fig. 2 Morphological characters of *Ophiostoma denticiliatum* (holotype) teleomorph structures. a. Ascoma; b. scanning electron micrograph (SEM) of ostiolar hyphae with denticulate apex, starting to form *Sporothrix*-like anamorph; c. ostiolar hyphae; d. ascospores. — Scale bars: a = 100 µm; b = 3 µm; c, d = 10 µm.

Sexuality — Heterothallic.

Host range — Associated with *Scolytus ratzeburgi* and their galleries in *Betula pendula* and *B. pubescens.*

Distribution — Presently known only from Southern Norway.

Specimens examined. Norway, Hobøl, on *Scolytus ratzeburgi* infesting *Betula* sp., July 2007, *R. Linnakoski*, holotype KUO 020520 (dried culture obtained from cross between CBS 124497 × CBS 124498, Herbarium of Kuopio Museum of Natural History, Finland), cultures ex-type CBS 124497, CBS 124498; paratype KUO 020521 (dried culture CBS 124497); paratype KUO 020522 (dried culture CBS 124498); paratype KUO 020523 (dried culture VTT D-091318).

Notes — *Ophiostoma denticiliatum* is morphologically similar to other hardwood species in the so-called *Ophiostoma piceae*-complex, especially to *O. quercus* and the Dutch elm disease fungi. Species in the complex are characterised by ascospores that are orange-section shaped, with *Pesotum* and *Sporothrix* synanamorphs that are often produced simultaneously in culture (Harrington et al. 2001). *Ophiostoma denticiliatum* can be distinguished from other species in the complex by its relatively long ascomatal necks and especially the ostiolar hyphae that rapidly transform into conidiogenous cells with distinct denticles that produce conidia. In species such as *O. quercus,* ostiolar hyphae in old perithecia occasionally transform into *Sporothrix*-like conidiogenous cells (Z.W. de Beer, pers. comm.) but in *O. denticiliatum* these structures are a consistent character with all of the 50 examined ascomata sharing this feature. The denticles at the apices of the ostiolar hyphae are formed as soon as the ostiolar hyphae appear, and within days they begin to produce conidia and elongate. A character shared by *O. denticiliatum* and the Dutch elm disease fungi is that they are associated with *Scolytus* species infesting hardwoods (Burges et al. 1979, Webber 1990, Hubbes 1999), but *O. denticiliatum* was not consistently isolated from *S. ratzeburgi*, suggesting that the beetle might be only a casual vector of the fungus.

DISCUSSION

Ophiostomatoid fungi were frequently isolated from the adult beetles of *Scolytus ratzeburgi* and their galleries on birch in Norway. Of the 99 fungal isolates collected, these represented six *Ophiostoma* species. The only species consistently isolated from every gallery and beetle sampled was *O. karelicum*. Other species isolated infrequently included *O. quercus* and four apparently undescribed taxa. Of these, *O. denticiliatum* was described as new and the remaining three taxa were not described formally because an inordinately small number of isolates was available for them.

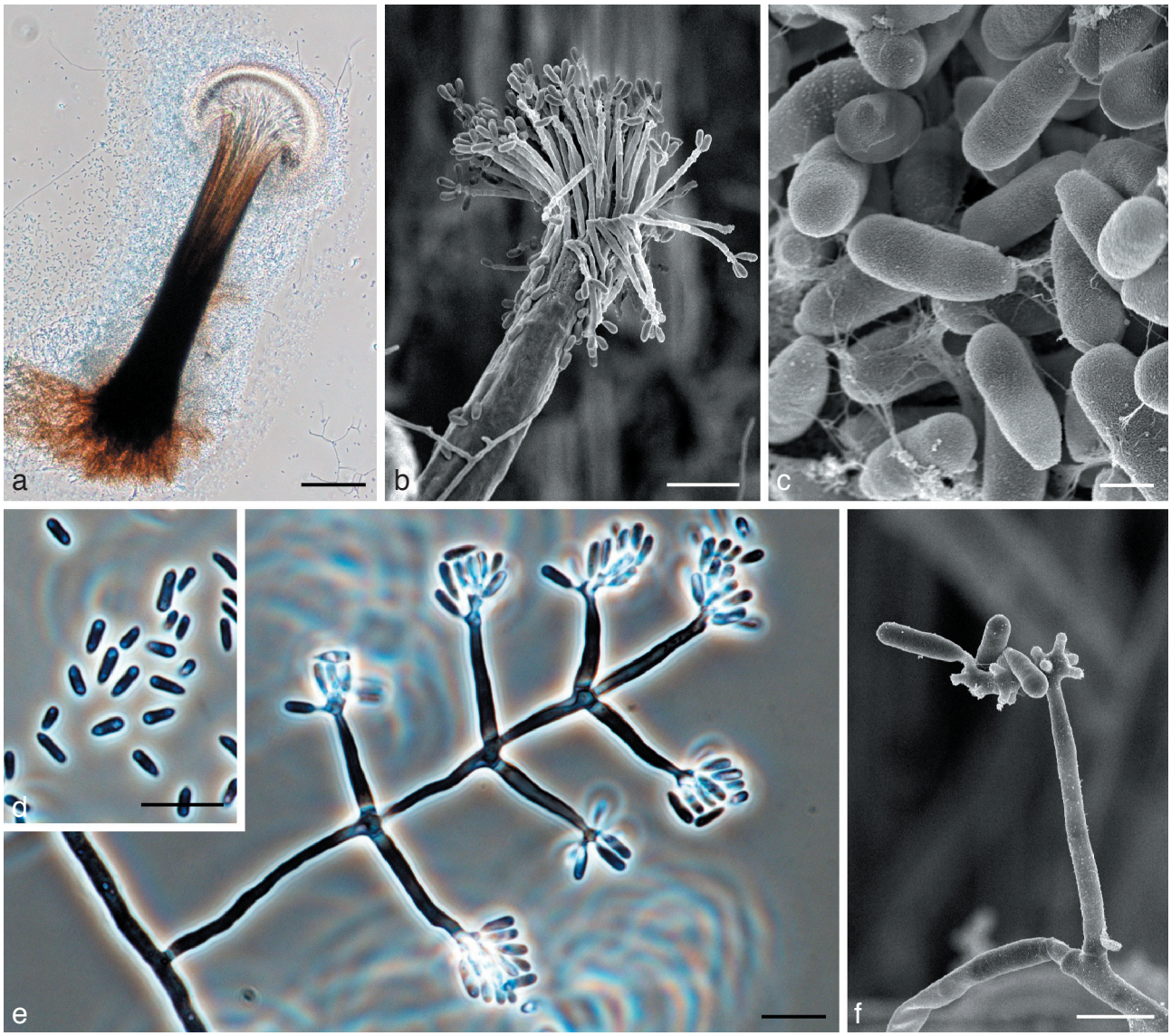


Fig. 3 Morphological characters of *Ophiostoma denticiliatum* (holotype) anamorph structures. a. *Pesotum* anamorph; b. scanning electron micrograph (SEM) of *Pesotum* anamorph; c. SEM of conidia of *Pesotum*; d. conidia of *Pesotum*; e. *Sporothrix*-like anamorph with conidia; f. SEM of *Sporothrix* anamorph with conidia. — Scale bars: a = 100 µm; b, d, e = 10 µm; c = 1 µm; f = 3 µm.

The results of this study are consistent with those of Linnakoski et al. (2008), who showed that *O. karelicum* is the predominant fungus associated with *S. ratzeburgi* in *B. pendula*. This fungus appears to have a specific association with the bark beetle species and its host tree. Our results further suggest that *O. karelicum* occurs across the geographic range of *S. ratzeburgi,* which includes various parts of Europe, Central and Eastern Russia, Mongolia and Japan (Postner 1974, Yanovskij 1996).

Ophiostoma quercus was found only occasionally associated with *S. ratzeburgi* in Norway, and this was also found previously for Finland and Russia (Linnakoski et al. 2008). The fungus was first described by Georgévitch (1926) from *Quercus pedunculata* (= *Q. robur*) in the present day Serbia. It is a common sap-staining agent on mostly hardwood hosts (de Beer et al. 2003, Geldenhuis et al. 2004, Kamgan Nkuekam et al. 2008, Grobbelaar et al. in press, Paciura et al. in press), but also causes economically relevant sap stain on commercially produced pine timber (Zhou et al. 2004, Thwaites et al. 2005, Kim et al. 2007). *Ophiostoma quercus* is morphologically and genetically highly diverse (Przybyl & Morelet 1993, Grobbelaar et al. 2009) with a worldwide distribution (Brasier & Kirk 1993, de Beer et al. 2003, Geldenhuis et al. 2004, Thwaites et al. 2005, Kamgan Nkuekam et al. 2008, Paciura et al. in press). It is vectored by many different insect species, especially bark beetles, but the association with these beetles appears to be casual (Brasier & Kirk 1993, Kirisits 2004, Zhou et al. 2004, 2006, Romón et al. 2007, Paciura et al. in press). The occasional occurrence of *O. quercus* with *S. ratzeburgi* is therefore not surprising.

DNA sequences for two gene regions of the new species described here as *O. denticiliatum*, confirmed that the species resides in a discrete, well-supported phylogenetic group in *Ophiostoma*. The fungus is related and morphologically similar to other *Ophiostoma* species from hardwoods, but closest to *Pesotum australiae* and *O. quercus*. *Pesotum australiae* is at present known only from wounds on the native hardwood, *Acacia mearnsii,* in Australia, and no teleomorph has been observed for it (Kamgan Nkuekam et al. 2008). Although it is likely that *P. australiae* is carried to wounds on trees by insects, no insect-fungus associations are known for it.

The identity of three morphologically distinct groups of isolates could not be resolved in this study. Two of these groups were represented by single isolates (CBS 124500 = CMW 29496 and CMW 29492), and were closely related to *O. flexuosum* and *O. multiannulatum*, respectively. The third group included two isolates (CMW 29490, CBS 124499 = CMW 29495), which are

related to, but clearly distinct from, *O. canum.* Due to the very low numbers of isolates of these three taxa, we have chosen not to provide names for them at the present time.

Several fungi vectored by bark beetles cause blue stain in timber, and some are serious forest pathogens. The best-known examples of such pathogens are the Dutch elm disease fungi, *Ophiostoma ulmi* and *O. novo-ulmi* subsp. *novo-ulmi* and subsp. *americana* (Burges et al. 1979, Hubbes 1999, Brasier & Kirk 2001). Both *O. karelicum* and *O. denticiliatum* are closely related to the Dutch elm disease fungi. Their relatedness to these highly pathogenic fungi suggests that *O. karelicum* and *O. denticiliatum* have the potential to cause a vascular disease. It will, therefore, be important to consider their virulence on *Betula pendula* and other *Betula* spp.

Several native *Betula* species occur, for example, in North America, but thus far *Scolytus ratzeburgi* has not been reported from that part of the world. Russian timber is exported to the USA (UNECE/FAO 2008) and the possibility of the insect and its associated fungi being introduced is relatively high. It would thus be useful to test the susceptibility of North American white birches to *S. ratzeburgi* and *O. karelicum*. Introduction of these fungi into North America would be analogous to the introduction of the Dutch elm disease pathogens, presumably from the Himalayas (Brasier 1983) into Europe and North America, to encounter tree hosts that had not evolved together with them. The resulting disease has been devastating and the same could occur for fungi such as *O. karelicum* if they are introduced into new environments.

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