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Ophiostomatoid fungi associated with bark beetles infesting broadleaved trees in Norway

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Preface

First of all, I which to thank my supervisor Halvor Solheim for introducing me to the fascinating world of ophiostomatoid fungi and for helping me throughout this thesis.
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Abstract

The diversity of ophiostomatoid fungi associated with coniferous trees in Europe is quite well known. However, far less is known about the species associated with broadleaved trees. This study investigated the diversity of ophiostomatoid fungi associated with bark beetles infesting broadleaved trees in Norway. A total of 671 adult bark beetles were collected from six different tree genera. Ten bark beetle species were found; *Anisandrus dispar*, *Dryocoetes alni, D. villosus, Hylesinus crenatus, H. varius, Scolytus intricatus S. laevis*, *S. ratzeburgi*, *Trypodendron domesticum* and *T. signatum.* The mycobiota associated with these beetles is largely unknown. Approximately 2200 isolates were obtained from the sampled beetles and were identified using morphology and DNA-sequencing. This resulted in identification of eighteen species of ophiostomatoid fungi, seven of which are known species: *Graphilbum fragrans, Grosmannia piceiperda, Ophiostoma karelicum, O. novo-ulmi, O. quercus, Sporothrix dentifunda* and *S. prolifera.* The remaining eleven species are most likely undescribed. Four of these species are closely related to *O. novo-ulmi*, within the *Ophiostoma ulmi* complex. Two of the species belong to *Sporothrix*. Three species form part art of *Leptographium sensu lato*. The last two species could not be accurately placed within a species complex, but are likely to belong in *Ophiostoma sensu lato*. Most of the bark beetles were only associated with 1-3 species of ophiostomatoid fungi. However, the number of new species discovered underlie the need for more studies regarding ophiostomatoid fungi associated with broadleaved trees, both in Norway and other European countries.

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1 Introduction

1.1 Insect-fungus interactions

Insect-fungus interactions exist in many forms, ranging from mutualistic, communalistic to antagonistic (Hofstetter & Moser 2014). Common for many insects that live in close association with fungi is that they feed on plant material that is difficult to digest and of poor nutritional value (Martin 1992). The fungi work as an external stomach, helping the insects to utilize the substrate more efficiently (Ayres et al. 2000; Hulcr & Dunn 2011). Dead wood is often a plentiful resource, but consists of compound difficult to break down and many wood boring insects have evolved close association with fungi capable of braking down these compound (Beaver 1989; Martin 1992; Farrell et al. 2001) On the other hand, many wood colonizing fungi rely on these insects for transportation and entering new hosts (Raffa et al. 2015). Bark beetles (Coleoptera: Curculionidae: Scolytinae) have evolved complex associations with a variety of fungi and these interactions have been a topic for scientific studies for more than 150 years (Kirisits 2004). However, many of the aspects regarding these associations are poorly understood (Six 2003). The most common fungal associates of bark beetles are the ophiostomatoid fungi (Ascomycetes) (Kirisits 2004). Some of these fungi have serious phytopathogens (Horntvedt et al. 1983; Brasier 1991) or may cause discoloration in timber (Harrington 2005), causing economically losses in forestry.

1.2 Bark beetles

Bark beetles are among the most important and most studied of forest insects (Paine et al. 1997; Kirisits 2004). They may infest many different plant parts, but most species infest the inner bark or the xylem of trees, in which they spend the majority of their lives (Kirkendall et al. 2015). Bark beetles have been quite thoroughly studied compared to other forest insects, mainly because some species are able of killing healthy trees. These bark beetles are often referred to as aggressive bark beetle or primary bark beetles and may cause large scale mass-attacks, killing millions of trees. These attacks have profound impacts on the environment of which they inhabit and may even alter fire regimes and carbon dynamics (Billings et al. 2004; Kurz et al. 2008). The majority of the about 6000 described species are nonaggressive and harmless to healthy trees, restricted to weakened, stressed or dead trees (Knížek & Beaver 2004). The non-aggressive bark beetles are very diverse and greatly outnumber the aggressive bark beetles (Six

2003; Krokene et al. 2013), which mainly infesting coniferios trees in boreal forest ecosystems (Krokene et al. 2013). Thus, the main research approach on bark beetle-fungi systems has been narrow both ecologically, geographically and on host level. This narrow approach may limit our understanding of bark beetle-fungal systems (Six 2003). The non-aggressive bark beetles may also play important roles, both ecologically and economically. They may be vectors of different forest pathogens. The most dramatic examples are the Dutch elm disease fungi (DED), *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier. which are mainly vectored by bark beetles infesting elm trees (*Ulmus* spp.) (Webber 2004). These fungi have killed millions of elm trees in Europe, North America and parts of Asia (Brasier 1991). Some non-aggressive bark beetles are able to kill weakened trees that normally would have survived if not attacked (Kirkendall et al. 2015) and some species have caused large damage when introduced to new environments (Hulcr & Dunn 2011). Non-aggressive bark beetles play important roles in ecosystem health by creating niches for other organisms and contribute to nutrient cycling (Raffa et al. 2015).

Bark beetles and symbiosis with fungi

A bark beetle species is typically associated with many different fungi. One example is *Ips typographus* (Linnaeus, 1758), which has been found to be associated with more than 20 different ophiostomatoid fungi (Kirisits 2004). The degree of dependency is highly variable between different bark beetle-fungus systems (Hofstetter et al. 2006). In most cases, only a few of the fungi are of importance to the bark beetle (Six 2003). The main benefit for the fungus is dispersion to a new host tree and protection (Batra 1967; Six 2003), but bark beetles may also profit from these associations. Some fungi serve as nutritional symbionts for bark beetles, mainly by enhancing the nitrogen availability in the galleries (Ayres et al. 2000). Fungi may also supply larvae or adults with vitamins, sterols and lipids (Beaver 1989). The best examples of bark beetles that live in nutritional symbiosis with fungi are found among the ambrosia beetles. These beetles live in obligate nutritional symbiosis with fungi often referred to as ambrosia fungi (Batra 1963). The ambrosia beetles breed in the xylem which is both of poor nutritional value and difficult to digest. Ambrosia beetles solve this problem in a very clever way by feeding on fungi they cultivate in their galleries. The fungi can reach a large area inside the xylem and produce nutrient rich mycelium covering the gallery walls, which serves as the main food source for both larvae and adults (Batra 1963). Phloem-breeding bark beetles may also feed on fungi, but many do not depend solely on fungi. Instead the phloem breeding bark

beetles often feed on a mix of both fungi and phloem (Harrington 2005). Fungi in association with bark beetles may also provide protection from antagonistic fungi (Klepzig & Wilkens 1997) and some yeasts have been shown to produce compound used in bark beetle communication (Brand et al. 1976; Leufvén et al. 1984). Some bark beetles have evolved mycangia for transportation of symbiotic fungi which works like fungal pockets, providing protection for spores and mycelium during dispersion to a new host (Batra 1963; Six 2003).

1.3 Ophiostomatoid fungi

Most ophiostomatoid fungi are saprophytes infesting dead plant parts, but this group also includes plant pathogens (Harrington 2005). Many colonize trees and are often vectored by bark beetles and some have evolved species specific associations with their vector (Six 2003). The hyphae are often melaninised giving colonized wood a blue-gray color. For this reason, ophiostomatoid fungi are often referred to as blue-stain fungi (Harrington 2005).

The ophiostomatoid fungi consist of a polyphyletic group with similar morphology, ecology and taxonomic history belonging to the Ascomycetes; *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Grosmannia* Goid, *Ophiostoma* Syd. & P. Syd. (Order Ophiostomatales) and Ceratocystis Ellis & Halst. (Order Microascales) (Six 2003). The group was previously believed to have a monophyletic origin because of the many similarities, but modern phylogenetic studies have shown that the ophiostomatoid fungi consist of different orders and that the similar adaptations have evolved by convergent evolution (Spatafora & Blackwell 1994; De Beer et al. 2013a). The most common morphological similarities are adaptations that facilitate dispersion by insects (Six 2003). Both conidia and ascospores are often produced in slimy droplets on top of long stalks, lifting the spores up from the substrate and increasing the possibility of attachment to wood-colonizing insects (Six 2003). Species of ophiostomatoid fungi are often difficult to recognize and distinguish using morphology, and this has led to a confusing taxonomy which has undergone many changes since the introduction of phylogenetic studies based on DNA sequencing (Zipfel et al. 2006). According to De Beer et al. (2013a), the ophiostomatoid fungi are now either placed in the order Ophiostomatales (Sordariomycetidae), which include one family (Ophiostomataceae) or in the order *Microascales (Hypocreomycetidae*), which include four families containing ophiostomatoid fungi (*Microascaceae, Ceratocystidaceae, Gondwanamycetaceae,* and *Graphiaceae*).

Identification of ophiostomatoid fungi

Identification and grouping of ophiostomatoid fungi is difficult and time consuming. Many characteristics have been used in identification and some examples are; shape of ascomata and ascospores, conidiogenous structures, growth rate, growth on different media and cell wall composition (Upadhyay 1981; Zipfel et al. 2006; De Beer & Wingfield 2013). Identification based solely on morphology can be very difficult since many similar characteristics have evolved independently in different taxa (Spatafora & Blackwell 1994; De Beer & Wingfield 2013). Many species have several anamorph states (*Hyalorhinocladiella*-like, *Raffaelea*-like, *Pesotum*-like, *Leptographium*-like and *Sprothrix*-like), which has resulted in many names for the same fungus (Zipfel et al. 2006). Different naming of different states of the same fungus is now considered outdated after the one fungus one name principle was introduced (Taylor 2011). During the last decades, molecular data has become more and more common and is now considered a very important part of modern fungal taxonomy (Zipfel et al. 2006; De Beer & Wingfield 2013). Use of molecular data has led to discovery of many new fungal species as old species concepts often have been proven too broad (Taylor et al. 2000). Studies dealing with molecular species identification use different gene regions for different purposes. The ribosomal small subunit (SSU) and the ribosomal large subunit (LSU) are often used to place species in species complexes and investigate the evolutionary relationship between groups (De Beer et al. 2013a). For species identification the ITS region is commonly used, but this region may in some cases be too conserved to separate closely related species within the ophiostomatoid fungi (Harrington et al. 2001) For this reason, it is common to also include protein coding genes with higher resolution, like β-tubulin, translation elongation factor 1-α or calmodulin. (Linnakoski et al. 2008; Linnakoski et al. 2009; De Beer et al. 2016). These protein coding gene regions are better to distinguish between closely related species, but often have lower PCR amplification success, compared to rDNA regions (Schoch et al. 2012).

1.4 Previous studies in Norway

Only six describes ophiostomatoid species have been found in association with broadleaved trees in Norway; *Ophiostoma borealis* Kamgan, H. Solheim & Z.W. de Beer., *Ophiostoma denticiliatum* Linnakoski, Z.W. de Beer & M.J. Wingf., *Ophiostoma karelicum* Linnakoski, Z.W. de Beer & M.J. Wingf., *Ophiostoma ulmi, Ophiostoma novo-ulmi,* and *Ophiostoma quercus* (Georgev.) Nannf. (Solheim & Hietala 2015). The best known of these species are

probably the fungi that causes DED (*Ophiostoma ulmi, O. novo-ulmi*). DED in Norway is mainly distributed around the Oslofjord region and seems to have a relatively low impact compared to other effected areas. (Solheim et al. 2011).

So far, *Scolytus ratzeburgi* (E. W. Janson, 1856) is the only hardwood-infesting bark beetle that has been investigated in Norway regarding associated ophiostomatoid fungi (Solheim & Hietala 2015). Linnakoski et al. (2009) found *Ophiostoma borealis*, *O. denticiliatum O. karelicum* and *Ophiostoma quercus* in association with this beetle infesting *Betula* spp. in southern Norway. *Ophiostoma karelicum* was constantly associated with this bark beetle and this was consistent with a previous study conducted in Finland and Russia (Linnakoski et al. 2008). A study investigating wounds of five different broadleaved tree genera in Norway and Sweden, found three species; *Ophiostoma borealis*, *O. denticiliatum* and *Ophiostoma quercus* (Kamgan Nkuekam et al. 2010). Thus, previous studies indicate that the diversity of ophiostomatoid fungi associated with broadleaved trees in Norway is low (Kamgan Nkuekam et al. 2010). However, many of the mentioned species were discovered for the first time and described as new species during these studies. This indicates that the diversity of ophiostomatoid fungi in Norway is only partially understood (Linnakoski et al. 2009; Kamgan Nkuekam et al. 2010).

1.5 Aims of the study

Only 22 species of ophiostomatoid fungi are known from Norway, and this is low compared to the other Nordic countries (Solheim & Hietala 2015). This study aims to increase the knowledge about ophiostomatoid fungi in Norway by doing isolation from adult bark beetles infesting broadleaved trees both in the southeastern and northern parts of Norway.

2 Materials and methods

2.1 Investigated bark beetles

A total of 671 bark beetles representing 10 species were sampled and studied for ophiostomatoid fungi. The nomenclature of the collected bark beetles follows Kvamme and Lindelöw (2014). Seven of the collected species breed in the phloem; *Dryocoetes alni* (Georg, 1856), *Dryocoetes villosus* (Fabricius, 1792), *Hylesinus crenatus* (Fabricius, 1787), *Hylesinus varius* (Fabricius, 1775), *Scolytus intricatus* (Ratzeburg,1837), *Scolytus laevis* Chapuis. 1869

and *Scolytus ratzeburgi*. And three species breed in the xylem (ambrosia beetles); *Anisandrus dispar* (Fabricius, 1792), *Trypodendron domesticum* (Linnaeus, 1758) and *Trypodendron signatum* (Fabricius, 1792). All of the investigated bark beetle species are native to Norway. Only adult beetles were investigated. The galleries were not investigated.

2.2 Sampling of bark beetles

Bark beetles were sampled from September 2015 until September 2016 at 16 locations in Norway (Table 1). The majority of the sampling sites were located in southeastern Norway, in the counties Akershus, Buskerud, Oslo and Vestfold, while two of the sampling sites were located in northern Norway, in Troms county. In general, two different sampling methods were used.

Sampling method 1: Beetles sampled directly from galleries.

Bark beetle species that hibernate as adults (*Hylesinus* spp, *Dryocoetes* spp. *Trypodendron* spp. and *A. dispar*) were sampled directly from galleries. During sampling of phloem-breeding beetles the bark was carefully removed around galleries using a knife and beetles were picked out by the use of tweezers and each specimen was stored in separate 2 ml Eppendorf tubes (Eppendorf, Hamburg, Germany). Tubes containing beetles were put in a freezer (-20°C) as soon as possible. Species that breed in the xylem (*Trypodendron* spp. and *A. dispar*) were sampled by cutting stems and branches into smaller pieces (10-50cm) that were cleaved with an axe or a chisel. The sampled beetles were treated as previously explained.

Sampling method 2: Use of emergence traps.

Most *Scolytus* specimens were sampled by the use of emergence traps around collected logs and stems containing larvae or pupae. Attacked material was cut into bolts of 50-70 cm, and placed in the institute's insectarium. Here the bolts were exposed to natural temperature fluctuations, but were not exposed to rain or directs sunlight. The bolts were hung vertically from the ceiling with a tread attached to a hook screw that was screwed into the end grain and then covered with an emergence trap. The trap consisted of a cylindrical fine mesh attached to a collection bottle underneath. The emergence traps were checked daily for emerging beetles until the material was assumed empty. Beetles present in the traps were stored as previously explained.

Table 1.

Overview of bark beetles sampled in this study.

Sampling site	Latitude	longitude	Municipality	County	Bark beetle species	N (beetles)	Host	Sampling method	Sampling date/period	Hatching date/period	Collector
1	69.068206	18.479447	Målselv	Troms	*T. signatum*	41	*Alnus incana*	1	29.8.16		GK
2	68.703988	16.309313	Kvæfjord	Troms	*D. alni*	25	*Alnus incana*	1	18.9.15-25.6.15		HS, LE
					T. domesticum	2	*Alnus incana*	1	25.9.15		HS, LE
3	60.148583	11.454051	Nes	Akershus	*D. alni*	22	*Alnus incana*	1	21.2.16		TA, PA
					T. domesticum	19	*Alnus incana*	1	21.2.16		TA, PA
4	59.891076	11.58073	Aurskog-Høland	Akershus	*S. ratzeburgi*	30	*Betula pubescens*	2	29.5.16	29.5.16-29.6.16	TA, PA
					T. domesticum	30	*Betula pubescens*	1	21.8.16		TA, PA
5	59.803741	10.788397	Kolbotn	Akershus	*D. alni*	7	*Corylus avellana*	1	16.4.16		TA
6	59.693703	10.747756	Ås	Akershus	*A. dispar*	3	*Quercus robur*	1	17.5.16-31.5.16		TA
					A. dispar	32	*Quercus robur*	1	2.7.16-14.9.16		TA
					S. intricatus	33	*Quercus robur*	1 & 2	17.5.16-19.6.16	3.6.16-18.6.16	TA
					T. domesticum	4	*Quercus robur*	1	14.9.16		TA
7	59.689109	10.752828	Ås	Akershus	*D. alni*	30	*Alnus incana*	1	7.2.16		TA
					T. domesticum	30	*Alnus incana*	1	8.2.16		TA
8	59.68102	10.777237	Ås	Akershus	*H. crenatus*	30	*Fraxinus excelsior*	1	15.12.15-10.1.16		TA, MEW
					H. crenatus	3	*Fraxinus excelsior*	1	7.7.16-15.7.16		TA
					H. varius	32	*Fraxinus excelsior*	1	15.12.15		TA, MEW
					T. domesticum	8	*Fraxinus excelsior*	1	15.8.16-27.8.16		TA
9	59.674146	10.842709	Ski	Akershus	*S. ratzeburgi*	4	*Betula sp.*	1	25.6.16-7.7.16		TA
10	59.599941	10.827877	Vestby	Akershus	*S. ratzeburgi*	8	*Betula sp.*	1 &2	12.6.16	22.6.16	TA
11	59.844763	10.788762	Oslo	Oslo	*S. laevis*	61	*Ulmus glabra*	2	21.5.16	18.6.16-2.7.16	TA, PA
12	59.55224	10.432011	Hurum	Buskerud	*S. intricatus*	42	*Quercus sp.*	1 & 2	21.5.16	6.6.16-28.6.16	TA, PA
					S. ratzeburgi	12	*Betula sp.*	1	25.6.16		TA, OVS
13	59.196958	9.918549	Larvik	Vestfold	*H. crenatus*	13	*Fraxinus excelsior*	1	29.6.16		TA
14	59.142153	10.02802	Larvik	Vestfold	*H. varius*	30	*Fraxinus excelsior*	1	29.6.16		TA, PA
15	59.060277	10.068521	Larvik	Vestfold	*D. alni*	30	*Alnus incana*	1	2.4.16		TA, PA
					T. domesticum	30	*Alnus incana*	1	2.4.16		TA, PA
16	58.976052	9.964013	Larvik	Vestfold	*D. villosus*	60	*Quercus robur*	1	28.8.16		TA, KDH

Collector: GK, Geir Kvammen; HS, Halvor Solheim; KDH, Kim Daniel Hansen; LE, Leif Evje; MEW, Max Emil Waalberg; OVS, Ola Våland Strandin; PA, Pål Aas; TA, Truls Aas. Sampling method; 1: Adult beetles sampled directly from galleries in the field or in the laboratory from collected branches and logs. 2: Bark beetles sampled by emergence traps around collecting logs and branches containing larvae or pupae.

2.3 Fungal isolation

Malt extract agar (MEA) was made by dissolving 6.25 g malt (Bacto malt extract, Beckton, Dickinson, Sparks, USA), 10 g agar (Bacto agar or agar powder from VWR International, Leuven, Belgium) and 0.5 l deionized water. The mixture was autoclaved for 20 minutes at 121°C. (HMC Hirayama, Saitama, Japan). Bark beetles were dissected and rinsed for phoretic mites (if present) using a stereo microscope, sterile tweezers and inoculation needles. Bark beetle parts were placed in 9 cm Petri dishes (Heger AS, Rjukan, Norway) containing malt extract agar. Three MEA-plates were used per beetle. The two elytra were placed on either side of one plate (about 2 cm from the edge). The front part, containing head and prothorax were placed in the center of a second plate. The rest of the beetle was split in two between abdomen and metathorax and placed on either side of a third plate (about 2 cm from the edge). All beetle-parts placed on MEA-plates were pushed into the medium so that all or most of it was submerged in the MEA. Each elytron was pushed 2-3 cm along the MEA-surface, with the outer surface facing down, before pushed into the medium. The main dishes (plates containing bark beetles) were inspected regularly and possible ophiostomatoid fungi were transferred to new MEA-plates. During the first month the plates were inspected three times (first inspection after two-four days, second after ca. two weeks, third after ca. one month). Later the plates were inspected more irregular, depending on the growth of the fungi present. All main dishes were inspected between 4 and 6 times. Both main dishes and isolates were stored at room temperature. Isolates were grouped according to morphological characteristics, which bark beetle species they were isolated from and sampling location. 1-5 isolates from each group were used for DNA extraction. These isolates were transferred to new MEA-plates covered with cellophane. After 4-10 days the colony were scraped of the cellophane with the use if a sterile toothpick and placed in 2 ml Eppendorf tubes. The tubes were stored in a freezer (-20˚C).

2.4 DNA extraction

Protocol 8# Isolation of DNA From Mouse Tails (Easy-DNA™ Kit; Invitrogen, San Diego, USA) was used to extract DNA. The description given here follows this protocol with minor changes. The maximum number of fungal isolates per batch was 24 (max load of centrifuge). About 0.03 g of sand (Seesand, purum, Fluka, Buchs, Switzerland) and 100 µl of lysis-buffer master mix was added to each tube containing mycelium. The lysis buffer contained per sample;

320 µl TE buffer, 20 µl Solution A, 10 µl Solution B and 5 µl Protein degrader. The mycelium was grinded into smaller pieces with a sterile pestle. After grinding, an additional load of 255 µl master mix was added to each tube. The tubes were vortexed using a Heidolph Reax Top-vortex mixer (Heidolph Instruments, Germany) and placed standing in an incubator shaker (INFORS HT, Bottmingen, Switzerland), or in a water shaking bath (GRANT, Cambridge, UK) at 200 rpm at 60˚C for 12-20 hours. The next day the tubes were centrifuged (Eppendorf Centrifuge 5415R: Eppendorf, Hamburg, Germany) for one minute at 1600 RCF. Thereafter, 300 µl of solution A, 120 µl of solution B was added to each tube. The tubes vortexed until the mixture was uniformly viscous. A volume of 750 µl of chloroform was added to each tube and vortexed until the mixture was homogeneous. The samples were then centrifuged (16100 RCF) for ten minutes at 4°C. The supernatant was transferred over to new 1.5 ml microtubes (Axygen, Scientific Inc., CA, USA). One ml of 96% ethanol was added to each tube, followed by incubation on ice for 30 minutes. The tubes were then centrifuged (16100 RCF) for 10 minutes at 4˚C. The ethanol was removed with the aid of a pipette, before adding 500 µl of 80% ethanol, followed by 4 minutes of centrifugation. The 80% ethanol was removed and the tubes were centrifuged for 2 minutes. Ethanol still present in the tubes was removed and the tubes were air dried (for about 15-20 minutes) in a laminar chamber until all the ethanol had evaporated. A volume of 50 µl of TE buffer was added to each tube, followed by vortexing and 1-minute centrifugation and storage at -20 ˚C.

2.5 PCR

The polymerase chain reaction (Kleppe et al. 1971; Mullis et al. 1986) was used to amplify DNA. The following regions were amplified; the internal transcriber spacers (ITS) and portions of the LSU rDNA region and portions of the β-tubulin, elongation factor-1 alpha (EF1-α) and calmodulin gene regions.

The ITS region, including ITS1, 5.8 gene and ITS2, were amplified using primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). The ITS2 and part of the LSU region were amplified using primers ITS3 and LR3 (White et al. 1990). The β-tubulin gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). Bt2a was replaced in some cases with primer T10 (O'Donnell & Cigelnik 1997). The EF1-α gene was amplified using primers EF1F and EF2R (Jacobs et al. 2004) or primers F-728F (Carbone & Kohn 1999) and

EF2 (O'Donnell et al. 1998). The calmodulin gene was amplified using primers CL2F and CL2R (Duong et al. 2012).

HotStar Taq Plus DNA Polymerase kit (Qiagen, Hilden, Germany) was used to make the reaction mixture. The reaction mixture was mixed in 0.2 ml PCR-strips (Axygen, Scientific Inc., CA, USA) and contained per sample; 1 µl of DNA-template, 5 µl 10x PCR buffer (containing 15 mM $MgCL_2$), 2 µl 25mM $MgCL_2$, 1 µl dNTP (10 mM of each), 5 µl BSA (0.4%), 5 µL TMACL (0.1 mM), 0.4 µl Hot Star Taq+, 1 µl of each primer (10 mM) (forward and reverse) and 27,6 µl RNase-free water. PCR was performed using a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The PCR settings for ITS were; an initial denaturation step for 5 minutes at 95 ˚C, followed by 35 cycles of 95 ˚C for 30 seconds, 53˚C for 30 seconds and 72 ˚C for 1 minute, followed by a final chain elongation at 72˚C for 10 minutes. The PCR conditions for ITS2-LSU (and in some cases EF1-α) were; an initial denaturation step for 3 minutes at 95˚C, followed by 35 cycles of 95˚C for 30 seconds, 58˚C for 45 seconds and 72˚C for 1 minute, followed by a final chain elongation at 72˚C for 8 minutes. The PCR-settings for β-tubulin were; an initial denaturation step for 5 minutes at 95 ˚C, followed by 35 cycles of 95 ˚C for 30 seconds, 56˚C for 30 seconds and 72 ˚C for 1 minute, followed by a final chain elongation at 72˚C for 10 minutes. The PCR conditions for calmodulin, EF1-α and when using primer T10 were; an initial denaturation step for 3 minutes at 95 ˚C, followed by 35 cycles of 95 ˚C for 30 seconds, 55˚C for 45 seconds and 72 ˚C for 1 minute, followed by a final chain elongation at 72˚C for 8 minutes. All programs had final storage at 4 °C.

2.6 Quality of PCR products

Success of PCR amplification was verified on 1% agarose gel stained with ethidium bromide. Five µl of each PCR-product and 1.1 µl loading buffer was mixed and added to the individual wells of the gel. The gel electrophoresis was performed in 1xTAE buffer in a Bio-rad Sub-cell GT horizontal electrophoresis gel apparatus (BIO-Rad, Laboratoties Inc., Hercules, CA, USA). The gel was running for 20-30 minutes at 80V, and thereafter visualized under UV-Light (Gene Genius Bioimaging Systems Syngene, Cambridge, UK). Samples that did not seem to contain any DNA were taken out.

2.7 Purification of PCR products

Illustra ExoProStar clean-up kit (GE Healthcare Life Science, Buckinghamshire, UK) was used to remove excess dNTPs and primers from the PCR products. A volume of 10 µl of each PCR product was mixed with 2 µl Shrimp Alkaline Phosphatase and 2 µl Exonuclease 1 in individual 1.5 ml Eppendorf tubes, followed by vortexing and 1-minute centrifugation (16100 RCF). The tubes were incubated for 5 minutes at 37˚C, and then 10 minutes at 80˚C using Techne Dri-Block heaters (Techne, Cambridge, UK). The tubes were then incubation on ice for 3 minutes to condensate the mixture and thereafter centrifuged for one minute. Each purified PCR product was transferred to two new 1.5 ml Eppendorf tubes (5 µl in each). Five µl Forward primer (5mM) was added to the first tubes and reverse primer (5mM) was added to the second tube. The tubes were then labelled and stored at -20°C.

2.8 DNA sequencing

Samples were sequenced by Sanger sequencing (Sanger et al. 1977) which was performed by use of the light run sequencing service of GATC Biotech (GATC Biotech, Konstanz, Germany).

2.9 Phylogenetic analyses

The forward and reverse sequence obtain from each isolate were aligned in CLC Main Workbench 7.7 (CLC Bio, Aarhus, Denmark). The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov) was used as a first step in identification of the sequences. Datasets based on the BLAST searches were compiled and did also include sequences from the GenBank (NCBI). Multiple sequences alignment was performed in MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) or in MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) to compare and group sequences. Sequences of high quality were used in the phylogenetic analyses which were performed in Molecular Evolutionary Genetics Analysis (MEGA) version 7 (Kumar et al. 2016). Neighbor joining analyses (Saitou & Nei 1987) were performed with bootstrap test (Felsenstein 1985) of 1000 replicates and the p-distance method (Nei & Kumar 2000) was used to estimate the evolutionary distance.

2.10 Growth studies

Optimal growth rate was determined for species believed to be undescribed. Three isolates of each species were used. Growth rate were determined at 5, 10, 15, 20, 25, 30, and 35°C. Agar plugs (5 mm) containing mycelium was transferred to new MEA-plates (same medium as described in 2.3). Growth was initiated at room temperature. After four days at room temperature the colony edge was marked with a permanent marker at two cross lines before placed at the given temperature. After five days at the given temperature in the dark the hyphal extension was measured (for measurements per culture). The mean radial growth was calculated for each species and is presented as mm/day.

2.11 Measurements and photos

Taxonomically informative structures of species believed to be undescribed were measured and photographed using a Leica DMR microscope (Leica, Heerbrugg, Switzerland) equipped with Leica DFC425 camera which was operated by Leica application suite software version 4.0. Mating of isolates and growth on sterilized twigs were in some cases preformed to stimulate production of fruiting structures. 1-3 strains were used in the description of each species. Fifty measurements were made of each observed structure. Measurements are presented in the format (minimum) mean minus standard deviation – mean plus standard deviation (maximum).

3 Results

Approximately 2200 isolates were taken during the course of this study. Ophiostomatoid-like isolates were believed to represent 19 different taxa based on morphology. Eleven groups produced *Pesotum* anamorphs, one group produced only ascomata, three produced *Leptographium* anamorphs and four groups produced *Sporothrix* anamorphs.

In total 283 strains were sequenced using ITS, 32 strains were sequenced using ITS2-LSU, 106 strains were sequenced using β-tubulin,75 strains were sequenced using EF1-α and 18 strains were sequenced using calmodulin. In some cases, the strains were of very poor quality when sequencing a region of a particular strain and could not be used for identification. The identity was then determined by sequencing a different region.

In most cases the sequence data corresponded well with the morphology and a total of 18 different taxa of ophiostomatoid fungi were found (Table 12) of which seven are known taxa; *Graphilbum fragrans* (Math.-Käärik) Z.W. De Beer, Seifert & M.J. Wingf., *Grosmannia piceiperda* (Rumbold) Goid., *Ophiostoma karelicum, O. novo-ulmi, O. quercus, Sporothrix dentifunda* (Aghayeva & M.J. Wingf.) Z.W. de Beer, T.A. Duong & M.J. Wingf., and *Sporothrix prolifera* (Kowalski & Butin) Z.W. de Beer, T.A. Duong & M.J. Wingf. Eleven of the species may be undescribed species. One of the new species, *Leptographium trypodendroni* R. Jankowiak, B. Strzałka & R. Linnakoski, is about to be described and the paper is submitted to the journal. This species is therefore not described here. The remaining ten species have been given a temporary name often associated with a related taxon and are treated as; *Leptographium* sp. 1, *Ophiostoma bacillisporum*-like, *O. brevicolle*-like, *O. catonianum*-like, *O. karelicum*-like, *O. quercus*-like, *Ophiostoma* sp. 1, *Sporothrix lunata*-like, *S. foliorum*-like and Sporothrix sp. 1, The morphological characteristics of these fungi are presented in section 3.3.

3.1 Frequencies of fungi

Phloem breeding bark beetles

3.1.1 Dryocoetes alni

Most of the *Dryocoetes alni* specimens were sampled from *Alnus incana* (L.) (107 specimens). In addition, seven specimens were found on *Corylus avellana* (L.), but no ophiostomatoid fungi were isolated from the beetles sampled from this host-tree.

Dryocoetes alni infesting *A. incana* was associated with eight ophiostomatoid fungi (Table 2). None of the species were isolated frequently. The highest number of species was found at the location in northern Norway (Kvæfjord). At all sites the most common species was *O. karelicum*-like, which was isolated from 22.3% of the beetles. *Graphilbum fragrans* and *Ophiostoma* sp. 1 were occasionally found, and was only isolated from beetles sampled from Nes and Kvæfjord. *Leptographium trypodendroni*, *O. brevicolle*-like, *Sporothrix* sp 1, *Leptographium* sp. 1 and *G. piceaperda* were only isolated between 1-3 times. *Leptographium trypodendroni* was found at all sites except Kvæfjord, while *Ophiostome brevicolle*-like was found at all sites except Larvik.

Table 2. Ophiostomatoid fungi isolated from *Dryocoetes alni* infesting *Alnus incana*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated specimens at each site (%).

Fungus/bark beetle	Dryocoetes alni									
	Larvik		Ås		Nes		Kvæfjord		Total	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Ophiostoma karelicum-like	6	(20.0)	7	(23.3)	6	(27.2)	5	(20.0)	24	(22.3)
Graphilbum fragrans					3	(13.6)	4	(16.0)	7	(6.5)
Ophiostoma sp. 1					3	(13.6)	3	(12.0)	6	(5.3)
Leptographium trypodendroni	1	(3.3)	1	(3.3)	1	(4.5)			3	(2.5)
Ophiostoma brevicolle-like			1	(3.3)	1	(4.5)	1	(4.0)	3	(2.5)
Sporothrix sp. 1	2	(6.7)							2	(1.8)
Leptographium sp. 1							1	(4.0)	1	(0.1)
Grosmannia piceiperda							1	(4.0)	1	(0.1)
Number of investigated beetles	30		30		22		25		107	

3.1.2 Dryocoetes villosus

Dryocoetes villosus was samples at only one site (Larvik) and three ophiostomatoid fungi were isolated from this species. The most common ophiostomatoid fungus was *O. quercus*-like, which was isolated from 26.7% of the investigated beetles. *Ophiostoma quercus* was isolated from 18.3% of the beetles and *S. dentifunda* was found on two of the total 60 investigated specimens (Table 3).

Table 3. Ophiostomatoid fungi isolated from *Dryocoetes villosus* infesting *Quercus robur*. Numbers represent the total number of individual beetles carrying the given fungus (N) and the percentage of individual beetles carrying the given fungus per total number of investigated specimens (%).

Fungus/bark beetle	*Dryocoetes villosus*	
	N	(%)
Ophiostoma quercus-like	16	(26.7)
Ophiostoma quercus	11	(18.3)
Sporothrix dentifunda	2	(3.3)
Number of investigated beetles	60	

3.1.3 Hylesinus crenatus

Hylesinus crenatus was sampled from *Fraxinus excelsior* L. and as associated with two ophiostomatoid fungi. *Ophiostoma catonianum*-like was the most common and was fond on 60.1% of the beetles. *Ophiostoma karelicum* was isolated only from two beetles, both sampled in Ås (Table 4).

Table 4. Ophiostomatoid fungi isolated from *Hylesinus crenatus* infesting *Fraxinus excelsior*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/bark beetle	*Hylesinus crenatus*					
	Larvik		Ås		Total	
	N	(%)	N	(%)	N	(%)
Ophiostoma catonianum-like	9	(69.2)	19	(57.6)	28	(60.1)
Ophiostoma karelicum			2	(6.1)	2	(4.2)
Number of investigated beetles	13		33		46	

3.1.4 Hylesinus varius

Hylesinus varius was sampled from *Fraxinus excelsior* and was only associated with one ophiostomatoid fungi. *Ophiostoma catonianum*-like was only found on one specimen collected in Ås (Table 5). It should be mentioned that *H. varius* was associated with three *Geosmithia* species and that 81% of the specimens were carrying at least one species of *Geosmithia.*

Table 5. Ophiostomatoid fungi isolated from *Hylesinus varius* infesting *Fraxinus excelsior*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/bark beetle	*Hylesinus varius*					
	Larvik		Ås		Total	
	N	(%)	N	(%)	N	(%)
Ophiostoma catonianum-like			1	(3.2)	1	(1.6)
Number of investigated beetles	30		31		61	

3.1.5 Scolytus intricatus

Three ophiostomatoid fungi were isolated from *S. intricatus* (Table 6). *Sporothrix foliorum*-like was the most common fungus and was isolated from 48% of the investigated beetles and had quite similar frequencies at the two sampling sites. *Ophiostoma quercus* was also quite commonly found and was present at 42.7% of the beetles. *Sporothrix prolifera* was present found on 24% of the beetles.

Table 6. Ophiostomatoid fungi isolated from *Scolytus intricatus* infesting *Quercus sp*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/bark beetle	*Scolytus intricatus*					
	Hurum		Ås		Total	
	N	(%)	N	(%)	N	(%)
Sporothrix foliorum-like	19	(45.2)	17	(51.5)	36	(48.0)
Ophiostoma quercus	20	(47.6)	12	(36.4)	32	(42.7)
Sporothrix prolifera	8	(19)	10	(30.3)	18	(24.0)
Number of investigated beetles	42		33		75	

3.1.6 Scolytus laevis

Scolytus laevis was only found at one location in Oslo. *Ophiostoma novo-ulmi* was transported by 50.8% of the investigated beetles and was the only ophiostomatoid fungi associated with this bark beetle (Table 6).

Table 6. Ophiostomatoid fungi isolated from *Scolytus laevis* infesting *Ulmus glabra*. Numbers represent the total number of individual beetles carrying the given fungus (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles (%).

Fungus/bark beetle	*Scolytus laevis*	
	N	(%)
Ophiostoma novo-ulmi	31	(50.8)
Number of investigated beetles	61	

3.1.7 Scolytus ratzeburgi

Scolytus ratzeburgi was sampled from four locations, but two of the locations (Ski and Ås) are very close and beetles sampled from these two sites were treated together. Two ophiostomatoid fungi were found. *Ophiostoma karelicum* was by far the most common, and was isolated from 90.7% of the investigated specimens. *Ophiostoma quercus* was only found on two beetles (Table 7).

Table 7. Ophiostomatoid fungi isolated from *Scolytus ratzeburgi* infesting *Betula* sp. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/ bark beetle	*Scolytus ratzeburgi*							
	Hurum		Ås/ski		Aurskog-Høland		Total	
	N	(%)	N	(%)	N	(%)	N	(%)
Ophiostoma karelicum	11	(91.7)	10	(83.3)	28	(93.3)	49	(90.7)
Ophiostoma quercus	1	(8.3)	1	(8.3)			2	(3.7)
Number of investigated beetles	12		12		30		54	

Xylem breeding bark beetles

3.1.8 Anisandrus dispar

Anisandrus dispar was only found at one location in Ås infesting *Q. robur*. Three ophiostomatoid species were isolated. (Table 8). *Sporothrix lunata*-like was the most common (48.6%). *Ophiostoma quercus* was also quite frequently found (31.4%) while *S. foliorum*-like was only occasionally isolated (8.6%).

Table 8. Ophiostomatoid fungi isolated from *Anisandrus dispar* infesting *Quercus robur*. Numbers represent the total number of individual beetles carrying the given fungus (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles (%).

Species/beetle	*Anisandrus dispar*	
	N	(%)
Sporothrix lunata-like	17	(48.6)
Ophiostoma quercus	11	(31.4)
Sporothrix foliorum-like	3	(8.6)
Number of investigated beetles	35	

3.1.9 Trypodendron domesticum

Trypodendron domesticum was sampled from seven sites and from four different host tree species. A total of 8 ophiostomatoid species were found; *O. karelicum*-like, *O. karelicum, O. quercus, O. brevicolle*-like, *Ophiostoma* sp. 1, *L. trypodendroni*, *G. fragrans* and *Sporothrix lunata*-like (Table 12). Most of the specimens were collected from *A. incana* (81 specimens) and the results for these beetles are presented in Table 9. The rest of the *T. domesticum* specimens were sampled from *Fraxinus excelsior* (8 specimens), *Quercus robur* and (4 specimens) and *Betula pubescens* (30 specimens) and are presented in Table 10.

Trypodendron domesticum infesting *A. incana*

Ophiostoma karelicum-like was the most common fungus associated with the specimens sampled from *A. incana* and was found on 100% of the specimens infesting this host at all sites except in Ås where the frequency was 76.7%. *Leptographium trypodendroni* was also commonly isolated and was found on 59.3% of the specimens. This species was only absent from the material sampled in Kvæfjord (only two investigated specimens). The highest frequency for this fungus was found on the material from Larvik (76.7%). *Graphilbum fragrans* was quite common on the material from Nes, were it was present at 42.1% of the beetles. This specie was only found on one of the beetles from Larvik and was absent from the rest of the locations. *Ophiostoma brevicolle*-like was only occasionally found but was present at all locations except Ås and was most frequently isolated from the beetles from Larvik. *Ophiostoma* sp. 1 was only transported by one beetle sampled in Nes.

Table 9. Ophiostomatoid fungi isolated from *Trypodendron domesticum* infesting *Alnus incana*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/bark beetle	*Trypodendron domesticum*									
	Larvik		Ås		Nes		Kvæfjord		Total	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Ophiostoma karelicum-like	30	(100)	23	(76.7)	19	(100)	2	(100)	74	(91.4)
Leptographium trypodendroni	23	(76.7)	13	(43.3)	12	(63.2)			48	(59.3)
Graphilbum fragrans	1	(3.3)			8	(42.1)			9	(11.1)
Ophiostoma brevicolle-like	4	(13.3)			1	(5.3)	1	(50.0)	6	(7.4)
Ophiostoma sp. 1					1	(5.3)			1	(1.2)
Number of investigated beetles	30		30		19		2		81	

Trypodendron domesticum infesting other host-tree species

Ophiostoma karelicum was also the most common fungus isolated from *T. domesticum* infesting *B. pubescens* (86.7%) and *F. excelsior* (37.5%), but it was not found on the beetles infesting *Q. robur* (only 4 specimens investigated). *Ophiostoma brevicolle*-like was found on beetles infesting *Q. robur* and *F. excelsior,* but was absent from the beetles infesting *B. pubescens. Ophiostoma karelicum* was occasionally found on beetles infesting *B. pubecense* (20%), but was also found on two *T. domesticum* specimens infesting *F. excelsior*. *S. lunata*-like and *O. quercus* were only found on beetles infesting *Q. robur* (Table 10).

Table 10. Ophiostomatoid fungi isolated from *Trypodendron domesticum* infesting *Fraxinus excelsior*, *Quercus robur* and *Betula pubescens*. Numbers represent the total number of individual beetles carrying the given fungus at each site (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles at each site (%).

Fungus/bark beetle	*Trypodendron domesticum*					
	Ås (*F. excelsior*)		Ås (*Q. robur*)		Aurskog-Høland (*B. pubescens*)	
	N	(%)	N	(%)	N	(%)
Ophiostoma karelicum-like	3	(37.5)			26	(86.7)
Leptographium trypodendroni	2	(25.0)			10	(33.3)
Ophiostoma karelicum	2	(25.0)			6	(20.0)
Ophiostoma brevicolle-like	2	(25.0)	1	(25.0)		
Sporothrix lunata-like			3	(75.0)		
Ophiostoma quercus			1	(25.0)		
Number of investigated beetles	8		4		30	

3.1.10 Trypodendron signatum

Trypodendron signatum was sampled from one location in Målselv and all specimens were collected from *A. incana*. Four ophiostomatoid fungi were found; *O. brevicolle*-like, *O. bacillisporum*-like, *O. karelicum*-like and *G. fragrans* (Table 11). The most common fungus was *O. brevicolle*-like, which was isolated from 95.1% of the investigated specimens. Another very common fungus associated with this bark beetle was *O. bacillisporum*-like, transported by 80.5% of the beetles. *Ophiostoma karelicum*-like was isolated from 24.4% of the beetles while *G. fragrans* was only occasionally found (12.2%).

Table 11. Ophiostomatoid fungi isolated from *Trypodendron signatum* infesting *Alnus incana*. Numbers represent the total number of individual beetles carrying the given fungus (N) and the percentage of individual beetles carrying the given fungus per total number of investigated beetles (%).

Species/bark beetle	*Trypodendron signatum*	
	N	(%)
Ophiostoma brevicolle-like	39	(95.1)
Ophiostoma bacillisporum-like	33	(80.5)
Ophiostoma karelicum-like	10	(24.4)
Graphilbum fragrans	5	(12.2)
Number of investigated beetles	41	

3.1.11 Overview of fungal species

Many of the fungal species were found associating with several beetle species. An overview of the ophiostomatoid fungi and their frequencies are presented in Table 12.

Table 12. Percentages of individual beetles carrying the respective fungus per total number of investigated bark beetles sampled from each host tree.

Fungu species/bark beetle	D. aln	T. dom	T. sig	T. dom	S. rat	D.aln	H. cre	H. var	T. dom	S. lae	A. dis	T. dom	S. int	D. vill
Graphilbum fragrans	6.5	11.1	12.2											
Grosmannia piceiperda	0.1													
Leptographium trypodendroni	2.5	59.3		33.3					25					
Leptographium sp 1.	0.1													
Ophiostoma bacillisporum-like			80.5											
O. brevicolle-like	2.5	7.4	95.1						25			25.0		
O. catonianum-like							60.9	1.6						
O. karelicum				20.0	90.7		4.3		25.0					
O. karelicum-like	22.3	91.4	24.4	86.7					37.5					
O. novo-ulmi										50.8				
Ophiostoma sp. 1	5.3	1.2												
O. quercus					3.7						31.4	25.0	42.7	18.3
O. quercus-like														26.7
Sporothrix dentifunda														3.3
Sporothrix lunata-like											48.6	75.0		
S. foliorum-like											8.6		48.0	
S. prolifera													24.0	
Sporothrix sp. 1	1.8													
Number of investigated beetles	107	81	41	30	54	7	46	62	8	61	35	4	75	60

Bark beetle: A. dis, *Anisandrus dispar*; D. aln, *Dryocoetes alni*; D. vill, *Dryocoetes villosus*; H. cre, *Hylesinus crenatus*; H. var, *Hylesinus varius*; S. int, *Scolytus intricatus*; *S. lae*, *Scolytus laevis*; S.rat, *Scolytus ratzeburgi*; T. dom, *Trypodendron domesticum*; T. sig, *Trypodendron signatum.*

Host-tree are indicated by color: Yellow; *Alnus incana*. Gray; *Betula sp*. Brown; *Corylus avellana* Blue; *Fraxinus excelsior*. Green; *Ulmus glabra*. Orange; *Quercus sp*

3.2 Phylogenetic analyses

Based on the identity matches in BLAST and multiple alignment comparison using the ITS and ITS2-LSU data the sequences were separated in five groups; species within the *Ophiostoma ulmi*-complex, species within *Sporothrix*, species within *Leptographium sensu lato*, species within *Graphilbum* and a group containing species with uncertain taxonomic position.

3.2.1 Species within the *Ophiostoma ulmi* complex

Nine of the morphological group were closely related to species within the *Ophiostoma ulmi* complex within the *Ophiostoma sensu stricto*. Neighbor joining analyses based on the ITS, β-tubulin, EF1-α and calmodulin sequences were performed for this species group (Figs 1 & 2).

The ITS analysis (Fig. 1) grouped the isolates in three clades. The clade treated here as the *O quercus* group was not resolved well and contained four morphological groups. This species complex was much better resolved using protein coding regions (Fig. 2 a & b). The analyses of the β-tubulin, EF1-α and calmodulin regions correspond well with the morphological grouping and separates the isolates in four groups, treated here as; *O. quercus*-like, O. *bacillisporum*-like, *O. catonianum*-like (named *O. catonianum* spp. in the β-tubulin analysis) and *O. quercus*. No calmodulin regions were available in the GenBank for species within this group, but the calmodulin analysis (Fig. 2 c) showed the same branching as β-tubulin (Fig. 2 a) and EF1-α (Fig. 2 b). The upper clade, named *O. quercus*-like in Fig. 2 ab & c, consists of strains isolated from *D. villosus*. These isolates did not group close to any other species in this complex, neither when using β-tubulin or EF1-α. Even in the ITS analysis these isolates resided in a distinct clade, but with bootstrap value lower than 70% (Fig. 1). These isolates were treated as *O. quercus*-like. A second group of isolates, grouped in the same clade as *Ophiostoma catonianum* Goid. in the β-tubulin dataset, but there were some differences between the *O. catonianum* and the isolates obtained in this study (Fig. 2 a). The EF1-α analysis showed higher resolution and placed the isolates in a clade with high support distinct from *O. catonianum* (Fig. 2 b), indicating that this might be a different species and the isolates were treated as *O. catonianum*-like. The third clade, named *O. bacillisporum*-like, consists of isolates originating from *T. signatum*. The strains grouped in a clade close but distinct to an unverified isolate *of Ophiostoma bacillisporum* (Butin & G. Zimm.) de Hoog & R.J. Scheff. from the GenBank (Fig. 2 a & b) and the isolates

were treated as *Ophiostoma bacillisporum*-like. The last group of isolates cluster in the same clade as *O. quercus*, but the genetic variability was quite large within this group (Fig. 2)

A second morphological group, isolated from *S. leavis*, grouped with *O. novo-ulmi* in the ITS analysis (Fig. 1). The identity was also confirmed by β-tubulin (Fig. 2 a).

The last four morphological groups belonged in clade with high support together with *O. karelicum* in the ITS analysis, treated her as the *O. karelicum* group (Fig 1). The isolates originated from many bark beetle species. The isolates showed large variation in color and were believed to represent different species. Isolates from *S. ratzeburgi* were always hyaline while the isolates originating from other spices varied from hyaline to dark brown. The β-tubulin and Ef1-α analyses separated the strains in two clades with high support, but the variability was relatively high within each group (Fig. 3). Both clads contained strains treated as *O. karelicum* from the GenBank but were still believed to represent two different species. The clade treated as *O. karelicum* mostly contained the hyaline isolates from bark beetles infesting *Betula* sp. and also many *O. karelicum* sequences from the GenBank and was treated *as O. karelicum*. The second clade treated as *O. karelicum*-like mainly contained isolates originating from bark beetles infesting as *A. incana*. These isolates showed large variation in color ranging from hyaline to dark brown. However, color was not found to be taxonomically informative and color was also later found to vary between isolates of the same strain. The *O. karelicum* sequences from the GenBank within this clade originates from an unpublished study regarding fungi associated with *Trypodendron lineatum* (Olivier, 1795) and is probably not *O. karelicum*. The isolates in this clade was treated as *O. karelicum*-like.

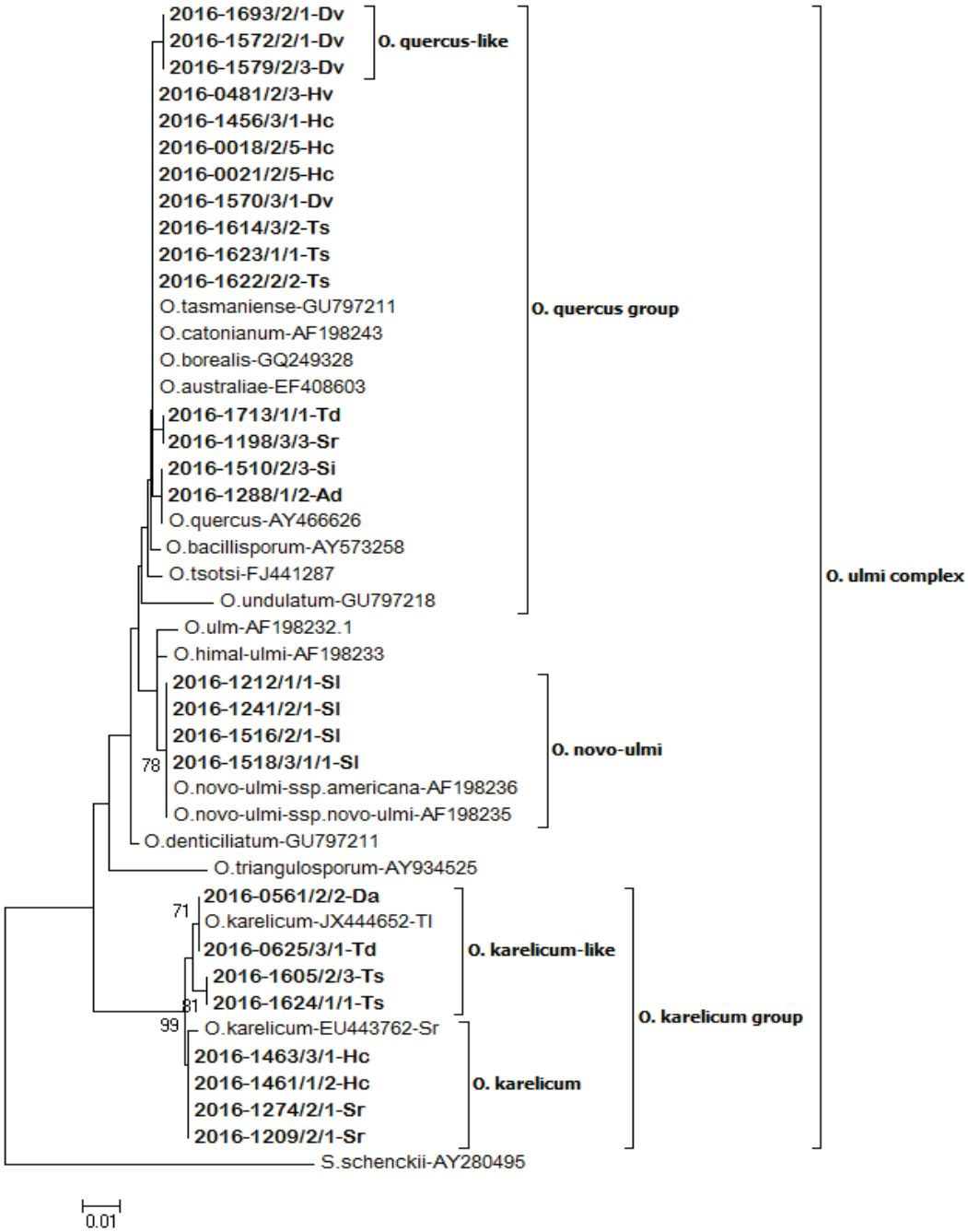

Figure 1. Phylogram based on ITS sequences of species in the O. novo-ulmi complex. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 363 positions in the final dataset. Isolates sequenced in this study are printed in bold. Bar= number of base pair differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Da, *Dryocoetes alni*; Dv, *Dryocoetes villosus*; Hc, *Hylesinus crenatus*; Hv, *Hylesinus varius*; Si, *Scolytus intricatus*; Sl, *Scolytus laevis*; Sr, *Scolytus ratzeburgi*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum*; Tl, *Trypodendron lineatum*.

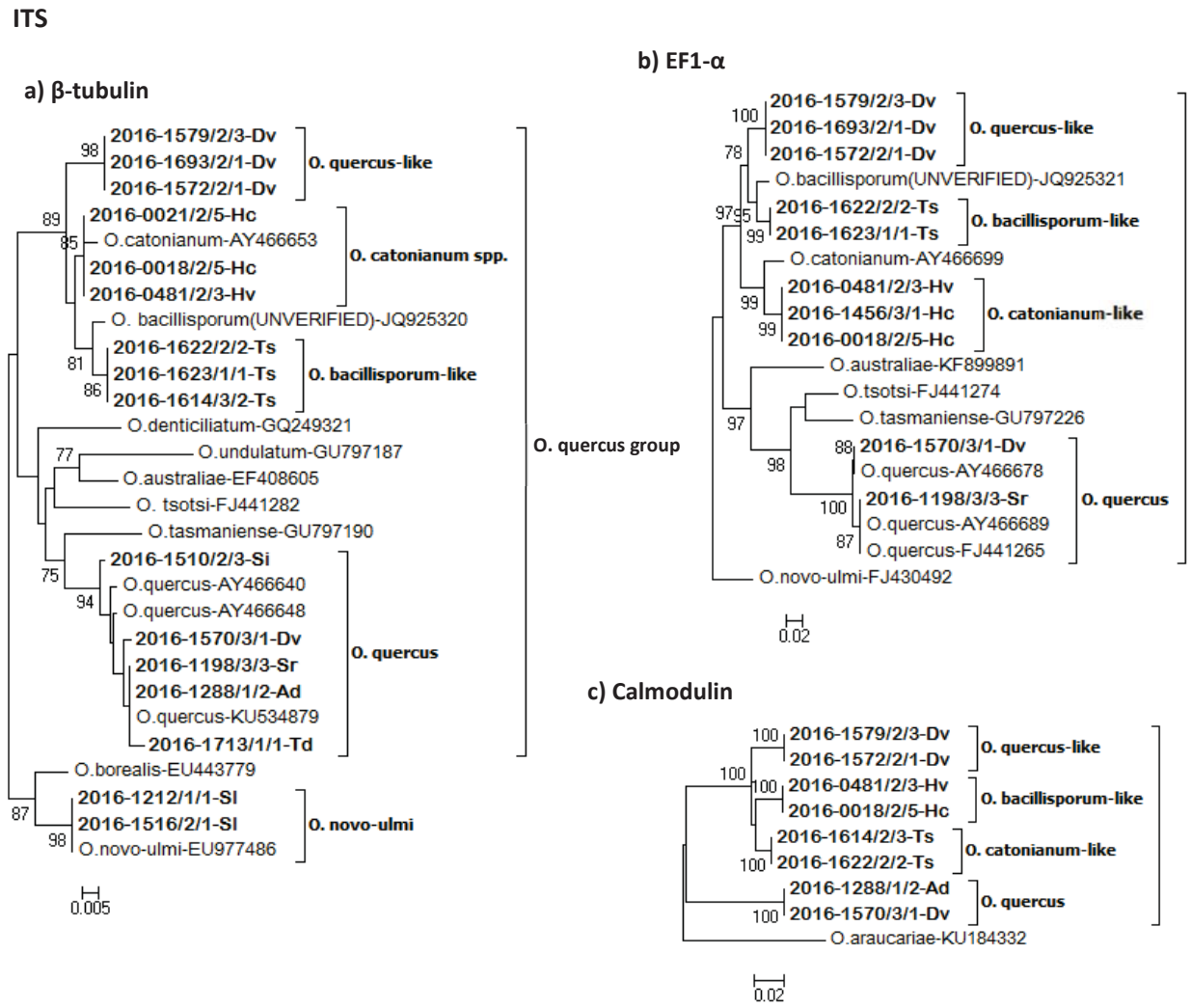

Figure 2. Phylogram based on (**a**) β-tubulin and (**b**) EF1-α and (**c**) calmodulin sequences of species in the *O. quercus* group rooted to (**a** & **b**) *O. novo ulmi* or to (**c**) *O. araucariae*. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 249 positions in the β-tubulin dataset, 190 positions in the EF1α dataset and 539 positions in the calmodulin dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Dv, *Dryocoetes villosus*; Hc, *Hylesinus crenatus*; Hv, *Hylesinus varius*; Si, *Scolytus intricatus*; Sl, *Scolytus laevis*; Sr, *Scolytus ratzeburgi*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum*

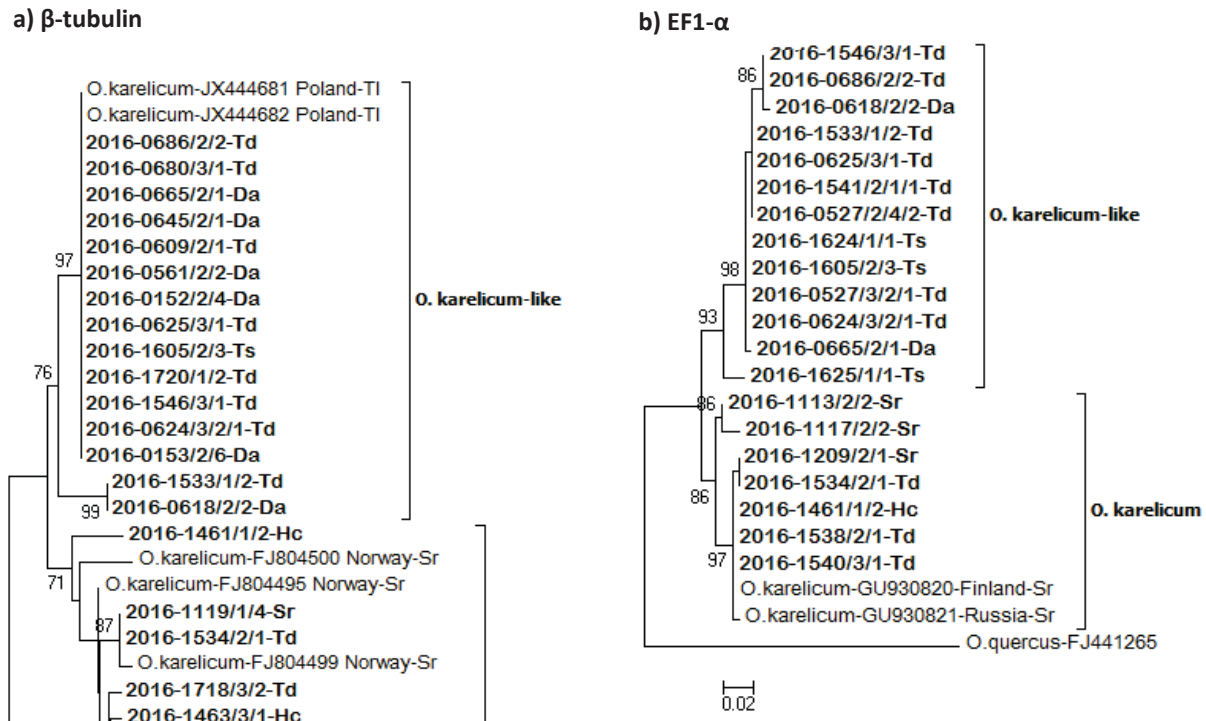

Figure 3. Phylogram based on (**a**) β-tubulin (**b**) EF1-α sequences of species in the *O. karelicum* group. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 320 positions in the β-tubulin dataset and 257 positions in the EF1-α dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate: Da, *Dryocoetes alni*; Hc, *Hylesinus crenatus*; Sr, *Scolytus ratzeburgi*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum*; Tl, *Trypodendron lineatum*.

3.2.2 Species within *Sporothrix*

The *Sporothrix* isolates consisted of three morphological groups, but the ITS analysis showed that one of the groups consisted of two different species and thus revealed four taxa (Fig. 4). Three of the taxa belonged in the *Sporothrix gossypina* complex defined by (De Beer et al. 2016)

One of the morphological groups was by sequencing divided in two, one in a well-supported clade with *Sporothrix prolifera* and the other in a clade with low support close to *Sporothrix lunata* (Aghayeva & M.J. Wingf.) Z.W. de Beer, T.A. Duong & M.J. Wingf. (Fig. 4). The separation of these isolates was vector specific. The isolates originating from *S. intricatus*

grouped with *S. prolifera* while the isolates close to *S. lunata* originated from ambrosia beetles (*A. dispar* and *T. domesticum*). The identity of the *S. prolifera*-isolates was also confirmed by β-tubulin and calmodulin (Fig. 5 a & b). The isolates close to *S. lunata* in the ITS analysis was not resolved well in the β-tubulin dataset (Fig. 5 a), but grouped in a well-supported clade close, but distinct from *S. lunata* in the calmodulin dataset. These isolates were treated as *S. lunata*-like.

A second morphological group (only represented by two isolates) clustered in a clade with low bootstrap value within the S. *gossypina complex* in the ITS analysis, named *Sporothrix* sp. 1 (Fig. 4). The β-tubulin dataset also showed low resolution for this species and the isolates were grouped together with the *S. lunata*-like isolates (Fig. 5 a). The two isolates grouped in well supported clade in the calmodulin dataset (Fig. 5 b), indicating that this might be a new species. The isolates were treated as *Sporothrix* sp. 1.

The last *Sporothrix* isolates (only represented by two isolates) clustered in a well-supported clade with *Sporothrix dentifunda* within the *Sporothrix inflata* complex in the ITS analysis (Fig. 4). The identity was also confirmed by β-tubulin (Fig. 5 a).

ITS

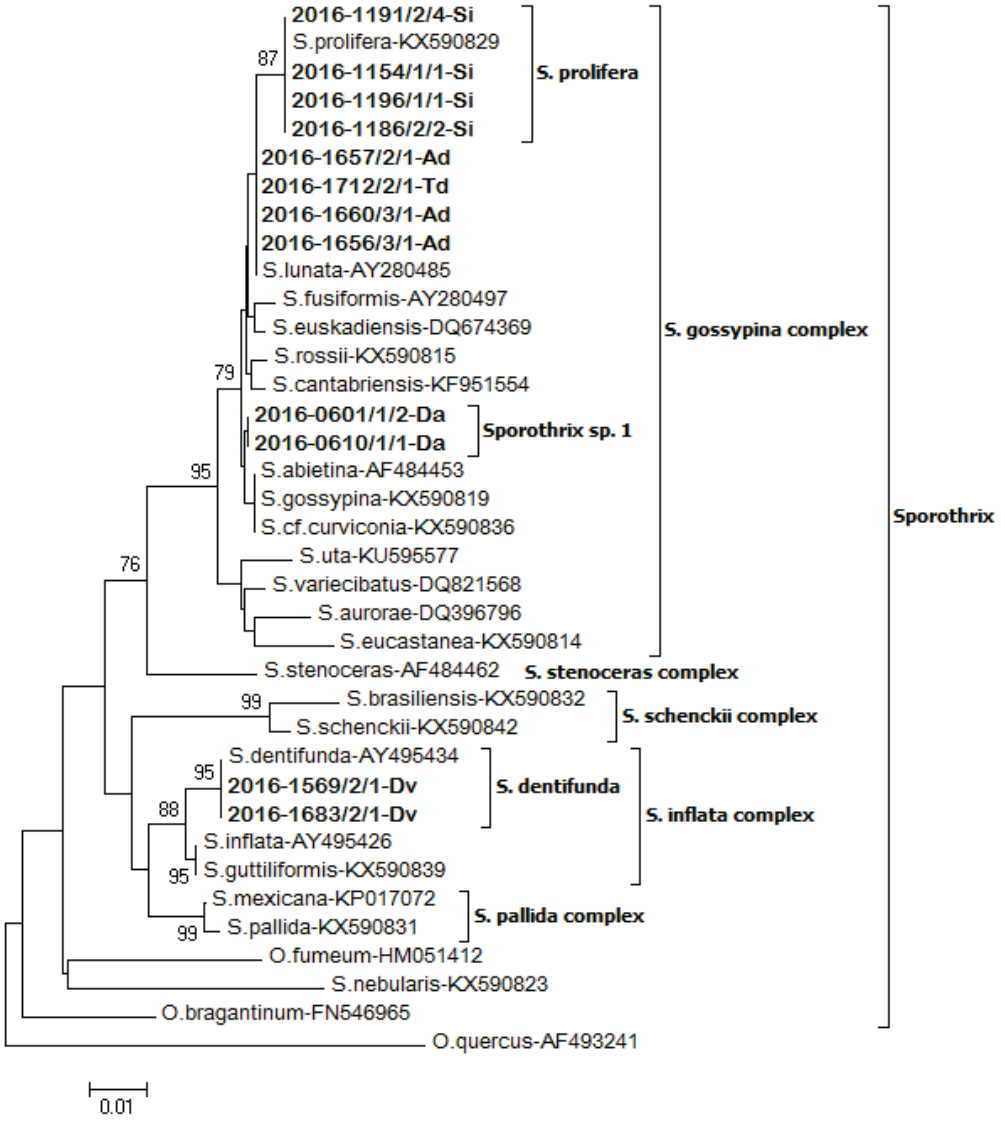

Figure 4. Phylogram based on ITS sequences of sporothrix species. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 380 positions in the final dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Da, *Dryocoetes alni*; Dv, *Dryocoetes villosus*; Si, *Scolytus intricatus*; Td, *Trypodendron domesticum.*

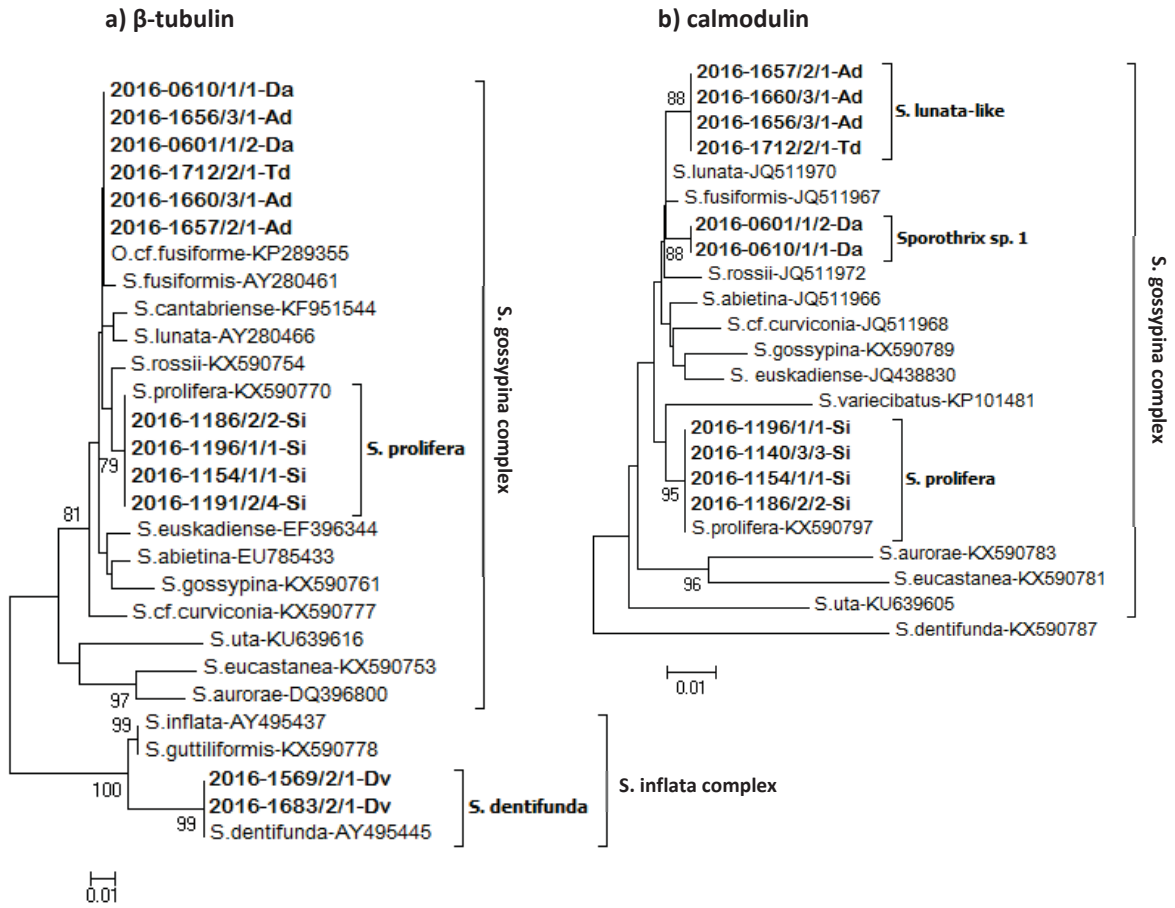

Figure 5. Phylogram based on (**a**) the β-tubulin gene and (**b**) the calmodulin gene of species in the *Sporothrix gossypina* complex. (**a**) also include the *Sporothrix inflata* complex. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 209 positions in the β-tubulin dataset and 383 positions in the calmodulin dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Da, *Dryocoetes alni*; Dv, *Dryocoetes villosus*; Si, *Scolytus intricatus*; Td, *Trypodendron domesticum.*

3.2.3 Species within *Leptographium sensu lato*

Four morphological groups (two of the groups were only represented by a single isolate) formed part of *Leptographium sensu lato*. The isolates were treated in a dataset containing ITS sequences and in a dataset containing ITS2-LSU sequences. Both analysis corresponded well with the morphological grouping and separated the isolates in four different clades (Fig. 6 a & b).

One of the isolates (2015-1559/3/2) was placed in the *G. piceiperda* complex in both datasets. (Fig. 6 a & b). The ITS analysis (Fig. 6 b) could not distinguish between the species in this complex, but the ITS2-LSU analysis showed more resolution and placed the isolate close to

Grosmannia piceiperda in a clade with low support (Fig. 6 b). The β-tubulin analysis placed the isolate in a clade together with *G. piceiperda*-isolates from Europe (Fig. 7). The EF1-α sequence was also most similar to a European isolate of *G. piceiperda* (99,8%), but the sequence was too short to be included in a similar analysis.

The second single isolate (2015-1559/3/4) was placed close to *Leptographium taigense* Linnakoski, Z.W. de Beer & M.J. Wingf. and *Leptographium. innermongolicum* W. Liu, Q. Lu & X.Y. Zhang in the ITS2-LSU analysis (Fig. 6 b). This species complex, treated here as as the *L. taigense* complex, could not be included in the ITS analysis because no ITS sequences were available. In the ITS analysis (Fig. 6 a) the isolate was placed close to *O. abieticola* Yamaoka & Masuya, which could not be included in the ITS2-LSU dataset. The β-tubulin analysis (Fig. 8 a) showed that the isolate is distinct from *O. abieticola,* and placed the isolate in a clade with low support together with an undescribed Grosmannia isolate from Japan (Grosmannia sp. TCL-2009). No EF1-α sequence was available for the undescribed *Grosmannia* species and it could not be included in the EF1-α analysis. The EF1-α analysis revealed more variation within this complex and separated *L. taigense* and *L. innermongolicum* (Fig. 8 b). The EF1-α analysis showed that the isolate is distinct from both *L. innermongolicum* and *L. taigense,* and was placed in a low supported clade together with an undescribed *Leptographium*-isolate from USA (Leptographium sp 3-SJT-2013). The identification of this isolate was inconclusive and was treated as *Leptographium* sp. 1.

Another morphological group clustered in a distinct clade both in the ITS analysis and the ITS2-LSU analysis, treated as *O. brevicolle*-like (Fig. 6 a & b). In the ITS analysis, the isolates grouped in a well-supported clade close to *Ophiostoma brevicolle* (R.W. Davidson) de Hoog & R.J. Scheff. and *Grosmannia francke-grosmanniae* (R.W. Davidson) de Hoog & R.J. Scheff., next to the *Grosmannia olivacea* complex (Fig. 6 a). These isolate also formed a distinct lineage next to the *G. olivacea* complex in the ITS2-LSU analysis (Fig. 6 b). However, *O. brevicolle* and *G. francke-grosmanniae* were placed very differently when analyzing this gene region (Fig. 6) The conflicting taxonomic placement of these two species is also mentioned by (De Beer & Wingfield 2013). The high bootstrap value in the ITS analysis indicate that the isolates found in this study are distinct from *O. brevicolle* (Fig. 6 a), but this could not be confirmed because no protein coding gene-regions are available for this species. The β-tubulin sequences revealed some differences between the isolates obtained in this study and separated the isolates in two clades (Fig 8 a). However, the bootstrap-values are low for both clades. One of the isolates from

each group was sequenced using EF1-α, and these were identical (Fig. 8 b). The isolates were treated as *Ophiostoma brevicolle*-like.

Another morphological group of *Leptographium*-isolates belonged in a well-supported clade together with *Leptographium pruni* Masuya & M.J. Wingfield and an undescribed species treated as *Leptographium* cf. *pruni* in the ITS analysis (Fig. 6 a). I have chosen to treat this complex as the *L. pruni* complex. The isolates also grouped in this complex in the ITS2-LSU analysis, but the *L.* cf. *pruni* could not be included (Fig. 6 b). The β-tubulin dataset reveled more variation at species level and showed that the isolates are close to *L.*cf. *pruni* and distinct from *L. pruni* (Fig. 8 a). No EF1-α sequences of *L.*cf. *pruni* or *L. pruni* were available, but the EF1-α analysis showed some variation between the isolates obtained in this study (Fig. 8 b). The ITS, ITS2-LSU, β-tubulin and EF1-α sequences have been sent to Dr. Robert Jankowiak who could confirm that this is *Leptographium trypodendroni*, which is about to be described (paper submitted).

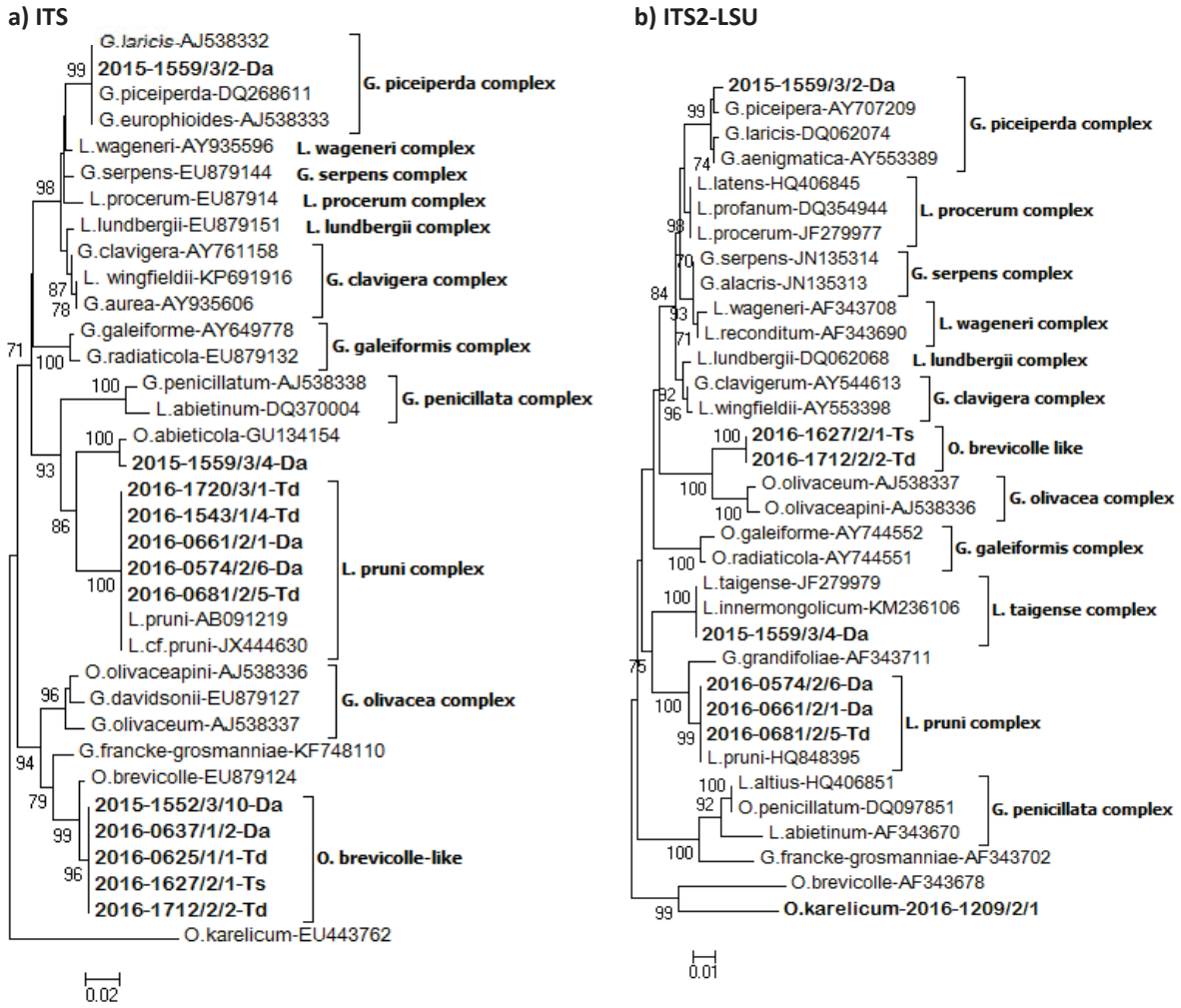

Figure 6. Phylogram based on (**a**) ITS and (**b**) ITS2-LSU sequences of *Leptographium* species. Bootstrap support values (1000 replicates) above 70% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 306 positions in the ITS dataset and 496 positions in the ITS2-LSU dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Da, *Dryocoetes alni*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum.*

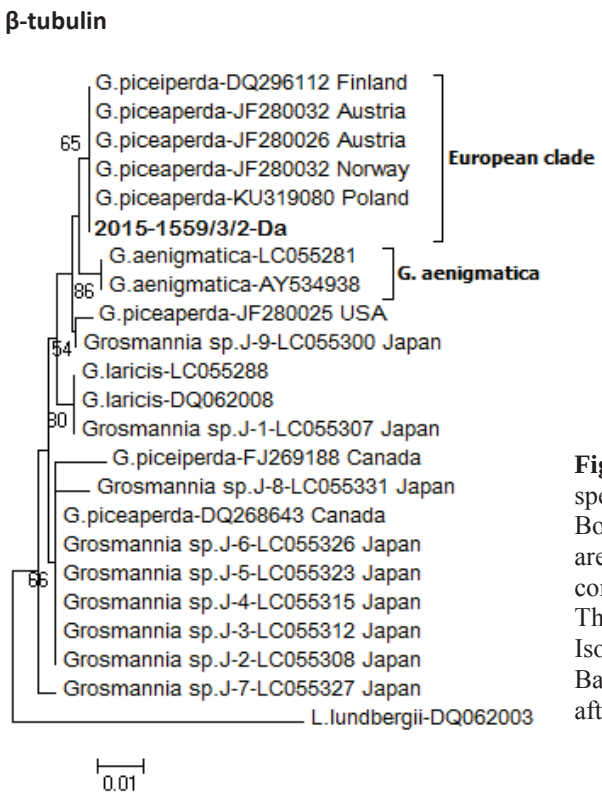

Figure 7. Phylogram based on β-tubulin sequences of species in the *Grosmannia piceiperda* complex. Bootstrap support values (1000 replicates) above 50% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 277 positions in the final dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; *Da*, *Dryocoetes alni*

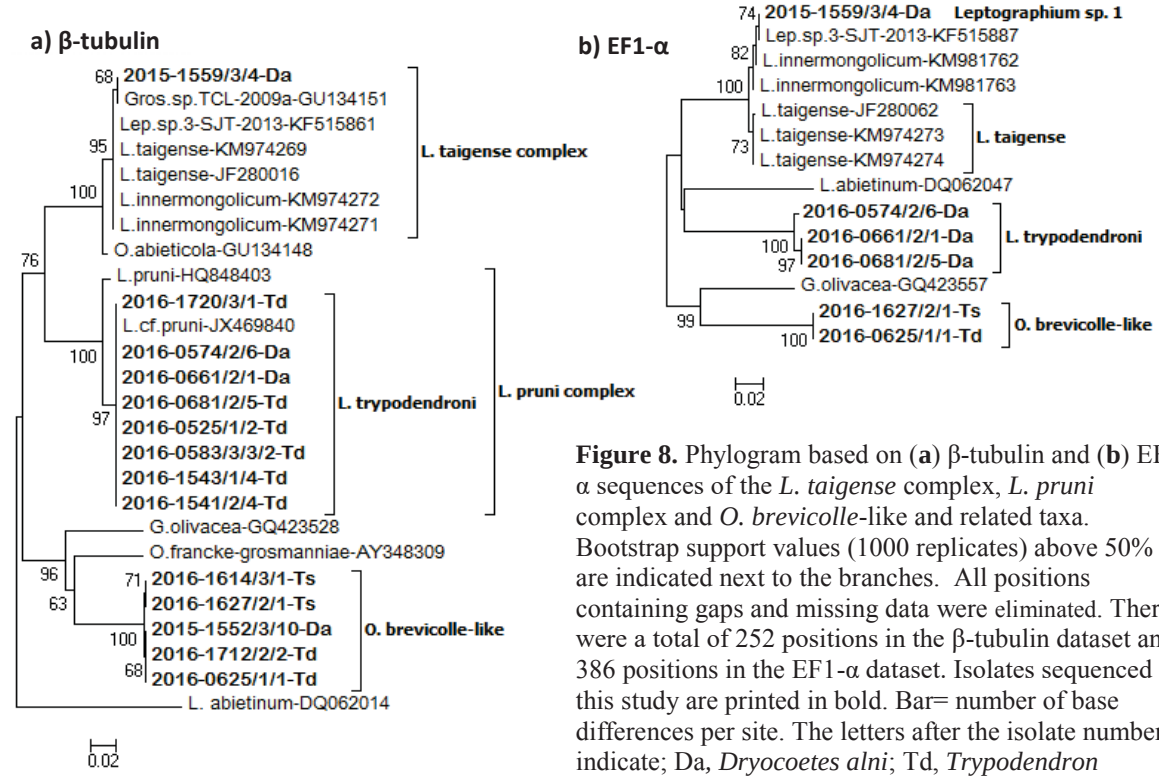

Figure 8. Phylogram based on (**a**) β-tubulin and (**b**) EF1-α sequences of the *L. taigense* complex, *L. pruni* complex and *O. brevicolle*-like and related taxa. Bootstrap support values (1000 replicates) above 50% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 252 positions in the β-tubulin dataset and 386 positions in the EF1-α dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Da, *Dryocoetes alni*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum.*

3.2.4 Species within *Graphilbum*

One morphological group was closely related to species within *Graphilbum*. The isolates were placed in a lineage with high bootstrap value with *Graphilbum fragrans* and *G. microcarpum* (Yamaoka & Masuya) Z.W. de Beer & M.J. Wingf. in the ITS analysis (Fig. 9 a). No protein coding sequences were available for *G. microcarpum*, so this species could not be included in the β-tubulin dataset which contained *G. fragrans* isolates from Poland and China. The sequences from this study grouped in a clade with low support with the *G. fragrans* isolate from Poland. (Fig. 9 b).

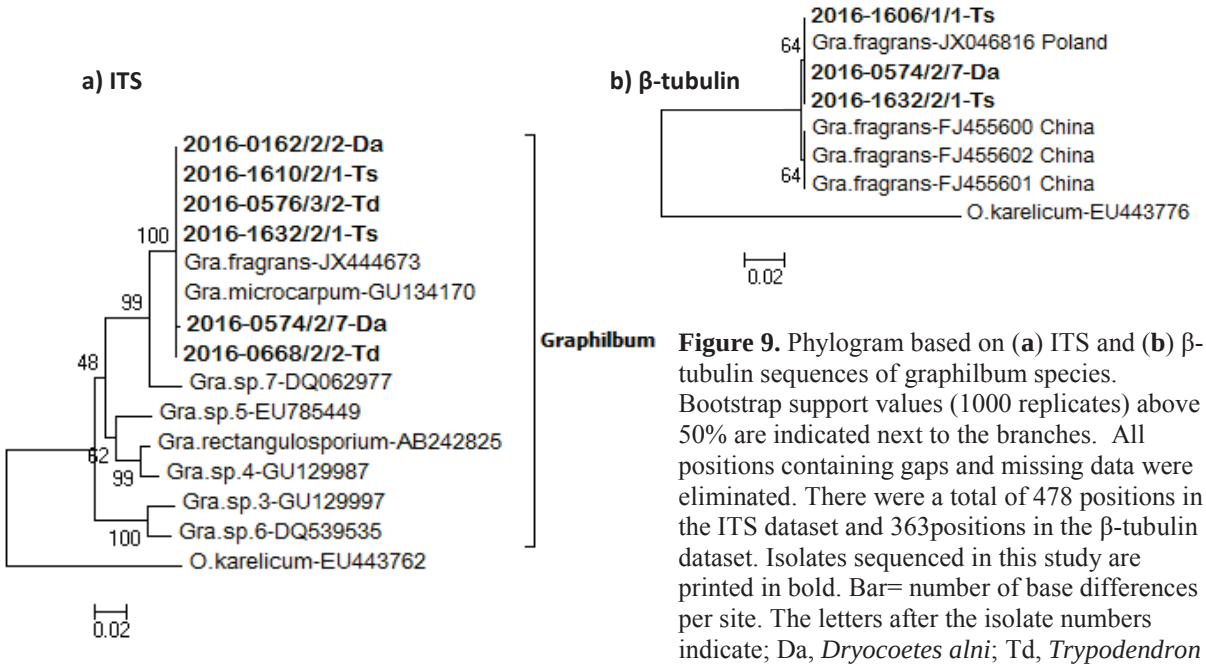

Figure 9. Phylogram based on (**a**) ITS and (**b**) β-tubulin sequences of graphilbum species. Bootstrap support values (1000 replicates) above 50% are indicated next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 478 positions in the ITS dataset and 363positions in the β-tubulin dataset. Isolates sequenced in this study are printed in bold. Bar= number of base differences per site. The letters after the isolate numbers indicate; Da, *Dryocoetes alni*; Td, *Trypodendron domesticum*; Ts, *Trypodendron signatum.*

3.2.5 Species with uncertain taxonomic position

Two morphological groups could not be placed in a species complex based on the BLAST searches. The species were included in an ITS analysis containing the major clades within the Ophiostomatales (Fig. 10). Both species were treated together when analyzing the β-tubulin and EF1-α regions. The phylogeny presented here is just meant as an indication of their taxonomic position. More conserved gene regions, like LSU, are probably needed to place these isolated more correctly according to neighboring clades.

One of the species was similar to *Sporothrix foliorum* J.J. Taylor in the ITS region (Fig. 10). However, the taxonomic position of *S. foliorum* is uncertain (De Beer et al. 2016). The ITS analysis indicated that *S. foliorum* and the isolates obtained in this study do not form part *Sporothrix*, but probably somewhere else in *Ophiostoma sensu lato*. The β-tubulin analysis did only contain the isolates obtained in this study because no β-tubulin sequences are available for *S. foliorum*. The analysis revealed some variation within the group and separated the isolates obtained from *Anisandrus dispar* and *Scolytus intricatus,* indicating that the isolates could represent two species (Fig. 11 a). However, the EF1-α analysis grouped the isolates differently, but showed that the isolates are distinct from *S. foliorum*. Because of the conflicting grouping the isolates were treated as one species, *Sporothrix foliorum*-like.

The second group of isolates had very low BLAST identities (<88%). The ITS analysis indicated that this species also forms a distinct clade within the *Ophiostoma sensu lato* (Fig. 10). Both the EF1-α and β-tubulin analyses (Fig. 11) showed little variability and this group of isolates were also considered to represent one species and was treated as *Ophiostoma* sp.1.

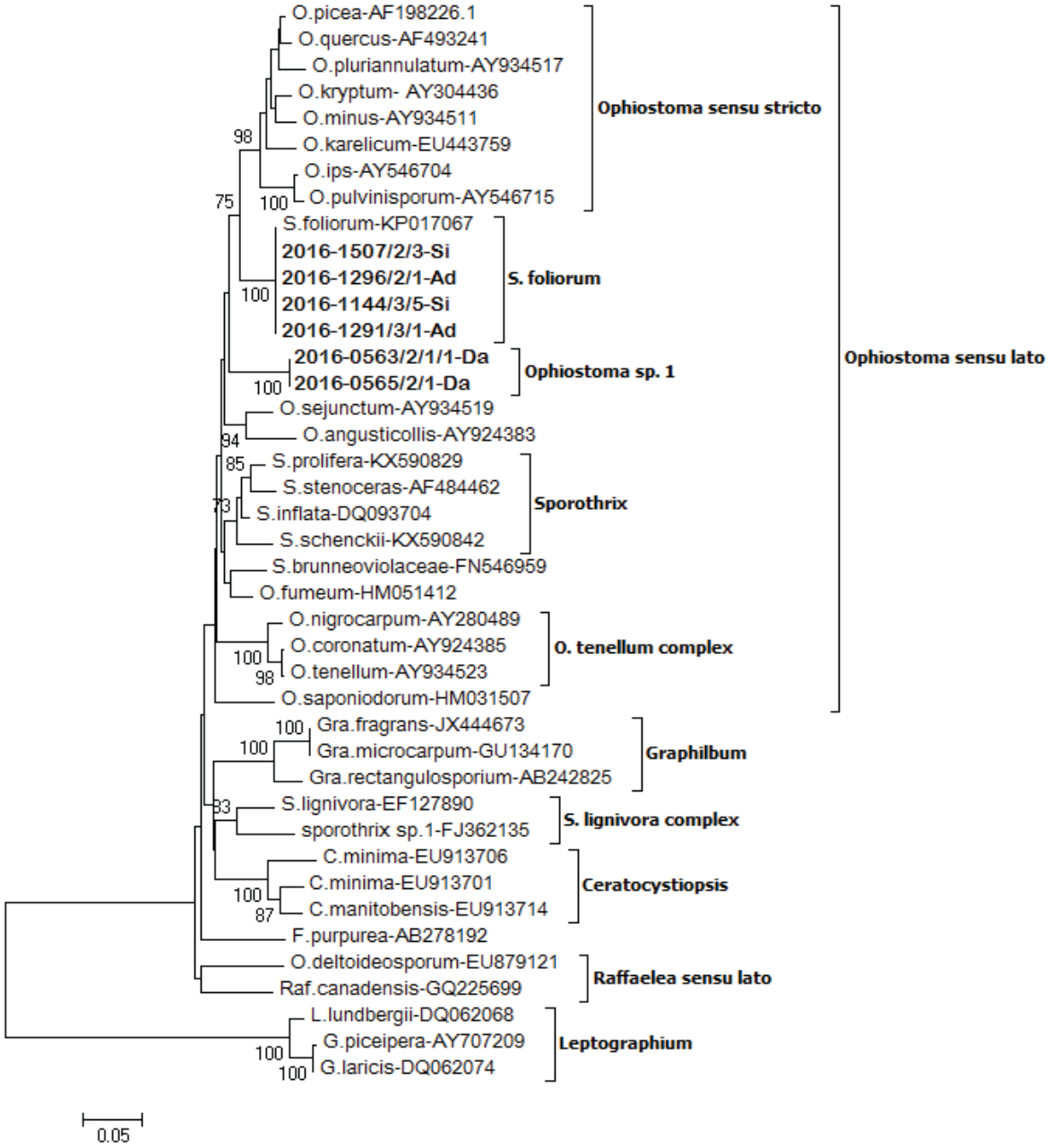

Figure 10. Phylogram based on ITS Sequences of the major clades within the *Ophiostomatales*. Bootstrap support values (1000 replicates) above 70% are indicate next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 303 positions in the final dataset. Isolates sequenced in this study are printed in bold. Bar= number of base pair differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Da, *Dryocoetes alni;* Si, *Scolytus intricatus.*

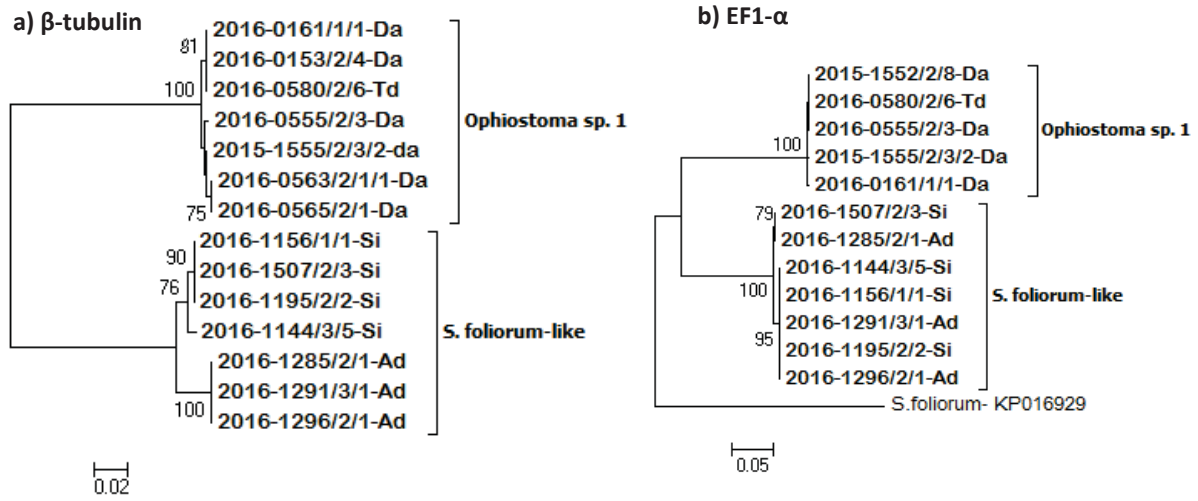

Figure 11. Phylogram based on (**a**) the β-tubulin gene and (**b**) the EF1-α gene of *Ophiostoma* sp.1 and S. foliorum-like. Bootstrap support values (1000 replicates) above 70% are indicate next to the branches. All positions containing gaps and missing data were eliminated. There were a total of 341 positions in β- tubulin dates and 321 positions in the EF1-α dataset. Isolates sequenced in this study are printed in bold. Bar= number of base pair differences per site. The letters after the isolate numbers indicate; Ad, *Anisandrus dispar*; Da, *Dryocoetes alni*; Si, *Scolytus intricatus;* Td, *Trypodendron domesticum*.

3.3 Morphology of new species

3.3.1 *Ophiostoma quercus*-like

Teleomorph: *Ascomata* (Fig. 12 a) often plentiful on MEA, developing after approximately 5-10 days, single or in groups, developing without the use of mating or use of sterilized twigs, indicating that this species is homothallic. *Ascomatal bases* black, globose, (100-)112-140(-158) µm in diameter, heavily ornamented with septate unbranched hypha of variable length (23-)37-67(-86) µm long, (2.0-)2.3-3.1(-3.8) µm wide at the base, (1.3-)1.7-2.2(-2.5) µm wide at the apex (Fig. 12 b). *Ascomatal necks* black, single, straight or slightly curved, apical taper, (22-)28-36(-44) µm wide at the base, (11-)13-17(-20) µm at the apex, (430-)464-540(-625) µm long excluded the ostiolar hypha. *Ostiolar hypha* (Fig. 12 c) pigmented, (20-)29-42(-51) µm long, (2.1-)2.4-3.2(3.6) µm at the base, (1.2-)1.4-1.7(-1.8) µm at the apex. *Asci* not observed. *Ascospores* (Fig. 12 d) one-celled, hyaline, (3.2-)3.7-4.3(-4.4) µm long, (1.3-)1.4-1.6(-1.7) µm wide, slightly allantoid shaped in side view, circular in end view, without visible sheath, accumulated in a hyaline to whitish droplet at the apex of the neck.

Anamorphs: *Pesotum*-like, developing after approximately 5-7 days. *Synnemata* **(**Fig. 12 e & g) single or in groups, black to brown, becoming paler toward the apex, (234-)252-312(-345)

µm long including capitulum, (40-)49-68(-82) µm at the base. *Conidia* (Fig. 12 f) hyaline, one celled, oval in side view, circular in end view, (3.0-)3.4-4.4(-6.3) µm long, (1.9-)2.0-2.3(-2.4) µm wide, accumulated in a hyaline droplet at the apex of the synnemata. *Sporothrix*-like anamorph was observed but not photographed or measured.

Colonies on MEA: (Fig. 12 h) Optimal growth 20°C, mean radial growth rate at optimum temperature 1.9 mm/day. No growth at 30°C or above, limited growth at 5°C. Colonies at first greenish to brown with hyaline edges, later becoming darker, reverse greenish to brown

Host tree: *Quercus robur*. Insect vector: *Dryocoetes villosus*

Comments: This is the most distinct of the possible new species in the *O. quercus* group. The closest related species from the GenBank is the unverified *O. bacillisporum* isolate. *Ophiostoma bacillisporum* produce very different ascospores (Upadhyay 1981).

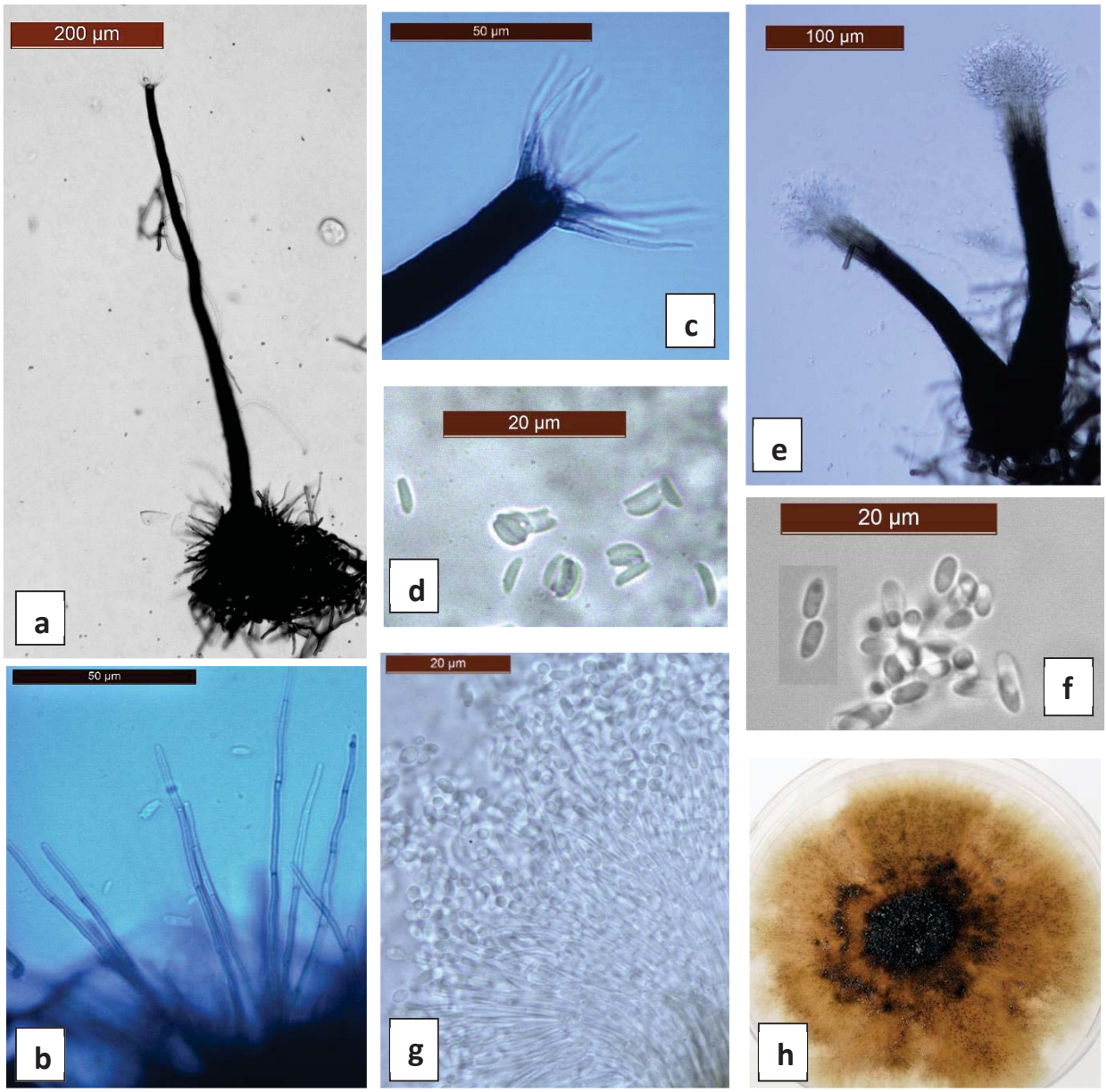

Figure 12. Morphological characters of *Ophiostoma quercus*-like. (**a-d**) Teleomorph (**a**) Ascocarp (**b**) Ornamentation of ascomatal base (**c**) Apex of ascomatal neck (**d**) Ascospores (**e-g**) *Pesotum* anamorph (**e**) Synnemata (**f**) Conidia (**g**) Capitulum (**h**) 30-day old culture.

3.3.2 *Ophiostoma bacillisporum*-like

Teleomorph: *Ascomata* (Fig. 13 a & b) developing after 5-7 days without the use of sterilized twigs or mating of different cultures, indicating that this species is homothallic. Superficially or completely embedded in the agar and often distributed in a circular pattern, single or in groups. *Ascomatal bases* globose, (121-)132-158(-171) µm in diameter, dark brown, sparsely ornamented with light brown hypha (4.9-)38-11(-69) µm long. *Ascomatal necks* dark brown at the base, single, becoming gradually paler towards the apex, straight or slightly curved, slight apical taper, (25-)31-42(-53) µm wide at the base, (14-)17-23(-28) µm wide at the apex, (173-

)264-383(-439) µm long excluding the ostiolar hyphae. *Ostiolar hyphae* (Fig. 13 c) (6.3-)7.5-17(-29) µm long, hyaline. *Asci* not observed. *Ascospores* (Fig. 13 d) hyaline (3.8-)4.0-5.0(-5.4) µm long, (1.2-)1.4-1.6(-1.9) µm wide, one-celled, allantoid in side view, circular in end view, without visible sheath, accumulated in a whitish to yellowish droplet at the apex of the neck.

Anamorphs: Not observed

Colonies on MEA: (Fig. 13 e) Optimal growth temperature 20°C, mean radial growth rate at optimal temperature 1.5 mm/day. Growth slightly reduced at 25°C. No growth at 35°C, almost no growth at 5°C. Colonies at first hyaline, later becoming brown or pale brown in areas where ascomata are formed, same color reverse. Brown protoperithecia-like structures were regularly observed (Fig. 13 b).

Host tree: *Alnus incana*. Insect vector: *Trypodendron signatum*

Comments: An unverified isolate of *O. bacillisporum* was the closest related strain according to the phylogenetic analyses. Both growth rate and shape of ascomata are quite similar as described for *O. bacillisporum* in (Upadhyay 1981), but *Ophiostoma bacillisporum* produce bacilliform conidia (5-12 µm long, 0.5-1 µm wide) and the ostiolar hypha are longer.

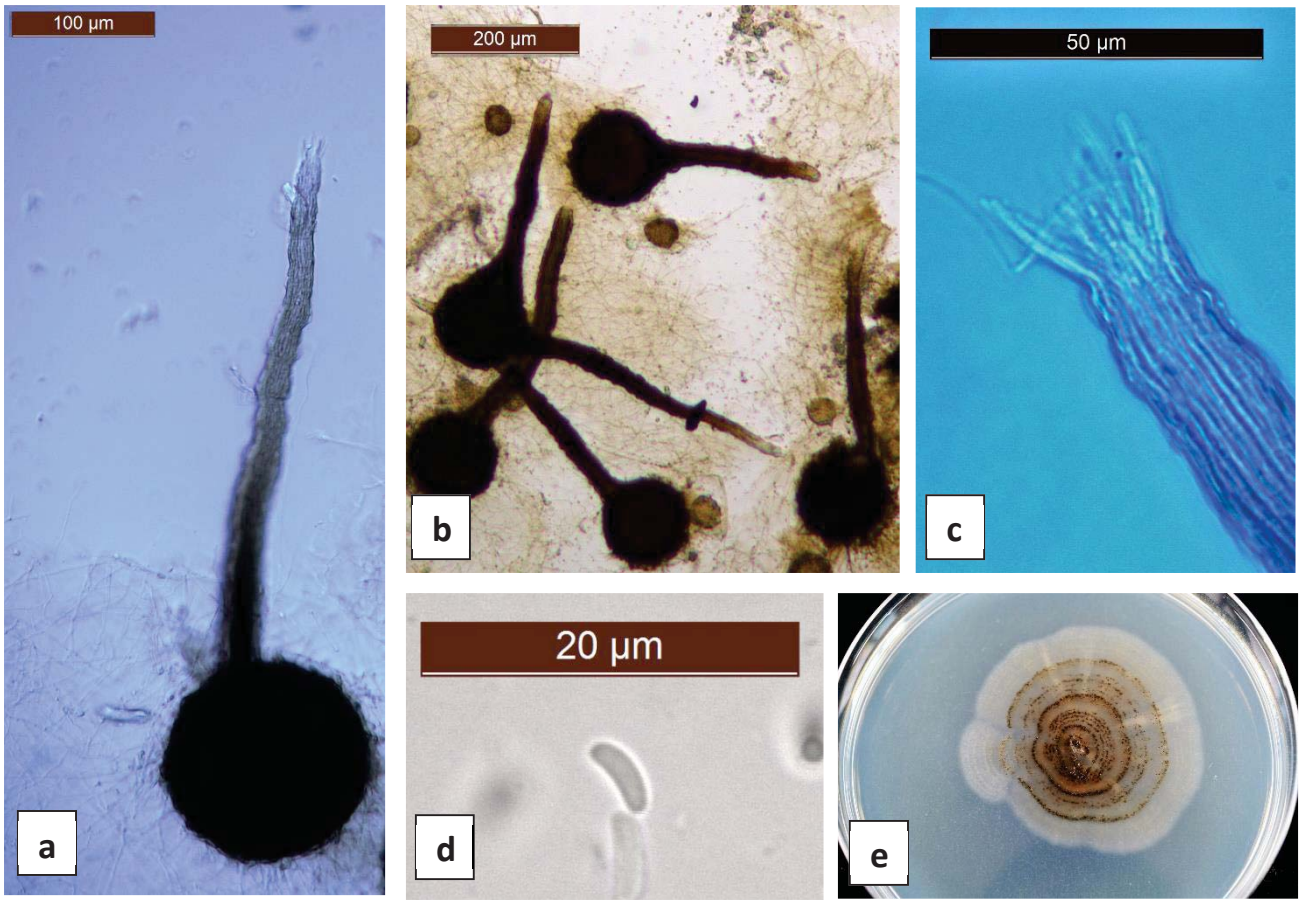

Figure 13. Morphological characters of *Ophiostoma bacillisporum*-like (**a**) Ascocarp (**b**) Ascomata and protoperithecia-like structures (**c**) Apex of ascomatal neck (**d**) Ascospore (**e**) 28-day old culture.

3.3.3 *Ophiostoma catonianum*-like

Teleomorph: Not observed. Mating of different cultures and growth on sterilized twigs did not result in production of ascomata.

Anamorphs: *Pesotum*-like, *synnemata* (Fig. 14 a) single or in groups, black at the base, becoming paler toward the apex, (192-)354-543(-641) µm long excluding capitulum, (31-)43-96(-146) µm wide at the base (Fig. 14 a). *Conidia* (Fig. 14 b) hyaline, one-celled, obovoid to oval, (3.2-)3.8-6.2(-7.8) µm long, (1.2-)1.4-2.0(-2.9) µm wide, accumulate in a hyaline to brownish droplet at the apex of the synnemata. *Sporothrix*-like anamorph, *conidia* (Fig. 14 c) hyaline, oval in side view, circular in end view, (3.1-)3.5-5.8(-9.7) µm long, (1.2-)1.6-2.5(-3.2) µm wide.

Colonies on MEA: (Fig. 14 d & e) Optimal growth temperature 25°C, mean radial growth 1.4 mm/day. No growth at 35°C, little growth at 5°C. Colonies at first white, floccose, *Sporothrix* anamorph dominating in young cultures, becoming more smooth with age and most of the

Sporothrix stage merge into the vegetative mycelium, *Pesotum* stage dominate in old cultures, old colonies hyaline with brownish spots, same color reverse.

Host tree**:** *Fraxinus excelsior*. Insect vectors: *Hylesinus crenatus* and *H. varius***.**

Comments: Characteristics of the *Pesotum* stage for *O. catonianum*-like is similar to the description of *O. catonianum* by Goidánich (1935), but the conidia produced by the *Sporothrix* anamorph of *O. catonianum* are fusiform and very different from the oval conidia produces by *O. catonianum*-like. The only existing isolate of *O. catonianum* has been studied by Harrington et al. (2001), but this isolate did only produce the *Sporothrix* anamorph, which was not described.

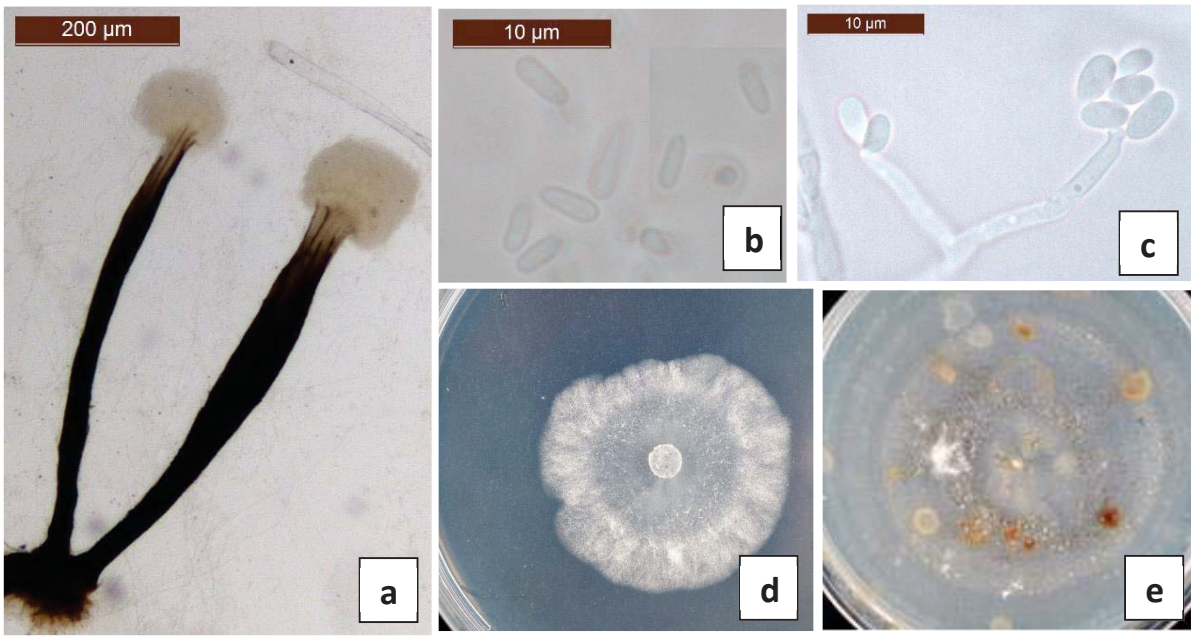

Figure 14. Morphological characters of *Ophiostoma catonianum*-like. (**a- b**) *Pesotum* anamorph (**a**) Synnemata (**b**) Conidia (**c**) *Sporothrix* anamorph with conidia (**d**) 17-day old colony (**e**) Old culture.

3.3.4 *Ophiostoma karelicum*-like

Teleomorph: Not observed. Mating of different cultures and growth on sterilized twigs did result in production of ascomata.

Anamorph: *Pesotum*-like, *synnemata* (Fig. 15 a b c & d) single or in groups, stipe sometimes hyaline, in other cases brown at the base, becoming hyaline towards the apex, (78-)98-139(-176) µm long including capitulum, (20-)29-65(-102) µm wide at the base. *Conidia* accumulated in a hyaline droplet at the apex of the synnema or in a whitish to greyish spore mass often covering the whole stipe. Two types of conidia were observed. *Ellipsoidal to oval conidia* (Fig.

15 e), hyaline, one-celled (3.3-)3.7-4.7(-5.9) µm long, (2.1-)2.5-3.1(-3.5) µm wide. *Obovoid conidia* (Fig. 15 f), hyaline, one celled, (2.7-)3.0-3.9(-4.9) µm long, (1.2-)1.4-1.8(-2.3) µm wide.

Colonies on MEA: Optimal growth temperature 25°C, mean radial growth 3.2 mm/day at optimum temperature, no growth at 35°C, little growth at 5°C. *Pesotum* anamorph in many cases lacking. Colonies at first hyaline, some isolates do not change much in color as they age, other isolates becoming light brown to dark brown. Brown protoperithecia-like structures were regularly observed (Fig. 15 a & b)

Host trees: *Alnus incana*, *Betula* sp. and *F. excelsior*. Insect vectors: *Dryocoetes alni Trypodendron domesticum* and *T. signatum*

Comments: The morphology described here for *O. karelicum*-like is quite different from the description of *O. karelicum* by Linnakoski et al. (2008). The obovoid conidia is similar to those described for *O. karelicum*, but no ellipsoidal to oval conidia are known to be produced by *O. karelicum*. Synnemata are shorter and the growth rate is slower for *O. karelicum*-like. Color is also different, only hyaline to grayish colonies are mentioned for *O. karelicum* (Linnakoski et al. 2008).

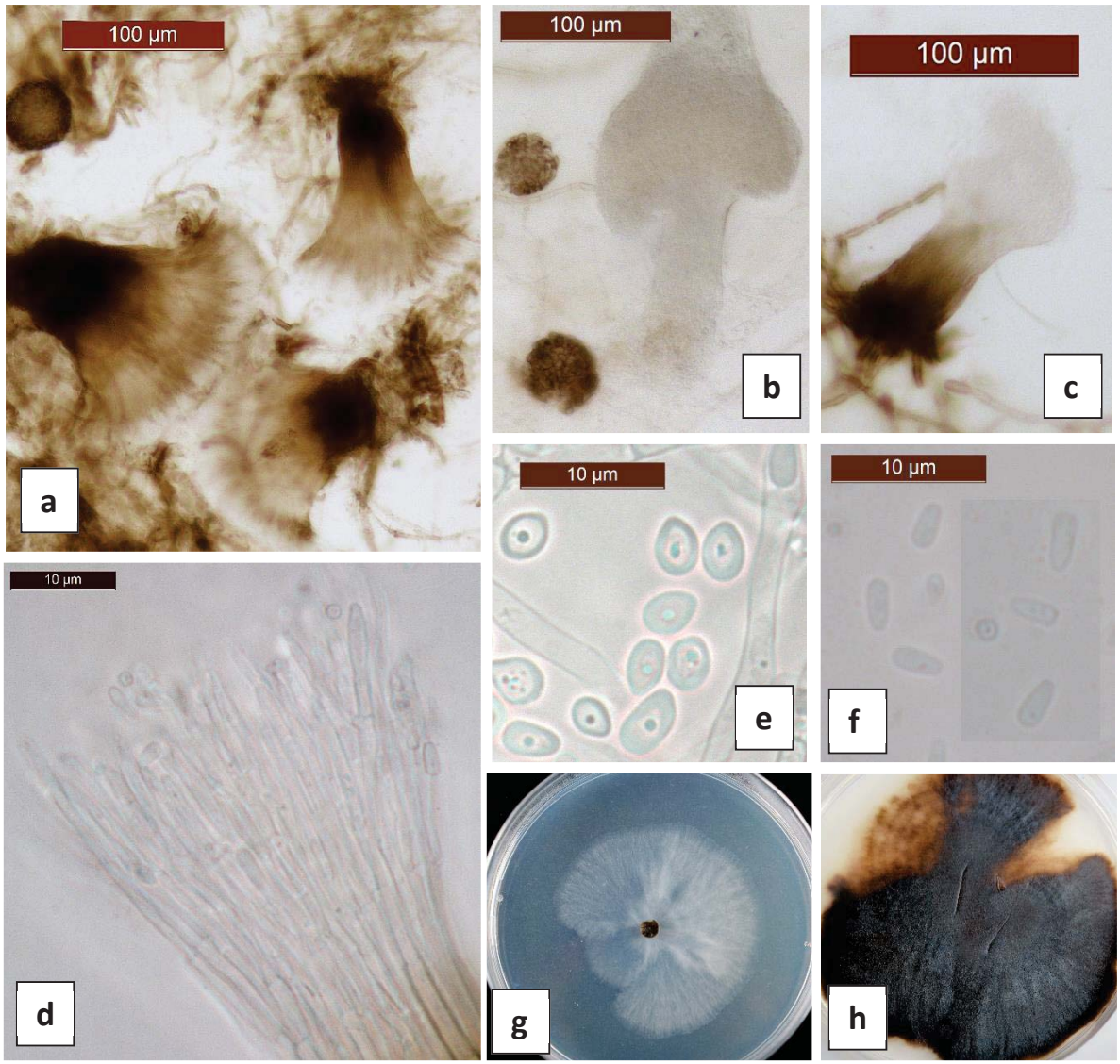

Figure 15. Morphological characters of *Ophiostoma karelicum*-like. (**a**-**g**) Pesotum anamorph (**a**) Synnemata and protoperithecia-like structure (**b**) Synnemata and protoperithecia-like structure (**c**) Synnemata (**d**) Capitulum (**e**) Globose conidia (**f**) Obovoid conidia (**g**) 9-day old colony (**h**) More than 3-month old colony (**g** and **h** is the same strain)

3.3.5 *Sporothrix lunata*-like

Teleomorph: *Ascomata* (Fig 16 a & b) black, single or in groups, developing late, partially or completely embedded in the agar, forms rarely on MEA (was only found in one of the cultures). Mating and growth on sterilized twigs was not performed. *Ascomatal bases* black, globose, (73-)96-175(-248) µm in diameter, heavily ornamented with brown septate hyphal hairs, (21-)50-99(-133) um long (Fig 16 ab & e). *Ascomatal necks* black, smooth, curved or waved, 1-2 necks per base, apical tapered, (21-)23-30(-35) µm wide at the base, (11-)12-16(-21) µm wide at the apex, (62-)100-629(-1162) µm long excluded the ostiolar hypha. *Ostiolar hypha* hyaline, (18-)30-51(-64) µm long. *Ascospores* (Fig. 16 c) hyaline, one celled, allantoid in side view, circular

in end view, (3.0-)3.4-4.0(-4.6) µm long, (1.0-)1.1-1.3(-1.4) µm wide, accumulated in a white to yellowish droplet at the apex of the ascomatal neck.

Anamorph: *Sporothrix*-like anamorph present in young cultures. *Conidia* **(**Fig. 16 f-h**)** one celled, hyaline, allantoid to oval, (2.9-)3.4-4.6(-5.6) µm long, (1.2-)1.4-1.8(-2.2) µm wide, produced singly from denticles directly along hypha or at hyphal tips, or in clusters at hyphal tips.

Colonies on MEA: Optimal growth temperature 25°C, mean radial growth at optimal temperature 1.6 mm/day, no growth at 5°C or 35°C. Colonies hyaline to white, reverse hyaline to slightly yellowish, forms concentric circles of growth

Host tree: *Quercus robur.* Insect vectors: *Anisandrus dispar* and *Trypodendron domesticum*

Comments: Closely related to *S. lunata* and *S. fusiforme.* Both of which are described by Aghayeva et al. (2004). Ascomatal base diameter and shape of conidia are different from *S. fusiforme*. The morphology is more or less identical to *S. lunata*, which also produce allantoid conidia and was the most related species according to the calmodulin dataset (Fig. 5 b). The teleomorph characters described here are only based on one isolate and more strains are needed to be investigated to get a more thorough description of *S. lunata*-like.

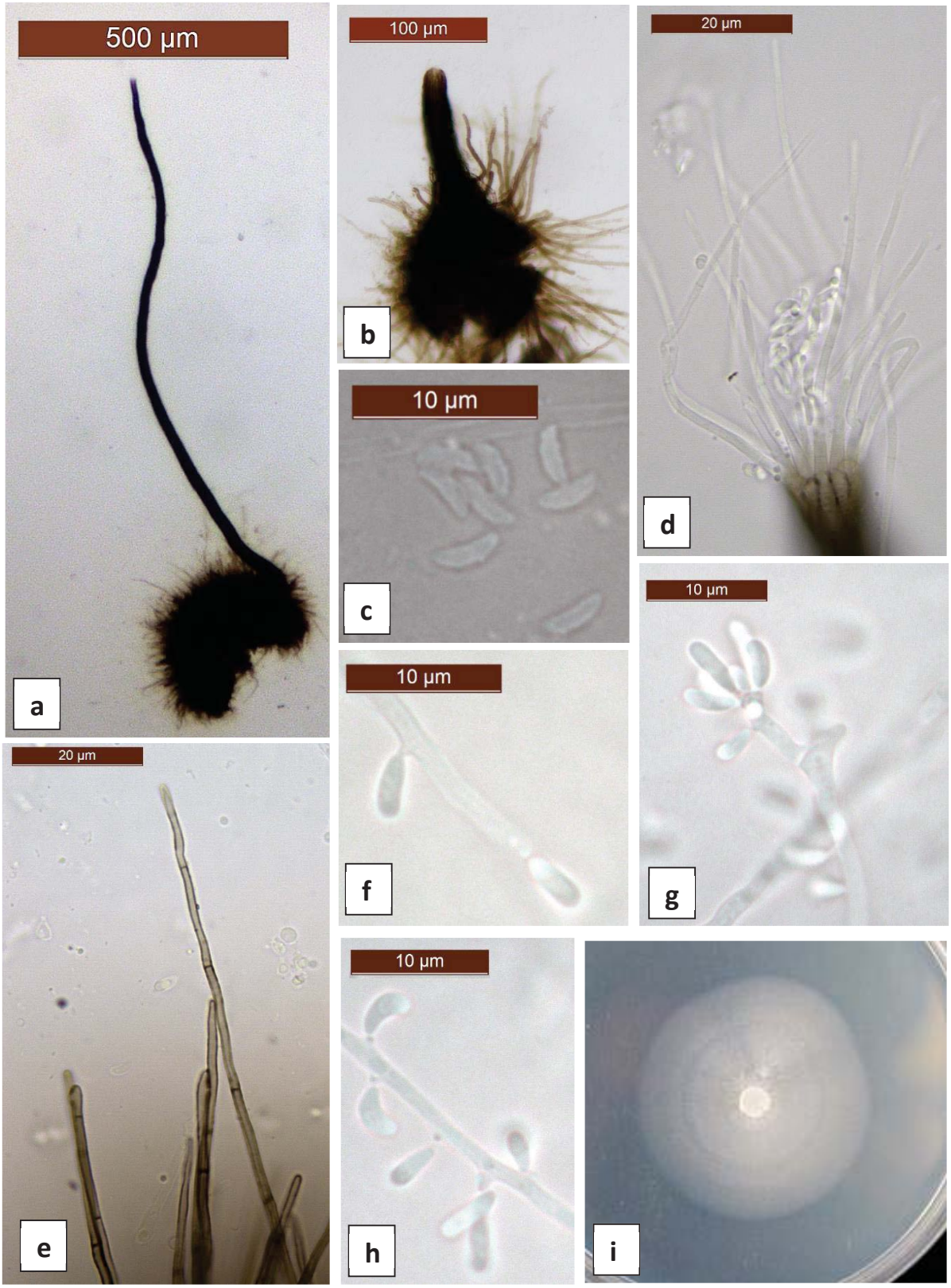

Figure 16. Morphological characters of *Sporothrix lunata*-like (**a-e**) Teleomorph (**a**) Ascoma (**b**) Young ascoma (**c**) Ascospores (**d**) Apex of ascomatal neck (**e**) Ornamentation of ascomatal base (**f-h**) Sporothrix anamorph and conidia (**i**) 17-day old colony.

3.3.6 *Sporothrix* sp. 1

Teleomorph: Not observed, mating or growth on sterilized twigs was not conducted.

Anamorph: *Sporothrix*-like anamorph (Fig. 17 a-c) present in young cultures. *Conidia* **(**Fig. 17 a-c) hyaline, one-celled, fusiform, (3.0-)3.5-4.6(-5.2) µm long, (1.2-)1.4-2.2(-3.1) µm wide, produced in smaller groups along the hypha or in larger groups at hyphal tips.

Colonies on MEA: (Fig. 17 d) Optimal growth temperature 25°C, mean radial growth at optimal growth temperature 1.2 mm/day, growth slightly reduced at 20°C, no growth at 5°C or 35°C (growth studies only based on two replicates). Colonies hyaline to white, smooth, reverse hyaline to slightly yellowish, forms concentric circles of growth.

Host tree: *Alnus incana*. Insect vector: *Dryocetes alni*

Comments: *Sporothrix. lunata*, *S. rossi, S. fusiforme* are closely related to *Sporothrix* sp. 1. *Sporothrix lunata* is the most distinct according to the calmodulin data. This species also produces different conidia (allantoid) (Aghayeva et al. 2004). *Sporothrix rossi* described by (Davidson 1971) (described as *Ceratocystis gossypina var. robusta*) has similar *Sporothrix* anamorph as *Sporothrix sp 1*, both producing obovoid conidia in clusters. *Sporothrix fusiforme* is the closest species according to the calmodulin analysis and produce similar conidia. However, the conidia are according to (Aghayeva et al. 2004) produced on long branches erected from hypha, which is different from *Sporothrix sp 1*.

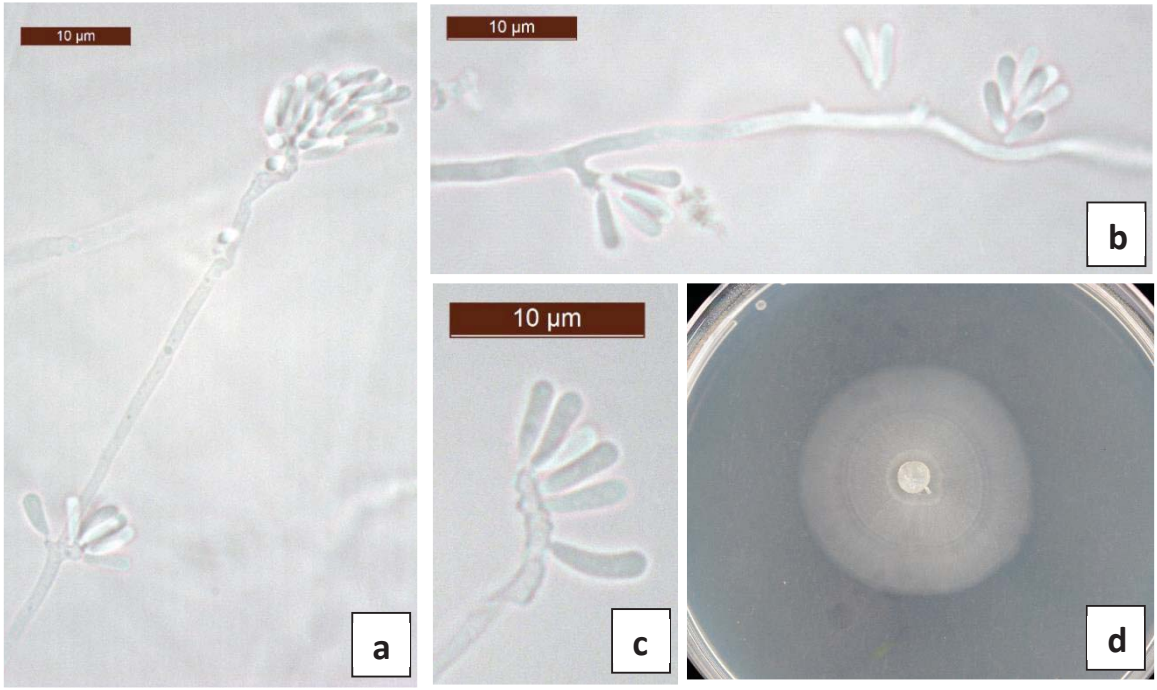

Figure 17. Morphological characters of *Sporothrix* sp. 1 (**a-c**) Sporothrix anamorph and conidia. (**d**) 17- day old colony.

3.3.7 *Leptographium* sp. 1

Teleomorph: Not observed. Mating or growth on sterilized twigs not conducted.
Anamorph: *Pesotum*-like, *Synnemata* (Fig. 18 a & b) plentiful on MEA after 4-6 days, single or on groups, dark brown, becoming paler toward the apex, (18-)70-192 (-348) µm long excluded capitulum, (14-)17-48(-85) µm wide at the base. *Conidia* (Fig. 16 c) hyaline, oval in side view, circular in end view, (2.6-)3.0-3.9(-4.8) µm long, (1.3-)1.4-1.7(-1.9) µm wide.
Colonies on MEA: (Fig. 18 d) Optimal temperature 20°C, mean radial growth rate at optimal temperature 2.5 mm/day, no growth at 35°C, little growth at 5°C, colony at first greenish with hyaline edges, becoming more brown with age, reverse greenish to brownish
Host tree: *Alnus incana*. Insect vector: *Dryocoetes alni*
Comments: Both synnemata, conidia and colony colors are very similar to the description of *Leptographium innermongolicum* described by Liu et al. (2017). Differences are the optimal growth temperature (optimal temperature for *L. innermongolicum* is 25°C) and that *Leptographium* sp. 1 has slightly faster growth rate.

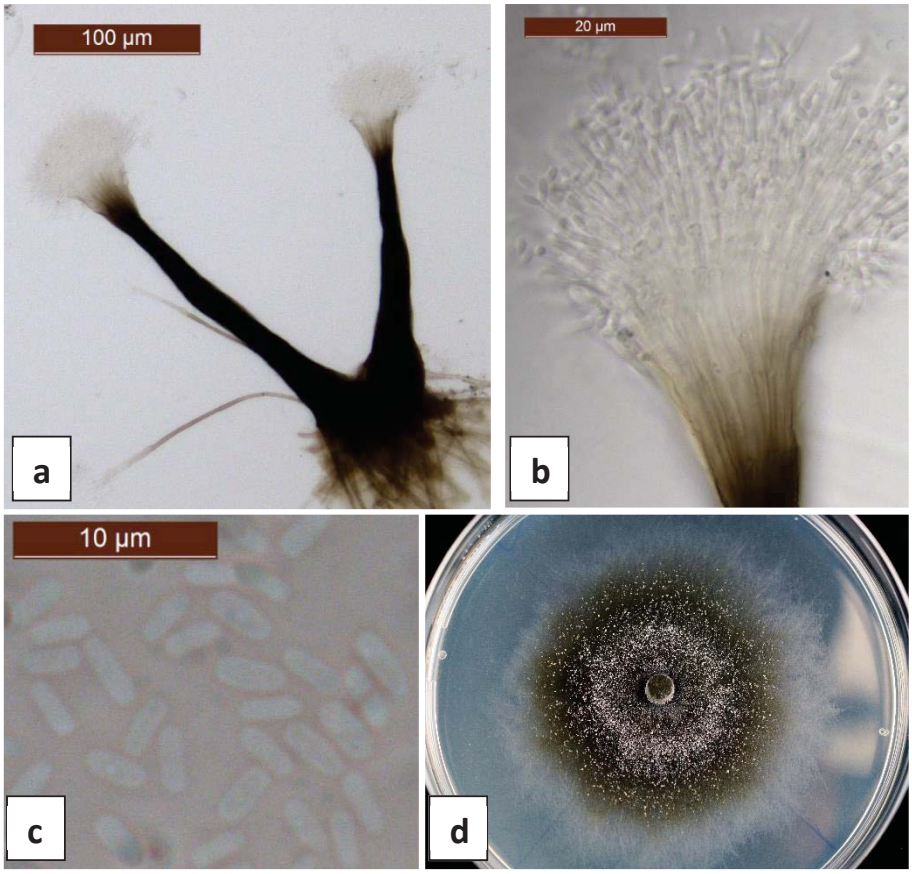

Figure 18. Morphological characters of *Leptographium* sp. 1 (**a-c**) Synnematous anamorph (**a**) Synnemata (**b**) Capitulum (**c**) Conidia (**d**) 17-day old colony

3.3.8 *Ophiostoma brevicolle*-like

Teleomorph: *Ascomata* (Fig. 19 a) developing late, but without the use of mating of different cultures or growth on sterilized twigs indicating a homothallic mating system, emerge in groups often in the center of the colony, on the surface or only slightly embedded in the agar. *Ascomatal bases* globose, brown, without ornamentation, (82-)107-147(-198) µm in diameter. *Ascomatal necks* brown at the base becoming paler toward the apex, no apical taper, (29-)34-40(-43) µm wide at the base, (29-)32-39(-45) µm wide at the apex, (122-)184-264(-294) µm long excluding the ostiolar hypha. *Ostiolar hypha* hyaline (38-)51-72(-85) µm long (Fig. 19 c). *Asci* not observed. *Ascospores* (Fig. 19 b) hyaline, orange sectioned, (3.4-)3.9-4.5(-4.9) µm long, (1.8-)1.8-2.2(-2.6) µm excluding *sheath* (0.5-)0.6-0.8(-1.0) µm thick, accumulating in a yellow droplet at the apex of the ascomatal neck.

Anamorph: *Conidiophores* (Fig. 19 d & e) often dominating on MEA, developing after 5-7 days, single or in groups, often in the center of the colony. *Stipe* brown, becoming paler toward apex, 4-9 septate, (80-)104-149(-191) µm long including conidiogenous apparatus, 2-4 primary branches, (27-)37-60(-78) µm long, conidiogenous cells hyaline to yellowish, (11-)12-16(-19) µm long (1.3-)1.5-1.9(-2.1) µm wide. *Conidia* (Fig. 19 e) hyaline, oval in side view, circular in end view, (4.2-)4.6-5.8(-6.6) µm long, (2.0-)2.3-2.8(-3.2) µm wide, accumulating in a hyaline droplet at the apex of the conidiophore.

Colonies on MEA: Optimal growth temperature 25°C, slow growing, mean radial growth 0.5 mm/day, growth slightly reduced at 20°C, no growth at 5°C or 35°C. Colonies first yellow, later becoming yellow to orange, same color reverse.

Host trees: *Alnus incana, Fraxinus excelsior* and *Quercus robur.* Insect vectors: *Dryocoetes alni, Trypodendron domesticum* and *T. signatum*

Comments: When compared to the description of *O. brevicolle* by Jacobs and Wingfield (2001) there are some similarities between the two species, but in general there are large morphological differences. *Ophiostoma brevicolle* produce olivaceous colored colonies, the ascomatal neck lack ostiolar hypha and the ascospores are allantoid shaped. The morphology is more similar to that of *Grosmannia francke-grosmannia* described by the same author. Considerable confusion exists regarding the taxonomy of both *G. francke-grosmannia* and *O. brevicolle* (De Beer & Wingfield 2013).

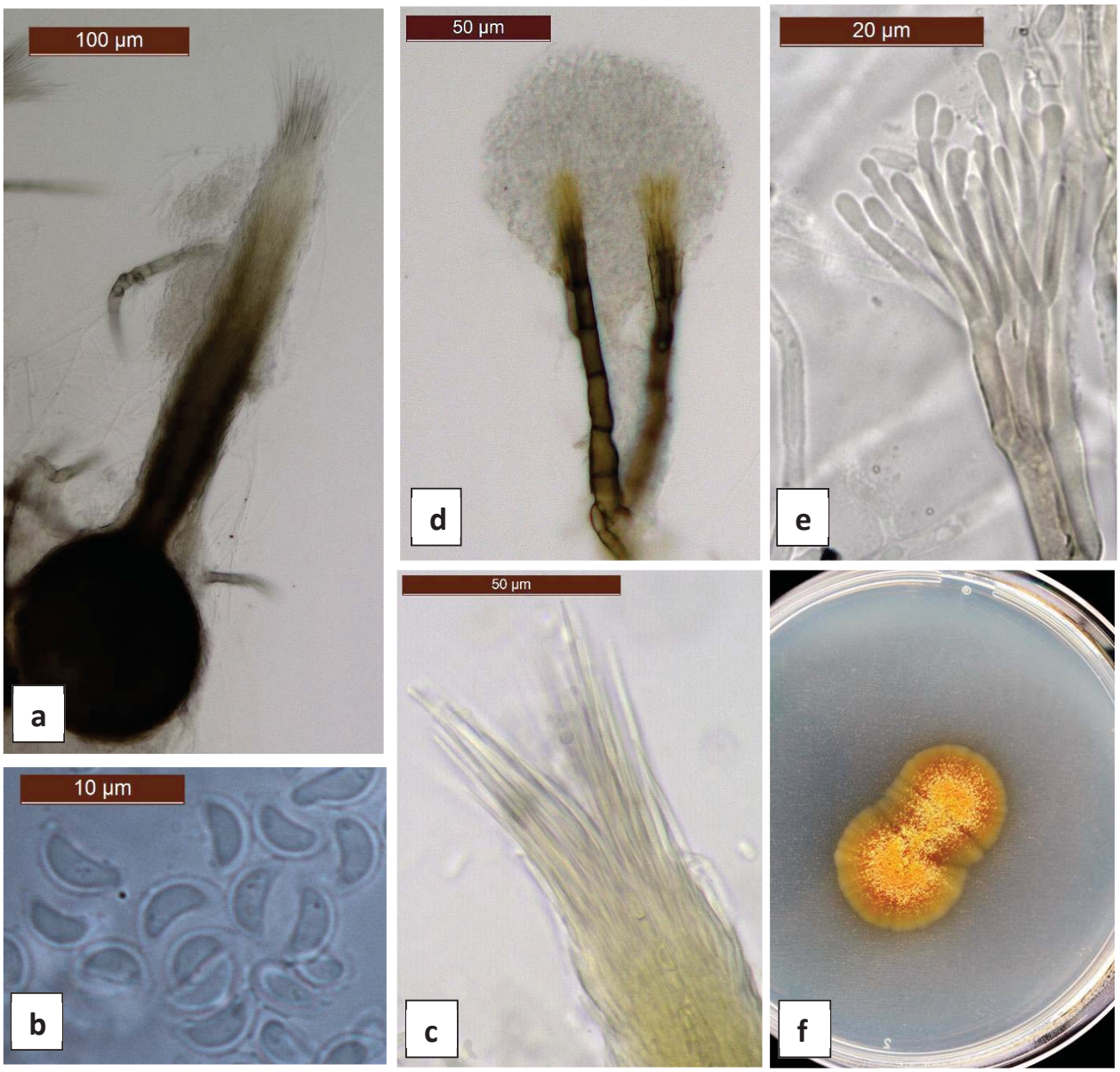

Figure 19. Morphological characters of *Ophiostoma brevicolle*-like (**a-c**) Teleomorph (**a**) Ascoma (**b**) Ascospores (**c**) Apex of ascomatal neck (**d**) Conidiophores (**e**) Conidiophore producing conidia (**f**) 25-day old culture

3.3.9 *Ophiostoma* sp. 1

Teleomorph: Not observed. Mating or growth on sterilized twigs was not conducted.
Anamorph: *Pesotum*-like, *Synnemata* (Fig. 20 a-c) single, developing late, hyaline to brown, becoming paler toward apex, (72-)116-238(-340) µm long including capitulum, (11-)16-35(-49) µm wide at the base. *Conidia* (Fig. 20 d-e) one celled, hyaline, oval in side view, circular in end view, (2.2-)2.6-3.1(-3.6) µm long, (1.1-)1.3-1.7(-2.0) µm wide, accumulated in a hyaline to white spore drop at the apex of the synnemata.
Colonies on MEA: Optimal growth temperature 20°C, slow growing, mean radial growth at optimal temperature 0.3 mm/day. Colonies at first hyaline to white, becoming brown in areas where synnemata are formed.
Host tree*: Alnus incana*. Insect vector: *Dryocoetes alni.*
Comments: This species is most likely a member of *Ophiostoma sensu lato*, but is very distinct from all other species based on the regions sequenced.

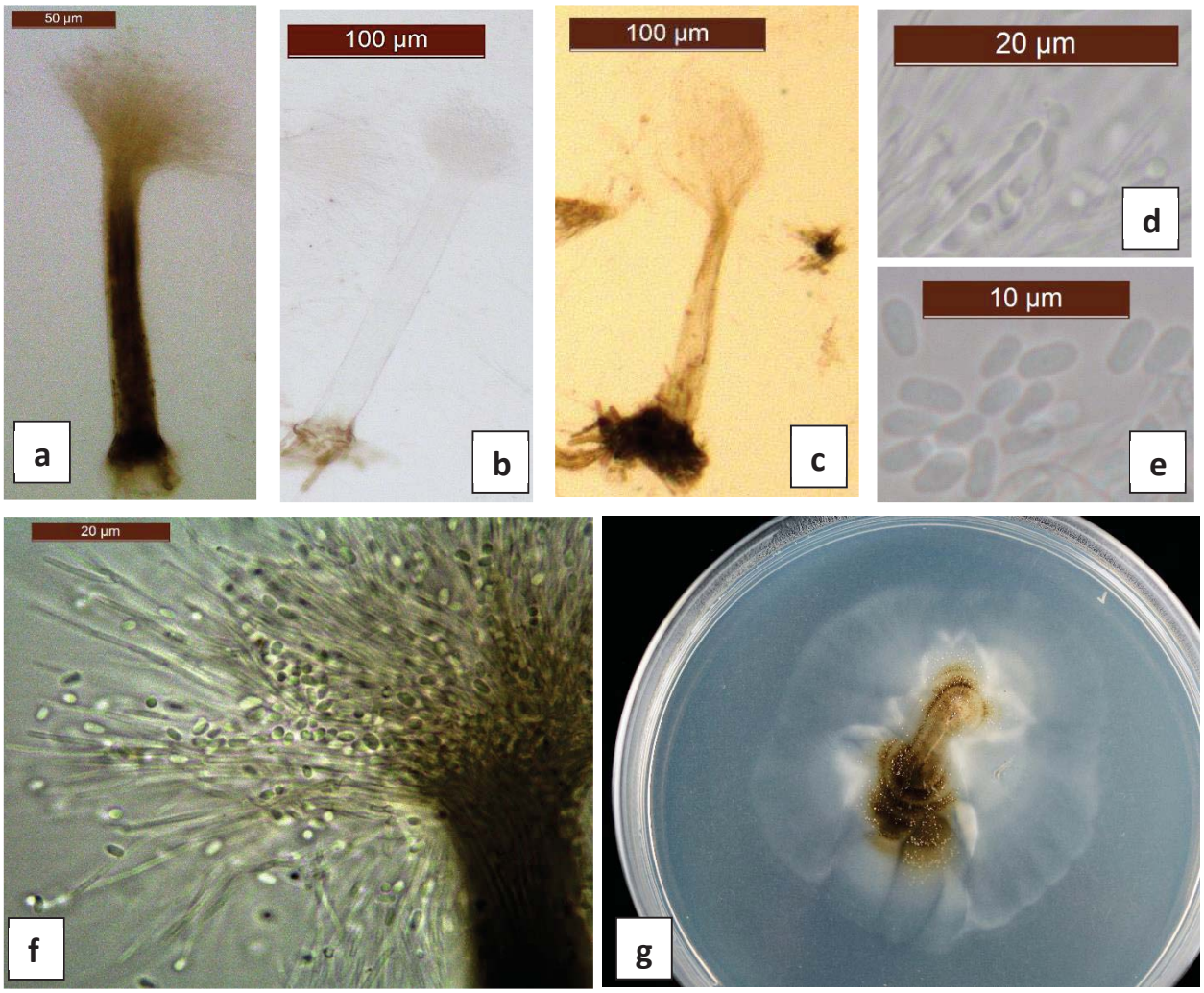

Figure 20. Morphological characters of *Ophiostoma* sp. 1 (**a-c**) Synnemata (**d**) Conidiogenous cell with conidium (**e**) Conidia (**f**) capitulum and conidia (**g**) More than 3-month old colony.

3.3.10 *Sporothrix foliorum*-like

Teleomorph: Not observed. Mating or growth on sterilized twigs was not conducted.
Anamorph: *Sporothrix*-like anamorph present on young cultures, but conidia may also be found in old cultures in slimy masses. Two types of conidia were observed. *Allantoid conidia*; (Fig. 20 a & b) hyaline, one celled, (3.0-)3.6-4.9(-6.5) µm long, (0.8-)1.0-1.5(-1.8) µm wide, produced in small groups at hyphal tips. *Obovoid/fusiform conidia*; (Fig. 20 c & d) hyaline, one celled, (3.6-)3.9-4.9(-5.3) µm long, (0.8-)1.4-2.2(-2.5) µm wide.
Colonies on MEA: (Fig. 20 d) Optimal growth temperature 25°C, mean radial growth at optimal growth temperature 1.3 mm/day, very little growth at 5°C and 35°C, colonies white, later becoming white to yellowish, same color reverse.
Host tree: *Quercus robur* Insect vector: *Scolytus intricatus* and *Anisandrus dispar*
Comments: *Sporothrix foliorum* is according to the ITS data the only closely related species. However, the EF1-α data indicated that the two species are very distinct. *Sporothrix foliorum* has much slower growth rate according the description by Hoog (1974). The conidia produced by *S. foliorum* are obovoid to ellipsoidal and are smaller than the obovoid to fusiform conidia produced by *S. foliorum*-like and no allantoid conidia are mentioned for *S. foliorum*.

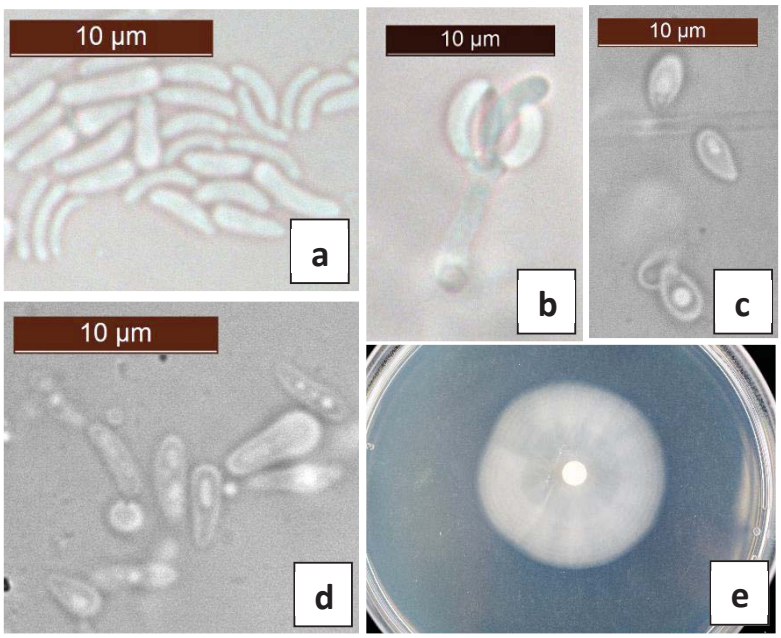

Figure 21. Morphological characters of *Sporothrix foliorum*-like (**a**) Allantoid conidia (**b**) Conidiogenous apparatus with conidia (**c**) Obovoid conidia (**d**) Obovoid to fusiform conidia (**e**) 17 day-old colony.

4 Discussion

4.1 Ophiostomatoid fungi

4.1.1 Species within the *Ophiostoma ulmi* complex

All species belonging to the *Ophiostoma ulmi* complex have most commonly been found on broadleaved trees (De Beer & Wingfield 2013). The complex has also been treated as the *O. quercus* complex (Kamgan Nkuekam et al. 2008) or the hardwood clade of the *O. piceae* complex (Grobbelaar et al. 2009). This study revealed seven species belonging to this complex.

Ophiostoma quercus-like

This species was found in association with *D. villosus* infesting *Q. robur*. This is the most distinct of the new species within the *O. quercus* group. The closest described species is *O. bacillisporum*-like, but the two species are very different both genetically and morphologically. Most species in the *O. ulmi* complex are heterothallic (Harrington et al. 2001), but all isolates of *O. quercus*-like produced ascomata, indicating a homothallic mating system. *Ophiostoma quercus*-like was only found in association with *D. villosus,* which may be indicating a species specific relationship. However, only 26.7% of the specimens carried this fungus and *D. villosus* was only found at one sampling site. Thus it is difficult to draw any conclusions about the association between *Ophiostoma quercus*-like and *D. villosus*.

Ophiostoma bacillisporum-like

Ophiostoma bacillisporum-like was commonly found in association with *T. signatum* infesting *A. incana* and was not isolated from any other bark beetle species. The only protein coding regions of *O. bacillisporum* available from the GenBank is from an unpublished study, and the GenBank have not verified that this is the type strain of *O. bacillisporum* (CBS 771.71). However, this is of little importance since the species obtained in this study is distinct from this strain whether it is *O. bacillisporum* or not. The morphology was still compared to *O. bacillisporum* and the two species share some similarities, but they produce very different ascospores. The high frequency of *O. bacillisporum*-like in relation to *T. signatum* (80.5%) indicates that this could be a consistent fungal associate, However, *T. signatum* was only

sampled from one location in northern Norway (Målselv) and more beetles from other locations are needed to confirm this.

Ophiostoma catonianum-like

This species was only found in association with *Hylesinus crenatus* and *H. varius*. Both these bark beetles breed in the phloem of newly dead or stressed *Fraxinus* spp. (Ehnström & Axelsson 2002). The obtained strains have ITS and β-tubulin regions very similar to *O. catonianum,* but the EF1-α gene is different. *Ophiostoma catonianum* has been found on *Pyrus communis* L. in Italy by Goidánich (1935) and the morphology of this species is similar to *Ophiostoma catonianum*-like. However, the conidia produced by the *Sporothrix* anamorph are much shorter for *O. catonianum*-like. This fungus was only isolated from bark beetles infesting *F. excelsior*, but was far more commonly associated with *H. crenatus*. This could perhaps be explained by the different breeding preference of these two beetles. *Hylesinus crenatus* breed in the thick bark on stems, while *H. varius* breeds in the thinner bark of smaller stem parts (Ehnström & Axelsson 2002). It could be that bark thickness influences the microclimatic conditions in the galleries and that the conditions in galleries of *H. varius* are less suitable for *O. catonianum*-like.

Ophiostoma quercus

This species was found in association with *D. villosus*, *S. intricatus S. ratzeburgi, A. dispar* and *T. domesticum*. This is a morphological and genetically diverse species (Grobbelaar et al. 2009). It has been found in many parts of the world, including, Europe, North and South America, Asia, Australia and Africa (Harrington et al. 2001; De Beer, Z. W. et al. 2003; Grobbelaar et al. 2009). It is possible that the distribution is even wider since many old strains treated as *O. piceae* might represent *O. quercus* (De Beer et al. 2003). The species has also been treated as *O. querc**i*** (Harrington et al. 2001), but *O. querc**us*** is now considered the correct name for this species (De Beer et al. 2003). It is most commonly found in association with broadleaved trees, but has also been isolated from coniferous trees (Grobbelaar et al. 2009). From the literature, it seems that *O. quercus* is a generalist most commonly found in low frequencies with various insect vectors (Carlier et al. 2006; Romon et al. 2007; Linnakoski et al. 2008; Linnakoski et al. 2009; Jankowiak & Bilański 2013). It has previously been found in Norway associated with *S. ratzeburgi* (Linnakoski et al. 2009) and has also been isolated from wounds of varius

broadleaved trees in southern Norway (Kamgan Nkuekam et al. 2010). In my study, *O. quercus* was found in association with both phloem breeding (*D. villosus*, *S. intricatus S. ratzeburgi)* and xylem breeding bark beetles (*A. dispar* and *T. domesticum*). It was quite commonly found in association with *S. intricatus* and less common on *A. dispar* and *D. villosus*. It was rarely found in association with *S. ratzeburgi* and *T. domesticum*. Considering the broad host- and vector-range of this species it was not surprising to find multiple vectors of this species. However, *O. quercus* was almost exclusively found in association with bark beetles infesting *Quercus* sp. The only exceptions were two isolates found on *S. ratzeburgi* infesting *Betula* sp. It is surprising that this species was not found on more host-tree species considering its large host-tree range.

Ophiostoma karelicum

Ophiostoma karelicum was very commonly isolated from *S. ratzeburgi* (90.7%), but was also found on *H. crenatus* and *T. domesticum* infesting *F. excelsior*. It is a genetically diverse species and has previously been found in Norway, Finland, Russia and Poland (Linnakoski et al. 2008; Linnakoski et al. 2009; Jankowiak 2011; Linnakoski 2011). It is considered as a highly consistent associate of *S. ratzeburgi* and this is also supported by this study. In my study, *O. karelicum* was also associated with *T. domesticum* infesting *B. pubescence* (20%). However, it is very likely that neighbouring galleries of *S. ratzeburgi* is the main source of this fungus since the logs were attacked by both species. *Ophiostoma karelicum* was also found in association with *H. crenatus* and *T. domesticum* infesting *F. excelsior*. Finding *O. karelicum* on a different host-tree than birch was not very surprising considering that this species also occasionally has been found on coniferous trees, indicating a broad host-tree range (Linnakoski 2011).

***Ophiostoma karelicum*-like**

This species was found in association with three bark beetle species (*T. domesticum*, *T. signatum* and *D. alni*) and three host-tree species (*A. incana, B. pubescence* and *F. excelsior*). It is clearly distinct from *O. karelicum* both in the β-tubulin and EF1-α gene regions. It is very genetically similar to strains obtained in an unpublished study by R. Jankowiak who isolated fungi from *Trypodendron lineatum* infesting *Pinus sylvestris* L. (These strains are treated as *O. karelicum* in GenBank). No sexual state was observed in any of the cultures, but large differences in colour were observed within this group and a lot of time and effort were used in

grouping these isolates to see if this was taxonomically informative. All cultures were hyaline as young, but some became coloured with age in various shades of brown, often after many weeks. Thus trying to group cultures of different age was confusing. However, colour was not found to be taxonomically informative and colour differed between cultures of the same strain. Genetic variation was found in this group especially at the EF1-α gene. However, subgrouping was not consistent between the β-tubulin analysis and the EF1-α analysis. Different subgrouping when using different gene regions has also been reported for other species, for example *O. quercus* (Grobbelaar et al. 2009). The genetic variability and the different conidia found among the *O. karelicum*-like isolates should be investigated further. *Ophiostoma karelicum*-like found both in southern and northern Norway indicating a wide geographical range. It was most commonly found in associated with *T. domesticum* infesting *A. incana*, and was found on 100% of the beetles at some locations. It was also very common on *T. domesticum* infesting *B. pubescence* (86.7%), indicating a close relationship. *Dryocoetes alni* was also occasionally transporting this fungus. However, it is very likely that this is the result of contamination from nearby *T. domesticum* galleries, as these bark beetles were often collected from the same stems and branches. Also *T. signatum* was associated with this fungus (24.4%). The results indicate that *Ophiostoma karelicum*-like seem to be primarily associated with bark beetles breeding in the xylem. However, more studies are needs to confirm this.

Ophiostoma novo-ulmi

Ophiostoma novo-ulmi was found in association with S. *laevis* (58.8%) infesting *U. glabra*. This is probably the best known fungus within the *Ophiostomatales* and it is the cause of the most destructive pandemic of Dutch elm disease (DED) in Europe and North America, killing millions of elm trees (Brasier 1991). DED is primarily vectored by *Scolytus* species infesting various elm species (Webber 2004). *Scolytus laevis* is considered the main vector of this disease in Norway (Hansen et al. 1998), but as far as I know, this is the first study which has actually isolated DED from this bark beetle species. *Ophiostoma novo-ulmi* consists of two subspecies, subspecies *novo-ulmi* and subspecies *americana* (Brasier & Kirk 2001), but the isolates obtained in this study were not resolved at subspecies level.

4.1.2 Species within *Sporothrix*

Four *Sporothrix* species were found of which three form part of the *Gossypina* complex and one is a member of the *S. inflata* complex. The *Gossypina* complex mostly contains species found in association with wood colonizing insects and the species is very closely related and almost genetically indistinguishable (De Beer et al. 2016). Many of the species within this complex are only represented by one strain, making it impossible to say anything about the variation within each species. Several strains per species are needed to get a better understanding of this complex (De Beer et al. 2016).

Sporothrix prolifera

Sporothrix prolifera was found in association with *S. intricatus* infesting *Q. robur*. *Sporothrix prolifera* can in contrast to many of the other species within the *Gossypina* complex quite easily be distinguished using molecular data. It has previously been found on *Q. robur* in Poland by Kowalski and Butin (1989) who described it as *Ceratocystis prolifera*. In the present study, *S. prolifera* was only found on bark beetles associated with *Q. robur,* indicating that this could be the main host-tree of this species. *Scolytus intricatus* was the only vector of this species, but the association seemed rather casual (24%). *Sporothrix prolifera* might have been found in association with *S. intricatus* in a study by Kubátová et al. (2002), which detected a species treated as *O.* cf. *prolifera*, but this study is only available as an abstract.

Sporothrix lunata-like

Sporothrix lunata-like was found in association with *A. dispar* and *T. domesticum* infesting *Q. robur*. This species also groups in the *Gossypina* complex, but is much more cryptic than *S. prolifera*. Only the calmodulin analysis had high enough resolution to distinguish between *Sporothrix lunata*-like and the very similar *S. lunata* and *S. fusiformis.* Both *S. lunata* and *S. fusiformis* have been described by Aghayeva et al. (2004) and both species have been found in association with both broadleaved and coniferous hosts. Another species who is very similar to *S. lunata*-like is a strain treated as *O.* cf. *fusiforme* from a study by Selochnik et al. (2015). This strain was isolated from *Q. robur* in Russia. Only the teleomorph characteristics of this strain was described and resemble that of *S. lunata*-like. Unfortunately, no calmodulin sequence was available and it could only be included in the β-tubulin analysis which showed low resolution

within this species complex. *Sporothrix lunata*-like was only found on *Q. robur* in association with xylem breeding bark beetles (*A. dispar* and *T. domesticum*). Thus it seems that *S. lunata*-like is more adapted to grow in galleries in the xylem and thus occupy a different niche than *S. prolifera* which seem to be more commonly found in galleries in the phloem on the same host-tree. However, mores studies are needed to confirm any preferences of these species.

***Sporothrix* sp. 1**

This species was only found in association with *D. alni* infesting *A. incana*. The species is closely related to many of the species in the *Gossypina* complex, but both shape of conidia and the calmodulin analysis indicate that *S. fusiformis* is the closest related species. Only two isolates were obtained of this species and more isolates are probably needed to resolve the identity of these isolates.

Sporothrix dentifunda

This species forms part of the *S. inflata* complex. It was rarely found in association with *D. villosus* infesting *Q. robur*. Very little is known about this species. According to Aghayeva et al. (2005) only two isolates of *S. dentifunda* are known, one from *Q. robur* in Poland and one from *Quercus* sp. in Hungary. Thus it is likely that oak is the main host of this species. *D. villosus* is the first insect to be found in association with this fungus as no other vector is previously known (Aghayeva et al. 2005). However, *S. dentifunda* was only found on two *D. villosus* specimens, indicating that this is a casual fungal associate.

4.1.3 Species within *Leptographium sensu lato*

Species within *Leptographium sensu lato* are common on varius coniferous trees (Jacobs & Wingfield 2001), but few species are known from broadleaved trees. *Grosmannia francke-grosmanniae* and *O. brevicolle* has been isolated from broadleaved trees (Davidson 1971; Jacobs & Wingfield 2001), but considerable confusion exists regarding the taxonomy of these two species (De Beer & Wingfield 2013). *Leptographium trypodendroni* isolated from *T. domesticum* infesting *Fagus sylvatica* L. and *Leptographium betulae* R. Jankowiak, B. Strzałka

& R. Linnakoski isolated from *Betula pendula* Roth. were recently found by Jankowiak et al. 2017.

Grosmannia piceiperda

Grosmannia piceiperda was found on one *D. alni* specimen infesting *A. incana* in Kvæfjord. This species groups in the *G. piceiperda* complex which consists of many cryptic species (De Beer & Wingfield 2013). Isolates treated as *G. piceiperda* has been found on many different coniferous trees in many parts of the world including; Europe, North America, Russia and Japan, but represent different lineages (Linnakoski et al. 2012; Ando et al. 2016). The *G. piceiperda* isolate obtained in this study grouped together with *G. piceiperda* isolates from Europe when analyzing the β-tubulin region. The European clade in this study has relatively low bootstrap support. However, other studies have shown that European isolates of *G. piceiperda* are distinct from the rest of the species within the *G. piceiperda* complex (Linnakoski et al. 2012; Ando et al. 2016). This is probably the first time *G. piceiperda* has been found in association with a broadleaved tree, but it appears that this species is very rarely associated with bark beetles treated in this study.

Leptographium trypodendroni

Leptographium trypodendroni form part of complex treated here as the *L. pruni* complex. No description is presented in this thesis since the Norwegian material is included in a submitted paper where the description is done (Jankowiak et al. 2017). In the study by Jankowiak et al. (2017) was *L. trypodendroni* commonly found in association with *T. domesticum* infesting *F. sylvatica*. No bark beetles from this host-tree were investigated in this study, but *L. trypodendroni* was commonly isolated from *T. domesticum* infesting *A. incana* and was also found on *T. domesticum* infesting *B. pubescence* and *F. excelsior* and seems to be adapted to grow in the xylem of many different tree species. This ability is probably an important trait for this fungus considering that *T. domesticum*, which seems to be the main vector of this species, may attack many different broadleaved tree species. *Leptographium trypodendroni* was also rarely found in association with *D. alni* but this is unlikely to be an important vector of this species.

***Leptographium* sp 1.**

Leptographium sp 1.was only found on one *D. alni* specimen sampled from northern Norway. Related species have been found in association with bark beetles infesting coniferous trees in many parts of the world, but very few isolates of each species have been found (Linnakoski et al. 2012; Taerum et al. 2013; Liu et al. 2017). No sexual states have been observed of any of the species within this complex (Linnakoski et al. 2012; Liu et al. 2017) nor was any sexual state produced by *Leptographium* sp 1. This complex consists of very similar species and were only separated in the EF1-α analysis, this is also mentioned by (Liu et al. 2017). *Leptographium* sp. 1 is according to the EF1-α data distinct from both *L. taigense* and *L. innermongolicum,* but is very similar to an undescribed *Leptographium* species found by Taerum et al. (2013) (*Leptographium* sp. 3 SJT-2013). This fungus was found in association with the bark beetle *Dendroctonus valens* LeConte 1860 in North America, but has not been described and only represented by a single isolate. It is difficult to say whether or not *Leptographium* sp. 1. and *Leptographium* sp. 3 SJT-2013 represent one or two species since so few isolates are known.

***Ophiostoma brevicolle*-like**

Ophiostoma brevicolle-like was found in association with *T. signatum*, *T. domesticum* and *D. alni*. This species forms part of a complex close to the *G. olivacea* complex. According to the ITS data the species is closely related to *O. brevicolle* and *G. francke-grosmannia* (*G. francke-grosmanniae* was also close when using β-tubulin). *Grosmannia francke-grosmannia* has been found on *Quercus* spp. in association with *Hylecoetus dermestoides* (Linnaeus, 1761) in Germany and *O. brevicolle* has been found on *Populus tremuloides* Michx infested by *Trypodendron retusus* (LeConte, 1868) in USA (Jacobs & Wingfield 2001). The morphology of both *G. francke-grosmannia* and *O. brevicolle* described by Jacobs and Wingfield (2001) resemble that of *O. brevicolle*-like. From the morphology and ITS data, it seems that these species form a clade of hardwood infesting species within *Leptographium.* However, the ITS2-LSU data indicated that both *O. brevicolle* and *G. francke-grosmannia* are very distantly related to *O. brevicolle*-like. The reason for the different results is that the ITS and ITS2-LSU gene regions originate from different strains and this is discussed by De Beer & Wingfield (2013). The ITS sequence of *G. francke-grosmannia* is obtained from the same strain used in the description by Jacobs and Wingfield (2001) thus it is likely that *G. francke-grosmannia* and *O. brevicolle* is part of the same complex. If *O. brevicolle* also is a member of this complex is more

uncertain as the ITS2-LSU sequence is the same strain used in the description of this species by Jacobs & Wingfield (2001). The strains treated as *O. brevicolle* and *G. francke-grosmannia* must be revisited in order to resolve the confusion regarding this potential species complex.

Ophiostoma brevicolle-like was sporadically found in southeastern Norway in association with *T. domesticum* and *D. alni*. It was found on beetles infesting *A. incana*, *F. excelsior* and *Q. robur* indicating that this species has a broad host range. The species was very commonly found in association with *T. signatum* sample in northern Norway and was also found on one *T. domesticum* specimens from the same region (only two investigated specimens). This species seems to be adapted to live in the xylem of various broadleaved trees much like *L. trypodendroni*. Whether this species is more common in northern Norway or more commonly associated with *T. signatum* was not resolved since *T. signatum* was not found in southern Norway.

4.1.4 Species within *Graphilbum*

Graphilbum is a very distant group in the Ophiostomatales that consist of many cryptic fungi often only known to produce anamorph states (De Beer & Wingfield 2013). *Graphilbum fragrans* was the only member of this complex found during this study.

Graphilbum fragrans

Graphilbum fragrans was found on *T. domesticum T. signatum* and *D. alni* infesting *A. incana* This species has no known sexual state, but the isolates are easy to recognize when grown on MEA because of the yellow colonies and long synnemata with brown stipes. These characteristics are typical for the species (Jacobs et al. 2003; Paciura et al. 2010). The β-tubulin sequences obtained in this study were identical to *G. fragrans* found in Poland by Jankowiak and Bilański (2013), but were also similar to isolates collected in China by Paciura et al. (2010). *Graphilbum fragrans* has previously been found on varius coniferous trees in association with many different insect vectors, and seems to have an almost worldwide distribution. It has previously been found in associated with *Ips typographus* infesting *Picea abies* Karst. in Norway (Solheim 1992) and also in other European countries in association with weevils and bark beetles infesting coniferous trees (Romon et al. 2007; Jankowiak & Bilański 2013) It has also been found in Canada, in association with *T. lineatum* and an introduced long horn beetle,

but *G. fragrans* is believed to be native to the region. (Jacobs et al. 2003). It has also been found in China associated with conifer-infesting weevils (Paciura et al. 2010) and on introduced bark beetles in south Africa. (Zhou et al. 2006). It has also been reported from USA, Australia and New Zealand (Harrington et al. 2001).

In this study *G. fragrans* was found in both southern and northern Norway, but was only isolated from bark beetles infesting *Alnus incana*. (*T. domesticum, T. signatum* and *D. alni*). This is probably the first record of *G. fragrans* in association with any of the mentioned bark beetles or any other bark beetles associated with broadleaved trees. It was in most cases only sporadically found and this is consistent with other studies (Jacobs et al. 2003; Romon et al. 2007; Jankowiak & Bilański 2013). The only location *G. fragrans* was quite commonly found was from the *T. domesticum* material sampled in Nes (42%). The large variety in both host and insect vector reported for this species, indicate that it is a generalist.

4.1.5 Species with unknown taxonomic position

***Sporothrix foliorum*-like**

This species was found in association with *A. dispar* and *S. intricatus* infesting *Quercus* sp. *Sporothrix foliorum*-like is according to the EF1-α analysis distinct from *Sporothrix foliorum*. It is probably not forming part of *Sporothrix*, but seems to groups within *Ophiostoma sensu lato*, but this must be confirmed by LSU sequences. In this study, the isolates are treated as one species. However, the β-tubulin analysis indicated that the isolates found could represent two closely related species, one associated with *A. dispar* and one associated with *S. intricatus*. This was, however, not supported by the EF1-α analysis. Different subgrouping with different primers has been reported for other species with large genetic variation, like *O. quercus* (Grobbelaar et al. 2009) A more thorough morphological survey is needed to determine the number of species within this group. *Sporothrix foliorum*-like was commonly associated with *S. intricatus* at both sampling sites and was in total isolated from 48% of the investigated specimens. It was only occasionally isolated from *A. dispar* found on the same log.

***Ophiostoma* sp. 1**

Ophiostoma sp. 1 was found in association with *D. alni* and *T. domesticum* infesting *A. incana*. The ITS analysis indicated that this species form part of *Ophiostoma sensu lato*, but LSU

sequences are needed to confirm this. It was only occasionally found in association with *D. alni* infesting alder (5.3%) and was only found on one specimens of *T. domesticum*, indicating that this species is a casual fungal associate of both species. It was found both in southern and northern Norway indicating that this species has a wide distribution. This species is very slow growing (0.3 mm/day at 20°C) and is probably easily overgrown by other fungi growing in the main Petri dishes. Thus this species might have been overlooked and the frequency of this species might be higher than what is observed in this study.

4.2 Bark beetles

Phloem breeding bark beetles

4.2.1 Dryocoetes alni

Dryocoetes alni is a common species on newly dead *A. incana A. glutinosa* and *C. avellana* and is common in most of Europe (Lekander et al. 1977). It was found to be vectoring eight ophiostomatoid fungi; *Gra. fragrans, Gro. piceiperda*, *O. brevicolle*-like, *O. karelicum*-like, *Ophiostoma* sp. 1, *Leptographium* sp. 1, *L. trypodendroni* and *Sporothrix* sp. 1. No ophiostomatoid fungi have previously been found in association with this beetle. All of the ophiostomatoid fungi were found in association with *D. alni* infesting *A.* incana, but very few specimens were sampled from *C. avellana.* All of the ophiostomatoid associates can be considered casual associates. *Dryocoetes alni* was associated with many of the same species found in association with *T. domesticum,* but the fungal species were often more commonly found on *T. domesticum*, indicating that many of the species are primarily transported by the latter vector. However, *Ophiostoma* sp. 1 was more commonly found on *D. alni* compared to *T. domesticum* and three ophiostomatoid fungi were only found in association with *D. alni* (*G. piceiperda, Leptographium* sp. 1, *Sporothrix* sp. 1), but these fungi were only found on 1-2 beetles. The results show that this bark beetles is capable of transporting many different ophiostomatoid species, but it is unlikely that ophiostomatoid fungi are of any major importance. This is also indicated by the absence of ophiostomatoid fungi from specimens infesting *C. avellana*.

4.2.2 Dryocoetes villosus

Dryocoetes villosus is mainly found on weakened *Quercus* spp. and may also infest *F. sylvatica*. It is considered as a rare species in the Nordic countries, but is found in whole Europe and in North Africa (Lekander et al. 1977). No ophiostomatoid fungi have previously been found in association with this beetle. Three ophiostomatoid fungi were found in this study: *Ophiostoma quercus*-like, *O. quercus,* and *S. dentifunda*. All of the species were found in relatively low numbers. The most common was *O. quercus*-like (26.7%) which only occurred in association with this bark beetle.

4.2.3 Hylesinus crenatus

Hylesinus crenatus mainly infests the thick bark of *F. excelsior*, but may also be found on other broadleaved trees and it is common in the whole of Europe (Lekander et al. 1977). Two ophiostomatoid fungi were found. *Ophiostoma catonianum* was commonly found on beetles from both sampling indicating that this fungus may be of some importance to *H. crenatus*. *Ophiostoma karelicum* was only rarely found. No ophiostomatoid fungi have previously been found in association with this beetle

4.2.4 Hylesinus varius

Hylesinus varius is common on *F. excelsior* throughout Europe, but may also rarely be found on other broadleaved trees (Lekander et al. 1977) This species was only associated with *O. catonianum*-like, which was present on one beetle, indicating that this bark beetle is only rarely associated with ophiostomatoid fungi. However, three species of *Geosmithia* were found in association with this beetle and 81% of the beetles carried at least one *Geosmithia* species. Kirschner (2001) investigated 123 specimens of *H. varius* of which none transported ophiostomatoid fungi, but 74% of the investigated beetles were associated with *Geosmithia putterillii* (Thom) Pitt. None of the *Geosmithia* species obtained in this study were closely related to *G. putterillii* (data not shown). Of the tree *Geosmithia* species found in this study, one was closely related *G. flava* M. Kolarik, Kubatova & Pazoutova, one was close to *Geosmithia* sp. 11 and the last was close to *Geosmithia* sp. 12. The two latter species are from Kolařík et al. (2008) who also found *Geosmithia* sp. 12 in association with *H. varius*. Thus it seems that *H. varius* primarily is associated with *Geosmithia* species.

4.2.5 Scolytus laevis

Scolytus laevis is found in southern and western Norway and southern Sweden but is not found in Finland. It is also distributed in other parts of Europe and Russia (Lekander et al. 1977). This species mainly infests *Ulmus* spp, but may occasionally be found on other broadleaved trees (Lekander et al. 1977). This bark beetle is considered the main vector of DED in Norway (Hansen et al. 1998). *Ophiostoma novo-ulmi* was the only ophiostomatoid fungi found in association with S. *laevis,* and the frequency was relatively high (50.8%). However, it is difficult to say anything about the effectiveness of *S. laevis* as a vector based solely on frequencies. The number of spores is an important factor influencing whether or not the DED-fungi enters and establish via the feeding grooves made by the beetles during maturation feeding (Sutherland & Brasier 1997). Large differences in spore loads have been found among different *Scolytus* species and is believed to be linked to where the beetles pupate (Webber 1990). Beetles that pupate in the phloem, which is where sporulation of DED-fungi most commonly occurs tend to have higher spore loads than beetles that pupate in the outer bark (Webber 1990). *Scolytus laevis* is considered as a less effective vector of DED, mainly because it pupates in the xylem (Webber 2004). Also indicating that *S. laevis* is not very effective in transferring this disease is that the impact of DED in Norway has been quite low (Solheim et al. 2011). *Ulmus glabra*, which is the only native *Ulmus* species in Norway is highly susceptible to *O. novo-ulmi,* and if an effective vector was associated with this tree species the impact should have been greater. (Webber 2004). According to (Moser et al. 2010) may some phoretic mites play important roles in the transmission of DED. Phoretic mites were only rarely found on *S. laevis* and all were removed to avoid them to be establish in the laboratory

The absence of other ophiostomatoid fungi found on *S. laevis* was surprising as *Scolytus* species usually are associated with several different ophiostomatoid fungi (Kirisits 2004; Linnakoski 2011). The sampling method used to collect *S. laevis* could have affected the number of fungal species. All the *S. laevis* specimens were collected by use of emergence traps and the bolts were shielded from rain for about one month. This has probably altered the moisture-content of the bolts which might have affected the number of ophiostomatoid fungi present on the beetles. Another possible explanation is that most ophiostomatoid fungi seldom sporulate in the xylem were the beetles pupate. It is also possible that DED has replaced many of the ophiostomatoid fungi naturally associated with bark beetles infesting *Ulmus* spp. (Brasier 1990). This sound

dramatic, but *O. novo-ulmi* has been shown to have rapidly replaced *O. ulmi* across Europe (Brasier et al. 2004).

4.2.6 Scolytus intricatus

This species mainly attacks *Quercus* species, but may also infest other broadleaved trees and is found in large parts of Europe (Lekander et al. 1977). It was associated with three ophiostomatoid fungi; *S. foliorum*-like *S. prolifera* and *O. quercus*. Both *S. prolifera* and *O. quercus* have probably been found in previous studies. *Sporothrix prolifera* was most likely found by Kubátová et al. (2002), but the species was treated as *O.*cf. *prolifera.* The same study also reported finding *O. peace* s.l. but this is very likely to be *O. quercus* or another species in the *O. ulmi* complex. Linnakoski et al. (2008) listed other ophiostomatoid fungi from older studies found in association with this bark beetles: *Ophiostoma kubanicum, O. roboris*, *O. valachicum* (these are all synonyms of *O. quercus* (Harrington et al. 2001; Grobbelaar et al. 2009)) and *O. stenoceras* (Robak) Nannf. Thus *Ophiostoma stenoceras* was most likely the only species previously reported in association with *S. intricatu*s which was not found in this study.

4.2.7 Scolytus ratzeburgi

Scolytus ratzeburgi is distributes in Europe, Siberia and Japan and is considered as a common species in Norway, and attacks weakened *Betula* species (Lekander et al. 1977). *Ophiostoma karelicum* and *O. quercus* were found in association with this beetle, of which *O. karelicum* was by far the most common. This corresponds well with other studies regarding *S. ratzeburgi* (Linnakoski et al. 2008; Linnakoski et al. 2009; Jankowiak 2011). However, previous studies have detected ophiostomatoid fungi not observed in this study. *Ophiostoma denticiliatum*, *O. borealis* and two undescribed ophiostomatoid fungi have been found to be causal associates (Linnakoski et al. 2009). However, some of them have been isolated from the galleries, not from the beetles and more species would probably have been found in this study if isolation of galleries had been included. The use of emergence traps used on *Scolytus* species in this study, may have reduced the fungal diversity by altering the moisture-content of the collected bolts.

Xylem breeding bark beetles

4.2.8 Anisandrus dispar

This species is found in Europe, Siberia and North Africa and infests various species of broadleaved trees (Lekander et al. 1977). It has also been introduced to North America (Haack & Rabaglia 2013). *Anisandrus dispar* was found on *Q. robur* and found to be associated with three ophiostomatoid fungi; *S. lunata*-like, *S. foliorum*-like and *O. quercus*, of which *S. lunata*-like was the most common. The biology of this bark beetle has been thoroughly studied (French & Roeper 1975), but only two ophiostomatoid fungi have previously been found. The ambrosia fungus *Ambrosiella hartigii* L.R. Batra and a species treated as *Ceratocystis* sp. (Batra 1967; Zimmermann 1973). The larvae depend on ambrosia fungi as a food source and are unable to gnaw wood (Francke-Grosmann 1967). However, no ambrosia fungus was found in association with this bark beetle or any of the other ambrosia beetles in this study. Many studies have been conducted on ambrosia fungi transported by bark beetles, but the result have often been divergent (Francke-Grosmann 1967). Growth of ambrosia fungi in culture is often very slow and the spores often fail to germinate (Batra 1963; Francke-Grosmann 1967). Studies focusing on ambrosia fungi use different and more advanced isolation techniques compared to this study and the growth media often contain streptomycin (Batra 1963; Mayers et al. 2015).

4.2.9 Trypodendron domesticum

Trypodendron domesticum is found throughout Europe and infest various species if broadleaved tress (Lekander et al. 1977). It was found to be associated with eight species of ophiostomatoid fungi: *O. karelicum*-like, *O. karelicum, O. quercus, O. brevicolle*-like, *Ophiostoma* sp. 1, *L. trypodendroni, G. fragrans* and *Sporothrix lunata*-like of which *O. karelicum*-like and *L. trypodendroni* can be considered to commonly associate with this beetle while the rest seem to be causal associates. *Ophiostoma quercus* has previously been found in association with *T. domesticum* infesting *F. sylvatica* by Carlier et al. (2006) and also most likely by Zimmermann (1973), who reported finding of *O. piceae* (this could very likely be *O. quercus*). Also *L. trypodendroni* has previously been found in association with this bark beetle (Jankowiak et al. 2017). Most of the ophiostomatoid fungi previously reported from *T. domesticum* were not found in my study. Two ambrosia fungi have been found in association with this beetle, *Ambrosiella ferruginea* (Math.-Käärik) L.R. Batra (Batra 1967) and a closely

related species treated as *Phialophoropsis* sp. by Mayers et al. (2015). *Ophiostoma arduennense* F.X. Carlier, Decock, K. Jacobs & Maraite and *O. bacillisporum* have been found in association with *T. domesticum* infesting *F. sylvatica* in Belgium (Carlier et al. 2006). *Ophiostoma bacillisporum* was also found by Zimmermann (1973) who in addition found *Ophiostoma torulosum* (Butin & G. Zimmermann) Georg Hausner, J. Reid & Klassen., *Graphium penicillioides* Corda and a species treated as *Graphium* sp. A study conducted in Scotland found *Ophiostoma ambrosium* (B.K. Bakshi) Georg Hausner, J. Reid & in association with *T. domesticum* infesting *B. pendula* (Bakshi 1950). This fungus has previously been treated as a synonym of *O. piliferum*, but is very distinct from this species (De Beer et al. 2013b) The reasons for the differences observed when comparing my results to previous studies are most likely numerous, but most important are probably that the studies have been conducted in other geographical areas and that most of the previous studies have investigated *T. domesticum* infesting *F. sylvatica*. Host-tree has been shown to have large influence on the fungal community found in associating with bark beetles (Kirisits 2004; Linnakoski 2011).

4.2.10 Trypodendron signatum

In Norway, this species is rather rear but is common in Finland and is distributed in most of Europe and infests various species of broadleaved trees (Lekander et al. 1977). *Trypodendron signatum* was associated with *O. brevicolle*-like *O. bacillisporum*-like, *O. karelicum*-like and *G. fragrans*. Both *O. brevicolle*-like and *O. bacillisporum*-like were commonly isolated and could be considered as consistent associates. However, *T. signatum* was only sampled from one location and more beetles from other regions are needed to confirm this. *Ophiostoma karelicum*-like and *G. fragrans* were both casual associates. The only fungus previously mention in association with *T. signatum* is the ambrosias fungus *Ambrosiella ferruginea* (Batra 1967), which most likely were unable to grow on the growth medium in this study.

4.3 Diversity of ophiostomatoid fungi associated with broadleaved trees

The majority of investigated bark beetle species were associated with 1-3 species of ophiostomatoid fungi and this is in contrast to most coniferous infesting bark beetles which often transport many species (Kirisits 2004; Linnakoski 2011). This difference could be due to the number of infesting bark beetle species, which is much higher in coniferous trees (Lekander et al. 1977). Thus a conifer-infesting bark beetles may come in contact with more

ophiostomatoid fungi usually transported by other species compared to bark beetles infesting broadleaved trees

The results of this study indicate that the diversity of ophiostomatoid fungi associated with broadleaved trees is relatively low in Norway and this is consistent to previous studies in this region (Linnakoski et al. 2009; Kamgan Nkuekam et al. 2010). However, only six ophiostomatoid fungi were known from Norway before this study (Solheim & Hietala 2015) and the number of species have now increased much. Three of the species previously reported from Norway were not found during this study (*O. borealis, O. denticiliatum* and *O. ulmi*), and indicate that also many unknown species might have been overlooked. Thus, the diversity of ophiostomatoid fungi in association with broadleaved trees in Norway is most likely higher. When also considering the great diversity of broadleaved tree species, one could expect high diversity among ophiostomatoid fungi infesting them, as each host species represent different environments and niches. That host-tree species influences the mycobiota is clearly seen in this study and also mentioned by others (Kirisits 2004; Linnakoski 2011).

4.4 General remarks

Comparison of the mycobiota found on bark beetles from different location should be done with care. The bark beetles have been sampled using an opportunistic sampling approach which has resulted in uneven sampling sizes, different sampling methods and sampling during different time of year. The majority of the investigated beetles were sampled during the later stages of hibernation and it is likely that many of the fungi found are so called “weed fungi” that are late colonizers of galleries. Also, the sampled material mostly consisted of beetles that have never dispersed. Dispersion flight may influence the number of spores transported by beetles as the spores get exposed to UV-radiation and desiccation, killing the spores (Beaver 1989). Wind may also probably loosen spores of certain “weed fungi” that are poorly adapted to be transported by insects. Thus, sampling during early colonization could have given different results.

Logs that were attacked by bark beetles were often attacked by more than one species and also often by other wood colonizing insects. The number of invertebrate species inhabiting a log is probably crucial for the species composition of ophiostomatoid fungi present. Galleries of different species of insects were often very close or overlapping and the risk of fungi being

transferred to neighboring galleries is probably high. Thus many of the casual fungal associates are probably primarily transported by other insects occupying the same tree.

The fungal isolation methods used in a study is crucial for the results, and all methods are selective (Kirisits 2004). The method used in this study did not involve use of antibiotics which may be mixed in the growth medium to exclude unwanted species. One example is the use of cycloheximide which are tolerated by *Ophiostoma* species, but inhibits many other fungi (Zipfel et al. 2006). Thus the number and frequencies of *Ophiostoma* species could have been higher if this was used. However, this is not suited for studies also searching for other ophiostomatoid fungi which are sensitive to this antibiotic. The methods used in this study favors fungi that grow well on the MEA or/and produce fruiting structures that are easy to observe, while fungi that are slow growing and do not produce synnemata or ascomata are easily overlooked and might be more common than what is reported. Ambrosia fungi have most likely been selected against in this study since no ambrosia fungi were detected even though three species of ambrosia beetles were investigated.

Many changes have occurred in fungal taxonomy after the introduction of DNA sequencing (Zipfel et al. 2006). Comparing new findings based on phylogenetic species recognition with older publications based on morphology is often problematic because the species concepts are different. This study clearly shows the importance of using sequence data when dealing with ophiostomatoid fungi. However, sequence data can often lead to more confusion and be misleading. The ITS sequences were in most cases far from variable enough to separate closely related species and even the β-tubulin gen was to conserved within some species complexes. EF1-α seem to have better resolution than β-tubulin and this is also mentioned by (Grobbelaar et al. 2009). However, more primer failure was associated with the primers used for this gene compared to the primers used for the β-tubulin gene. Calmodulin seem to have very high resolution and the primers used in this study seem to work well. However, few ophiostomatoid fungi have been sequenced using this gene region except from species within *Sporothrix* (De Beer et al. 2016). I believe that this gene region will be used more in future studies also when dealing with ophiostomatoid fungi outside of *Sporothrix*.

5 Conclusion

The results show that several species of ophiostomatoid fungi are associated with bark beetles infesting broadleaved trees in Norway, but that most of the bark beetle species only have a few ophiostomatoid fungal associates. Some of the ophiostomatoid fungi appear to be relatively consistently associated with one bark beetle species. Others seem to be generalists, occurring with variable frequencies with several bark beetle species while some seem to be casual associates.

Even though the diversity of ophiostomatoid fungi was relatively low, several new taxa were found and this study clearly shows that this is a topic that needs more investigation. Future studies regarding ophiostomatoid fungi associated with broadleaved trees are likely to discover many new species.

6 References

Aghayeva, D. N., Wingfield, M. J., de Beer, Z. W. & Kirisits, T. (2004). Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. *Mycologia*, 96: 866-878.

Aghayeva, D. N., Wingfield, M. J., Kirisits, T. & Wingfield, B. D. (2005). *Ophiostoma dentifundum* sp. nov. from oak in Europe, characterized using molecular phylogenetic data and morphology. *Mycological Research*, 109: 1127-1136.

Ando, Y., Masuya, H., Motohashi, K., Linnakoski, R. & Yamaoka, Y. (2016). Phylogenetic relationship of Japanese isolates belonging to the *Grosmannia piceiperda* complex (Ophiostomatales). *Mycoscience*, 57: 123-135.

Ayres, M. P., Wilkens, R. T., Ruel, J. J., Lombardero, M. J. & Vallery, E. (2000). Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. *Ecology*, 81: 2198-2210.

Bakshi, B. K. (1950). Fungi associated with ambrosia beetles in Great Britain. *Transactions of the British Mycological Society*, 33: 111-120.

Batra, L. R. (1963). Ecology of ambrosia fungi and their dissemination by beetles. *Transactions of the Kansas Academy of Science*, 66: 213-236.

Batra, L. R. (1967). Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. *Mycologia*, 59: 976-1017.

Beaver, R. (1989). Insect-fungus relationships in the bark and ambrosia beetles. In Wilding, N., Collins, N. M., Hammond, P. M. & Webber, J. F. (eds) *Insect-fungus interactions*, pp. 121-143. London: Academic Press.

Billings, R. F., Clarke, S. R., Mendoza, V. E., Cabrera, P. G., Figueroa, B. M., Campos, J. R., & Baeza, G. (2004). Bark beetle outbreaks and fire: a devastating combination for Central America's pine forests. *Unasylva* 217.

Brand, J., Bracke, J., Britton, L., Markovetz, A. & Barras, S. (1976). Bark beetle pheromones: Production of verbenone by a mycangial fungus of *Dendroctonus frontalis*. *Journal of Chemical Ecology*, 2: 195-199.

Brasier, C. (1990). China and the origins of Dutch elm disease: an appraisal. *Plant Pathology*, 39: 5-16.

Brasier, C. (1991). *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. *Mycopathologia*, 115: 151-161.

Brasier, C., Buck, K., Paoletti, M., Crawford, L. & Kirk, S. (2004). Molecular analysis of evolutionary changes in populations of *Ophiostma novo-ulmi*. *Forest Systems*, 13: 93-103.

Brasier, C. M. & Kirk, S. A. (2001). Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. *Mycological Research*, 105: 547-554.

Carbone, I. & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, 91: 553-556.

Carlier, F.-X., Decock, C., Jacobs, K. & Maraite, H. (2006). *Ophiostoma arduennense* sp. nov.(*Ophiostomatales, Ascomycota*) from *Fagus sylvatica* in southern Belgium. *Mycological research*, 110: 801-810.

Davidson, R. W. (1971). New species of Ceratocystis. *Mycologia*, 63: 5-15.

De Beer, Z., Glen, H., Wingfield, B. & Wingfield, M. (2003). *Ophiostoma quercus* or *Ophiostoma querci*? *Mycotaxon* 86: 211-214.

De Beer, Z. W., Wingfield, B. D. & Wingfield, M. J. (2003). The *Ophiostoma piceae* complex in the Southern Hemisphere: a phylogenetic study. *Mycological Research*, 107: 469-476.

De Beer, Z. W., Seifert, K. A. & Wingfield, M. J. (2013a). The ophiostomatoid fungi: their dual position in the Sordariomycetes. In Seifert, K. A., Wilhelm De Beer, Z. & Wingfield, M. J. (eds) *The ophiostomatoid fungi: expanding frontiers*, *CBS Biodiversity Series,* vol. 12. pp. 1-19. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

De Beer, Z. W., Seifert, K. & Wingfield, M. J. (2013b). A nomenclator for ophiostomatoid genera and species in the *Ophiostomatales* and *Microascales*. In Seifert, K. A., Wilhelm De Beer, Z. & Wingfield, M. J. (eds) *The ophiostomatoid fungi: expanding frontiers*, *CBS Biodiversity Series,* vol. 12. pp. 245-322. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

De Beer, Z. W. & Wingfield, M. J. (2013). Emerging lineages in the *Ophiostomatales*. In Seifert, K. A., Wilhelm De Beer, Z. & Wingfield, M. J. (eds) *The ophiostomatoid fungi: expanding frontiers*, *CBS Biodiversity Series,* vol. 12. pp. 21-46. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

De Beer, Z. W., Duong, T. A. & Wingfield, M. J. (2016). The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. *Studies in Mycology*, 83: 165-191.

Duong, T. A., de Beer, Z. W., Wingfield, B. D. & Wingfield, M. J. (2012). Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. *Mycologia*, 104: 715-732.

Ehnström, B. & Axelsson, R. (2002). *Insektsgnag i bark och ved*: ArtDatabanken SLU, Uppsala, Sweden.

Farrell, B. D., Sequeira, A. S., O'Meara, B. C., Normark, B. B., Chung, J. H. & Jordal, B. H. (2001). The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, 55: 2011-2027.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
Francke-Grosmann, H. (1967). Ectosymbiosis in wood-inhabiting insects. In Henry, S. M. (ed.) *Symbiosis* vol. 2. New York Academic Press: 141-205.
French, J. & Roeper, R. (1975). Studies on the biology of the ambrosia beetle *Xyleborus dispar* (F.)(Coleoptera: Scolytidae). *Journal of Applied Entomology*, 78: 241-247.
Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular ecology*, 2: 113-118.
Glass, N. L. & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, 61: 1323-1330.
Goidánich, G. (1935). Una nuova specie di "*Ophiostoma*" vivent sul pero ed alcune osservazioni sull'esatta posizione sistematica della forma ascofora e delle forme metagenetiche del genere. *Bollettino della Stazione di Patologia Vegetale di Roma*, 15: 122-168.
Grobbelaar, J. W., Aghayeva, D. N., de Beer, Z. W., Bloomer, P., Wingfield, M. J. & Wingfield, B. D. (2009). Delimitation of *Ophiostoma quercus* and its synonyms using multiple gene phylogenies. *Mycological Progress*, 8: 221-236.
Haack, R. A. & Rabaglia, R. J. (2013). Exotic bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in the United States: potential and current invaders. In Peña, J. E. (ed) *Potential invasive pests of agricultural crop species*. CAB International, Wallingford, UK: 48-74.
Hansen, L. O., Ligaard, S. & Sagvolden, B. (1998). Notes on Norwegian Coleoptera. 4. *Fauna Norvegica Serie B*, 45: 77-82.
Harrington, T. C., McNew, D., Steimel, J., Hofstra, D. & Farrell, R. (2001). Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. *Mycologia*, 93: 111-136.
Harrington, T. C. (2005). Ecology and evolution of mycophagous bark beetles and their fungal partners. In Vega, F. E. & Blackwell, M (eds) *Insect-fungal associations: ecology and evolution*. pp. 257-291. Oxford University Press, Oxford.
Hofstetter, R. & Moser, J. (2014). The role of mites in insect-fungus associations. *Annual review of entomology*, 59: 537-557.
Hofstetter, R. W., Cronin, J. T., Klepzig, K. D., Moser, J. C. & Ayres, M. P. (2006). Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. *Oecologia*, 147: 679-691.
Horntvedt, R., Christiansen, E., Solheim, H. & Wang, S. (1983). Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. *Meddelelser fra Norsk institutt for skogforskning*, 38(4): 1-20.
Hulcr, J. & Dunn, R. R. (2011). The sudden emergence of pathogenicity in insect–fungus symbioses threatens naive forest ecosystems. *Proceedings of the Royal Society of London B: Biological Sciences*, 278: 2866-2873.
Jacobs, K., Bergdahl, D. R., Wingfield, M. J., Halik, S., Seifert, K. A., Bright, D. E. & Wingfield, B. D. (2004). *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological research*, 108: 411-418.
Jacobs, K., Seifert, K. A., Harrison, K. J. & Kirisits, T. (2003). Identity and phylogenetic relationships of ophiostomatoid fungi associated with invasive and native *Tetropium* species (Coleoptera: Cerambycidae) in Atlantic Canada. *Canadian Journal of Botany*, 81: 316-329.

Jacobs, K. & Wingfield, M. J. (2001). *Leptographium species: tree pathogens, insect associates, and agents of blue-stain*. American Phytopathological Society (APS Press).

Jankowiak, R. (2011). First report of *Ophiostoma karelicum* from birch stands in Poland. *Phytopathologia*, 59: 55-58.

Jankowiak, R. & Bilański, P. (2013). Diversity of ophiostomatoid fungi associated with the large pine weevil, *Hylobius abietis*, and infested Scots pine seedlings in Poland. *Annals of forest science*, 70: 391-402.

Jankowiak, R. Strzałka, B. Bilański, P. Linnakoski, R. Aas, T. Solheim, H. Groszek, M. de Beer Z. W. (2017) Two new *Leptographium* spp. reveal an emerging complex of hardwood-infecting species in the Ophiostomatales. (submitted to Antonie van Leeuwenhoek)

Kamgan Nkuekam, G., Jacobs, K., De Beer, Z., Wingfield, M. & Roux, J. (2008). *Ceratocystis* and *Ophiostoma* species including three new taxa, associated with wounds on native South African trees. *Fungal Diversity*, 29: 37-59.

Kamgan Nkuekam, G., Solheim, H., Grobbelaar, J. W. & Jacobs, K. (2010). *Ophiostoma* species, including *Ophiostoma borealis* sp. nov., infecting wounds of native broad-leaved trees in Norway. *Cryptogamie Mycologie*, 31: 285–303.

Kirisits, T. (2004). Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C. & Evans, H. F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*, pp. 181-236. Dordrecht, The Netherlands: Springer.

Kirkendall, L. R., Biedermann, P. H. & Jordal, B. H. (2015). Evolution and diversity of bark and ambrosia beetles. In Vega, F. E. & Hofstetter, R. W. (eds) *Bark beetles: biology and ecology of native and invasive species.* pp. 85-156. San Diego, California: Academic Press.

Kirschner, R. (2001). Diversity of filamentous fungi in bark beetle galleries in central Europe. In Misra, J. K. & Horn, B. W. (eds) *Trichomycetes and other fungal groups*, pp. 175-196. Enfield, NH, USA: Professor Robert W Lichtwardt Commemoration Volume Science Publishers.

Kleppe, K., Ohtsuka, E., Kleppe, R., Molineux, I. & Khorana, H. (1971). Studies on polynucleotides: XCVI. Repair replication of short synthetic DNA's as catalyzed by DNA polymerases. *Journal of molecular biology*, 56: 341-361.

Klepzig, K. D. & Wilkens, R. T. (1997). Competitive interactions among symbiotic fungi of the southern pine beetle. *Applied and Environmental Microbiology*, 63: 621-627.

Knížek, M., and Beaver, R. 2004. Taxonomy and systematics of bark and ambrosia beetles. In Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C. & Evans, H. F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*, pp. 41-54. Dordrecht, The Netherlands: Springer.

Kolařík, M., Kubátová, A., Hulcr, J. & Pažoutová, S. (2008). *Geosmithia* fungi are highly diverse and consistent bark beetle associates: evidence from their community structure in temperate Europe. *Microbial Ecology*, 55: 65-80.

Kowalski, T. & Butin, H. (1989). Taxonomie bekannter und neuer *Ceratocystis*-Arten an Eiche (*Quercus robur* L.). *Journal of phytopathology*, 124: 236-248.

Krokene, P., Nagy, N. E., Fossdal, C. G. & Solheim, H. (2013). Conifer defense against insects and fungi. In Seifert, K. A., Wilhelm De Beer, Z. & Wingfield, M. J. (eds) *The ophiostomatoid fungi: expanding frontiers*, *CBS Biodiversity Series,* vol. 12. pp. 141-153. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Kubátová, A., Novotný, D. & Prášil, K. (2002). *Microscopic fungi associated with oak bark beetle (*Scolytus intricatus*) in the Czech Republic. IMC7 Book of Abstracts: 300*. International Mycological Congress, Oslo.

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33: 1870-1874.

Kurz, W. A., Dymond, C., Stinson, G., Rampley, G., Neilson, E., Carroll, A., Ebata, T. & Safranyik, L. (2008). Mountain pine beetle and forest carbon feedback to climate change. *Nature*, 452: 987-990.

Kvamme, T. & Lindelöw, Å. (2014). Corrections and comments to the Norwegian part of the Palaearctic list of bark beetles (Coleoptera, Curculionidae, Scolytinae). *Norwegian Journal of Entomology*, 61: 201-212.

Lekander, B., Bejer-Petersen, B., Kangas, E. & Bakke, A. (1977). The distribution of bark beetles in the nordic countries. *Acta Entomologica Fennica*, 32: 1-37.

Leufvén, A., Bergström, G. & Falsen, E. (1984). Interconversion of verbenols and verbenone by identified yeasts isolated from the spruce bark beetle *Ips typographus*. *Journal of Chemical Ecology*, 10: 1349-1361.

Linnakoski, R. (2011). *Bark beetle-associated fungi in Fennoscandia with special emphasis on species of* Ophiostoma *and* Grosmannia. PhD thesis, Faculty of Science and Forestry School of Forest Sciences, University of Easteren Finland, Joensuu.

Linnakoski, R., De Beer, Z. W., Rousi, M., Niemelä, P., Pappinen, A. & Wingfield, M. J. (2008). Fungi, including *Ophiostoma karelicum* sp. nov., associated with *Scolytus ratzeburgi* infesting birch in Finland and Russia. *Mycological research*, 112: 1475-1488.

Linnakoski, R., De Beer, Z. W., Rousi, M., Solheim, H. & Wingfield, M. J. (2009). *Ophiostoma denticiliatum* sp. nov. and other *Ophiostoma* species associated with the birch bark beetle in southern Norway. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 23: 9-15.

Linnakoski, R., de Beer, Z. W., Duong, T. A., Niemelä, P., Pappinen, A. & Wingfield, M. J. (2012). *Grosmannia* and *Leptographium* spp. associated with conifer-infesting bark beetles in Finland and Russia, including *Leptographium taigense* sp. nov. *Antonie Van Leeuwenhoek*, 102: 375-399.

Liu, X.-W., Wang, H.-M., Lu, Q., Decock, C., Li, Y.-X. & Zhang, X.-Y. (2017). Taxonomy and pathogenicity of *Leptographium* species associated with *Ips subelongatus* infestations of *Larix* spp. in northern China, including two new species. *Mycological Progress*, 16: 1-13.

Martin, M. M. (1992). The evolution of insect-fungus associations: from contact to stable symbiosis. *American Zoologist*, 32: 593-605.

Mayers, C. G., McNew, D. L., Harrington, T. C., Roeper, R. A., Fraedrich, S. W., Biedermann, P. H., Castrillo, L. A. & Reed, S. E. (2015). Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. *Fungal biology*, 119: 1075-1092.

Moser, J. C., Konrad, H., Blomquist, S. R. & Kirisits, T. (2010). Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease? *Naturwissenschaften*, 97: 219-227.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, 51: 263-273.

Nei, M. & Kumar, S. (2000). *Molecular evolution and phylogenetics*. New York, Oxford university press.

O'Donnell, K. & Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the Fungus *Fusarium* Are nonorthologous. *Molecular phylogenetics and evolution*, 7: 103-116.

O'Donnell, K., Kistler, H. C., Cigelnik, E. & Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences*, 95: 2044-2049.

Paciura, D., Zhou, X., De Beer, Z., Jacobs, K., Ye, H. & Wingfield, M. (2010). Characterisation of synnematous bark beetle-associated fungi from China, including *Graphium carbonarium* sp. nov. *Fungal Diversity*, 40: 75-88.

Paine, T. D., Raffa, K. F. & Harrington, T. C. (1997). Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology*, 42: 179–206.

Raffa, K. F., Gregoire, J.-C. & Lindgren, B. S. (2015). Natural history and ecology of bark beetles. In Vega, F. E. & Hofstetter, R. W. (eds) *Bark beetles: biology and ecology of native and invasive species.* pp. 1-40. San Diego, California: Academic Press.

Romon, P., Zhou, X., Iturrondobeitia, J. C., Wingfield, M. J. & Goldarazena, A. (2007). *Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. *Canadian Journal of Microbiology*, 53: 756-767.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4: 406-425.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the national academy of sciences*, 74: 5463-5467.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Bolchacova, E., Voigt, K. & Crous, P. W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109: 6241-6246.

Selochnik, N. N., Pashenova, N. V., Sidorov, E., Wingfield, M. J. & Linnakoski, R. (2015). Ophiostomatoid fungi and their roles in *Quercus robur* die-back in Tellermann forest, Russia. *Silva Fenn*, 49: doi.org/10.14214/sf.1328

Six, D. L. (2003). Bark beetle-fungus symbioses. In Bourtzis, K. & Miller, T. (eds) *Insect symbiosis*. pp. 97-114. CRC Press, Boca Raton, FL.

Solheim, H. (1992). Fungal succession in sapwood of Norway spruce infested by the bark beetle *Ips typographus*. *European Journal of Forest Pathology*, 22: 136-148.

Solheim, H., Eriksen, R. & Hietala, A. M. (2011). Dutch elm disease has currently a low incidence on wych elm in Norway. *Forest Pathology*, 41: 182-188.

Solheim, H. & Hietala, A. 2015. Ophiostomatoid fungi in Norway. Agarica, 36: 75-88.

Spatafora, J. W. & Blackwell, M. (1994). The polyphyletic origins of ophiostomatoid fungi. *Mycological Research*, 98: 1-9.

Sutherland, M. & Brasier, C. (1997). A comparison of thirteen d-factors as potential biological control agents of *Ophiostoma novo-ulmi*. *Plant Pathology*, 46: 680-693.

Taerum, S. J., Duong, T. A., De Beer, Z. W., Gillette, N., Sun, J.-H., Owen, D. R. & Wingfield, M. J. (2013). Large shift in symbiont assemblage in the invasive red turpentine beetle. *PLoS One*, 8: e78126.

Taylor, J. W. (2011). One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. *IMA fungus*, 2: 113-120.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S. & Fisher, M. C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal genetics and biology*, 31: 21-32.

Upadhyay, H. P. (1981). *A monograph of Ceratocystis and Ceratocystiopsis*: Athens, Georgia: The University of Georgia Press

Webber, J. (2004). Experimental studies on factors influencing the transmission of Dutch elm disease. *Forest Systems*, 13: 197-205.

Webber, J. F. (1990). Relative effectiveness of *Scolytus scolytus*, *S. multistriatus* and *S. kirschi* as vectors of Dutch elm disease. *Forest Pathology*, 20: 184-192.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18: 315-322.

Zhou, X., de Beer, Z. W. & Wingfield, M. J. (2006). DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa. *Studies in Mycology*, 55: 269-277.

Zimmermann, G. (1973). *Vergleichende ökologisch-physiologische Untersuchungen an Ambrosiapilzen, assoziierten Bläuepilzen und Luftbläuepilzen*. PhD thesis, Georg-August-Universität Göttingen, Göttingen, Germany.

Zipfel, R. D., De Beer, Z. W., Jacobs, K., Wingfield, B. D. & Wingfield, M. J. (2006). Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Studies in mycology*, 55: 75-97.

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