

**Ophiostomatoid fungi associated with conifer-infesting
bark beetles in China**

by

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Declaration

I, Runlei Chang, declare that the dissertation, which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Preface

The ophiostomatoid fungi is a group of sapwood staining fungi that includes several species that are tree and human pathogens. Based on their similar morphology and ecology, the taxonomy of these species was confused for many years, but during the past two decades phylogenetic inferences based on DNA sequence data have shown that these species can be separated in several distinct genera in two orders, the Ophiostomatales and Microascales (Ascomycota) (De Beer et al., 2013). These fungi are specifically adapted for spore dispersal by arthropods. Of these, bark and ambrosia beetles (Scolytinae and Platypodinae, Coleoptera) and their phoretic mites (Acari) are the most common vectors.

The first ophiostomatoid fungus, *Ophiostoma piliferum*, was described from stained pine wood in Sweden almost two centuries ago (Fries, 1823). Since that time the bulk of research on these fungi has focused on the species associated with conifer-infesting bark beetles in the Europe, North America, and to a lesser extent, Japan (Wingfield et al., 1993; Seifert et al., 2013), with a growing body of research on hardwood infesting ambrosia beetles and their fungi that emerged over the past decade (Hulcr & Stelinski, 2017). From China, more than 160 bark beetle species has been recorded, but the knowledge about their fungal assemblages is scant and nothing has been published about the fungal symbionts of bark beetle associated mites from this country. The overarching aim of this thesis was thus to expand our knowledge on the diversity of fungal species and mites associated with conifer-infesting bark beetles in four provinces in China (**Figure 1**). I focused on beetles infesting conifer trees in Yunnan and their mites and fungal assemblages, the fungi associated with *Ips typographus* in Heilongjiang and Jilin Provinces, and the fungal associates of mites phoretic on beetles in Qinghai.

Ips typographus is one of the most aggressive bark beetles in Europe, mainly attacking spruce. In Google Scholar searches with the words '*Ips typographus*' picked up the second most hits of all the well-known bark beetles after *Scolytus scolytus*, the Elm Bark Beetle vector of the Dutch Elm Disease fungi. Although some aspects about *I. typographus* has been reviewed during the past 20 years, the last comprehensive review on this beetle was in 1988 (Christiansen & Bakke, 1988). Rather than to write another broad review (of which there are several) on bark beetles and their fungal associates, I chose *I. typographus* as a model, and in **Chapter 1**, I provide a comprehensive review about this beetle. The review focuses on the literature of the past 20 years, and especially the impact of DNA based technologies on the knowledge base of the beetle. First, I provide general information about *I. typographus* including its taxonomy and life cycle. This is followed by sections on the geographical distribution and host range, population dynamics, and chemical ecology of the beetle. Next, ecological interactions with tree hosts, fungi, bacteria, entomopathogens, nematodes, mites, parasitoids and predators are summarized. The last section centers around the management of the beetle, and it culminates with some conclusions about remaining gaps in the knowledge of the beetle.

Compared to other parts of China, Yunnan in Southwestern China (**Figure 1**) has a high biodiversity because of its unique geography, and the province forms part of the second largest forest area in China. However, to date only 13 ophiostomatoid species have been reported from Yunnan and all these species were reported as associates of bark beetles. In **Chapter 2**, I further explored the diversity of ophiostomatoid fungi associated with conifer-infesting bark beetles and their mites in this province. Samples including beetles, mites and fungi were collected from different sites and tree hosts. Beetles and mites were identified by morphological characters, while fungal species were identified based on morphological characters and DNA sequences.

From the review on *Ips typographus* in **Chapter 1**, it became evident that the fungal assemblages of *I. typographus* were extensively studied in Europe and Japan. More than 40 species have been reported from

Europe and 13 from Japan. From China, only two species have been reported to date, even though the beetle has been causing severe damage to spruce forests in northeastern China for many years. In **Chapter 3** I present the results from the first systematic survey of fungi associated with *I. typographus* and its galleries from trees in Heilongjiang and Jilin provinces (**Figure 1**). Fungal species were identified by morphological characters and DNA sequences of multiple gene regions. The list of fungal associates of *I. typographus* in China were then compared to those from Europe and Japan in an attempt to see whether the fungal assemblages in different geographical regions reflect the genetic population structure of the beetle as has been explored in previous studies.

Qinghai province (**Figure 1**) is one of the world's biodiversity hotspots because of its diverse landscapes and complex geological and climatic history. However, only five species of ophiostomatoid fungi have been described in association with bark beetles from Qinghai, and nobody has studied the fungal symbionts of phoretic mites in this province. In the final chapter of thesis, **Chapter 4**, I explored the diversity of ophiostomatoid fungi with mites phoretic on four bark beetle species in Qinghai. We collected samples from two forest regions in the province and identified species with multiple gene phylogenies and morphological characters.

This thesis focused on ophiostomatoid fungi associated with bark beetles and mites on conifer hosts in China. I have collected and identified more than 1500 fungal isolates representing 52 species from 22 beetle species and 34 mite species. I report 13 ophiostomatoid species for the first time from China, and have described 19 new species. These represent 37 % of the total number of 87 ophiostomatoid species known from China. As such, this thesis makes a substantial contribution to our understanding of the diversity of fungi associated with bark beetles in East Asia.

The research herein helps us to understand the fungal assemblages in different part of China with different beetle and mite species. Also, it has contributed to understanding biological diversity of China.

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Figure 1 The provinces of China. Bold names indicate the four provinces where bark beetles, mites and fungi were collected for the different chapters of this thesis.

Summary

Ophiostomatoid fungi are causal agents of blue stain in wood and are commonly associated with bark beetles and their phoretic mites. Some ophiostomatoid species are tree pathogens and together with their beetle vectors they can dramatically change forest structures. Although the majority of these fungi are not pathogens, they may reduce the value of timber because of the stain they cause. More than 350 ophiostomatoid species have been described globally, of which 48 have been reported from China. China has in excess of 2 million km² of forests, accounting for 22% of the country's surface area. These forests harbour over 2800 tree species. The main hypothesis of this thesis was that Chinese forests harbour many as yet undescribed species of ophiostomatoid fungi. The aim was thus to explore and characterize the diversity of these fungi in China, focusing on conifer-infesting bark beetle ecosystems in four provinces.

As background to the thesis, the first chapter consists of a literature review that focused on the Eurasian spruce bark beetle, *Ips typographus*. This is the best studied conifer-infesting bark beetle on earth but the latest comprehensive review on this species was published in 1988. The body of research about this beetle is immense and it thus serves as model system for bark beetles in general. One of the major conclusions of this chapter was that in view of available DNA sequence data, the currently accepted synonymy of *Ips typographus japonicus* with *Ips typographus* should be reconsidered.

In the first experimental chapter, 340 fungal isolates were obtained from 17 beetle and 13 mite species in Yunnan. The isolates represented 19 taxa based on multiple gene sequences, and five new species were described. Although the numbers of beetles and mites were insufficient to provide statistical inferences, the study confirmed that mites are important vectors of the Ophiostomatales in China, and are most likely responsible for horizontal transfer of fungal species between galleries of different beetle species.

In the second experimental chapter, 1046 fungal isolates were obtained from 145 *Ips typographus* beetles and 178 of their galleries from forests in Heilongjiang and Jilin. A total of 23 ophiostomatoid species were identified based on multigene sequences, including 11 novel species that were described in this chapter. Comparisons of the fungal symbionts of *I. typographus* from China, Europe and Japan suggested that the fungal assemblages of the bark beetle in the different geographical areas do not reflect the population structure of the beetle.

In the final chapter, the fungal diversity of mites phoretic on four bark beetle species in Qinghai province was examined. A total of 135 fungal isolates were obtained from 173 mite individuals belonging to 18 species. Based on DNA sequences of four gene regions, the isolates could be classified into 14 taxa, all species of the Ophiostomatales. Three of these were described as new species. The results highlighted the fact that mites should not be ignored in pest risk assessments of bark beetles, which often focus only on the beetles and their fungi.

Chapter 1

The European spruce bark beetle, *Ips typographus*: a review

ABSTRACT

Ips typographus (Coleoptera: Scolytinae) is a conifer-infesting bark beetle also known as the Eurasian spruce bark beetle. This beetle species is considered as one of the most destructive forest pests in Europe and Asia, mainly attacking *Picea* species and killing millions of hectares of trees during the course of the 20th Century. As such, it is one of the best studied bark beetles globally, with more than 10000 research papers dedicated to specific aspects of its biology, ecology, and management. This review synthesizes the major areas of research on this model species. The first section considers the taxonomic status of the beetle. By combining and re-analyzing DNA sequence data from various studies, we show that the Far East Asian form of the beetle, previously treated as *I. typographus* f. *japonicus* or simply *I. japonicus*, but currently considered a synonym of *I. typographus*, probably represents a distinct species. The name *I. japonicus* should be reinstated for it. Furthermore, the review highlights the fact that nothing is known about the fungal and mite assemblages of *I. typographus* in China. Other biotic interactions between the beetle and trees, parasitoids, nematodes, and predators, as well as the impact of abiotic factors such as climate change, drought and wind on beetle dynamics are also discussed. Similarly, the chemical ecology as well as previously and currently applied management strategies are briefly considered. The study provides an exemplar narrative of one bark beetle species, but also highlights the many remaining gaps in our current knowledge of the ecology of this beetle that could guide researchers working on this and other bark beetle ecosystems in the future.

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INTRODUCTION

Ips typographus (L.) (Coleoptera: Scolytinae) was one of the first five bark beetle species to be formally described (Linnaeus, 1758). It is a conifer-infesting bark beetle, commonly known as the eight spined spruce bark beetle (Stauffer et al., 1992), the European spruce bark beetle (Wermelinger, 2004), or the Eurasian spruce bark beetle (Viiri & Lieutier, 2004). As the latter name suggests, the species is distributed throughout Eurasia (**Fig. 1**), attacks primarily spruce trees in which it constructs breeding galleries in the cambial layer under the bark and where eggs are laid and overwinter. These galleries are complex ecosystems where the beetles co-exist with many symbionts, including fungi and other microbes, as well as other arthropods (Hofstetter et al., 2015). The beetle generally establishes in dying or stressed trees, but when population numbers become high during outbreaks, they can also infest healthy trees, causing tree mortality (Kirkendall et al., 2015).

The Eurasian spruce bark beetle is considered one of the most destructive forest pests in Europe, and has been responsible for heavy losses in spruce stands in several European countries during the 1990's (Grégoire & Evans, 2004). In the same time period, the beetle also caused serious damage to spruce stands in Japan (Yamaoka et al., 1997) and China (Sun et al., 2007). The species has caused tree mortality only in its native range in Eurasia although its accidental introduction to other parts of the world could have devastating implications.

Specific aspects of the biology, impact and management of *I. typographus* have been treated regularly by researchers, in especially Europe and Japan over the past few decades. Google Scholar searches (15 June 2018, excluding patents and citations) using Scolytine bark beetle species names as search criteria, revealed that, after *Scolytus scolytus*, the Elm Bark Beetle vector of the Dutch Elm Disease fungi with 10700 hits, *I. typographus* had the second most hits at 10100. This was more than for all the other well-known conifer-infesting bark beetle pests such as *Dendroctonus frontalis* (Southern Pine Beetle) with 9990 hits, *D. ponderosae* (Mountain Pine Beetle) with 9070 hits, *Tomicus piniperda* (Pine Shoot Beetle) with 4540 hits, and *D. valens* (Red Turpentine Beetle) with 3120 hits. These figures underpin the economic importance and potential risk that *I. typographus* poses. However, the last comprehensive review on *I. typographus* was published in 1988 (Christiansen & Bakke, 1988), with subsequent reviews focusing on specific aspects such as its ecology and management (Wermelinger, 2004), a detailed list of its fungal associates in Europe (Kirisits, 2004), chemical ecology (Sun et al., 2006), population dynamics (Kausrud et al., 2012), possible impacts of climate change on outbreaks (Økland et al., 2015), and diapause and overwintering behavior (Schebeck et al., 2017). With more than 60% (i.e. 6100) of the *I. typographus* publications listed in Google Scholar published after 2006, the present review aims to provide an up to date synthesis of the most important aspects of research related to *I. typographus*. Special emphasis is placed on research published in the past decade based on DNA sequences and other modern technologies that have provided novel insights into the taxonomy and population genetics of the beetle and its fungal associates.

TAXONOMY

Until the late 1990's, the classification of Scolytine beetles at all levels of taxonomy was based on external morphological characters, especially those of the head, thorax and elytra (Knížek & Beaver, 2004). Together

with gallery structure, host tree and locality, these characters are still useful for preliminary identifications of species such as *I. typographus*. However, for the non-specialist, morphological characteristics do not provide easy resolution of *Ips* from other Ipini genera, or of *I. typographus* from closely related species. During the past two decades, DNA sequence-based phylogenies have become the norm in taxonomic studies on this group (Cognato & Sun, 2007; Cognato, 2013), and robust topologies based on sequences of multiple genes and supported by morphology, are enabling faster and more accurate identifications of most *Ips* species, including *I. typographus* (Cognato, 2015).

The genus *Ips* DeGeer

Linnaeus (1758) described *I. typographus* initially as *Dermestes typographus* (L.). The genus *Ips* DeGeer was later erected for seven scolytine species, of which *I. typographus* was listed first (DeGeer, 1775). However, during the course of the next century, the genus was treated by different authors as synonym of genera such as *Bostrichus* and *Tomicus* (Wood & Bright, 1992; Cognato, 2015). *Ips* only became accepted in the literature after Bergroth (1884) re-instated the name and explicitly designated *I. typographus* as type species. Despite this, the delineation of *Ips* and closely related genera and their higher classification remained problematic until phylogenetic studies based on DNA sequences resolved most of these issues. Initially, only mitochondrial cytochrome oxidase I (COI) sequences were used to address genus level questions (Stauffer et al., 1997; Cognato, 2000; Cognato & Sperling, 2000). However, multiple gene regions such as mitochondrial 16S rRNA (16S), nuclear elongation factor-1 α (EF-1 α) (Cognato & Vogler, 2001; Cognato & Sun, 2007) nuclear 28S rRNA (28S) and multifunctional nuclear protein coding gene (CAD) (Cognato, 2013; Cognato, 2015) have become common when delineating Scolytine genera (Hulcr et al., 2015).

Based on current phylogenetic data, the genus *Ips* belongs to the tribe Ipini, subfamily Scolytinae, weevil family Curculionidae, in the order Coleoptera (**Fig. 2**) (Hulcr et al., 2015; Pistone, 2018). The tribe Ipini contains the *Ips* group that includes the genera *Ips*, *Orthotomicus*, *Pityogenes* and *Pityokteines*, as well as *Acanthotomicus*, *Premnobius* and *Premnophilus* (**Fig. 3**) (Cognato, 2013). Within the genus *Ips*, four monophyletic subgenera are currently recognized: *Ips* (*Ips*) DeGeer, *Ips* (*Bonips*) Cognato, *Ips* (*Cumatotomicus*) Ferrari, and *Ips* (*Granips*) Cognato (Cognato & Vogler, 2001; Cognato & Sun, 2007; Cognato, 2015). *Ips typographus* belongs to the first of these sub-genera (**Fig. 4**). There are at present 37 accepted *Ips* species that are distributed throughout conifer forests of the northern hemisphere, with 23 species occurring only in North and Central America, and the remaining 14 species in Eurasia (Cognato, 2015).

Seven main morphological characters are used to define the genus *Ips* (Cognato, 2015). These are: (1) the antennae consist of five funicular segments and an oval club; (2) the antennal club is compressed and three sutures divide the anterior face; (3) the sutures vary in shape from straight, bisinuate, to acutely angulate; (4) the elytral declivity originally defined the genus and is concave with spines along the lateral margins and ending in an expanded marginal apex; (5) female spines are reduced in size and spines never occur on the face of the declivity; (6) the parts of the male genitalia include the median lobe, median struts, internal sac, tegmen, seminal trough, and the speculum gastrale; and (7) the seminal trough is variable in shape but consistently represented as two rods either held parallel or crossing each other and ending in an acute or broad tip.

Morphology of *Ips typographus*

The morphological characters that distinguish *I. typographus* from closely related species are the four spines on the elytral declivity (**Fig. 5**). It differs from other Eurasian species by its dull elytral declivity and impunctate

on the basal half of the elytral disc. It can also be distinguished from the Himalayan and North American spruce-feeding species, as well as *I. woodi*, by the presence of a major medial frontal tubercle (Cognato, 2015). The colour, size and shape of *I. typographus* individuals can vary between localities and years, and this has led to some confusion in the past (Sallé et al., 2005).

The morphological differences between males and females of *I. typographus* are minor and difficult to observe, even for experienced taxonomists. In the past, several external morphological characters (e.g. density of bristles on the pronotum) were used to distinguish the sexes, but over time most of these were shown to be unreliable (Schlyter & Cederholm, 1981). The most reliable method is genital dissection but in the process specimens are damaged and this is consequently not an ideal approach (Zahradníková, 2014).

Synonyms of *Ips typographus*

In the latest nomenclator listing all species in the genus *Ips* (Cognato, 2015), two formal heterotypic synonyms are listed for *I. typographus* as follows:

Ips typographus (Linnaeus) 1758

Bostrichus octodentatus Paykull 1800

Ips japonicus Niisima 1909

Paykull (1800) described *Bostrichus octodentatus* from Sweden. He recognized the similarities with *I. (Bostrichus) typographus*, but distinguished between the species based on minor differences in size and the number of spines on the elytra. According to Wood & Bright (1992), Thomson (1965) considered the two species as synonyms, a treatment that has been widely accepted (Titovšek, 1983; Wood & Bright, 1992; Cognato, 2015).

Niisima (1909) described *I. japonicus* from Yezo spruce and Sachalin spruce in Japan, distinguishing it from *I. typographus* based on the elytra that are opaque versus the more shiny elytra of *I. typographus*. Kono & Tamanuki (1939) found these differences to be inconsistent and reduced *I. japonicus* to a subspecies of *I. typographus* for which the trinomial *I. typographus japonicus* had often been used (Nobuchi, 1974; Furuta et al., 1996; Lawson et al., 1997; Moser et al., 1997b), while other authors referred to the Japanese form as *I. typographus* f. *japonicus* (Christiansen & Bakke, 1988; Yamaoka et al., 1997; Yamaoka et al., 2000; Masuya et al., 2003; Økland et al., 2011). However, Sokanovskii (1954) considered the Japanese population as conspecific with *I. typographus* s. str., and thus treated it as a synonym of the latter species. Wood & Bright (1992) accepted the synonymy, although subsequent publications continued to treat the Japanese population as a distinct form or sub-species (Furuta et al., 1996; Lawson et al., 1997; Moser et al., 1997; Yamaoka et al., 1997, 2000; Masuya et al., 2003; Økland et al., 2011). Despite the currently accepted synonymy of *I. japonicus* with *I. typographus*, genetic studies in the past 20 years, which have compared beetle populations from Europe, China and Japan, suggest that the synonymy should be reconsidered. This is discussed in some detail in the following section.

Population genetics and within species variation

Initial attempts to study the diversity in *I. typographus* populations from Austria, using allozyme patterns, were rather inconclusive (Stauffer et al., 1992). Later, the first DNA sequences produced for *I. typographus* were used together with allozyme markers to determine the phylogenetic relatedness of the species with six other European *Ips* species (Stauffer et al., 1997). Sequences of the mitochondrial cytochrome oxidase I (COI)

gene were generated for nine *I. typographus* individuals from Austria and revealed two haplotypes among them (Stauffer et al., 1997). In a subsequent study, Stauffer et al. (1999) sequenced three gene regions (COI, COII, and a non-transcribed region between the COI and tRNA-leu genes) in a population study on 17 European populations from locations in southwestern France to as far east as the Moscow region. One specimen, labelled as *I. typographus* ssp. *japonicus* from Jilin Province in China, was included as outgroup. The allozyme analyses did not show geographical structure, and the COII and non-coding region sequences did not reveal polymorphic sites. However, in the COI gene region sequences, eight haplotypes were detected among the European isolates, and in a cladogram, the Chinese specimen grouped basal to the European haplotypes.

In the early 2000's, microsatellite markers were commonly employed to determine population structure for various organisms (Vignal et al., 2002; Varshney et al., 2005; Werth, 2010). Five microsatellite markers were thus developed using 30 individuals of *I. typographus* from France (Salle et al., 2003). The markers were later used to study 28 populations from across Europe, but again results were rather inconclusive with a lack of genetic structure across Europe. However, one population (12 specimens) from Shanghai, China, and one (11 specimens) from Japan, were included. The data suggested significant differences between these two populations and those of Europe, but the two East Asian populations were also fairly well differentiated from each other.

Cognato & Sperling (2000) used COI to reconstruct phylogenies based on 39 *Ips* species, showing that the genus is polyphyletic. Although this was thus not a population study, they included three specimens labelled as *I. typographus*: one from the Czech Republic, one from western Russia (St. Petersburg), and one from far eastern Russia (Khabarovsk, Russia), just across the border from Heilongjiang Province, China (Cognato & Sperling, 2000). The latter specimen grouped in a well-supported clade with the other two specimens, but interestingly, it differed in several base pair positions from those collections.

In an unpublished MSc study, Zhang (2009) explored the genetic diversity of seven *I. typographus* specimens from seven sites in Jilin and Heilongjiang provinces in northern China. His Chinese specimens grouped in an east Asian clade with three subclades. The first of these included five of his Chinese specimens; the second with two of his Chinese specimens and the far eastern Russian specimen used by Cognato & Sperling (2000). The third sub-clade included the published sequence of the Chinese '*I. typographus* ssp. *japonicus*' specimen reported in Stauffer et al. (1997) and two unpublished sequences from the same authors. In GenBank, the latter three sequences are labelled as *I. typographus* haplotypes IX, X, and XI, while their paper mentioned only haplotypes I to VIII from Europe. Zhang (2009) concluded that there was a clear distinction between the European and Chinese/ East Russian populations of *I. typographus*.

Bertheau et al. (2011) showed that nuclear copies of mitochondrial DNA (NUMT) containing COI sequences, might lead to the erroneous interpretation of haplotype richness and thus population diversity in *I. typographus*. They identified 16 NUMT haplotypes from 11 European populations totaling 421 individuals. Nine of these NUMT haplotypes were unique, while seven corresponded with authentic mitochondrial haplotypes. The authors outlined several strategies to identify NUMTs, but suggested caution in the interpretation of within species diversity based on COI sequences. Bertheau et al. (2013) followed this study with a reconsideration of *I. typographus* diversity in Europe by analyzing COI and nuclear ribosomal internal transcribed spacer 2 (ITS2) sequences of 394 specimens from across Europe. Analyses of COI sequences revealed 32 haplotypes separating into three major haplogroups, while ITS2 sequences were less variable and

only four alleles were observed. No specimens from East Asia were included in their analyses.

The most recent population study on *I. typographus* (Mayer et al., 2015) included 296 individuals collected from 108 localities in Europe and Asia. However, in terms of East Asian specimens, the numbers were low in comparison to those from Europe. Four specimens from the Central Siberian Plateau were included. Ten of these were from the Russian Far East, 18 from Japan and none were from China. Sequences for five molecular markers were determined, including COI, arginine kinase (AK), EF-1 α , glucose-6-phosphate dehydrogenase (G-6-PD) and a protein referred to as Wingless (Wg). The results showed for the first time that there was a genetic separation between northern and southern European populations. However, a deep genealogical divergence was again observed between the Eurasian and Far East Asian populations, this time supported by multiple loci (**Fig. 6**). These were also the first DNA sequences published for specimens from Japan.

From the studies discussed above, it is clear that the population structure of *I. typographus* reveals interesting patterns. Even with data available for only a small number of East Asian specimens, it is evident that those populations are genetically very distinct from the European populations. Although all the available data cannot be compared because different studies employed different technologies and sequences from different gene regions, when the available COI sequences of these studies are analysed together (**Fig. 7**), the East Asian (Far East Russia, China and Japan) specimens form a well-supported subclade within the larger *I. typographus* clade. In view of the problems with NUMTs as mentioned above, COI sequences should be considered with caution (**Fig. 7**). But the fact that the East Asian-European split is also supported by the four additional gene regions sequenced by Mayer et al. (2015), demands further consideration. In order to make inferences on population genetics and to reach a final conclusion about the taxonomic placement of the East Asian clade, sequences of several gene regions (see Table 2.3, Hulcr et al., 2015) for a greater number of specimens (i.e. populations) from different localities in Eastern Russia, China, Japan and South Korea should be analysed.

LIFE CYCLE

Like in all other *Ips* species, the greatest part of the *I. typographus* life cycle occurs within the cambium and phloem beneath the tree bark. Similar to the standard life cycle of most bark beetles, in this life-cycle Sauvard (2004) includes the reproduction, development, maturation and dispersal phases (**Fig. 8**). Depending on the altitude and temperature, the life cycle of *I. typographus* is typically completed in between 7 and 11 weeks, but it can be interrupted by overwintering (Sauvard, 2004). Up to three generations can be produced annually (Wermelinger, 2004). Selected details regarding the biology and life cycle of *I. typographus* are summarized in **Table A**.

The reproduction phase (**Fig. 8**) of *I. typographus* starts when mature insects arrive on a tree, and the male bores through the bark to initiate gallery construction by excavating a mating (nuptial) chamber in the phloem (Sauvard, 2004; Čičková & Kozánek, 2015). Aggregation pheromones are then emitted, attracting conspecifics of both sexes to the site (Christiansen & Bakke, 1988). In the progradation phase (i.e. the beginning of an outbreak), the sex ratio is skewed towards much more than 50% females, but towards the retrogradation phase (i.e. the end of an outbreak) the ratio normalizes towards 1:1 (Wermelinger, 2004). Females that arrive on the bark compete through “pushing behavior” and the “winner(s)” enter the nuptial gallery and mate with the waiting male (Paynter et al., 1990). All this can occur within 24 hours after the

male has colonized the tree (Čičková & Kozánek, 2015). Being polygamous, the male can have a up to five females, each of which then bores in a different direction, creating breeding galleries radiating outwards from the mating chamber (Schlyter & Zhang, 1996) (**Fig. 9**). The females chew out well defined egg niches in the tunnel wall at regular intervals and lay one egg per niche (Schlyter & Zhang, 1996). At low attack densities, each female can lay up to 80 eggs on the sides of the breeding gallery, but the numbers of eggs, the sides on which they are laid and the general shape of the breeding gallery varies based on the density of attack and thus competition from neighboring galleries (Wermelinger, 2004). During this process, the male does not assist in construction of breeding galleries, but clears away frass (Sauvard, 2004). After about three weeks, the parental beetles leave their first brood and initiate a second, and sometimes even a third brood. The second and third egg-laying periods are characterized by short and often curved egg galleries (Annala, 1969).

The development phase (**Fig. 8**) is entirely endophytic as larvae and pupae of bark beetles cannot survive outside the host tree (Sauvard, 2004). Larvae of *I. typographus* feed on phloem, generally boring individual galleries more or less perpendicular to the maternal gallery. The larvae moult in the galleries. Pupation takes place in little individual pupal chambers, created by the older larvae at the terminal ends of their individual tunnels in the phloem (Sauvard, 2004).

After the imaginal moult, teneral beetles need a period of maturation (**Fig. 8**) before being able to reproduce. During this time cuticle sclerotization takes place, and the beetles remain in the galleries where development occurred, consuming the remaining phloem around the galleries. When mature, adults emerge from the brood material using the maternal entrance hole, any other hole in the bark, or if necessary by perforating it (Sauvard, 2004).

The beetles disperse (**Fig. 8**) in search of a suitable tree for reproduction right after emergence. *Ips typographus* individuals can fly for up to six hours, easily covering distances of more than 500 m at a time (Forsse & Solbreck, 1985). Dispersal of *I. typographus* from 19-50 km within a single generation has been recorded (Botterweg, 1982; Forsse & Solbreck, 1985; Franklin & Gregoire, 1999). The beetles can encounter more than one suitable host tree for reproduction during the dispersal flight. The manner in which beetles locate and accept susceptible host trees is discussed in the *Chemical Ecology* section of this review.

Like many other insects, *I. typographus* has the ability to enter a diapause as a strategy to survive under unfavourable conditions. The diapause is a period of suspended development during the life cycle, characterized by a reduced metabolic rate, a suspension of reproduction and development, and increased resistance to stress (Schebeck et al., 2017). The onset of diapause is triggered by low temperatures and a reduction in photoperiod (Table A), and the beetles are consequently completely reliant on external conditions. This results in inconsistency and unpredictability of life cycle activities from one year to the next (Schebeck et al., 2017). Development of beetles ceases when the temperature drops below 8 °C and adults will die below -32 °C. The flight period is also sensitive to temperature. The first flight takes place when the air temperature reaches above 16.5 °C (Lobinger, 1994). Overwintering occurs in particular locations: underneath the snow cover in the soil or forest litter in northern Europe and underneath the bark in central Europe (Annala, 1969; Botterweg, 1982; Faccoli, 2002). When the first low temperatures occur, the beetles move from brood galleries to the overwintering location. The number of generations per year is thus largely determined by temperature. Higher temperatures allow for rapid oviposition, development and maturation, thus short generation times, and earlier swarming in the spring (Sauvard, 2004). Apart from thermal conditions, voltinism also varies with other ecological factors such as the photoperiod, population density, seasonal

variation in mortality, and resource availability (Öhrn, 2012). In northern Europe and Scandinavia, *I. typographus* has one generation and one (or several) sister broods, while in central Europe it typically has two generations and several sister broods. In the southern Alps, it usually has three generations per year. In China it has one generation in Heilongjiang province, while further south in Jilin province, it has up to three generations (Zhang, 2009). The minimum temperatures and daylength that determines the onset of the diapause has been determined in some of these areas (**Table 1**).

GEOGRAPHICAL DISTRIBUTION AND HOST RANGE

Ips typographus occurs widely in spruce and pine forests throughout Europe and Asia (**Fig. 1**) (Cognato, 2015) in the following countries:

Europe: Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Moldova, Montenegro, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, and Ukraine.

Western Asia: Georgia, Russia (Volga, Northwest, Central, South, North Caucasus), Turkey.

Central Asia: Kazakhstan, Russia (Siberia, Ural), Tajikistan.

East Asia: Mongolia, Russia (Far East, Sakhalin Islands), China (Heilongjiang, Henan, Inner Mongolia, Jilin, Qinghai, Sichuan, Shanxi and Xinjiang province), Japan, North Korea and South Korea.

Africa: Algeria.

Ips typographus poses a substantial threat to spruce and other conifers grown in countries such as the USA, Canada, Australia and New Zealand, where it had been intercepted frequently, but had not yet established (Humphreys & Allen, 1999; Haack, 2001; Brouckhoff et al., 2006; Økland et al., 2011; Lawson et al., 2018).

The main tree host of *I. typographus* in Europe is *Picea abies* (Norway spruce) (**Table 2**). In China, the main hosts are *Picea koraiensis*, *P. jezoensis* and *P. schrenkiana*, while in Japan it primarily infests *Picea jezoensis* and *Picea glehnii* (Yamaoka et al., 1997). During population eruptions, attacks on nonhost genera sometimes occur (Huber et al., 2009), and such episodes may initiate host switches that could potentially become stable and lead to speciation (Lindgren & Raffa, 2013).

POPULATION DYNAMICS

Population dynamics is the study of the change in abundance, dispersion, and age structure of bark beetles (Weed et al., 2015). For economically important beetles such as *I. typographus*, an understanding of the factors contributing to decline and growth of bark beetle population abundance over time and space, forms the foundation for management strategies. These include pest control, harvesting practices and species conservation (Weed et al., 2015). Although these are very difficult to predict (Weed et al., 2015), there are some factors that are considered most important drivers of population dynamics (Raffa et al., 2015).

Bottom-up forces such as host tree availability and host quality are thought to be primary drivers of bark beetle population dynamics (Reeve, 1997). Bark beetles face a high risk of attack failure on healthy trees because of multifaceted defense mechanisms, and a lower risk of attack failure on weakened or stressed

trees because of the low defense levels (Raffa et al., 2015). Many studies on *I. typographus* showed that availability of breeding substrates in the form of storm-felled trees act as the main outbreak trigger (Öhrn, 2012; Marini et al., 2013; Kärnemo et al., 2014).

Intraspecific and interspecific competition of bark beetles also drive the population dynamics of *I. typographus* (Raffa et al., 2015). Although male and female beetles respond positively to aggregation pheromones produced by their counterparts (Bakke et al., 1977), *I. typographus* populations are regulated by a negative density feedback, suggesting that intraspecific competition is probably an important driver for population density (Økland & Berryman, 2004; Marini et al., 2013). Similarly, *I. typographus* preferentially colonizes uninfested logs or parts of logs, rather than those occupied by competitors for the same niche such as *Pityogenes chalcographus* (Byers, 1993) or *I. duplicatus* (Schlyter & Anderbrant, 1993).

Top-down controls such as parasitism or predation also play a role, but are not considered as important drivers in regulating *I. typographus* population dynamics (Marini et al., 2013). Their impact is most apparent in low abundance populations (Weed et al., 2015) in a manner discussed in other parts in this review (Wermelinger & Seifert, 1998; Wermelinger, 2002; Jonsson et al., 2009; Jonsson et al., 2011; Podlesnik et al., 2017).

Except for biotic drivers, some abiotic factors, such as temperature, are also considered as constraints or those that promote the build-up of bark beetle populations (Raffa et al., 2015). As discussed above, high temperatures can speed up *I. typographus* development and thus influence voltinism (Sauvard, 2004; Hof & Svahlin, 2016). Temperature (e.g. seasonal fluctuations) also affects the diversity, composition and abundance of *I. typographus* symbionts such as fungi and mites (Linnakoski et al., 2016), which might in turn impact on bark beetle development (Six & Bentz, 2007). However, Marini et al. (2013) argued that temperature is not amongst the most important drivers of population dynamics.

Factors favoring outbreaks

A variety of factors have been suggested that might contribute to the enhanced breeding success of *I. typographus*, that in turn increase the severity of outbreaks. These factors include: (1) short and flexible reproductive cycles that allow for up to three generations in particularly warm years, helping the species to quickly respond to climate variations (Botterweg, 1982; Jurc et al., 2006; Jonsson et al., 2011); (2) the reproduction of massive numbers of offspring, estimated to reach up to 35 000 pre-emergent adults in each cubic meter of an infested tree (Gonzalez et al., 1996); (3) the release of efficient aggregation pheromones that allowing rapid mass colonization (Byers, 2004; Andersson et al., 2009); (4) a strong association with blue stain fungi that might help them overcome tree defense systems or serve as food source (Kirisits, 2004; Six & Wingfield, 2011); and (5) a highly efficient dispersal capacity that would facilitate the location of suitable hosts (Forsse & Solbreck, 1985).

Strong winds during storms are one of the main factors triggering outbreaks of *I. typographus*. Many outbreaks have occurred after storms in both Europe and Japan, where serious damage to forests has been reported (Trägårdh & Butovitsch, 1935; Butovitsch, 1938a; Butovitsch, 1941; Moser et al., 1997a; Grégoire & Evans, 2004; Nilsson et al., 2004; Anon, 2006, 2007). The mechanisms of this pattern (Botterweg, 1983) can be explained as follows: After storms the density of egg galleries is lower than under normal conditions because of the large numbers of damaged and fallen trees with weakened or non-existent defenses. This results in an increased number of progeny per female as well as on the vigor of the progeny. The progenies

were bigger in size with relatively higher fat content compared to *I. typographus* progeny from lower attack densities. Consequently, the population densities are high enough to overcome the defense mechanisms of living trees.

Drought and relatively higher temperatures are other factors that trigger outbreaks of *I. typographus* (Rouault et al., 2006; Netherer et al., 2015; Seidl et al., 2016). Summer precipitation deficits and increased temperature were found to be significantly correlated with *I. typographus* attacks (Baier et al., 2007; Faccoli, 2009; Marini et al., 2012; Marini et al., 2013). Warm dry summers in 1933 to 1937 were recorded after the severe storms in 1932 in Sweden. From 1935 to 1937 about 3 750, 50 000 and 70 000 infested trees were felled per annum (Butovitsch, 1938b). After large storms hit southern and central Sweden in the autumn of 1969, a long period of outbreak of *I. typographus* followed that killed about 4.5 million m³ trees (Eidmann, 1983; Risberg, 1985). Several warmer and dryer summers were considered as contributing to this long outbreak. Another warm and dry summer occurred in 1992, which caused increased *I. typographus* activity in Southern and central Sweden (Samuelsson & Örlander, 2001). The Swedish Forest Agency estimated that about 1.5 million m³ trees were killed by the beetle during this outbreak (Karvemo & Schroeder, 2010).

A feature that distinguishes *I. typographus* from other *Ips* spp., is the fact that it can maintain epidemics for years even when windthrow events cease (Kausrud et al., 2012). Sometimes epidemics can be prolonged by drought (Marini et al., 2013). Drought influences outbreaks of *I. typographus* by a so-called “carbon starvation” mechanism (Allen et al., 2010; McDowell et al., 2011). Persistent droughts decrease the water potential in the xylem that increases the risk of cavitation and embolism in the conducting tissues (Tyree & Sperry, 1989; Cruiziat et al., 2002; Cochard et al., 2009). To avoid or delay this situation, Norway spruce trees restrict transpiration via stomatal closure (Rothe et al., 2002; Schume et al., 2004). The reduced ascent of water and leaf atmosphere gas exchange causes reductions in photosynthetic production but continued respiration consumption of carbon (Bréda et al., 2006; Rennenberg et al., 2006). The carbohydrates available for synthesis of defense structures and compounds are reduced significantly under drought conditions because of the depletion of stored carbon (McDowell et al., 2008).

Usually dry conditions are accompanied by higher temperatures that impact on voltinism and population sizes of the beetles. During warmer periods *I. typographus* develops more rapidly and reproduces more successfully than under normal conditions (Hof & Svahlin, 2016). For example, an additional generation was observed in the extremely hot year that was accompanied by a dramatically increased population size (Fleischer et al., 2016).

Climate change

Several recent studies have considered the potential impact of a warming climate on aspects of *I. typographus* population dynamics. Økland et al. (2015) reviewed the body of work relating to climate change in an attempt to make predictions about the consequences of climate change on the propensity of outbreaks across European forests. These authors concluded that warmer and drier summers, and more frequent extreme winds may trigger both population growth and susceptibility of spruce stands to attacks (Økland et al., 2015; Fleischer et al., 2016). Such epidemics may exhaust susceptible tree species. In addition, a warmer climate is expected to lead to a northern expansion of the area experiencing two to three generations of *I. typographus* per year (Økland et al., 2015). This could result in an ecological disaster similar to that in Canada where the northern migration of the Mountain Pine Beetle (*Dendroctonus ponderosae*) has killed millions of hectares of pine forests (Kurz et al., 2008; Kausrud et al., 2012; Raffa et al., 2015).

CHEMICAL ECOLOGY

The role of chemicals in the ecology of bark beetles spans almost every facet of the beetles' existence, and as such, almost every part of this review intersects with aspects related to chemicals. The chemical ecology of bark beetles in general have been extensively reviewed (Wood, 1982; Trapp & Croteau, 2001; Byers, 2004; Six, 2013; Raffa, 2014; Krokene, 2015), including that relating to *I. typographus* (Sun et al., 2006). This section thus covers the topic only in broad terms, as details on specific chemical interactions are elsewhere in the document.

Beetles detect chemicals via olfactory receptors on the antennae (Holighaus & Rohlfs, 2016). For *I. typographus*, plant volatile responses and pheromone responses are elicited from the same olfactory receptor neurons (Andersson et al., 2009). Andersson et al. (2013) assembled the antennal transcriptomes from next-generation sequencing of *I. typographus* and identified 80 members of the major chemosensory multi-gene families. Identification of these proteins is important as they help to elucidate biochemical pathways and might represent new targets for population control.

If the chemical ecology of *I. typographus* is considered more or less according to the order of events and interactions during the typical life cycle (**Fig. 8**) (Borden, 1989), the first question would relate to how the beetle locates a suitable host tree. The beetles locate damaged or diseased trees over several meters based on volatile monoterpene constituents of oleoresin produced and released by the trees (McMullen & Atkins, 1962). Nutritional quality and composition of deterrents and toxins determines the suitability of the tree host to the beetles (Byers, 2004). The recognition of nonhost trees is equally important as these most likely provide fewer nutrients to a particular beetle than its regular hosts. In addition, toxins in the non-host probably cannot be detoxified by the beetle (Byers, 2004). *Ips typographus*, for example, avoids volatiles of non-host birch trees (Byers et al., 1998) and will leave a birch tree after landing on it due to a relatively high concentration of repellent nonhost volatiles on the bark surface (Byers, 2004). Recent studies have suggested that conophthorin, produced by fungi or bacteria (Beck et al., 2012; Citron et al., 2012) can inhibit attraction of both gymnosperm- and angiosperm-attacking bark beetles to their aggregation pheromones (Unelius et al., 2014). The conophthorin-specific odorant receptor neurons have been characterized in the *I. typographus* olfactory organ (Andersson et al., 2009). This receptor most likely enables *I. typographus* to avoid non-host trees and find host trees (Holighaus & Rohlfs, 2016).

Bakke (1970) was the first to show that *I. typographus* males produce aggregation pheromones to synchronize mass attacks when the first male successfully starts gallery construction on a new host tree. The pheromones are synthesized from components of host oleoresin and attract both males and females of the species (Borden, 1989; Sun et al., 2006). Once the optimal attack density has been obtained, the attacks are redirected to adjacent, uninhabited host trees by anti-aggregation pheromones (Borden, 1989; Sun et al., 2006).

Host tree defense against *I. typographus* attacks occurs via production of oleoresin at the wound site. This consists of a complex mixture of mono-, sesqui-, and diterpenoids toxic to the bark beetle and fungal associates (Trapp & Croteau, 2001; Sun et al., 2006). The resistance of the host can be overcome only by a successful mass attack that follows aggregation (Sun et al., 2006).

Some sources suggest that *I. typographus* may find weakened and susceptible host trees by orientating to volatiles produced by competing species during colonization. These include for example exo-brevicom produced by *Dendroctonus micans* and *Dryocoetes* species (Tommeras & Mustaparta, 1984; Borden et al., 1987). However, bark beetles usually avoid competition. For example, *I. duplicatus* can produce a pheromone component, ipsdienol, that inhibits response of *I. typographus* (Byers et al., 1990; Schlyter et al., 1992; Byers, 1993). Small amounts of ipsdienol and ipsenol produced by mated males of *I. typographus* during colonization might function in avoiding competition (Birgersson et al., 1984; Birgersson & Leufven, 1988). Ipsdienol functions by either inactivating or inhibiting attraction of *I. typographus* to *cis*-verbenol and methyl butenol, the most potent aggregation pheromone components (Schlyter et al., 1987a, b).

BIOLOGICAL INTERACTIONS

The interactions between *I. typographus* and the other biological entities in its ecosystem are most often not only linear (e.g. beetle + entity 1), but often intertwined in a complex network where the other entities also interact with each other. For the purpose of clarity, the entities are considered individually with a focus on their linear interactions with the beetle.

Host trees

The most obvious and important interaction is between *I. typographus* and its conifer host trees, which are primarily spruce, but can also include pine species (**Table 2**). Conifers are long-lived organisms with wide distribution ranges. Most often only weakened and dying trees are attacked by *I. typographus* (Krokene, 2015). Effective defense strategies against bark beetles and other insects have contributed to their evolutionary success and wide distribution (Franceschi et al., 2005). Two types of defense strategies are involved against the diversity of potential attackers: constitutive defenses and inducible defenses (Franceschi et al., 2005). Both of these include chemical and anatomical components, and there are no clear boundaries between the two defense types (Krokene, 2015). Conifer defenses against bark beetles were reviewed by Franceschi et al. (2005) and Krokene (2015). In addition to pheromones that bark beetles such as *I. typographus* employ to overcome host defenses, they have evolved several morphological adaptations. These include a conically shaped head, compressed antennae, spined tarsi, and orientation to thin bark crevices (Raffa et al., 2016).

Fungi

A second, well-known interaction that *I. typographus* has, is its association with a suite of fungi. Although the beetle has no specialized organs for the transmission of its fungal associates, the galleries are heavily colonised by fungi. and it has been known for a long time that fungal spores are transmitted in the digestive tracts and on the integument (Siemaszko, 1939), especially in pits on the pronota and elytra (Furniss et al., 1990). However, the absence of specialized mycangia is indicative of the fact that *I. typographus* does not have consistent fungal partners and is not likely to rely on fungi for nutrition (Kirisits, 2004; Six & Wingfield, 2011; Giordano et al., 2013; Six, 2013). Several of the fungal associates of the beetle have been suggested to be pathogens that assist the beetle in overcoming host responses and in killing the trees (Krokene & Solheim, 1998). However, the fact that fungal associates of the beetle vary considerably between hibernation environments, localities, and time of the life cycle (Solheim, 1991, 1992b), together with the low frequencies of pathogenic fungi carried by the beetle during outbreaks, suggest that the beetle does not require them to

attack or kill trees (Persson et al., 2009; Six & Wingfield, 2011).

The assemblage of fungi associated with *I. typographus* is dominated by the ophiostomatoid fungi that have been well studied in Europe and Japan (**Table 3**). The ophiostomatoid associates normally reside in the genera *Endoconidiophora* (Microascales), *Ceratocystiopsis*, *Grosmannia*, *Graphium*, *Leptographium* and *Ophiostoma* (Ophiostomatales) (Yamaoka et al., 1997; Yamaoka et al., 1998; Viiri & Lieutier, 2004; Jankowiak, 2005; Jankowiak & Hilszczanski, 2005; Linnakoski et al., 2010; Linnakoski et al., 2012). At present, about 40 species have been reported associated with *I. typographus* in Europe, and about 13 in Japan. Only two species have been reported associated with *I. typographus* from China (Paciura et al., 2010). Seven species have been isolated from the insect in both Europe and Japan. However, it is important to note that most of the identifications in these studies were conducted based on morphological characters (**Table 3**), which implies that some of the identifications could have been incorrect.

Endoconidiophora polonica is one of the species that has been reported with *I. typographus* in both Europe and Japan (**Table 3**). It is a blue stain fungus and can cause serious damage on *Picea abies* in Europe and on *P. jezoensis* and *P. glehnii* in Japan (Christiansen & Solheim, 1990; Visser et al., 1995; Yamaoka et al., 1997). However, not all isolates of *E. polonica* were highly pathogenic (Kirisits & Anglberger, 1999). A population study on the fungus showed that there was a strong genetic separation between European and Japanese populations of the fungus, which could have arisen by geographic isolation (Marin et al., 2009). This result was consistent with studies that have compared beetle populations from Europe and the far east, as was discussed earlier in this review. *Endoconidiophora polonica* is not only associated with *I. typographus* but also with other *Ips* species, such as *I. amitinus* and *I. duplicatus*, and is occasionally vectored by other bark beetles in Europe (Kirisits, 2004).

Although *I. typographus* is not necessarily dependant on fungi such as *E. polonica* for successful infestation, the beetle could still benefit indirectly from reduced tree defenses caused by fungal resource uptake. Phloem non-structural carbohydrates and sapwood lipids were shown to decline over time after the inoculation of *E. polonica* (Lahr & Krokene, 2013). The fungus consumed sugars that the tree would otherwise need for resin synthesis (Lee, 2006). Less resin was found in trees that were inoculated with *E. polonica* (Repe et al., 2015). Phenolics are one of the important chemicals that confer resistance against bark beetles and their associated fungi (Krokene, 2015). Stilbene is one of these soluble phenolics. However, induction of stilbenes following infection with bark beetles and the associated fungi were not strongly detected, although stilbene biosynthesis was up-regulated in the tree. One of the reasons is that *E. polonica* converts the stilbenes to other compounds that prevent the stilbenes from accumulating in the bark (Hammerbacher et al., 2013). Recent studies have also shown that stilbenes and flavonoids are used as a carbon source by *E. polonica* (Wadke et al., 2016).

Fungi can play a role in enhancing the attraction of *I. typographus* to the tree. Zhao (2011) showed that the enantiomeric ratio of (-)- α -pinene increased after *E. polonica* inoculation (Zhao, 2011). (-)- α -pinene can increase the attraction of *I. typographus* to its aggregation pheromone. Studies on other fungal symbionts of *I. typographus* also showed that those fungi have a similar function. For example, *Grosmannia penicillata* and *G. europhioides* can produce 2-methyl-3-buten-2-ol which is the main component of aggregation pheromones of the beetle (Zhao et al., 2015).

Fungi can enhance tree resistance to beetles subsequent to *E. polonica* attacks (Christiansen & Krokene, 1999;

Krokene et al., 1999; Evensen et al., 2000; Krokene et al., 2001; Krokene et al., 2003; Novak et al., 2014). Research has shown that the inoculation of *E. polonica* can directly or indirectly affect the suitability of the trees to infestation by *I. typographus* (Zhao et al., 2010). After fungal inoculation, increases in the absolute amount and relative proportion of (+)-3-carene and the enantiomeric ratio of (-)- α -pinene and (-)-limonene were observed in the tree (Evensen et al., 2000; Zhao et al., 2010). In this regard, terpenes such as 3-carene and limonene are toxic to insects and microorganisms.

Apart from filamentous fungi, yeasts are also often associated with bark beetles (Six, 2013; Davis, 2015). Nine yeast species had been reported with *I. typographus*, the most frequent of which was *Pichia holstii* (Davis, 2015). These yeasts are found in the frass, intestinal tract, macerated adults, and oviposition galleries (Davis, 2015). The yeasts can affect their host beetles in two indirect ways (Six, 2013). One of these is by changing the tree chemistry. The change of chemistry of tree host can affect the growth of nutritional filamentous fungi and the colonization patterns of conspecific and heterospecific beetles in a host tree (Hunt & Borden, 1990; Byers, 1995; Paine et al., 1997). Another manner in which yeasts might affect the beetles is by affecting the growth of filamentous fungi. For example, *Pichia holstii* outcompetes *E. polonica*, which may have an effect on beetle performance, because *E. polonica* could play a role in the success of *I. typographus* infestations as discussed above (Furniss et al., 1990; Solheim, 1992a).

Like filamentous fungi, yeasts can also affect *I. typographus* in a direct way. Several yeasts associated with *I. typographus* have displayed an ability to convert the aggregative pheromone component cis-verbenol to verbenone (Leufven et al., 1984), which is an anti-aggregative pheromone. They can also produce monoterpenes such as α -terpineol and borneol grown in Sabouraud's medium (Leufven et al., 1984). In a recent study, α -terpineol showed a high GC-EAD response to the bark beetle parasitoid *Roptrocercus xylophagorum* (Pettersson et al., 2000), which implies that some yeasts might have a detrimental effect on *I. typographus*.

Bacteria

Bacteria are very common symbionts of beetles (Six, 2013) and are found on the exoskeletons, in oral secretions and guts, in frass, and on the walls of their galleries (Cardoza et al., 2006; Adams et al., 2008; Morales-Jimenez et al., 2012). The interactions between bacteria and beetles are diverse, ranging from antagonism to commensalism to mutualism and from obligate to facultative (Janson et al., 2008; Douglas, 2009; Morales-Jimenez et al., 2012). Because of advances in technology and an increasing recognition of the importance of bacterial symbioses in insect ecology and evolution, the bacterial communities associated with bark beetles are only now being studied in earnest (Clark et al., 2010; Duron & Hurst, 2013). Interestingly, compared to other insect groups, particularly other wood-feeding insects, conifer infesting bark beetles possess considerably less bacterial species (Delalibera et al., 2005; Morales-Jiménez et al., 2009; Adams et al., 2010; Sevim et al., 2012).

The relationships between bacteria and bark beetles had barely been studied, and this is especially true in the case of *I. typographus*. They may have roles in cellulose breakdown, or the provision of vitamins, sterols, and/or amino acids to the beetles (Klepzig et al., 2009). The gut bacteria can synthesize essential nutrients, fix nitrogen, recycle uric acid, assist in digestion, produce pheromones, and metabolise toxins for the beetle's development and survival (Nakabachi & Ishikawa, 1999; Genta et al., 2006; Morales-Jimenez et al., 2012). Some bacteria associated with bark beetles may act as biological control agents (see section on Entomopathogens). To date, only eight species of bacteria have been identified in association with *I.*

typographus, of these, *Serratia liquefaciens* significantly increased mortality in healthy adults (Muratoglu et al., 2011).

Entomopathogens

Pathogens of bark beetles can include viruses, fungi, bacteria and protozoa (**Table 4**), and can be transmitted to these insects horizontally and vertically (Wegensteiner, 2004). Horizontal transmission includes contact, ingestion, and transmission by vectors. Pathogens of bark beetles can be classified as potential pathogens, facultative pathogens, and obligate pathogens. Some pathogens produce exotoxins which open the hosts for infections, while others can release endotoxins confined with the infectious unit with the same function. Fungi invade through the integument, whereas viruses, bacteria and protozoa enter their host primarily through the mouth parts (Wegensteiner, 2004).

Viruses are obligate pathogens of beetles and most of them are relatively host specific. *Ips typographus* Entomopoxvirus (*ItEPV*) was reported several times in *I. typographus*, occurring in the cells of midgut epithelium (Wegensteiner & Weiser, 1995; Wegensteiner et al., 2014; Wegensteiner et al., 2015a). *ItEPV* is a common pathogen of *I. typographus*, which develops completely intracellularly in the host and is suspected to be relatively virulent (Wegensteiner et al., 2014).

The majority of bacteria do not cause diseases in beetles, but some can be important pathogens (Wegensteiner, 2004). Bacterial infections in insects can be classified as bacteremia, septicemia and toxemia. Many insect-pathogenic bacteria occur in the family Bacillaceae, such as *Bacillus thuringiensis*, which was reported in *I. typographus* (Lipták et al., 2013). However, infection experiments in field tests with *B. thuringiensis* var. *tenebrionis* against *I. typographus* showed that the bacteria caused no significant mortality in adult *I. typographus* (Novotný & Turčáni, 2000).

Entomopathogenic fungi constitute a large group including representatives from the Zygomycota and Ascomycota (Wegensteiner, 2004). Most of them form superficial growth on the surface of their hosts where they produce conidia. Abiotic factors, especially high humidity and adequate temperature, play an important role in fungal growth and development (Zimmermann, 2007a, b; Lipták et al., 2013). Some entomopathogenic fungi are host specific and others can infect a broad range of insect hosts (Shah & Pell, 2003; Shahid et al., 2012). The generalist pathogen *Beauveria bassiana* is found not only on *I. typographus*, but also on many other bark beetle species. This fungus resulted in high levels of mortality in adult *I. typographus*, but seemingly does not affect their larvae (Wegensteiner, 2004).

Previously, microsporidia were considered to be closely related to the Protozoa, but are now known to be fungi (Corradi & Keeling, 2009). They produce two types of spores: thick-walled forms that are environmental spores and thin-walled forms known as primary spores. Microsporidia enter the host beetles through the mouth parts with food, and infection is initiated in the midgut epithelium (Solter et al., 2012). *Chytridiopsis typographi* was the first microsporidia detected in the gut epithelium of *I. typographus* (Weiser, 1954a, b). *Ips typographus* appears to be highly susceptible to infection by this species (Wegensteiner et al., 2015b).

Entomopathogenic protozoans are members of the protozoan phyla Rhizopoda, Apicomplexa, Zoomastigina and Ciliophora (Wegensteiner, 2004). The first two protozoan species described in *I. typographus* were *Telosporidium typographi* and *Gregarina typographi* (Fuchs, 1915). *Malamoeba scolyti* can inhibit manifestation of disease caused by nematodes in the gut of *I. typographus* (Kirchhoff & Führer, 1990).

Malamoeba scolyti is also a common protozoan in *I. typographus* and other bark beetles (Wegensteiner, 2004).

Nematodes

Between 40 000 and 500 000 species of insect-associated nematodes are thought to exist, with relationships ranging from commensalism, to mutualism to parasitism (Giblin-Davis et al., 2013). Thirteen species have been reported to be associated with *I. typographus* (Table 5). Among these, *Contortylenchus diplogaster* was the first nematode found associated with bark beetles (Grucmanova & Holusa, 2013). The females in genus *Contortylenchus* can penetrate the beetles directly through the integument, and/or enter through the oral or anal openings (Wegensteiner et al., 2015b). Endoparasitic nematodes can alter bark beetle behavior, fecundity, longevity, or delay emergence (Kaya, 1984). Some of these nematodes can also have a slight effect on the flight duration of *I. typographus* (Forsse, 1987). Parasitic nematodes of *I. typographus*, such as *Tylenchus contortus typographi* (*Contortylenchus diplogaster*) and *T. dispar typographi* (*Parasitylenchus dispar*), can lower general activity, and in particular the egg-laying capacity of the beetle (Fuchs, 1914).

Mites

Mites are some of the better known organisms associated with bark beetles, especially those in the superfamily Uropodoidea (Zach et al., 2016). More than 270 mite species have been reported as associates of bark beetles and more than 60 of these are associated with *I. typographus* (Hofstetter et al., 2015) (Table 6). Their feeding behavior ranges from predacious to mycetophagous and omnivorous. The host specificity of mites varies depending on the particular mite species. Some mite species, such as *Ameroseius sculptilis*, have been reported only from *I. typographus*. Others, such as *Dendrolaelaps quadrisetus*, are found not only on *I. typographus*, but also on other beetle species (Hofstetter et al., 2015).

Because of their small size, most mites are phoretic or attach to other organisms for dispersal (Hofstetter et al., 2013). There are five types of phoresy in the Acarina. Some Macrochelidae use their chelicerae or legs to attach to the vector, some Parasitidae attach using their claws, some Uropodina attach with a specialized anal pedicel, while highly heteromorphic deutonymphs possess specialized structures for clinging to their vectors, and some species possess dimorphic females (Farish & Axtell, 1971). In the latter case, the specialized morph for phoresy is termed phoretomorph, and these are found in *Siteroptes graminum* (Farish & Axtell, 1971; Moser & Cross, 1975). The juvenile developmental stages of mites, such as deutonymphs, are the main stage that attach to bark beetle adults (Bajerlein & Bloszyk, 2004; Gwiazdowicz et al., 2012; Pernek et al., 2012).

The presence of mites that associate with bark beetles varies between seasons. For example, fewer mites have been observed with *I. typographus* during the flight season, than during spring and autumn (Linnakoski et al., 2016). This could be due to the fact that mites can inhibit the ability of the beetle to disperse effectively (Linnakoski et al., 2016).

Mites play an important role in the dispersion of fungi and other microbes in bark beetle ecosystems (Pernek et al., 2008). Some species even have a specialized structure, known as sporothecae, to transmit fungal spores (Bridges & Moser, 1983). Several fungal species have been reported from the mites phoretic on *I. typographus*. In Japan, seven species of fungal spores were found associated with mites phoretic on *I. typographus japonicus*, including *E. polonica*, the pathogenic fungus and common symbiont of *I. typographus* (Moser et al., 1997b). In Finland, twelve fungal species, including *E. polonica* have been isolated from mites carried by these beetles (Linnakoski et al., 2016).

Mites can directly impact on biology and population dynamics of bark beetles. Mites associated with bark beetles include some predacious species, which are predators and parasitoids of the bark beetles' immature stages, especially the eggs and early larval stages (Hofstetter et al., 2015; Vissa & Hofstetter, 2017). *Iponemus gaebleri* is a known parasitoid species, preying on the eggs of *Ips* species (Gabler, 1947). This mite species is dominant on *I. typographus*, both in Japan and Europe, representing 26% in the vial sediments in Japan and 18% in Sweden respectively (Moser et al., 1989; Moser et al., 1997b). This mite species could increase the mortality of *I. typographus*.

Mites can also indirectly impact on bark beetle biology and population dynamics. *Dendrolaelaps quadrisetus* is a regular associate of *I. typographus* in Japan and Europe (Moser et al., 1997b; Cejka & Holusa, 2014; Gwiazdowicz et al., 2015). This mite species preys on nematodes (Kinn, 1967) and increases mortality of the beetles (Penttinen et al., 2013). The reason why it causes beetle death remains uncertain, but it might be linked to the nematodes on which this mite preys that possibly fulfill an important beneficiary role to the beetles (Penttinen et al., 2013).

Parasitoids

Parasitic wasps are some of the most abundant natural enemies of *I. typographus* (Wermelinger, 2004). There are four parasitoid guilds found on Scolytinae. These include egg parasitoids, egg-larval endoparasitoids, larval ectoparasitoids and adult endoparasitoids. In *I. typographus*, most parasitoids are larval ectoparasitoids and adult endoparasitoids (**Table 7**). Host specificity differs among these parasitoids. Adult and the egg-larval parasitoids are usually more specific than the larval ectoparasitoids. Adult endoparasitoids such as *Ropalophorus clavicornis* are specifically associated with *I. typographus*, whereas the larval ectoparasitoid, *Dendrosoter middendorfi*, has been reported to infest at least 17 bark beetle species (Kenis et al., 2004).

Host location mechanisms are key to understanding parasitoid host range. New control methods may result from a better understanding of host location mechanisms. There are two steps involved in this process. Firstly, the parasitoid must locate the host habitat, and secondly, the parasitoid must be able to locate a particular host at a suitable developmental stage. Aggregation pheromones play an important role in the process of host location. For example, *Tomicobia seitneri* adults are attracted by the aggregation pheromone of *I. typographus* under field conditions (Kenis et al., 2004).

Predators

The most abundant natural enemies of *I. typographus* are predatory beetles (Coleoptera) and flies (Diptera) (**Table 8**). Beetles are among the most important and most intensively investigated predators of scolytids. Among the coleopterans the Cleridae, Rhizophagidae, and Trogossitidae are important families, while the most relevant dipteran families that predate on *I. typographus* are the Dolichopodidae and Lonchaeidae. Most predatory dipterans feed on bark beetles in their larval stages. Apart from the Coleoptera and Diptera, other *I. typographus* predators include bugs in the heteropteran family Anthocoridae, some mite species, and woodpeckers (Kenis et al., 2004).

Many of the *I. typographus* predators locate their prey by semiochemicals, which include pheromones from the beetles and volatiles from the tree hosts. The functions of pheromones and volatiles in this process are different. Many predators are attracted by prey-emitted pheromones, modulated by tree volatiles. In addition, predators can detect anti-aggregation pheromones such as verbenone emitted by bark beetles.

Predator antennae have receptors keyed to a high number of prey pheromones, which causes high catches in pheromone traps. Apart from chemical attraction, predators also respond to visual cues to particular parts of the trees (Kenis et al., 2004; Wegensteiner et al., 2015b).

IMPACT OF *IPS TYPOGRAPHUS* OUTBREAKS

During the 20th century, several outbreaks of *I. typographus* occurred for which impact data are available. In some cases, the driving forces underlying the outbreaks are also known. Outbreaks for which such information is available are listed in **Table 9**. In most of these outbreaks, defective human harvesting and other forestry related practices, extended periods of drought and heat, or damaging wind storms, have triggered or contributed to the severity of the outbreaks.

MANAGEMENT

The impact of *I. typographus* during outbreaks can be reduced substantially through management practices. These have been adapted or changed over the years as research has generated novel scientific data, but also as values and opinions regarding the problem have changed. Wermelinger (2004) reviewed the evolution of these practices employed over the past century. The ultimate aim of management during bark beetle outbreaks is to minimize the number of attacks on living trees. The use of pesticides in most European countries is banned, and this form of control is therefore not applied or considered here. Several other strategies that have been applied to *I. typographus* outbreaks in the past, are briefly discussed.

Early detection and accurate delineation of areas affected by wind and subsequent outbreaks are crucial in guiding management decisions (Tanase et al., 2018). Traditionally, detection was dependent on human observations in the field. However, remote sensing technologies that enable faster and more comprehensive detection of outbreaks have become an important research focus in recent years (Latifi et al., 2014). The application of this technology in assessing bark beetle outbreaks was recently reviewed by Senf et al. (2017). Passive or active sensors can be used in remote sensing. Passive sensors were most commonly used in the past (Tanase et al., 2018), of which satellite images was the most utilized technology (Näsi et al., 2018). With the development of new technologies such as airborne-, mobile-, and terrestrial laser scanning, stereo imagery, and unmanned airborne vehicle platforms, are now available for detection of the green attack stage, or of single dead trees (Näsi et al., 2015; White et al., 2016; Näsi et al., 2018; Stoyanova et al., 2018; Tanase et al., 2018). Active sensors using L-band space-borne synthetic aperture radar, can be used over larger areas and different ecosystem types in the temperate and boreal regions, to detect and delineate damaged areas (Tanase et al., 2018). Training dogs to detect semiochemicals emitted from infested trees provides another option to survey infested areas (Johansson et al., 2018). These dogs can rapidly detect single, first attacked trees and enable their removal before offspring emergence.

As a first step after detection, sanitation (i.e. the removal and destruction of infested material from the forests) has been considered for centuries as the most effective management to reduce the impact of outbreaks (Fettig & Hilszczański, 2015). As mentioned above, windthrown timber after storms is most often the primary trigger for *I. typographus* outbreaks. If possible, infested wood, including weakened trees, and even fallen bark, should be removed from the windthrow areas before the following midsummer to avoid

completion of the *I. typographus* life cycle (Gothlin et al., 2000; Dworschak et al., 2014; Fettig & Hilszczański, 2015; Økland et al., 2016; Grodzki & Fronek, 2017; Havašová et al., 2017; Mezei et al., 2017; de Groot et al., 2018; Kärhä et al., 2018). Although sanitation can be very effective, it has disadvantages due to the financial costs and the intensity of required labour (Mezei et al., 2017). Furthermore, some recent studies have suggested that not all infested or dead trees should be removed. This is because the retention of some material is beneficial for natural enemies and other dead-wood dependent species (Weslien, 1992; Hilszczański, 2008; Thorn et al., 2014; Kärvemo, 2015). Apart from arthropods, these can include larger animals such as protected bat species (Wilcox, 2017). In addition, the so-called 'sun effect' that can result from sanitary logging causes favourable conditions for insect dispersal and higher emission of primary attractants, especially at forest edges (Peltonen, 1999; Kautz et al., 2013). For these reasons, sanitation strategies have become a major point of dispute between foresters and ecologists (Lindenmayer et al., 2017; Schiermeier, 2017; Stokstad, 2017; Konarzewski et al., 2018). Ecologists argue that the impacts of salvage logging also disrupt the natural assembly process and decreases species richness (Lindenmayer & Noss, 2006; Thorn et al., 2014; Beudert et al., 2015; Thorn et al., 2016).

Traps are thought to be a reasonable measure to protect living trees from *I. typographus* attacks. The traditional method is to use trap trees (Skuhravý, 2002). Broken and windthrown trees are used to lure *I. typographus* away from healthy trees (Raty et al., 1995; Eriksson et al., 2005). Large diameter trees, located on non-shaded sites should be felled or ring-barked just before beetles emerge or if conditions have been relatively warm and snowless, but during cooler conditions with regular snow it can be felled further in advance (Holuša et al., 2017). After oviposition those trap trees should be destroyed or debarked before adults emerge (Bakke, 1983). An alternative method is to utilise pheromone-baited traps (Fettig & Hilszczański, 2015) (**Fig. 10**), but the efficiency is lower than trap trees (Raty et al., 1995). Compared to trap trees, lure-baited insecticide-treated tripod traps have two advantages. Firstly, any spruce timber can be used to attract *I. typographus* and secondly, they require significantly less surveillance (Lubojacký & Holuša, 2014). 2-methyl-3-buten-2-ol and cis-verbenol are key components of the pheromone lures, and a mixture of these two compounds resulted in significant trap catches in field experiments (Bakke, 1976; Birgersson et al., 1984). The addition of α -pinene and limonene increase the effectivity of barrier traps (Stepanycheva et al., 2017). Environmental and local conditions that include temperature, sun exposure, and competition from nearby woody debris, slash, log stacks, windthrows, and susceptible trees, can influence trapping efficiency (Lobinger, 1995; Grodzki, 2004).

Ips typographus has many natural enemies and pathogens as described above. Some of these have the potential of being applied as biological control agents. Among these, different strains of *B. bassiana* have been tested using different application methods (Vaupel & Zimmermann, 1996; Kreutz et al., 2004a; Kreutz et al., 2004b; Grodzki & Kosibowicz, 2015; Draganova et al., 2017; Barta et al., 2018). Trials have shown that the production of females was reduced, but the fungus was not passed on to their progeny (Grodzki & Kosibowicz, 2015). In other studies, *B. pseudobassiana* was tested with positive results (Kocacevik et al., 2016). Another important natural biological control agent is woodpeckers, but no guidelines are available regarding human-mediated implementation of these birds (Fayt et al., 2005). Studies on the use of parasitoids in *I. typographus* control showed contradictory results. Surveys conducted during short-term (2–3 years) and long-term (>10 years) outbreaks showed low levels of predation and/or parasitism, suggesting that natural enemies like parasitoids did not have a significant impact on *I. typographus* populations (Holuša & Lukášová, 2017). However, other studies showed parasitism rates of up to 23%, with a substantial impact on the population dynamics (Podlesnik et al., 2017).

Another potential strategy for control could be to interfere in the reproduction of the beetle. A so-called sterile insect technique (SIT) has been tested on *I. typographus* in laboratory trials. Laboratory-reared males treated with γ -radiation and mated with untreated females significantly affected the number of viable offspring (Turcani & Vakula, 2007; Čičková et al., 2018). Several studies have investigated the application of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) as a possible means to edit target genes in insects with the ultimate aim of pest control (Sun et al., 2017; McFarlane et al., 2018). To the best of our knowledge, this technology has not yet been applied to bark beetles, but it should certainly be considered for important bark beetle pests such as *I. typographus*.

As genetic homogeneity of host trees increases the risk of spruce bark beetle infestations, trees with resistance should be selected and genetic variability within the host tree populations should be maintained (Christiansen & Bakke, 1988). In addition to genetic homogeneity, species homogeneity in forests also increases the risk of infestation, and tree mortality has been shown to be highest in areas with spruce volumes of more than 200 m³ ha⁻¹ (Kärvemo, 2015). Increasing the proportions of mixed forest may thus decrease the rates of tree mortality caused by *I. typographus* (Hilszczahski et al., 2006) and the risk to spruce from storm felling (Valinger & Fridman, 2011).

Wermelinger (2004) recommended that by managing the age of forests, the impact of spruce beetle outbreaks can be reduced. Norway spruce should not be maintained until old age (> 60 years), and interspersing of age groups and species will reduce habitat continuity (Christiansen & Bakke, 1988). Stand edges should also be maintained to achieve maximum stability, natural windbreaks and well-rooted trees.

CONCLUSIONS

It is clear that *I. typographus* is one of the best studied bark beetle species globally. This is primarily due to its economic impact in Europe, Japan and China (**Table 9**). Although this beetle serves as a model organism for all bark beetles this review has also revealed some areas of research where knowledge is lacking.

The generic placement of the genus *Ips* within the Scolytinae has been confirmed phylogenetically based on the placement of its type species, *I. typographus*. Similarly, the delineation of the subgenus *Ips*, and the relatedness of *I. typographus* with its sister species has been resolved. However, the currently accepted synonymy of *I. japonicus* with *I. typographus* needs to be reconsidered. As has been outlined in the discussion above, beetle specimens from Japan, China and Far East Russia differ substantially from European and Western and Central Asian specimens when applying several genetic markers in various population studies. But in all these studies, the numbers of specimens included from Russia, China and Japan were inordinately low to make definitive conclusions about genetic distance and gene flow between these populations. A comprehensive population genetics study is thus needed that includes sufficient numbers of specimens from the Far East to resolve this question. Based on the currently available data, we predict that such a study will confirm that the Far Eastern populations represent a species distinct from *I. typographus*, and that the name *I. japonicus* should be re-instated for them.

From reports on outbreaks and other studies on the population dynamics of *I. typographus*, there seems to be a strong association between outbreaks and factors causing stress on trees, like wind damage, drought and

extreme temperatures. In view of climate change, future studies therefore would need to include extensive monitoring of possible range expansions and host shifts of *I. typographus*. This is especially true in Central and East Asia where knowledge in this regard is limited.

A wide range of factors have been shown to be involved in the chemical ecology of *I. typographus*. The rapid development of DNA and RNA sequencing technologies will provide many opportunities to gain a better understanding of the genetic basis for host resistance, and the chemical communication between beetles, their hosts and their symbionts. This will emerge from studies of the transcriptomes of the beetles, the host trees, fungi and possibly other associated organisms.

Studies on the symbiont assemblages of *I. typographus* have been mostly focused on fungi and mites from Europe and Japan, and they have relied mainly on culture-dependent methods. Nothing is known about the symbiont assemblages of the beetle in China. Much can be learned from comparisons of symbiont assemblages from different geographical areas, but discrepancies between sampling techniques and strategies make such comparisons difficult at present. DNA metabarcoding has already been widely used to study symbionts of insects, but only a single study from Finland explored this technique to study the succession of fungal associates in galleries of *I. typographus*. Applying this technology to study the fungal, mite and bacterial assemblages of *I. typographus* on a global scale in a more systematic manner will provide substantial insights into the biology of *I. typographus*.

The gene editing technology CRISPR is being explored as an aid to introduce desirable genetic elements that will spread through wild populations of many organisms, including insects (Sun et al., 2017; McFarlane et al., 2018). The introduction of genes that could negatively impact on bark beetle population dynamics has the potential to assist in controlling pests such as *I. typographus*, should certainly be explored.

Traditional methods of controlling and managing the impact of *I. typographus* outbreaks have been severely criticized in the past few years. A growing body of forest ecologists and environmentalists argue that removal of trees infested by *I. typographus* from natural forests during outbreak cause more damage than good to natural ecosystems (Stokstad, 2017). The resolution of this dispute will likely emerge from additional research on the longer-term impact of human interference in forests during outbreaks versus areas where nature is left to follow its course.

It is evident that much has yet to be learned in terms of the biology and control of *I. typographus*. The focus of this thesis is solely on the fungal associates of the beetle and some other conifer-infesting bark beetle species in China, as well as the fungal assemblages of mites associated with these beetles. As such, these studies will hopefully resolve some of the questions relating to the bark beetle ecology.

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Table 1 Selected data regarding the biology and life cycle of *Ips typographus* (Wermelinger, 2004; Schebeck et al., 2017).

Feature	Data
Minimum temperature for development	6–8.3 °C
Minimum temperature for oviposition	11.4 °C
Optimum temperature for development and oviposition	29–30 °C
Fecundity	Up to 80 eggs/female
Sex ratio (% females)	50% (retrogradation) 50% (progradation)
Overwintering mortality	50%
Optimum breeding density	500 maternal galleries m ²
Optimum harem size	Three females
Minimum temperature for flight (threshold)	16.5 °C
Optimum flight temperature	22–26 °C
Main flight time	Noon, early afternoon
Facultative diapause induced:	
Central Europe	Day length < 15 h; constant temp. < 23 °C
Northern Sweden	Day length < 19 h
Southern Sweden	Day length < 16-17 h
Minimally required number of days with temperatures	
>flight threshold for successful attack on living trees	3–4 days in a row
Active flight distance	>500 m

Table 2 Tree hosts of *Ips typographus*.

	China	Europe	Japan	Reference
<i>Picea abies</i>		○		
<i>Picea glehnii</i>			○	Yamaoka et al., 1997
<i>Picea jezoensis</i>	○		○	Yamaoka et al., 1997; Zhang, 2009
<i>Picea koraiensis</i>	○			Zhang, 2009
<i>Picea obovata</i>		Δ		Allen et al., 2010
<i>Picea orientalis</i>		○		Akkuzu et al., 2009
<i>Picea schrenkiana</i>	○			Zhang, 2009
<i>Pinus koraiensis</i>	○			Ahan, 2018
<i>Pinus sylvestris</i>	Δ	Δ		Zhang, 2009
<i>Pinus thunbergii</i>	Δ			Zhang, 2009
<i>Larix qmelinii</i>	○			Ahan, 2018

○: Main host; Δ: occasional host

Table 3 Ophiostomatoid fungi associated with *Ips typographus*.

Fungal species	Europe	Japan	China	References
Ophiostomatales				
<i>Ceratocystiopsis alba</i>	✓			Kirisits 2004
<i>Cop. minuta</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997
<i>Cop. minuta-bicolor</i>		✓		Yamaoka et al. 1997
<i>Grosmannia aenigmatica</i>		✓		Yamaoka et al. 1997
<i>G. cucullata</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997; Linnakoski et al. 2012
<i>G. europhioides</i>	✓			Linnakoski et al. 2012
<i>G. olivacea</i>	✓			Linnakoski et al. 2012
<i>G. olivaceapini</i>	✓			Linnakoski et al. 2012
<i>G. penicillata</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997
<i>G. piceaperda</i>	✓			Kirisits 2004; Linnakoski et al. 2012
<i>G. piceaperda-D</i>		✓		Ando et al. 2016
<i>G. serpens</i>	✓			Kirisits 2004
<i>L. curviconidium</i>			✓	Paciura et al. 2010
<i>L. euphyes</i>	✓			Kirisits 2004
<i>L. gracile</i> as (<i>L. latens</i>)			✓	Paciura et al. 2010
<i>L. lundbergii</i>	✓			Kirisits 2004
<i>L. obscurum</i>	✓			Kirisits 2004
<i>L. taigense</i>	✓			Linnakoski et al. 2012
<i>Leptographium</i> sp.	✓			Kirisits 2004
<i>Ophiostoma ainoae</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997; Linnakoski et al. 2010
<i>O. albidum</i>		✓		Aoshima 1965
<i>O. araucariae</i>	✓			Kirisits 2004
<i>O. arborea</i>	✓			Kirisits 2004
<i>O. bicolor</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997; Linnakoski et al. 2010
<i>O. brunneo-ciliatum</i>	✓			Linnakoski et al. 2010
<i>O. brunneolum</i>	✓			Linnakoski et al. 2016
<i>O. cainiii</i>	✓			Kirisits 2004
<i>O. flexuosum</i>	✓			Kirisits 2004
<i>O. floccosum</i>	✓			Kirisits 2004; Linnakoski et al. 2010
<i>O. fuscum</i>	✓			Linnakoski et al. 2010
<i>O. japonicum</i>		✓		Kirisits 2004; Yamaoka et al. 1997
<i>O. karelicum</i>	✓			Linnakoski et al. 2010
<i>O. macroclavatum</i>	✓			Linnakoski et al. 2016
<i>O. minus</i>	✓			Kirisits 2004
<i>O. neglectum</i>	✓			Kirisits 2004
<i>O. piceae</i>	✓	✓		Kirisits 2004; Yamaoka et al. 1997
<i>O. piliferum</i>	✓			Linnakoski et al. 2010
<i>O. pluriannulatum</i>	✓			Kirisits 2004
<i>O. saponiodorum</i>	✓			Linnakoski et al. 2010
<i>O. tetropii</i>	✓			Kirisits 2004
<i>O. tapionis</i>	✓			Linnakoski et al. 2012

Fungal species	Europe	Japan	China	References
Ophiostomatales (continued)				
<i>O. truncicola</i>		√		Aoshima 1965
<i>Ophiostoma</i> sp.	√			Kirisits 2004
<i>Ophiostoma (Pesotum)</i> sp.	√			Kirisits 2004
<i>Sporothrix stenoceras</i>	√			Kirisits 2004
Microascales				
<i>Endoconidiophora polonica</i>	√	√		Kirisits 2004
<i>Graphium fimbriisporum</i>	√			Kirisits 2004
<i>Gr. pseudormiticum</i>	√			Kirisits 2004
Number of species reported	40	13	2	

Table 4 Entomopathogens of *I. typographus*.

Organism	Species	Reference
Bacteria	<i>Bacillus thuringiensis</i> var. <i>insectus</i>	Wegensteiner, 2004
	<i>Bacillus thuringiensis</i> var. <i>tenebrionis</i>	Wegensteiner, 2004
Fungi	<i>Beauveria bassiana</i>	Wegensteiner, 2004; Wegensteiner et al., 2015a
	<i>Beauveria caledonica</i>	Wegensteiner, 2004; Wegensteiner et al., 2015a
	<i>Isaria farinosa</i>	Wegensteiner et al., 2015a
	<i>Metschnikowia typographi</i>	Wegensteiner, 2004
	<i>Paecilomyces farinosus</i>	Wegensteiner, 2004
	<i>Paecilomyces fumosoroseus</i>	Wegensteiner, 2004
	<i>Verticillium lecanii</i>	Wegensteiner, 2004
Microsporidia	<i>Chytridiopsis typographi</i>	Holusa et al., 2009; Wegensteiner et al., 2015a
	<i>Nosema typographi</i>	Holusa et al., 2009
	<i>Unikaryon montanum</i>	Weiser et al., 1998; Wegensteiner, 2004; Wegensteiner et al., 2015a
Protozoa	<i>Gregarina typographi</i>	Holusa et al., 2009; Wegensteiner et al., 2015a
	<i>Malamoeba scolyti</i>	Wegensteiner, 2004
	<i>Mattesia</i> cf. <i>schwenkei</i>	Wegensteiner et al., 2014
	<i>Menzbieria chalcographi</i>	Holusa et al., 2009
	<i>Telosporidium typographi</i>	Wegensteiner, 2004
Viruses	<i>Entomopoxvirus typographi</i>	Wegensteiner et al., 2014; Wegensteiner et al., 2015a

Table 5 Nematodes associated with *Ips typographus* in Europe (Grucmanova & Holusa, 2013; Wegensteiner et al., 2015b).

Family	Nematode species
Aphelenchoididae	<i>Cryptaphelenchus macrogaster macrogaster</i>
Contortylenchidae	<i>Contortylenchus diplogaster</i>
Diplogasteroididae	<i>Fuchsnema halleri</i>
Ektaphelenchidae	<i>Ektaphelenchus typographi</i>
Mononchidae	<i>Clarkus papillatus</i>
Mononchidae	<i>Prionchulus muscorum</i>
Neodiplogastridae	<i>Micoletzkyia buetschlii</i>
Neodiplogastridae	<i>Pristionchus lheritieri</i>
Neotylenchidae (syn. Sychnotylenchidae)	<i>Neoditylenchus major</i>
Panagrolaimidae	<i>Panagrolaimus chalcographi</i>
Parasitaphelenchidae	<i>Bursaphelenchus eidmanni</i>
Parasitylenchidae	<i>Parasitylenchus dispar</i>
Rhabditidae	<i>Parasitorhabditis obtuse</i>

Table 6 Mites species associated with *Ips typographus* (Gwiazdowicz et al., 2015; Hofstetter et al., 2015)

Order	Family	Species	Feeding Behavior
Heterostigmata	Acarophenacidae	<i>Aethiophenax (Paracarophenax) ipidarius</i>	Predacious
Mesostigmata	Ameroseiidae	<i>Amblyseius rademacheri</i>	Mycetophagous
	Ameroseiidae	<i>Ameroseius sculptilis</i>	Mycetophagous
	Ascidae	<i>Arctoseius longispinosus</i>	?
	Celaenopsidae	<i>Pleuronectocelaeno barbara</i>	?
	Digamasellidae	<i>Dendrolaelaps cornutus</i>	Predacious
	Digamasellidae	<i>Dendrolaelaps disetus</i>	Predacious
	Digamasellidae	<i>Dendrolaelaps nostricornumtus</i>	Predacious
	Digamasellidae	<i>Dendrolaelaps pini</i> Hirschmann	Predacious
	Digamasellidae	<i>Dendrolaelaps quadrisetus</i>	Predacious
	Digamasellidae	<i>Dendrolaelaps zwoelferi</i>	Predacious
	Digamasellidae	<i>Insectolaelaps quadrisetus</i>	?
	Laelapidae	<i>Hirstionyssus isabellinus</i>	
	Macrochelidae	<i>Macrocheles glaber</i>	Predacious
	Melicharidae	<i>Proctolaelaps eccoptogasteris</i>	Predacious
	Melicharidae	<i>Proctolaelaps fiseri</i>	Predacious
	Melicharidae	<i>Proctolaelaps scolyti</i>	?
	Mesoplophoridae	<i>Phthiracarus nitens</i>	?
	Micreremidae	<i>Micreremus brevipes</i>	?
	Neotenogyniidae	<i>Pleuronectocelaeno austriaca</i>	Predacious
	Neotenogyniidae	<i>Pleuronectocelaeno barbara</i> (= <i>drymoecetes</i>)	Predacious
	Neotenogyniidae	<i>Pleuronectocelaeno drymoecetes</i>	Predacious
	Neotenogyniidae	<i>Pleuronectocelaeno japonica</i>	Predacious
	Ologamasidae	<i>Cyrtolaelaps mucronatus</i>	
	Oppiidae	<i>Oppia splendens</i>	Predacious
	Oribatellidae	<i>Oribatella calcarata</i>	?
	Oribatulidae	<i>Chamobates borealis</i>	Mycetophagous
	Oribatulidae	<i>Phauloppia rauschenensis</i>	?
	Pachylaelapidae	<i>Pseudoparasitus vitthumi</i>	?
	Parasitidae	<i>Cornigamasus lunaris</i>	
	Parasitidae	<i>Leptogamasus suecicus</i>	?
	Parasitidae	<i>Paraleius consanguineous</i>	?
	Parasitidae	<i>Poecilohirus carabi</i>	?
	Parasitidae	<i>Poecilochirus davydovae</i>	?
	Parasitidae	<i>Schizosthetus lyriformis</i>	?
	Parasitidae	<i>Schizosthetus simulatrix</i>	?
	Peloppiidae	<i>Ceratoppia bipilis</i>	Predacious
	Trachytidae	<i>Uroseius acuminatus</i>	
	Trematuridae	<i>Trichouropoda hirsuta</i>	Omnivorous
	Trematuridae	<i>Trichouropoda ovalis</i>	?
	Trematuridae	<i>Trichouropoda parisiiana</i>	?
	Trematuridae	<i>Trichouropoda polonica</i>	?

Order	Family	Species	Feeding Behavior
	Trematuridae	<i>Trichouropoda polygraphi</i>	?
	Trematuridae	<i>Trichouropoda polytricha</i>	?
	Urodinychidae	<i>Uroobovella ipidis</i>	?
	Urodinychidae	<i>Uroobovella nova</i>	?
	Urodinychidae	<i>Uroobovella obovata</i>	?
	Urodinychidae	<i>Uroobovella pulchella</i>	?
	Urodinychidae	<i>Uroobovella vinicolora</i>	?
	Urodinychidae	<i>Uroobovella</i> sp.	?
Mestigmata	Ascidae	<i>Antennoseius</i> sp.	
	Ascidae	<i>Lasioseius ometes</i>	Predacious
Parasitiformes	Halolaelapidae	<i>Halolaelaps</i> sp.	
	Parasitidae	<i>Vulgarogamasus oudemansi</i>	?
Sacroptiformes	Acaridae	<i>Boletoglyphus boletophagi</i>	Mycetophagous
	Carabodidae	<i>Carabodes labyrinthicus</i>	Mycetophagous
	Cepheidae	<i>Cepheus latus</i>	Omnivorous
	Oribatulidae	<i>Zygoribatula exilis</i>	?
	Acaridae	<i>Schweibea</i> spp.	?
	Acaridae	<i>Thyreophagus corticalis</i>	Mycetophagous
	Autognetidae	<i>Conchogneta raegardhi</i>	Omnivorous
	Hemileiidae	<i>Siculobata leontonycha</i>	?
	Histiostomatidae	<i>Bonomoia pini</i>	Omnivorous
	Histiostomatidae	<i>Histiostoma piceae</i>	?
	Histiostomatidae	<i>Histiostoma vitzthumi</i> (=serrata)	?
	Humerobatidae	<i>Diapterobates humerlis</i>	?
	Oribatulidae	<i>Eporibatula gessneri</i>	?
	Oribatulidae	<i>Paraleius leontonychus</i>	?
Trombidiformes	Cheyletidae	<i>Mexeches virginiensis</i>	Predacious
	Ereynetidae	<i>Ereynetes propescutulis</i>	Predacious
	Ereynetidae	<i>Ereynetes scutulis</i>	Predacious
	Pyemotidae	<i>Pyemotes dryas</i>	?
	Pygmephoridae	<i>Bakerdania hylophila</i>	Mycetophagous
	Pygmephoridae	<i>Pseudopygmephorus bogenschutzi</i>	?
	Scutacaridae	<i>Scutacarus scolysi</i>	?
	Tarsonemidae	<i>Iponemus gaebleri</i>	Predacious
	Tarsonemidae	<i>Tarsonmeus subcorticalis</i>	Mycetophagous
	Cheyletidae	<i>Chelacheles michalskii</i>	Predacious

Table 7 Parasitoids of *Ips typographus* (Kenis et al., 2004)

Family	Species	Guild
Braconidae	<i>Bracon palpebrator</i>	Larval ectoparasitoid
	<i>Bracon stabilis</i>	Larval ectoparasitoid
	<i>Coeloides abdominalis</i>	Larval ectoparasitoid
	<i>Coeloides bostrichorum</i>	Larval ectoparasitoid
	<i>Coeloides scolyticida</i>	Larval ectoparasitoid
	<i>Coeloides sordidator</i>	Larval ectoparasitoid
	<i>Cosmophorus klugi</i>	Adult endoparasitoid
	<i>Cosmophorus regius</i>	Adult endoparasitoid
	<i>Dendrosoter middendorfi</i>	Larval ectoparasitoid
	<i>Dendrosoter mutilator</i>	Larval ectoparasitoid
	<i>Dendrosoter pomarius</i>	Larval ectoparasitoid
	<i>Doryctes leucogaster</i>	Larval ectoparasitoid
	<i>Ecphyllus hylesini</i>	Larval ectoparasitoid
	<i>Lestricus secalis</i>	?
	<i>Ropalophorus clavicornis</i>	Adult endoparasitoid
	<i>Spathius brevicaudis</i>	Larval ectoparasitoid
	<i>Spathius exarator</i>	Larval ectoparasitoid
Eupelmidae	<i>Eupelmus urozonus</i>	Larval ectoparasitoid (Essentially hyperparasitoid)
Eurytomidae	<i>Eurytoma abieticola</i>	Larval ectoparasitoid ?
	<i>Eurytoma arctica</i>	Larval ectoparasitoid
	<i>Eurytoma blastophagi</i>	Larval ectoparasitoid
	<i>Eurytoma crassinervis</i>	Larval ectoparasitoid ?
	<i>Eurytoma morio</i>	Larval ectoparasitoid
	<i>Eurytoma polygraphi</i>	Larval ectoparasitoid ?
Pteromalidae	<i>Cheilopachus quadrum</i>	Larval ectoparasitoid
	<i>Dinotiscus aponius</i>	Larval ectoparasitoid
	<i>Dinotiscus eupterus</i>	Larval ectoparasitoid
	<i>Heydenia pretiosa</i>	Larval ectoparasitoid
	<i>Mesopolobus typographi</i>	Adult endoparasitoid (Essentially hyperparasitoid)
	<i>Metacolus unifasciatus</i>	Larval ectoparasitoid
	<i>Psychophagus omnivorus</i>	(Essentially hyperparasitoid) ?
	<i>Rhopalicus guttatus</i>	Larval ectoparasitoid
	<i>Rhopalicus tutela</i>	Larval ectoparasitoid
	<i>Roptrocerus mirus</i>	Larval ectoparasitoid, cryptoparasitoid
	<i>Roptrocerus xylophagorum</i>	Larval ectoparasitoid, cryptoparasitoid
	<i>Tomicobia seitneri</i>	Adult endoparasitoid

Table 8 Predators of *Ips typographus* (Kenis et al., 2004). Killing rate denotes prey consumption or killing by the respective predator (A= adult; L= larva).

Predator family	Predator species	Tree host	Killing rate
Acarophenacidae	<i>Paracarophenax ipidarius</i>		
Anthoridae	<i>Scoloposcelis pulchella</i>	<i>Picea, Pinus</i>	39-144 larvae/L
Asilidae	<i>Choerades (Laphria) gilva</i>	<i>Picea</i>	7 beetles/A/day
	<i>Laphria flava</i>	<i>Picea</i>	
	<i>Tolmerus (Machimus) atricapillus</i>	<i>Picea</i>	
Chrysopidae	<i>Chrysoperla carnea</i>	<i>Picea</i>	
Cleridae	<i>Thanasimus femoralis</i>		44-57 larvae/L 2.9 adults/A/day 74-109 adults/A
	<i>Thanasimus formicarius</i>	<i>Picea, Fraxinus</i>	
	<i>Thanasimus rufipes</i>	<i>Picea</i>	
Dolichopodidae	<i>Medetera adjaniae</i> (=breviseta)	<i>Picea</i>	0.5-10 larvae/L/day
	<i>Medetera ambigua</i>	<i>Picea, Pinus</i>	
	<i>Medetera dendrobaena</i>	<i>Picea, Fagus</i>	
	<i>Medetera dichrocera</i>	<i>Picea, Pinus</i>	
	<i>Medetera excellens</i>	<i>Picea, larix</i>	
	<i>Medetera infumata</i>	<i>Picea, Pinus</i>	
	<i>Medetera pinicola</i> (=nuortevai)	<i>Picea, Pinus</i>	
	<i>Medetera signaticornis</i>	<i>Picea, Pinus</i>	
	<i>Medetera thunebergi</i>	<i>Picea, Pinus</i>	
	<i>Medetera vagans</i> (=fennica)	<i>Picea</i>	
	<i>Medetera</i> spp.	<i>Picea, Pinus, Abies</i>	7-20 larvae+pupae/L 1 larval/L/day
Fringillidae	<i>Fringilla coelebs</i>		
Histeridae	<i>Platysoma angustatum</i>	<i>Picea, Pinus</i>	
	<i>Platysoma lineare</i>	<i>Picea, Pinus</i>	
	<i>Plegaderus vulneratus</i>	<i>Picea, Pinus</i>	
Lonchaeidae	<i>Lonchaea bruggeri</i>	<i>Conifers</i>	
	<i>Lonchaea fugax</i>	<i>Picea</i>	
	<i>Lonchaea helvetica</i>	<i>Picea</i>	
	<i>Lonchaea scutellaris</i>	<i>Picea</i>	
	<i>Lonchaea zetterstedti</i>	<i>Picea, Pinus</i>	
Muscidae	<i>Phaonia gobertii</i>	<i>Pinus</i>	
	<i>Phaonia</i> sp.		
Nitidulidae	<i>Epuraea marseuli</i> (=E. pusilla)	<i>Pinus</i>	
	<i>Epuraea pygmaea</i>	<i>Picea</i>	
	<i>Epuraea rufomarginata</i>	<i>Pinus</i>	
	<i>Epuraea thoracica</i>	<i>Picea, Pinus</i>	
	<i>Epuraea</i> spp.	<i>Picea, Pinus</i>	
	<i>Glischrochilus hortensis</i>	<i>Picea</i>	
	<i>Pityophagus ferrugineus</i>	<i>Pinus, Fagus</i>	
Pallopteridae	<i>Palloptera ustulata</i>		
Picidae	<i>Picoides tridactylus</i>	Conifers	1200 adults/day

Predator family	Predator species	Tree host	Killing rate
Pyemotidae	<i>Pyemotes dryas</i>		
Raphidiidae	<i>Dichrostigma (Raphidia) flavipes</i>	<i>Picea</i>	
	<i>Phaeostigma (Raphidia) notata</i>	<i>Picea</i>	
Rhizophagidae	<i>Rhizophagus bipustulatus</i>	<i>Picea, Pinus, Fraxinus</i>	
(=Monotomidae)	<i>Rhizophagus cribratus</i>	<i>Picea, Pinus</i>	
	<i>Rhizophagus depressus</i>	<i>Picea, Pinus</i>	14 larvae/L 1 larvae/A/day 16 eggs/A/day
	<i>Rhizophagus dispar</i>	<i>Picea, Pinus</i>	15 eggs/L/day
	<i>Rhizophagus ferrugineus</i>	<i>Picea, Pinus</i>	79 eggs/A
Salpingidae	<i>Rabocerus foveolatus</i>	<i>Picea</i>	
	<i>Rabocerus gabrieli</i>	<i>Picea</i>	
	<i>Salpingus (Rhinosimus) planirostris</i>	<i>Picea</i>	
	<i>Salpingus (Rhinosimus) ruficollis</i>	<i>Picea</i>	
	<i>Sphaeriestes (Salpingus) castaneus</i>	<i>Picea</i>	
Staphylinidae	<i>Aleochara sparsa</i>	<i>Picea</i>	
	<i>Nudobius lentus</i>	<i>Picea, Pinus, Larix</i>	
	<i>Phloeonomus</i> spp.	<i>Picea, Pinus</i>	
	<i>Phloeopora testacea</i>	<i>Picea, Pinus</i>	
	<i>Placusa depressa</i>	<i>Picea</i>	2 eggs/L/day
	<i>Placusa tachyporoides</i>		
	<i>Quedius laevigatus</i>	<i>Picea</i>	7 larvae/A/day
	<i>Quedius plagiatus</i>	<i>Picea</i>	
	Staphylinidae spp.	<i>Picea, Pinus, Larix</i>	
Stratiomyidae	<i>Zabrachia minutissima</i>		
Tarsonemidae	<i>Iponemus gaebleri</i>		
Tenebrionidae	<i>Corticeus. (Hypophloeus) linearis</i>		
Trogossitidae	<i>Nemosoma elongatum</i>	<i>Picea, Pinus, Alnus, Fagus</i>	1-2 beetles/A/day 30-45 larvae + pupae + teneral beetles/L

Table 9 Impact of *Ips typographus* outbreaks

Period	Duration	Continent	Country	Region	Contributing factors	Damage	Tree species	Reference
17 th -18 th century	30 years	Europe	Germany	Harcynian Mountains	Early forestry practices	Recurring outbreaks		Christiansen & Bakke 1988
1850-1900	50 years	Europe	Norway	Southeastern Norway	Excessive harvesting, wind, drought		<i>P. abies</i> - managed forests	Christiansen & Bakke 1988
1944-1951	5-7 years	Europe	Germany	Northwestern Germany	Harvesting, wind-felling, management	2 million m ³	<i>P. abies</i> artificial stands	Christiansen & Bakke 1988
1942-1949	7 years	Europe	Germany	Southern Germany	Dry, hot summers, intense harvesting	<30 million m ³	<i>P. abies</i> artificial stands	Christiansen & Bakke 1988
1953		East Asia	China	Jilin		30 000 ha		Liu et al., 2015
1971-1982	9 years	Europe	Norway	Southeastern Norway	Wind, drought, accumulation of mature forest, timber with bark left in forests	5 million m ³	<i>P. abies</i> - managed forests	Christiansen & Bakke 1988
1990's		Europe	Central Europe		'Vivian/Wiebke' & 'Lothar' storms in February/March 1990	>32 million m ³		Grégoire and Evans 2004; Wermelinger 2004
1990's		East Asia	Japan	Hokkaido	Strong winds		<i>Picea jezoensis</i> , <i>P. glehnii</i>	Yamaoka et al., 1997
2004-2014		Europe	Slovak Republic	Tatra National Park		20 000 ha		Fleischer et al., 2016
1990's, early 2000's		East Asia	China	Jilin; Qinghai		160 000 ha; 13300 ha	<i>Picea asperata</i> ; <i>Abies fabri</i>	Anonymous 2010; Sun et al., 2007
2012		East Asia	China	Qinghai		8094		Anonymous 2012

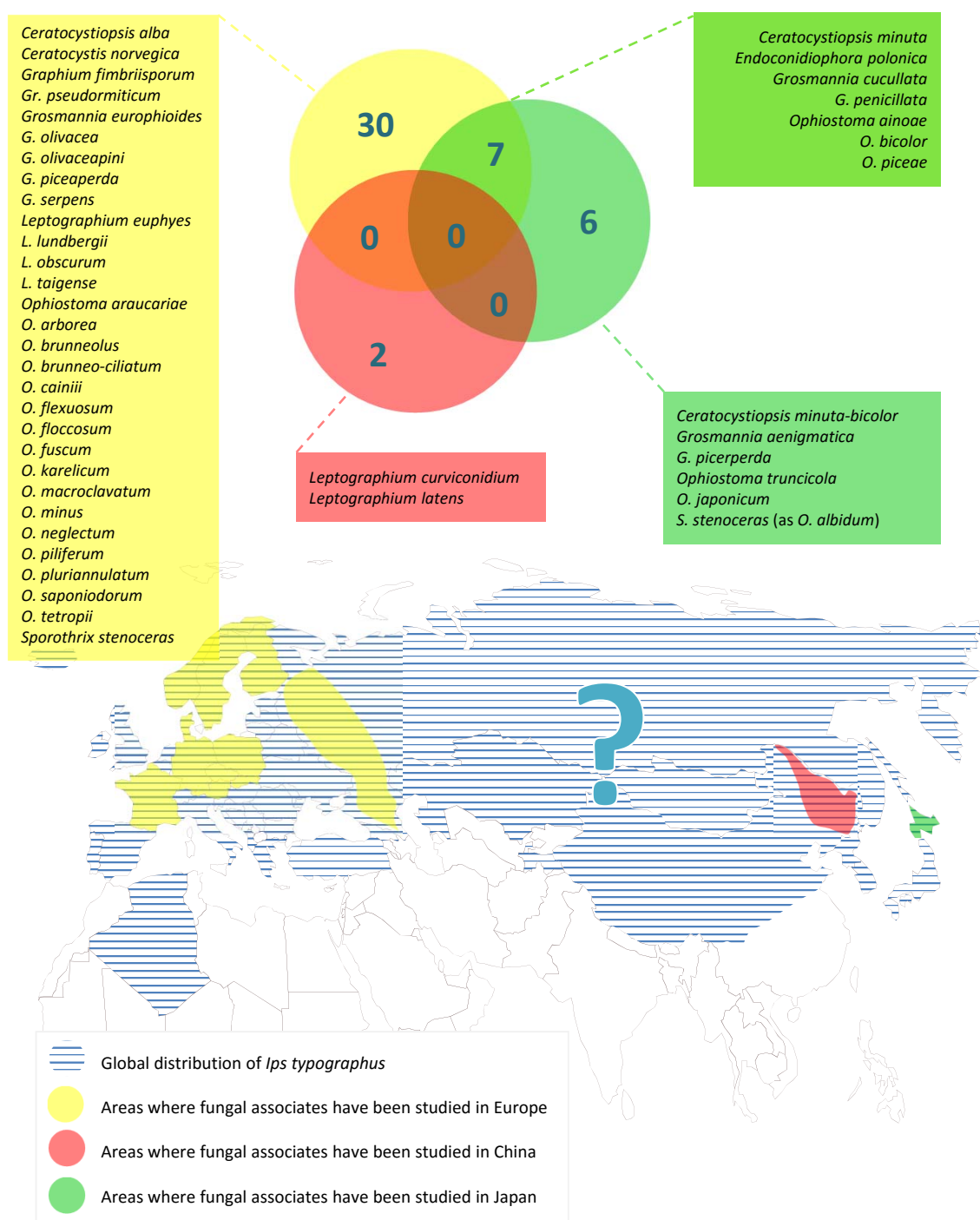


Fig. 1 Eurasian distribution of *Ips typographus* and the ophiostomatoid fungi reported from the beetle and its galleries from different regions.

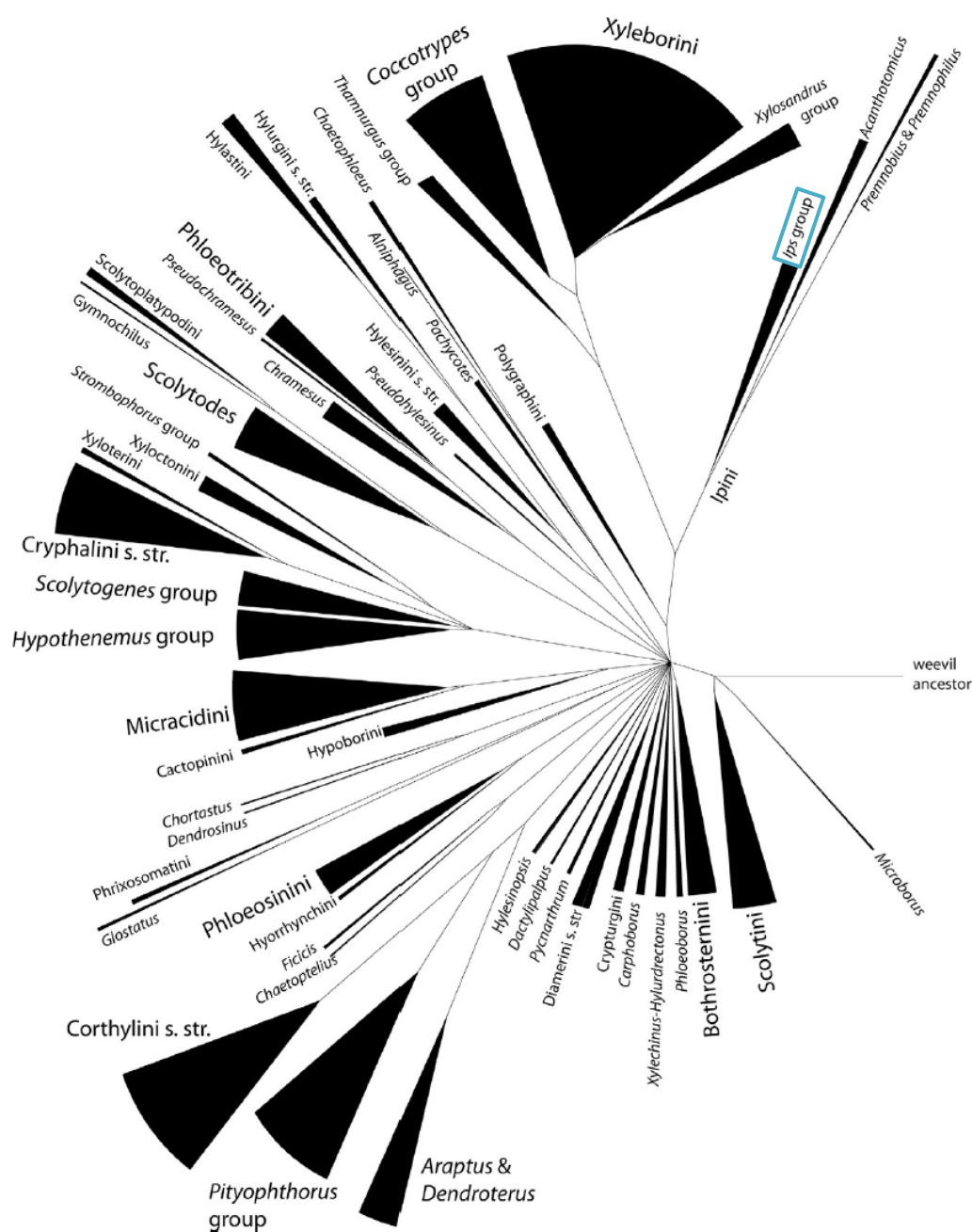


Fig. 2 Schematic representation of the evolution and diversity of Scolytine bark and ambrosia beetles modified from Hulcr et al. (2015). The genus *Ips* is highlighted.

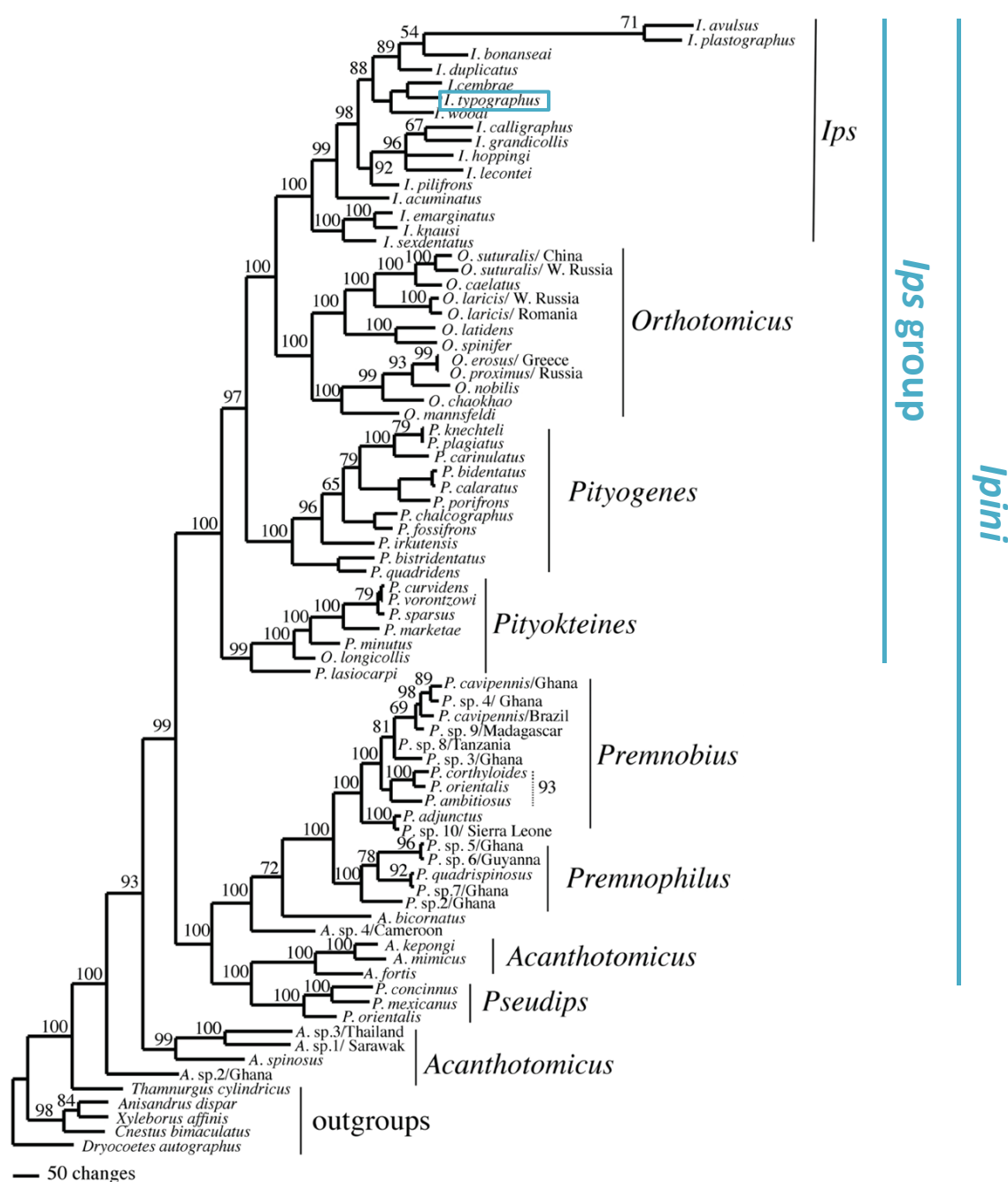


Fig. 3 Phylogeny of Ipini genera based on a Bayesian analysis of nucleotides from four gene regions (COI, 16S, 28S, and CAD, ~2640 characters), showing the phylogenetic placement of *Ips typographus* and the generic boundaries of the genus *Ips* in relation to other genera in the tribe. Numbers represent posterior probabilities (Cognato, 2013).

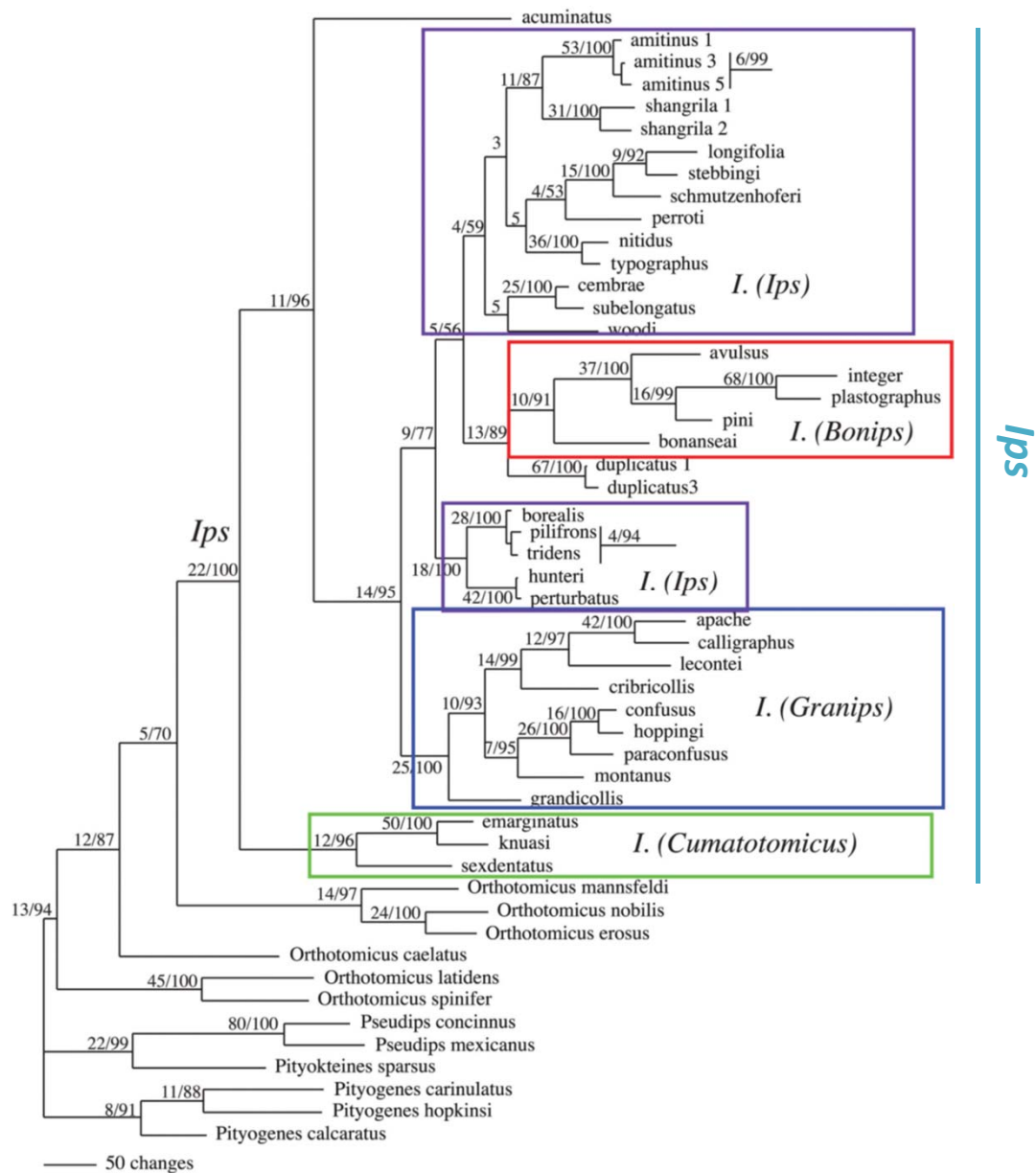


Fig. 4 Phylogeny of the genus *Ips* based on parsimony analysis of molecular and morphological data, showing the four subgenera as defined by Cognato & Vogler (2001) and illustrated by Cognato (2015). Numbers represent Bremer support/bootstrap values.



Fig. 5 The view of type specimen (A-B), dorsal view (C) of *Ips typographus*, with an enlarged illustration of its characteristic elytra with spines (D), lateral view of elytral declivity with four spines on one side (E), and related distinct species *Ips perturbatus* declivity (F), *Ips pini* declivity (G), *Ips hunteri* declivity (H), *Ips perroti* declivity (I), *Ips woodi* declivity (J), *Ips integer* declivity (K), *Ips plastographus* declivity (L) (Benzel, 2015).

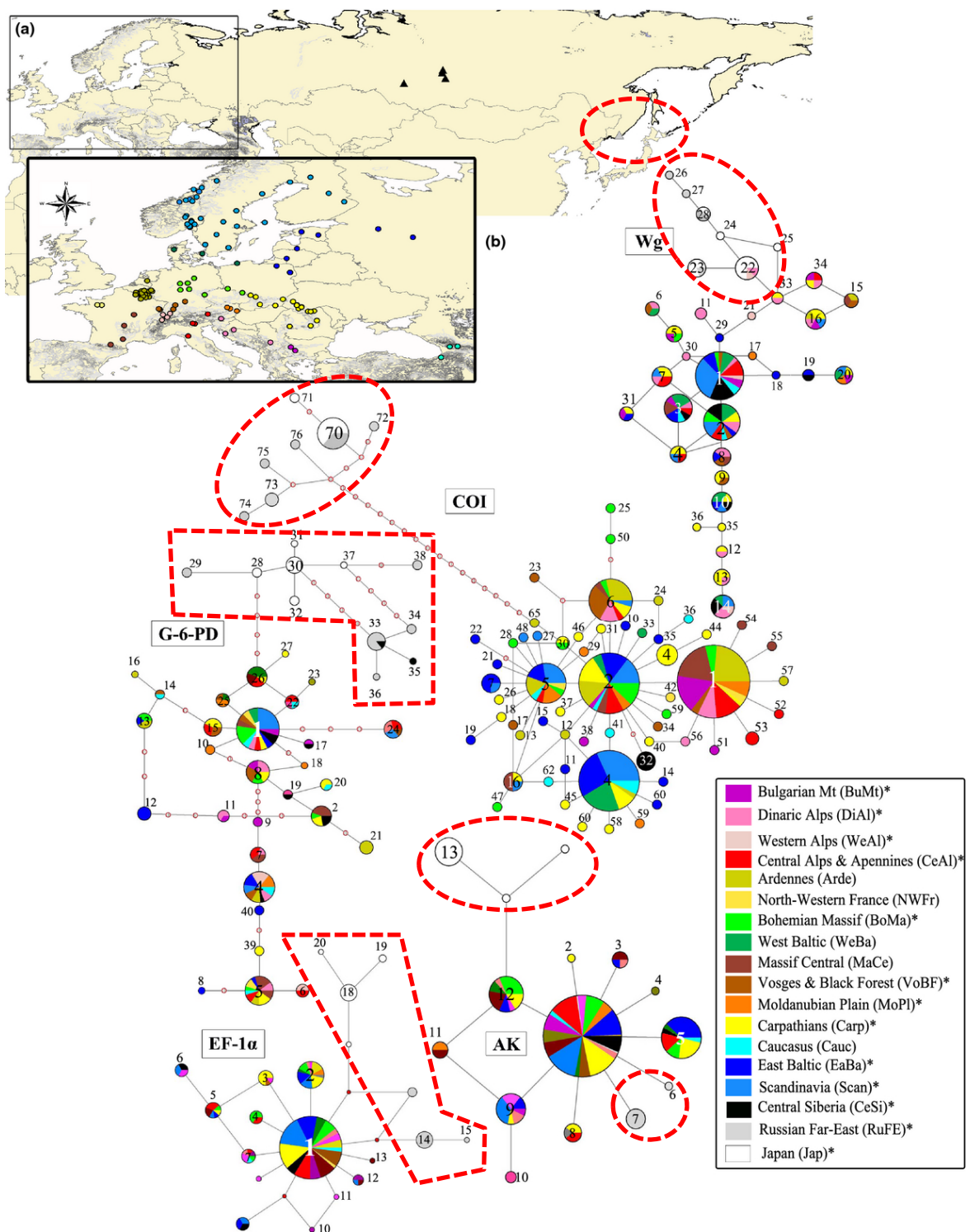
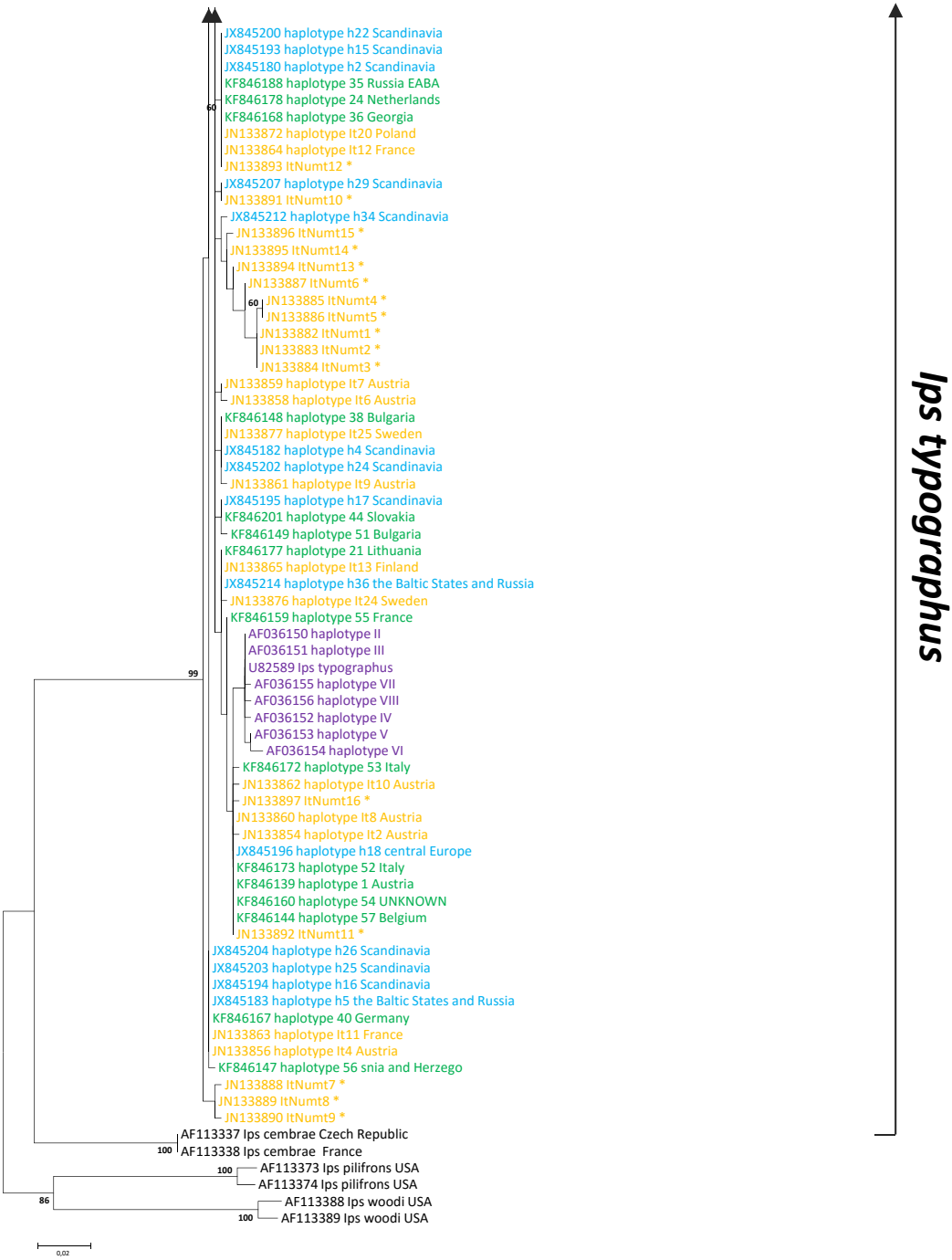


Fig. 6 Median-joining networks for the Arginine kinase, Cytochrome oxidase I, Elongation factor-1 α , Glucose-6-phosphate dehydrogenase and Wingless gene for *Ips typographus* (Mayer et al. 2015). Far East and Asian haplotypes are highlighted by red dotted line cycles.

Fig. 7 (Following pages) Phylogeny of *Ips typographus* based on a Maximum likelihood analysis of COI gene sequences from different studies as indicated in the tree. Tree was drawn by MEGA 6 with GTR substitution matrix and a 1000 rapid bootstrap analysis.



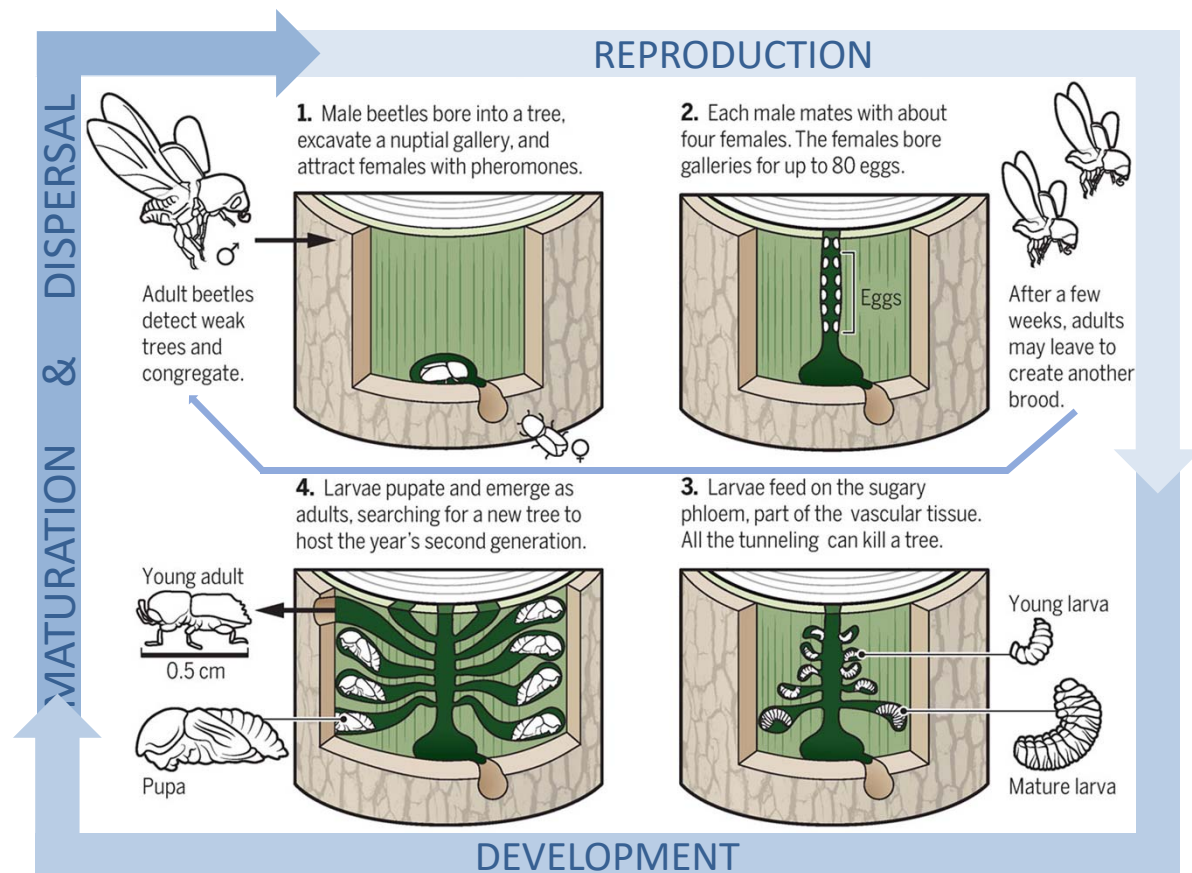


Fig. 8 Basic bark beetle life cycle adapted from Sauvard (2004) and Schebeck et al. (2017).

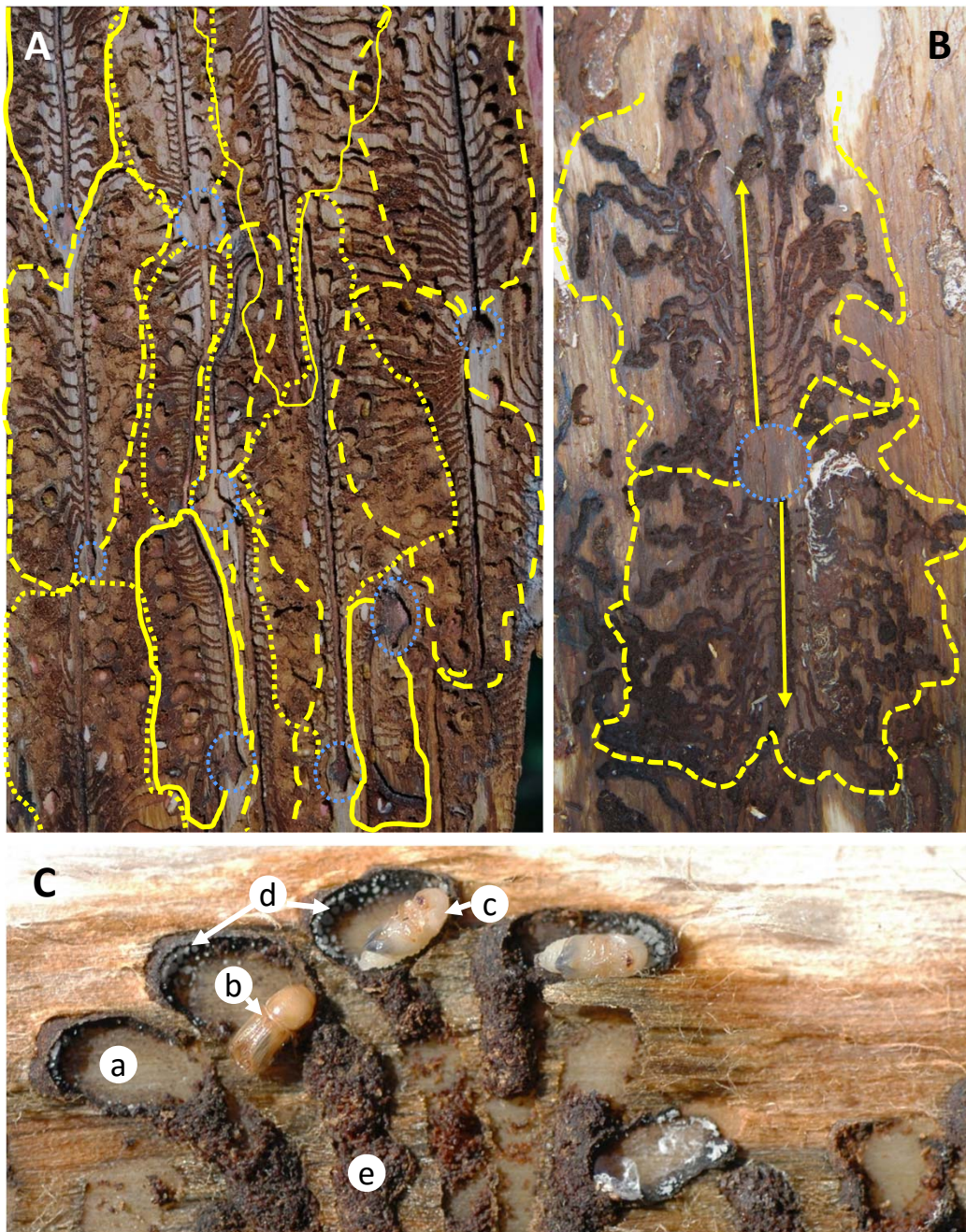


Fig. 9 Gallery structure of *Ips typographus* on spruce in China (A, B) and Western Russia (C). (A) Multiple gallery systems adjacent to each other, encircled by solid and dotted yellow lines to distinguish between individual galleries. Nuptial chambers are indicated in blue dotted lines. (B) Yellow arrows showing breeding galleries excavated in opposite directions by two females mated by one male. (C) Larval galleries showing (a) a pupal chamber, (b) light coloured teneral beetle, (c) pupae, (d) white spore drops of ophiostomatoid fungi growing in the pupal chambers, and (e) larval galleries widening towards the pupal chamber as the larvae grows and moves radially away from the breeding gallery. The older larval galleries are filled with frass behind the larvae as it constructs the pupal chamber (Photos A, C: Z.W. de Beer; Photo B: SF Chen).



Fig. 10 Impact and management of *Ips typographus* in northern China. (A) Trees killed by the beetles. (B) Setting up traps and (C) beetles caught in the traps (Photos: Z.W. de Beer)

Chapter 2

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Ophiostomatoid fungi associated with conifer-infesting beetles and their phoretic mites in Yunnan, China

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Abstract

The Ophiostomatales is an Ascomycete order of fungi that accommodates several tree pathogens and many species that degrade wood. These fungi are commonly vectored by Scolytine bark and ambrosia beetles. In recent years it has also been shown that hyperphoretic mites on these beetles can vector some Ophiostomatales. Little is known regarding the Ophiostomatales in China and we have consequently explored the diversity of these fungi associated with conifer-infesting beetles and mites in Yunnan province. Galleries and beetles were collected for 17 beetle species, while 13 mite species were obtained from six of these beetle species. Collectively, 340 fungal isolates were obtained, 45 from beetles, 184 from mites, 56 from galleries and 55 isolates where the specific niche was not clear. DNA sequences for five gene regions (ITS, LSU, BT, EF, and CAL) were determined for fungal isolates representing different morphological groups. Phylogenetic analyses confirmed the presence of 19 fungal taxa, including five novel species described here as *Ophiostoma acarorum* **sp. nov.**, *Ophiostoma brevopilosi* **sp. nov.**, *Graphilbum kesiya* **sp. nov.**, *Graphilbum puerense* **sp. nov.**, and *Leptographium ningerense* **sp. nov.** *Ophiostoma ips* was the most frequently isolated species, representing approximately 31% of all isolates. Six of 19 taxa were present on mites, beetles and in the galleries of the beetles, while three species were found on mites and galleries. Two species were found only on mites and one species only on a beetle. Although the numbers of beetles and mites were insufficient to provide statistical inferences, this study confirmed that mites are important vectors of the Ophiostomatales in China. We hypothesize that these mites are most likely responsible for horizontal transfer of fungal species between galleries of different beetle species. The fact that half of the fungal species found were new to science, suggests that the forests of east Asia include many undescribed Ophiostomatales yet to be discovered.

Keywords

Symbiont, species diversity, fungal vector, *Ophiostoma quercus*

Introduction

The ophiostomatoid fungi represent a polyphyletic group of tree- or wood-infecting fungi, most often staining freshly exposed sapwood and thus lowering the value of timber (Seifert 1993). Some of the species are important tree pathogens that have dramatically impacted natural forests and caused major losses to forestry companies during the course of the last century (Harrington 1993, Wingfield et al. 2017). The sexual and often asexual spore-bearing structures of the ophiostomatoid fungi are specifically adapted for dispersal by arthropods, producing spores in sticky droplets that attach to the bodies of their vectors (Malloch and Blackwell 1993). However, these morphological and biological traits have evolved more than once in the Ascomycota and phylogenetic analyses have shown that these fungi reside in two different orders, the Ophiostomatales and Microascales (De Beer et al. 2013). The Ophiostomatales currently accommodates a single family, the Ophiostomataceae, that includes ten genera (Bateman et al. 2017, De Beer et al. 2016a, De Beer et al. 2016b, Van der Linde et al. 2016), while the Microascales comprises of five families, of which the Ceratocystidaceae, Gondwanamycetaceae and Graphiaceae include 14 genera of ophiostomatoid fungi (De Beer et al. 2014, 2017, Mayers et al. 2015, Nel et al. 2017).

Bark beetles (Coleoptera: Scolytinae) are well known vectors of ophiostomatoid fungi (Harrington 2005, Kirisits 2004, Six 2003). Other than a few bark beetle species that are considered to be primary pests causing significant economic losses, most species of bark beetles are secondary invaders, which colonize injured or stressed trees (Harrington 2005, Six and Wingfield 2011). Some bark beetle species have specialized structures known as mycangia that carry fungal conidia or ascospores, but most of these insects carry fungi on their exoskeletons (Six 2003). They construct their galleries, where the ophiostomatoid fungi grow, in the phloem and cambial layers under the bark of trees (Paine et al. 1997). The nature of the relationships between fungi and bark beetles is recognized as being very diverse. Some beetle-fungus symbioses appear to be obligate, while others may be facultative (Kirisits 2004). The role of the fungi can vary from their being nutritional symbionts of the beetles to saprophytes in the galleries, although in most cases the exact nature of the symbiosis is not well understood (Six and Wingfield 2011).

Along with the fungi, mites are also common symbionts of bark beetles (Hofstetter and Moser 2014, Roets et al. 2009). Due to their small body size and low mobility, many mite species rely on beetles or other animals for dispersal (Mitchell 1970). The relationship between mites and their vectors is usually commensal, but in some cases these are antagonistic or even mutualistic (Pfammatter et al. 2013). The phoretic mites of only a limited number of bark beetles have been studied in detail, including mostly North American species of *Scolytus*, *Dendroctonus* and *Ips* (Hofstetter et al. 2013).

As is the case with bark beetles, their mite associates can also vector ophiostomatoid fungi (Hofstetter and Moser 2014). Some mites such as *Tarsonemus* spp. possess sporothecae that are specialized structures of the integument that serve to carry fungal spores (Hofstetter et al. 2015). Ophiostomatoid fungi associated with mites include genera such as *Ceratocystis*, *Knoxdaviesia*, *Ceratocystiopsis*, *Ophiostoma*, *Leptographium*, *Grosmannia* and *Sporothrix* (Hofstetter et al. 2013). The interactions between mites and fungi are dynamic and vary with tree host condition and climate (Klepzig and Hofstetter 2011). It is for example known that mites can promote some fungal growth by increasing fungal transmission, reproduction, and survival, or by reducing the abundance of antagonistic fungi (Hofstetter and Moser 2014). Mites can also negatively affect some fungi because of exploitative/interference competition, predation, or encouragement of antagonistic fungi (Hofstetter et al. 2006).

Research on the interactions between ophiostomatoid fungi and bark beetles have been ongoing for more than a century in North America (Harrington 2005, Hedgcock 1906), Europe (Kirisits 2004, Münch 1907), and Japan (Masuya et al. 2013, Nisikado and Yamauti 1933). The associations of ophiostomatoid fungi with mites have been studied only in North America and Europe (Hofstetter and Moser 2014, Hofstetter et al. 2013), with a single report from Japan (Moser et al. 1997) and with ongoing studies on *Protea* spp. in Southern Africa (Roets et al. 2009, Roets et al. 2007). It is only more recently that the fungal associates of bark beetles have been explored in China, despite the fact that more than 160 bark beetle species have been recorded from that country (Yin et al. 1984).

The first new beetle associated ophiostomatoid species described from China for which the description was supported by DNA sequence data, was *Grosmannia yunnanensis* (Zhou et al. 2000, Yamaoka et al. 2008). Additional new species described during the past decade from a variety of conifer-infesting beetles in China, include one *Graphium* (Paciura et al. 2010a), 12 *Leptographium* (Liu et al. 2017, Lu et al. 2008, Paciura et al. 2010b, Yin et al. 2015), and five *Ophiostoma* (Yin et al. 2016) species. The most comprehensive study on the fungal associates of a specific beetle species in China has been on *Dendroctonus valens*, an economically important invasive bark beetle that was introduced to China from the USA and is attacking native pine species (Lu et al. 2008, 2009a, b, Taerum et al. 2013). Together with the fungal associates of *D. valens*, almost 40 ophiostomatoid species have been recorded and described from conifers in China (Liu et al. 2017, Paciura et al. 2010a, b, Yin et al. 2015, 2016, Zhou et al. 2013). Most of these are from bark beetles or their galleries. The fungal associates of mites have not been studied in this country (Hofstetter et al. 2013, Zhou et al. 2013).

Yunnan province forms part of in the southwestern forest zone, the second largest forest area in China (<http://www.china.org.cn/english/shuzi-en/en-shuzi/gq/htm/zrzy-land-sl.htm>). This province has a unique geography where three climatic regions meet: the eastern Asia monsoon region, the Tibetan plateau region, and the tropical monsoon region of southern Asia and Indo-China. As a result, species diversity in Yunnan province is high when compared to other parts of China. For example, more than 18000 plant species and 1836 vertebrate species are found here which represent 51.6% and 54.8% respectively of total species numbers in China (Yang et al. 2004). However,

only 13 ophiostomatoid species have been reported from Yunnan (Zhou et al. 2013). Based on the high diversity of trees and vertebrates, but the relatively low number of ophiostomatoid species reported from Yunnan to date, we hypothesize that there are many undiscovered species from this group of fungi associated with bark beetles and mites in this province. The aim of this study was thus to further explore the diversity of ophiostomatoid fungi associated with conifer-infesting beetles and mites in Yunnan.

Material and methods

Collection of bark beetles and mites

Three surveys were conducted in Yunnan during the flight period of bark beetles in July 2001, July 2002, and between June and September 2010 (Table 1, Suppl. material 2: Figure S1). Surveys were conducted both in sawmill log-yards and forest areas. During the 2001 and 2002 surveys, only galleries and beetles were considered from pine species in conifer and mixed forests in central and northwestern Yunnan (Table 1, Suppl. material 2: Figure S1). During the 2010 survey, some collections were conducted using *Pinus kesiya* log traps in the pine forests around Pu'er city in southwestern Yunnan. Both adult bark beetles and their galleries were collected and stored in Eppendorf tubes and re-sealable bags, respectively. Mites were carefully removed from these beetles and galleries in the laboratory and individually placed in Eppendorf tubes. After the isolation of fungi, beetles and mites were stored in 75% ethanol for later identification. Beetles and mites were identified based on the morphology by Dr R. Beaver (Chiang Mai University, Thailand) and Dr E.A. Ueckermann (Plant Protection Research Institute, Agricultural Research Council, South Africa) respectively.

Isolation of fungi

Fungi were isolated from the bark beetles and their galleries following the methods described by Linnakoski et al. (2008). At the time of collection, living mites collected from the galleries were placed on the surface of 2% MEA medium (20 g Difco agar, 20 g Difco Bacto™ malt extract [Becton, Dickinson & Company], 1 L deionized water) and allowed to crawl over the plate for 24 hours. The mites were then removed and stored in Eppendorf tubes for identification. MEA plates were monitored daily for fungal growth and hyphal tips of emerging colonies were transferred to fresh MEA plates in order to purify the fungi. Pure cultures were grouped based on culture morphology, and representatives from each group were selected for DNA sequencing. All isolates used in this study were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa. Isolates representing types of new species were also deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.

Table 1. Conifer-infesting bark and ambrosia beetles (Scolytinae), weevils (Molytinae), true weevils (Cossoninae), cylindrical bark beetles (Colydiinae), and mites (Acar) collected from Yunnan in this study. Numbers in the table refer to number of mite individuals collected.

Beetle species	Family	Subfamily	Tree host	Origin	Collection dates	Mite species													
						a	b	c	d	e	f	g	h	i	j	k	l	m	Total
A	<i>Coccotrypes cyperi</i>	Curculionidae	<i>Pinus kesiya</i>	Ning'er	Sep. 2010	16	3	9	0	0	0	0	2	4	0	0	0	3	37
B	<i>Cyrtogenius luteus</i>	Curculionidae	<i>P. kesiya</i>	Ning'er	Sep. 2010	0	2	10	0	0	0	0	0	0	0	0	0	0	12
C	<i>Hylurgops major</i>	Curculionidae	<i>Pinus yunnanensis</i>	Zixishan	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	<i>Ips acuminatus</i>	Curculionidae	<i>P. kesiya</i>	Ning'er, Puer	Jun. 2010	0	0	0	6	0	0	0	0	0	0	0	0	2	8
E	<i>Lasconotus</i> sp.	Zopheridae	<i>P. kesiya</i>	Ning'er	Jun. 2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F	<i>Orthotomicus angulatus</i>	Curculionidae	<i>P. kesiya</i>	Ning'er	Sep. 2010	0	0	4	13	0	0	0	0	0	1	0	0	1	19
G	<i>Pissodes</i> sp.	Curculionidae	<i>Pinus armandii</i>	Lijiang, Midu	Jul. 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H	<i>Polygraphus aterrimus</i>	Curculionidae	<i>P. kesiya</i>	Ning'er	Jun. 2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	<i>Polygraphus</i> sp.	Curculionidae	<i>P. kesiya</i>	Ning'er	Jun. 2010	0	0	0	6	0	4	0	0	0	0	0	0	0	10
J	<i>P. szemaensis</i>	Curculionidae	<i>P. kesiya</i>	Ning'er, Simao	Jun. 2010	0	0	0	14	1	0	0	0	0	2	2	0	1	20
K	<i>P. verrucifrons</i>	Curculionidae	<i>P. yunnanensis</i>	Lufeng	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	<i>Stenoscelis</i> sp.	Curculionidae	<i>P. kesiya</i>	Ning'er	Jun. 2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	<i>Tomicus minor</i>	Curculionidae	<i>P. yunnanensis</i>	Zixishan	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	<i>T. piniperda</i>	Curculionidae	<i>P. yunnanensis</i>	Lufeng, Zixishan, Changhu	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	<i>T. brevipilobus</i>	Curculionidae	<i>P. kesiya</i>	Ning'er	Jun. 2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	Unknown sp.1	Curculionidae	<i>P. semanensis</i>	Chuxiong	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q	Unknown sp.2	Curculionidae	<i>Tsuga</i> sp.	Dali	Jul. 2002	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total						16	5	23	39	1	4	2	4	1	2	2	1	6	106

Mite species: a = *Dendrolaelaps* sp. 1 (Mesostigmata, Digamasellidae); b = *Dendrolaelaps* sp. 2 (Mesostigmata, Digamasellidae); c = *Histioglyphus* cf. *sapromyzarum* (Sarcoptiformes, Histioglyphidae); d = *Insectolaelaps* sp. 1 (Mesostigmata, Digamasellidae); e = *Lasioeius* sp. 1 (Mesostigmata, Blattisociidae); f = *Proctolaelaps* nr. *hystrix* (Mesostigmata, Melicharidae); g = *Rhizoglyphus* sp. 1 (Sarcoptiformes, Acaridae); h = *Schwiebia taiwanensis* (Sarcoptiformes, Acaridae); i = Unknown sp.1 (Mesostigmata); j = Unknown sp.2 (Oribatei); k = Unknown sp.3 (Mesostigmata, Uropodidae); l = Unknown sp.4 (Mesostigmata); m = Unknown sp.5.

DNA sequencing and phylogenetic analyses

Isolates were grown on 2% MEA in Petri dishes. DNA was extracted using PrepMan Ultra Sample Preparation reagent (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. The primer pairs ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used to amplify internal transcribed spacer regions (ITS), the primary barcode of fungi (Schoch et al. 2012). LR0R and LR5 (Vilgalys and Hester 1990) were used to amplify nuclear large subunit (LSU). For ITS2-LSU used in *Leptographium*, ITS and LSU sequences were combined. EF2F (Marincowitz et al. 2015) and EF2R (Jacobs et al. 2004) were used to amplify the elongation factor 1- α (EF) gene, the secondary barcode of fungi (Stielow et al. 2015). BT2A and BT2B (Glass and Donaldson 1995) were used to amplify part of the β -tubulin (BT) gene, and CL2F and CL2R (Duong et al. 2012) for the calmodulin (CAL) gene. PCR and sequencing were conducted using the methods described by Duong et al. (2012).

The sequences obtained using the forward and reverse primers were aligned in Geneious Pro v. 7.1.4 (Biomatters, Auckland, New Zealand). ITS sequences were submitted to BLAST searches in NCBI Genbank for preliminary identifications. Based on these results, the ITS data were separated into different data sets according to genus. ITS2-LSU data were used for analyses of taxa residing in *Leptographium s.l.*, while ITS was used for analyses of taxa belonging to the other genera. The BT, EF and CAL data were separated into smaller data sets based on the species complexes as defined by De Beer and Wingfield (2013). All sequences obtained in this study were submitted to GenBank (Table 2). Alignments were done online with MAFFT v. 7 (Kato and Standley 2013). Maximum likelihood (ML), maximum parsimony (MP) and Bayesian Inference (BI) were conducted on the datasets as described in Duong et al. (2012). For haplotype analyses of *O. quercus* and *O. tsotsi*, the online server ElimDupes was used (<http://www.hiv.lanl.gov/content/sequence/ELIMDUPES/elimdupes.html>).

Growth studies

Growth studies were conducted on three isolates of each novel taxon. Mycelium-covered agar plugs were transferred from the actively growing margins of one-week-old cultures and placed at the centers of 90 mm Petri dishes containing 2% MEA. Cultures were incubated in the dark at temperatures ranging from 5–35 °C at 5 °C intervals. Three replicates were used for each isolate at each temperature. Colony diameters were measured every day until hyphae reached the edges of the Petri dishes. Optimum and maximum growth temperatures were calculated for each species.

Morphological studies

To facilitate morphological descriptions of new taxa, asexual and sexual structures (where present) were mounted in lactophenol on glass slides, covered with a coverslip

Taxon	Species	Isolate number ^{1,2}		Host	Location	Beetle	G/B/ M ³	GenBank number ⁴			
		CMW	CBS					ITS/ITS2- LSU ⁵	BT	EF	CAL
4	<i>O. ips</i>	41653		<i>P. kesiya</i>	Ning'er	<i>P. szemaonensis</i>	G	=MG205658	MG205688	–	–
		41695		<i>P. kesiya</i>	Simao	<i>P. szemaonensis</i>	M	=MG205658	=MG205688	–	–
		41697		<i>P. kesiya</i>	Simao	<i>P. szemaonensis</i>	B	=MG205658	=MG205688	–	–
		41709		<i>P. kesiya</i>	Simao	<i>P. szemaonensis</i>	B	=MG205658	=MG205688	–	–
		41745		<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	=MG205658	=MG205688	–	–
		41916		<i>P. kesiya</i>	Ning'er	<i>P. szemaonensis</i>	G	=MG205658	=MG205688	–	–
5	<i>Ophiostoma</i> sp. B	41993		<i>P. kesiya</i>	Ning'er	<i>I. acuminatus</i>	M	=MG205658	=MG205687	–	–
		12032		<i>Pinus semaonensis</i>	Chuxiong	Unknown sp.1	*	MG205659	MG205689	MG205731	–
6	<i>O. brevipilos</i> sp. nov.	41624	139661	<i>P. kesiya</i>	Ning'er	<i>Tomitus brevipilosus</i>	B	=MG205660	MG205690	MG205732	–
		41662 ^H	139659	<i>P. kesiya</i>	Ning'er	<i>T. brevipilosus</i>	B	MG205660	=MG205690	=MG205732	–
		41760		<i>P. kesiya</i>	Ning'er	<i>T. brevipilosus</i>	B	=MG205660	=MG205690	=MG205732	–
		41873	139660	<i>P. kesiya</i>	Ning'er	<i>T. brevipilosus</i>	B	=MG205660	=MG205690	=MG205732	–
		41932		<i>P. kesiya</i>	Ning'er	<i>T. brevipilosus</i>	B	=MG205660	=MG205690	=MG205732	–
		41995		<i>P. kesiya</i>	Ning'er	<i>T. brevipilosus</i>	B	=MG205660	=MG205690	=MG205732	–
7	<i>O. setosum</i>	12152		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	MG205661	MG205691	MG205733	–
		12192		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=MG205661	=MG205691	MG205734	–
		12337		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=MG205661	=MG205691	MG205735	–
		11747		<i>P. yunnanensis</i>	Lufeng	<i>Polygraphus verrucifrons</i>	*	=AF198238	=FJ455565	MG205736	–
8	<i>O. quercus</i>	11748		<i>P. yunnanensis</i>	Lufeng	<i>P. verrucifrons</i>	*	=AF198238	=FJ455565	=MG205736	–
		11756		<i>P. yunnanensis</i>	Lufeng	<i>P. verrucifrons</i>	*	MG205662	MG205692	MG205737	–
		11806		<i>P. yunnanensis</i>	Changhu	<i>T. piniperda</i>	*	=FJ434947	=FJ455565	MG205738	–
		11807		<i>P. yunnanensis</i>	Changhu	<i>T. piniperda</i>	*	=FJ434947	=AY466647	=MG205738	–
		11981		<i>Abies</i> sp.	Chuxiong	Unknown sp.1	*	=FJ434947	=FJ455570	MG205739	–
		12015		<i>P. semaonensis</i>	Chuxiong	Unknown sp.1	*	=AY466624	MG205693	MG205740	–
		12037		Unknown	Chuxiong	Unknown sp.1	*	=AY466624	MG205694	MG205741	–
		12039		Unknown	Chuxiong	Unknown sp.1	*	=FJ434947	MG205695	MG205742	–

Taxon	Species	Isolate number ^{1,2}		Host	Location	Beetle	G/B/ M ³	GenBank number ⁴			
		CMW	CBS					ITS/ITS2- LSU ⁵	BT	EF	CAL
8	<i>O. quercus</i>	12122		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	MG205696	MG205743	—
		12146		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	=MG205696	=MG205743	—
		12185		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	MG205697	=MG205740	—
		12195		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	=MG205697	=MG205740	—
		12286		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	=FJ455570	MG205744	—
		12350		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=FJ434947	MG205698	MG205745	—
		12359		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=AF198238	=FJ455570	MG205746	—
		12364		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=AF198238	MG205699	MG214780	—
		12370		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=AF198238	=MG205698	MG205747	—
		12382		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	=AF198238	=FJ455570	MG205748	—
		41659		<i>P. kesiya</i>	Pu'er	<i>I. acuminatus</i>	G	MG205664	MG205700	MG205749	—
		41693		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	G	=MG205664	=MG205700	=MG205749	—
		41715		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	B	=MG205664	=MG205700	=MG205749	—
9	<i>O. tsotsi</i>	41718		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	B	=MG205664	=MG205700	=MG205749	—
		41724		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	B	MG205665	MG205701	MG205750	—
		41732		<i>P. kesiya</i>	Ning'er	<i>Cy. luteus</i>	G	=MG205664	=MG205700	=MG205749	—
		41730		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	G	=FJ441284	MG205704	MG205755	—
		41731		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	G	=FJ441284	MG205705	MG205756	—
		41733		<i>P. kesiya</i>	Ning'er	<i>Cy. luteus</i>	G	=FJ441284	=MG205704	=MG205755	—
		41734		<i>P. kesiya</i>	Ning'er	<i>Cy. luteus</i>	G	=FJ441284	=MG205704	=MG205755	—
		41735		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	=FJ441284	=MG205704	=MG205755	—
		41742		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	=FJ441284	=MG205705	=MG205756	—
		41746		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	=FJ441284	=MG205704	=MG205755	—
		41758		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	=FJ441284	=MG205705	=MG205756	—
		12150		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	MG205666	MG205709	MG205762	—
		11	<i>Graphilbum fragrans</i>	11778		<i>P. yunnanensis</i>	Zixishan	<i>Tomicu minor</i>	*	MG205667	MG205710
12	<i>Gra. kesiyae</i> sp. nov.	41626		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	=MG205669	—	—	
		41657	139639	<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	MG205668	=MG205714	—	

Taxon	Species	Isolate number ^{1,2}		Host	Location	Beetle	G/B/ M ³	GenBank number ⁴			
		CMW	CBS					ITS/ITS2- LSU ⁵	BT	EF	CAL
12	<i>Gra. kesiyae</i> sp. nov.	41686	139641	<i>P. kesiyae</i>	Simao	<i>P. szemaensis</i>	M	=MG205668	MG205711	–	–
		41691	139642	<i>P. kesiyae</i>	Simao	<i>P. szemaensis</i>	M	=MG205669	=MG205713	–	–
		41703		<i>P. kesiyae</i>	Simao	<i>P. szemaensis</i>	G	–	MG205712	–	–
		41716	139657	<i>P. kesiyae</i>	Simao	<i>P. szemaensis</i>	M	=MG205669	=MG205712	–	–
		41729 ^H	139652	<i>P. kesiyae</i>	Ning'er	<i>P. szemaensis</i>	G	MG205669	MG205713	–	–
		41774	139653	<i>P. kesiyae</i>	Ning'er	<i>Polygraphus aterrimus</i>	B	MG205668	MG205714	–	–
13	<i>Gra. puerense</i> sp. nov.	46468		<i>P. kesiyae</i>	Ning'er	<i>P. aterrimus</i>	G	MG205668	=MG205714	–	–
		46469		<i>P. kesiyae</i>	Ning'er	<i>P. aterrimus</i>	B	MG205668	=MG205714	–	–
		41619		<i>P. kesiyae</i>	Ning'er	<i>P. szemaensis</i>	B	=MG205670	MG205715	–	–
		41667	139651	<i>P. kesiyae</i>	Pu'er	<i>I. acuminatus</i>	G	MG205670	MG205716	–	–
		41670		<i>P. kesiyae</i>	Ning'er	<i>I. acuminatus</i>	B	=MG205670	–	–	–
		41671		<i>P. kesiyae</i>	Ning'er	<i>I. acuminatus</i>	B	=MG205670	MG205717	–	–
		41673	139640	<i>P. kesiyae</i>	Ning'er	<i>I. acuminatus</i>	B	=MG205670	MG205718	–	–
		41942 ^H	139650	<i>P. kesiyae</i>	Ning'er	<i>P. szemaensis</i>	G	MG205671	MG205719	–	–
		41971		<i>P. kesiyae</i>	Ning'er	<i>P. szemaensis</i>	M	=MG205671	MG205720	–	–
		41996		<i>P. kesiyae</i>	Ning'er	<i>I. acuminatus</i>	M	=MG205671	MG205721	–	–
14	<i>Leptographium gracile</i>	41998		<i>P. kesiyae</i>	Ning'er	<i>I. acuminatus</i>	G	=MG205670	=MG205715	–	–
		12305		<i>Pinus armandii</i>	Lijiang	<i>Pisodes</i> sp.	*	MG205672	MG205722	MG205763	MG205782
		12397		<i>P. armandii</i>	Midu	<i>Pisodes</i> sp.	*	=MG205672	=MG205722	=MG205763	=MG205782
		12399		<i>P. armandii</i>	Midu	<i>Pisodes</i> sp.	*	=MG205672	=MG205722	=MG205763	=MG205782
		12404		<i>P. armandii</i>	Midu	<i>Pisodes</i> sp.	*	=MG205672	=MG205722	=MG205763	=MG205782
		12407		<i>P. armandii</i>	Midu	<i>Pisodes</i> sp.	*	=MG205672	=MG205722	=MG205763	=MG205782
		12412		<i>P. armandii</i>	Midu	<i>Pisodes</i> sp.	*	=MG205672	=MG205722	=MG205763	=MG205782
		12323		<i>Tsuga</i> sp.	Dali	Unknown sp.2	*	MG205673	MG205723	MG205764	–
15	<i>Grosmannia radiaticola</i>	41773		<i>P. kesiyae</i>	Ning'er	<i>Co. cyperi</i>	M	–	=MG205724	MG205765	=MG205783
16	<i>L. ningerense</i> sp. nov.	41786 ^H	139663	<i>P. kesiyae</i>	Ning'er	<i>Co. cyperi</i>	M	MG205674	MG205724	=MG205765	MG205783
		41831	139664	<i>P. kesiyae</i>	Ning'er	<i>O. angulatus</i>	M	MG205675	=MG205724	=MG205765	=MG205783

Taxon	Species	Isolate number ^{1,2}		Host	Location	Beetle	G/B/ M ³	GenBank number ⁴				
		CMW	CBS					ITS/ITS2- LSU ⁵	BT	EF	CAL	
<i>G. yunnanensis</i>		41622		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	B	MG205676	MG205725	MG205766	–	
		41627		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i> or <i>I. acuminatus</i>	M	–	=MG205725	–	–	
		41633		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i> or <i>I. acuminatus</i>	M	–	MG205726	–	–	
		41635		<i>P. kesiya</i>	Ning'er	<i>Lasconotus</i> sp.	B	–	=MG205726	MG205767	–	
		41636		<i>P. kesiya</i>	Ning'er	<i>Lasconotus</i> sp.	B	–	=MG205726	MG205768	–	
		41666		<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	–	=MG205725	–	–	
		41687		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	M	–	=MG205725	MG205769	–	
		41694		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	M	–	=MG205725	–	–	
		41707		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	B	–	=MG205725	MG205770	–	
		41720		<i>P. kesiya</i>	Simao	<i>P. szemaensis</i>	G	–	=MG205725	MG205771	–	
		41721		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	–	=MG205725	MG205772	–	
		41726		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	–	=MG205725	–	–	
		41728		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	–	=MG205726	MG205773	–	
		41777		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	–	MG205727	MG205774	–	
		41778		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	–	MG205728	=MG205774	–	
		41781		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	–	=MG205727	=MG205774	–	
		41783		<i>P. kesiya</i>	Ning'er	<i>Co. cyperi</i>	M	–	=MG205725	MG205775	–	
		41805		<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	–	=MG205725	–	–	
		41814		<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	–	=MG205725	–	–	
		41858		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	G	–	–	MG205725	MG205776	–
		41863		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	G	MG205677	=MG205725	MG205777	–	
		41945		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	–	=MG205725	–	–	
		41963		<i>P. kesiya</i>	Ning'er	<i>P. szemaensis</i>	M	–	=MG205725	–	–	
		41990		<i>P. kesiya</i>	Ning'er	<i>I. acuminatus</i>	G	–	–	=MG205725	=MG205776	–

Taxon	Species	Isolate number ^{1,2}		Host	Location	Beetle	G/B/ M ³	GenBank number ⁴			
		CMW	CBS					ITS/ITS2- LSU ⁵	BT	EF	CAL
17	<i>G. yunnanensis</i>	41992		<i>P. kesiya</i>	Ning'er	<i>I. acuminatus</i>	G	–	=MG205725	MG205778	–
		41999		<i>P. kesiya</i>	Ning'er	<i>P. szemaoensis</i>	B	–	=MG205725	–	–
		42000		<i>P. kesiya</i>	Ning'er	<i>P. szemaoensis</i>	B	–	–	=MG205778	–
18	<i>L. conjunctum</i>	11782		<i>P. yunnanensis</i>	Zixishan	<i>Hylargops major</i>	*	MG205678	MG205729	MG205779	–
		41761		<i>P. kesiya</i>	Ning'er	<i>Polygraphus</i> sp.	M	MG205679	MG205730	MG205780	–
Microascales											
19	<i>Graphium pseudormiticum</i>	41665		<i>P. kesiya</i>	Pu'er	<i>I. acuminatus</i>	M	MG205680	–	MG205781	–

¹ The culture collection (CBS) of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW/ Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

² H = ex-holotype isolate

³ G = Gallery; B = Beetle; M = Mire; * = Unknown

⁴ ITS = internal transcribed spacer regions 1 and 2 of the nuclear ribosomal DNA operon, including the 5.8S region; ITS2–LSU = the internal transcribed spacer 2 region and partial large subunit of the nrDNA operon; BT = beta-tubulin; EF = translation elongation factor 1–alpha; CAL = Calmodulin

and examined with a Zeiss Axioskop2 Plus compound microscope or a Zeiss Discovery V12 dissection microscope with an Axiocam digital camera (Axiovision 3.1) (München-Hallbergmoos, Germany). Measurements were made for each taxonomically characteristic structure. The measurements are presented in the format (minimum–) mean minus standard deviation–mean plus standard deviation (–maximum). For reference to asexual states that resemble morphological features of well-known asexual genera, we followed the reference style (e.g. hyalorhinocladiella-like) suggested by Hawksworth (2011). Descriptions of morphological features of *Leptographium* species were based on the style of Jacobs and Wingfield (2001).

Frequency of isolation

Frequencies of isolation of the ophiostomatoid species in all samples were calculated as follows: $F = (NF/NT) \times 100$, where F represents the frequency of isolation (%), NT represents the total number of isolates collected, and NF represents the number of isolates of a particular fungal species.

Results

Bark beetles and mites

Collectively, 17 beetle species belonging to four sub-families were collected from conifer hosts at 10 sites (Table 1). Seven of the species were collected in 2001 and 2002 and ten species in 2010. In total, 106 mites were collected from galleries of six Scolytine bark beetle species during the 2010 survey. One hundred of these mites represented 12 mite species belonging to eight families (Table 1). The remaining six mites (Table 1, Column m) could not be identified. The abundance of each mite species collected varied considerably, with *Insectolaelaps* sp. 1, *Histiostoma* cf. *sapromyzae* and *Dendrolaelaps* sp. 1 being the most abundant overall. The other species were present in very low numbers. Among the most abundant mites, *Insectolaelaps* sp. 1 was found on four beetle species, and *Histiostoma* cf. *sapromyzae* was found on three beetle species, while *Dendrolaelaps* sp. 1 was associated with only one beetle species. *Coccotrypes cyperi* was the most common beetle vector as it was phoretized with 34 mites representing eight species, including three unknown species. Nineteen mites representing five species (including one unknown) came from *Polygraphus szemaoensis*, and *Orthotomicus angulatus* vectored 19 mites representing four species.

Fungal isolation

A total of 340 fungal isolates were obtained, 54 from beetles in 2001 and 2002, and 286 isolated from beetles, galleries and mites in 2010 (Tables 3 and Suppl. material 1: Table S1).

Table 3. Numbers of fungal isolates per species obtained from beetles (B), their galleries (G) or mite (M) associates

Year of survey →		2010																		2001		2002													
Host tree species ¹ →		I																		II		III													
Beetle species ² →	Taxon. Fungus species ³	A		B		D		E		F		H		I		J		L		O		G	C		K		M		N		P		Q		Total
		B	G	M	B	G	M	B	G	M	B	G	M	B	G	M	B	G	M	B	G	M	*	*	*	*	*	*	*	*	*	*	*		
	1. <i>Sporothrix</i> sp. A	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	2. <i>S. nebularis</i>	0	0	4	0	0	7	0	1	0	0	0	0	0	0	0	5	1	3	2	1	0	0	1	0	0	0	0	0	0	1	1	0	45	
	3. <i>Ophiostoma acaronum</i> sp. nov.	0	0	21	0	0	2	0	2	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	
	4. <i>O. ips</i>	0	0	1	0	1	17	6	11	14	0	0	0	0	0	13	0	1	0	2	9	15	5	0	0	0	0	0	0	3	0	4	4	0	106
	5. <i>Ophiostoma</i> sp. B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1		
	6. <i>O. brevipilos</i> sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8		
	7. <i>O. setosum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5		
	8. <i>O. quercus</i>	0	1	0	0	1	7	0	1	0	0	0	0	0	0	0	0	6	1	6	0	0	0	0	0	0	0	0	3	0	2	4	11	43	
	9. <i>O. tsotsi</i>	0	2	0	0	2	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12		
	10. <i>Ophiostoma</i> sp. C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1		
	11. <i>Graphilbum fragrans</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1		
	12. <i>Gn. kesiya</i> sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	2	0	2	5	0	0	0	0	0	0	0	0	0	0	0	0	11	
	13. <i>Gn. puerense</i> sp. nov.	0	0	0	0	0	4	2	2	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	11	
	14. <i>Leptographium gracile</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	
	15. <i>G. radiaticola</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	
	16. <i>L. ningense</i> sp. nov.	0	0	2	0	0	8	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	
	17. <i>G. yunnanensis</i>	0	0	0	0	0	6	0	2	0	0	0	0	0	0	0	3	4	3	17	0	0	0	0	0	0	0	0	0	0	1	1	0	39	
	18. <i>L. conjunctum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2		
	19. <i>Graphium pseudorniticum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Total	0	3	28	0	4	56	10	19	18	2	0	0	0	34	2	0	0	12	21	28	36	1	0	9	0	10	1	6	1	8	12	17	340	

¹ Tree host species: I = *Pinus kesiya*; II = *P. armandii*; III = *P. yunnanensis*; IV = *P. semnaensis*; V = *Tsuga* sp.

² Beetle species: A = *Coccotrypes cyperi*; B = *Cyrtogenius luteus*; C = *Hylurgops major*; D = *Ips acuminatus*; E = *Lasconotus* sp.; F = *Orbhotomicus angulatus*; G = *Pisodes* sp.; H = *Polygraphus aterrimus*; I = *Polygraphus* sp.; J = *P. szemaensis*; K = *P. verrucifrons*; L = *Tomiscus minor*; M = *Tomiscus minor*; N = *T. piniperda*; O = *T. brevipilosus*; P = Unknown sp.1; Q = Unknown sp.2.

* = information about specific niche not available.

Bark beetle niches in this study include the beetles themselves, their galleries or the mites associated with a particular bark beetle or its galleries. The numbers of fungal isolates collected from the different bark beetle niches varied substantially. For example, 85 isolates were collected from the *P. szemaoensis* niche, 60 isolates from that of *C. cyperi*, but only two and four isolates from the niches of *Lasconotus* sp. and *P. aterrimus* respectively.

DNA sequencing and phylogenetic analyses

From the total of 340 isolates obtained in the study, DNA sequences were generated for 134 isolates (Table 2), representing all the morphological groups. Based on preliminary BLAST results, ITS data generated in the present study were separated into two data sets, the first including *Ophiostoma*, *Sporothrix* and *Graphilbum* in the Ophiostomatales, and the second including *Graphium* spp. in the Microascales. Because amplification of the ITS1 and 2 regions is often problematic for *Leptographium* s.l. species (De Beer and Wingfield 2013), a reference data set consisting of ITS2-LSU data was compiled to determine the species complexes in *Leptographium* s.l. to which the isolates from China belonged.

The 340 isolates were separated in 19 taxa (Taxa 1 to 19) based on DNA sequences (Table 2) and culture morphology. Taxa 1 and 2 (Fig. 1) represented species of *Sporothrix*, Taxa 3 to 10 resided in five species complexes in *Ophiostoma* s.l., and Taxa 11 to 13 belonged to *Graphilbum*. Taxa 14 to 18 belonged to four species complexes in *Leptographium* s.l. (Fig. 2). One taxon (Taxon 19) resided in the genus *Graphium* (Suppl. material 2: Figure S1).

Two species of *Sporothrix* were collected (Suppl. material 2: Figure S3). Taxon 1 was represented by a single isolate and both ITS and BT data suggested that this was of an undescribed species. Taxon 2 included 45 isolates, eight for which sequence data were included in our analyses (Suppl. material 2: Figure S3). In the ITS tree, these isolates grouped in a monophyletic clade that included the ex-type isolates of *S. nebularis* and *S. nigrograna*. In the BT tree these isolates again grouped with *S. nebularis*.

Taxon 3 included 29 isolates (Table 3), 12 of which (Table 2) were included in the analyses. These isolates grouped with a number of *Ophiostoma* spp. peripheral to the well-defined complexes in *Ophiostoma* s.str., and they were treated as Group A in *Ophiostoma* s.l. (Fig. 1). In both the ITS and BT trees (Fig. 3), the Yunnan isolates formed a well-supported lineage, closest to but clearly distinct from *O. pallidulum* and *O. saponiodorum*.

Taxon 4 formed part of the *O. ips* complex (Fig. 1). This included 106 isolates (Table 3), nine of which were included in the analyses (Table 2). ITS and BT data (Suppl. material 2: Figure S4) confirmed that these isolates all represented *O. ips*.

Taxon 5, including only one isolate, together with Taxon 6 that included eight isolates (Table 3), six for which sequences were produced, formed part of the *O. clavatulum* complex (Fig. 1). BT and EF data confirmed that both these taxa grouped distinct from all known species in the complex (Fig. 4).

Five isolates comprised Taxon 7 (Tables 2 and 3), which formed part of the *O. piceae* complex (Fig. 1). Both BT and EF analyses confirmed that these isolates grouped

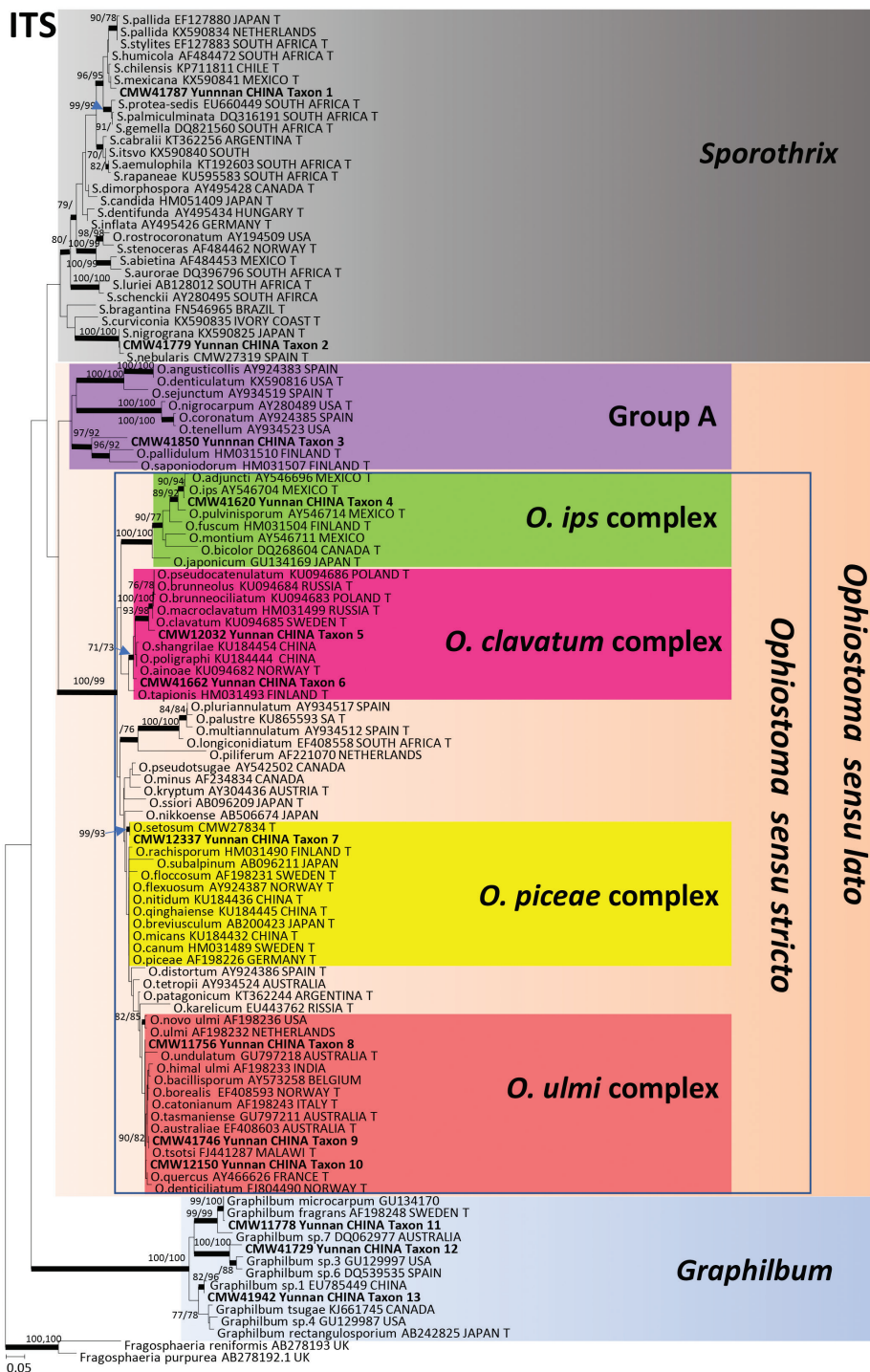


Figure 1. ML tree of the ITS region of *Ophiostoma*, *Sporothrix*, *Graphilbum*. Novel sequences obtained in this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP T = ex-type isolates.

ITS2-LSU

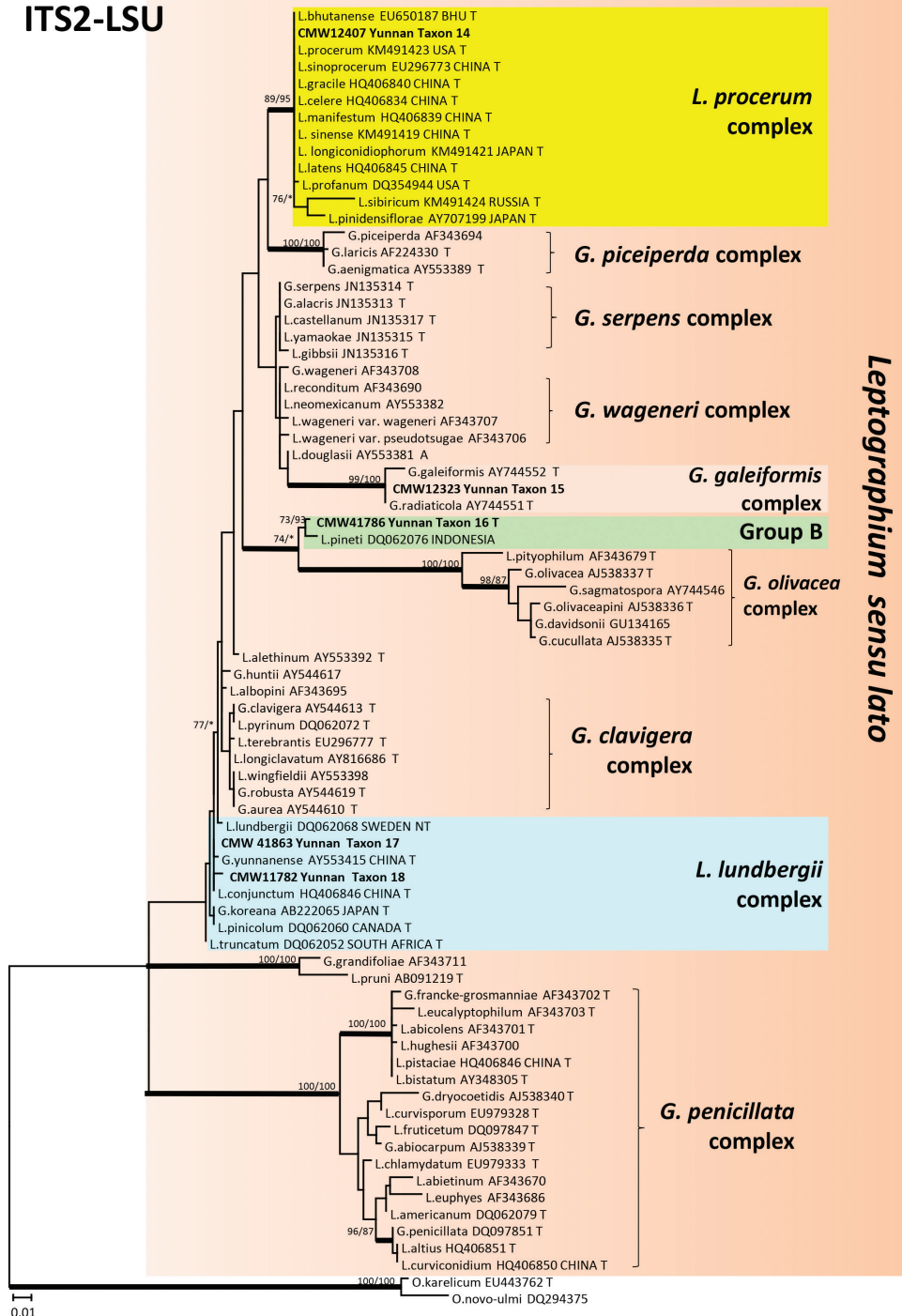


Figure 2. ML tree of the ITS2-LSU region of *Leptographium*. Bold branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

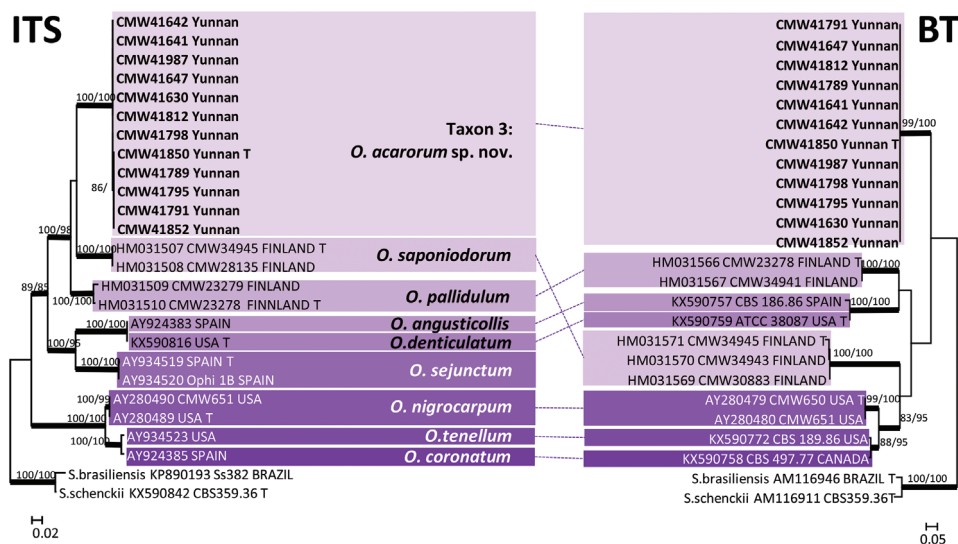


Figure 3. ML trees of Group A generated from DNA sequences of ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

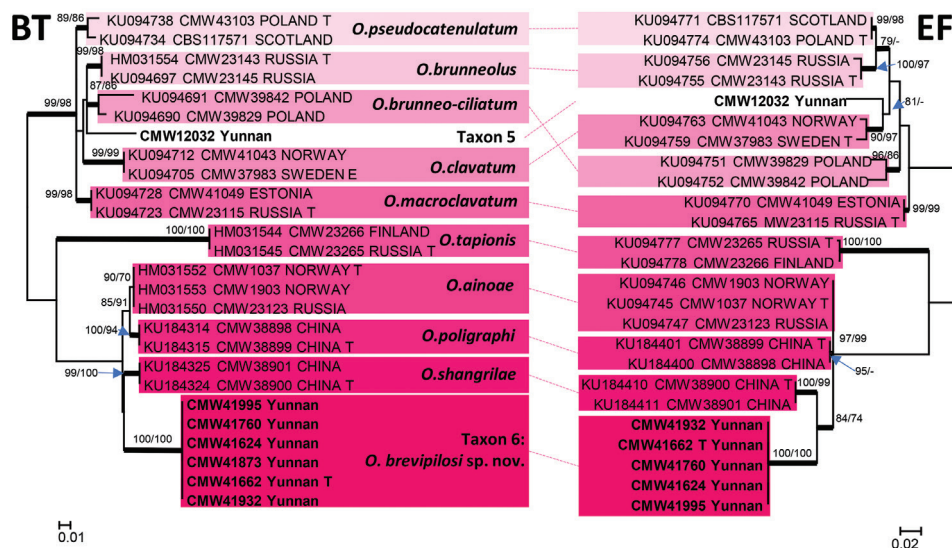


Figure 4. ML trees of the *O. clavatum* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

with *O. setosum* isolates previously identified from Canada, Korea and China (Suppl. material 2: Figure S5).

There were 55 isolates (Table 3) belonging to the *O. ulmi* complex (Fig. 1). The sequences for these isolates were quite variable. Thus more detailed analyses, including all

available related sequences from Genbank generated in previous studies dealing with the variable haplotypes of these taxa (Grobelaar et al. 2009, Kamgan Nkuekam et al. 2010), were required. Taxon 8 represented *O. quercus* and Taxon 9 *O. tsotsi* based on ITS (Suppl. material 2: Figure S6), BT (Suppl. material 2: Figure S7), and EF (Suppl. material 2: Figure S8). Sequences of the 36 putative *O. quercus* isolates were very variable and respectively represented 13, 23 and 28 haplotypes for the ITS, BT and EF gene regions (Suppl. material 1: Table S2). When combined, 51 unique haplotypes were found (Suppl. material 1: Table S2). The 18 isolates representing Taxon 9 grouped with isolates of *O. tsotsi* (Suppl. material 2: Figures S6, S7, S8). The ITS data for *O. tsotsi* was less variable than those of *O. quercus* (Suppl. material 2: Figure S6), including only one haplotype. However, the BT (Suppl. material 2: Figure S7) and EF (Suppl. material 2: Figure S8) of *O. tsotsi* had 14 and 12 unique haplotypes respectively. The combined ITS, BT and EF data, represented 20 unique haplotypes (Suppl. material 1: Table S3). Based on BT (Suppl. material 2: Figure S7) and EF (Suppl. material 2: Figure S8) data, the isolate representing Taxon 10 grouped distinct from all other lineages and could represent an undescribed species.

For *Graphilbum*, results from the analyses of the ITS and BT sequences (Fig. 5) suggested that the Yunnan isolates resided in three taxa. These included one isolate of *Gra. fragrans* (Taxon 11) and two distinct, well-supported lineages (Taxa 12 and 13) each including 11 isolates (Table 3), representing undescribed species.

In *Leptographium s.l.* (Fig. 2), ten isolates (Taxon 14) grouped in the *L. procerum* complex (Table 3). Although both BT and EF sequences (Suppl. material 2: Figure S9) of this taxon differed in 1 bp from those of *L. gracile*, CAL sequences were identical to those of *L. gracile* and we conclude that Taxon 14 represents the latter species. However, species delineation in this complex requires reconsideration because sequences of several species in the complex are very similar.

A single isolate (Taxon 15) grouped in the *Grosmannia galeiformis* complex (Fig. 2, Table 2). Both the BT and EF sequences placed this isolate among those of *G. radiatocola* (Suppl. material 2: Figure S10).

Taxon 16 (Fig. 2) included 13 isolates (Table 3) that grouped closest to *L. pineti*, peripheral to the *G. olivacea* complex. BT, EF and CAL analyses (Fig. 6) showed that Taxon 16 was distinct from *L. pineti* and represented a novel species.

Forty one isolates (Tables 2 and 3) belonged to the *L. lundbergii* complex (Fig. 2). The analyses of BT and EF sequences (Suppl. material 2: Figure S11) showed that these isolates separated in two groups, respectively aligning with *G. yunnanensis* (Taxon 17) and *L. conjunctum* (Taxon 18).

A single isolate represented Taxon 19 (Table 2) in the genus of *Graphium*. Both the ITS and EF sequences of this isolate grouped with *Graphium pseudormiticum* isolates (Suppl. material 2: Figure S1).

Frequencies of isolation

The origin and sources of the 340 isolates representing 19 taxa are presented in Tables 2, 3 and Suppl. material 1: S1. The 54 isolates collected from beetles and their

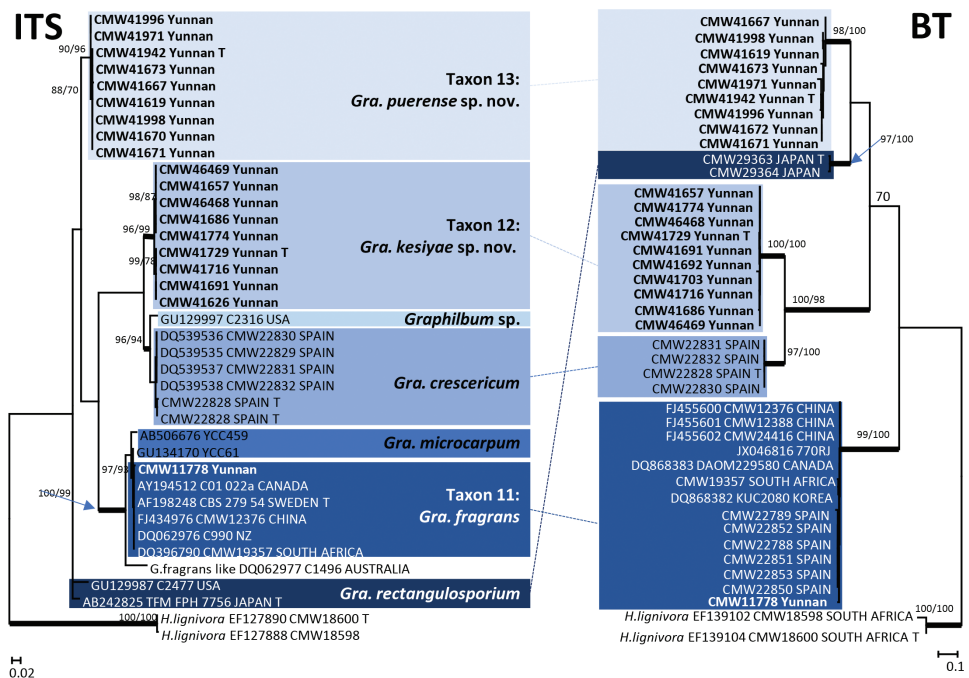


Figure 5. ML trees of the *Graphilbum* generated from DNA sequences of ITS and BT regions. Bold branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

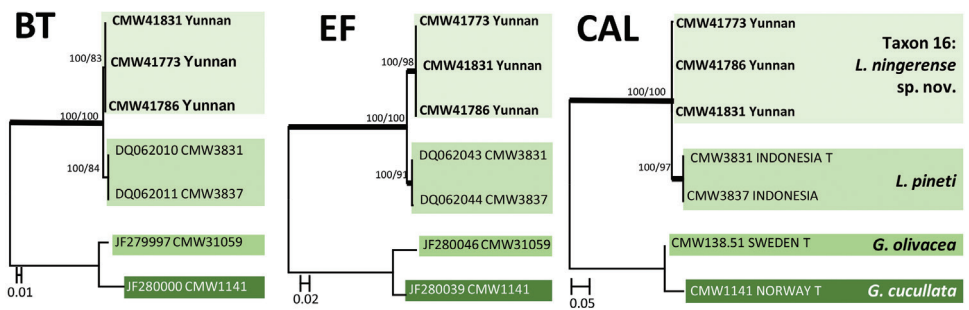


Figure 6. ML trees of the *L. pineti* generated from DNA sequences of BT, EF and CAL regions. Bold branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

galleries in 2001 and 2002 belonged to 11 taxa (Table 3). During the 2010 survey, 286 isolates belonging to 12 taxa were collected (Table 3). The 45 isolates collected directly from six bark beetle species belonged to seven taxa, the 184 isolates from mites to 11 taxa, and the 57 isolates from galleries represented nine taxa. Six taxa were present on beetles, galleries and mites, three taxa on galleries and mites, one taxon only on beetles,

and two taxa only on mites. No taxa were found only in galleries that were not also found on beetles and/or mites. Four taxa were collected from both the 2001–2002 survey and the 2010 survey.

Grosmannia yunnanensis, *O. ips*, *O. quercus*, *S. nebularis* and Taxon 3 were the most frequently isolated species, representing 12.5%, 31.2%, 12.6%, 13.2% and 8.5% of the isolated fungi respectively. The remaining species were isolated only occasionally. *O. ips* was isolated from the niches (beetles, galleries, mites) of ten different bark beetle species. However, most of the *O. ips* were found associated with niches of *I. acuminatus*, *P. szemaoensis* and *C. cyperi* representing approximately 9.7%, 8.5% and 5.3%, respectively. *S. nebularis* was also isolated from the niches of ten beetle species and the highest frequency of isolation was 5.5%, associated with the niche of *O. angulatus*.

The number of fungal species isolated from different beetle or weevil niches varied between different species. There were ten fungal species, representing about 27.9% of total fungal isolates associated with the niche of *P. szemaoensis*. Among them, *O. ips* and *L. yunnanense* were the most frequently isolated, representing about 8.5% and 7.1%, respectively. There were eight fungal species, representing about 15.3%, associated with *Co. cyperi* and of these, the most frequently isolated fungus was *O. ips* with the frequency of 5.3%.

Only 13.2% of the total number of isolates were obtained directly from beetles (Table 3). Of these, 6.2% was obtained from *P. szemaoensis*. Most of the isolates from beetles were identified as *O. ips* (2.6%) and *O. quercus* (1.8%), while 2.9% and 2.6% of isolates were collected from *I. acuminatus* and *T. brevipilosus* respectively. Among these Taxon 6, representing 2.4%, was isolated only from *T. brevipilosus*. The other beetles vectored very low numbers of fungi. *O. ips* was the fungus most frequently isolated from the beetles, representing 4.4%, followed by Taxon 6 representing 2.4 % of the isolates.

Approximately 54% of all isolates were collected from mites (Table 3, Suppl. material 1: Table S1). The fungi associated with mites varied between the different mite species. Ten fungal species were isolated from *Insectolaelaps* sp. 1, representing 21.2% of the total. From some mite species, such as *Lasioseius* sp. 1, *Dendrolaelaps* sp. 2 and the unknown species in the families of Mesostigmata and Oribatei, only one fungal species was found, representing a frequency of isolation of 0.3%, 1.8%, 1.2% and 1.5%, respectively. *O. ips*, *S. nebularis* and *G. yunnanensis* were the three most frequently isolated species associated with mites, representing 15.3%, 10.6% and 7.9%, respectively.

Taxonomy

Eight of the 19 taxa obtained in the present study represented undescribed species. For three of these, only a single isolate was obtained and we have chosen not to formally describe these. The remaining five taxa including two *Ophiostoma*, two *Graphilbum*, and one *Leptographium* species, are described as follows:

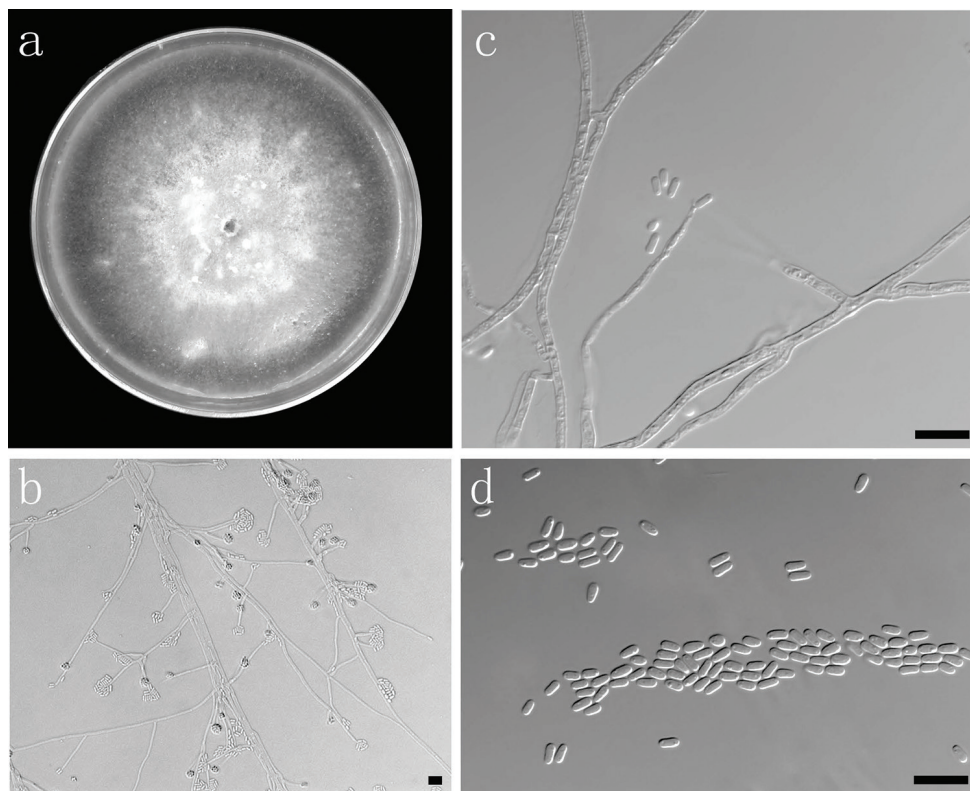


Figure 7. Morphological characters of asexual structures of *Ophiostoma acarorum* sp. nov. **a** fourteen days old culture on OA **b–c** Hyalorhinocladiella-like asexual state **d** conidia. Scale bars: **a–d** = 10 μ m.

Taxon 3

Ophiostoma acarorum R.Chang & Z.W.de Beer, sp. nov.

Mycobank MB 823693

Fig. 7

Etymology. The epithet *acarorum* refers to the subclass Acari in the Arachnida to which all mite species belong from which 25 of the 29 isolates of this species were isolated.

Description. Sexual state not observed. Hyalorhinocladiella-like asexual state: *conidiophores* (7–) 18–76.5 (–140) μ m long; *conidiogenous cells* arising directly from the hyphae, (10.5–) 13.5–24.5 (–31) $\times$ (1–) 1.5–2 (–2) μ m; *conidia* hyaline, smooth, oblong, (3–) 3.5–5 (–6.5) $\times$ (0.7–) 1–1.5 (–2.5) μ m.

Culture characteristics. Colonies hyaline at the beginning, becoming white to dark brown with age. Mycelium superficial on the 3% OA. Colony margin smooth. Colonies on 2% MEA flat, reaching 69 mm diam in 13 d at 30 °C. No growth observed at 5 °C. Optimal temperature for growth 25 °C.

Type material. CHINA, Yunnan Province, Puer City, from *Insectolaelaps* sp. 1 in *Orthotomicus angulatus* gallery on *Pinus kesiya* bark, 17 Sep. 2010, S.J.Taerum,

herbarium specimen of dried culture, PREM 61539 (holotype), CMW41850 = CBS139748 (ex-holotype culture).

Additional specimens examined. CHINA, Yunnan Province, Puer City, from *Histiostoma* cf. *sapromyzae* in *Cyrtogenius luteus* gallery on *Pinus kesiya* bark, 16 Sep. 2010, S.J. Taerum, PREM 61540, CMW41812 = CBS139658; from *Histiostoma* cf. *sapromyzae* in *Cyrtogenius luteus* gallery on *Pinus kesiya* bark, 16 Sep. 2010, S.J. Taerum, CMW41798 = CBS139643.

Host. *Pinus kesiya*.

Beetle vectors. *Ips acuminatus*, *Polygraphus szemaoensis*.

Mite vectors. *Histiostoma* cf. *sapromyzae* (phoretic on *Cyrtogenius luteus*), *Insectolaelaps* sp. 1 (phoretic on *Ips acuminatus* and *Orthotomicus angulatus*).

Distribution. At present known only from Yunnan, China.

Notes. The hyalorhinocladia-like asexual state of *O. acarorum* resembles that of *O. pallidulum* (Linnakoski et al. 2010), one of its two closest relatives based on phylogeny (Fig. 3). *Ophiostoma saponiodorum*, the other close relative has a similar hyalorhinocladia-like state, but can be distinguished based on the presence of a second, synnematus asexual state (Linnakoski et al. 2010).

Taxon 6

Ophiostoma brevopilosi R.Chang & Z.W.de Beer, sp. nov.

Mycobank MB 823694

Fig. 8

Etymology. The epithet *brevopilosi* refers to the bark beetle vector *Tomicus brevipilosus* from which all eight isolates of this taxon were obtained.

Description. Sexual state not observed. Pesotum-like macronematal asexual state predominant. *Synnemata* simple, dark brown at the base, (179.5–) 227–468 (–667) µm long including *conidiogenous apparatus*, (22–) 32.5–58 (–69) µm wide at base; *conidiogenous cells* (13–) 16–26 (–32.5) µm long, *conidia* hyaline, 1-celled, smooth, oblong, (3–) 3–4.5 (–5.5) × (1.5–) 1.5–2.5 (–3) µm. Hyalorhinocladia-like asexual state: *conidiophores* (14.5–) 33–115 (–145) µm long; *conidiogenous cells* arising directly from the hyphae, (12–) 15–38 (–47) × (1.1–) 1.5–2 (–2.5) µm; *conidia* hyaline, smooth, obovoid, (2.5–) 3–5.5 (–8) × (1.5–) 2–2.5 (–3) µm.

Culture characteristics. Colonies hyaline at the beginning, then becoming white to dark. Mycelium superficial on the 3% OA. Colony margin smooth. Colonies on 2% MEA flat, reaching 67 mm diam in 11 d at 25 °C. No growth observed at 5 and above 30 °C. Optimal temperature for growth 20 and 25 °C.

Type material. CHINA, Yunnan Province, Puer City, from *Tomicus brevipilosus* on *Pinus kesiya* bark, 27 Jun. 2010, S.J. Taerum, herbarium specimen of dried culture, PREM 61537 (holotype), CMW41873 = CBS139660 (ex-holotype culture).

Additional specimens examined. CHINA, Yunnan Province, Puer City, from *Tomicus brevipilosus* on *Pinus kesiya* bark, 27 Jun. 2010, S.J. Taerum, PREM 61538,

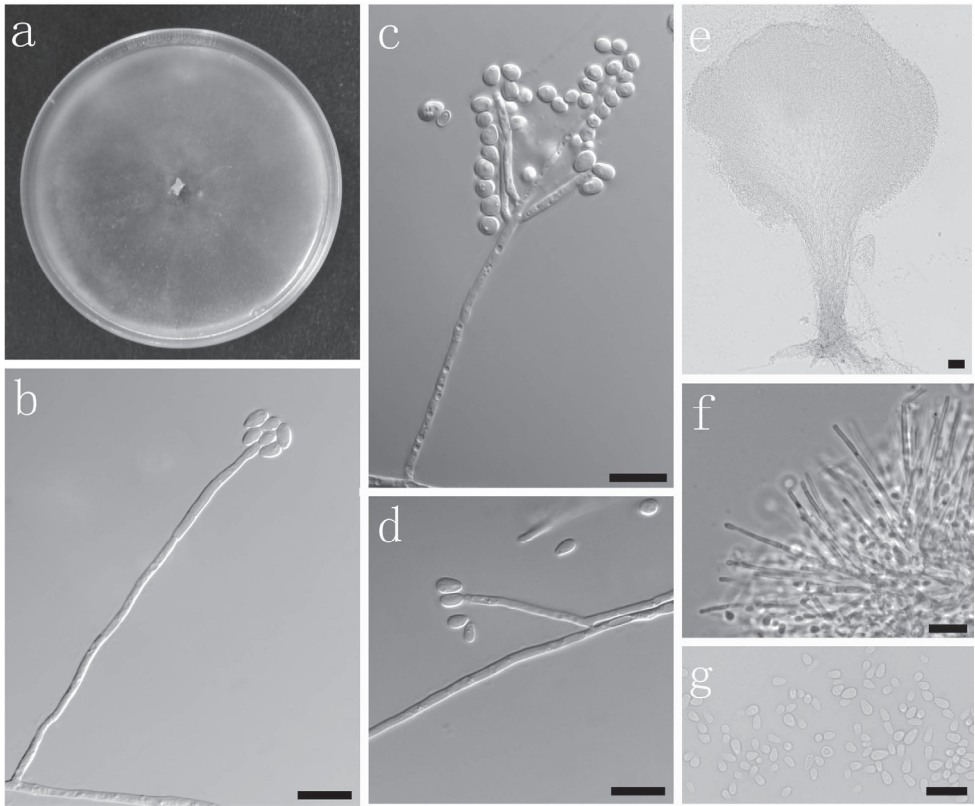


Figure 8. Morphological characters of asexual structures of *Ophiostoma brevopilosi* sp. nov. **a** fourteen days old culture on OA **b–d** Hyalorhinocladia-like asexual state and conidia **e** Pesotum-like macrone-matal asexual state **f** conidiogenous cells of Pesotum-like macrone-matal asexual state **g** conidia. Scale bars: **a–g** = 10 μ m.

CMW41624 = CBS139661; CHINA, Yunnan Province, Puer City, from *Tomicus brevipilosus* on *Pinus kesiya* bark, 27 Jun. 2010, S.J.Taerum, CMW41662 = CBS139659.

Host. *Pinus kesiya*.

Beetle vector. *Tomicus brevipilosus*.

Distribution. At present known only from Yunnan, China.

Notes. The synnematus asexual state of *O. brevopilosi* corresponds with similar structures of *O. brunneo-ciliatum* as described by Linnakoski et al. (2016). The hyalorhinocladia state resembles those of *O. brunneolum*, *O. macroclavatum*, *O. pseudocatenulatum* (Linnakoski et al. 2016) and *O. poligraphi* (Yin et al. 2016). However, the morphology of these structures is not sufficient to distinguish between the species in the complex, and DNA sequences of the BT and EF gene regions are recommended for accurate species identification.

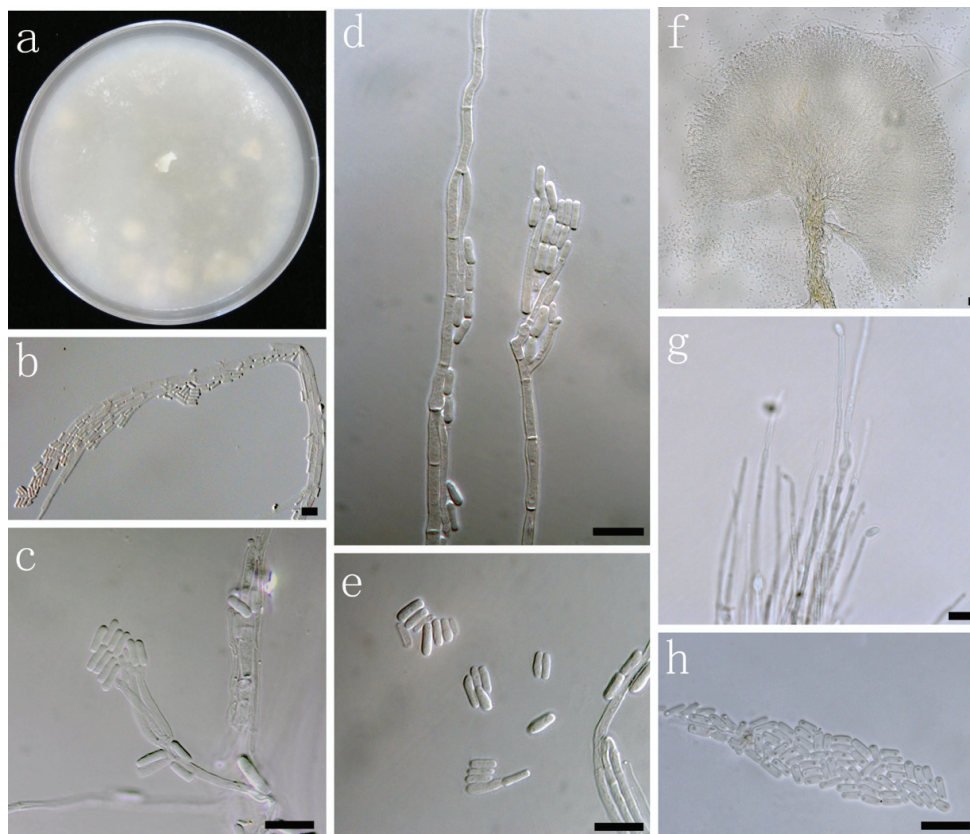


Figure 9. Morphological characters of asexual structures of *Graphilbum kesiyae* sp. nov. **a** fourteen days old culture on OA **b–e** Hyalorhinocladiella-like asexual state and conidia **f** Pesotum-like macronematal asexual state **g** conidiogenous cells of Pesotum-like macronematal asexual state **h** conidia. Scale bars: **a–h** = 10 µm.

Taxon 12

Graphilbum kesiyae R.Chang & Z.W.de Beer, sp. nov.

Mycobank MB 823695

Fig. 9

Etymology. The epithet *kesiyae* refers to the tree host of all beetles and mites from which the 12 isolates of this species were collected.

Description. Sexual state not observed. Pesotum-like macronematal asexual states predominant. *Synnemata* simple, dark brown at the base, (85.5–) 112.5–173 (–203) µm long including *conidiogenous apparatus*, (9–) 14–45.5 (–65.5) µm wide at base; *conidiogenous cells* (8.5–) 10–18.5 (–25.5) µm long; *conidia* hyaline, 1-celled, smooth, oblong, (3.5–) 4–5 (–5.5) × (1.5–) 1.5–2 (–2.5) µm. Hyalorhinocladiella-like asexual

state: *conidiophores* (22–) 38–101.5 (–166) μm long; *conidiogenous cells* arising directly from the hyphae, (10–) 12–27(–40) $\times$ (1.2–) 1.5–2 (–2.5) μm ; *conidia* hyaline, smooth, obovoid, (3.5–) 4–5.5 (–8.5) $\times$ (1–) 1.5–2 (–3) μm .

Culture characteristics. Colonies hyaline. Mycelium superficial on the 3% OA. Colony margin smooth. Colonies on 2% MEA flat, reaching 85 mm diam in 10 d at 25 °C. No growth observed at 5 and 35 °C. Optimal temperature for growth 25 °C.

Type material. CHINA, Yunnan Province, Puer City, from *Polygraphus szemaoensis* gallery on *Pinus kesiya* bark, 12 Aug. 2010, S.J. Taerum, herbarium specimen of dried culture, PREM 61541 (holotype), CMW41729 = CBS139652 (ex-holotype culture).

Additional specimens examined. CHINA, Yunnan Province, Puer City, from *Insectolaelaps* sp. 1 in *Polygraphus szemaoensis* gallery on *Pinus kesiya* bark, 10 Aug. 2010, S.J. Taerum, CMW41691 = CBS 139642; CHINA, Yunnan Province, Puer City, from *Proctolaelaps* nr. *hystrix* in *Polygraphus szemaoensis* gallery on *Pinus kesiya* bark, 11 Aug. 2010, S.J. Taerum, PREM 61542, CMW41716 = CBS139657.

Host. *Pinus kesiya*.

Beetle vectors. *Polygraphus aterrimus*, *Polygraphus szemaoensis*.

Mite vectors. *Proctolaelaps* nr. *hystrix* (phoretic on *Polygraphus szemaoensis*), *Insectolaelaps* sp. 1 (phoretic on *Polygraphus szemaoensis*).

Distribution. At present known only from Yunnan, China.

Notes. *Graphilbum kesiya* and *Gra. puerense* can be distinguished from *Gra. crescericum* by the presence of both synnematus and hyalorhinocladia-like asexual states in culture. *Gra. crescericum* produces only the hyalorhinocladia-like asexual state. The optimal temperature for growth of *Gra. puerense* is 30 °C while that for *Gra. kesiya* is 25 °C, and synnemata of *Gra. puerense* reach double the length of those of *Gra. kesiya*.

Taxon 13

Graphilbum puerense R.Chang & Z.W.de Beer, sp. nov.

MycoBank MB 823696

Fig. 10

Etymology. The epithet *puerense* refers to the city from which this species was collected.

Description. Sexual state not observed. Pesotum-like macronematal asexual states predominant. *Synnemata* simple, dark brown at the base, (187.5–) 206– 357(–437.5) μm long including *conidiogenous apparatus*, (12–) 15.5–45 (–61) μm wide at base; *conidiogenous cells* (15.5–) 18.5–30.5 (–34) μm long, *conidia* hyaline, 1-celled, smooth, oblong, (4–) 4–5 (–5.5) $\times$ (1–) 1.5–2 (–2.5) μm . Hyalorhinocladia-like asexual state: *conidiophores* (17–) 3–140 (–232.5) μm long; *conidiogenous cells* arising directly from the hyphae, (6.5–) 10–25.5 (–42.5) $\times$ (1–) 1–2 (–3) μm ; *conidia* hyaline, smooth, obovoid to oblong, (3.5–) 4–8 (–12) $\times$ (1–) 1.5–2.5 (–3) μm .

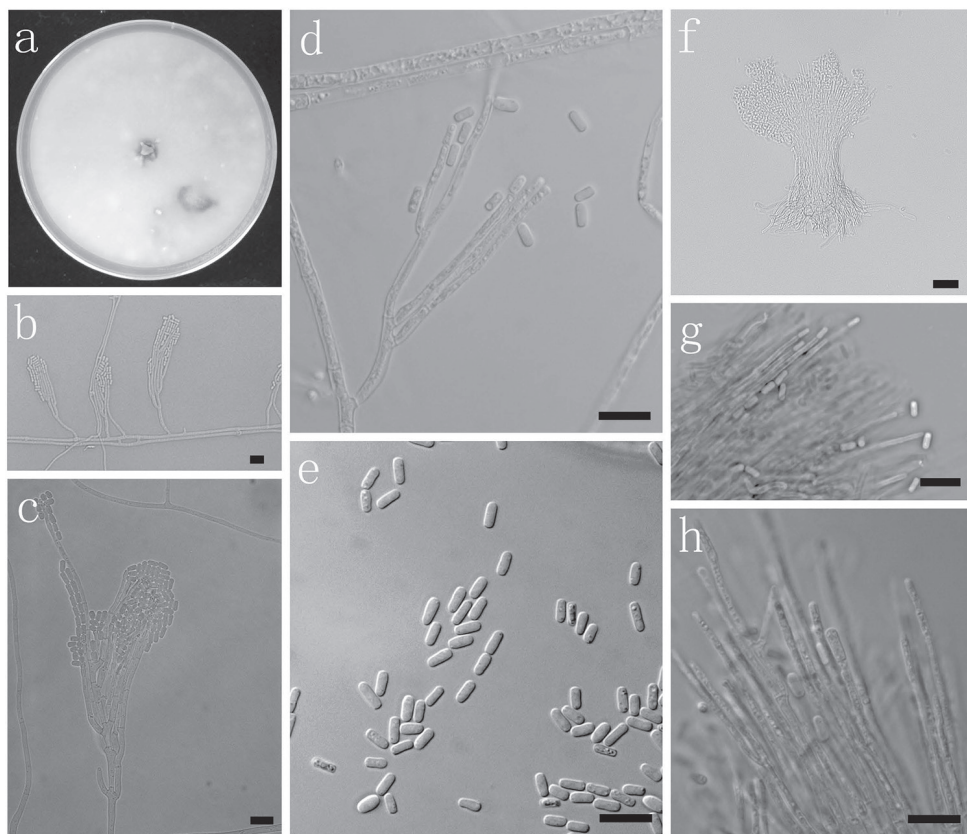


Figure 10. Morphological characters of asexual structures of *Graphilbum puerense* sp. nov. **a** fourteen days old culture on OA **b–d** Hyalorhinocladiella-like asexual state **e** conidia **f** Pesotum-like macronematal asexual state **g–h** conidiogenous cells of Pesotum-like macronematal asexual state and conidia. Scale bars: **a–h** = 10 μ m.

Culture characteristics. Colonies hyaline. Mycelium superficial on the 3% OA. Colony margin smooth. Colonies on 2% MEA flat, reaching 76 mm diam in 5 d at 30 °C. No growth observed at 5 °C. Optimal temperature for growth 30 °C.

Type material. CHINA, Yunnan Province, Puer City, from *Polygraphus szemaoensis* gallery on *Pinus kesiya* bark, 29 Jun. 2010, S.J. Taerum, herbarium specimen of dried culture, PREM 61543 (holotype), CMW41673 = CBS139640 (ex-holotype culture).

Additional specimens examined. CHINA, Yunnan Province, Puer City, from *Ips acuminatus* gallery on *Pinus kesiya* bark, 4 Jul. 2010, S.J. Taerum, PREM 61544, CMW41667 = CBS139651; CHINA, Yunnan Province, Puer City, from *Ips acuminatus* gallery on *Pinus kesiya* bark, Jul 2010, S.J. Taerum, CMW41942 = CBS139650.

Host. *Pinus kesiya*.

Beetle vectors. *Ips acuminatus*, *Polygraphus szemaoensis*.

Mite vectors. *Proctolaelaps nr. hystrix* (phoretic on *Ips acuminatus*), *Insectolaelaps* sp. 1 (phoretic on *Ips acuminatus*), and *Uropodoidea* sp. 2 (phoretic on *Polygraphus szemaoensis*).

Distribution. At present known only from Yunnan, China.

Notes. See comparison between *Gra. kesiyae* and *Gra. puerense* above under notes of *Gra. kesiyae*.

Taxon 16

Leptographium ningerense R.Chang & Z.W.de Beer, sp. nov.

MycoBank MB 823697

Fig. 11

Etymology. The epithet *ningerense* refers to the Ning'er county where all isolates of this taxon were collected.

Description. Sexual state not observed. Asexual state, *conidiophores* occurring singly or in groups of up to 3, macronematous, mononematous, erect, arising directly from the mycelium, (93.5–) 141.5–195.5 (–210.5) μm long. Rhizoids present. *Stipes* dark olivaceous, 4–6 septa, not constricted at septa, (66–) 119.5–142 (–159) μm long. *Apical* cells not swollen at apex, (3–) 5–6.5 (–7) μm wide. *Basal cells* occasionally swollen at apex, (5.5–) 7–10 (–11.5) μm wide. *Conidiogenous apparatus* (28–) 35–58 (–70) μm long, excluding the conidial mass, with multiple series of cylindrical branches. *Primary branches* olivaceous, smooth, cylindrical, not swollen at apex, aseptate, arrangement of primary branches was Type B—two or more branches, (12.5–) 14.5–18 (–19.5) $\times$ (3.5–) 4–5.5 (–6.5) μm . *Secondary branches* light olivaceous, frequently swollen at apex, aseptate, (6.5–) 9–13 (–15) $\times$ (3.5–) 4–5 (–5.5) μm . *Tertiary branches* light olivaceous, aseptate, (7–) 8–10 (–12) $\times$ (3–) 3.5–4.5 (–5) μm . *Conidiogenous cells* discrete, hyaline, 2–3 per branch, aseptate, cylindrical, tapering slightly at the apex, (10.5–) 12–17.5 (–20.5) $\times$ (2–) 2–2.5 (–3) μm . *Conidia* hyaline, aseptate, elliptical, (3–) 3.5–5.5 (–6.5) $\times$ (1.5–) 2–3 (–4) μm .

Culture characteristics. Colonies on 3% OA flat, hyaline at the beginning, then becoming light olivaceous to dark olivaceous. Colonies hyaline at the beginning, then becoming dark olivaceous. Mycelium superficial on the 3% OA with olivaceous aerial mycelium. Colony margin smooth. Conidiophores forms abundantly in clusters on OA. Colonies on 2% MEA flat, reaching 76 mm diam in 10 d at 25 °C. No growth observed at 5 and 35 °C. Optimal temperature for growth 25 °C, reaching 30.5 mm in diam. in 7 days.

Type material. CHINA, Yunnan Province, Puer City, from *Schwiebea (Jacotietta) taiwanensis* hyperphoretic on *Coccotrypes cyperi* on *Pinus kesiya* bark, 16 Sep. 2010, S.J.Taerum, herbarium specimen of dried culture, PREM 61545 (holotype), CMW41786 = CBS139663 (ex-holotype culture).

Additional specimens examined. CHINA, Yunnan province, Puer City, from *Insectolaelaps* sp. 1 in *Orthotomicus angulatus* on *Pinus kesiya* bark, 17 Sep. 2010, S.J.Taerum, PREM 61546, CMW41831 = CBS139664.

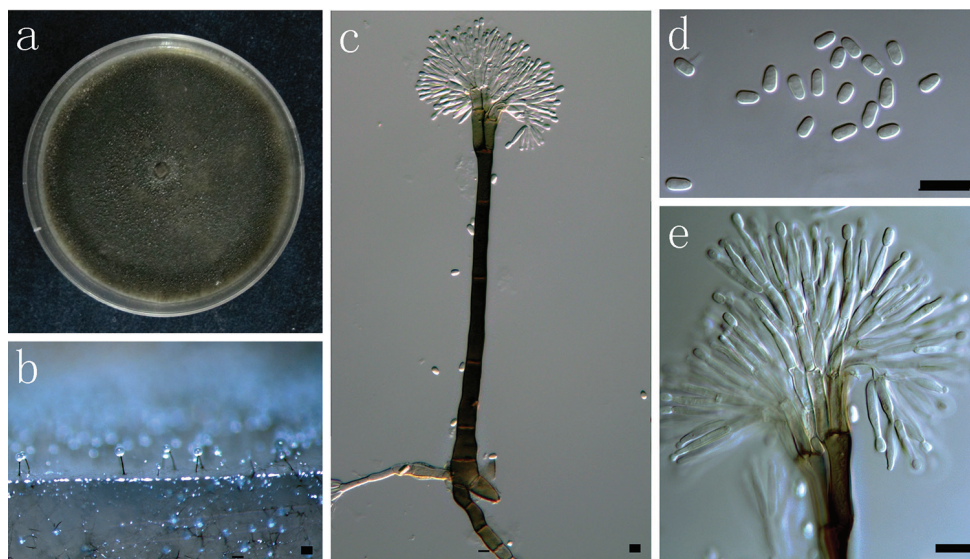


Figure 11. Morphological characters of asexual structures of *Leptographium ningerense* sp. nov. **a** fourteen days old culture on OA **b** mononematous asexual morph on wood tissue on WA **c** conidiophore **d** conidia **e** conidiogenous apparatus. Scale bars: **a–e** = 10 µm.

Host. *Pinus kesiya*.

Beetle vectors. *Polygraphus szemaoensis*.

Mite vectors. *Dendrolaelaps* sp. 1 (phoretic on *Coccotrypes cyperi*), *Dendrolaelaps* sp. 2 (phoretic on *Coccotrypes cyperi* and *Cyrtogenius luteus*), *Schwiebea* (*Jacotietta*) *taiwanensis* (phoretic on *Coccotrypes cyperi*), *Insectolaelaps* sp. 1 (*Orthotomicus angulatus*).

Distribution. At present known only from Yunnan, China.

Notes. *L. ningerense* is morphologically similar to *L. pineti*, but grows much more rapidly, reaching 30 mm in 7 d on 2% MEA at 25 °C while *L. pineti* reaches a diameter of only 15 mm in 6 d. However, the two species are best distinguished with BT, EF and CAL sequences.

Discussion

This study resulted in a total of 340 fungal isolates of ophiostomatoid fungi obtained from the beetles and weevils, their galleries and phoretic mites in a province of China where these fungi are poorly known. The fungi resided in the two phylogenetically unrelated Microascales and Ophiostomatales and included a species of *Graphium* (Microascales) as well as species of *Sporothrix*, *Graphilbum*, *Leptographium* and *Ophiostoma* (Ophiostomatales). Analysis showed that these isolates belonged to 19 distinct taxa, eight of which represented undescribed species of which five were provided with names. Of the remaining 11 species, 10 had previously been reported from China, with only *S. nebularis* representing a new report for this country. This study also in-

cludes the first records of fungi associated with the Scolytine beetle species *Coccotrypes cyperi*, *Cyrtogenius luteus*, and *Tomicus brevilopus* and it is the first time that fungal associates are reported from beetle-associated mites in China.

Surveys in this study aimed to explore fungal diversity in conifer-infesting beetle ecosystems in Yunnan. This is in contrast to determining the specificity of these interactions where a much more focused and systematic sampling would have been required. The exact nature of the interactions between the beetles and mites, mites and fungi, beetles and fungi, and all of these with their host trees, thus remains largely unknown. The discussion below therefore focuses on the fungi collected in these surveys, with limited notes on the hosts, beetles and mites and some general observations.

The two *Sporothrix* species collected in this study were primarily from mites. *Sporothrix nebularis* was the second most abundant species and 36 of the 45 isolates came from five different mite species phoretic on five different beetle species. The fungus was first described from *Hylastes attenuatus* infesting *Pinus radiata* in Spain (Romón et al. 2014b) and was later also found on *Hylastes ater* and *Hylurgops palliatus* from the same host and country (Romón et al. 2014a). Our results suggest that *S. nebularis* has a much wider Eurasian distribution and host range than previously realized. It is also possible that in Europe, many of the previous reports of *S. stenoceras* from nine bark beetle species (Kirisits 2004), actually represent *S. nebularis*. This is particularly because the two taxa are morphologically almost indistinguishable, and *S. nebularis* might have been misidentified as *S. stenoceras* where only morphology was considered (Romón et al. 2014b). The single isolate of *Sporothrix* sp. A was from a mite on *Cyrtogenius luteus*. Overall, the results support the suggestion by De Beer et al. (2016a) that the majority of *Sporothrix* species are mite associates.

Ophiostoma acarorum grouped peripheral to the well-known species complexes in *Ophiostoma s.str.*, and closest to, but distinct from *O. pallidulum* and *O. saponiodorum*. *O. pallidulum* is known from ten different bark beetle and one weevil species infesting *Pinus sylvestris* in Finland (Linnakoski et al. 2010), Poland (Jankowiak and Bilański 2013a, b, c), and the Ukraine (Davydenko et al. 2017). Interestingly, *O. pallidulum* was also isolated from dead *P. sylvestris* roots in Poland in the absence of beetle attack (Jankowiak et al. 2012). *O. saponiodorum* is known from two spruce-infesting beetles in Finland and Russia (Linnakoski et al. 2010), pine-infesting *Hylastes* and *Pissodes* spp. in Poland and Spain (Jankowiak and Bilański 2013a, c, Romón et al. 2014a), and *Orthotomicus erosus* caught in traps in Italy (Malacrinò et al. 2017). Similar to *O. pallidulum* and *O. saponiodorum*, *O. acarorum* does not appear to be beetle-specific as it was isolated from three mite species phoretic on four beetle species, and from the galleries of two beetle species. However, the fact that 25 of 29 isolates in the present study were from mites, suggests that *O. acarorum* is a symbiont of mites on conifer-infesting beetles, rather than the beetles themselves. The wide range of beetle species from which *O. pallidulum* and *O. saponiodorum* have been isolated in Europe, always in relatively low abundance, also suggests that these two species are mite rather than beetle symbionts.

Ophiostoma ips was the species most frequently isolated in the study, constituting 31% of the 340 isolates. It was also the fungus found in association with the largest

number of beetle species i.e. 10 of 17, and with three of the five host trees in central and southern Yunnan. This is consistent with current knowledge of the fungus that is known to have a global distribution (Zhou et al. 2007) and is associated with a wide range of conifer-infesting bark beetles in various genera (Kirisits 2004, Rumbold 1931, Upadhyay 1981). Shortly after the description of *O. ips* (Rumbold 1931), Leach et al. (1934) had already shown that ascospores of the fungus were vectored by mites associated with two *Ips* spp. Furthermore, Stone and Simpson (1990) observed that mycetophagous mites associated with *Ips grandicollis* fed directly on *O. ips* in the galleries of the beetle. About half of the 106 *O. ips* isolates from Yunnan came from mites associated with six of the bark beetle species. The results thus provide additional weight to the hypothesis that *O. ips* is primarily a mite associate, rather than a bark beetle associate.

Two taxa from Yunnan formed part of the *O. clavatum* complex as recently defined by Linnakoski et al. (2016). These authors showed that most of the species in the complex appear to be specific to one or two closely related *Ips* spp. on either a pine or spruce host from Europe. The only exception is *O. macroclavatum*, that is known from on three *Ips* spp. and *Pityogenes chalcographus* on both pine and spruce. Yin et al. (2016) subsequently described two additional species in the complex from *Polygraphus poligraphus* and *Ips shangrila* respectively, on spruce in Qinghai Province. Both fungi were also isolated from *Dendroctonus micans* in the same area. One of the species that we collected, *O. brevipilosi*, was from *Tomicus brevipilosus* beetles infesting *P. kesiya*. It is thus the first species in the complex associated with a *Tomicus* species. The other species from Yunnan, *Ophiostoma* sp. B (Taxon 5) was represented by only one isolate from the gallery of an unknown beetle on *P. semanensis*.

Only a single species of the *O. piceae* complex, *O. setosum*, was obtained during the surveys. Five isolates of this species were from *Pissodes* galleries on *Tsuga dumosa*, confirming a previous report from the same host in China (Paciura et al. 2010b). The fungus was originally described from *Tsuga heterophylla* in Canada (Uzunovic et al. 2000). It was also reported from *Tsuga* and *Pseudotsuga* in the USA (Harrington et al. 2001), from *Hylastes ater* on pine in New Zealand (Reay et al. 2005) and pine logs imported from New Zealand to Korea (Kim et al. 2005a). In Bhutan, this species is associated with *Ips schmutzenhoferi* on *Picea spinulosa* and *Pinus wallichiana* (Kirisits et al. 2012). It thus seems as if *O. setosum* is a generalist associate of beetles infesting conifers in native Northern Hemisphere forests, and was most likely introduced into New Zealand.

The third most abundant species collected in this study was *O. quercus* that forms part of the *O. ulmi* species complex (De Beer and Wingfield 2013). This fungus is generally considered as hardwood-infecting with many reports from wounds and stained wood of well-known tree genera such as *Quercus*, *Betula*, *Populus*, *Eucalyptus*, *Acacia*, and *Nothofagus* from North and South America, Europe, Africa, Australasia, and Central and East Asia (De Beer et al. 2003, De Errasti et al. 2016, Grobbelaar et al. 2009, Harrington et al. 2001, Paciura et al. 2010b). Little is known regarding the insect vectors of *O. quercus* apart from reports that the fungus is vectored by nitidulid beetles on

oak (Juzwik et al. 1998) and eucalypts (Kamgan Nkuekam et al. 2012). Unlike most other species in the *O. ulmi* complex, there is a growing body of evidence suggesting that *O. quercus* is also a successful colonist of conifer wood. It has been reported from stained pine wood in the USA, Sweden, Australia, New Zealand and South Africa (De Beer et al. 2003, Harrington et al. 2001), but also in association with pine bark beetles such as *Tomicus yunnanensis* in China (Paciura et al. 2010b), *Hylastes plumbeus* in Japan (Masuya et al. 2009), as well as *Ips sexdentatus* and *Hylobius abietus* in Poland (Jankowiak 2012, Jankowiak and Bilański 2013b). Furthermore, it has been found with *Pissodes* weevils on *Tsuga* in China (Paciura et al. 2010b) and pine in Poland (Jankowiak and Bilański 2013c).

The 43 *O. quercus* isolates obtained in this study came from four of the host trees distributed in eight of the ten study sites in both central and southern Yunnan. The *O. quercus* isolates were found in association with eight beetle species, with 13 of the isolates coming from three mite species. To the best of our knowledge, this represents the first records of this fungus from mites. However, spores of the closely related and morphologically similar *O. novo-ulmi* have been observed in the guts and sporothecae of mites phoretic on *Scolytus* beetle vectors of the Dutch Elm Disease fungi (Brasier 1978, Moser et al. 2010). The difference between the latter group of fungi and *O. quercus* is that they are specific to elm trees and elm-infesting beetles. In contrast, *O. quercus* is probably of all ophiostomatoid species the one with the widest array of hosts and the plasticity to utilize as a vector, any arthropod passing a wound or gallery in which it is growing. It is thus not surprising that *O. quercus* is also one of the most genetically diverse ophiostomatoid species (Grobbelaar et al. 2009, Kamgan Nkuekam et al. 2010), which is supported by the large variety of haplotypes revealed in analyses of the BT and EF gene regions in this study.

Ophiostoma tsotsi was the second species in the *O. ulmi* complex obtained in this study. This species, which is morphologically indistinguishable from *O. quercus*, is known from wounds on planted *Eucalyptus* and *Acacia mearnsii* trees in Africa (Grobbelaar et al. 2010), Australia (Kamgan Nkuekam et al. 2011) and China (Grobbelaar et al. 2011), as well as native mangroves in South Africa (Osorio et al. 2016). The only insects from which the fungus has previously been reported are nitidulid beetles (Kamgan Nkuekam et al. 2012). We isolated *O. tsotsi* from galleries of two pine bark beetles, as well as from two mite species associated with one of these beetles. This was an unexpected result because, much like *O. quercus*, the assumption has been that *O. tsotsi* is an exclusively hardwood-infesting member of the *O. ulmi* complex. This complex was initially referred to as the ‘hardwood lineage’ of the *O. piceae* complex (De Beer et al. 2003, Harrington et al. 2001) before De Beer and Wingfield (2013) distinguished between the *O. piceae* and *O. ulmi* complexes. Although *O. tsotsi* is known from fewer hosts and locations than *O. quercus*, it also displays a relatively high level of genetic diversity as was evident from our BT and EF sequence analyses.

A single isolate referred to as *Ophiostoma* sp. C (Taxon 10) came from an unknown beetle gallery on *Tsuga*. This isolate was clearly distinct from all other species in the *O. ulmi* complex. Although we have chosen not to describe it as new, together with *O.*

quercus and *O. tsotsi*, this is only the third of the 18 species currently recognized in the complex to be reported from conifer hosts.

Three of the taxa collected in the surveys resided in the genus *Graphilbum* that includes 10 known species and several undescribed taxa (De Beer and Wingfield 2013, Lu et al. 2009a, Reid and Hausner 2015, Romón et al. 2014b). All the known species are from conifers (*Abies*, *Picea*, *Pinus*, *Tsuga*) and mostly from bark beetles or their galleries, and in some cases stained wood. Only two undescribed species are from hardwoods (Geldenhuis et al. 2004, Kamgan Nkuekam et al. 2008). *Graphilbum fragrans* is by far the most common species with one report from bark beetles on spruce and fir in Canada (Jacobs et al. 2003). The remainder of the species have been collected from a variety of pine bark beetles and weevils from Europe (Mathiesen-Käärik 1953, Romón et al. 2007), the USA, New Zealand and Australia (Harrington et al. 2001), South Africa (Zhou et al. 2006), Japan (Masuya et al. 2009) and Korea (Kim et al. 2007).

Graphilbum fragrans found in this study had previously been reported from *T. yunnanensis* on *Pinus yunnanensis*, and *Pissodes* spp. on *Tsuga dumosa* and *P. armandii* in China (Paciura et al. 2010b, Zhou et al. 2013). In the present study, the fungus was isolated from galleries of *Tomicus minor* on *P. kesiya*. We collected 11 isolates each for *Gra. kesiya* (Taxon 12) and *Gra. puerense* (Taxon 13). *Gra. kesiya* was found in association with three beetle species with seven isolates from mites. *Gra. puerense* was from two beetle species with three isolates from mites. These are the first reports of any *Graphilbum* species isolated from mites.

Five species of fungi residing in *Leptographium sensu lato* were collected in this study, including one novel species. Ten isolates representing Taxon 14 were from *Pissodes* sp. on *P. armandii* and were shown to represent *L. gracile*. This species was described from the same insects and tree host as the isolates in the present study, as well as from *Ips typographus* on *Picea koraiensis* (Paciura et al. 2010a). Four other species grouped very closely to *L. gracile* in the *L. procerum* complex. *Leptographium sinoprocerum* and *L. sinense* were both described from China where they were associated with the introduced bark beetle *Dendroctonus valens* that infests and kills native *P. tabuliformis* (Lu et al. 2008), and native *Hylobitelus xiaoi* on non-native *P. elliotii* (Yin et al. 2015). *Leptographium longiconidiophorum* was originally isolated from *P. densiflora* in Japan (Yin et al. 2015) and *L. bhutanense* from *Hylobitelus chenкупдоржii* on *Pinus wallichiana* in Bhutan (Zhou et al. 2008).

A single isolate from the gallery of an unknown bark beetle on *Tsuga* grouped with *Grossmannia radiaticola*. This species was originally described from stained *Pinus radiata* wood imported from New Zealand to Korea (Kim et al. 2005b). However, it has been reported from a variety of pine root-infesting bark beetles from South Africa, Chile, Sweden (Linnakoski et al. 2012), California (Kim et al. 2011) and Poland (Jankowiak and Bilański 2013a, b). In China, it has been reported from a gallery of the invasive *D. valens* on *P. tabuliformis* in Shanxi, which might have resulted in the impression that the fungus was introduced with the beetle into China (Lu et al. 2009b). However, the present report from another conifer host in a distant province suggests that the fungus could be native across Eurasia.

Of the 13 isolates of *L. ningerense* collected in this study one isolate was from the gallery of *Polygraphus szemaoensis* on *Pinus kesiya*, while the remaining 12 isolates were from four mite species associated with *Coccotrypes cyperi*, *Cyrtogenius luteus* and *Orthotomicus angulatus*. This species grouped closest to *L. pineti*, which was described from the gallery of an *Ips* sp. under the bark of *Pinus merkusii* growing on the island of Sumatra, Indonesia (Jacobs et al. 2000). The fact that no isolates were obtained from beetles might indicate that this species is a preferential symbiont of mites.

The fourth most prevalent species collected in this study was *G. yunnanensis* including 39 isolates, 27 of which were vectored by seven mite species. *G. yunnanensis* was originally described from *Tomicus yunnanensis* on *P. yunnanensis* in China (Zhou et al. 2000), and was subsequently reported from *Polygraphus major* on *Pinus kesiya* in Thailand (Yamaoka et al. 2007), and four other pine bark beetle species on *P. densiflora* in Japan (Masuya et al. 2009). Results of the present study have added an additional three beetle species to this list including *Cryphales luteus*, *Polygraphus szemaoensis* and an unidentified *Polygraphus* sp. This seemingly promiscuous relationship of *G. yunnanensis* with nine different beetle species in East Asia, together with the fact that more than two thirds of the isolates in our study were from mites, suggests an association of this fungus with mites, rather than beetles.

Two isolates of *L. conjunctum*, including one from a mite, were collected in the present study. *L. conjunctum* has not previously been reported from any location other than in the case of the original study in which it was described from *Hylurgops major* on *Pinus yunnanensis* in China (Paciura et al. 2010a). Our one isolate was from *H. major*, while the mite from which the second isolate came, was collected from the unknown *Polygraphus* sp.

The single *Graphium* isolate arising from this study was from a mite on *Ips acuminatus*, and had identical sequences to *Gr. pseudodormiticum*. This fungus was first described from South Africa in association with *Orthotomicus erosus* on pine bait logs (Mouton et al. 1994). It was subsequently found in Sweden associated with *Ips typographus* on an unknown host tree, in Austria with *Tomicus minor* on *Pinus sylvestris* (Lackner and de Hoog 2011), and in China associated with *Pissodes* sp. on *Tsuga dumosa* (Paciura et al. 2010b). This is the first report of a *Graphium* species associated with a mite. The low frequency with which it was encountered could result from the fact that *Graphium* species are unable to grow on media including the antibiotic cycloheximide, which is commonly used as in a selective medium for the Ophiostomatales in surveys of bark beetle fungi.

Conclusions

Three surveys of pine-infesting bark beetles in Yunnan revealed several new fungal species and new beetle-fungus associations. This supports the view that the diversity of fungi associated with bark beetles in China is high and that it deserves further exploration. The results of the 2010 survey that included isolations from mites, revealed

that many of the ophiostomatoid species often considered as beetle associates can also be isolated frequently from mites. It has been suggested that mycetophagous mites are often generalists with the ability to feed on and vector several fungal species (Hofstetter et al. 2013). It is also known that phoretic mites are promiscuous in terms of their beetle vectors, and that they will infest virtually any beetle species in order to reach their tree hosts (Hofstetter et al. 2013). Our results underscore these views although structured surveys supported by statistical analyses should be undertaken to better understand these relationships.

One of the most surprising and intriguing outcomes of the study was that fungal species such as *O. quercus* and *O. tsotsi*, which were considered to be primarily hardwood-infecting species, are prevalent on several of the pine-specific beetles and their associated mites. Bark beetles commonly have broad host ranges on either the hardwoods or conifers, but not across these groups. The question thus arises how these hardwood-infecting fungi can move between these host groups. One possibility is that they simply move with mites from one tree in a forest to another, crossing the forest floor and carrying the fungi with them. However, it is also known that most bark beetle-associated mites are not monospecific and may be common on other non-bark beetle hosts as well (e.g. Tenebrionidae, Cleridae, Histeridae, Elateridae) (Hofstetter et al. 2013). Some of these often predatory beetles, could thus vector the mites and their fungi across the otherwise hardwood-conifer barrier. The determining factor would then be whether the fungus has the ability to survive in the new environment. The results of this study suggest that *O. quercus* and *O. tsotsi* are the rare exceptions among the ophiostomatoid fungi that are equally fit in both the angiosperm and gymnosperm environments.

None of the known fungal species collected in the present study are considered pathogens based on current published knowledge, and we have not found any evidence that any of the novel taxa are pathogens. However, the study underscores the possibility that many ophiostomatoid fungi that have been considered beetle associates in the past, might actually have a closer association with mites, or that mites at least have the ability to vector them. Generally, bark beetles and their fungal associates are considered serious threats with invasive potential when they are introduced into new environments through the movement of wood and wood products (Wingfield et al. 2017). However, most pest risk analyses consider only the beetles and the fungi. Our results suggest that even in cases where beetles do not successfully establish and reproduce in a new environment, mites could vector potential fungal pathogens and introduce them into established beetle ecosystems. The role of mites in mixing fungal assemblages of different beetle species on a single tree or log, especially in the case of invasive versus native beetle species, deserves further exploration.

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Supplementary material I

Table S1–S3

Authors: Runlei Chang, Tuan A. Duong, Stephen J. Taerum, Michael J. Wingfield, Xudong Zhou, Z. Wilhelm de Beer

Data type: species data

Explanation note:

Table S1. Numbers of ophiostomatoid fungal isolates obtained from different mite species in this study.

Table S2. Haplotypes of *Ophiostoma quercus*.

Table S3. Haplotypes of *Ophiostoma tsotsi*.

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Link: <https://doi.org/10.3897/mycokeys.28.21758.suppl1>

Supplementary material I

Figure S1–S11

Authors: Runlei Chang, Tuan A. Duong, Stephen J. Taerum, Michael J. Wingfield, Xudong Zhou, Z. Wilhelm de Beer

Data type: occurrence

Explanation note:

Figure S1. Map of Yunnan Province in China showing the sites and tree hosts from which samples were collected during the three surveys in 2001, 2002 and 2010.

Figure S2. ML trees of the genus *Graphium* generated from DNA sequences of the ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S3. ML trees of the genus *Sporothrix* generated from DNA sequences of ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S4. ML trees of the *O. ips* complex generated from DNA sequences of ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S5. ML trees of the *O. piceae* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S6. ML tree of the *O. quercus* complex generated from DNA sequences of ITS region. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S7. ML tree of the *O. quercus* complex generated from DNA sequences of BT region. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S8. ML tree of the *O. quercus* complex generated from DNA sequences of EF region. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S9. ML trees of the *L. procerum* complex generated from DNA sequences of BT, EF and CAL regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S10. ML trees of the *G. galeiformis* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S11. ML trees of the *L. lundbergii* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

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Table S1 Numbers of ophiostomatoid fungal isolates obtained from different mite species in this study

Host tree species →		<i>Pinus kesiya</i>													Total
Taxon	Fungal species↓	Mite species ¹ →	a	b	c	d	e	f	g	h	i	j	k	m	
1.	<i>Sporothrix</i> sp. A		0	0	0	0	0	0	0	1	0	0	0	0	1
2.	<i>S. nebularis</i>		6	0	8	17	0	0	1	0	4	0	0	0	36
3.	<i>Ophiostoma acarorum</i> sp. nov.		0	0	21	2	0	0	0	0	0	0	0	2	25
4.	<i>O. ips</i>		7	0	7	28	0	1	2	4	0	0	0	3	52
5.	<i>Ophiostoma</i> sp. B		0	0	0	0	0	0	0	0	0	0	0	0	0
6.	<i>O. brevipilosi</i> sp. nov.		0	0	0	0	0	0	0	0	0	0	0	0	0
7.	<i>O. setosum</i>		0	0	0	0	0	0	0	0	0	0	0	0	0
8.	<i>O. quercus</i>		0	0	7	4	0	0	0	0	0	0	2	0	13
9.	<i>O. tsotsi</i>		0	0	6	0	0	0	0	0	0	0	0	2	8
10.	<i>Ophiostoma</i> sp. C		0	0	0	0	0	0	0	0	0	0	0	0	0
11.	<i>Graphilbum fragrans</i>		0	0	0	0	0	0	0	0	0	0	0	0	0
12.	<i>Gra. kesiya</i> sp. nov.		0	0	0	5	0	1	0	0	0	0	0	0	6
13.	<i>Gra. puer</i> sp. nov.		0	0	0	1	0	1	0	0	0	0	1	0	3
14.	<i>Leptographium gracile</i>		0	0	0	0	0	0	0	0	0	0	0	0	0
15.	<i>Grosmannia radiaticola</i>		0	0	0	0	0	0	0	0	0	0	0	0	0
16.	<i>L. ningerense</i> sp. nov.		3	6	0	2	0	0	0	1	0	0	0	0	12
17.	<i>Gro. yunnanensis</i>		6	0	0	12	1	1	0	0	0	5	1	1	27
18.	<i>L. conjunctum</i>		0	0	0	0	0	0	0	0	0	0	0	0	0
19.	<i>Graphium pseudormiticum</i>		0	0	0	1	0	0	0	0	0	0	0	0	1
Total			22	6	49	72	1	4	3	6	4	5	4	8	184

¹ Mite species: a = *Dendrolaelaps* sp. 1 (Mesostigmata, Digamasellidae); b = *Dendrolaelaps* sp. 2 (Mesostigmata, Digamasellidae); c = *Histiostoma* cf. *sapromyzarum* (Sarcoptiformes, Histiostomatidae); d = *Insectolaelaps* sp. 1 (Mesostigmata, Digamasellidae); e = *Lasioseius* sp. 1 (Mesostigmata, Blattisociidae); f = *Proctolaelaps* nr. *Hystrix* (Mesostigmata, Melicharidae); g = *Rhizoglyphus* sp. 1 (Sarcoptiformes, Acaridae); h = *Schwiebea taiwanensis* (Sarcoptiformes, Acaridae); i = Unknown sp. 1 (Mesostigmata); j = Unknown sp. 2 (Oribatei); k = Unknown sp. 3 (Mesostigmata, Uropodoidea); l = Unknown sp. 4 (Mesostigmata); m = Unknown sp. 5

Table S2 Haplotypes of *Ophiostoma quercus*. Numbers of isolates originating from China are printed in bold type.

Isolate	ITS ACC ¹	ITS HAP ²	BT ACC ³	BT HAP ⁴	EF ACC ⁵	EF HAP ⁶	COMB ⁷
CMW2467*	AY466626	HQI1	AY466647	HQB1	AY466693	HQE1	HQIBE1
CMW11747	=AF198238	HQI2	=FJ455565	HQB2	MG205736	HQE2	HQIBE2
CMW11748	=AF198238	HQI2	=FJ455565	HQB2	=MG205736	HQE2	HQIBE2
CMW11756	MG205662	HQI3	MG205692	HQB3	MG205737	HQE3	HQIBE3
CMW11806	=FJ434947	HQI4	=FJ455565	HQB2	MG205738	HQE4	HQIBE4
CMW11807	=FJ434947	HQI4	=AY466647	HQB1	=MG205738	HQE4	HQIBE5
CMW11981	=FJ434947	HQI4	=FJ455570	HQB4	MG205739	HQE5	HQIBE6
CMW12015	=AY466624	HQI5	MG205693	HQB5	MG205740	HQE6	HQIBE7
CMW12037	=AY466624	HQI5	MG205694	HQB6	MG205741	HQE7	HQIBE8
CMW12039	=FJ434947	HQI4	MG205695	HQB7	MG205742	HQE8	HQIBE9
CMW12122	=FJ434947	HQI4	MG205696	HQB8	MG205743	HQE9	HQIBE10
CMW12146	=FJ434947	HQI4	=MG205696	HQB8	=MG205743	HQE9	HQIBE10
CMW12185	=FJ434947	HQI4	MG205697	HQB9	=MG205740	HQE6	HQIBE11
CMW12195	=FJ434947	HQI4	=MG205697	HQB9	=MG205740	HQE6	HQIBE11
CMW12286	=AF198238	HQI2	=FJ455570	HQB4	MG205744	HQE10	HQIBE12
CMW12286	=FJ434947	HQI4	=FJ455570	HQB4	MG205751	HQE11	HQIBE13
CMW12287	FJ434947	HQI4	FJ455563	HQB10	=MG205740	HQE6	HQIBE14
CMW12294	=AF198238	HQI2	=FJ455570	HQB4	MG205752	HQE12	HQIBE15
CMW12295	FJ434944	HQI4	FJ455560	HQB11	MG205753	HQE13	HQIBE16
CMW12299	=FJ434947	HQI4	=FJ455570	HQB4	=MG205740	HQE6	HQIBE17
CMW12332	FJ434948	HQI4	FJ455565	HQB2	=MG205740	HQE6	HQIBE18
CMW12350	=FJ434947	HQI4	MG205698	HQB12	MG205745	HQE14	HQIBE19
CMW12354	=FJ434947	HQI4	=FJ455570	HQB4	MG205754	HQE15	HQIBE20
CMW12357	=AF198238	HQI2	=FJ455570	HQB4	=MG205740	HQE6	HQIBE21
CMW12359	=AF198238	HQI2	=FJ455570	HQB4	MG205746	HQE11	HQIBE12
CMW12359	=AF493241	HQI6	=FJ455570	HQB4	=MG205742	HQE8	HQIBE22
CMW12360	=FJ434947	HQI4	=MG205696	HQB8	=MG205740	HQE6	HQIBE23
CMW12364	=AF198238	HQI2	MG205699	HQB13	MG214780	HQE16	HQIBE24
CMW12370	=AF198238	HQI2	=MG205698	HQB12	MG205747	HQE17	HQIBE25
CMW12371	FJ434952	HQI4	FJ455570	HQB4	=MG205752	HQE12	HQIBE26
CMW12382	=AF198238	HQI2	=FJ455570	HQB4	MG205748	HQE18	HQIBE27
CMW17585	=AF493241	HQI6	=HM041866	HQB14	=FJ441267	HQE19	HQIBE28
CMW19192	MG205663	HQI12	GQ249310	HQB15	FJ441267	HQE19	HQIBE29
CMW2463	AF493239	HQI9	AY466644	HQB1	AY466690	HQE20	HQIBE30
CMW24638	=FJ434947	HQI4	HM041866	HQB14	HM041898	HQE21	HQIBE31
CMW2464	AY466624	HQI5	AY466645	HQB16	AY466691	HQE22	HQIBE32
CMW24643	=FJ434947	HQI4	HM041868	HQB14	HM041900	HQE23	HQIBE33
CMW2465	AY466625	HQI10	AY466646	HQB1	AY466692	HQE24	HQIBE34
CMW2520	AF493241	HQI6	AY466648	HQB17	AY466694	HQE25	HQIBE35
CMW27058	HM051398	HQI5	HM041859	HQB18	=HM041900	HQE23	HQIBE36
CMW27172	=FJ434947	HQI4	MG205702	HQB7	=HM041898	HQE21	HQIBE37
CMW27173	HM051397	HQI4	HM041856	HQB14	HM041897	HQE23	HQIBE38
CMW27192	HM051399	HQI4	HM041858	HQB17	HM041893	HQE21	HQIBE39
CMW29734	HM051407	HQI4	HM041862	HQB19	HM041896	HQE23	HQIBE40
CMW3119	AF493244	HQI6	AY466650	HQB17	AY466696	HQE26	HQIBE41
CMW41659	MG205664	HQI7	MG205700	HQB22	MG205749	HQE27	HQIBE49
CMW41693	=MG205664	HQI7	=MG205700	HQB22	=MG205749	HQE27	HQIBE49

CMW41715	=MG205664	HQI7	=MG205700	HQB22	=MG205749	HQE27	HQIBE49
CMW41718	=MG205664	HQI7	=MG205700	HQB22	=MG205749	HQE27	HQIBE49
CMW41724	MG205665	HQI8	MG205701	HQB23	MG205750	HQE28	HQIBE50
CMW41732	=MG205664	HQI7	=MG205700	HQB22	=MG205749	HQE27	HQIBE49
CMW5679	HQ131894	HQI11	HQ131893	HQB1	FJ441265	HQE19	HQIBE42
CMW7650	AF198238	HQI2	AY466651	HQB1	AY466697	HQE26	HQIBE43
CMW9255	AY466612	HQI1	AY466632	HQB20	AY466678	HQE1	HQIBE44
CMW9257	AY466614	HQI1	AY466634	HQB16	AY466680	HQE26	HQIBE45
CMW9262	AY466617	HQI1	AY466637	HQB18	AY466683	HQE26	HQIBE46
CMW9267	AY466619	HQI1	AY466639	HQB20	AY466685	HQE20	HQIBE47
CMW9474	AY466620	HQI1	AY466640	HQB21	AY466686	HQE26	HQIBE48

¹ ITS ACC: ITS GenBank accession number

² ITS HAP: Haplotypes based on the ITS sequences

³ BT ACC: BT GenBank number

⁴ BT HAP: Haplotypes based on the BT sequences

⁵ EF ACC: EF GenBank number

⁶ EF HAP: Haplotypes based on the EF sequences

⁷ COMB: Haplotypes based on the combination of ITS, BT and EF sequences

* Ex-type isolate

Table S3 Haplotypes of *Ophiostoma tsotsi*. Numbers of isolates originating from China are printed in bold type.

Strains	ITS ACC ¹	ITS HAP ²	BT ACC ³	BT HAP ⁴	EF ACC ⁵	EF HAP ⁶	COMB ⁷
CMW3117*	FJ441284	HTI1	FJ441277	HTB1	FJ441269	HTE1	HTIBE1
CMW15239	FJ441287	HTI1	FJ441280	HTB2	MG205757	HTE8	HTIBE2
CMW16779	FJ441288	HTI1	FJ441281	HTB3	FJ441273	HTE2	HTIBE3
CMW17573	=FJ441284	HTI1	FJ441255	HTB3	=FJ441273	HTE2	HTIBE3
CMW17606	=FJ441284	HTI1	FJ441256	HTB4	=FJ441270	HTE3	HTIBE4
CMW17618	=FJ441284	HTI1	FJ441257	HTB5	MG205758	HTE4	HTIBE5
CMW17682	=FJ441284	HTI1	=FJ441256	HTB4	MG205759	HTE5	HTIBE6
CMW17686	=FJ441284	HTI1	=FJ441255	HTB3	MG205760	HTE6	HTIBE7
CMW18134	FJ441289	HTI1	FJ441282	HTB3	FJ441274	HTE7	HTIBE8
CMW24802	=FJ441284	HTI1	FJ441258	HTB6	=FJ441269	HTE1	HTIBE9
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CMW24822	=FJ441284	HTI1	FJ441263	HTB8	=FJ441269	HTE1	HTIBE11
CMW24954	=FJ441284	HTI1	MG205706	HTB9	=FJ441270	HTE3	HTIBE12
CMW24978	=FJ441284	HTI1	MG205707	HTB10	MG205761	HTE9	HTIBE13
CMW24982	=FJ441284	HTI1	MG205708	HTB11	=MG205761	HTE9	HTIBE14
CMW24983	=FJ441284	HTI1	=FJ441257	HTB5	=FJ441270	HTE3	HTIBE15
CMW24988	=FJ441284	HTI1	=MG205706	HTB9	=MG205761	HTE9	HTIBE16
CMW25042	=FJ441284	HTI1	=FJ441257	HTB5	=FJ441270	HTE3	HTIBE15
CMW3116	FJ441285	HTI1	FJ441278	HTB12	FJ441270	HTE3	HTIBE17
CMW41730	=FJ441284	HTI1	MG205704	HTB13	MG205755	HTE10	HTIBE18
CMW41731	=FJ441284	HTI1	MG205705	HTB14	MG205756	HTE11	HTIBE19
CMW41733	=FJ441284	HTI1	=MG205704	HTB13	=MG205755	HTE10	HTIBE18
CMW41734	=FJ441284	HTI1	=MG205704	HTB13	=MG205755	HTE10	HTIBE18
CMW41735	=FJ441284	HTI1	=MG205704	HTB13	=MG205755	HTE10	HTIBE18
CMW41742	=FJ441284	HTI1	=MG205705	HTB14	=MG205756	HTE11	HTIBE19
CMW41746	=FJ441284	HTI1	=MG205704	HTB13	=MG205755	HTE10	HTIBE18
CMW41758	=FJ441284	HTI1	=MG205705	HTB14	=MG205756	HTE11	HTIBE19

¹ ITS ACC: ITS GenBank accession number² ITS HAP: Haplotypes based on the ITS sequences³ BT ACC: BT GenBank number⁴ BT HAP: Haplotypes based on the BT sequences⁵ EF ACC: EF GenBank number⁶ EF HAP: Haplotypes based on the EF sequences⁷ COMB: Haplotypes based on the combination of ITS, BT and EF sequences

* Ex-type isolate

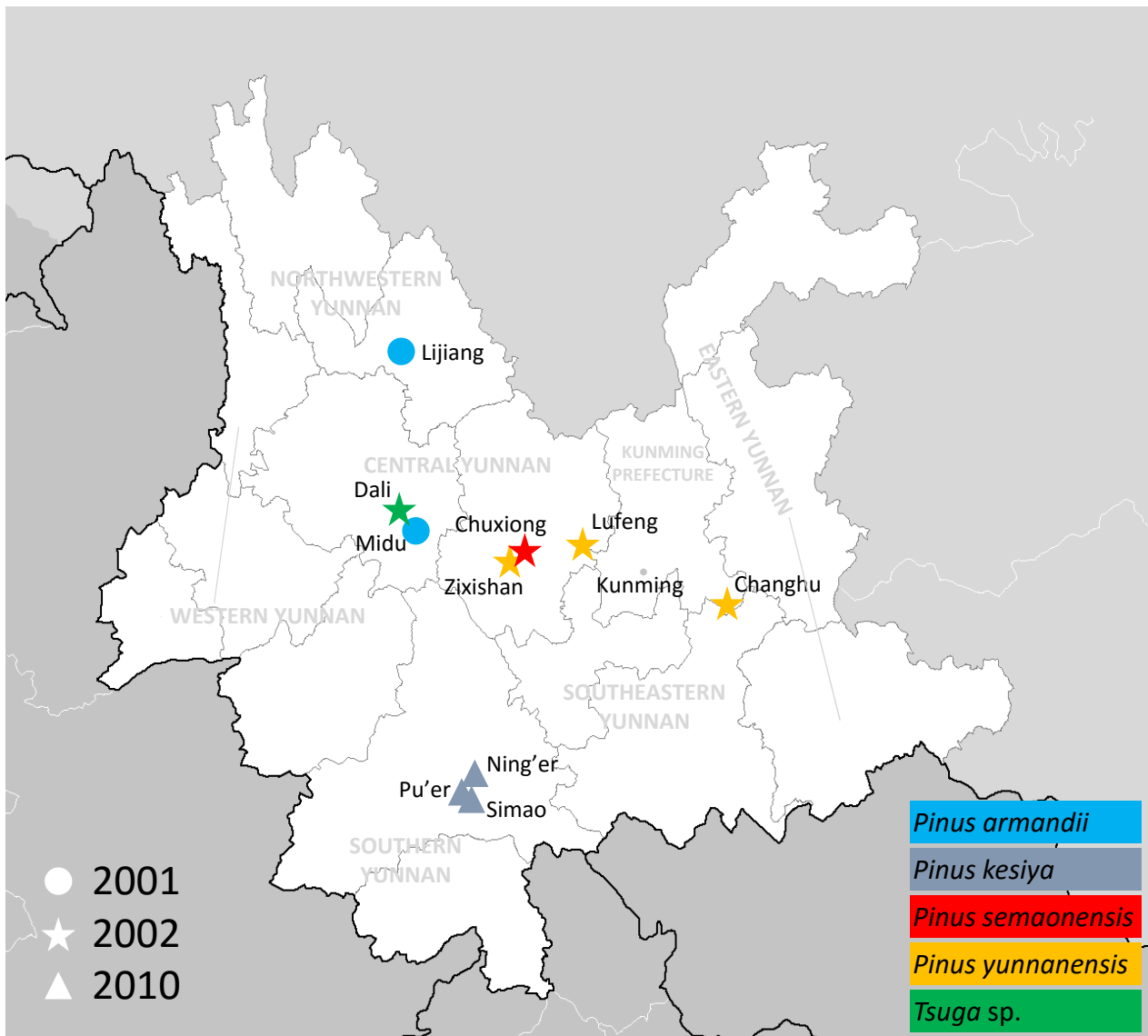


Figure S1. Map of Yunnan Province in China showing the sites and tree hosts from which samples were collected during the three surveys in 2001, 2002 and 2010.

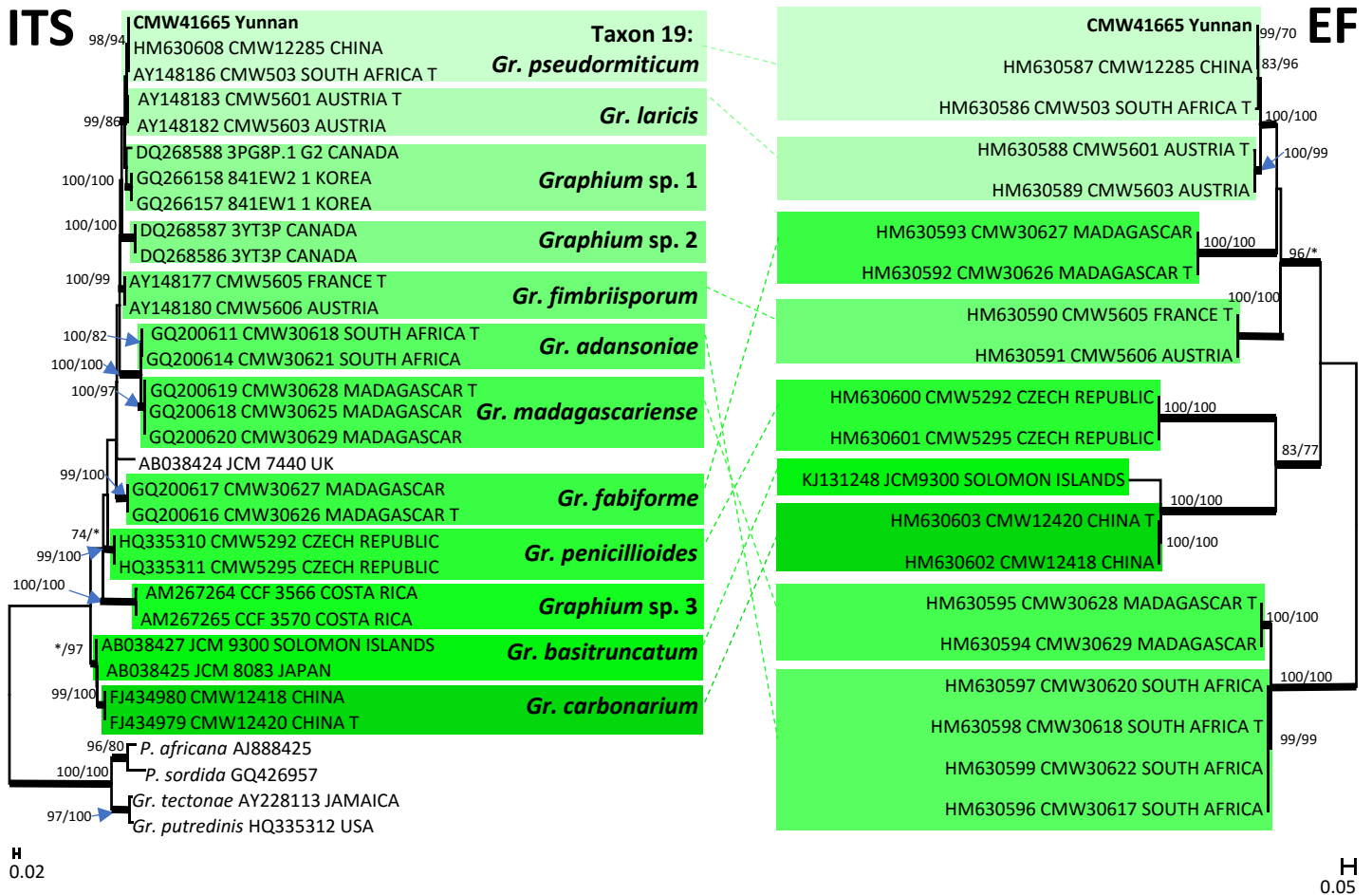


Figure S2. ML trees of the genus *Graphium* generated from DNA sequences of the ITS and BT regions. *Bold* branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

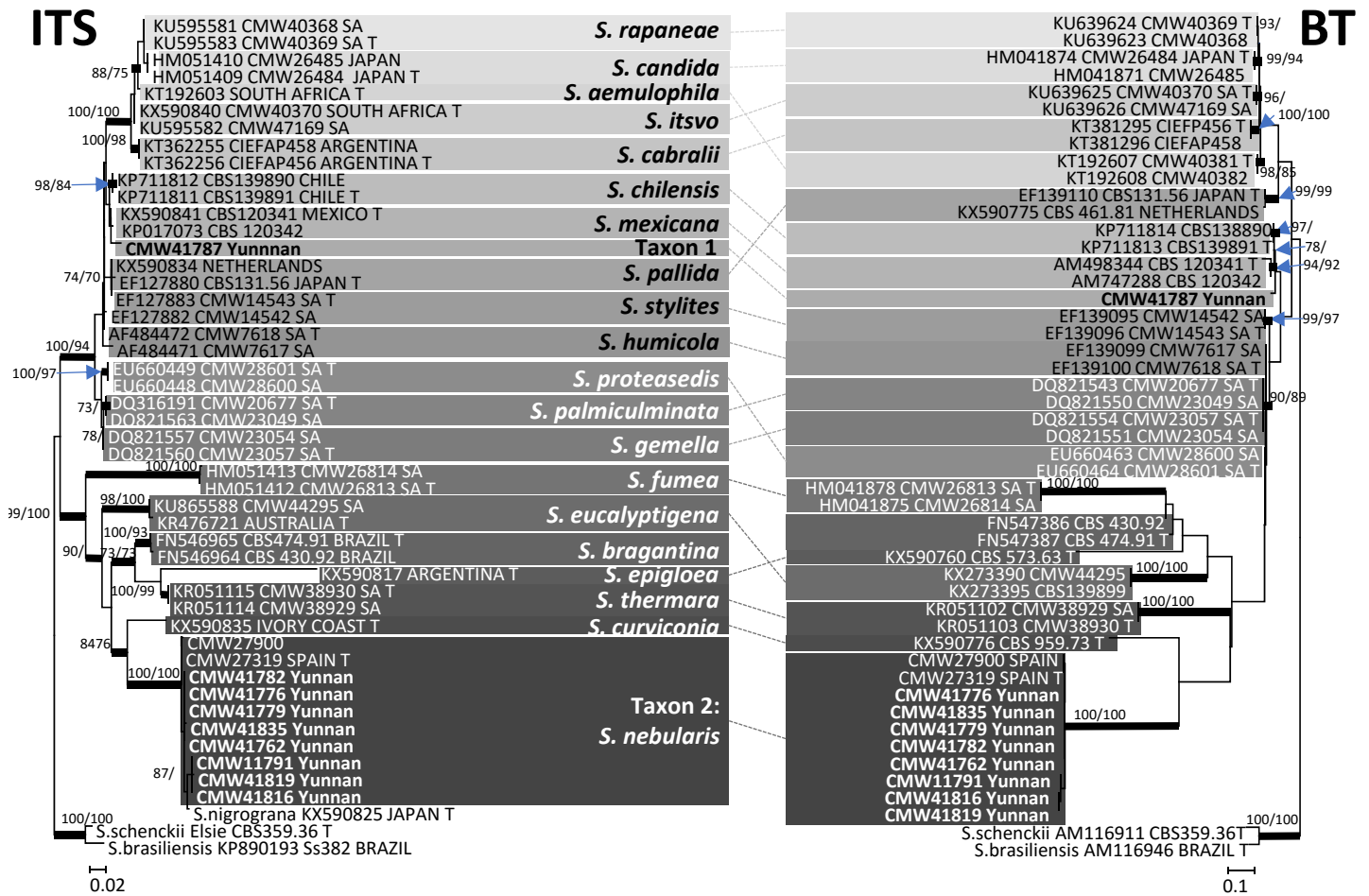


Figure S3. ML trees of the genus *Sporothrix* generated from DNA sequences of ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

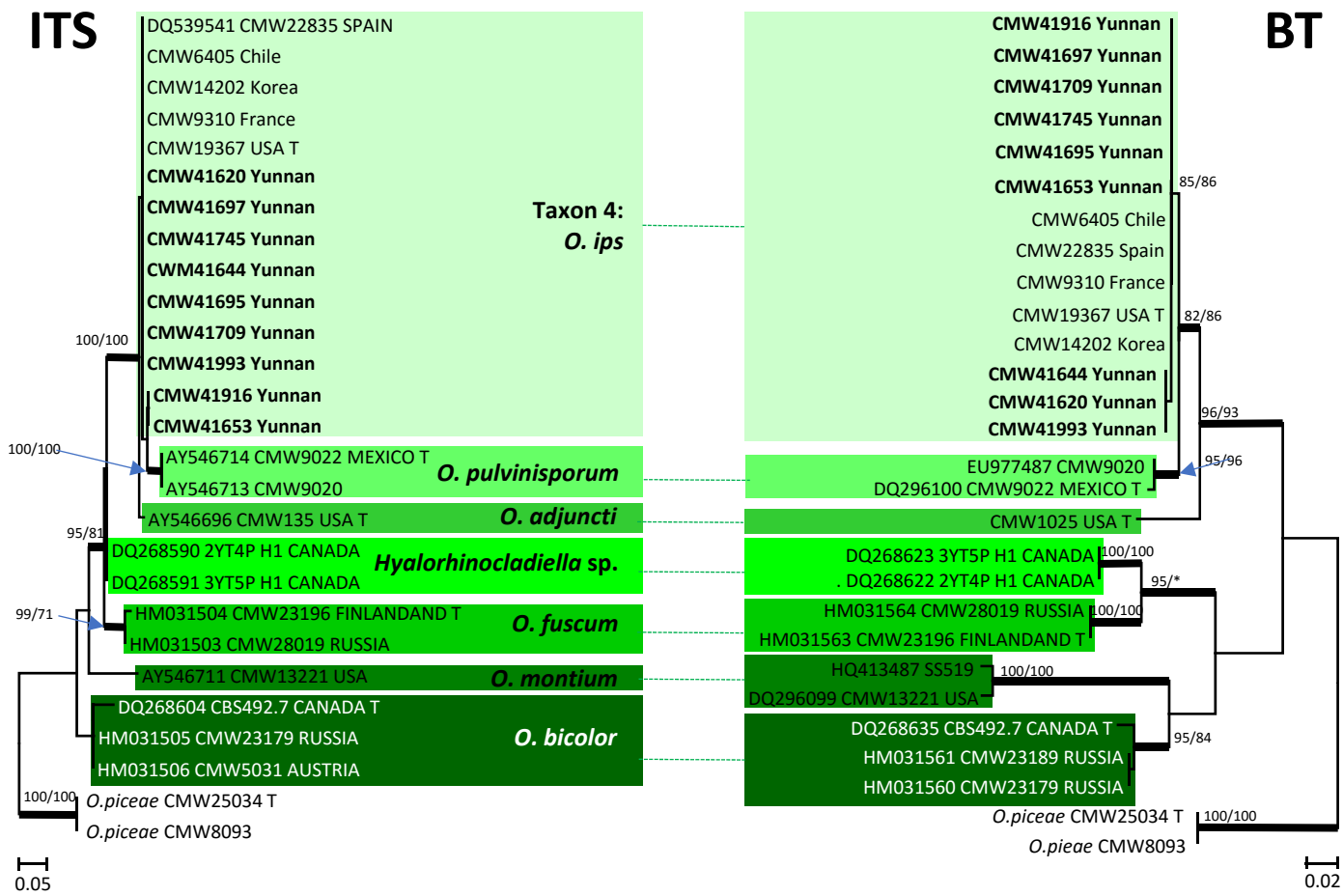


Figure S4. ML trees of the *O. ips* complex generated from DNA sequences of ITS and BT regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

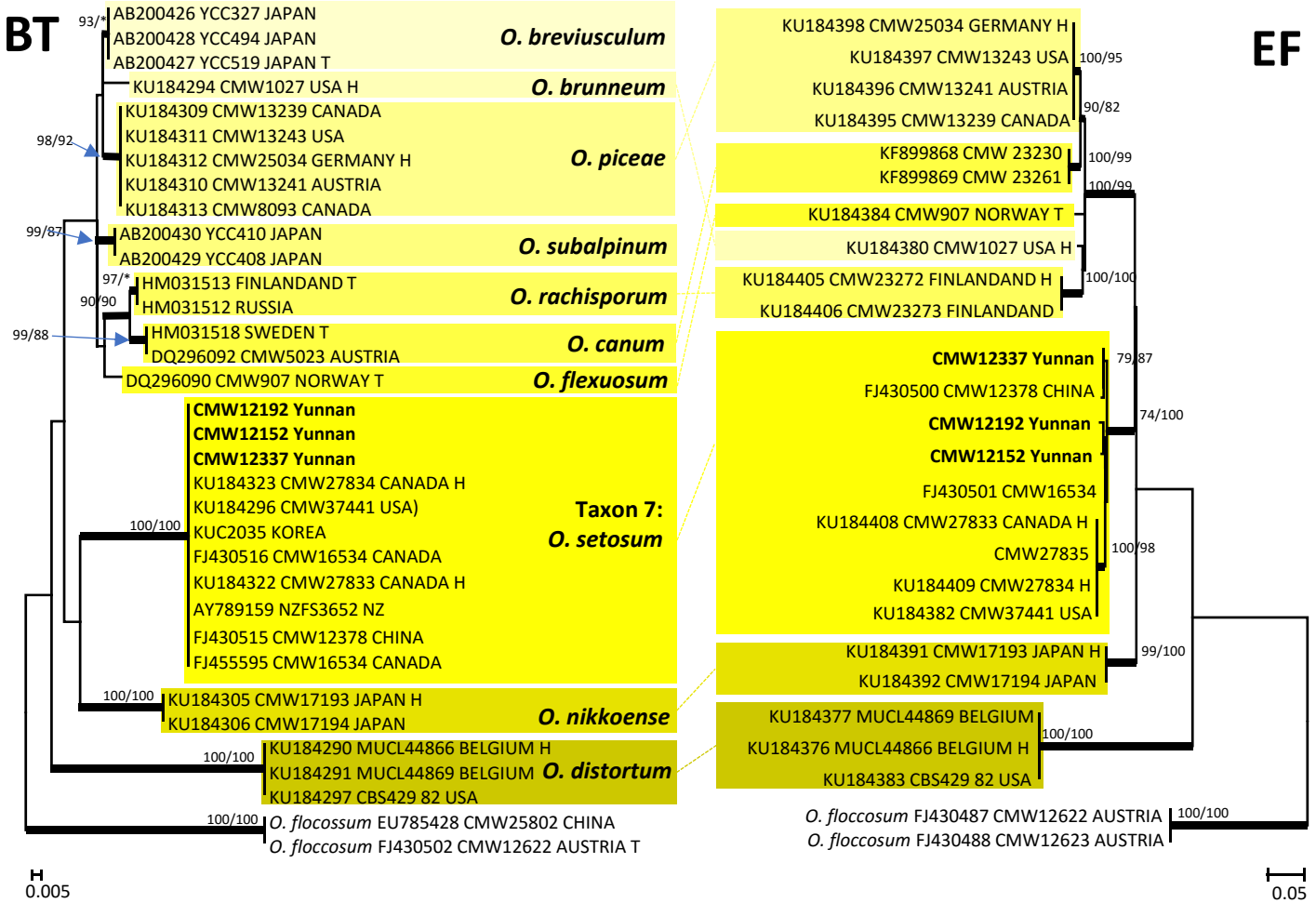


Figure S5. ML trees of the *O. piceae* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Figure S6. ML tree of the *O. quercus* complex generated from DNA sequences of ITS region. *Bold* branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

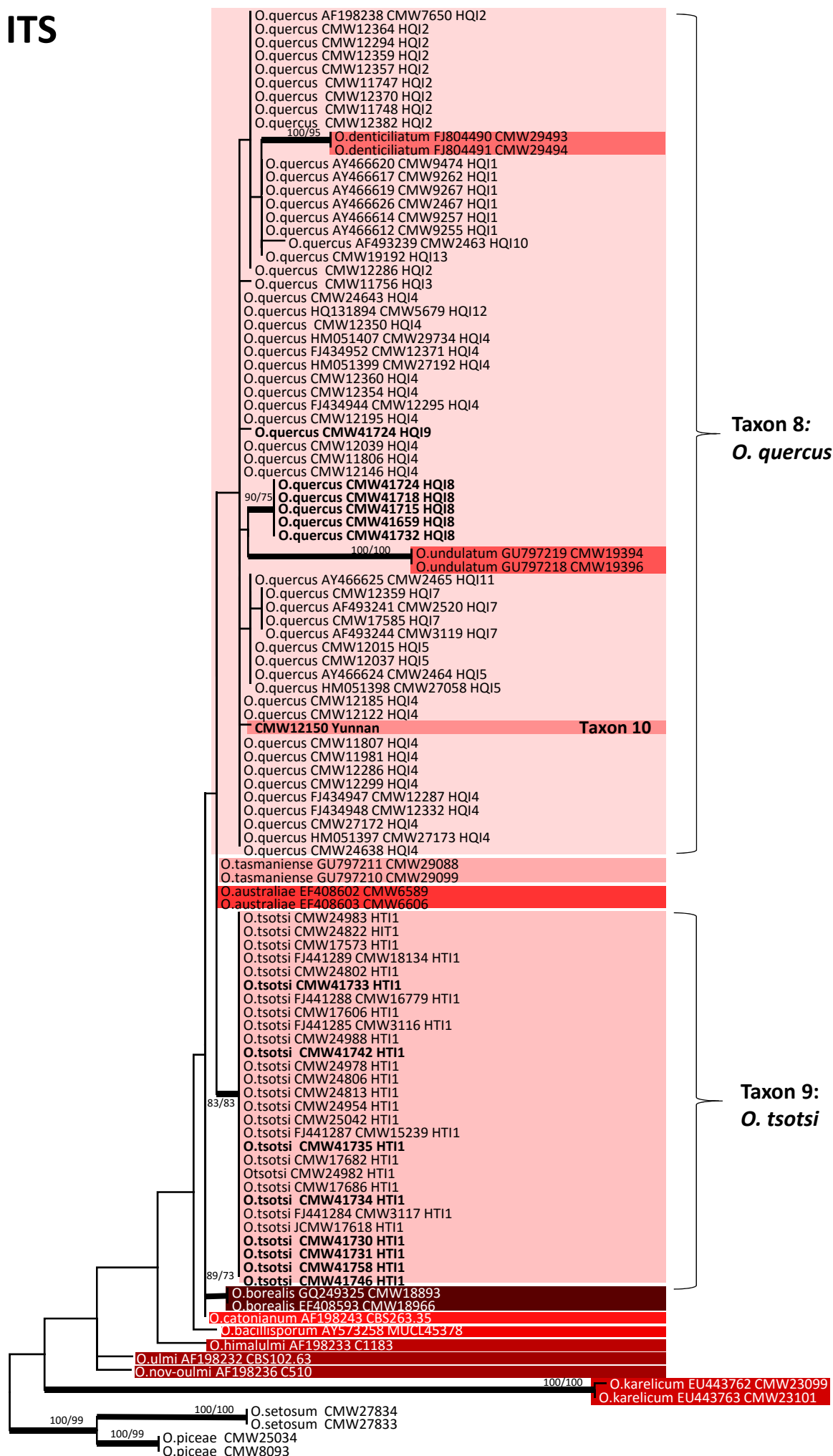


Figure S7. ML tree of the *O. quercus* complex generated from DNA sequences of BT region. *Bold* branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

BT

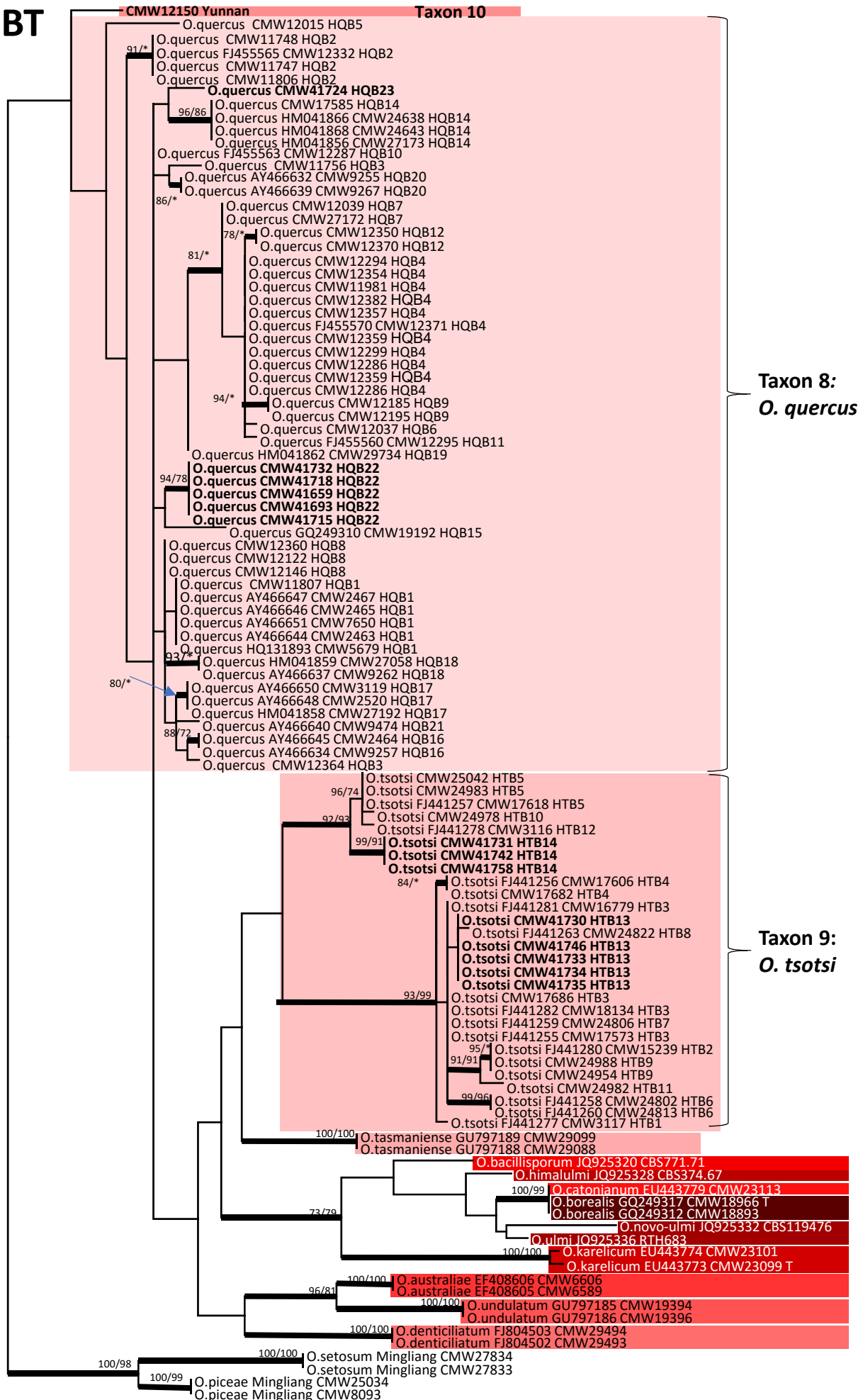
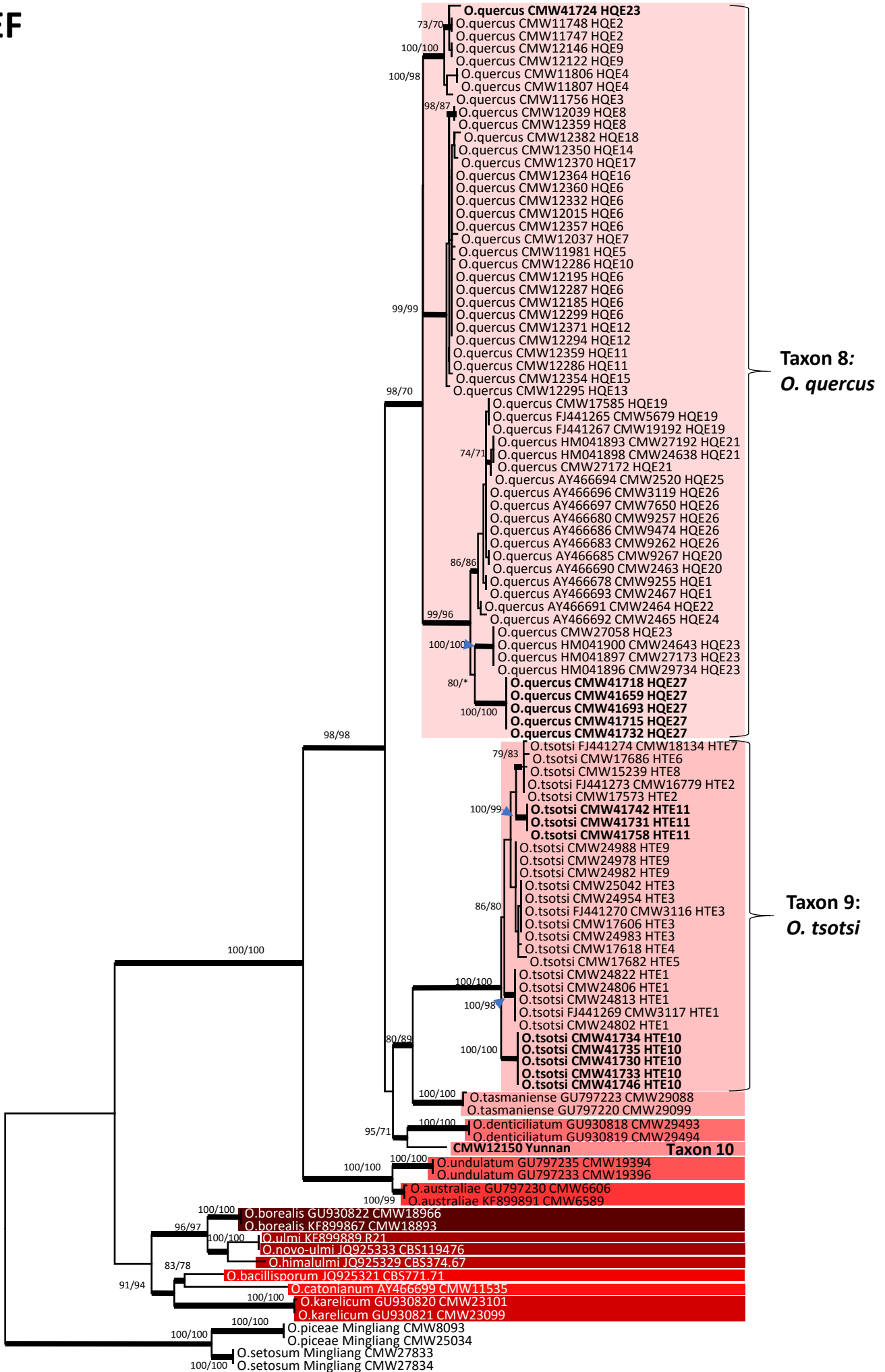
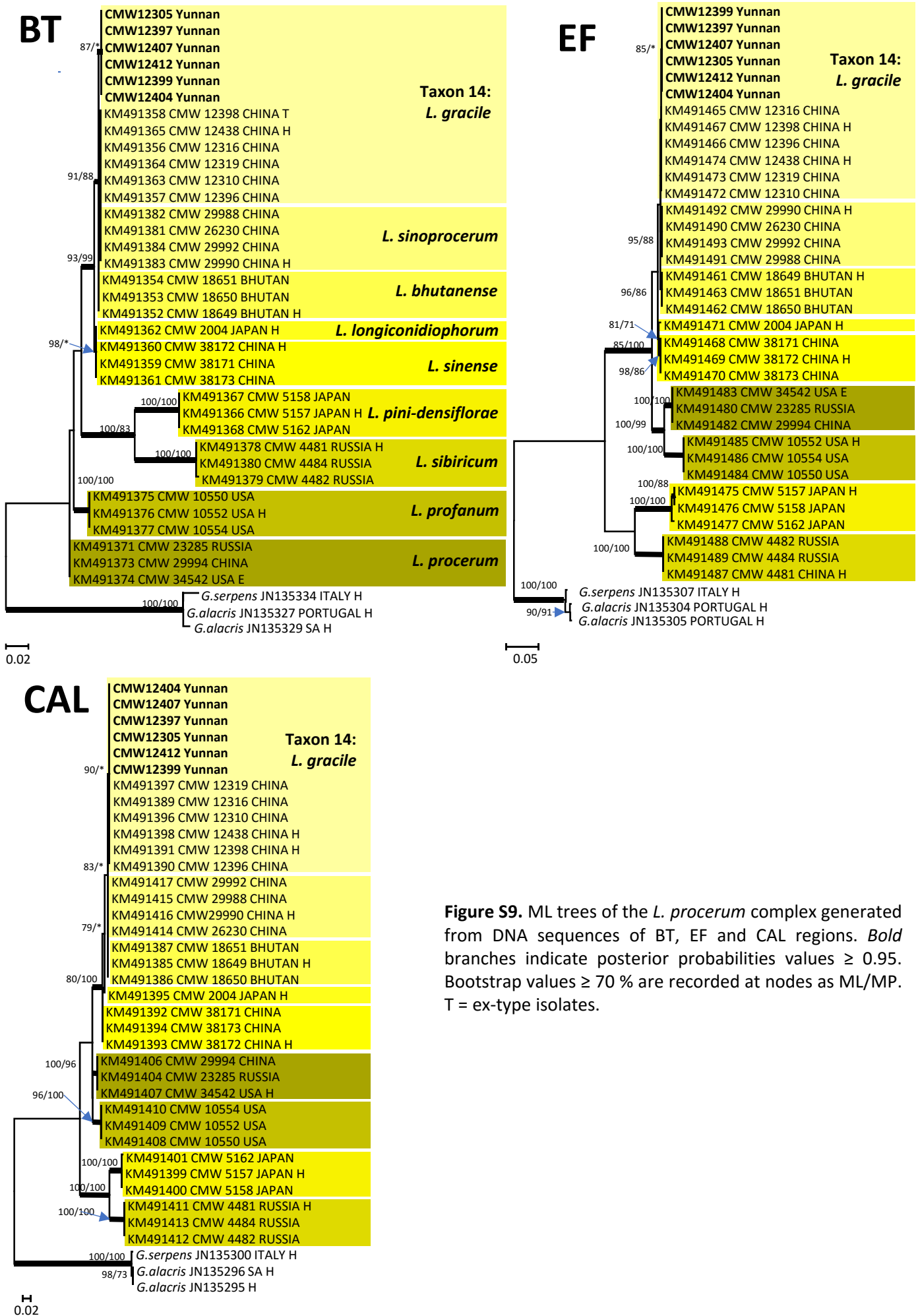


Figure S8. ML tree of the *O. quercus* complex generated from DNA sequences of EF region. *Bold* branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

EF





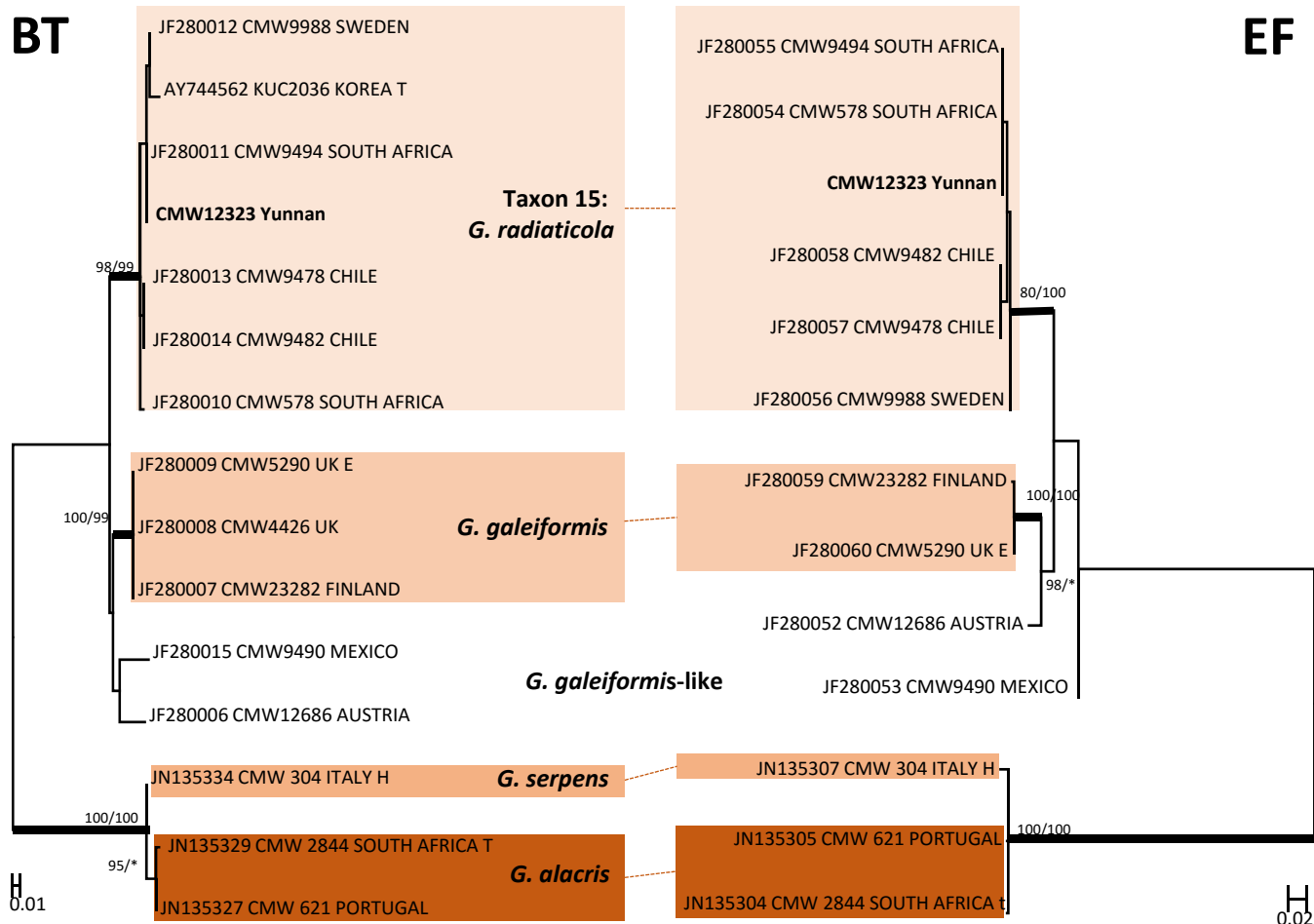


Figure S10. ML trees of the *G. galeiformis* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

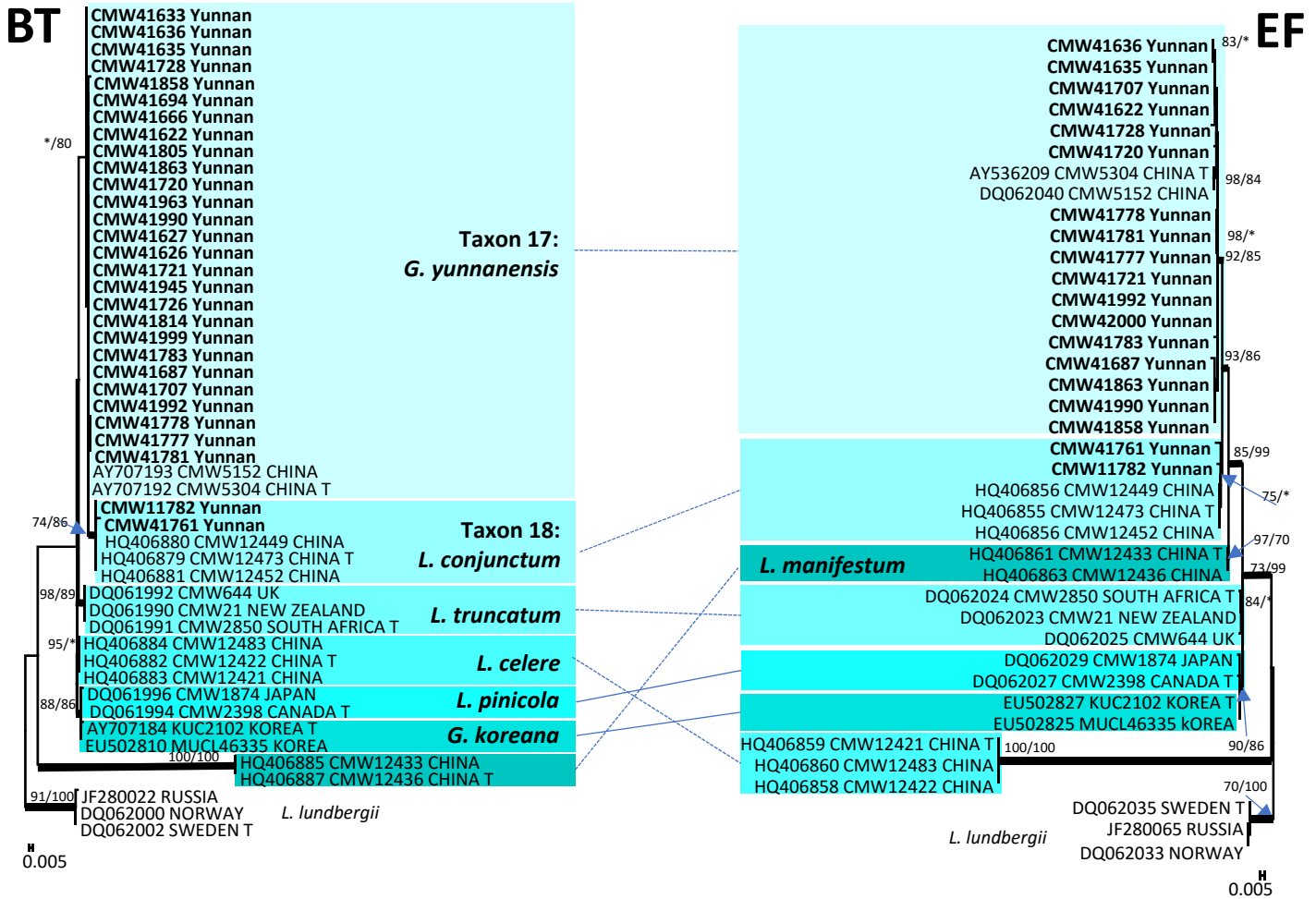


Figure S11. ML trees of the *L. lundbergii* complex generated from DNA sequences of BT and EF regions. **Bold** branches indicate posterior probabilities values ≥ 0.95 . Bootstrap values $\geq 70\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Chapter 3

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Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus*, including 11 new species from China

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Key words

11 new taxa
Ophiostomatales
Microascales
vector
Scolytinae

Abstract *Ips typographus* (Coleoptera, Scolytinae) is a spruce-infesting bark beetle that occurs throughout Europe and Asia. The beetle can cause considerable damage, especially when colonized trees are stressed and beetle populations increase. Although some studies have shown that populations of *I. typographus* in Europe, China and Japan are genetically distinct, these populations are biologically similar, including a strong association with ophiostomatoid fungi. To date, only two *Leptographium* spp. have been reported from the beetle in China, while 40 species have been reported from Europe and 13 from Japan. The aims of this study were to identify the ophiostomatoid fungal associates of *I. typographus* in north-eastern China, and to determine whether the fungal assemblages reflect the different geographical populations of the beetle. Field surveys in Jilin and Heilongjiang provinces yielded a total of 1046 fungal isolates from 145 beetles and 178 galleries. Isolates were grouped based on morphology and representatives of each group were identified using DNA sequences of the ribosomal LSU, ITS, β -tubulin, calmodulin and elongation factor 1- α gene regions. A total of 23 species of ophiostomatoid fungi were identified, including 12 previously described species and 11 novel species, all of which are described here. The dominant species were *Ophiostoma bicolor*, *Leptographium taigense* and *Grosmannia piceiperda* D, representing 40.5 %, 27.8 % and 17.8 % of the isolates, respectively. Comparisons of species from China, Europe and Japan are complicated by the fact that some of the European and all the Japanese species were identified based only on morphology. However, assuming that those identifications are correct, five species were shared between Europe, Japan and China, two species were shared between China and Japan, five between Europe and China, and two between Europe and Japan. Consequently, *Ips typographus* populations in these different geographic areas have different fungal assemblages, suggesting that the majority of these beetle-associations are promiscuous. The results also suggested that the symbionts of the bark beetle do not reflect the population structures of the beetle. The use of fungal symbiont assemblages to infer population structures and invasion history of its vectors should thus be interpreted with circumspection.

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INTRODUCTION

Symbionts of insects can dramatically impact the ecology, evolution and physiology of their vectors, sometimes with detrimental effects on human health, food security and industry (Klepzig et al. 2009). When molecular data provide ambiguous results regarding the movement history of an insect species, comparisons between symbiont assemblages at various locations can provide clues regarding the movement patterns of the insect. They can also reflect geographical barriers that limit gene flow between populations of the insect (Taerum et al. 2013). For example, when the Formosan subterranean termite (*Coptotermes formosanus*) invaded the United States from China, bacterial symbionts were useful in determining the source population of the insect (Husseneder et al. 2010). However, there are few studies on geographical variation in symbiont assemblages of insects, including those on bark beetles and their associated ophiostomatoid fungi (Jacobs et al. 2003, Taerum et al. 2013). Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a highly diverse group of insects that occur in most forested regions of the world (Raffa et al. 2015). Some species can form large tree-killing outbreaks, which are considered to be major forest

disturbances (Grégoire et al. 2015). Bark beetles are important vectors of microbial and animal symbionts between host trees (Six & Wingfield 2011, Hofstetter et al. 2013). Among the most common associates of these beetles is a group of fungi, commonly referred to as the ophiostomatoid fungi, which includes members of the Ophiostomatales and Microascales (Wingfield et al. 1993, De Beer et al. 2013, 2014). Most of these fungi cause blue stain in the wood of host trees, but a few are important pathogens, such as *Ophiostoma ulmi* and *O. novo-ulmi*, the causal agents of Dutch Elm Disease (Brasier 1988).

In symbioses between bark beetles and microbes, there is frequently evidence for coevolution between the arthropods and microbes. Many species of ophiostomatoid fungi produce long-necked ascomata or conidiophores with sticky ascospores or/and conidia. These characters are thought to be adaptations for dispersal between tree hosts by arthropods (Malloch & Blackwell 1993). In addition, some bark beetle species possess specialised structures, known as mycangia, which facilitate the transport of fungi between trees (Batra 1963). All these adaptations contribute to the maintenance of bark beetle-fungus symbioses.

The patterns of association between fungi and bark beetles is usually not 'one fungus-one insect' (Kirisits 2004). Most bark beetle species are associated with several species of fungi. For example, *Endoconidiophora polonica*, *O. ainoae*, *O. bicolor*, *Grosmannia penicillata* and *G. piceiperda* have all been isolated from *Ips typographus*. Similarly, *O. piceae* is an example of an

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individual fungal species that can be associated with numerous bark beetle species such as *Cryphalus abietis*, *Crypturgus cinereus*, *Crypturgus pusillus*, *Dryocoetes autographus*, *Hylastes cunicularius* as well as other beetle vectors (Kirisits 2004). Due to differences in tolerance to various environmental conditions, including host tree defensive chemistry, and moisture and oxygen requirements, the fungal symbioses can differ over time or vary within and among locations (Solheim 1991, Viiri 1997, Klepzig et al. 2004, Hofstetter et al. 2006, Adams & Six 2007, Six & Bentz 2007, Bleiker & Six 2009a, b).

Associations between fungal symbionts and their bark beetle vectors range from antagonistic to mutualistic (Six & Wingfield 2011). Antagonistic symbionts can affect vector fitness either directly or indirectly. *Beauveria bassiana* is an example of a direct antagonist; this fungus is a pathogen of several bark beetles and other insect species (Wegensteiner et al. 2015). An example of indirect antagonism is the association between the fungus *O. minus* and the southern pine beetle, *Dendroctonus frontalis*. *Ophiostoma minus* is commonly associated with *D. frontalis*, and has negative impacts on the fitness of the beetle, outcompeting the nutritional mutualists of *D. frontalis* within the phloem of host trees (Klepzig 1998). Conversely, mutualistic symbionts can aid their bark beetle vectors in colonizing tree hosts.

Ophiostomatoid fungi have been hypothesized to aid their vectors in overcoming tree defences, thus allowing the beetles to establish colonies in their tree hosts (Lieutier et al. 2009), although this view has been challenged (Six & Wingfield 2011). However, it has been shown that the fungus *G. clavigera*, a symbiont of the mountain pine beetle *Dendroctonus ponderosae*, can detoxify defensive chemicals produced by the host tree (DiGiustini et al. 2011). There are also many examples where fungi associated with bark beetles act as nutritional mutualists, by concentrating nutrients that are essential for brood development (Lee et al. 2005, 2006, Bleiker & Six 2007).

Ips typographus is an aggressive bark beetle species that is native to Eurasia. In Europe, the insect mainly attacks Norway spruce (*Picea abies*), while in Japan where it is known as *I. typographus japonicus*, it mainly attacks Yezo spruce (*P. jezoensis*) and Sachalin spruce (*P. glehnii*) (Yamaoka et al. 1997, Stauffer et al. 2001). Outbreaks of *I. typographus* can result in dramatic host mortality (Krascsenitsova et al. 2013). In China, *I. typographus* is mainly distributed in Heilongjiang, Jilin, Sichuan, Qinghai and Xinjiang provinces, and primarily damages Korean spruce (*P. koraiensis*), Yezo spruce (*P. jezoensis*) and Asian spruce (*P. schrenkiana*). The beetle also incurs costs because it necessitates sanitation felling and the clearing of wind throw areas (Wermelinger 2004). Population genetics studies on *I. typographus* have suggested that the Chinese and Japanese populations are more similar to each other than either population is to the European population (Stauffer et al. 1992, Stauffer et al. 1999, Salle et al. 2007, Bertheau et al. 2013, Mayer et al. 2015).

The ophiostomatoid fungal symbionts of *I. typographus* have been well identified in Europe and Japan. The first fungal associate of *I. typographus* to be recorded was *G. penicillata* in Germany (Grosmann 1930, Solheim 1986). Later, several additional species of ophiostomatoid fungi in the genera *Endoconidiophora* (previously treated as *Ceratocystis*), *Ceratocystis*, *Grosmannia*, *Leptographium* and *Ophiostoma* were reported from Japan and Europe (Yamaoka et al. 1997, 1998, Viiri & Lieutier 2004, Jankowiak 2005, Jankowiak & Hilszczanski 2005). *Endoconidiophora polonica*, a highly pathogenic fungal associate of *I. typographus* has been reported from Europe and Japan (Marin et al. 2005). However, research on the association between ophiostomatoid fungi and *I. typographus*, in China is limited, with only *L. curviconidium* and *L. gracile* (as *L. latens*) having been reported as associates of the beetle to date (Paciura et al. 2010, Zhou et al. 2013). In this study, we surveyed the ophiostomatoid fungal associates of *I. typographus* in north-eastern China, and based on published records,

Table 1 Isolates of ophiostomatoid fungi associated with *Ips typographus* in China.

Genus	Species complex	Species	Numbers of isolates		Percentages (%)		Total percentage
			Heilongjiang	Jilin	Heilongjiang	Jilin	
Ophiostomatales							
<i>Ophiostoma</i> s.lat.	Group A	<i>O. floccosum</i> (Taxon 1)	0	8	0.0	0.8	0.8
	<i>O. piceae</i>	<i>O. typographi</i> sp. nov. (Taxon 2)	3	1	0.3	0.1	0.4
	<i>O. minus</i>	<i>O. wuyingense</i> sp. nov. (Taxon 3)	3	0	0.3	0.0	0.3
	<i>O. clavatum</i>	<i>O. jiamusiensis</i> sp. nov. (Taxon 4)	0	1	0.0	0.1	0.1
		<i>O. songshui</i> sp. nov. (Taxon 5)	2	0	0.2	0.0	0.2
		<i>O. ainoae</i> (Taxon 6)	18	2	1.7	0.2	1.9
		<i>O. brunneolum</i> (Taxon 7)	47	0	4.5	0.0	4.5
	<i>O. ips</i>	<i>O. japonicum</i> (Taxon 8)	14	1	1.3	0.0	1.3
		<i>O. bicolor</i> (Taxon 9)	423	0	40.5	0.0	40.5
	Group B	<i>O. jilinense</i> sp. nov. (Taxon 10)	0	2	0.0	0.2	0.2
<i>Leptographium</i> s.lat.	<i>Grosmannia galeiformis</i>	<i>L. bachii</i> sp. nov. (Taxon 11)	1	0	0.1	0.0	0.1
	<i>L. lundbergii</i>	<i>L. shansheni</i> sp. nov. (Taxon 12)	1	0	0.1	0.0	0.1
	<i>G. piceiperda</i>	<i>L. heilongjiangense</i> sp. nov. (Taxon 13)	2	0	0.2	0.0	0.2
		<i>G. piceaperda</i> -D (Taxon 14)	158	28	15.1	2.7	17.8
	<i>L. procerum</i>	<i>L. yichunense</i> sp. nov. (Taxon 15)	4	0	0.4	0.0	0.4
	<i>G. olivacea</i>	<i>G. cucullata</i> (Taxon 16)	13	0	1.2	0.0	1.2
		<i>G. olivacea</i> (Taxon17)	3	0	0.3	0.0	0.3
		<i>L. duchongi</i> sp. nov. (Taxon 18)	1	0	0.1	0.0	0.1
		Group C	<i>L. taigense</i> (Taxon 19)	288	2	27.6	0.2
	<i>G. penicillata</i>	<i>G. fenglinhense</i> sp. nov. (Taxon 20)	1	0	0.1	0.0	0.1
		<i>G. penicillata</i> (Taxon 21)	4	0	0.4	0.0	0.4
Microascales							
<i>Endoconidiophora</i>		<i>E. polonica</i> (Taxon 22)	7	0	0.7	0.0	0.7
<i>Graphium</i>		<i>Gr. fimbriisporum</i> (Taxon 23)	6	2	0.6	0.2	0.8
Total			999	47	95.7	4.5	

Table 2 Isolates of ophiostomatoid fungi obtained from *Ips typographus* in Heilongjiang and Jilin and used in this study.

Taxon	Species	Isolate number ^{1,2}		Host	Locality	GenBank number ³			
		CMW	CBS			ITS/ITS2-LSU	BT	EF	CAL
Ophiostomatales									
1	<i>Ophiostoma floccosum</i>	40505		<i>Picea</i> sp.	Jilin	MH144054	MH124247	MH124332	MH124417
		40518		<i>Picea</i> sp.	Jilin	MH144055	MH124248	MH124333	MH124418
		40519		<i>Picea</i> sp.	Jilin	MH144056	MH124249	MH124334	MH124419
		40525		<i>Picea</i> sp.	Jilin	MH144057	MH124250	MH124335	–
		40526		<i>Picea</i> sp.	Jilin	MH144058	MH124251	MH124336	MH124420
2	<i>O. typographi</i> sp. nov.	44483 ^H	141709	<i>Pinus koraiensis</i>	Heilongjiang	MH144059	MH124252	MH124337	–
		44484	141710	<i>P. koraiensis</i>	Heilongjiang	MH144060	MH124253	MH124338	–
		44586	141711	<i>P. koraiensis</i>	Heilongjiang	–	MH124254	MH124339	–
3	<i>O. wuyingense</i> sp. nov.	44474 ^H	141706	<i>P. koraiensis</i>	Heilongjiang	MH144061	MH124255	MH124340	–
		44475	141753	<i>P. koraiensis</i>	Heilongjiang	MH144062	MH124256	MH124341	–
		44476	141754	<i>P. koraiensis</i>	Heilongjiang	MH144063	MH124257	MH124342	–
4	<i>O. jiamusiensis</i> sp. nov.	40512 ^H	141893	<i>Picea</i> sp.	Jilin	MH144064	MH124258	MH124343	MH124421
	5	<i>O. songshui</i> sp. nov.	44473 ^H	141707	<i>P. koraiensis</i>	Heilongjiang	MH144065	MH124259	MH124344
44602			141708	<i>P. koraiensis</i>	Heilongjiang	MH144066	MH124260	MH124345	–
6	<i>O. ainoae</i>	40496		<i>Picea</i> sp.	Jilin	MH144067	MH124261	MH124346	MH124422
		40511		<i>Picea</i> sp.	Jilin	MH144068	MH124262	MH124347	MH124423
		44496		<i>P. koraiensis</i>	Heilongjiang	MH144069	MH124263	MH124348	–
		44497		<i>P. koraiensis</i>	Heilongjiang	MH144070	MH124264	MH124349	–
		44498		<i>P. koraiensis</i>	Heilongjiang	MH144071	MH124265	MH124350	–
		44499		<i>P. koraiensis</i>	Heilongjiang	MH144072	MH124266	MH124351	–
		44500		<i>P. koraiensis</i>	Heilongjiang	MH144073	MH124267	MH124352	–
		44501		<i>P. koraiensis</i>	Heilongjiang	MH144074	MH124268	MH124353	–
		44502		<i>P. koraiensis</i>	Heilongjiang	MH144075	MH124269	MH124354	–
		44503		<i>P. koraiensis</i>	Heilongjiang	MH144076	MH124270	MH124355	–
7	<i>O. brunneolum</i>	44584		<i>P. koraiensis</i>	Heilongjiang	–	MH124271	MH124356	–
		44477		<i>P. koraiensis</i>	Heilongjiang	MH144077	MH124272	MH124357	–
		44478		<i>P. koraiensis</i>	Heilongjiang	MH144078	MH124273	MH124358	–
		44479		<i>P. koraiensis</i>	Heilongjiang	MH144079	MH124274	MH124359	–
		44480		<i>P. koraiensis</i>	Heilongjiang	MH144080	MH124275	MH124360	–
		44481		<i>P. koraiensis</i>	Heilongjiang	MH144081	MH124276	MH124361	–
		44482		<i>P. koraiensis</i>	Heilongjiang	MH144082	MH124277	MH124362	–
		44467		<i>P. koraiensis</i>	Heilongjiang	–	MH124278	MH124363	–
		44468		<i>P. koraiensis</i>	Heilongjiang	MH144083	MH124279	–	–
		44469		<i>P. koraiensis</i>	Heilongjiang	MH144084	MH124280	–	–
8	<i>O. japonicum</i>	44470		<i>P. koraiensis</i>	Heilongjiang	MH144085	MH124281	MH124364	–
		44592		<i>P. koraiensis</i>	Heilongjiang	MH144086	MH124282	MH124365	–
		44471		<i>P. koraiensis</i>	Heilongjiang	MH144087	MH124283	MH124366	–
		44472		<i>P. koraiensis</i>	Heilongjiang	MH144088	MH124284	MH124367	–
		44597		<i>P. koraiensis</i>	Heilongjiang	MH144089	MH124285	–	–
9	<i>O. bicolor</i>	44598		<i>P. koraiensis</i>	Heilongjiang	MH144090	MH124286	–	–
		44599		<i>P. koraiensis</i>	Heilongjiang	MH144091	MH124287	MH124368	–
		44600		<i>P. koraiensis</i>	Heilongjiang	MH144092	MH124288	–	–
		44601		<i>P. koraiensis</i>	Heilongjiang	MH144093	MH124289	MH124369	–
		40491 ^H	141894	<i>Picea</i> sp.	Jilin	MH144094	MH124290	MH124370	–
		40492	141716	<i>Picea</i> sp.	Jilin	MH144095	MH124291	MH124371	–
		44461 ^H	141898	<i>P. koraiensis</i>	Heilongjiang	MH144096	MH124292	MH124372	–
		44462 ^H	141895	<i>P. koraiensis</i>	Heilongjiang	MH144097	MH124293	MH124373	–
		44456 ^H	141702	<i>P. koraiensis</i>	Heilongjiang	MH144098	MH124294	MH124374	–
		45083	141703	<i>P. koraiensis</i>	Heilongjiang	MH144099	MH124295	MH124375	–
14	<i>Grosmannia piceiperda</i>	40498		<i>Picea</i> sp.	Jilin	MH144100	MH124296	MH124376	–
		40499		<i>Picea</i> sp.	Jilin	MH144101	MH124297	MH124377	–
		40501		<i>Picea</i> sp.	Jilin	MH144102	MH124298	MH124378	–
		40506		<i>Picea</i> sp.	Jilin	MH144103	MH124299	MH124379	–
		40507		<i>Picea</i> sp.	Jilin	MH144104	MH124300	MH124380	–
		40510		<i>Picea</i> sp.	Jilin	MH144105	MH124301	MH124381	–
		40524		<i>Picea</i> sp.	Jilin	MH144106	MH124302	MH124382	–
		40529		<i>Picea</i> sp.	Jilin	MH144107	MH124303	MH124383	–
		40532		<i>Picea</i> sp.	Jilin	MH144108	MH124304	MH124384	–
		44457		<i>P. koraiensis</i>	Heilongjiang	MH144109	MH124305	MH124385	–
15	<i>L. yichunense</i> sp. nov.	44458		<i>P. koraiensis</i>	Heilongjiang	MH144110	MH124306	MH124386	–
		44459		<i>P. koraiensis</i>	Heilongjiang	MH144111	MH124307	MH124387	–
		44460		<i>P. koraiensis</i>	Heilongjiang	MH144112	MH124308	MH124388	–
		44580		<i>P. koraiensis</i>	Heilongjiang	MH144113	MH124309	MH124389	–
		44464 ^H	141705	<i>P. koraiensis</i>	Heilongjiang	MH144114	MH124310	MH124390	MH124424
16	<i>G. cucullata</i>	44465	141752	<i>P. koraiensis</i>	Heilongjiang	MH144115	MH124311	MH124391	MH124425
		44485		<i>P. koraiensis</i>	Heilongjiang	MH144116	MH124312	MH124392	–
		44486		<i>P. koraiensis</i>	Heilongjiang	MH144117	MH124313	MH124393	–
		44487		<i>P. koraiensis</i>	Heilongjiang	MH144118	MH124314	MH124394	–
		44578		<i>P. koraiensis</i>	Heilongjiang	MH144119	MH124315	MH124395	–
17	<i>G. olivacea</i>	44488		<i>P. koraiensis</i>	Heilongjiang	MH144120	MH124316	MH124396	–
		44489		<i>P. koraiensis</i>	Heilongjiang	MH144121	MH124317	MH124397	–
		44455 ^H	141897	<i>P. koraiensis</i>	Heilongjiang	MH144122	MH124318	MH124398	–
18	<i>L. duchongi</i> sp. nov.	40490		<i>Picea</i> sp.	Jilin	MH144123	MH124319	MH124399	–
		40528		<i>Picea</i> sp.	Jilin	MH144124	MH124320	MH124400	–
		44493		<i>P. koraiensis</i>	Heilongjiang	MH144125	MH124321	MH124401	–
		44494		<i>P. koraiensis</i>	Heilongjiang	MH144126	MH124322	MH124402	–
		44495		<i>P. koraiensis</i>	Heilongjiang	MH144127	MH124323	MH124403	–

Table 2 (cont.)

Taxon	Species	Isolate number ^{1,2}		Host	Locality	GenBank number ³			
		CMW	CBS			ITS/ITS2-LSU	BT	EF	CAL
20	<i>G. fenglinhense</i> sp. nov.	44579 ^H	141896	<i>P. koraiensis</i>	Heilongjiang	MH144128	MH124324	MH124404	–
21	<i>G. penicillata</i>	44490		<i>P. koraiensis</i>	Heilongjiang	MH144129	MH124325	MH124405	–
		44491		<i>P. koraiensis</i>	Heilongjiang	MH144130	MH124326	MH124406	–
		44492		<i>P. koraiensis</i>	Heilongjiang	MH144131	MH124327	MH124407	–
		44581		<i>P. koraiensis</i>	Heilongjiang	MH144132	MH124258	MH124369	–
Microascales									
22	<i>Endoconidiophora polonica</i>	43236		<i>P. koraiensis</i>	Heilongjiang	MH144133	MH124328	MH124408	–
		43237		<i>P. koraiensis</i>	Heilongjiang	MH144134	MH124329	MH124409	–
		43238		<i>P. koraiensis</i>	Heilongjiang	MH144135	MH124330	MH124410	–
		43239		<i>P. koraiensis</i>	Heilongjiang	MH144136	MH124331	MH124411	–
23	<i>Graphium fimbriisporum</i>	40517		<i>Picea</i> sp.	Jilin	–	–	–	–
		43240		<i>P. koraiensis</i>	Heilongjiang	MH144137	–	MH124412	–
		43241		<i>P. koraiensis</i>	Heilongjiang	MH144138	–	MH124413	–
		43242		<i>P. koraiensis</i>	Heilongjiang	MH144139	–	MH124414	–
		43243		<i>P. koraiensis</i>	Heilongjiang	MH144140	–	MH124415	–
		44504		<i>P. koraiensis</i>	Heilongjiang	MH144141	–	MH124416	–

¹ The culture collection (CBS) of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

² H = ex-holotype isolate.

³ ITS = internal transcribed spacer regions 1 and 2 of the nuclear ribosomal DNA operon, including the 5.8S region; ITS2-LSU = the internal transcribed spacer 2 region and partial large subunit of the nrDNA operon; BT = beta-tubulin; EF = translation elongation factor 1- α ; CAL = Calmodulin.

compared their diversity with those in Japan and Europe. Based on these comparisons, we considered whether the fungal assemblages reflect the different geographical populations of the beetle.

MATERIALS AND METHODS

Collection of beetles and isolation of fungi

Ips typographus was sampled in June 2010 in Jilin province, and in August 2014 in Heilongjiang province, China. Galleries from trees infested with *I. typographus* adults were collected and stored individually in sealable bags. Adult *I. typographus* were individually placed in Eppendorf tubes. Both adult beetles and galleries were stored at 4 °C until fungal isolations were made.

Beetles were crushed on the surface of 2 % malt extract agar (MEA) that contained 0.05 % streptomycin. In addition, fungal mycelium and/or spore masses from *I. typographus* galleries were transferred to MEA medium. Where no mycelium was visible, galleries were incubated in humid chambers in the dark at 25 °C and inspected every 2 d for fungal structures or mycelial masses. When mycelia and/or spore masses appeared during the incubation period, these were transferred to MEA plates amended with streptomycin. All isolates used in this study were deposited in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa. Isolates representing types of new species were also deposited in the culture collection (CBS) of Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

DNA extraction, PCR and sequencing

All the isolates were initially grouped based on morphological characters and only representative isolates of these groups were sequenced. DNA was extracted following the method used by Linnakoski et al. (2010). The internal transcribed spacer (ITS) regions (ITS1 and ITS2, including the 5.8S gene), parts of the β -tubulin (BT) and the elongation factor 1- α (EF) genes were amplified and sequenced for taxa residing in the *Ophiostoma* s.l. For fungal taxa in *Leptographium* s.l., the BT, calmodulin (CAL) and EF gene regions were also amplified, as well as ITS2 and part of the ribosomal large subunit 28S (ITS2-LSU).

The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the ITS1-ITS2 region, and Bt2a and Bt2b (Glass & Donaldson 1995) were used for the BT region. T10 (O'Donnell & Cigelnik 1997) was used in place of Bt2a when initial amplification with Bt2a and Bt2b primer combination failed. Primers EF2F (Marincowitz et al. 2015) and EF2R (Jacobs et al. 2004) were used to amplify the EF region, and ITS3 and LR3 (White et al. 1990) were used for the ITS2-LSU region. CL2F and CL2R (Duong et al. 2012) were used for amplifying the CAL.

The PCR reaction mixture consisted of 2.5 μ L 10 $\times$ KAPA Taq Buffer A, 0.5 U KAPA Taq DNA Polymerase, 1.0 μ L dNTP mix (10 mM), 0.5 μ L of each primer (10 mM) and PCR grade water to the final volume of 25 μ L. PCR conditions were as follows: an initial denaturation step at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min and a final elongation step at 72 °C for 10 min. PCR products were cleaned using EXO-SAP (Exonuclease I - Shrimp Alkaline Phosphatase, Thermo, USA) following the manufacturer's recommendations.

The primers used for the sequencing PCR were the same as those used for PCR. The reaction mixture included 0.5 μ L of BigDye Terminator v. 3.1 ready reaction mixture (Applied Biosystems, Foster City, California, USA), 2 μ L sequencing buffer, 1 μ L of either forward or reverse primer, 1 μ L cleaned PCR product and 5.5 μ L of PCR grade water. The reaction conditions were: 30 cycles at 95 °C for 10 s, 55 °C for 5 s and 4 min at 60 °C. Sequencing products were cleaned using ethanol/salt precipitation. Sequencing products were analysed on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Phylogenetic analyses

Geneious Pro v. 7.1.7 (Biomatters, Auckland, New Zealand) with Geneious Alignment was used to construct consensus sequences from forward and reverse reads. Preliminary identification of the isolates was made using the BLAST tool on GenBank (<http://blast.ncbi.nlm.nih.gov>). Data sets were compiled based on the species complex or genera based on BLAST analyses. Sequence alignments were done online with MAFFT v. 7 (Katoh & Standley 2013).

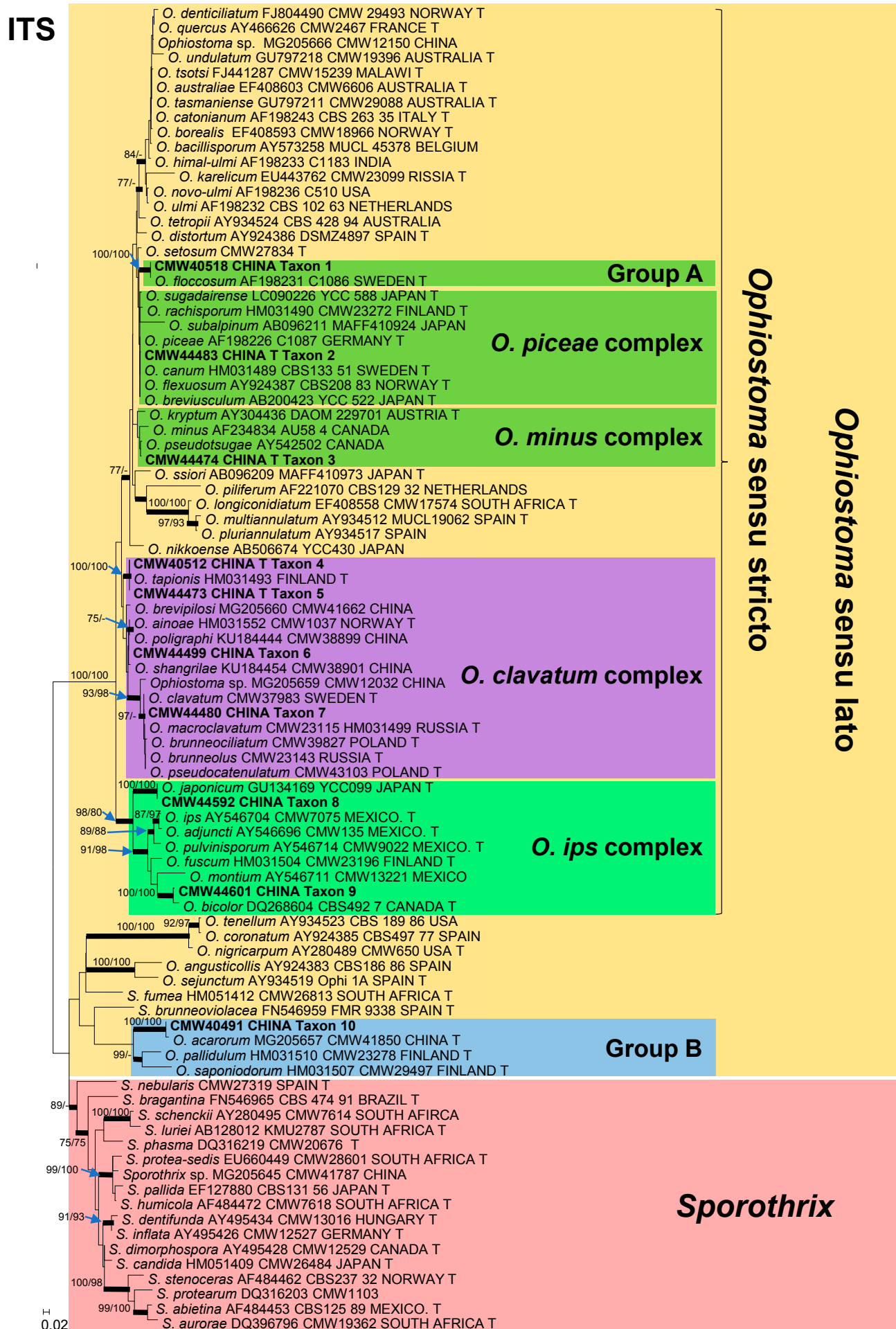


Fig. 1 ML tree of *Ophiostoma* s.l. and *Sporothrix* generated from the ITS DNA sequence data. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes. T = ex-type isolates.

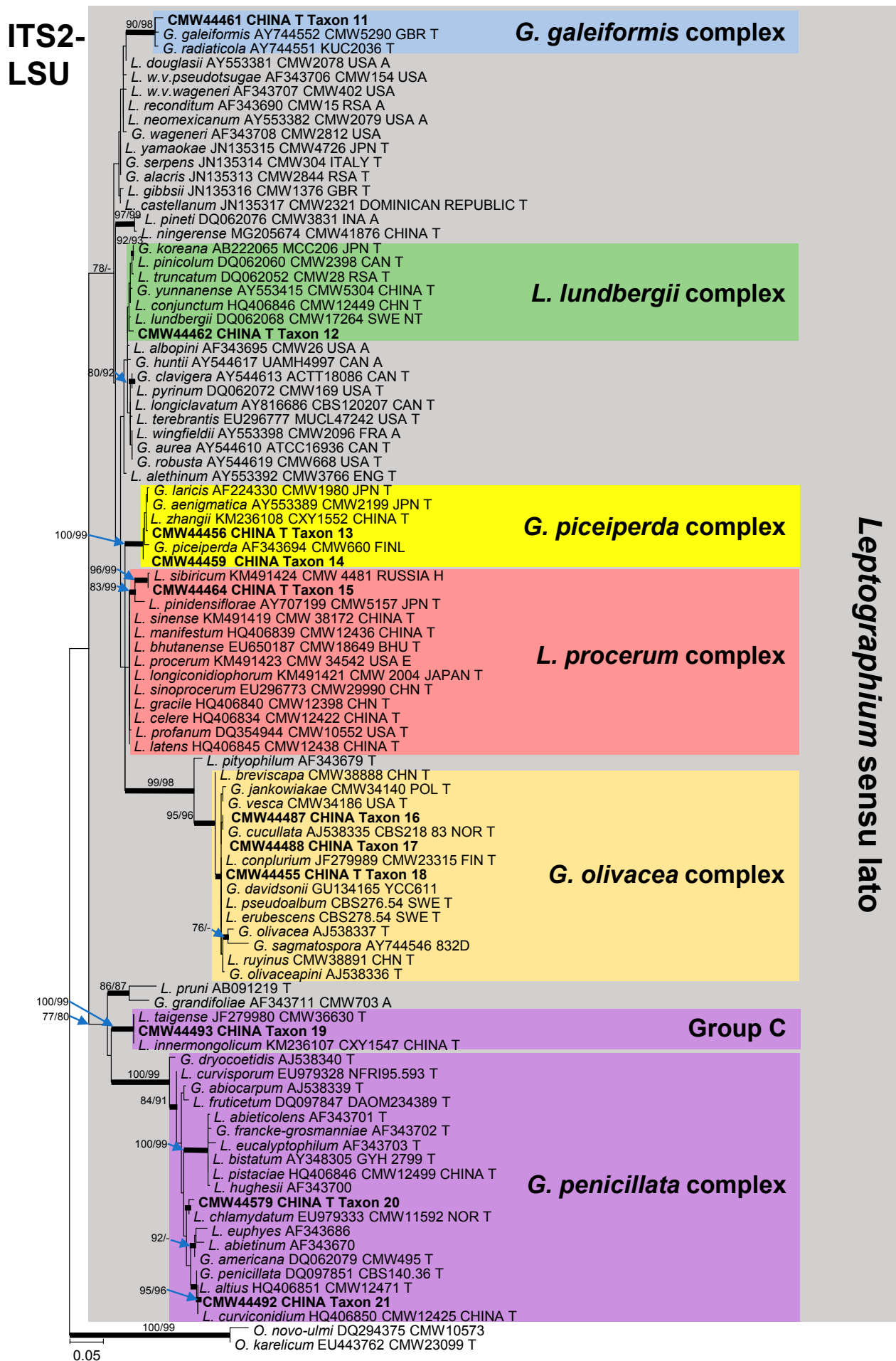


Fig. 2 ML tree of *Leptographium* s.lat. generated from the ITS2-LSU DNA sequence data. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes. T = ex-type isolates.



Fig. 3 ML tree of *Ophiostoma piceae*, *O. minus* complex and Group A generated from DNA sequences of *BT* and *EF* regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

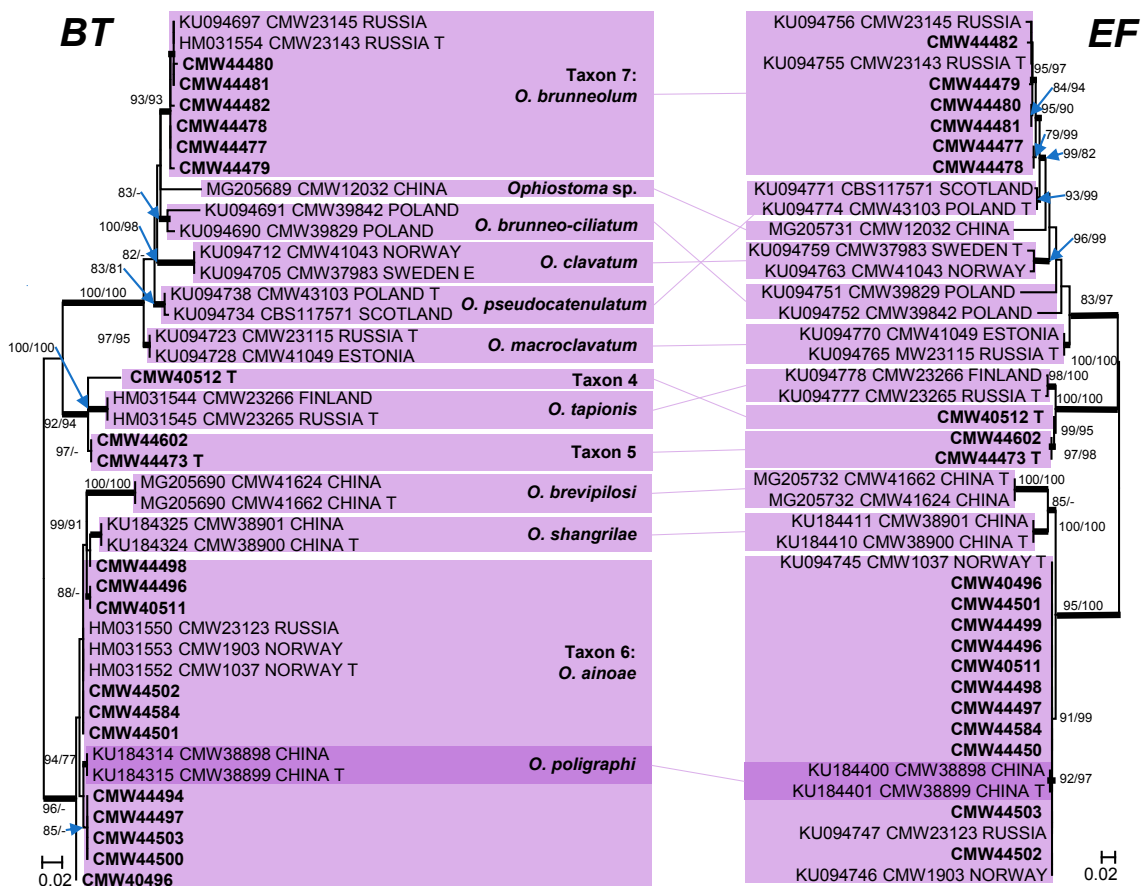


Fig. 4 ML tree of *Ophiostoma clavatum* complex generated from DNA sequences of *BT* and *EF* regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

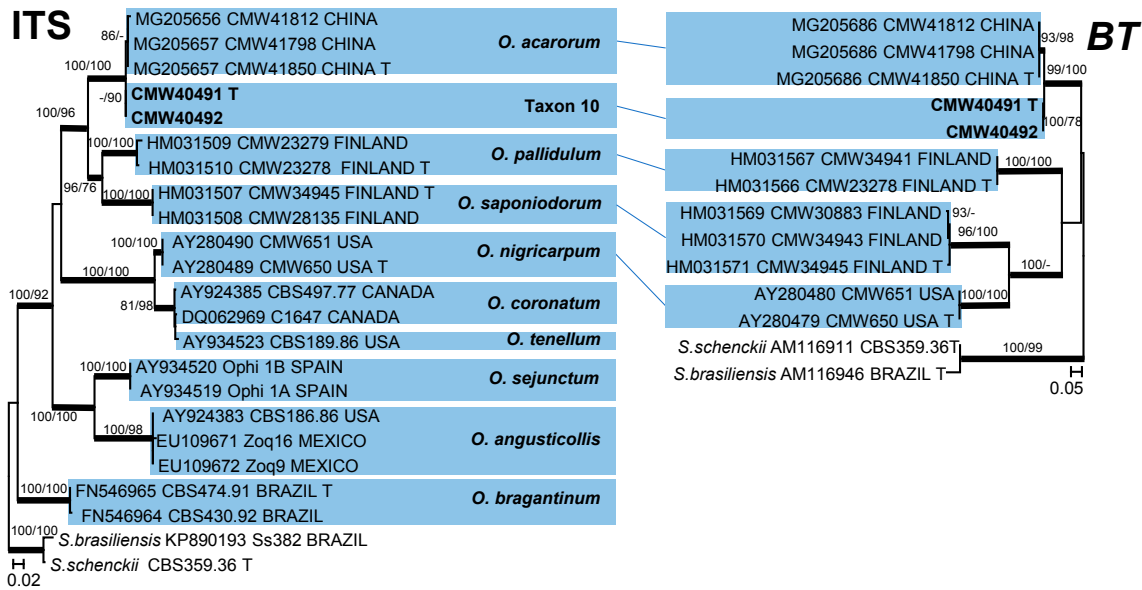


Fig. 5 ML tree of Group B generated from DNA sequences of ITS and BT regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

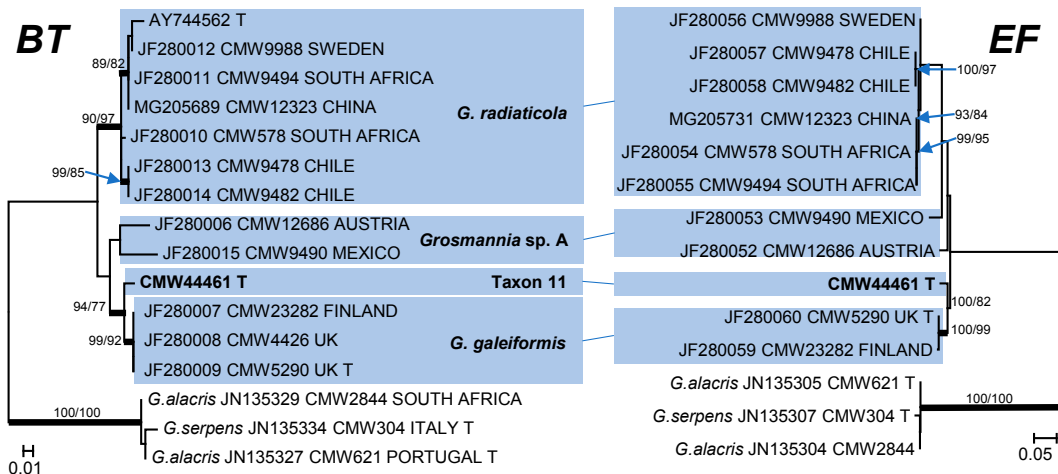


Fig. 6 ML tree of *G. galeiformis* complex generated from DNA sequences of BT and EF regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

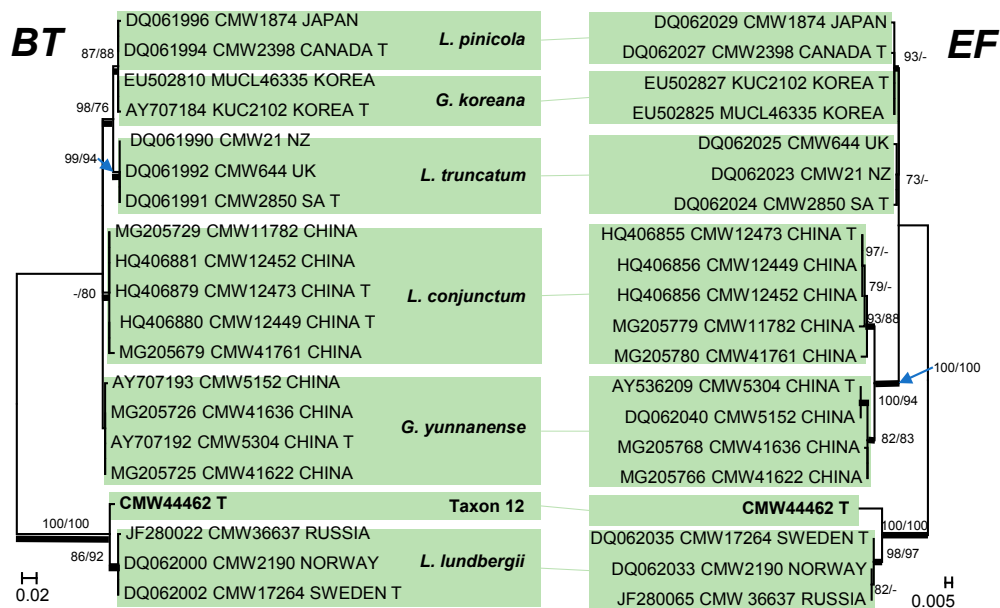


Fig. 7 ML tree of *L. lundbergii* complex generated from DNA sequences of BT and EF regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

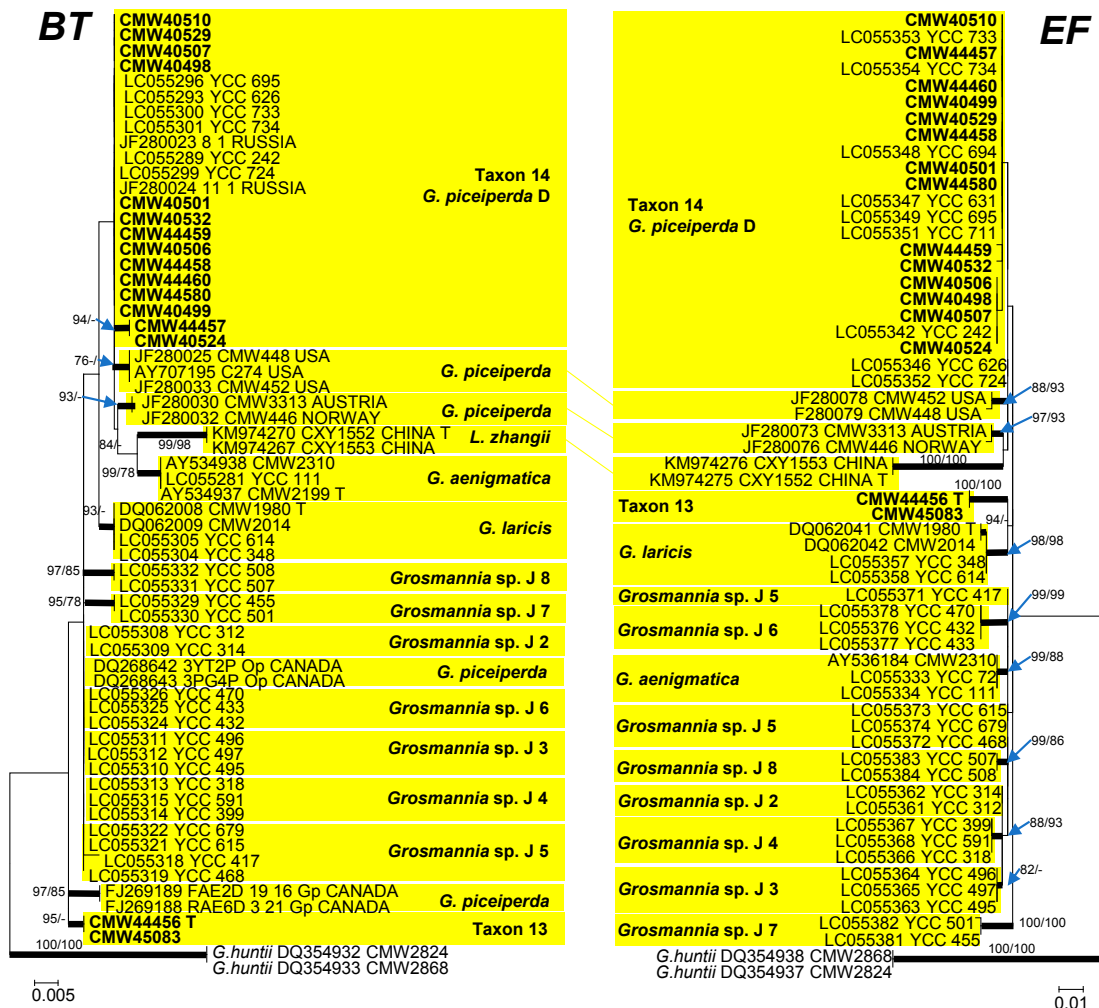


Fig. 8 ML tree of *G. piceiperda* complex generated from DNA sequences of *BT* and *EF* regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

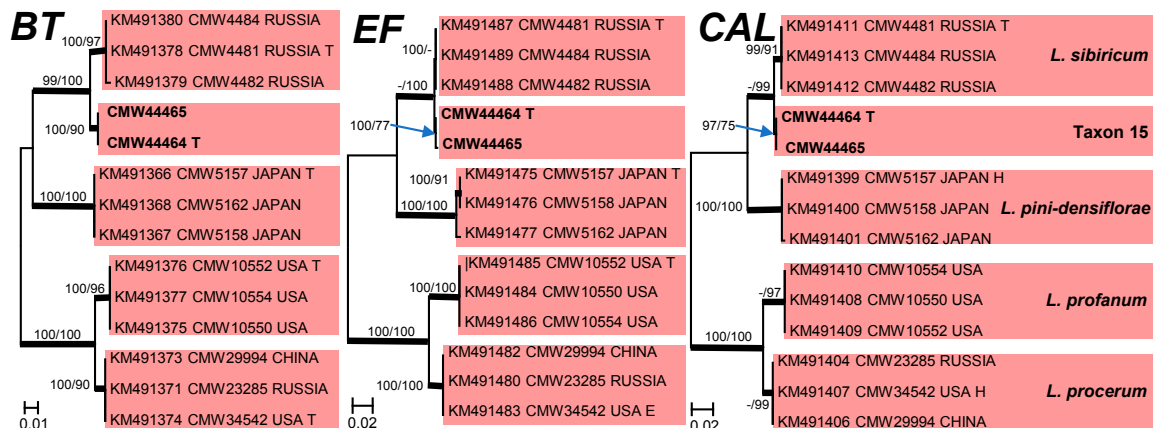


Fig. 9 ML tree of *L. procerum* complex generated from DNA sequences of *BT*, *EF* and *CAL* regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

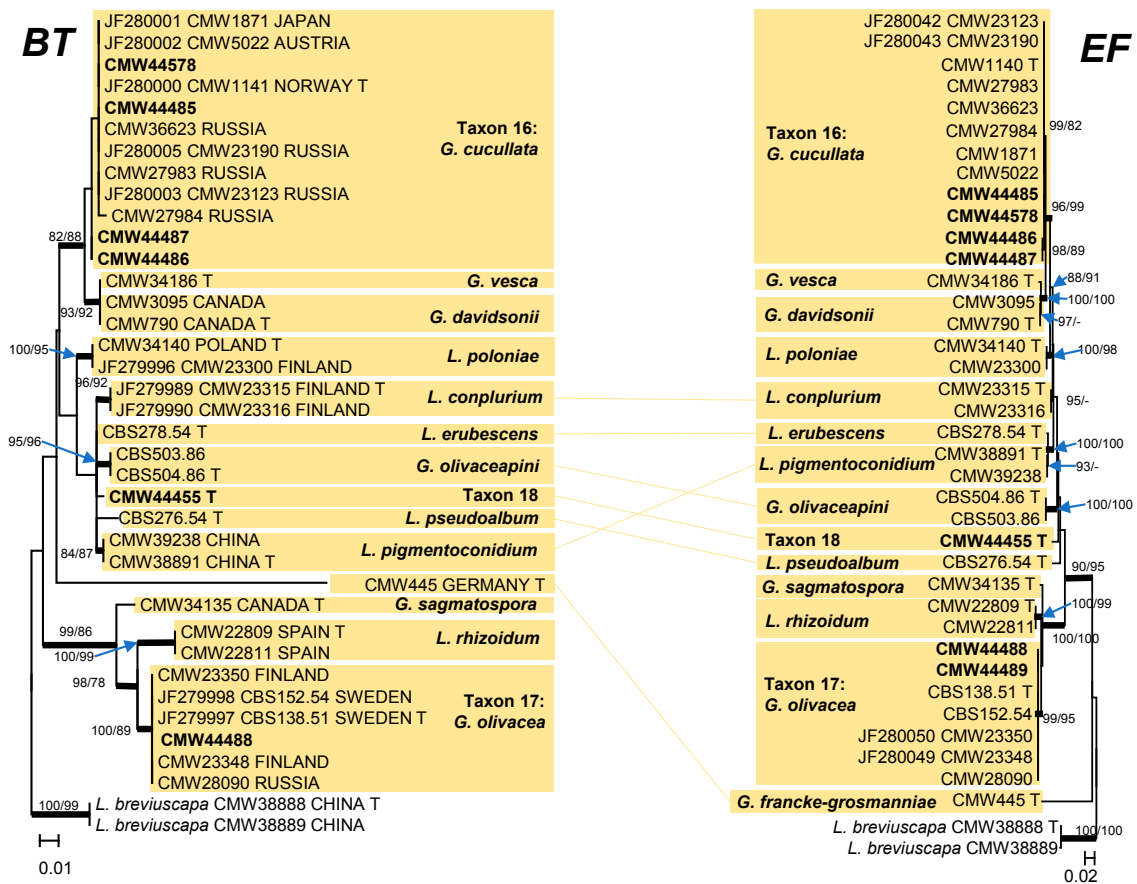


Fig. 10 ML tree of *G. olivacea* complex generated from DNA sequences of BT and EF regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

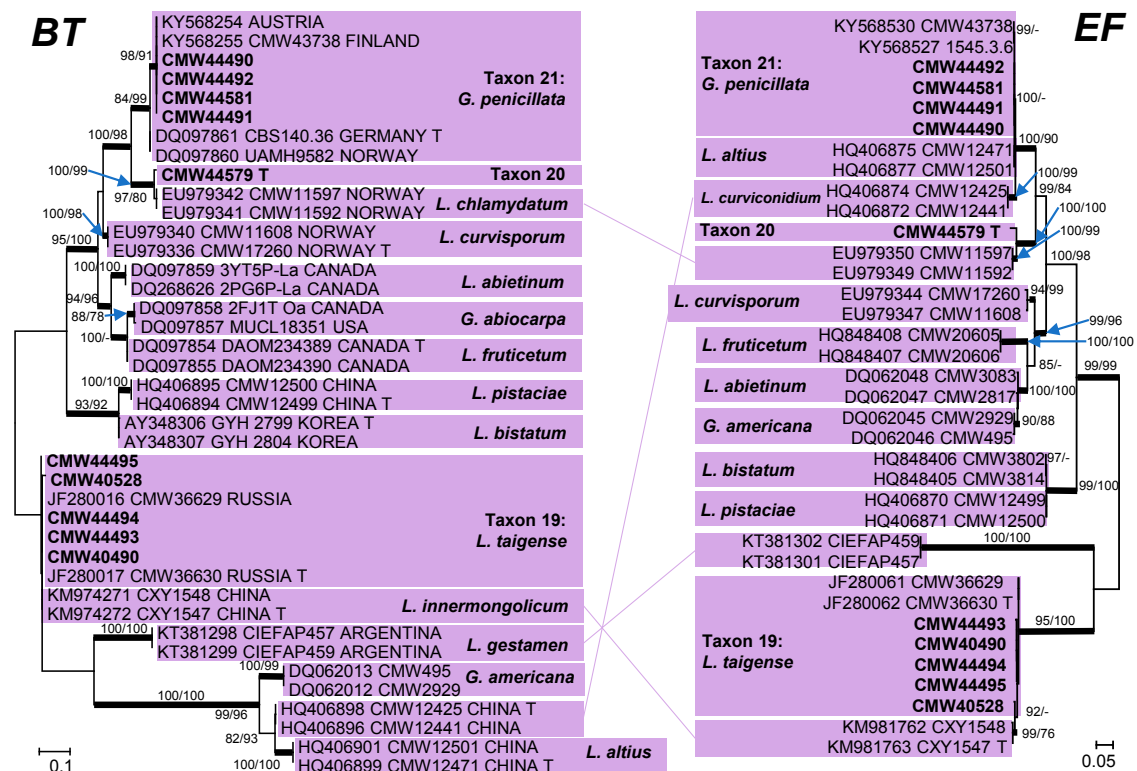


Fig. 11 ML tree of Group C and *G. penicillata* complex generated from DNA sequences of BT and EF regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Phylogenetic analyses were conducted using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI). ML analyses were conducted using RaxML v. 8.2.4 (Stamatakis 2014) on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The best-scoring ML tree searching with GTR substitution matrix and a 1000 rapid bootstrap analysis were conducted. MP analyses were performed using PAUP v. 4.0b10 (Swofford 2002). Gaps were treated as a fifth character. The best substitution models for each data set were determined using jModelTest v. 2.1.6 (Darriba et al. 2012) on the CIPRES Science Gateway v. 3.3. BI analyses were conducted using MrBayes v. 3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway v. 3.3. Four MCMC chains were run from a random starting tree for five million generations, trees were sampled every 100 generations. Twenty five percent of trees sampled were discarded as burn-in and the remaining trees were used to construct majority rule consensus trees.

Comparisons of species from China, Europe and Japan

Results from the present study were compared with those from previously published studies from China (Paciura et al. 2010, Zhou et al. 2013), Japan (Yamaoka et al. 1997, Masuya et al. 2013) and Europe (Kirisits 2004, Viiri & Lieutier 2004, Jankowiak 2005, Jankowiak & Hilszczanski 2005, Linnakoski et al. 2010, 2012a, b, 2016b). A summary of these comparisons are presented in Table 3.

RESULTS

Collection of beetles and isolation of fungi

In total, 1046 fungal isolates were obtained from beetles and galleries of *Ips typographus* (Table 1). Of these, 999 isolates were from Heilongjiang province and 47 isolates were from Jilin province.

Phylogenetic analyses

DNA sequence data for 92 isolates, representing all the morphological groups were generated (Table 2). Blast analyses of the ribosomal DNA sequences placed isolates in *Ophiostoma* s.lat., *Leptographium* s.lat., *Endoconidiophora* and *Graphium*. In total, 1031 isolates were species of *Ophiostomatales* and only 15 isolates resided in the *Microascales* (Table 1). Based on phylogenetic analyses of the ITS (Fig. 1), ITS2-LSU (Fig. 2), *BT*, *CAL* and *EF* gene regions (Fig. 3–11, Appendix 1–3), the isolates represented 23 species, which included ten *Ophiostoma* s.lat. (Taxa 1–10), 11 *Leptographium* s.lat. species (Taxa 11–21), and one species each of *Endoconidiophora* (Taxon 22) and *Graphium* (Taxon 23).

Ophiostoma s.lat.

The majority of isolates in *Ophiostoma* s.lat. resided in four species complexes, while two taxa grouped outside any currently recognized species complex (Fig. 1). These are treated in Groups A and B below.

Taxon 1 included eight isolates from Jilin province, five of which were included in the analyses. In the ITS tree, this taxon grouped peripheral to the *O. piceae* complex in Group A (Fig. 1), together with the ex-type isolate of *O. floccosum*. Also in the *BT* and *EF* trees (Fig. 3), these isolates formed a monophyletic clade with authenticated isolates of *O. floccosum*. Taxon 2 grouped in the *O. piceae* complex (Fig. 1). In the *BT* and *EF* trees (Fig. 3), these isolates form a well-supported lineage, distinct from *O. piceae* and all the other known species in the complex. Taxon 3 grouped in the *O. minus* complex (Fig. 3) and included three isolates forming a well-supported lineage

distinct from *O. minus* and *O. pseudotsugae* in both *BT* and *EF* trees (Fig. 3).

Taxa 4–7 included a total 70 isolates and belonged in the *O. clavatum* complex (Fig. 1; Table 1). Taxon 4 included only one isolate from *Picea* in Jilin, together with Taxon 5 that included two isolates collected from *Pinus koraiensis* in Heilongjiang. These two taxa grouped with the ex-type isolate of *O. tapioinis* based on ITS (Fig. 1). However, both these taxa represented novel species in the phylogenetic analyses based on *BT* and *EF* data (Fig. 4). Taxon 6 included 11 isolates. In the *BT* tree, these isolates separated into several smaller clades and grouped with isolates of both *O. ainoae*, and *O. poligraphi*. However, the *EF* data showed that they formed part of only one monophyletic clade, suggesting that Taxon 6 represented *O. ainoae*. Six of 47 isolates of Taxon 7 were used in the analyses. *BT* and *EF* data confirmed that those isolates all represented *O. brunneolum* (Fig. 4).

There was a total of 438 isolates belonging to the *Ophiostoma ips* complex (Fig. 1; Table 1). These included 15 of *O. japonicum* (Taxon 8), and 423 that were conspecific with *O. bicolor* (Taxon 9). Five of the *O. japonicum* isolates and seven of the *O. bicolor* isolates were included in the analyses. ITS and *BT* data confirmed that those isolates represented *O. japonicum* and *O. bicolor* (Appendix 1).

Two isolates (Taxon 10) formed a lineage with *O. acarorum*, closely related to *O. pallidulum* and *O. saponiodorum*, apart from any currently defined *Ophiostomatales* species complex and labelled here as Group B (Fig. 1). Analyses of ITS and *BT* gene regions confirmed that these isolates from Jilin represented an undescribed species (Fig. 5).

Leptographium s.lat.

Isolates from *I. typographus* belonging to *Leptographium* s.lat. resided in six currently recognized species complexes (Fig. 2). An exception was found for Taxon 19 that grouped outside any defined species complex in a lineage defined here as Group C.

One isolate (Taxon 11) grouped in the *G. galeiformis* complex, distinct from other known species based on *BT* and *EF* data (Fig. 2, 6). Another single isolate resided in the *L. lundbergii* complex (Taxon 12). It grouped close to, but distinct from the ex-type isolate of *L. lundbergii* in the *BT* and *EF* trees (Fig. 7), suggesting that this represented an undescribed species.

Taxon 13 grouped together with Taxon 14 in the *G. piceiperda* complex (Fig. 2; Table 1). The two isolates of Taxon 13 from Heilongjiang formed a well-supported lineage in both *BT* and *EF* trees (Fig. 8) distinct from all other species in the complex. Taxon 14 included 186 isolates from Jilin and Heilongjiang provinces (Table 1) of which 15 were used in the analyses. In both *BT* and *EF* trees (Fig. 8), Taxon 14 grouped with *G. piceiperda* D as defined by Ando et al. (2016).

Two of the four isolates in Taxon 15 were included in the analyses and formed part of the *L. procerum* complex (Fig. 2). These two isolates formed a well-supported clade, closest to but clearly distinct from *L. sibiricum* in the *BT*, *EF* and *CAL* trees (Fig. 9).

Isolates from China collected in the present study grouped in the *Grosmannia olivacea* complex and were comprised of three taxa, Taxa 16, 17 and 18 (Fig. 2, 10). Four of 13 isolates (Table 1) representing Taxon 16 were used in the analyses. *BT* and *EF* data confirmed that these isolates grouped with ex-type isolate of *G. cucullata* (Fig. 10). Taxon 17 isolates grouped with ex-type isolate of *G. olivacea* in *BT* and *EF* trees (Fig. 10). One isolate (Taxon 18) grouped closely with *G. olivacea-pini* and *L. album* in the *BT* tree (Fig. 10), but in the *EF* tree, Taxon 18 as well as *G. olivacea-pini* and *L. album* formed separate clades, suggesting that Taxon 18 represented an undescribed species.

Group C (Fig. 2) included 290 isolates (Taxon 19; Table 1), five of which were used in our analyses. In the *BT* tree (Fig. 11), these isolates grouped with *L. taigense* and the newly described species, *L. innermongolicum*, but these could not be separated from each other. In the *EF* tree, our isolates again grouped with *L. taigense*, while the two *L. innermongolicum* sequences formed a well-supported lineage (Fig. 11). We thus treat the Taxon 19 isolates as conspecific with *L. taigense*.

The remaining isolates in *Leptographium* resided in the *G. penicillata* complex (Fig. 2). Taxon 20 was represented by a single isolate that was closest to *L. chlamydatum*, but distinct from this species based on the *BT* and *EF* data (Fig. 11). Four isolates (Taxon 21) formed a clade with *G. penicillata* isolates in both the *BT* and *EF* analyses (Fig. 11). The *BT* sequences for these isolates had 4 bp differences with the ex-type isolate of *G. penicillata*, but the sequences were the same as isolates from Austria and Finland. The *EF* sequences had 3 bp differences with those from Austria and Finland. The *EF* sequence for the ex-type isolate of *G. penicillata* was not available for study and our isolates could thus not be confirmed to be different from *G. penicillata*.

Seven isolates from Heilongjiang grouped in the genus *Endoconidiophora*. Four of these isolates were included in the an-

alyses (Appendix 2). The ITS, *BT* and *EF* sequences confirmed that these isolates were of *E. polonica*.

Eight isolates of which six were from Heilongjiang and two from Jilin, resided in *Graphium*. Analyses of the ITS and *EF* sequences of these six isolates showed that they were identical to those of the ex-type isolate of *Gr. fimbriisporum* (Appendix 3).

At the genus level, most of the isolates from China belonged to *Ophiostoma* s.lat. and *Leptographium* s.lat. and were represented by similar numbers of isolates. The three most frequently isolated taxa were *O. bicolor* (Taxon 9), representing 40.5 % of all isolates, *L. taigense* (Taxon 19), representing 27.8 %, and *G. piceiperda* D (Taxon 14), representing 17.8 % of all isolates. Five of the taxa were collected only once in the study. These include Taxa 4, 11, 12, 18 and 20. Six species were found in both Heilongjiang and Jilin provinces, while 14 species were found in Heilongjiang province only, and three species only in Jilin province (Table 1).

Taxonomy

Eleven of 23 taxa obtained in this study were of undescribed species. These included five *Ophiostoma* spp. and six *Leptographium* spp. They are described as follows:

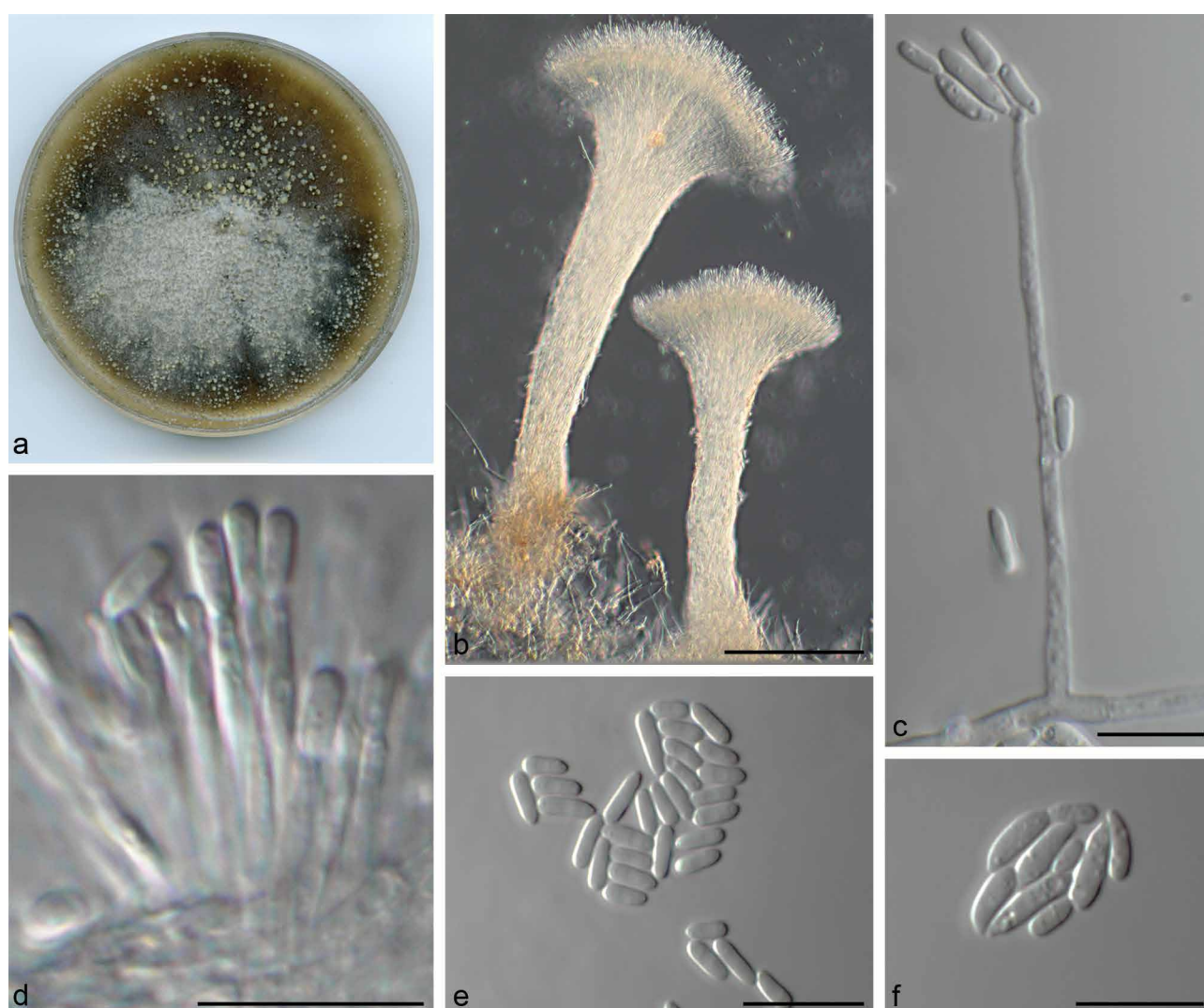


Fig. 12 Morphological characters of asexual structures of *Ophiostoma typographi* sp. nov. (Taxon 2). a. Fourteen-d-old cultures on MEA; b. pesotum-like asexual morph; c. sporothrix-like asexual morph and conidia; d. conidiogenous cells of pesotum-like macronematal asexual morph; e. conidia of pesotum-like asexual morph; f. conidia of sporothrix-like asexual morph. — Scale bars: b = 100 μ m; c–f = 10 μ m.

Taxon 2

Ophiostoma typographi R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825082; Fig. 12

Etymology. The name refers to the bark beetle vector of this species, *Ips typographus*.

Colonies dark brown. Mycelium superficial on the agar, white. Optimal temperature for growth 25 °C, reaching full plates in 14 d. No growth observed at 5 °C and 35 °C. *Sexual morph* not observed. *Asexual morph* synnematosus, macronematous, erect, (281–)370–848(–1182) × (32–)37–90(–128) µm. *Conidia* hyaline, 1-celled, smooth, oblong, clavate or obovoid

(3.5–)4–4.5(–5.5) × (1–)1.5(–2) µm. Sporothrix-like asexual morph, erect, arising directly from the mycelium, conidiophore (16–)25–51.5(–69) × (32.5–)37–90(–128.5). *Conidia* hyaline, 1-celled, smooth, oblong, clavate or obovoid (4–)5–8.5(–13) × (1.3–)1.5–1.8(–2) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from the gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61565 (herbarium specimen of dried culture), CMW 44483 = CBS 141709 (ex-holotype culture); Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61566 (herbarium specimen of dried culture), CMW 44484 = CBS 141710 (ex-paratype culture); Heilongjiang province,

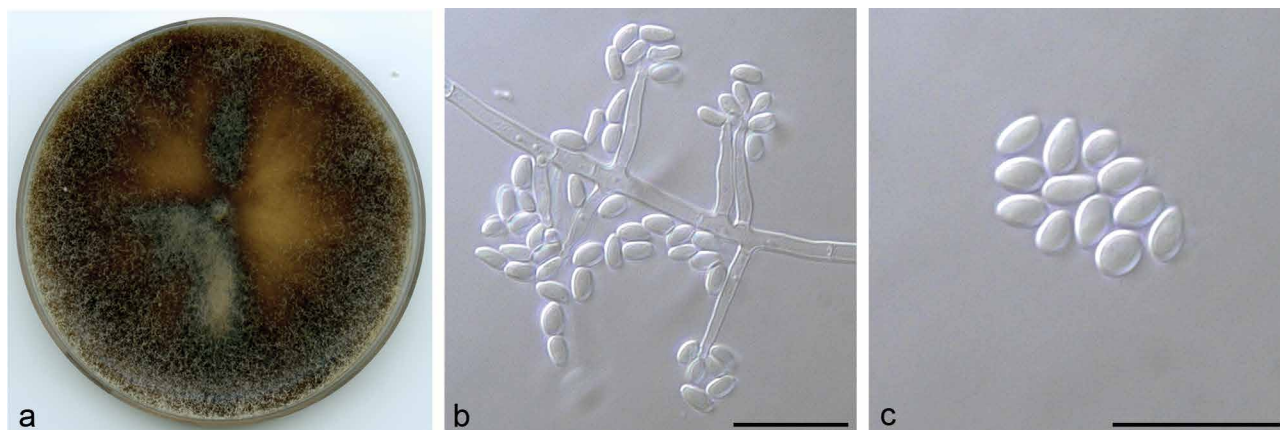


Fig. 13 Morphological characters of asexual structures of *Ophiostoma wuyingense* sp. nov. (Taxon 3). a. Fourteen-d-old cultures on MEA; b–c. hyalorhinocladia-like asexual morph and conidia. — Scale bars: b–c = 10 µm.

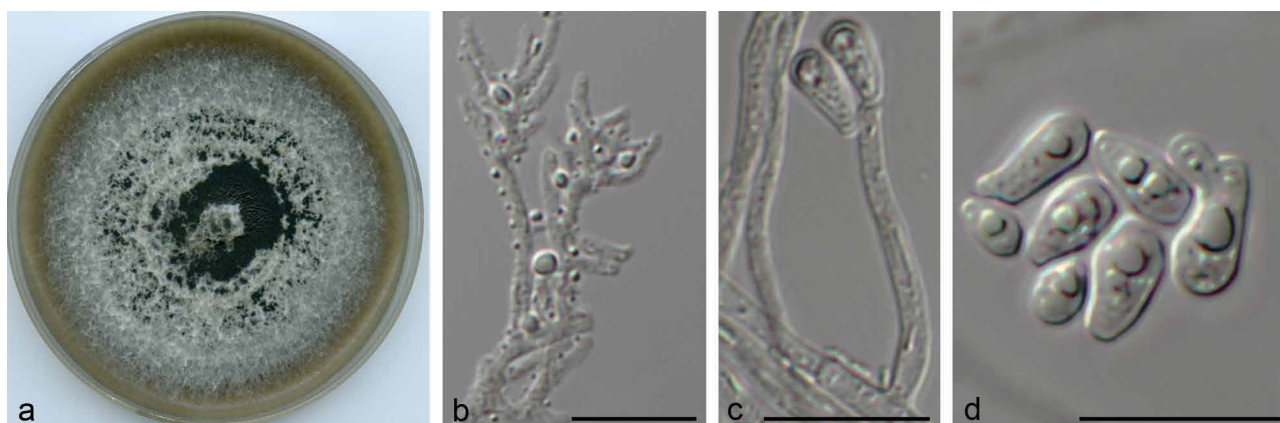


Fig. 14 Morphological characters of asexual structures of *Ophiostoma jiamusiensis* sp. nov. (Taxon 4). a. Thirty-d-old cultures on MEA; b. hyphae; c–d. hyalorhinocladia-like asexual morph and conidia. — Scale bars: b–d = 10 µm.

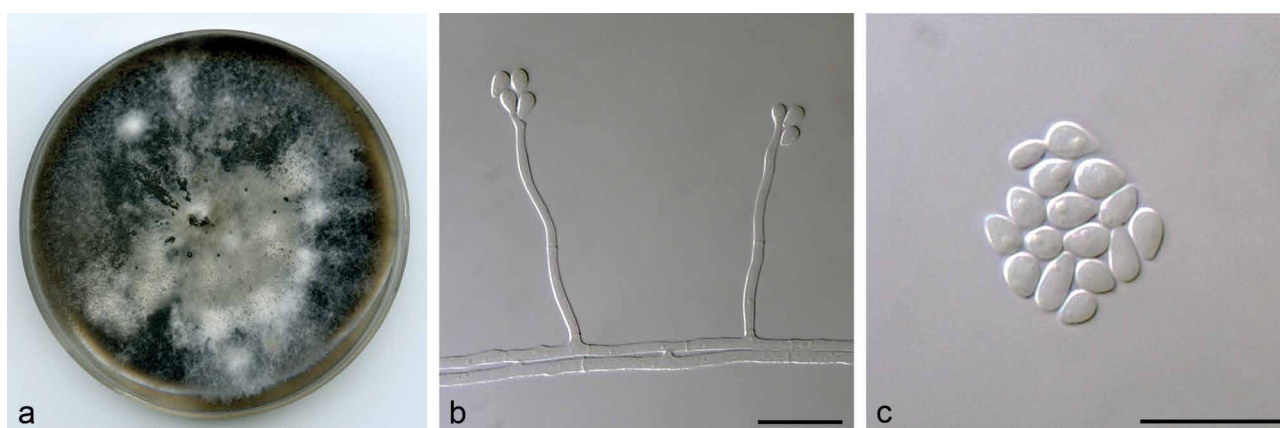


Fig. 15 Morphological characters of asexual structures of *Ophiostoma songshui* sp. nov. (Taxon 5). a. Fourteen-d-old cultures on MEA; b–c. hyalorhinocladia-like asexual morph and conidia. — Scale bars: b–c = 10 µm.

Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61567 (herbarium specimen of dried culture), CMW 44586 = CBS 141711 (ex-paratype culture).

Notes — Both pesotum-like and sporothrix-like asexual morphs were present. *Ophiostoma typographi* resembles *O. breviusculum* (Chung et al. 2006) that is one of its close relatives. However, the DNA sequences of *BT* and *EF* (Fig. 3) clearly suggested that *O. typographi* is distinct from *O. breviusculum*, *O. piceae* and *O. brunneum*.

Including *O. typographi* there are 10 species now described in the newly defined *O. piceae* complex (Yin et al. 2015), and all of these occur on conifers (Chung et al. 2006, Yin et al. 2016). Species in this complex with sexual morphs are all characterised by unsheathed, allantoid ascospores and most produce pronounced pesotum-like synnemata and sporothrix-like asexual morphs (Yin et al. 2016).

Taxon 3

Ophiostoma wuyingense R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825083; Fig. 13

Etymology. The name refers to Wuying district where this fungus was collected.

Colonies hyaline to dark brown. White aerial mycelium superficial on agar. Colonies flat on 2 % MEA. Optimal temperature for growth 25–30 °C, reaching 90 mm diam in 4 d. No growth observed at 5 °C and 35 °C. **Sexual morph** not observed. **Asexual morph** hyalorhinocladia-like, erect, arising directly from mycelium (6–)8.5–14.5(–21) × (1–)1.5(–2) µm. **Conidia** hyaline, 1-celled, smooth, oblong, clavate (2.5–)3–3.5(–4) × (1–)1.5–3(–4) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61552 (herbarium specimen of dried culture), CMW 44474 = CBS 141706 (ex-holotype culture); Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61554 (herbarium specimen of dried culture), CMW 44476 = CBS 141754 (ex-paratype culture); Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61553 (herbarium specimen of dried culture), CMW 44475 = CBS 141753 (ex-paratype culture).

Notes — The growth rate of *O. wuyingense* (22.5 mm/d) is more rapid than those of its closest relatives (*O. pseudotsugae*, 7.3 mm/d and *O. kryptum*, 4.3 mm/d) (Gorton & Webber 2000, Jacobs & Kirisits 2003).

Ophiostoma wuyingense, together with *O. kryptum*, *O. minus* and *O. pseudotsugae* reside in the *O. minus* complex, which is close to the *O. piceae* complex. Species in this complex are characterized by ascomata with short necks, elongated ascospores and hyalorhinocladia-like asexual morphs (Linnakoski et al. 2010). All the species in the *O. minus* complex are found on conifers (Gorton et al. 2004).

Taxon 4

Ophiostoma jiamusiensis R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825084; Fig. 14

Etymology. Name refers to Jiamusi, the city where the samples were collected.

Colonies hyaline without asexual structures or dark brown when asexual structures form. Mycelium superficial on the agar. Colonies slow growing, reaching 46 mm diam in 10 d at 20 °C. Growth reduced at 5 °C. Growth not observed at 30 °C and 35 °C. Optimal temperature for growth 20 °C. **Sexual morph**

not observed. **Asexual morph**, hyalorhinocladia-like erect, arising directly from the mycelium. **Conidia** hyaline, 1-celled, smooth, oblong, clavate or obovoid (3.5–)4.5–6.5(–8) × (1.5–)2–3(–3.5) µm.

Specimens examined. CHINA, Jilin province, Jiamusi, from gallery of *Ips typographus* on *Pinus koraiensis*, June 2010, X.D. Zhou, holotype PREM 61570 (herbarium specimen of dried culture), CMW 40512 = CBS 141893 (ex-holotype culture).

Notes — See comparisons between *O. jiamusiensis*, *O. songshui* and *O. tapionis* below the description of *O. songshui* (Taxon 5).

Taxon 5

Ophiostoma songshui R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825085; Fig. 15

Etymology. Name refers to the Chinese name (songshu) of pine, the tree host from which this fungus was isolated.

Colonies dark brown to black with white aerial mycelium, superficial on agar. No growth observed at 5 °C and 35 °C. Optimal temperature for growth 25 °C, reached 90 mm diam in 14 d. **Sexual morph** not observed. **Asexual morph**, hyalorhinocladia-like erect, arising directly from the mycelium (8.5–)9–151(–450) × (1.5–)2.5–3(–4) µm. **Conidia** hyaline, 1-celled, smooth, oblong, clavate or obovoid (3–)4.5(–5.5) × (1.5–)2–2.5(–3) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61555 (herbarium specimen of dried culture), CMW 44473 = CBS 141707 (ex-holotype culture); Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, PREM 61556 (herbarium specimen of dried culture), CMW 44602 = CBS 141708 (ex-paratype culture).

Notes — The hyalorhinocladia-like asexual morph of *O. songshui* resembles those of *O. jiamusiensis* and *O. tapionis* (Linnakoski et al. 2010), its two closest relatives based on phylogeny. These three species are best distinguished from one another using *BT* and *EF* sequences (Fig. 4).

The recently defined *O. clavatum* complex (Linnakoski et al. 2016a) accommodates 13 species, which are all vectored by conifer-infesting bark beetles, most notably of beetles in the genus *Ips* (Linnakoski et al. 2016a, Yin et al. 2016, Chang et al. 2017). Only five species in this complex are known to have sexual morphs. These are characterized by brown, spirally coiled ostiolar hyphae and cylindrical to rectangular ascospores, sometimes covered by a thin sheath (Linnakoski et al. 2016a). The asexual morphs include hyalorhinocladia- and in some species also pesotum-like forms. *Ophiostoma songshui* groups closest to *O. jiamusiensis* and *O. tapionis* in a well-supported clade (Fig. 1, 4) in all gene regions.

Taxon 10

Ophiostoma jilinense R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825086; Fig. 16

Etymology. Name refers to Jilin, the province where this species was collected.

Colonies dark brown. White mycelium superficial on agar. Colonies slow growing, reaching 61 mm diam in 10 d at 30 °C. Growth reduced at 5 °C. Growth observed at 35 °C, reaching 54 mm at 10 d. Optimal temperature for growth 30 °C. **Sexual morph** not observed. **Asexual morph**, hyalorhinocladia-like erect, arising directly from the mycelium. **Conidia** hyaline, 1-celled, smooth, oblong, clavate or obovoid, (3.5–)4–4.5(–5.5) × (1–)1.5–2(–2.5) µm.

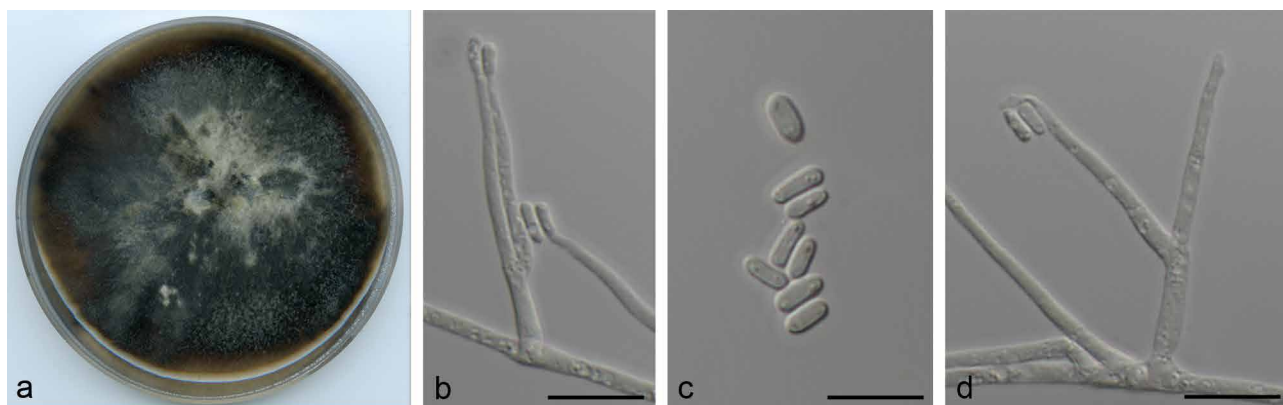


Fig. 16 Morphological characters of asexual structures of *Ophiostoma jilinense* sp. nov. (Taxon 10). a. Fourteen-d-old cultures on MEA; b–d. hyalorhinocladia-like asexual morph and conidia. — Scale bars: b–d = 10 μ m.



Fig. 17 Morphological characters of asexual structures of *Leptographium koraiensis* sp. nov. (Taxon 11). a. Fourteen-d-old cultures on MEA; b. leptographium-like asexual morph; c–d. conidiogenous cells and conidia. — Scale bars: b–d = 10 μ m.



Fig. 18 Morphological characters of asexual structures of *Leptographium shansheni* sp. nov. (Taxon 12). a. Fourteen-d-old cultures on MEA; b–c. leptographium-like asexual morph; d–e. conidiogenous cells and conidia. — Scale bars: b–d = 10 μ m.

Specimens examined. CHINA, Jilin province, Jiamusi, from gallery of *Ips typographus* on *Pinus koraiensis*, June 2010, X.D. Zhou, holotype PREM 61568 (herbarium specimen of dried culture), CMW 40491 = CBS 141894 (ex-holotype culture); Jilin, Jiamusi, from gallery of *Ips typographus* on *Pinus koraiensis*, June 2010, X.D. Zhou, paratype PREM 61569 (herbarium specimen of dried culture), CMW 40492 = CBS 141716 (ex-paratype culture).

Notes — The hyalorhinocladia-like asexual morph of *O. jilinense* resembles that of *O. acarorum*, which is its closest relative based on phylogeny. The other close relatives, *O. pallidulum* and *O. saponiodorum* also have hyalorhinocladia-like asexual morphs (Linnakoski et al. 2010). Both the ITS and *BT* sequences of *O. jilinense* have six bp differences from *O. acarorum* (Fig. 5).

Ophiostoma jilinense grouped in an unsupported clade in *Ophiostoma* s.lat., peripheral to both *Ophiostoma* s.str. and *Sporothrix* based on ITS phylogeny (Fig. 1). Most of the species grouping between these two genera occur on conifer hosts (De Beer et al. 2016). Those that have sexual morphs have unsheathed, allantoid ascospores. Asexual morphs include hyalorhinocladia-like, pesotum-like and sporothrix-like types (Linnakoski et al. 2010).

Taxon 11

Leptographium koraiensis R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825087; Fig. 17

Etymology. Name refers to *Pinus koraiensis*, the tree host from which this species was collected.

Colonies hyaline at first, becoming dark brown in centre with age. Mycelium superficial on the agar. Colonies slow growing, reaching 23 mm diam in 10 d at 20–25 °C. Growth reduced at 5 °C and 30 °C. No growth observed at 35 °C. Optimal temperature for growth 20–25 °C. *Sexual morph* not observed. *Asexual morph* synnematosus, macronematous, erect, conidiophores (125–)167–227(–272) µm. *Conidiogenous cells* discrete, cylindrical, tapering slightly at the apex, (11.5–)24–41(–50.5) µm long, (1.5–)2–2.5(–3.5) µm wide. *Conidia* hyaline, aseptate, elliptical, (5.5–)6.5–9(–10.5) × (2.5–)3–4(–4.5) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61574 (herbarium specimen of dried culture), CMW 44461 = CBS 141898 (ex-holotype culture).

Notes — The synnematosus asexual morph of *L. koraiensis* resembles that of *G. galeiformis* (Zhou et al. 2004). For the four taxa in the *G. galeiformis* complex, the sexual morphs of only *G. galeiformis* and *G. radiaticola* are known, and are characterized by ascospores lacking ostiolar hyphae and kidney shaped ascospores surrounded by a sheath (Kim et al. 2005). Isolates from Austria and Mexico were labelled (Linnakoski et al. 2012a) as *G. galeiformis* A, and in our analyses these also group distinct from the other species based on the *BT* and *EF* sequences (Fig. 6). Although *L. koraiensis* groups close to *G. galeiformis*, in the *BT* and *EF* sequences it differs from the latter species in 4 bp and 19 bp, respectively.

Apart from *L. koraiensis* from China, the other species in the *G. galeiformis* complex include *G. galeiformis* from Europe, *G. galeiformis* A from Europe and Mexico (although these might represent distinct taxa) and *G. radiaticola* from Europe, China South America and South Africa. All the species in this complex are associated with bark beetles infesting conifers (Kim et al. 2005, Linnakoski et al. 2012a).

Taxon 12

Leptographium shansheni R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825088; Fig. 18

Etymology. Specimens of this fungus were collected in the Lesser Khingan Mountains, where many locals believe in the existence of Shanshen, the 'God of the mountains'.

Colonies hyaline at first, becoming dark brown with age. Mycelium superficial on the agar. Colonies fast growing, reaching 75 mm diam in 4 d at 25 °C. Growth reduced at 5 °C. No growth observed at 35 °C. Optimal temperature for growth 25 °C. *Sexual morph* not observed. *Asexual morph* leptographium-like. *Conidiophores* macronematous, mononematous, erect, arising directly from the mycelium, (36–)59–110(–139) µm in length, rhizoid-like structures absent. *Stipes* light olivaceous, simple, 1-septate, apical cell swollen or not swollen, (9–)12–18(–23) × (3–)4–6(–10) µm. *Conidiogenous apparatus* (33–)46.5–95(–113.5) µm long, excluding the conidial mass, with multiple series of cylindrical branches. *Primary branches* 2–3, light olivaceous, cylindrical, (6.5–)10.5–19(–27.5) × (1.5–)3–5(–7) µm, arrangement of the primary branches on the stipes-type B. *Other branches* hyaline to light olivaceous, (9–)12–19(–23.5) × (2–)2.5–5(–8) µm. *Conidiogenous cells* discrete, cylindrical, tapering slightly at the apex, (9.5–)14.5–24(–31) × (1.5–)2–3(–4) µm. *Conidia* hyaline, aseptate, elliptical, (8–)9–13(–16) × (3.5–)4–5(–6) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61571 (herbarium specimen of dried culture), CMW 44462 = CBS 141895 (ex-holotype culture).

Notes — The leptographium-like asexual morphs observed both in *L. shansheni* and its closest relative, *L. lundbergii* broadly resemble each other. They do not have rhizoids, which are common in some *Leptographium* spp. (Jacobs et al. 2005). The DNA sequences for the *BT* and *EF* regions confirmed that *L. shansheni* is a novel species (Fig. 7).

Leptographium shansheni grouped in the *L. lundbergii* complex that is defined by *L. lundbergii*, the type species of *Leptographium* known from Europe. All species in this complex are found on conifers (Jacobs et al. 2005, Linnakoski et al. 2012a).

Taxon 13

Leptographium heilongjiangense R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825089; Fig. 19a

Etymology. Name refers to Heilongjiang, the province where this species was collected.

Colonies dark brown. Mycelium superficial on the agar. No spore-forming structures present. Optimal temperature for growth 25 °C, reaching 90 mm in 5 d. No growth observed at 5 °C and 35 °C. No reproductive structures were observed.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61547 (herbarium specimen of dried culture), CMW 44456 = CBS 141702 (ex-holotype culture); Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61548 (herbarium specimen of dried culture), CMW 45083 = CBS 141703 (ex-paratype culture).

Notes — No spore forms were observed for *L. heilongjiangense*. DNA sequences for the *BT* and *EF* gene regions showed that this is a novel species in the *G. piceiperda* complex.

Leptographium heilongjiangense grouped in *G. piceiperda* complex with four other species. All of these species are characterized by cucullate ascospores and typical a leptographium-like asexual morph (De Beer & Wingfield 2013). Ando et al. (2016) identified seven lineages in this complex and considered these

to represent novel species from Japan. Some of our isolates grouped with their '*G. piceiperda* D'.

Taxon 15

Leptographium yichunense R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825090; Fig. 19b–d

Etymology. Name refers to Yichun, the city where this species was collected.

Colonies dark brown to black. Mycelium superficial on the agar. Optimal temperature for growth 25–30 °C, reaching 90 mm in 7 d. No growth observed at 5 °C and 35 °C. **Sexual morph**

not observed. **Asexual morph** hyalorhinocladia-like. **Conidiophores** micro- to macronematous, mononematous, erect, arising directly from the mycelium, (8.5–)2.5–83(–228.5) × (1.5–)2(–3) µm. **Conidia** hyaline, aseptate, elliptical, (4–)4.5–7(–9.5) × (1.5–)2–2.5(–3) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61550 (herbarium specimen of dried culture), CMW 44464 = CBS 141705 (ex-holotype culture); Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, paratype PREM 61551 (herbarium specimen of dried culture), CMW 44465 = CBS 141706 (ex-paratype culture).

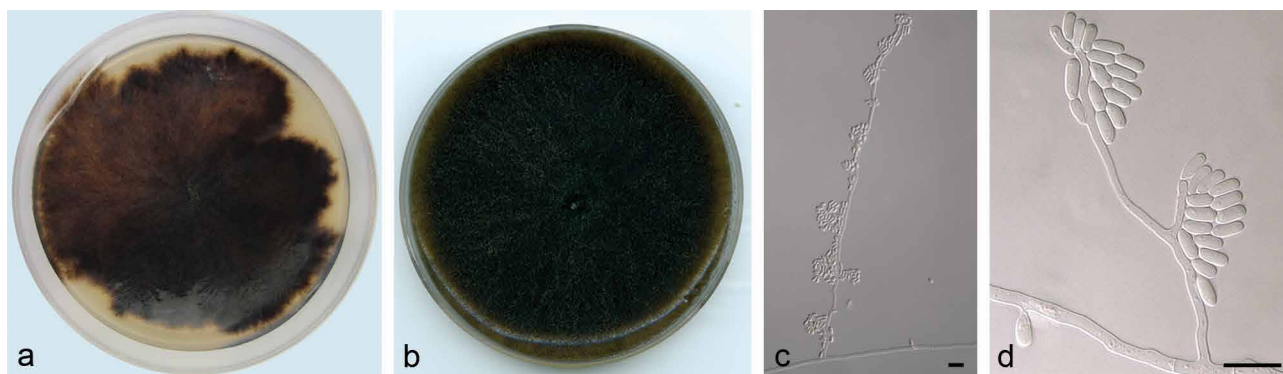


Fig. 19 a–b. Culture characteristics of fourteen-d-old cultures on MEA of a. *Leptographium heilongjiangense* sp. nov. (Taxon 13); b. *Leptographium yichunense* sp. nov. (Taxon 15). c–d. Morphological characters of asexual structures of *L. yichunense* sp. nov. c. Hyalorhinocladia-like asexual morph; d. conidia. — Scale bars: b–d = 10 µm.

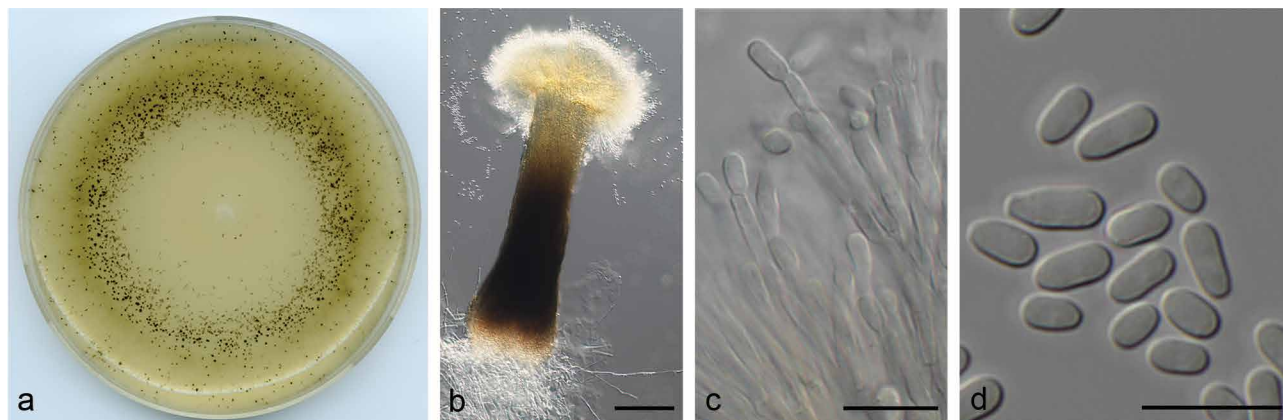


Fig. 20 Morphological characters of asexual structures of *Leptographium duchongi* sp. nov. (Taxon 18). a. Fourteen-d-old cultures on MEA; b. pesotum-like asexual morph; c. conidiogenous cells of pesotum-like macronematous asexual morph; d. conidia. — Scale bars: b = 100 µm; c–d = 10 µm.

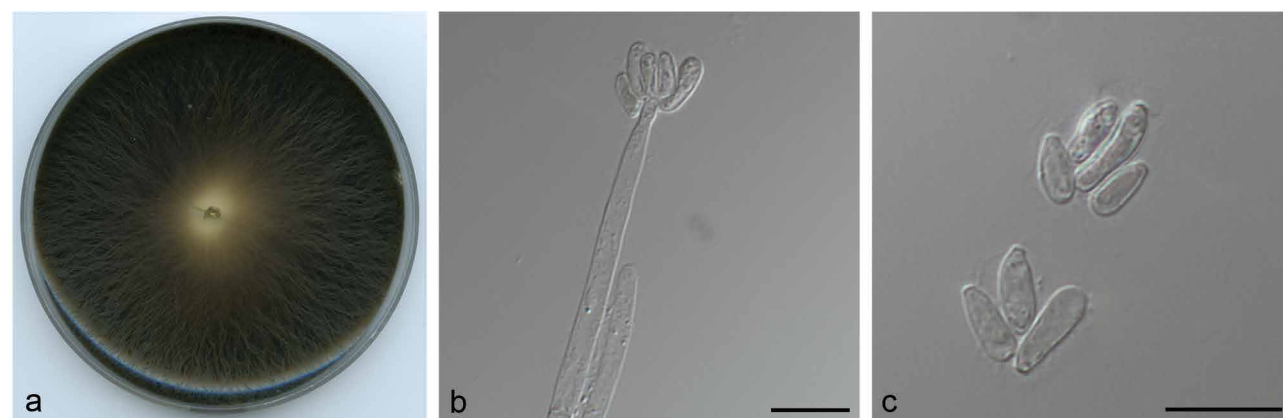


Fig. 21 Morphological characters of asexual structures of *Leptographium fenglinhense* sp. nov. (Taxon 20). a. Fourteen-d-old cultures on MEA; b. sporothrix-like asexual morph; c. conidia. — Scale bars: b–c = 10 µm.

Notes — The hyalorhinocladia-like asexual morph (Fig. 19b–d) of *L. yichunense* is different to that of *L. sibiricum* (Jacobs et al. 2000), its closest relative based on phylogeny, that has a leptographium-like asexual morph. The conidia of *L. yichunense* are also larger than those of *L. sibiricum*. The optimal temperature for growth of *L. yichunense* lies between 25 °C and 30 °C, while that for *L. sibiricum* is 25 °C.

Leptographium yichunense resides in the *L. procerum* complex with ten other species, most of which have conifer hosts. The new Chinese taxon was isolated from *I. typographus* infested pines, while *L. sibiricum* came from egg chambers of *Monochamus urussovi* in the phloem of *Abies sibirica* (Jacobs et al. 2000). No sexual morph has been found for any species in this complex even though some have been shown to be genetically heterothallic, and isolates of opposing mating types have been grown together in culture (Duong et al. 2013). Three lineages have been defined in the *L. procerum* complex and these correspond to the geographical origin of the isolates (Yin et al. 2015). *Leptographium yichunense*, *L. pini-densiflorae* and *L. sibiricum* reside in a single lineage and all have been recorded only from Asia.

Taxon 18

Leptographium duchongi R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825091; Fig. 20

Etymology. The name refers to the Chinese word for bark beetles, 'du-chong'.

Colonies hyaline at first, becoming dark brown with time. Mycelium superficial on agar. Colonies slow growing, reaching 62 mm diam in 10 d at 25 °C. Growth reduced at 5 °C, 30 °C and 35 °C. Optimal temperature for growth 25 °C. **Sexual morph** not observed. **Asexual morph**, macronematous, synnematus, erect, (389.5–)453–608.5(–634.5) × (19–)27.5–106.5(–257.5) µm. **Conidia** hyaline, aseptate, elliptical, (3.5–)4–5.5(–7) × (2–)2.5(–3) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Wuying National Forest Park, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61573 (herbarium specimen of dried culture), CMW 44455 = CBS 141897 (ex-holotype culture).

Notes — *Leptographium duchongi* has a pesotum-like asexual morph, which is similar to closely related species such as *L. pseudoalbum*, *L. conplurium*, *G. olivaceapini* and *L. pigmentoconidium* (Yin et al. submitted). The conidia of these species are also similar. DNA sequences of the *BT* and *EF* regions supported the distinction between these species (Fig. 10).

Leptographium duchongi resides in the *G. olivacea* complex. All the species in this complex have been collected from *Pinus*, *Picea* and *Pseudotsuga*. Globose ascomata with long necks, terminating in prominent ostiolar hyphae, orange-section shaped ascospores with cucullate gelatinous sheaths are the main characters of species in this complex (Yin et al. submitted).

Taxon 20

Leptographium fenglinhense R. Chang, Z.W. de Beer & M.J. Wingf., sp. nov. — MycoBank MB825092; Fig. 21

Etymology. Name Fenglinhe refers to the forest farm, where samples representing this species were collected.

Colonies hyaline at first, becoming dark brown with age. Mycelium superficial on agar. Colonies fast growing, reaching 75 mm diam in 6 d at 25 °C. Growth reduced at 5 °C and 30 °C. No growth observed at 35 °C. Optimal temperature for growth 25 °C. **Sexual morph** not observed. **Asexual morph**, hyalorhinocladia-like, micronematous, mononematous, erect, arising directly from the mycelium. **Conidia** hyaline, aseptate, elliptical, (4–)4.5–7(–10.5) × (1.5–)2–3(–4) µm.

Specimens examined. CHINA, Heilongjiang province, Yichun city, Wuying district, Fenglinhe, from gallery of *Ips typographus* on *Pinus koraiensis*, Sept. 2014, R. Chang, holotype PREM 61572 (herbarium specimen of dried culture), CMW 44579 = CBS 141896 (ex-holotype culture).

Notes — Although a leptographium-like asexual morph characterizes all known species in the *G. penicillata* complex, we did not observe this morph in *L. fenglinhense*. However, its closest relative, *L. chlamydatum*, produces a hyalorhinocladia-like asexual morph similar to that of *L. fenglinhense*, in addition to its leptographium-like morph (Jacobs et al. 2010). DNA sequences for the *BT* and *EF* regions distinguished *L. fenglinhense* from *L. chlamydatum* and the other species in the complex (Fig. 19).

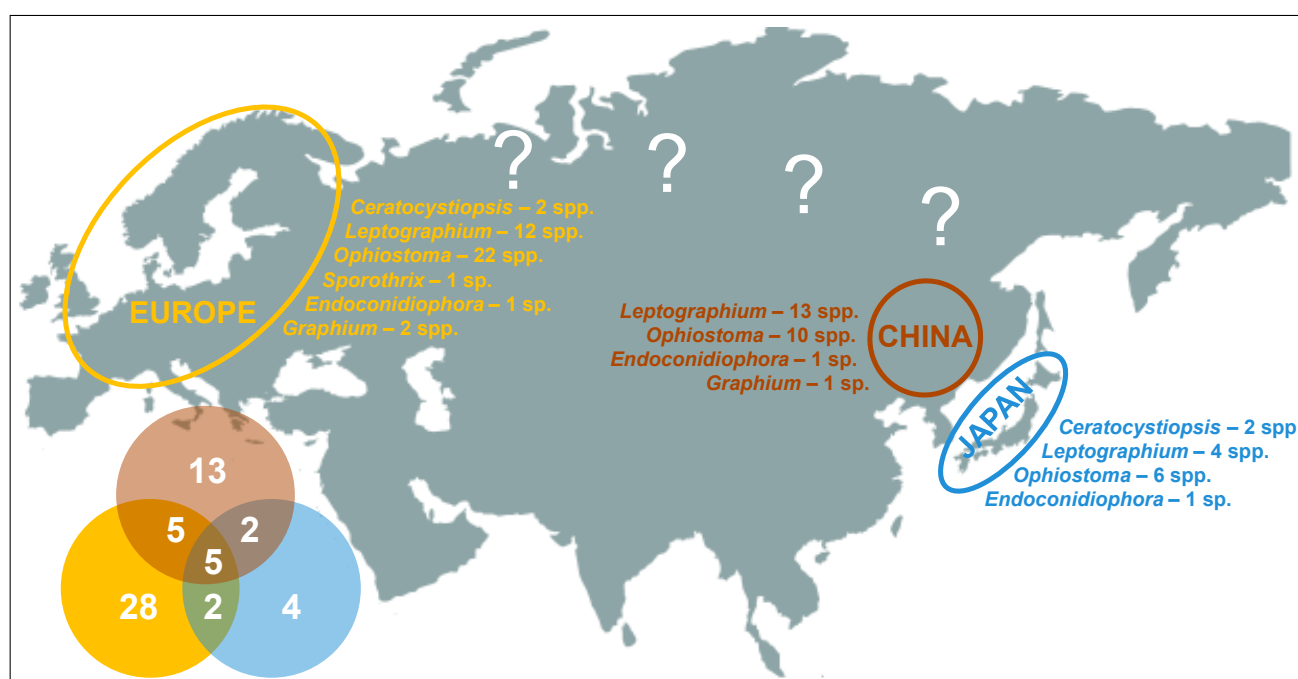


Fig. 22 Comparison of composition of fungal assemblages of *Ips typographus* in Europe, China and Japan. The Venn diagram illustrates the numbers of fungal species overlapping in the different regions. White question marks highlight the absence of data on fungal associates of *Ips typographus* in the boreal forests of Siberia, which forms an ecological corridor for the beetle between Europe and East Asia.

Table 3 Comparison of fungal associates of *Ips typographus* in Europe, Japan and China.

Fungal species	Europe	Japan	China
Ophiostomatales			
<i>Ceratocystiopsis alba</i>	✓		
<i>Cop. minuta</i>	✓	✓	
<i>Cop. minuta-bicolor</i>		✓	
<i>Grosmannia aenigmatica</i>		✓	
<i>G. cucullata</i> (Taxon 16)	✓	✓	✓
<i>G. europhioides</i>	✓		
<i>G. olivacea</i> (Taxon 17)	✓		✓
<i>G. olivaceapini</i>	✓		
<i>G. penicillata</i> (Taxon 21)	✓	✓	✓
<i>G. piceaperda</i>	✓		
<i>G. piceaperda-D</i> (Taxon 14)		✓	✓
<i>G. serpens</i>	✓		
<i>Leptographium curviconidium</i>			✓
* <i>L. duchongi</i> (Taxon 18)			✓
<i>L. euphyes</i>	✓		
* <i>L. fenglinhense</i> (Taxon 20)			✓
<i>L. gracile</i> (as <i>L. latens</i>)			✓
* <i>L. heilongjiangense</i> (Taxon 13)			✓
* <i>L. koraiensis</i> (Taxon 11)			✓
<i>L. lundbergii</i>	✓		
<i>L. obscurum</i>	✓		
* <i>L. shansheni</i> (Taxon 12)			✓
<i>L. taigense</i> (Taxon 19)	✓		✓
* <i>L. yichunense</i> (Taxon 15)			✓
<i>Leptographium</i> sp.	✓		
<i>Ophiostoma ainoae</i> (Taxon 6)	✓	✓	✓
<i>O. albidum</i>		✓	
<i>O. araucariae</i>	✓		
<i>O. arborea</i>	✓		
<i>O. bicolor</i> (Taxon 9)	✓	✓	✓
<i>O. brunneo-ciliatum</i>	✓		
<i>O. brunneolum</i> (Taxon 7)	✓		✓
<i>O. cainii</i>	✓		
<i>O. flexuosum</i>	✓		
<i>O. floccosum</i> (Taxon 1)	✓		✓
<i>O. fuscum</i>	✓		
* <i>O. japonicum</i> (Taxon 8)		✓	✓
* <i>O. jiamusiensis</i> (Taxon 4)			✓
* <i>O. jilinense</i> (Taxon 10)			✓
<i>O. karelicum</i>	✓		
<i>O. macroclavatum</i>	✓		
<i>O. minus</i>	✓		
<i>O. neglectum</i>	✓		
<i>O. piceae</i>	✓	✓	
<i>O. piliferum</i>	✓		
<i>O. pluriannulatum</i>	✓		
<i>O. saponiodorum</i>	✓		
* <i>O. songshui</i> (Taxon 5)			✓
<i>O. tetropii</i>	✓		
<i>O. tapionis</i>	✓		
<i>O. truncicola</i>		✓	
* <i>O. typographi</i> (Taxon 2)			✓
* <i>O. wuyingense</i> (Taxon 3)			✓
<i>Ophiostoma</i> sp.	✓		
<i>Ophiostoma</i> (Pesotum) sp.	✓		
<i>Sporothrix stenoceras</i>	✓		
Microascales			
<i>Endoconidiophora polonica</i> (Taxon 22)	✓	✓	✓
<i>Graphium fimbriisporum</i> (Taxon 23)	✓		✓
<i>Gr. pseudormiticum</i>	✓		
Number of species reported	40	13	25

* Species discovered for the first time from China in the present study.

Species in the *G. penicillata* complex with known sexual morphs are characterised by globose ascomata with medium to long necks and allantoid, sheathed ascospores (Yin et al. submitted). All species have been isolated from spruce or spruce-infesting bark beetles in Europe and East Asia. *Leptographium fenglinhense* is the only species in the group that occurs on *Pinus*.

Comparisons of species from *I. typographus* in China, Europe and Japan

A comparison of the results of the present study with to published studies from Japan and Europe showed that the fungal assemblages of *I. typographus* from China are distinct from those elsewhere in the world (Table 3; Fig. 22). Overall, 58 ophiostomatoid species, including 11 new species resolved in this study, have been found in association with this beetle (Table 3). In terms of species diversity, *I. typographus* from Europe has the largest number of species (i.e., 40). China is second with the 25 species revealed in the present study. Only 13 species are known from *I. typographus* in Japan. Five species, namely *E. polonica*, *G. cucullata*, *G. penicillata*, *O. ainoae* and *O. bicolor*, were shared between all three regions. Two species, namely *Ceratocystiopsis minuta* and *O. piceae*, were shared between Europe and Japan. Five additional species, namely *Graphium fimbriisporum*, *G. olivacea*, *L. taigense*, *O. brunneolum* and *O. floccosum*, were shared between Europe and China. Two species, *O. japonicum* and *G. piceaperda* D were exclusively shared between China and Japan. *Ips typographus* from Europe also had the highest number of unique species (28), followed by China (13) and Japan (3).

DISCUSSION

In this study, 1046 ophiostomatoid fungal cultures representing 23 different taxa, were isolated and identified from *I. typographus* and its galleries in China. Eleven of these taxa represented undescribed species. Most of these isolates resided in the *Ophiostoma* s.lat. and *Leptographium* s.lat. as defined by De Beer & Wingfield (2013). The three species most frequently isolated were *O. bicolor*, *L. taigense* and *G. piceaperda* D. A comparison was made between the species assemblage found in this study with those previously published from Japan (Yamaoka et al. 1997, Masuya et al. 2013) and Europe (Kirisits 2004, Viiri & Lieutier 2004, Jankowiak 2005, Jankowiak & Hilszczanski 2005, Linnakoski et al. 2012a, 2016a, b). These comparisons revealed interesting patterns, most importantly that there are clear differences and some similarities between the fungi associated with *I. typographus* in the three areas considered.

This is the first study focused exclusively on the fungal diversity associated with *I. typographus* in China. Although Paciura et al. (2010) described two new species, *L. curviconidium* and *L. gracile* (as *L. latens*), associated with *I. typographus* in Yunnan and Jilin, the focus of that study was on the taxonomy of *Leptographium* spp. from various hosts and beetles, and included only a small number of randomly sampled isolates from *I. typographus*. Neither of the two species reported by Paciura et al. (2010) were isolated in the present study.

Leptographium taigense (Taxon 19) was the only species found in this study that has previously been recorded in China. This species has previously been described from various conifer-infesting bark beetles in Russia (Linnakoski et al. 2012a), and recently reported from *Ips subelongatus* infesting *Larix gmelinii* in Inner Mongolia in China (Liu et al. 2017). *Ophiostoma floccosum* (Taxon 1), *O. ainoae* (Taxon 6), *O. bicolor* (Taxon 9), *G. cucullata* (Taxon 16), *G. olivacea* (Taxon 17), *G. penicillata* (Taxon 21), *E. polonica* (Taxon 22) and *Gr. fimbriisporum* (Taxon

23) have a wide distribution in Eurasia, where they are not only associated with *I. typographus*, but also with other bark beetles (Yamaoka et al. 1997, Kirisits 2004, Linnakoski et al. 2010). *Ophiostoma brunneolum* (Taxon 7) was recently described from Europe associated with *Ips* spp. on *Picea abies* (Linnakoski et al. 2016a). *Ophiostoma japonicum* (Taxon 8) was previously known only from Japan associated with *I. typographus* (Yamaoka et al. 1997). Similarly, *G. piceiperda* D (Taxon 14) has previously been known only from Japan, where it is associated with several bark beetles (Ando et al. 2016).

Several species found in this study have also been isolated from mites associated with *I. typographus* (Moser et al. 1989, 1997). *Ophiostoma bicolor*, the most commonly encountered species in this study was also the most common species associated with mites phoretic on *I. typographus* in both Sweden and Japan (Moser et al. 1989, 1997). *Endoconidiophora polonica* and *G. penicillata* have also been isolated from mites in Sweden and Japan (Moser et al. 1989, 1997). In the only study of ophiostomatoid fungi vectored by hyperphoretic mites on bark beetles in China, Chang et al. (2017) isolated 19 fungal species from 13 mite species on 17 beetle species in Yunnan, China. However, *I. typographus* was not included in their study. Although mites were not specifically considered in the present study, the fact that *O. bicolor* was the dominant fungus corresponds to its common occurrence on mites in Sweden and Japan. Future studies to consider the fungal diversity of mites phoretic on *I. typographus* in China would be interesting and could for example show whether species such as *O. bicolor* are predominantly a mite-associated fungus, rather than associates of the beetles.

Of all the species found in this study, *E. polonica* is the only fungus known to have a high level of pathogenicity (Christiansen & Solheim 1990). In this regard, it is known to cause serious damage to *P. abies* in Europe and *P. jezoensis* and *P. glehnii* in Japan (Christiansen & Solheim 1990, Visser et al. 1995, Yamaoka et al. 1997). Some authors (Baier 1996, Franceschi et al. 2005, Repe et al. 2015) have suggested that *E. polonica* plays a role in the success of *I. typographus* attacks. However, the role of fungal associates such as *E. polonica* in tree mortality has been questioned (Six & Wingfield 2011). This is for example because the prevalence of this fungus is highly variable between outbreaks and it has been shown that *I. typographus* can kill trees in the absence of *E. polonica* (Solheim 1986, Krokene & Solheim 1996, Viiri 1997, Kirisits 2004, Viiri & Lieutier 2004, Jankowiak 2005, Sallé et al. 2005).

Comparisons of species associated with *I. typographus* occurring in China, Europe and Japan are complicated by the fact that some of the European and all the Japanese species were identified based only on morphology. However, assuming at least the dominant and well-known species were correctly identified, some trends can be inferred. For example, only five species are associated with *I. typographus* in all three regions, while five species were shared exclusively between China and Europe, two species exclusively between Europe and Japan and two species exclusively between China and Japan. Of these three regions, Japan had the lowest while Europe had the highest ophiostomatoid fungus diversity. This could reflect more extensive sampling in Europe than in China and Japan. However, the fact that Japan is made up of geographically isolated islands could restrict horizontal transfer of fungi between beetle species.

It was interesting that the five species of ophiostomatoid fungi shared among China, Japan and Europe (i.e., *E. polonica*, *G. cucullata*, *G. penicillata*, *O. ainoae* and *O. bicolor*) were not necessarily among the most frequently isolated species in these areas. In the present study, *O. bicolor*, *L. taigense* and

G. piceiperda D were the most dominant fungi in north-eastern China where they represented 40.5 %, 27.8 % and 17.8 % of the isolates, respectively. Of these, *O. bicolor* was the only species that was also among the most prevalent species in Japan (Yamaoka et al. 1997), Poland (Jankowiak 2005, Jankowiak & Hilszczanski 2005) and western Russia, but not in Finland (Linnakoski et al. 2010). In Japan, the dominant species are *O. ainoae*, *O. piceae* and *G. penicillata* (Yamaoka et al. 1997), which is consistent with some reports from Europe (Jankowiak 2005, Jankowiak & Hilszczanski 2005). However, Linnakoski et al. (2010) reported that an *O. canum*-like species was most dominant in Finland and *O. brunneolum* (reported as *O. brunneociliatum*) was most common in western Russia. *Ophiostoma ainoae* has never been found in Finland (Linnakoski et al. 2010), while *G. cucullata* was found only in low numbers in that country (Linnakoski et al. 2012a). The differences in composition and dominant fungal species associated with *I. typographus* at the three locations considered, suggests a casual relationship between *I. typographus* and its fungal associates, rather than a specific association with any particular species.

The fact that only few taxa were shared between Europe, Japan and China is consistent with the population genetic data for *I. typographus*. These suggest that the populations from China, Japan and Europe are very distinct (Stauffer et al. 1992, Stauffer et al. 1999, Salle et al. 2007, Bertheau et al. 2013, Mayer et al. 2015). In contrast, only six species of ophiostomatoid fungi were shared between China and Japan when compared with the 10 species shared between China and Europe, and seven between Europe and Japan. This finding is inconsistent with beetle population studies that have suggested a closer genetic relationship between *I. typographus* populations from China and Japan when compared to Europe. Overall, the results of comparisons emerging from the present study showed that the symbionts of bark beetle fail to reflect the population structure of the bark beetle. It is also clear that the use of fungal symbiont assemblages to infer population structures and invasion history of its vectors need to be carefully interpreted. This is especially because factors such as sampling strategy, age of galleries and identification techniques must be taken into consideration.

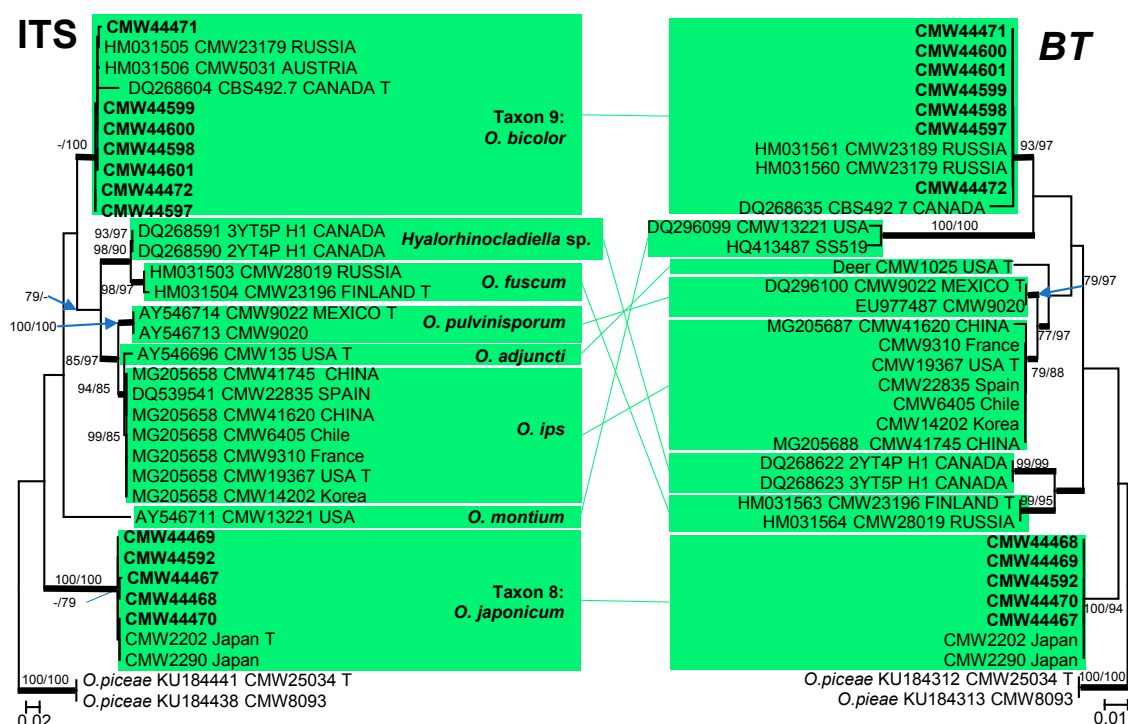
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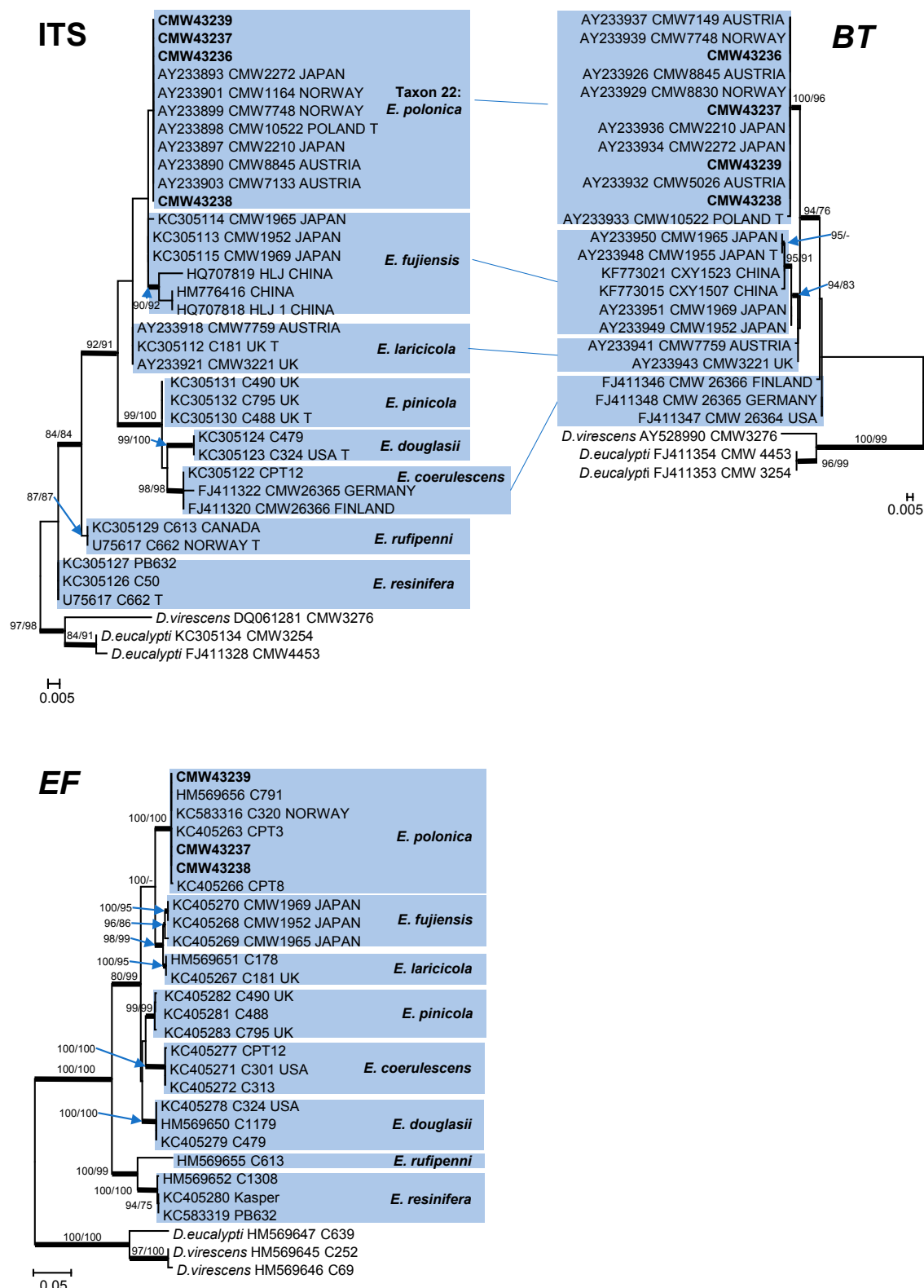
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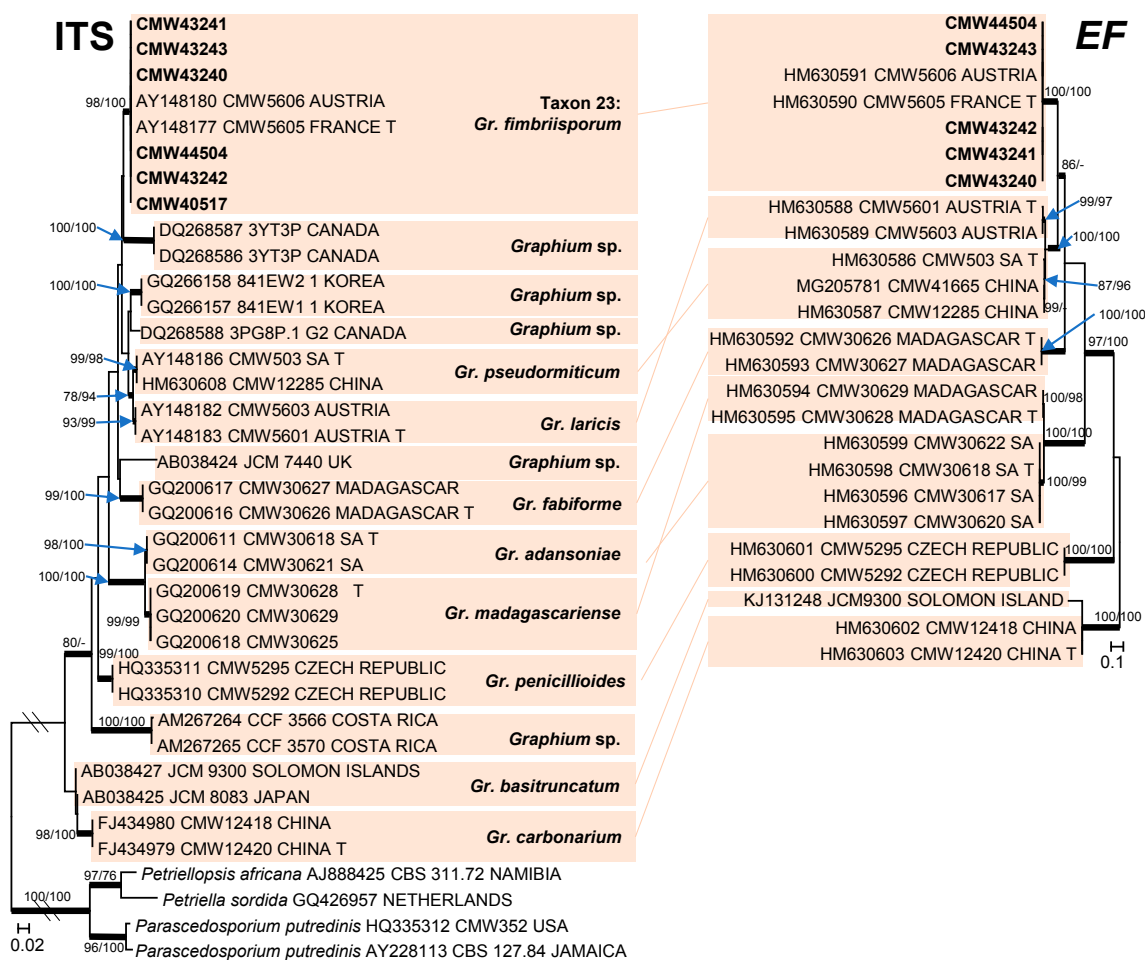
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Appendix 1 ML tree of *Ophiostoma ips* complex generated from DNA sequences of ITS and BT regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.



Appendix 2 ML tree of *Endoconidiophora* generated from DNA sequences of ITS, BT and EF regions. Sequences generated from this study are printed in bold type. Bold branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.



Appendix 3 ML tree of *Graphium* generated from DNA sequences of ITS and EF regions. Sequences generated from this study are printed in **bold** type. **Bold** branches indicate posterior probabilities values ≥ 0.9 . Bootstrap values of ML $\geq 75\%$ are recorded at nodes as ML/MP. T = ex-type isolates.

Chapter 4

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Ophiostomatoid fungi associated with mites phoretic on bark beetles in Qinghai, China.
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Ophiostomatoid fungi associated with mites phoretic on bark beetles in Qinghai, China

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Abstract: Bark beetle galleries are complex ecosystems where many microbes and arthropods co-exist with the beetles. Fungi isolated from these galleries are often referred to as ‘beetle associates’, but the nature of these associations are poorly understood. The possibility that many of these fungi might in fact be mite associates is often overlooked. Several recent studies explored the diversity of fungi from conifer-infesting bark beetles and their galleries in China, but only one study considered phoretic mites and their fungi from conifer-infesting bark beetles in Yunnan, southwestern China. We studied the mites and fungi from galleries of four spruce-infesting bark beetle species in the high altitude forests of Qinghai province, western China. Mites were identified based on morphological characteristics, and fungi based on DNA sequences of four gene regions. In total, 173 mite individuals were collected belonging to 18 species in 11 genera. A total of 135 fungal isolates were obtained from the mites, representing 14 taxa from the *Ophiostomatales*. Three new species are described as *Grosmannia zekuensis* sp. nov., *Ophiostoma manchongi* sp. nov. and *Ophiostoma kunlunense* sp. nov. The most frequently isolated fungus was *Ophiostoma nitidum*, which represented 23.5 % of the total isolates. More fungal species were found from fewer mites and bark beetle species than from the study in Yunnan. Although we could not elucidate the exact nature of interactions between mites and their fungi, our results re-enforce that these organisms should not be ignored in pest risk assessments of bark beetles, that often focus only on the beetles and their fungi.

Keywords:

spruce, symbiosis, ascomycetes, Scolytinae, new taxa

Introduction

Mites (*Arachnida*, *Acari*) are commonly associated with bark beetles (*Insecta*, *Coleoptera*, *Scolytinae*) and their galleries (Hofstetter et al., 2015). Because of their small size, these mite species rely on the bark beetles for dispersal between plant hosts (Hofstetter et al., 2013). To date, about 270 mite species have been identified as associates of a limited number of bark beetle species, and many more are likely to be discovered (Hofstetter et al., 2013; Hofstetter et al., 2015).

The ecological roles of very few bark beetle-associated mites are well understood, but the different classes of feeding behavior (Hofstetter et al., 2013) suggests that their impacts in gallery ecosystems could be broad. Some of these mites, such as *Pyemotes dryas*, predate upon or parasitize the eggs and early larval instars of bark beetles (Wegensteiner et al., 2015) and thus have a direct effect on population dynamics of the beetles. However, the impacts of many mites on bark beetle behavior and population growth are indirect. A few species are known to be predators of nematodes, while many of the mite species are omnivores and feed on fungi, nematodes and dead arthropods in the galleries. Other species are exclusively mycetophagous, utilizing fungi as their only source of nutrition (Hofstetter et al., 2013).

It appears that some mycetophagous mites have preference for one or a few specific species of fungi. One such an example is *Tarsonemus krantzi*, associated with the southern pine beetle, *Dendroctonus frontalis*, that vector and feeds on *Ophiostoma minus* (Lombardero et al., 2000). Other mites such as *Histiogaster* spp. are generalists that can feed on several different fungal species (Hofstetter & Moser, 2014). The most common fungi associated with bark beetles and their mites are the ophiostomatoid fungi (Hofstetter & Moser, 2014). This is a polyphyletic group of fungi that includes several genera in the *Microascales* and *Ophiostomatales*, that are characterized by spores produced in sticky droplets to facilitate dispersal by bark beetles and mites (De Beer et al., 2013). Many bark beetles have specialized structures known as mycangia in which to carry the spores of these fungi (Six, 2012). In the case of mites, most fungi appear to be vectored on their exterior surfaces (Moser et al., 1989b), but some mite species have evolved specialized structures known as, sporothecae, to ensure the successful transmission of specific fungal symbionts between hosts (Moser, 1985).

Although the impact of most fungi on the fitness of the mites or their beetle vectors remains largely unknown, the three-way interaction between beetles, mites and fungi, has been elucidated in the case of the southern pine beetle, *Dendroctonus frontalis*, and its associated *Tarsonemus* mites. The larvae of *D. frontalis* are obligately dependent on two fungal species, *Ceratocystiopsis ranaculosus* and *Entomocorticium* sp. A, for nutrition (Ayres et al., 2000). These fungi are outcompeted by *Ophiostoma minus* (Barras, 1970; Bridges, 1983; Goldhammer et al., 1990), the nutritional fungal mutualist of *Tarsonemus* mites, when the mites are present in the galleries of *D. frontalis* (Hofstetter et al., 2013). As a result of these interactions, large populations of *Tarsonemus* mites can suppress the reproduction of *D. frontalis* (Hofstetter et al., 2006).

The relationships between mites and their beetle vectors are generally unspecific, and a single species of mite can use many different beetle species as vector in order to reach its tree hosts (Hofstetter et al., 2013). Similarly, many ophiostomatoid fungi are promiscuous in their association with beetle species, and a single beetle species can vector a number of different fungal species (Kirisits, 2004; Linnakoski et al., 2012; Taerum et al., 2013). In addition, one mite species can carry any of a number of ophiostomatoid fungi (Chang et al., 2017). It thus becomes extremely difficult to resolve specificity between any of these organisms. However,

where galleries of different bark beetle species might be restricted to different parts of a specific tree and thus not overlap, it has been suggested that mites might facilitate the movement of fungal species between galleries of different beetle species (Chang et al., 2017).

There are several serious tree pathogens amongst the ophiostomatoid fungi. These include the well-known Dutch Elm Disease fungi, *Ophiostoma ulmi* and two varieties of *O. novo-ulmi* (Brasier, 1990; Brasier, 1991). These pathogens are vectored by *Scolytus* bark beetles (Webber, 2004), but it has been shown that phoretic mites also vector the pathogens and might contribute to the spore load and the high efficiency of *S. scolytus* in spreading Dutch elm disease (Moser et al., 2010). *Leptographium wingfieldii* is a mildly pathogenic ophiostomatoid fungus vectored by the pine shoot beetle, *Tomicus piniperda*, in its native range in Europe (Solheim, 1991). The beetle was first detected in the USA in the early 1990's, and it was later shown that *L. wingfieldii* was introduced with the beetle, and that the fungus has subsequently become an associate of two native north American beetles, *Dendroctonus valens* and *Ips pini* (Jacobs et al., 2004). Although the mechanism by which *L. wingfieldii* was transferred between the beetle species was not considered (Jacobs et al., 2004), it is likely that mites facilitated the transfer. Such novel associations between ophiostomatoid fungi, bark- or ambrosia beetles and trees, can pose serious threats to both natural and commercial forests, as well as tree crops. The role of mites in the establishment of these associations are poorly studied, most probably underestimated, and can only be understood if their associations with fungi are explored in natural ecosystems.

To date, the majority of studies on interactions between fungi, bark beetles and mites have been conducted on *Dendroctonus*, *Ips* and *Dryocetes* spp. in North America (Klepzig & Hofstetter, 2011; Hofstetter et al., 2013; Hofstetter & Moser, 2014; Hofstetter et al., 2015), and *Scolytus*, *Ips* and *Pityokteines* spp. in Europe (Levieux et al., 1989; Moser et al., 1989a; Moser et al., 2005; Moser et al., 2010; Linnakoski et al., 2016b). In southern Africa, ongoing studies focus on the interactions between mites and ophiostomatoid fungi in infructescences of *Protea* spp. (Roets et al., 2007, 2009; Roets et al., 2011). There have been only two studies reporting on the fungal associates of mites from east Asia. In these cases, Moser et al. (1997) reported on the fungal associates of mites on *Ips typographus* in Japan, and Chang et al. (2017) described fungi from mites associated with various conifer-infesting bark beetles in Yunnan, China.

In recent years, 76 ophiostomatoid species, including 25 new species, have been reported in association with bark beetles from China (**Table S1**) (Lu et al., 2009a; Lu et al., 2009b; Paciura et al., 2010a; Paciura et al., 2010b; Zhou et al., 2011; Taerum et al., 2013; Zhou et al., 2013; Yin et al., 2015; Wang et al., 2016; Yin et al., 2016; Chang et al., 2017; Liu et al., 2017). The study by Chang et al. (2017) was the first to report ophiostomatoid fungi associated with phoretic mites. They reported 11 species from mites associated with bark beetles infesting *Pinus kesiya*, of which four were described as new species. Only one of the studies from China, was conducted in Qinghai province (Yin et al., 2016). This province, which is located on the Qinghai–Tibetan Plateau, is one of the world's biodiversity hotspots because of its diverse landscapes, and complex geological and climatic history (Li et al., 2012). Yin et al. (2016) described five new *Ophiostoma* spp. from four spruce-infesting bark beetles, two of which are *Ips* spp. that cause severe damage to spruce trees in this area (Liu et al., 2008). Apart from these five species, no other ophiostomatoid fungi have been reported from Qinghai. Furthermore, nothing is known regarding the fungal associates of mites phoretic on spruce-infesting beetles in China. In this study, we addressed the following questions: 1) which mite species are associated with spruce-infesting bark beetles in Qinghai, and 2) which species of ophiostomatoid fungi are associated with these mites?

Material and methods

Collection of mites and fungi

surveys were conducted on *Picea crassifolia* and *Picea purpurea* in July 2010, during the flight period of bark beetles, in the Maixiu and Xianmin forest farms in Qinghai province, China. Bark beetle galleries were collected and stored in re-sealable plastic bags at 4 °C until isolations could be made. Living mites were collected from the galleries under a dissecting microscope. Each individual mite was placed on a separate Petri dish containing malt extract agar (MEA, 20 g Difco agar, 20 g Difco Bacto™ malt extract [Becton, Dickinson & Company], 1 L deionized water) medium. After the plates were sealed, the mites were allowed to crawl over the plates for 24 hours. The mites were then removed and stored in 1.5 mL Eppendorf tubes containing 75 % Ethanol for later identification by Dr E.A. Ueckermann (Plant Protection Research Institute, Agricultural Research Council, South Africa).

The MEA plates were incubated at 20 °C until fungal growth was evident. The hyphal tips of colonies were transferred to fresh MEA plates to obtain pure cultures. All isolates used in this study were deposited into the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa. Isolates representing types of new species were deposited in the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.

DNA sequencing and phylogenetic analyses

Isolates were grown on 2 % MEA medium. DNA was extracted using PrepMan ultra sample preparation reagent (Applied Biosystems, Foster City, CA) following the manufacturer's recommendations. The internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S region, were amplified using the primers ITS1F and ITS4 (White et al., 1990; Gardes & Bruns, 1993), the β -tubulin (*BT*) gene was amplified using the primer pair of Bt2a and Bt2b (Glass & Donaldson, 1995), and the elongation factor 1- α (*EF*) gene was amplified using the primer pair of EF2F (Marincowitz et al., 2015) and EF2R (Jacobs et al., 2004). In addition, the nuclear large subunit (LSU) was amplified with the primer pair LR0R and LR5 (Vilgalys & Hester, 1990) for fungi that reside in *Leptographium* sensu lato. PCR and sequencing were conducted following the protocols described by Duong et al. (2012).

The sequences obtained with the forward and reverse primers were aligned and contigs constructed using the program Geneious pro v. 7.1.4 (Biomatters, Auckland, New Zealand). All sequences obtained in this study were deposited in GenBank. BLAST searches of the ITS sequences were conducted in NCBI GenBank for preliminary identifications. Based on the BLAST results, sequence data for other markers were separated according to the relevant species complexes. For taxa residing in *Leptographium* sensu lato, the ITS2-LSU regions were used to determine generic placement, unlike the case for *Ophiostoma* spp. where the ITS1 and 2 regions were used for this purpose. The *BT* and *EF* data sets were analyzed separately for each species complex. Alignments were made using an online version of MAFFT v. 7 (Katoh & Standley, 2013). All aligned sequence datasets were submitted to TreeBase (No. 23181).

Phylogenetic analyses including maximum likelihood (ML) and Bayesian inference (BI) were conducted for all datasets. The best substitution models for each data set were determined using jModelTest v. 2.1.6 (Darriba et al., 2012) on the CIPRES Science Gateway v. 3.3 (Miller et al., 2010). ML analyses were conducted using RaxML v. 8.2.4 on the CIPRES Science Gateway v. 3.3 (Stamatakis, 2014) with default GTR substitution matrix and 1000 rapid bootstraps. BI analyses were conducted using MrBayes v. 3.2.6 (Ronquist et al., 2012) on the

CIPRES Science Gateway v. 3.3. Four MCMC chains were run from a random starting tree for five million generations and trees were sampled every 100th generation. 25 % of trees sampled were discarded as burn-in and the remaining trees were used to construct majority rule consensus trees.

Growth studies

Mycelium-covered agar plugs were transferred from the actively growing margins of one-week-old cultures and placed at the centers of 90 mm Petri dishes containing 2 % MEA. The optimal temperatures for growth were determined at temperatures ranging from 5 - 35 °C at 5 °C intervals and there were three replicate plates for each temperature. Cultures were incubated in the dark. Colony diameters were measured every two days until hyphae reached the edges of the Petri dishes, at which point the temperatures for optimum growth were noted.

Morphological studies

Asexual and/or sexual structures were mounted in lactophenol on glass slides, covered with coverslips and examined with a Zeiss Axioskop 2 Plus compound microscope or a Zeiss Discovery V12 dissection microscope with an Axiocam digital camera (Axiovision 3.1) (München-Hallbergmoos, Germany). Fifty measurements were made for each taxonomically informative structure. The measurements were given in the format (minimum-) mean minus standard deviation-mean plus standard deviation (-maximum).

Frequency of isolation

The following formula was used to calculate the frequencies of isolation of the ophiostomatoid species : $F = (NF/NT) \times 100$, where F represented the frequency of isolation (%), NT represented the total number of isolates obtained, and NF represented the number of isolates for each particular taxon.

Results

Collection of mites and fungi

In total, 173 mite individuals representing 18 mite species residing in 11 genera were collected from galleries of four bark beetle species on two host tree species (**Table 1**). The bark beetles were *Dendroctonus micans*, *Ips shangrila*, *Ips nitidus* and *Polygraphus polygraphus*. All *D. micans*, *I. nitidus* and *P. polygraphus* were collected from *P. crassifolia*, and *I. shangrila* was collected from *P. purpurea*. Three mite species were associated with *D. micans*, 13 mite species with *I. nitidus*, five mite species with *I. shangrila*, and three mite species with *P. polygraphus*. Most mite species were found only in the galleries of a single bark beetle species. Exceptions were for *Insectolaelaps* sp. 1, *Uropodoidea* sp. 4, *Uropodoidea* sp. 6, and *Winterschmidtidae* sp., which were found in the galleries of more than one beetle species (**Table S2**).

In total, 135 ophiostomatoid fungal isolates were obtained from 65 mite individuals (**Table S3**). Seventy isolates were collected from 13 mite species in galleries of *I. nitidus*, 33 isolates were collected from three mite species in galleries of *D. micans*, 19 isolates were collected from five mite species in galleries of *I. shangrila*, and 13 isolates were collected from three mite species in *P. polygraphus*. Twenty-eight isolates were collected from one mite species in the family *Winterschmidtidae* and 23 isolates collected from *Uropodoidea* sp. 4. More than 10 isolates were collected from each of *Insectolaelaps* sp. 2, *Insectolaelaps* sp. 1 and *Uropodoidea* sp. 6.

DNA sequencing and phylogenetic analysis

Based on analysis of ITS sequence data, 87 isolates resided in *Ophiostoma* sensu stricto (**Fig. 1**), while 48 isolates were of *Leptographium* sensu lato (**Fig. 2**). Sequences for other gene regions (as discussed below) separated the isolates in 14 distinct taxa (**Table 2**).

In *Ophiostoma* s.str., two taxa (Taxa 1 and 4) represented by four and three isolates respectively, grouped peripheral to the *O. piceae* complex (**Fig. 1**). *BT* and *EF* sequences data (**Fig. 3**) showed that Taxon 1 grouped with *O. tetropii* isolates, and that Taxon 4 represented a novel species. Taxa 2 and 3, represented by 32 and 11 isolates respectively, grouped with *O. nitidus* and *O. qinghaiense* in the *O. piceae* complex.

Taxa 5 and 6 grouped in the *O. clavatum* complex (**Fig. 1**) and were represented ten and 11 isolates respectively. Based on the *BT* and *EF* sequence data (**Fig. S1**) the Taxon 5 isolates grouped in a single clade with the ex-type isolates of *O. ainoae* and *O. poligraphi*, suggesting that these represented a single species. Taxon 6 isolates grouped with sequences of *O. shangrilae*.

Taxa 7 and 8 resided in the *O. ips* complex (**Fig. 1**) and were represented ten and six isolates respectively. Based on ITS and *BT* data (**Fig. 4**) Taxon 7 was closest to, but clearly distinct from *O. japonicum* and thus represented a novel species. Taxon 8 isolates grouped in a monophyletic lineage with several isolates of *O. bicolor*.

Taxon 9 represented by only one isolates grouped in Group A of *Leptographium* s.l. together with *L. pineti* and *L. ningerensis* (**Fig. 2**). *BT* and *EF* sequences confirmed this Taxon was conspecific with *L. ningerensis* (**Fig. S2**).

Taxon 10 grouped in the *G. olivacea* complex (**Fig. 2**). *BT* and *EF* sequences confirmed that this isolate represented *L. breviscapa* (**Fig. S2**).

Taxon 11 grouped peripheral to the *G. penicillata* complex with *L. taigense* (**Fig. 2**). The identity of the species was confirmed as *L. taigense* with *BT* and *EF* sequences (**Fig. 5**).

Taxa 12, 13, and 14 grouped in the *G. penicillata* complex (**Fig. 2**). Based on *BT* and *EF* sequences (**Fig. 5**), Taxon 12 was identified as *G. purpurea*, Taxon 13 as a novel species, and Taxon 14 as *G. xianmiense*.

Frequencies of isolation

The most frequently collected mite species were *Uropodoidea* sp. 4 and *Uropodoidea* sp. 6 each of which represented 21.2 % of all mites (**Table 1**). The next most frequently collected species was *Insectolaelaps* sp. 2 which represented 13.9 % of the mites. A few species were collected at very low frequencies, such as *Uropodoidea* sp. 7 and *Uropodoidea* sp. 8 that only represented 0.6 % of the collections.

The most frequently isolated ophiostomatoid fungi were *O. nitidum* (Taxon 2) and *L. taigense* (Taxon 11), which represented 23.5 % and 22.8 % of the total isolates respectively (**Table S3**). This was followed by *O. qinghaiense* (Taxon 3, 8.1 %) and *O. shangrilae* (Taxon 6, 8.1 %), and *O. ainoae* (Taxon 5, 7.4 %) and Taxon 8 (7.4 %). The fungi with the lowest frequency of isolations were *L. breviscapa* (Taxon 10) and *L. ningerensis* (Taxon 9), both of which represented 0.7 % of total isolates. The remaining fungi were found at frequencies lower than 5 %.

The number of fungal isolates collected from different mite species differed substantially (**Table S3**). About 20 % of the fungi were isolated from mites in the family *Winterschmidtidae*. Twenty-three isolates collected from *Uropodoidea* sp. 4 represented 17 % of the total isolates. This was followed by 12.1 % of the isolates collected from *Insectolaelaps* sp. 2, 10 % isolates collected from *Insectolaelaps* sp. 1, 10.6 % isolates collected from *Uropodoidea* sp. 6 (**Table S3**).

The 33 isolates collected from the mites in *D. micans* galleries represented 23.4 % of the total number of isolates, while 75 isolates from the mites in *I. nitidus* galleries represented 53.2 %, 19 isolates from the mites in *I. shangrila* galleries represented 14.2 %, and the remaining 13 isolates from mites in *P. polygraphus* galleries represented 9.2 % of the isolates.

Taxonomy

Taxon 4

Ophiostoma kunlunense R.L. Chang & Z.W. de Beer, **sp. nov.**

Mycobank MB 827335

(**Fig. 6**)

Etymology. Name refers to the Kunlun mountains, which is one of the longest mountain ranges in Asia, that ends in Qinghai province from where this fungus was first isolated.

Description: Sexual state not observed. Pesotum-like asexual state, occurring singly or in groups of up to 15, macronematous, erect, (511–) 705–1729.5 (–1301) μm long, including condigenous apparatus. Conidia hyaline, 1-celled, smooth, oblong, clavate or obovoid (3.5–) 3–6 (–8) $\times$ 1.5–2 (–2.5) μm .

Culture characteristics: colonies hyaline or dark brown when synnemata form. Mycelium superficial on the agar. Pesotum-like asexual state dominant in the cultures. Optimal temperature for growth 20 °C, reaching 56.6 mm diam in 10 d. No growth was observed at 5 °C or 30 °C and above.

Specimens examined. CHINA, Qinghai province, Maixiu Forest Farm, from *Uropodoidea* sp. in gallery of *Ips shangrila* on *Picea purpurea*, 8 Aug. 2010, S. J. Taerum, PREM61583 (herbarium specimen of dried culture), holotype, CMW41927 = CBS141903 (ex-holotype culture). CHINA, Qinghai province, Maixiu Forest Farm, from *Insectolaelaps* sp. in gallery of *Ips shangrila* on *Picea purpurea*, 8 Aug. 2010, S. J. Taerum, PREM61584 (herbarium specimen of dried culture), paratype, CMW48853 = CBS141904 (ex-paratype culture). CHINA, Qinghai province, Maixiu Forest Farm, from *Uropodoidea* sp. in gallery of *Ips shangrila* on *Picea purpurea*, 8 Aug. 2010, S. J. Taerum, PREM61585 (herbarium specimen of dried culture), paratype, CMW48854 = CBS141905 (ex-paratype culture).

Notes: *Ophiostoma kunlunense* produces a pesotum-like asexual state similar to all other species in *O. piceae* complex, and species peripheral to the complex such as *O. nikkoense* and *O. setosum*. However, the species lacks the sporothrix-like synasexual state that characterizes *O. piceae* complex species (Harrington et al., 2001), and is thus more similar to species in *O. clavatum* and *O. ips* complex than those in *O. piceae* complex and species peripheral to the complex.

Taxon 5

Ophiostoma ainoae H. Solheim, Nord. J. Bot. 6:201 (1986). MB 103626.

= *Ophiostoma poligraphi* M.L. Yin, Z.W. de Beer & M.J. Wingf., Fungal Biol. 120:464 (2016). MB 814785

Notes: When *BT* and *EF* sequences produced for several isolates obtained in the present study were analysed together with sequences of three *O. ainoae* isolates from the study of Linnakoski et al. (2016a) and two *O. poligraphi* isolates from the study of Yin et al. (2016), it became clear that the latter two groups of isolates represented geographically isolated populations of the same species. Sequences of our isolates did not group consistently with isolates in either of the two clades (**Fig. S1**). For the *BT* region, our isolates grouped between the two 'species', while in the *EF* analyses, they all grouped with *O. poligraphi*. There are confirmed reports of *O. ainoae* from *Ips typographus* and *Pityogenes chalcographus* on *Picea abies* in Europe (Linnakoski et al., 2016a), while *O. poligraphi* was described from *Polygraphus poligraphus* and *Dendroctonus micans* on *Picea crassifolia* in Qinghai (Yin et al., 2016). Our isolates were from mites on *Polygraphus poligraphus* and *Ips nitidus*, also from *Picea crassifolia* in Qinghai, and this is consistent with the host and beetle vectors of both species. Although a sexual state has not been described for *O. poligraphi*, the broad synnemata of this species also correspond with those described for *O. ainoae* (Solheim, 1986), synonymy of the two species.

Taxon 7

Ophiostoma manchongi R.L. Chang & Z.W. de Beer, **sp. nov.**

Mycobank MB 827336

(**Fig. 7**)

Etymology. Name refers to *manchong*, the Chinese word for mite.

Description: Sexual state not observed. Sporothrix-like asexual state, erect, arising directly from the mycelium. Conidia hyaline, 1-celled, smooth, oblong, obovoid (3–) 4–5.5 (–7) x (1.5–) 2–3 (–4.5) μm .

Culture characteristics: colonies at first hyaline, later becoming dark brown at the centre. Mycelium superficial on the agar. Optimal temperature for growth 25 °C, reaching 29.6 mm diam in 10 d. No growth at 5 °C or 35 °C.

Specimens examined. CHINA, Qinghai province, Xianmi Forest Farm, from *Uropodoidea* sp. in gallery of *Ips shangrila* on *Picea purpurea*, 8 Aug. 2010, S. J. Taerum, Holotype PREM61580 (herbarium specimen of dried culture), holotype, CMW41954 = CBS141906 (ex-holotype culture). CHINA, Qinghai province, Xianmi Forest Farm, from *Insectolaelaps* sp. in gallery of *Ips nitidus* on *Picea crassifolia*, 8 Aug. 2010, S. J. Taerum, PREM61581 (herbarium specimen of dried culture), paratype, CMW42975 = CBS141907 (ex-paratype culture). CHINA, Qinghai province, Xianmi Forest Farm, from *Uropodoidea* sp. in gallery of *Ips shangrila* on *Picea purpurea*, 8 Aug. 2010, S. J. Taerum, PREM61582 (herbarium specimen of dried culture), paratype, CMW41979 = CBS141908 (ex-paratype culture).

Notes: The sporothrix-like asexual state of *Ophiostoma manchongi* is different from its closest relative, *O. japonicus*, which has a light coloured synnematus asexual state, described at the time as *Graphilbum*-like

(Yamaoka et al., 1997). The conidia in these two species are also very different. Conidia of *O. japonicus* are cylindrical or clavate (Yamaoka et al., 1997), while those of *Ophiostoma manchongi* are obovoid. Both ITS and BT DNA sequences can also be used to distinguish *O. manchongi* from *O. japonicus*.

Taxon 13

Grosmannia zekuensis R.L. Chang & Z.W. de Beer, **sp. nov.**

Mycobank MB 827337

(Fig. 8)

Etymology. Name refers to Zeku, the county where samples were collected.

Description: Sexual state not observed. Conidiophores macronematous, mononematous, erect, arising directly from the mycelium, (98–) 114–182 (–269) μm long. Rhizoids present. Stipes olivaceous, 2–4 septate, not constricted at septa, (67–) 73.5–125.5 (–182) μm long. Apical cells occasionally swollen at apex, (4–) 5–7.5 (–10.5) μm wide. Basal cells not swollen, (5.5–) 7–10 (–11) μm wide. Conidiogenous apparatus (16–) 30.5–49 (–67) μm long, excluding the conidial mass, with multiple series of cylindrical branches. Primary branches olivaceous, smooth, cylindrical, not swollen at apex, aseptate, arrangement of primary branches was Type B—more than two branches, (7.5–) 10–16 (–16) $\times$ (3–) 3.5–4.5 (–5.5) μm . Secondary branches light olivaceous, frequently swollen at apex, aseptate, (6–) 8–11 (–12.5) $\times$ (2–) 2.5–4 (–5) μm . Tertiary branches light olivaceous, aseptate, (6–) 7–10 (–11) $\times$ (2–) 2.5–3 (–3) μm . Conidiogenous cells discrete, hyaline, 2–3 per branch, aseptate, cylindrical, tapering slightly at the apex, (6–) 8–12 (–14.5) $\times$ (1.5–) 2–3 (–3) μm . Conidia hyaline, aseptate, elliptical, (3.5–) 4–5.5 (–8) $\times$ (2–) 2.5–3 (–3.5) μm .

Culture characteristics: colonies hyaline without asexual structures or dark brown when asexual structures form. Mycelium superficial on the agar. Optimal temperature for growth 25 °C. Some isolates grow faster, reaching 73.3 mm diam in 8 d at 25 °C and no growth at 30 °C and above. However, some isolates grow slow only reach 46.3 mm diam in 8 d and slow growth observed at 35 °C.

Specimens examined. CHINA, Qinghai province, Xianmi Forest Farm, from *Bakerdania* sp. in gallery of *Ips nitidus* on *Picea crassifolia*, 8 Aug. 2010, S. J. Taerum, PREM61579 (herbarium specimen of dried culture), holotype, CMW41876 = CBS141901 (ex-holotype culture). CHINA, Qinghai province, Xianmi Forest Farm, from *Insectolaelaps* sp. in gallery of *Ips nitidus* on *Picea crassifolia*, 8 Aug. 2010, S. J. Taerum, PREM61578 (herbarium specimen of dried culture), paratype, CMW48852 = CBS141902 (ex-paratype culture). CHINA, Qinghai province, Xianmi Forest Farm, from *Schwiebea wainsteini* in gallery of *Ips nitidus* on *Picea crassifolia*, 8 Aug. 2010, S. J. Taerum, PREM61577 (herbarium specimen of dried culture), paratype, CMW41870 = CBS141900 (ex-paratype culture).

Note: *Grosmannia zekuensis* is morphologically similar to *G. purpurea*, but the two species can be distinguished based on differences in BT and EF sequences.

Discussion

In this study, we collected 173 mites representing 18 species from spruce-infesting bark beetles in Qinghai

province of China. *Uropodoidea* sp. 4 and *Uropodoidea* sp. 6, were most abundant among the mites. We obtained 135 fungal isolates from 65 mite individuals. Based on DNA sequence data, 14 fungal species were identified, eight species of *Ophiostoma* and six of *Leptographium*. Among these species, three were recognized as novel taxa and were thus described and provided with names. Of the remaining 11 species, ten had previously been reported from China. *Ophiostoma tetropii* is reported for the first time from China.

The only previous study to report on ophiostomatoid fungi associated with phoretic mites in China was conducted on mite associates of six pine-infesting bark beetles in Yunnan (Chang et al., 2017). The species diversity of mites collected in the present study from Qinghai was higher than that found in Yunnan (18 species vs. 13), despite the fact that only four beetle species were sampled in Qinghai as opposed to six in Yunnan. However, more mite individuals (173) were collected in Qinghai from two host trees than the 106 mites from only *Pinus kesiya* in Yunnan (Chang et al., 2017). Only *Insectolaelaps* sp. 1 was collected from both Qinghai and Yunnan. This species was the most frequently collected species in Yunnan and the fourth most frequently collected species in Qinghai, suggesting that it is widely distributed on conifer hosts in western China. The fact that only one species was shared between the two regions is perhaps not surprising because the mites were collected from different bark beetle species, different tree species, and different climatic zones.

Together with the previous report from Yunnan (Chang et al., 2017), a total of 30 species of mites associated with ten bark beetles have now been reported from western China. This number is much less than 270 mite species that have been reported in association with more 110 bark beetle species from the many studies in North America and Europe (Hofstetter et al., 2015)., twelve mite species were reported from *Ips typographus* in Japan, of which only three species did not occur in Europe (Moser et al., 1997). Because most of the mites in our study have not been identified to species level, and because our sampling strategy was aimed at isolating fungi and not only the collection of mites, it is not possible to compare the presence or absence of mite species with other. However, at a higher taxonomic level, the most frequent mite order associated with bark beetles was the *Mesostigmata* in both China and collections collectively from Europe and North America, where it is represented by 20 and 140 species respectively (Hofstetter et al., 2015).

The fungal species diversity from mites in Qinghai was somewhat higher when compared with the diversity of fungal species from mites in Yunnan (Chang et al., 2017), with 12 species reported from Yunnan versus 14 species in Qinghai, although the total number of fungal isolates was smaller in Qinghai. The fungi collected in Qinghai were all belonged to only two genera in the *Ophiostomatales*, *Ophiostoma* and *Leptographium* s.l., while isolates from Yunnan belonged to five genera, four (*Graphilbum*, *Leptographium* s.l., *Ophiostoma* and *Sporothrix*) in the *Ophiostomatales*, and *Graphium* in the *Microascales*. The fungal community collected from bark beetle-associate mites in Qinghai was very different to that in Yunnan. Only one fungal species, *L. ningerensis*, was shared between Qinghai and Yunnan.

A comparison of the fungal species from spruce infesting bark beetles in Qinghai (Yin et al., 2016) with those from mites in our study, revealed that eight species were found in both studies. These included : *L. breviscapa*, *G. purpurea*, *G. xianmiense*, *O. nitidum*, *O. qinghaiense*, *O. ainoae*, *O. shangrilae* and *O. tetropii*. Not surprisingly, a large number of fungal species were also shared between galleries and mites in Yunnan province (Chang et al., 2017).

Apart from the three new species discovered in this study, we also collected and identified *O. tetropii* for the

first time from China. This fungus was first described from spruce tree attacked by *Tetropium* sp., in Sweden (Mathiesen, 1951), and was subsequently also reported from *Picea abies* attacked by *Ips typographus* and *Pityogenes chalcographus* in Finland (Linnakoski et al., 2010) and *Picea rubens* attacked by *Tetropium fuscum* in Canada (Harrison & Smith, 2013). Its presence on mites in China is surprising because it was considered as an association of beetles such as Harrison & Smith (2013) suggest that the presence of the fungus as the indicator of *Tetropium fuscum*.

Conclusions

The results of this study conducted in China reaffirmed the findings elsewhere in the world showing that there are close associations between bark beetle-associated mites and ophiostomatoid fungi. The sampling area, beetle species and hosts considered was relatively limited. Yet many undescribed species of mites and various novel taxa in *Ophiostoma* and *Leptographium* emerged from the study. This reflects how little is known regarding the ecology of bark beetles and their symbionts. This is not only in China but also in many other parts of the world. Although relatively high numbers of mites and fungal species were found in this study, the survey covered a relatively small geographical area. Larger surveys with more systematic sampling are needed in the future to elucidate the ecological roles and specificity of fungus-vector relationships. However, what is clear is that mites may act as important vectors of fungal species other than those vectored by the bark beetles. These mites and their fungi should therefore also be considered in pest risk assessments which often only focus on the bark beetles and their fungi.

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Table 1. Mites (*Acari*) collected from bark beetle galleries on spruce in Qinghai in this study.

Mite	Order/Family Name	Species Name	NI ¹	NI/TN ²	NMCF ³	NMCF/NI ⁴
M 1	<i>Acaridae</i>	<i>Horstia</i> sp.	1	0.0061	0	0.000
M 2	<i>Acaridae</i>	<i>Schwiebea wainsteini</i>	2	0.0121	2	1.000
M 3	<i>Ascidae</i>	<i>Diseius cf. ulmi</i>	3	0.0182	1	0.333
M 4	<i>Digamasellidae</i>	<i>Dendrolaelaps</i> sp.	1	0.0061	0	0.000
M 5	<i>Digamasellidae</i>	<i>Insectolaelaps</i> sp. 2	23	0.1394	7	0.304
M 6	<i>Digamasellidae</i>	<i>Insectolaelaps</i> sp. 1	15	0.0909	7	0.467
M 7	<i>Ereynetidae</i>	<i>Ereynetes</i> sp.	3	0.0182	2	0.667
M 8	<i>Ereynetidae</i>	sp.	1	0.0061	0	0.000
M 9	Unknown	sp. 1	6	0.0364	1	0.167
M 10	<i>Melicharidae</i>	<i>Proctolaelaps</i> nr. <i>hystrix</i>	10	0.0606	5	0.500
M 11	<i>Mesostigmata</i>	sp. 1	1	0.0061	1	1.000
M 12	<i>Mesostigmata</i>	sp. 2	1	0.0061	0	0.000
M 13	<i>Mesostigmata</i>	sp. 3	1	0.0061	1	1.000
M 14	<i>Mesostigmata</i>	sp. 4	2	0.0121	1	0.500
M 15	Unknown	sp. 2	6	0.0364	1	0.167
M 16	<i>Pygmephoridae</i>	<i>Bakerdania</i> sp.	5	0.0303	1	0.200
M 17	<i>Tarsonemidae</i>	<i>Tarsonemus</i> sp.	7	0.0424	1	0.143
M 18	<i>Uropodoidea</i>	sp. 4	35	0.2121	13	0.371
M 19	<i>Uropodoidea</i>	sp. 5	1	0.0061	0	0.000
M 20	<i>Uropodoidea</i>	sp. 6	35	0.2121	9	0.257
M 21	<i>Uropodoidea</i>	sp. 7	1	0.0061	1	1.000
M 22	<i>Uropodoidea</i>	sp. 8	1	0.0061	0	0.000
M 23	<i>Winterschmidtidae</i>	sp.	11	0.0667	10	0.909
M 24	<i>Zerconidae</i>	<i>Zercon</i> sp.	1	0.0061	1	1.000
			173		65	0.376

¹ NI = Number of mite individuals² NI/TN = Number of mite individuals/Total number of mite individuals³ NMCF = Number of mites carrying fungi⁴ NMCF/NI = Number of mites carrying fungi/ Number of mite individuals

Table 2. Isolates of ophiostomatoid fungi obtained from different mites in Qinghai.

Taxon	Species	Isolate number ^{1,2}		Host	Beetle	Mite ³	Locations	GenBank number ⁴		
		CMW	CBS					ITS/ITS2-LSU	BT	EF
1	<i>Ophiostoma tetropii</i>	41891		<i>Picea crassifolia</i>	<i>Dendroctonus micans</i>	M11	Maixiu	MH121623	MH124426	MH124490
		41892		<i>P. crassifolia</i>	<i>D. micans</i>	M11	Maixiu	MH121624	MH124427	MH124491
		41893		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121625	MH124428	MH124492
		41938		<i>P. crassifolia</i>	<i>Ips nitidus</i>	M7	Xianmi	MH121626	MH124429	MH124493
2	<i>O. nitidum</i>	41883		<i>P. crassifolia</i>	<i>Polygraphus polygraphus</i>	M6	Maixiu	MH121627	MH124430	MH124494
		41874		<i>P. crassifolia</i>	<i>I. nitidus</i>	M9	Maixiu	MH121628	MH124431	MH124495
		41886		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M6	Maixiu	MH121629	MH124432	MH124496
		41895		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121630	MH124433	MH124497
		41898		<i>P. crassifolia</i>	<i>D. micans</i>	M3	Maixiu	MH121631	MH124434	MH124498
		41899		<i>P. crassifolia</i>	<i>D. micans</i>	M3	Maixiu	MH121632	MH124435	MH124499
		41901		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121633	MH124436	MH124500
		41902		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121634	MH124437	MH124501
		41911		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121635	MH124438	MH124502
		41917		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121636	MH124439	MH124503
		41918		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121637	MH124440	MH124504
		41923		<i>P. purpurea</i>	<i>Ips shangrila</i>	M6	Maixiu	MH121638	MH124441	MH124505
		41933		<i>Picea purpurea</i>	<i>I. shangrila</i>	M17	Maixiu	MH121639	MH124442	MH124506
		41934		<i>P. purpurea</i>	<i>I. shangrila</i>	M17	Maixiu	MH121640	MH124443	MH124507
		41939		<i>P. crassifolia</i>	<i>I. nitidus</i>	M20	Xianmi	MH121641	MH124444	MH124508
3	<i>O. qinghaiense</i>	41900		<i>P. crassifolia</i>	<i>D. micans</i>	M3	Maixiu	MH121642	MH124445	MH124509
		41903		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121643	MH124446	MH124510
		41905		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121644	MH124447	MH124511
		41906		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121645	MH124448	MH124512
		41907		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121646	MH124449	MH124513
		41915		<i>P. crassifolia</i>	<i>D. micans</i>	M23	Maixiu	MH121647	MH124450	MH124514
4	<i>O. kunlunense</i>	41927	141903 ^H	<i>P. purpurea</i>	<i>I. shangrila</i>	M19	Maixiu	MH121648	MH124451	MH124515

		48853	141904	<i>P. purpurea</i>	<i>I. shangrila</i>	M6	Maixiu	MH121649	MH124452	MH124516
		48854	141905	<i>P. purpurea</i>	<i>I. shangrila</i>	M18	Maixiu	MH121650	MH124453	-
5	<i>O. ainoae</i>	41882		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M20	Maixiu	MH121651	MH124454	MH124517
		41890		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M14	Maixiu	MH121652	MH124455	MH124518
		41958		<i>P. crassifolia</i>	<i>I. nitidus</i>	M18	Xianmi	MH121653	MH124456	MH124519
		41881		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M20	Maixiu	MH121654	MH124457	MH124520
		41950		<i>P. crassifolia</i>	<i>I. nitidus</i>	M10	Xianmi	MH121655	MH124458	MH124521
6	<i>O. shangrilae</i>	41885		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M6	Maixiu	MH121656	MH124459	MH124522
		41930		<i>P. purpurea</i>	<i>I. shangrila</i>	M18	Maixiu	MH121657	MH124460	MH124523
		41968		<i>P. crassifolia</i>	<i>I. nitidus</i>	M24	Xianmi	MH121658	MH124461	MH124524
		41983		<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121659	MH124462	MH124525
		41984		<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121660	MH124463	MH124526
7	<i>O. manchongi</i>	41872		<i>P. crassifolia</i>	<i>I. nitidus</i>	M10	Maixiu	MH121661	MH124464	MH124527
		41954	141906 ^H	<i>P. crassifolia</i>	<i>I. nitidus</i>	M18	Xianmi	MH121662	MH124465	-
		41975	141907	<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121663	MH124466	MH124528
		41978		<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121664	MH124467	MH124529
		41979	141908	<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121665	MH124468	-
8	<i>O. bicolor</i>	41861		<i>P. crassifolia</i>	<i>I. nitidus</i>	-	Maixiu	MH121666	MH124469	-
		41877		<i>P. crassifolia</i>	<i>I. nitidus</i>	M15	Maixiu	MH121667	MH124470	MH124530
		41878		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M20	Maixiu	MH121668	MH124471	MH124531
		41949		<i>P. crassifolia</i>	<i>I. nitidus</i>	M18	Xianmi	MH121669	MH124472	MH124532
		41957		<i>P. crassifolia</i>	<i>I. nitidus</i>	M18	Xianmi	MH121670	MH124473	MH124533
		41965		<i>P. crassifolia</i>	<i>I. nitidus</i>	M20	Xianmi	MH121671	MH124474	MH124534
9	<i>Leptographium ningerensis</i>	41868		<i>P. crassifolia</i>	<i>I. nitidus</i>	M6	Maixiu	MH121672	MH124475	MH124535
10	<i>L. breviuscapa</i>	41887		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M6	Maixiu	MH121673	MH124476	MH124536
11	<i>L. taigense</i>	41864		<i>P. crassifolia</i>	<i>I. nitidus</i>	-	Xianmi	MH121674	MH124477	MH124537
		41970		<i>P. crassifolia</i>	<i>I. nitidus</i>	M2	Xianmi	MH121675	MH124478	MH124538
		41977		<i>P. crassifolia</i>	<i>I. nitidus</i>	M5	Xianmi	MH121676	MH124479	MH124539
12	<i>Grosmannia purpurea</i>	41928		<i>P. purpurea</i>	<i>I. shangrila</i>	M18	Maixiu	MH121677	MH124480	MH124540

12	<i>G. purpurea</i>	41922		<i>P. purpurea</i>	<i>I. shangrila</i>	M18	Maixiu	MH121678	MH124481	MH124541
13	<i>L. zekuensis</i>	41862		<i>P. crassifolia</i>	<i>I. nitidus</i>	-	Maixiu	MH121679	MH124482	MH124542
		41870	141900	<i>P. crassifolia</i>	<i>I. nitidus</i>	M2	Maixiu	MH121680	-	MH124543
		41871		<i>P. crassifolia</i>	<i>I. nitidus</i>	M6	Maixiu	MH121681	MH124483	MH124544
		41875		<i>P. crassifolia</i>	<i>I. nitidus</i>	M9	Maixiu	MH121682	MH124484	MH124545
		41876	141901 ^H	<i>P. crassifolia</i>	<i>I. nitidus</i>	M16	Maixiu	MH121683	MH124485	MH124546
		48852	141902	<i>P. crassifolia</i>	<i>I. nitidus</i>	M6	Maixiu	MH121684	MH124486	MH124547
14	<i>G. xianminense</i>	41866		<i>P. crassifolia</i>	<i>I. nitidus</i>	M13	Maixiu	MH121685	MH124487	MH124548
		41867		<i>P. crassifolia</i>	<i>I. nitidus</i>	M18	Maixiu	MH121686	MH124488	MH124549
		41880		<i>P. crassifolia</i>	<i>P. polygraphus</i>	M20	Maixiu	MH121687	MH124489	MH124550

¹ The culture collection (CBS) of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

² H = ex-holotype isolate

³ Mite species, see Table 1

⁴ ITS = internal transcribed spacer regions 1 and 2 of the nuclear ribosomal DNA operon, including the 5.8S region; ITS2-LSU = the internal transcribed spacer 2 region and partial large subunit of the nrDNA operon; *BT* = beta-tubulin; *EF* = translation elongation factor 1-alpha.

Fig. 1 (Following pages) Phylogram obtained from ML analyses of the ITS region of *Ophiostoma*. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

ITS

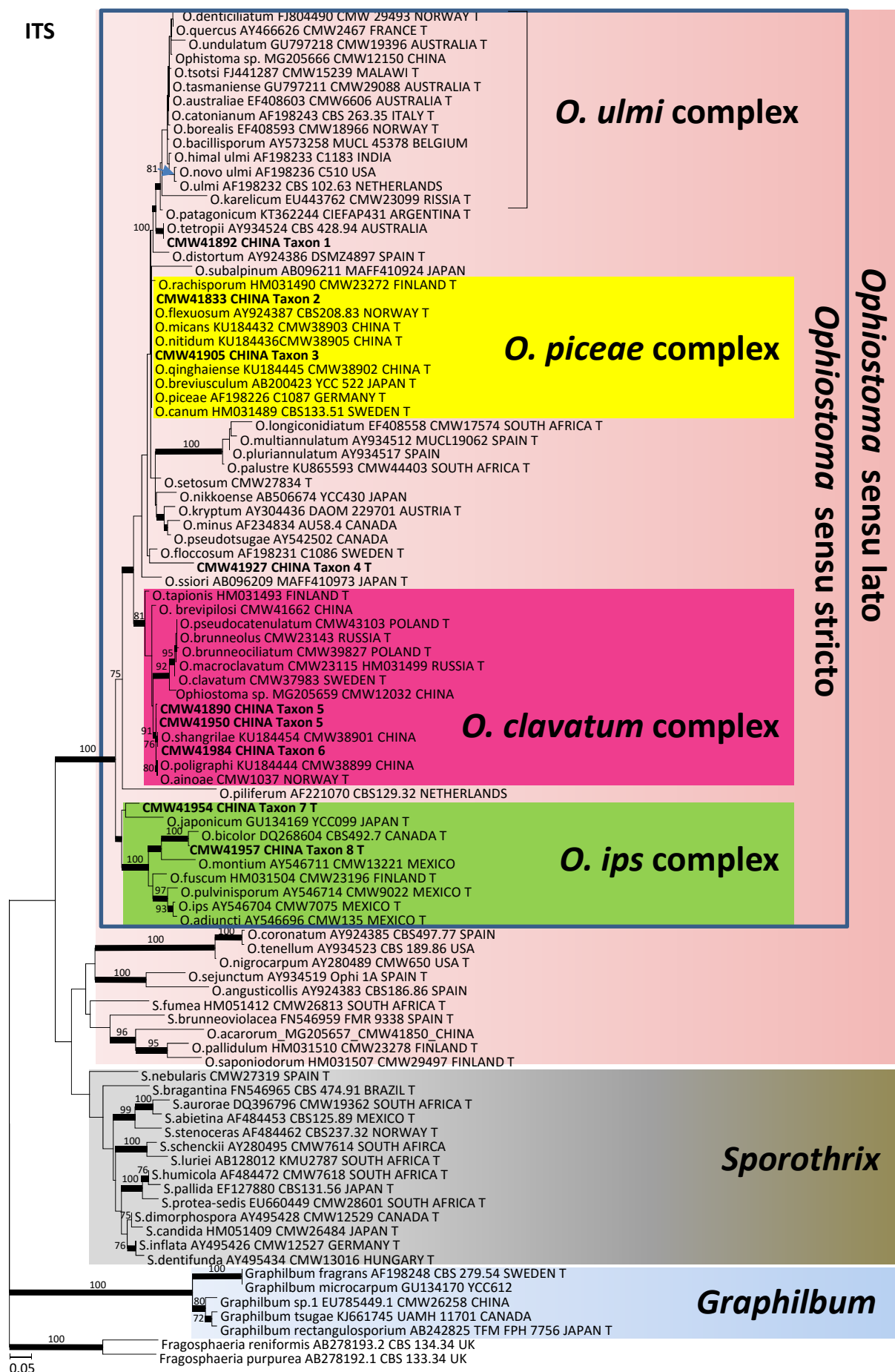
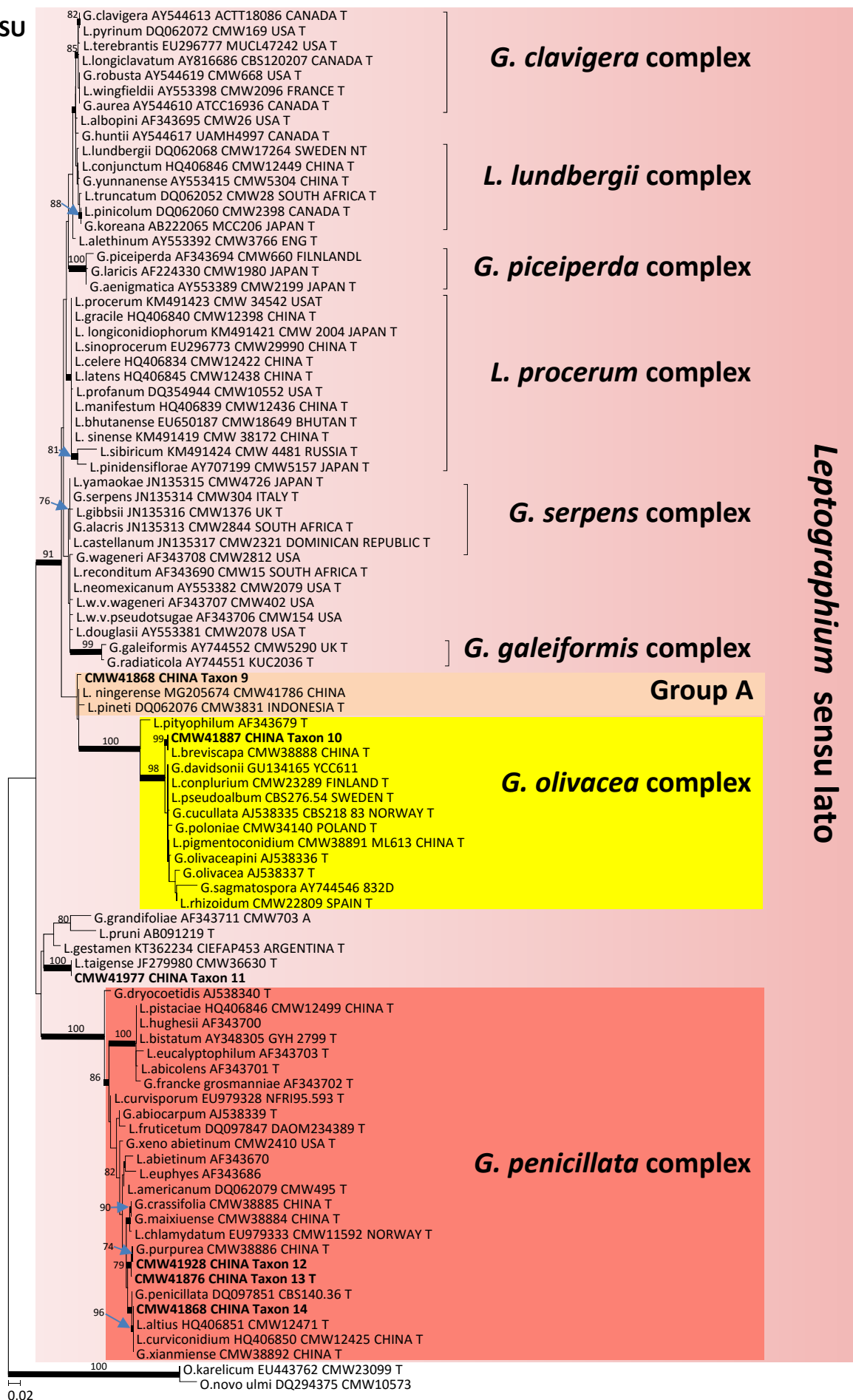


Fig. 2 (Following pages) Phylogram obtained from ML analyses of the ITS2-LSU region of *Leptographium*. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

ITS2-LSU



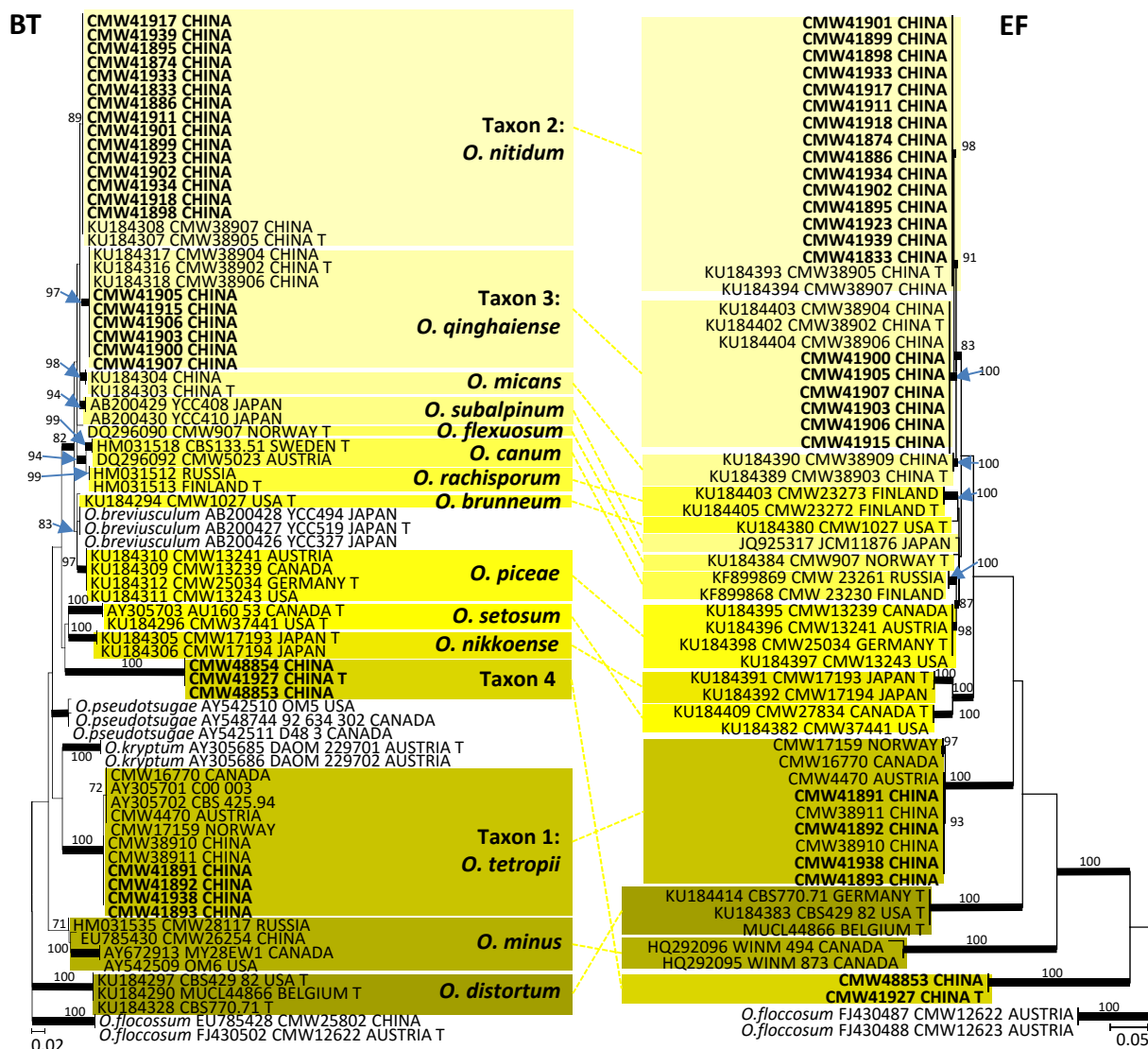


Fig. 3 Phylogram obtained from ML analyses of the partial *BT* and *EF* gene of *O. piceae* complex. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

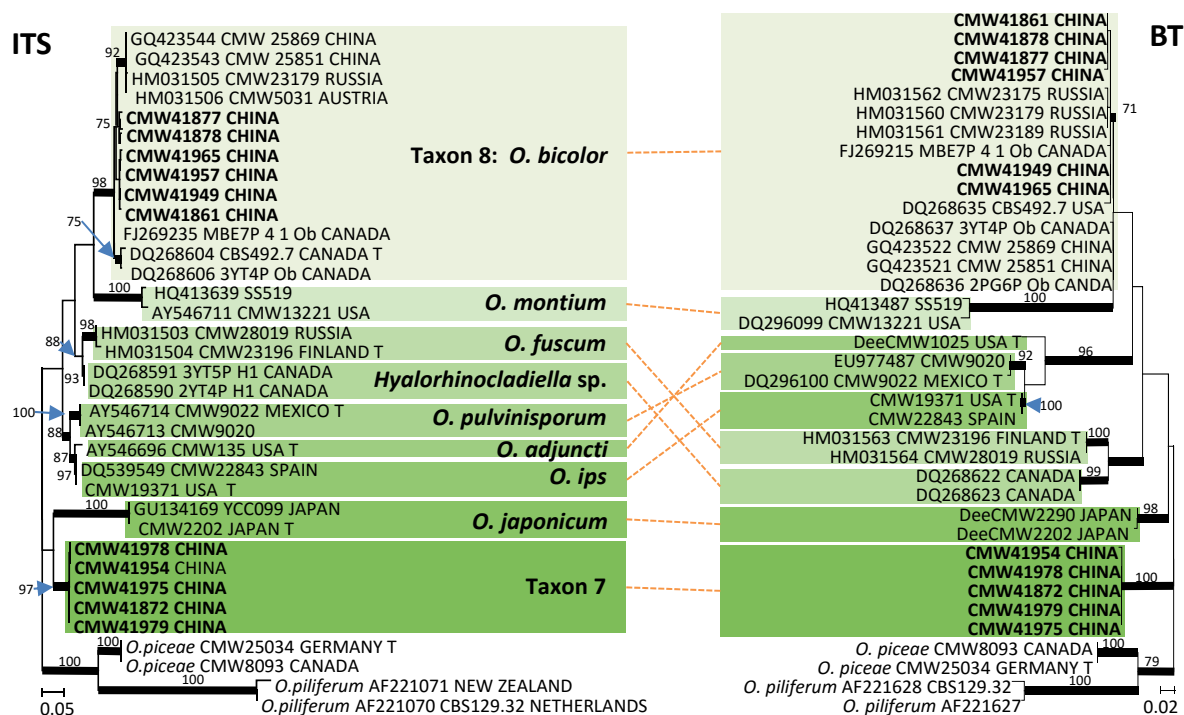


Fig. 4 Phylogram obtained from ML analyses of the ITS region and the partial *BT* gene of *O. ips* complex. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

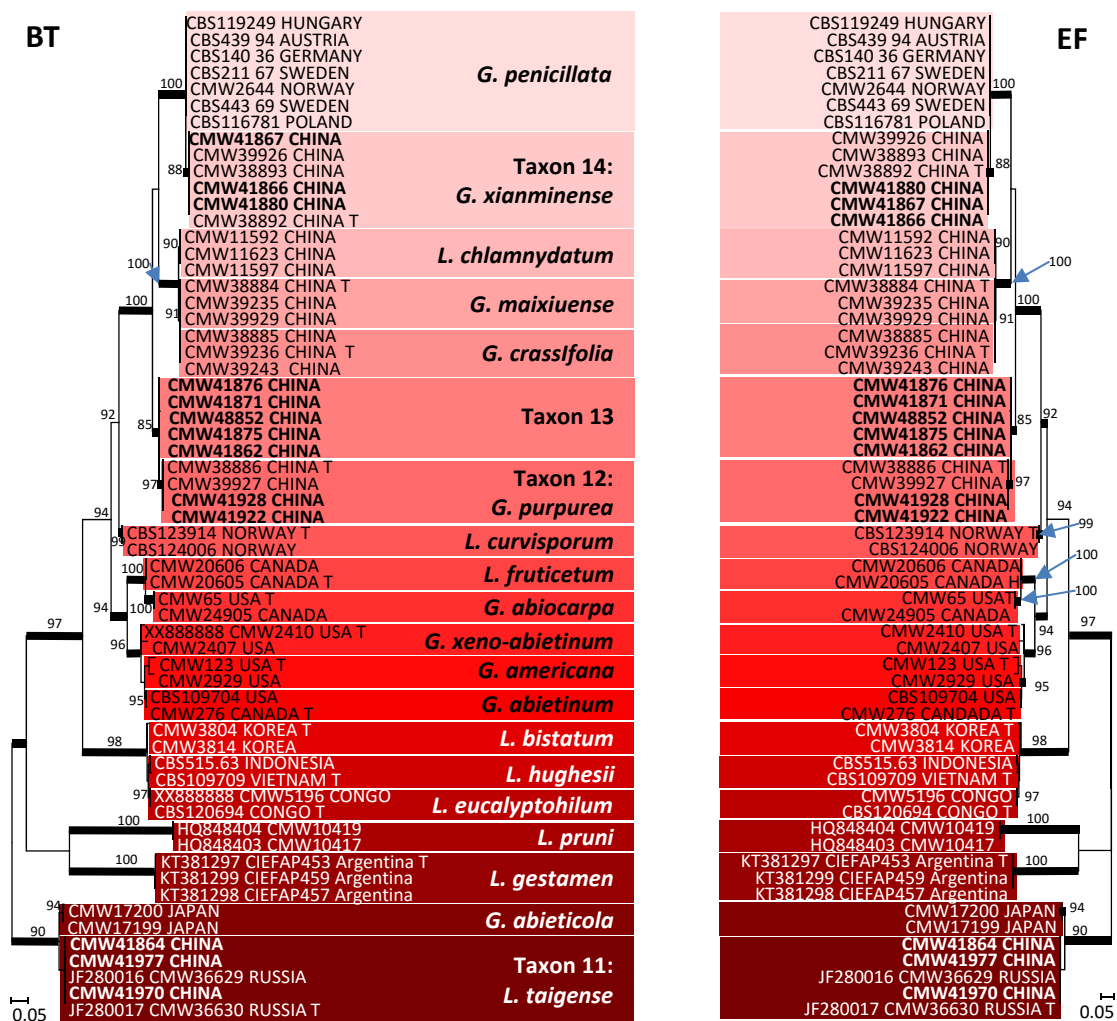


Fig. 5 Phylogram obtained from ML analyses of the partial *BT* and *EF* gene of *G. penicillata* complex. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

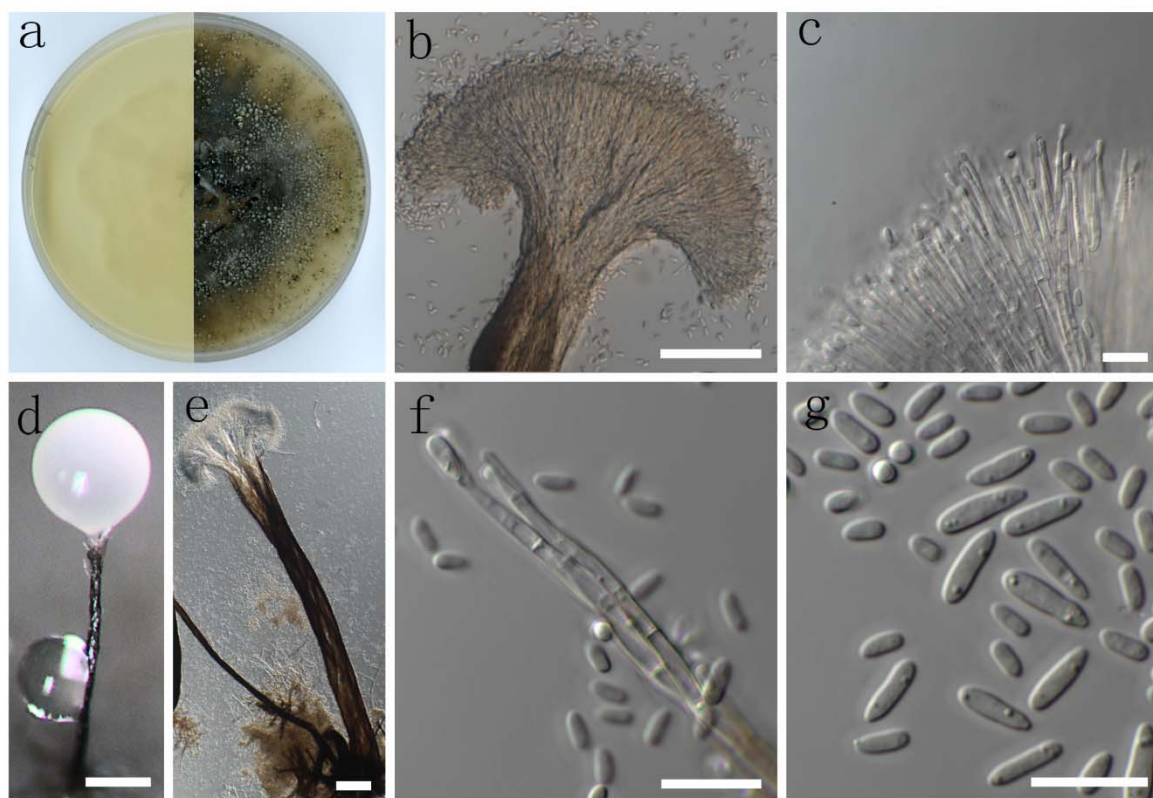


Fig. 6 Morphological characters of asexual structure of *Ophiostoma kunlunense* sp. nov. a. Cultures on malt extract agar (MEA); b, d, e. Pesotum asexual state; c, f. conidiogenous cells; g. conidia. –Scale bars: b = 50 µm; c, f, g = 10 µm; d = 200 µm; e = 100 µm.

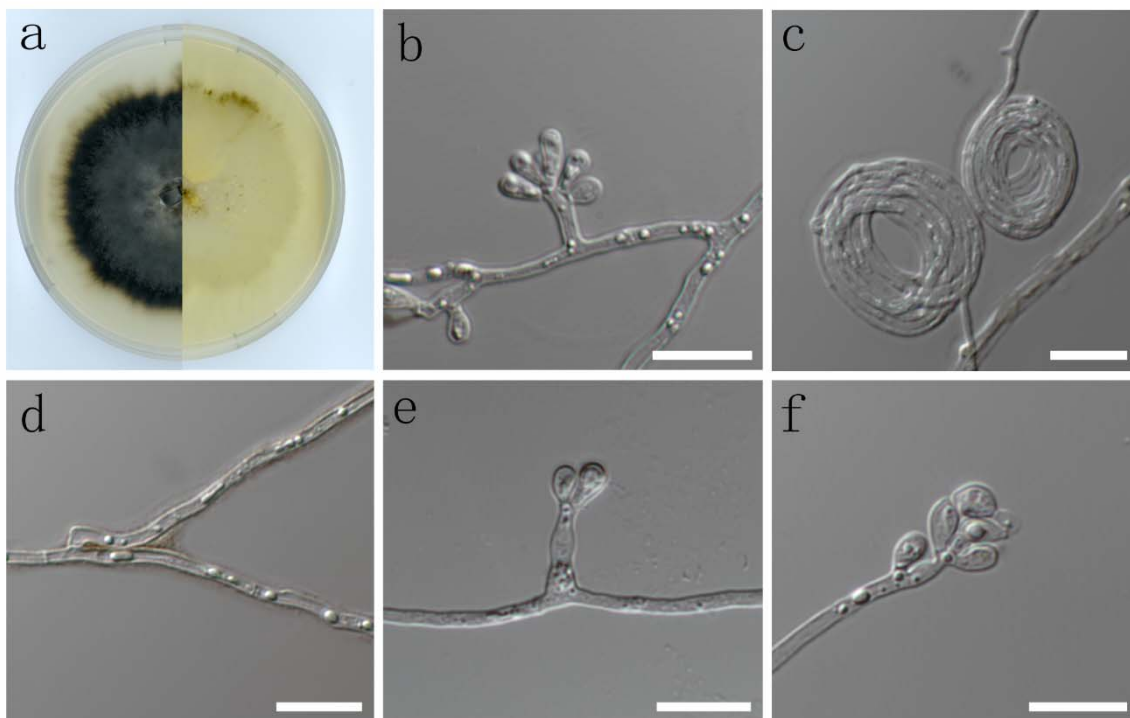


Fig. 7 Morphological characters of asexual structure of *Ophiostoma manchongi* sp. nov. a. Cultures on malt extract agar (MEA); b, e, f. conidiogenous cells and conidia; c, d mycelium. – Scale bars: 10 μ m.

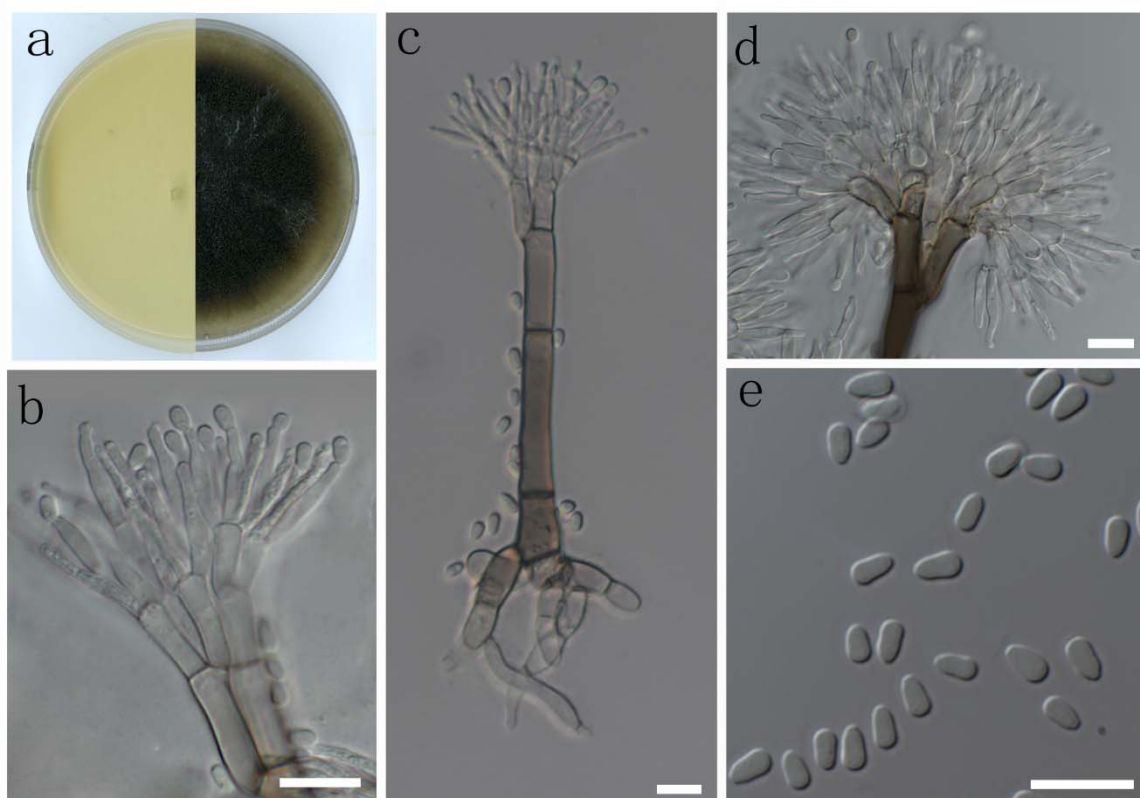


Fig. 8 Morphological characters of asexual structure of *Grosmannia zekuensis* sp. nov. a. Cultures on malt extract agar (MEA); b, d. conidiogenous apparatus; c. conidiophore; e conidia. Scale bars: 10 µm.

Table S1. Ophiostomatoid fungi reported from China.

Fungal species	Location	Tree host	Beetle vector	Reference
<i>Endoconidiophora fujiensis</i>	Inner Mongolia	<i>Larix gmelinii</i>	<i>Ips subelongatus</i>	Zhou et al., 2011
<i>E. polonica</i>	Heilongjiang	<i>Pinus koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>Graphilbum fragrans</i>	Yunnan	<i>Pi. yunnanensis</i>	<i>Tomicus minor</i>	Paciura et al., 2010b; Zhou et al., 2013; Chang et al., 2017
		<i>Tsuga dumosa</i>	<i>T. yunnanensis</i>	
		<i>Pi. armandii</i>	<i>Pissodes</i> sp.	
<i>Gra. kesiya</i>	Yunnan	<i>Pi. kesiya</i>	<i>Polygraphus szemaoensis</i>	Chang et al., 2017
			<i>P. aterrimus</i>	
			<i>Polygraphus</i> sp.	
<i>Gra. puerense</i>	Yunnan	<i>Pi. kesiya</i>	<i>P. szemaoensis</i>	Chang et al., 2017
			<i>I. acuminatus</i>	
<i>Graphilbum</i> sp.	Shanxi	<i>Pi. tabuliformis</i>	<i>Dendroctonus valens</i>	Taerum et al., 2013
<i>Graphium carbonarium</i>	Yunnan	<i>Salix babylonica</i>	<i>Pissodes</i> sp.	Paciura et al., 2010b
<i>Gr. cf. rectangulosporium</i>	Zhejiang	<i>P. massoniana</i>	<i>Monochamus alternatus</i>	Wang et al., 2018
<i>Gr. fimbriisporum</i>	Jilin	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
	Heilongjiang	<i>Picea</i> sp.		
<i>Gr. pseudormiticum</i>	Yunnan	<i>Pi. kesiya</i>	<i>I. acuminatus</i>	Paciura et al., 2010b; Chang et al., 2017
		<i>T. dumosa</i>	<i>Pissodes</i> sp.	
<i>Grosmannia radiaticola</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>G. koreana</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
	Shannxi			
<i>G. cucullata</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>G. olivacea</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>G. piceiperda</i>	Jilin	<i>Picea</i> sp.	<i>I. typographus</i>	Chang et al., 2019
<i>G. radiaticola</i>	Yunnan	<i>Tsuga</i> sp.	Unknown	Chang et al., 2017

<i>G. yunnanensis</i>	Yunnan	<i>Pi. kesiya</i>	<i>P. szemaoensis</i> <i>Lasconotus</i> sp. <i>I. acuminatus</i> <i>Polygraphus</i> sp. <i>Coccotrypes cyperi</i>	Chang et al., 2017
<i>Leptographium alethinum</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>L. altius</i>	Jilin	<i>L. olgensis</i> <i>Pi. koraiensis</i>	<i>I. cembrae</i>	Paciura et al., 2010a
<i>L. bachii</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. celere</i>	Yunnan	<i>Pi. kesiya</i>	–	Paciura et al., 2010a
	Jilin	<i>Pinus</i> sp.		
<i>L. conjunctum</i>	Yunnan	<i>Pi. yunnanensis</i> <i>Pi. kesiya</i>	<i>Hylurgops major</i>	Paciura et al., 2010a; Zhou et al., 2013; Chang et al., 2017
<i>L. curviconidium</i>	Jilin	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Paciura et al., 2010a
<i>L. duchongii</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. fenglinhensis</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. gracile</i>	Yunnan	<i>Pi. armandii</i>	<i>Pissodes</i> sp.	Paciura et al., 2010a; Zhou et al., 2013; Chang et al., 2017
<i>L. heilongjiangii</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. innermongolicum</i>	inner mongolia	<i>L. gmelinii</i>	<i>I. subelongatus</i>	Liu et al., 2017
<i>L. manifestum</i>	Jilin	<i>L. olgensis</i>	<i>I. subelongatus</i>	Paciura et al., 2010a
	Yunnan	<i>Pi. yunnanensis</i>	<i>P. verrucifrons</i>	
<i>L. ningerense</i>	Yunnan	<i>Pi. kesiya</i>	<i>Co. cyperi</i> <i>Orthotomicus angulatus</i>	Chang et al., 2017
<i>L. penicillata</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. pineti</i>	Yunnan	<i>Pi. kesiya</i>	–	Paciura et al., 2010a

<i>L. pinicola</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>L. pini-densiflorae</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
	Shannxi			
<i>L. pistaciae</i>	Yunnan	<i>Pistacia chinensis</i>	—	Paciura et al., 2010a
<i>L. procerum</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Lu et al., 2009b; Taerum et al., 2013
	Shannxi	<i>Pi. bungeana</i>		
	Henan			
<i>L. shanshensis</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. sinense</i>	Jiangxi	<i>Pi. elliottii</i>	<i>Hylobitelus xiaoi</i>	Yin et al., 2015
<i>L. sinoprocerum</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Lu et al., 2009b; Taerum et al., 2013
	Hebei	<i>Pi. bungeana</i>		
<i>L. taigense</i>	Inner Mongolia	<i>L. gmelinii</i>	<i>I. subelongatus</i>	Liu et al., 2017
<i>L. truncatum</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Lu et al., 2009b; Taerum et al., 2013
<i>L. yichunensis</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>L. zhangii</i>	heilongjiang	<i>L. gmelinii</i>	<i>I. subelongatus</i>	Liu et al., 2017
<i>Ophiostoma abietinum</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>O. acarorum</i>	Yunnan	<i>Pi. kesiya</i>	<i>I. acuminatus</i>	Chang et al., 2017
			<i>P. szemaoensis</i>	
			<i>Cy. luteus</i>	
			<i>O. angulatus</i>	
			<i>Co. cyperi</i>	
<i>O. ainoae</i>	Jilin	<i>Picea</i> sp.	<i>I. typographus</i>	Chang et al., 2019
	Heilongjiang	<i>Pi. koraiensis</i>		
<i>O. album</i>	Zhejiang	<i>P. massoniana</i>	<i>Monochamus alternatus</i>	Wang et al., 2018
<i>O. bicolor</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>O. brevipilosi</i>	Yunnan	<i>Pi. kesiya</i>	<i>T. brevipilosus</i>	Chang et al., 2017

<i>O. brunneolum</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>O. cf. deltoideosporum</i>	Shangdong	<i>P. thunbergii</i>	<i>Monochamus alternatus</i>	Wang et al., 2018
<i>O. floccosum</i>	Shanxi	<i>P. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Lu et al., 2009b; Taerum et al., 2013
<i>O. ips</i>	Shanxi	<i>Pi. kesiya</i>	<i>I. acuminatus</i>	Lu et al., 2009a; Taerum et al., 2013; Zhou et al., 2013; Chang et al., 2017; Wang et al., 2018
	Yunnan	<i>P. tabuliformis</i>	<i>P. szemaoensis</i>	
			<i>Polygraphus</i> sp.	
			<i>D. valens</i>	
	Zhejiang and	<i>P. thunbergii</i>	<i>Monochamus alternatus</i>	
	Shandong	<i>P. massoniana</i>		
<i>O. japonicum</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>O. jiamusi</i>	Jilin	<i>Picea</i> sp.	<i>I. typographus</i>	Chang et al., 2019
<i>O. jilinensis</i>	Jilin	<i>Picea</i> sp.	<i>I. typographus</i>	Chang et al., 2019
<i>O. massoniana</i>	Zhejiang	<i>P. massoniana</i>	<i>Monochamus alternatus</i>	Wang et al., 2018
<i>O. micans</i>	Qinghai	<i>Pic. crassifolia</i>	<i>D. micans</i>	Yin et al., 2016
<i>O. minus Europe</i>	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>O. nitidum</i>	Qinghai	<i>Pic. crassifolia</i>	<i>I. nitidus</i>	Yin et al., 2016
<i>O. olgensis</i>	Heilongjiang	<i>L. gmelini</i>	<i>I. subelongatus</i>	Wang et al., 2016
	Inner Mongolia	<i>L. olgensis</i>		
<i>O. piceae</i>	Jilin	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Paciura et al., 2010b; Taerum et al., 2013
	Shanxi	<i>L. olgensis</i>	<i>I. subelongatus</i>	
<i>O. poligraphi</i>	Qinghai	<i>Pic. crassifolia</i>	<i>P. poligraphus</i>	Yin et al., 2016
<i>O. qinghaiense</i>	Qinghai	<i>Pic. crassifolia</i>	<i>P. poligraphus</i>	Yin et al., 2016
			<i>D. micans</i>	
<i>O. quercus</i>	Yunnan	<i>Pi. yunnanensis</i>	<i>I. acuminatus</i>	Paciura et al., 2010b; Zhou et al., 2013; Chang et al., 2017
		<i>Abies</i> sp.	<i>P. szemaoensis</i>	
		<i>Pi. semaonensis</i>	<i>P. verrucifrons</i>	
		<i>Tsuga</i> sp.	<i>T. piniperda</i>	
		<i>Pi. kesiya</i>	Unknown spp.	
		<i>T. dumosa</i>	<i>Pissodes</i> sp.	

S. babylonica

<i>O. setosum</i>	Yunnan	<i>Tsuga</i> sp. <i>Abies</i> sp. <i>T. dumosa</i>	Unknown <i>Pissodes</i> sp.	Paciura et al., 2010b; Zhou et al., 2013; Chang et al., 2017
<i>O. shangrilae</i>	Qinghai	<i>Pic. purpurea</i>	<i>I. shangrila</i>	Yin et al., 2016
<i>Ophiostoma</i> sp. 1	Jilin	<i>L. olgensis</i>	<i>I. subelongatus</i>	Zhou et al., 2013
	Yunnan	<i>T. dumosa</i>	<i>Pissodes</i> sp.	
<i>Ophiostoma</i> sp. 2	Jilin	<i>L. olgensis</i>	<i>I. subelongatus</i>	Zhou et al., 2013
<i>Ophiostoma</i> sp. 3	Shanxi	<i>Pi. tabuliformis</i>	<i>D. valens</i>	Lu et al., 2009a; Taerum et al., 2013
<i>Ophiostoma</i> sp. 4	Jilin	<i>Pi. koraiensis</i>	–	Zhou et al., 2013
<i>Ophiostoma</i> sp. 5	Yunnan	<i>Pi. yunnanensis</i> <i>Abies</i> sp. <i>Pi. kesiya</i>	<i>H. major</i>	Zhou et al., 2013
<i>Ophiostoma</i> sp. 6	Yunnan	<i>Pi. semaonensis</i>	Unknown	Chang et al., 2017
<i>Ophiostoma</i> sp. 7	Yunnan	<i>Tsuga</i> sp.	Unknown	Chang et al., 2017
<i>O. tsotsi</i>	Yunnan	<i>Pi. kesiya</i>	<i>Co. cyperi</i> <i>Cyrtogenius luteus</i>	Chang et al., 2017
<i>O. typographus</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>O. wuyingii</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019
<i>O. yunshanensis</i>	Heilongjiang	<i>Pi. koraiensis</i>	<i>I. typographus</i>	Chang et al., 2019

<i>Sporothrix nebularis</i>	Yunnan	<i>Pi. yunnanensis</i> <i>Pi. kesiya</i>	<i>T. piniperda</i> <i>Co. cyperi</i> <i>Cy. luteus</i> <i>Or. angulatus</i> <i>Polygraphus</i> sp.	Chang et al., 2017
<i>S. zhejiangensis</i>	Zhejiang	<i>P. massoniana</i>	<i>Monochamus alternatus</i>	Wang et al., 2018
<i>Sporothrix</i> sp. A	Yunnan	<i>Pi. kesiya</i>	<i>Co. cyperi</i>	Chang et al., 2017

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Table S2. Numbers of mite individuals were collected and numbers of mite individuals carrying fungi in this study.

Beetle	Mite species ^{1, 2}																																															
species	M1		M2		M3		M4		M5		M6		M7		M8		M9		M10		M11		M12		M13		M14		M15		M16		M17		M18		M19		M20		M21		M22		M23		M24	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B		
<i>D. micans</i>	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	9	0	0
<i>I. nitidus</i>	0	0	2	2	0	0	1	0	23	7	11	3	3	2	0	0	6	1	10	5	0	0	0	0	1	1	0	0	0	0	5	1	0	0	29	8	0	0	21	6	0	0	0	0	2	1	1	1
<i>I. shangrila</i>	1	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	7	1	6	5	0	0	11	1	1	1	1	0	0	0	0	
<i>P. polygraphus</i>	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0	0	0	0	0	0	0	1	0	3	2	0	0	0	0	0	0	0	

¹ Mite species see Table 1² A = Number of mite individuals in beetle galleries; B = Number of mite individuals carrying fungi

[illegible]

M 14	0	0	0	0	4	0	0	0	0	0	0	0	0	0
M 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 20	0	0	0	0	2	0	0	1	0	0	0	0	0	1
M 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	4	32	11	3	10	11	10	6	1	1	31	3	6	6

¹ Mite species see **Table 1**

² Fungal species see **Table 2**

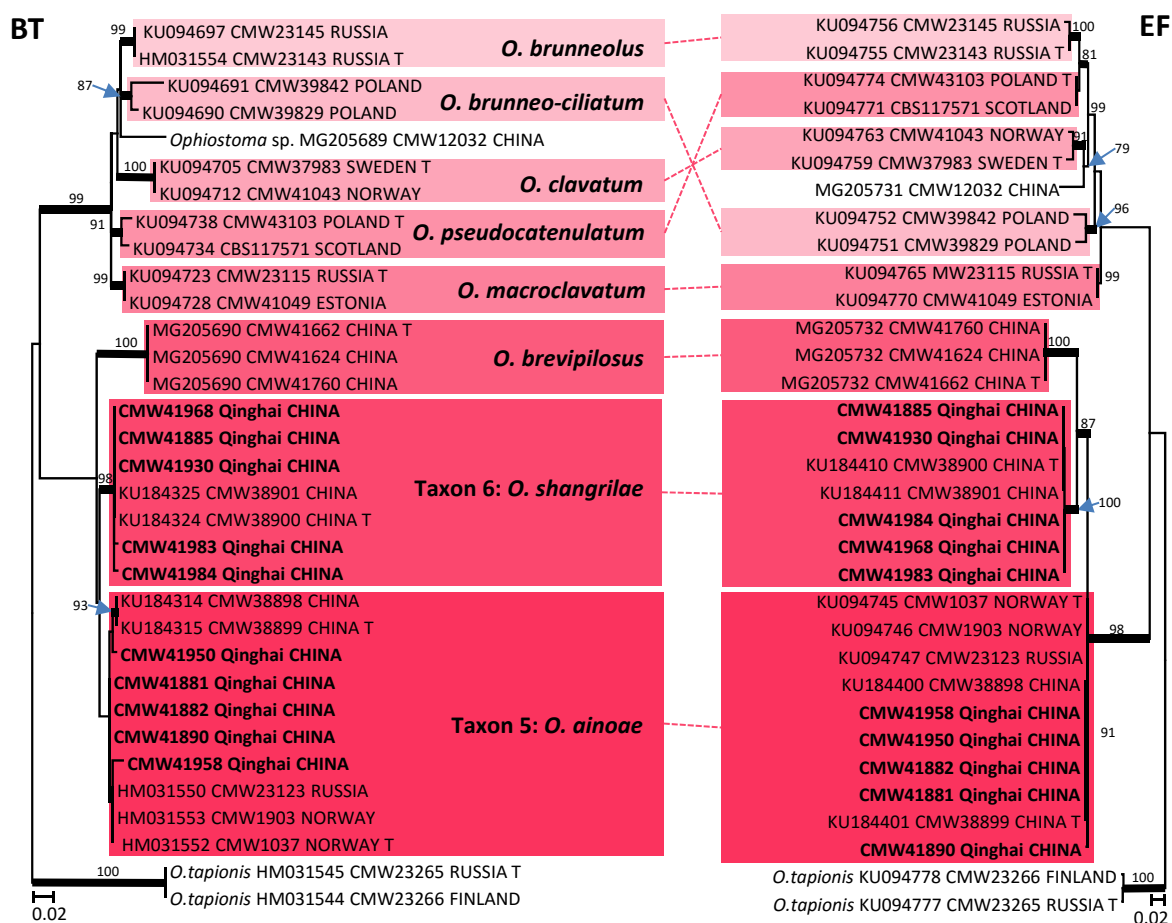


Fig. S1 Phylogram obtained from ML analyses of the partial *BT* and *EF* gene of *O. clavatum* complex. Sequences obtained in this study are printed in bold type. ML and MP bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.

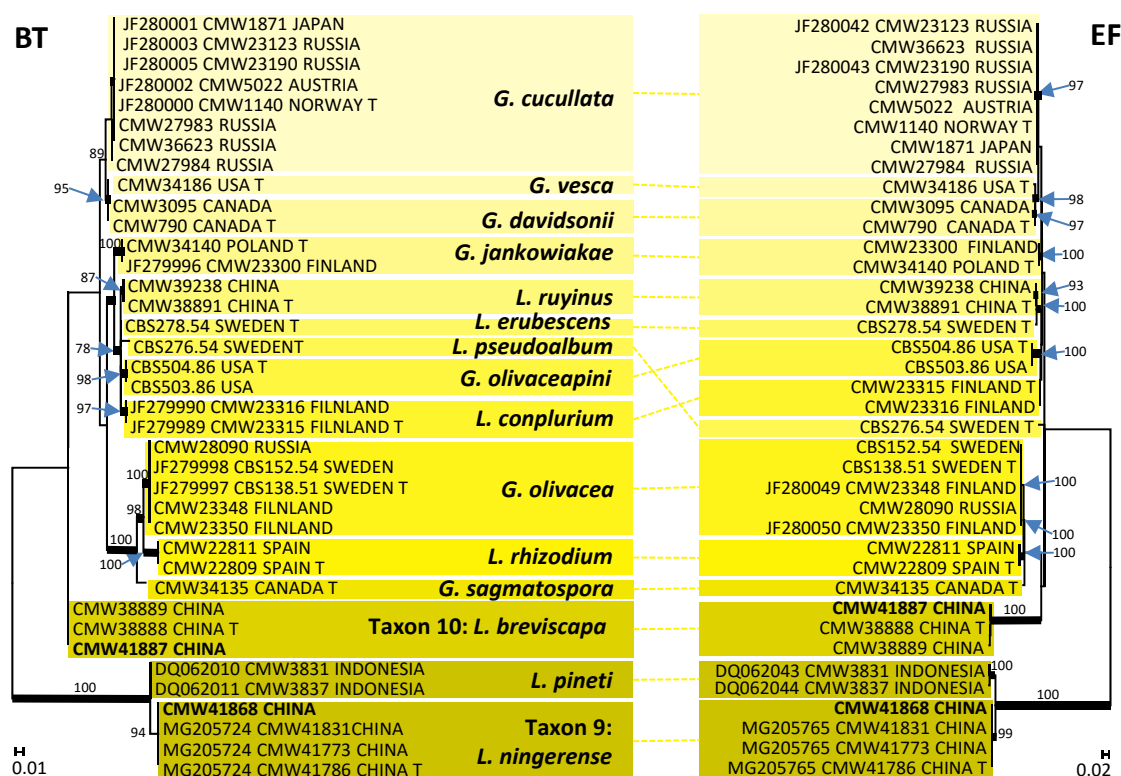


Fig. S2 Phylogram obtained from ML analyses of the partial *BT* and *EF* gene of *G. olivacea* complex. Sequences obtained in this study are printed in bold type. ML bootstrap support values (1000 replicates, normal type) above 75 % are indicated at the nodes. Posterior probabilities (above 0.9) obtained from BI are indicated by bold lines at the relevant branching points. T= ex-type cultures. Scale bar = total nucleotide difference between taxa.