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ORIGINAL ARTICLE

Taxonomy and pathogenicity of *Leptographium* species associated with *Ips subelongatus* infestations of *Larix* spp. in northern China, including two new species

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Abstract The larch bark beetle (*Ips subelongatus*), which occurs in larch plantations over a vast area of eastern Asia, infects both dying and fallen trees. When its population reaches a high density, the beetle may also infect healthy trees, resulting in tree decline and, eventually, death. *Leptographium* spp., in both their sexual and asexual states, are mainly associated with conifer-infesting bark beetles; some species are important tree pathogens. The aims of this study were to identify the *Leptographium* spp. associated with *I. subelongatus* infestations of *Larix* spp. in northern China and to examine their pathogenicity towards the tree. Morphological studies and phylogenetic approaches based on multilocus DNA sequence data (ITS2- partial r28S, partial β-tubulin, and EF-1α gene regions) showed that three *Leptographium* species occur in association with *I. subelongatus* in the areas investigated: *Leptographium taigense*, which is recorded in China for the first time, and two new species, namely *L. innermongolicum* sp. nov. and *L. zhangii* sp. nov. *Leptographium innermongolicum* is closely related to *L. taigense*, whereas *L. zhangii* belongs to the *Grosmannia piceaperda* species complex. The pathogenicity of these *Leptographium* species towards mature *Larix* spp. was tested by stem inoculation in forests. All inoculations only resulted in small lesions on the inner bark; therefore, the three *Leptographium* species were not considered to be pathogenic.

Keywords DNA multilocus · *Leptographium innermongolicum* · *Leptographium taigense* · *Leptographium zhangii*

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Introduction

Ips subelongatus Motschulsky (Scolytidae, Coleoptera) is an important pest that mainly infects larches (*Larix* spp., Pinacae) in eastern Asia. The bark beetle can infect dying or fallen trees and may also infect healthy trees at high population densities, with subsequent morbidity or death (Yin et al. 1984; Van der Westhuizen et al. 1995). Because of their morphological similarities, *I. subelongatus* and several other eight-spined larch bark beetles were considered synonyms of *Ips cembrae* Heer (Wood and Bright 1992; Pfeffer 1995). However, mitochondrial gene sequence analysis indicates that the European and

Asian populations of *I. cembrae* are genetically diverse and encompass several haplotypes (Stauffer et al. 2001); at species level, the East Asian haplotypes correspond to *I. subelongatus*. Bark beetles cause serious damage; therefore, these species have been added to the European and Mediterranean Plant Protection Organization (EPPO) alert list A2 (http://www.eppo.int/QUARANTINE/listA2.htm).

In China, *I. subelongatus*, which mainly occurs in northern areas, infects several species of *Larix*: *Larix gmelinii* in the Da Hinggan and Xiao Hinggan mountain ranges in the Inner Mongolia autonomous region and the Heilongjiang Province, *Larix olgensis* in south-eastern Heilongjiang Province and the Chang Bai mountain range in Liaoning Province, and *Larix principis-ruprechtii* in middle Inner Mongolia, as well as some areas of Beijing, Hebei, and Shanxi Provinces, with allopatric distribution, consequently threatening local plantations (Yang et al. 2007). *Ips subelongatus* is commonly found in association with ophiostomatoid fungi, including species of *Ophiostoma*, *Grosmannia*/*Leptographium*, *Ceratocystiopsis*, *Ceratocystis*, and *Graphilbum* (Aoshima 1965; Yamaoka et al. 1997, 1998, 2009; Paciura et al. 2010; De Beer and Wingfield 2013). At present, six species of *Grosmannia*/*Leptographium* have been recorded in association with *I. subelongatus* in eastern Asia, viz. *Grosmannia laricis* Zipfel et al., *Grosmannia olivacea* Zipfel et al., *Leptographium altius* Paciura et al., *Leptographium manifestum* Paciura et al., and two unnamed species (Yamaoka et al. 1998, 2009; Paciura et al. 2010).

Grosmannia, which was discovered in 1936 (Goidánich 1936), has historically been considered a synonym of the genus *Ophiostoma*. It was reconsidered as an independent genus on the basis of data from multilocus DNA sequence analysis (Zipfel et al. 2006). *Grosmannia* species are characterized by ascomata with a globose base ending in a neck of variable length and ascospores embedded in a mucilaginous sheath (Jacobs and Wingfield 2001; Zipfel et al. 2006). *Leptographium*, the main asexual form of *Grosmannia* species, was first described in 1927 as a fungus that causes a blue stain on timber (Lagerberg et al. 1927; Zipfel et al. 2006). It is characterized by dematiaceous, erect conidiophores terminating in penicillated branches that give rise to conidiogenous cells. These cells produce single-celled conidia that accumulate in mucilaginous drops (Kendrick 1962; Jacobs and Wingfield 2001).

Species of *Leptographium* are mainly associated with conifer-infesting bark beetles (Grosmann 1931; Harrington and Cobb 1988; Wingfield et al. 1993; Jacobs and Wingfield 2001; Kirisits 2004; Paciura et al. 2010). Only a few species of this genus are known to be associated with non-coniferous hosts (Jacobs and Wingfield 2001; Jacobs et al. 2006; Paciura et al. 2010). Some *Leptographium* species, such as *L. wageneri* Zipfel et al., which causes the black-stain root disease (Wagener and Mielke 1961; Harrington and Cobb 1988), or *Grosmannia serpens* Goid, which is linked to pine decline in the USA (Eckhardt et al. 2007), are primary pathogens that cause significant economic losses (Lagerberg et al. 1927; Wingfield et al. 1988; Seifert et al. 1993).

In recent years, several species of *Grosmannia* and their *Leptographium* asexual forms have been recorded in association with various bark beetles in China: *G. koreana* Q Lu et al., *L. procerum* K Jacobs et al., and *L. sinoprocerum* Q Lu et al. associated with *Dendroctonus valens* LeConte (Lu et al. 2008, 2009a, b); *G. yunnanense* Tsiang associated with *Tomicus yunnanensis* Kirkendall and Faccoli (Zhou et al. 2000; Kirkendall et al. 2008; Yamaoka et al. 2008); and *L. sinense* Lour associated with *Hylobitelus xiaoi* Zhang (Yin et al. 2015). However, to date, few investigations on *Grosmannia*/*Leptographium* associated with *I. subelongatus* have been carried out: only two species, namely *L. manifestum* and *L. altius* (Paciura et al. 2010), have been reported in China.

In a recent survey of ophiostomatoid fungi associated with *I. subelongatus* and their galleries in northern larch forests in China, several strains of *Leptographium* were isolated. The primary aim of this study was to identify these strains using a combination of morphological observations and multilocus DNA sequence data. This study additionally evaluated the pathogenicity of the identified species via inoculation tests in the field.

Materials and methods

Collection of samples and isolation of fungi

Samples of *I. subelongatus* and their galleries were collected from *L. gmelinii* in the Heilongjiang Province and the Inner Mongolia autonomous region in China. Fungi were isolated from galleries as described by Seifert et al. (1993) and incubated at 25 °C. Fungi were also isolated from young adult beetles by crushing them onto the surface of 2 % malt extract agar (MEA) with 0.05 % cycloheximide. All strains were purified by hyphal tip isolation. Representative cultures were deposited in the BCCM/MUCL culture collection and the culture collection of the Chinese Academy of Forestry (CXY).

Cultural and morphological studies

The strains of *Leptographium* spp. were grown on 2 % MEA and oatmeal agar (OA) (Gams et al. 1998) at 25 °C for 20 days. All microscopic measurements were performed in 85 % lactic acid. Fifty measurements were performed for each morphological character.

The optimal growth temperature of the various strains was determined by placing a 5-mm (diameter) plug from an actively growing fungal colony at the center of MEA plates for three replicates. Plates were incubated in the dark at temperatures

ranging from 5 to 35 °C, at 5 °C intervals. Colony diameters on each dish were measured along two perpendicular lines and the averages were calculated for each of the seven temperatures. Colony color was determined according to the method of Rayner (1970).

DNA extraction, PCR, and sequencing

The fungal strains were grown in liquid malt at 25 °C in the dark for 7 days. DNA was extracted using an Invisorb Spin Plant Mini Kit (Invitek, Berlin), following the manufacturer's instructions. Three gene regions, viz. the internal transcribed spacer 2 (ITS2) and part of the 28S (containing domains D1 and D2), partial β-tubulin, and partial elongation factor 1-α (EF1-α) were amplified. The ITS2 and 28S regions were amplified using primers ITS3 and LR3 (White et al. 1990). The primers Bt2a and Bt2b (Glass and Donaldson 1995) were used to amplify part of the β-tubulin gene region. The transcription elongation factor-1α gene region was amplified with primers EF1F and EF2R (Jacobs et al. 2004).

Polymerase chain reaction (PCR) assays were performed in 25-μL volumes (2.5 mM $MgCl_2$, 1 × PCR buffer, 0.2 mM dNTP, 0.2 mM of each primer, and 2.5 U Taq polymerase enzyme). The PCR conditions for the ITS2 and 28S gene regions were: an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 54 °C, and 1 min at 72 °C, and a final chain elongation at 72 °C for 8 min. The partial β-tubulin and EF1-α genes were amplified using a denaturation step at 95 °C for 2 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C, and 1 min at 72 °C, and a final chain elongation at 72 °C for 8 min. PCR products were cleaned using an MSB Spin PCRapace Kit (250) (Invitek, Berlin), following the manufacturer's instructions.

Sequencing reactions were performed with a CEQ DTCS Quick Start Kit (Beckman Coulter), following the manufacturer's instructions, with the same PCR primers as above. Nucleotide sequences were determined with a CEQ 2000XL capillary automated sequencer (Beckman Coulter).

Phylogenetic analyses

BLAST searches were conducted for preliminary identification, after which datasets were compiled including published GenBank sequences. Datasets were aligned using MAFFT 6 (Katoh et al. 2002). Phylogenetic analyses were performed using maximum parsimony (MP) as implemented in PAUP* 4.0b10 (Swofford 2003), Bayesian inference (BI) as implemented in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001), and maximum likelihood (ML) using RAxML 7.0.4 (Stamatakis 2006).

For phylogenetic inferences based on the ITS2-28S dataset, *O. piliferum* Syd et al., *O. karelicum* Linnakoski et al., and *O. novo-ulmi* Brasier were used as the outgroup (Linnakoski et al. 2012). The phylogenetic inferences based on the β-tubulin and EF1-α datasets were performed without outgroups (unrooted trees).

ML analyses were performed using RAxMLv7.0.4 (Stamatakis et al. 2006) assuming the GTR + G Substitution model, and run on the CIPRES cluster at the San Diego Supercomputer Center. Supports for the nodes were estimated from 1000 bootstrap replicates. MP was performed using PAUP* version 4.0b10 (Swofford 2001), with gaps treated as fifth base. The most parsimonious trees were identified using heuristic searches with random addition sequence (1000), with MAXTREES set to 200, and further evaluated by bootstrap analysis, retaining clades compatible with the 50 % majority rule in the bootstrap consensus tree. The analysis conditions were tree bisection reconnection branch swapping (TBR); starting tree obtained via stepwise addition; steepest descent not in effect; MULTREES effective.

BI was carried out using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). The most appropriate substitution models were selected using the Akaike information criterion (AIC) in MrModeltest v2.3. In order to calculate posterior probabilities, BI analyses were performed using the Markov chain Monte Carlo (MCMC) approach with 5,000,000 generations.

Pathogenicity tests

The pathogenicity of three *Leptographium* species was tested by artificial inoculation of mature larch trees in the field. The possible host specificity of the fungi was additionally assessed by cross-inoculation between two larch species. Two separated forest plots were selected for inoculation, viz. the Genhe forest farm (north-eastern Inner Mongolia, N: 50°54′18.9″, E: 121°29′59.9″) and the Huangangliang forest farm (middle Inner Mongolia, N: 43°36′24.5″, E: 117°30′45.6″). The local larch species inoculated were *L. gmelinii* and *L. principis-ruprechtii*, respectively.

Three strains were grown on 2 % MA in 9-cm Petri dishes at 25 °C for 2 weeks before being used as inoculum. Twelve 25-year-old healthy trees were selected for inoculation, with three trees used for each of the three *Leptographium* strains. Sterile MEA was inoculated as the control for each experiment. Tree stems were inoculated a height of 150 cm above the ground, and 6-mm-diameter holes were drilled horizontally up to the sapwood. A plug of 5-mm-diameter mycelium disk, cut from the actively growing margin of the colony, was inserted into the bark hole using a sterilized toothpick, according to the method described by Yamaoka et al. (1998). Sterilized Eppendorf tube caps were used to cover the inoculation holes to prevent invasion by insects and air contamination.

Field inoculations were performed on 4 July 2014. After periodic inspection for the development of external symptoms over 10 weeks, the trees were cut in September. The lesions

Table 1 Fungal strains obtained from various bark beetle species and used in this study

Species	Isolate numbers	Origin	Host	Insect vector	GenBank no.			References
					ITS2-28S	β-tubulin	EF1-α	
Leptographium zhangii sp. nov.	CXY1552T, MUCL55162	China, Heilongjiang	*L. gmelinii*	*I. subelongatus*	**KM236108**	**KM974270**	**KM974275**	
	CXY1553, MUCL55163	China, Heilongjiang	*L. gmelinii*	*I. subelongatus*	**KM236109**	**KM974267**	**KM974276**	
L. innermongolicum sp. nov.	CXY1547T, MUCL55158	China, Inner Mongolia	*L. gmelinii*	*I. subelongatus*	**KM236107**	**KM974272**	**KM981763**	
	CXY1548, MUCL55159	China, Inner Mongolia	*L. gmelinii*	*I. subelongatus*	**KM236106**	**KM974271**	**KM981762**	
L. abietinum	CMW2817	USA	*Picea engelmannii*		DQ062080	DQ062014	DQ062047	Jacobs et al. (2005)
	CMW3083	British Columbia	*Picea* sp.		DQ062081	DQ062015	DQ062048	Jacobs et al. (2005)
L. aenigmatica	CMW2199T				AY553389	AY534937	AY536183	Jacobs et al. (2004)
	CMW2310				AY553390	AY534938	AY536184	Jacobs et al. (2004)
L. altius	CMW12501	China, Jilin	*Picea koraiensis*		HQ406853	HQ406901	HQ406877	Paciura et al. (2010)
	CMW12471T	China, Jilin	*Picea koraiensis*		HQ406851	HQ406899	HQ406875	Paciura et al. (2010)
L. americana	CMW495T	USA	*L. decidua*		DQ062079	DQ062013	DQ062046	Jacobs et al. (2005)
	CMW2929				DQ062078	DQ062012	DQ062045	Jacobs et al. (2005)
L. aurea	ATCC16936T	Canada, BC	*P. contorta*		AY544610	AY263187	AY544633	Lim et al. (2004)
	CMW714	Canada	*P. contorta* var. *latifolia*		AF343699	DQ062005	DQ062038	Jacobs et al. (2001)
L. bhutanense	CMW18649T	Bhutan	*P. wallichiana*	*Hylobitelus chenkupdorjii*	EU650187	EU650191	EU650195	Zhou et al. (2008)
	CMW18650	Bhutan	*P. wallichiana*	*Hylobitelus chenkupdorjii*	EU650186	EU650190	EU650194	Zhou et al. (2008)
L. chlamydatum	CMW37213	Finland Punkaharju	*Picea*sylvestris	*Pityogenes chalcographus*	JF279966	JF280027	JF280083	Linnakoski et al. (2012)
	CMW11592T	Norway		*D. autographus*	EU979333	EU979341	EU979349	Jacobs et al. (2010)
L. curviconidium	CMW12425T	China, Jilin	*P. koraiensis*	*I. typographus*	HQ406850	HQ406898	HQ406874	Paciura et al. (2010)
	CMW12486	China, Jilin	*P. koraiensis*	*I. typographus*	HQ406849	HQ406897	HQ406873	Paciura et al. (2010)
L. curvisporum	CMW17260T	Norway	*Picea abies*	*Dryocetes autographus*	EU979328	EU979336	EU979344	Jacobs et al. (2010)
	CMW11608	Norway	*Picea abies*	*D. autographus*	EU979332	EU979340	EU979348	Jacobs et al. (2010)
L. gracile	CMW12396	China, Yunnan	*P. armandii*	*Pissodes* sp.	HQ406841	HQ406889	HQ406865	Paciura et al. (2010)
	CMW12398T	China, Yunnan	*P. armandii*	*Pissodes* sp.	HQ406840	HQ406888	HQ406864	Paciura et al. (2010)
L. latens	CMW12319	China, Yunnan	*Picea koraiensis*	*I. typographus*	HQ406844	HQ406892	HQ406868	Paciura et al. (2010)
	CMW12438T	China, Yunnan	*Picea koraiensis*	*I. typographus*	HQ406845	HQ406893	HQ406869	Paciura et al. (2010)
L. laricis	CMW1980T	Japan	*Larix* sp.	*I. subelongatus*	DQ062074	DQ062008	DQ062041	Jacobs et al. (2005)
	CMW2014				DQ062075	DQ062009	DQ062042	Jacobs et al. (2005)
L. lundbergii	CMW217	Europe	*Pinus* sp.		DQ062065	DQ061999	DQ062032	Jacobs et al. (2005)
	CMW17264T	Sweden	*P. sylvestris*		DQ062068	DQ062002	DQ062035	Jacobs et al. (2005)

Table 1 (continued)

Species	Isolate numbers	Origin	Host	Insect vector	GenBank no.			References
					ITS2-28S	β-tubulin	EF1-α	
L. piceaperda	CMW2811	USA	*Picea rubens*		AY707209	AY707195	JF280077	Kim et al. (2005)
L. procerum	CMW25627	China	*P. tabuliformis*	*Dendroctonus valens*	EU785393	EU785360	EU785418	Lu et al. (2009b)
	CMW13	USA	*P. resinosa*		JF279977	EU296783	EU296790	Linnakoski et al. (2012)
L. pyrinum	CMW169T	USA	*P. ponderosa*		DQ062072	DQ062006	DQ062039	Jacobs et al. (2005)
	CMW509				AY553414	AY534962	AY536208	Jacobs et al. (2004)
L. robusta	CMW668T	USA, Idaho	*P. ponderosa*	*Ambrosia* and *Dendroctonus*	AY544619	AY534945	AY536191	Jacobs et al. (2005)
	CMW2805	USA, Idaho	*P. ponderosa*		AF343705	AY534944	AY536190	Jacobs et al. (2001)
L. sinoprocerum	MUCL46352T	China, Hebei	*P. tabuliformis*	*Dendroctonus valens*	EU296773	EU296779	EU296786	Lu et al. (2008)
	MUCL46331	China, Shanxi	*P. tabuliformis*	*Dendroctonus valens*	EU296772	EU296778	EU296785	Lu et al. (2008)
L. taigense	CMW36629	Russia, Lisino-Corpus	*Picea abies*	*Ips typographus*	JF279979	JF280016	JF280061	Linnakoski et al. (2012)
	CMW36630T	Russia, Lisino-Corpus	*Picea sylvestris*	*Hylurgops palliatus*	JF279980	JF280017	JF280062	Linnakoski et al. (2012)
	CXY1549	China, Inner Mongolia	*Larix gmelinii*	*I. subelongatus*	**KM236104**	**KM974268**	**KM974273**	
	CXY1554, MUCL55160	China, Inner Mongolia	*L. gmelinii*	*I. subelongatus*	**KM236105**	**KM974269**	**KM974274**	
L. terebrantis	CBS337.70	USA, Louisiana	*P. taeda*	*Dendroctonus terebrans*	EU296777	EU296784	EU296791	Lu et al. (2008)
	CMW9	USA, Minnesota	*P. sylvestris*	*Hylobius pales*	AY553384	EU652698	EU652700	Jacobs et al. (2004)
	CMW9a		*Pinus* spp.	*H. radicis* and *H. rhizophagus*	EU652697	EU652699	EU652701	Zhou et al. (2008)
L. truncatum	CMW2402	Canada	*Pinus resinosa*		DQ062051	DQ061985	DQ062018	Jacobs et al. (2005)
	CMW28	South Africa	*P. taeda*		DQ062052	DQ061986	DQ062019	Jacobs et al. (2005)
L. wingfieldii	CMW2095	Europe	*P. strobus*	*Tomicus piniperda*	AY553400	AY534948	AY536194	Jacobs et al. (2004)
	CMW2096	Europe	*P. sylvestris*	*T. piniperda*	AY553398	AY534946	AY536192	Jacobs et al. (2004)
L. yunnanense	CMW5152T	China, Yunnan	*P. yunnanensis*	*Tomicus piniperda*	DQ062073	DQ062007	DQ062040	Jacobs et al. (2005)
	CMW5304	China, Yunnan	*P. yunnanensis*	*Tomicus piniperda*	AY553415	AY534963	AY536209	Jacobs et al. (2004)

GenBank accession numbers of sequences obtained in the present study are indicated in **bold type**

T: ex-holotype strain, CXY: culture collection of the Chinese Academy of Forestry; MUCL: part of the Belgian Coordinated Collections of Microorganisms, BCCM; CMW: culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria; ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

formed in the inner bark around the inoculation points were measured. The stems were then split at the center of each inoculated area in order to evaluate the extent of the stained area inside the wood. Tissues at the margin of the reaction zone were collected for reisolation of the fungi under aseptic conditions. The data for the lesions were then analyzed using one-way analysis of variance (ANOVA).

Results

Fungal strains and phylogenetic analyses

Six strains of *Leptographium* were obtained from bodies or galleries of *I. subelongatus*, at relatively low frequencies (≤5 % of the total isolates) (Table 1).

Amplification of the ITS2-28S region, partial β-tubulin gene, and partial EF1-α gene yielded fragments of 900 bp, 410 bp, and 820 bp, respectively. For each of the sequence datasets, the topologies of MP and Bayesian tree were similar to that of ML.

Phylogenetic inferences based on the ITS2-LSU dataset grouped the strains isolated from the larch trees into two clades (Fig. 1). Together with *G. aenigmatica* Zipfel et al., *G. piceaperda* Goid et al., and *G. laricis*, the strains CXY1552 (=MUCL 55162) and CXY1553 (=MUCL 55163) formed a well-supported clade (the *G. piceaperda* clade). The strains CXY1547 (=MUCL 55158), CXY1548 (=MUCL 55159), CXY1549, and CXY1554 (=MUCL 55160) grouped together with *L. taigense* Linnakoski et al. to form a second well-supported clade; the ITS2-LSU sequences of these four

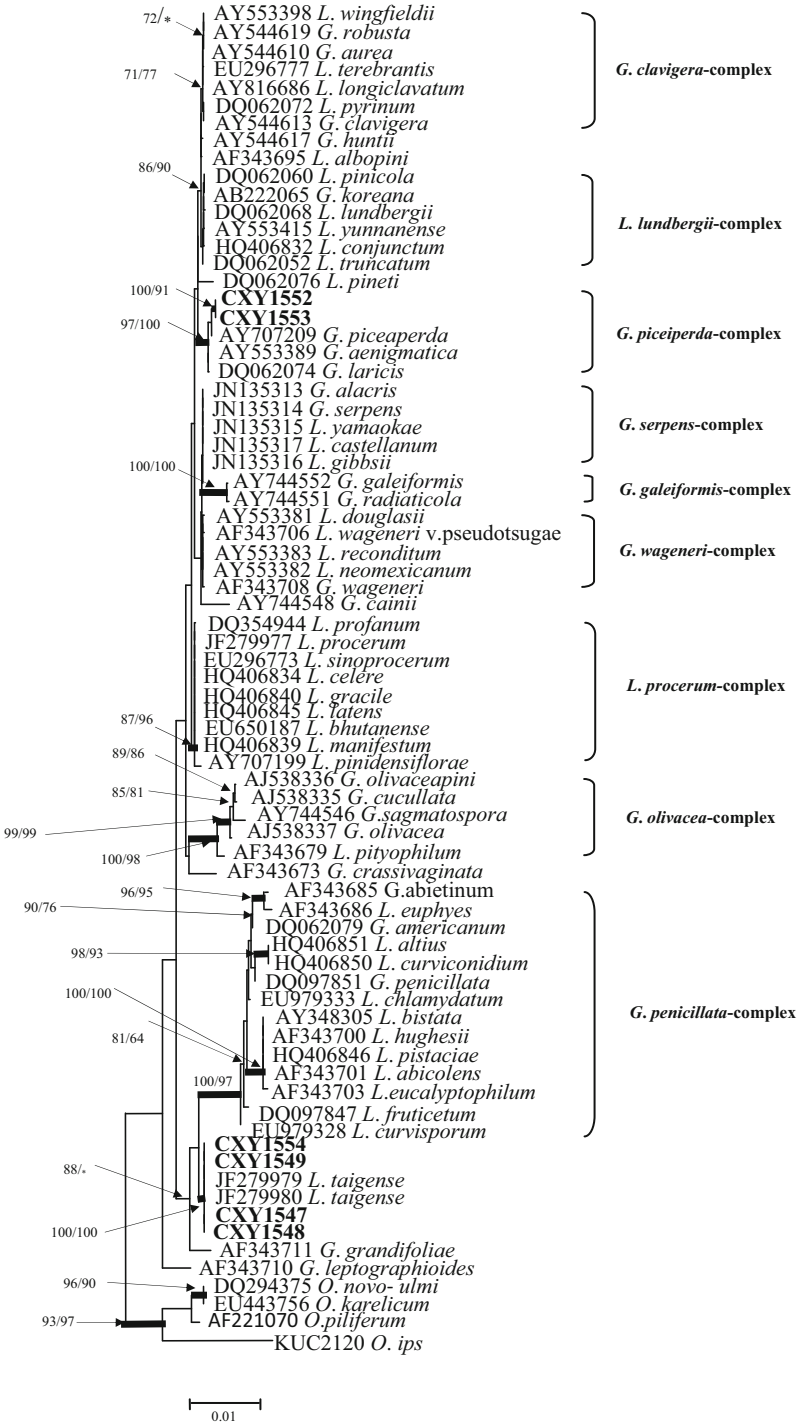

Fig. 1 Phylogram obtained from ML analyses of the ITS2 and 28S regions; novel sequences obtained in this study are indicated in **bold type**. ML bootstrap support values (1000 replicates) (normal type) and MP jackknife values (10,000 replicates) (**bold type**) above 70 % are indicated at the nodes. Posterior probabilities (above 95 %) obtained from BI are indicated by **bold lines** at the relevant branching points. Values less than 70 % are indicated by an asterisk

strains were found to be 100 % identical to those of *L. taigense*.

Analysis of the β-tubulin dataset yielded trees (Fig. 2) with a topology similar to that obtained from the ITS2-28S dataset (Fig. 1). The present strains isolated from *Larix* also clustered into two clades, viz. the *G. piceaperda* and the *L. taigense* clades. The strains CXY1552 and CXY1553 were resolved as a distinct lineage within the *G. piceaperda* clade, with very high bootstrap support (Fig. 2), suggesting a possibly distinct phylogenetic species. The β-tubulin sequence of the strains CXY1547, CXY1548, CXY1549, and CXY1554 was identical to that of *L. taigense*.

Phylogenetic relationships inferred from the EF-1α dataset yielded a tree topology (Fig. 3) similar to that obtained from the analysis of the β-tubulin dataset (Fig. 2). The strains CXY1552 and CXY1553 were also resolved as a distinct lineage within the *G. piceaperda* clade, with very high bootstrap support (Fig. 3). The strains CXY1549 and CXY1554 still grouped together with the type strain of *L. taigense* (Fig. 3). However, the strains CXY1547 and CXY1548 were resolved as a distinct clade with strong support (Fig. 3).

Culture morphology and characteristics

On the basis of cultural characteristics and micromorphology, the present strains from *I. subelongatus* and their galleries could be grouped into three distinct morphological groups or phenotypes. Group 1 comprised of the strains CXY1552 and CXY1553, group 2 the strains CXY1547 and CXY1548, and group 3 the strains CXY1549 and CXY1554.

The strains belonging to group 1 produced fertile perithecia on MEA within 8 days. However, the asexual form was not observed. The colony color of these strains was white, with abundant aerial mycelium. Perithecia were not formed by strains from groups 2 and 3 on MEA. However, strains of both groups 2 and 3 developed a dark brown synnematous asexual form, whose conidia differed between strains of the two groups. The colony color of strains from groups 2 and 3 was white, becoming respectively dark celadon and light brown with age on MEA.

The optimal temperature of growth for all three groups was 25 °C, with no growth observed at 5 °C and 35 °C. Strains of group 1 grew much faster than those of groups 2 and 3, reaching 76-mm diameters after 6 days at 25 °C. In comparison, colonies of strains from groups 2 and 3 reached 24 and 23 mm, respectively, after 6 days of growth at 25 °C (Fig 4).

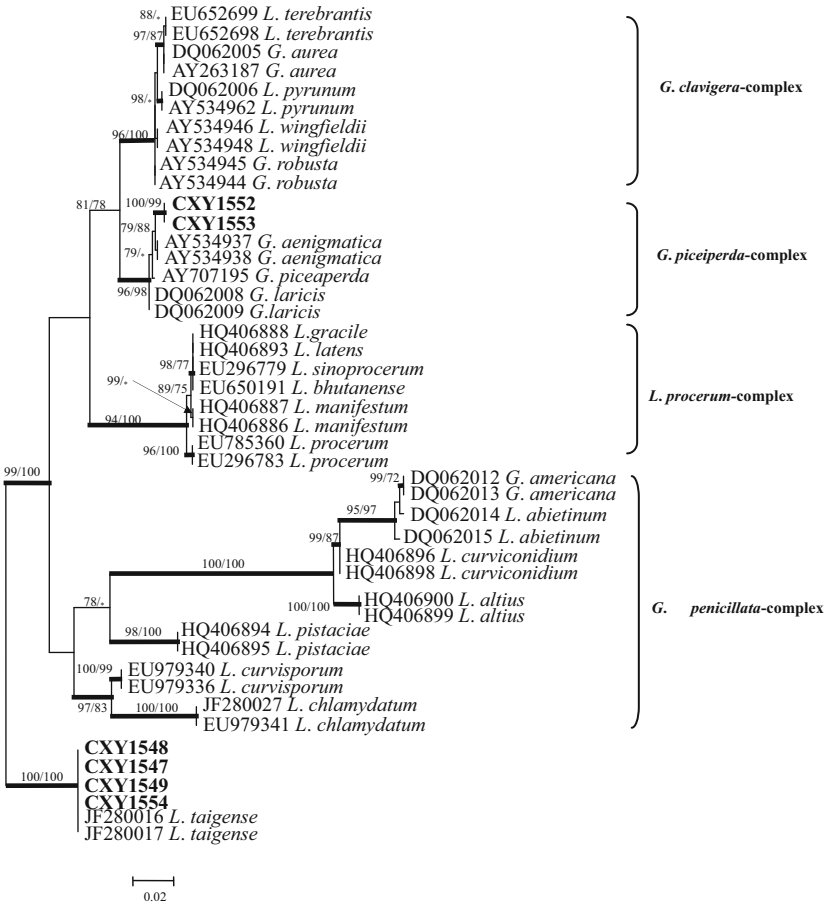

Fig. 2 Phylogram obtained from ML analyses of the β-tubulin gene regions; novel sequences obtained in this study are indicated in **bold type**. ML bootstrap support values (1000 replicates) (normal type) and MP jackknife values (10,000 replicates) (**bold type**) above 70 % are indicated at the nodes. Posterior probabilities (above 95 %) obtained from BI are indicated by **bold lines** at the relevant branching points. Values less than 70 % are indicated an asterisk

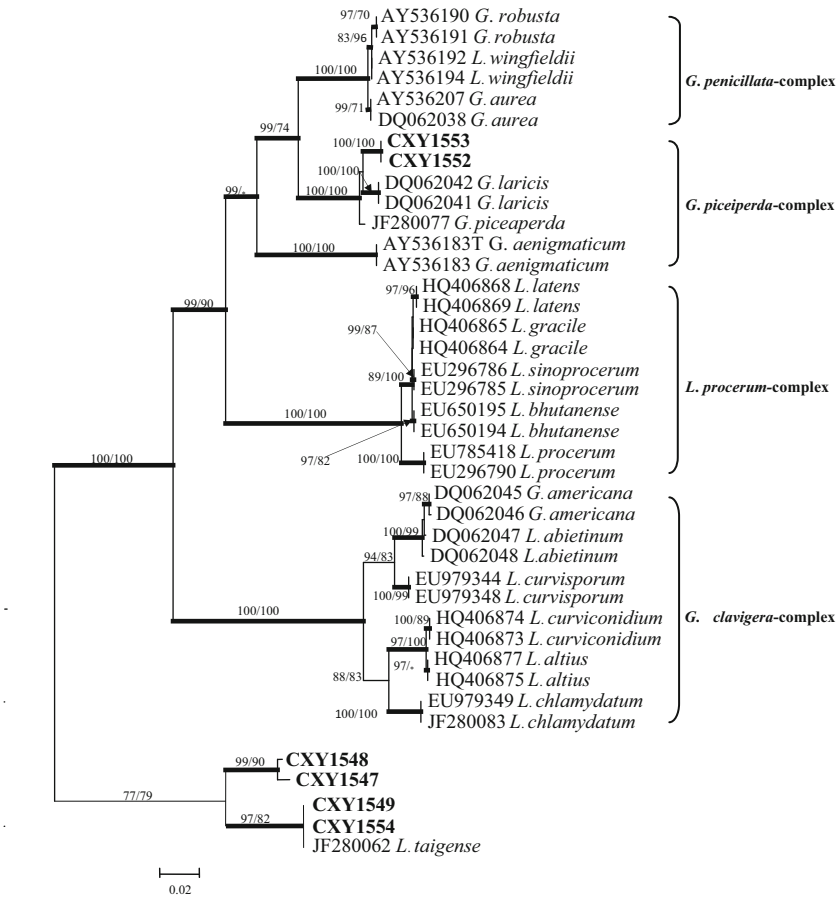

Fig. 3 Phylogram obtained from ML analyses of the EF1-α gene region; novel sequences obtained in this study are indicated in **bold lines**. ML bootstrap support values (1000 replicates) (normal type) and MP jackknife values (10,000 replicates) (MP Jackknife values) above 70 % are indicated at the nodes. Posterior probabilities (above 95 %) obtained from BI are indicated by **bold lines** at the relevant branching points. Values less than 70 % are indicated by an asterisk

Taxonomy

Leptographium zhangii X. W. Liu, Q. Lu & X. Y. Zhang, sp. nov. Fig. 5

MycoBank: MB 811205

Etymology: "zhangii" (L), named in honor of Prof. Xingyao Zhang, the senior author of the paper.

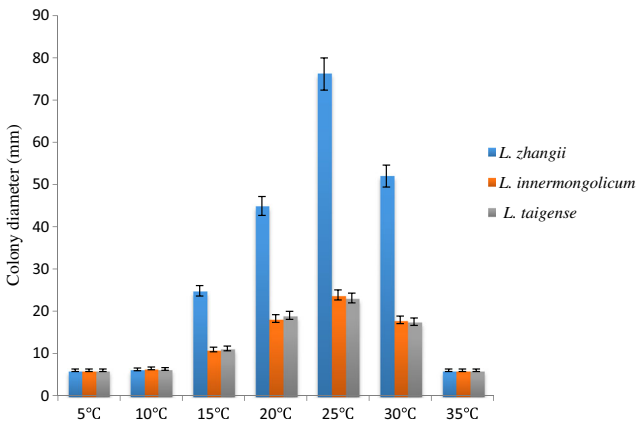

Fig. 4 Average colony diameters (mm) of the strains from larch growing on MEA at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C after 6 days

Colonies on 2 % MEA were fast growing, reaching 84 mm in diameter in 7 days at the optimal growth temperature of 25 °C; no growth at 5 °C and 35 °C; colonies on malt extract agar white, with abundant aerial mycelium, hyaline on OA.

Perithecia appearing over the colony surface, more abundant at its edge, after 8 days; perithecia with a globose base, (126–)141–215(−316) μm diameter, dark, ornamented with hyphae, ending in black, smooth, straight to slightly curved perithecial necks, (190–)200–430(−445) μm long, (37–)42–69(−78) μm wide at the base down to (24–)29–37(−43) μm at the apex; ostiolar hyphae absent; ascospores with hyaline gelatinous sheets, hyaline, aseptate, oblong, 5.5–10.0 × 2.7–3.8 μm (excluding the mucilaginous sheath). Asexual form unknown.

Type material: Holotype CXY1552 (dried culture), CHINA, Heilongjiang, Mohe, from *I. subelongatus* infecting *L. gmelinii*, 2012, collected by X. Liu, ex-holotype culture CXY1552 = MUCL55162.

Hosts/substrate: *L. gmelinii*

Known distribution: China

Leptographium innermongolicum X. W. Liu, Q. Lu & X. Y. Zhang, sp. nov. Fig. 6

MycoBank: MB 811204

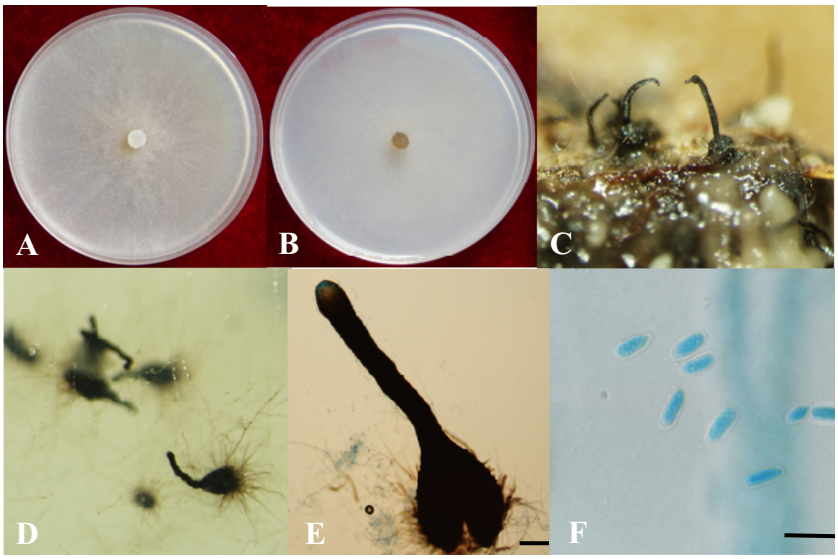

Fig. 5 *Leptographium zhangii*. A–C: Growing on 2 % MEA, OA, and WA-twigs, respectively. D–E: Perithecium (bar = 100 μm). F: Ascospores (bar = 10 μm)

Etymology: "innermongolicum" (L), in reference to the type locality of this species, Inner Mongolia.

Colonies on 2 % MEA reaching 29 mm in diameter in 7 days at the optimal growth temperature of 25 °C; no growth observed at 5 °C and 35 °C; colony color on MEA white, becoming dark celadon with age; colony color on OA hyaline, becoming dark brown with age.

Sexual form unknown. Synnematous anamorph predominant in culture; single or in groups, dark brown, (80–)120–272(−385) μm high, (12–)20–39(−48) μm wide at the base, with rhizoid-like structures occasionally present; mononematous *Leptographium*-like synanamorph present, but sparse on MEA, soon aggregated to form synnematous structures; stipes hyaline to light brown, cylindrical, (15–)60–165(−250) μm long and (1.3–)2–2.0(−2.5) μm wide, apical cell not swollen, basal cell not swollen. Primary branches 2–3, cylindrical, 0–1-septate, (7–)9–22(−35) μm long and (1–)1.5–2(−2.5) μm wide. Secondary branches occasionally swollen, (4–)6–11(−14) μm long and (1–)1.5–2.5(−3.5) μm wide. Tertiary branches sometimes observed, typically swollen, (6–)7–12(−12.5) μm long and (2–)2.5–4(−4.5) μm wide. Conidiogenous cells discrete, 2–7 per branch, cylindrical, tapering slightly at the apex, (4–)10–15(−21) μm long and 1.0–2.0 μm wide. Conidia hyaline, aseptate, oblong, 2.2–3.7 × 0.9–2.2 μm.

Type material: Holotype CXY1547 (dried culture), CHINA, Inner Mongolia, Genhe wood reservation station from *I. subelongatus* infecting *L. gmelinii*, 2010, collected by Q. Lu, ex-holotype culture CXY1547 = MUCL55158.

Hosts/substrate: *L. gmelinii*

Known distribution: China

Pathogenicity tests

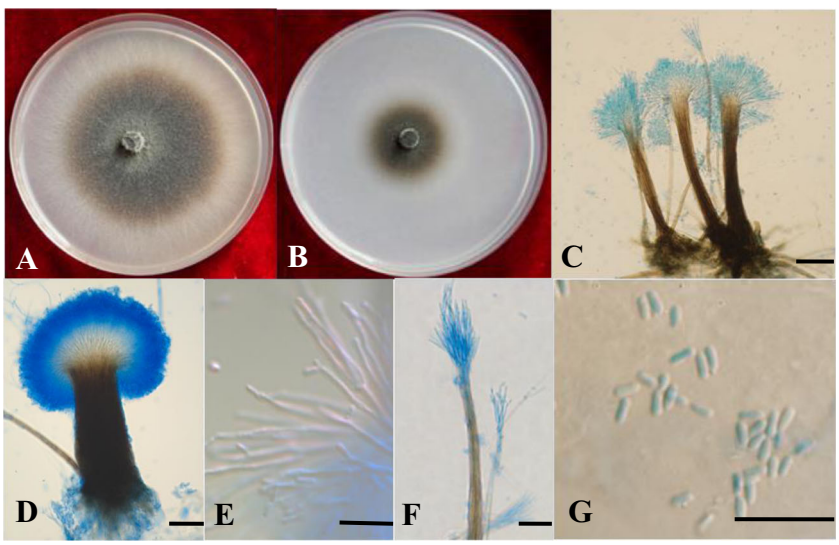

Fig. 6 *Leptographium innermongolicum*. A–B: Growing on 2 % MEA and OA, respectively. C–D: Rhizoids, conidiophores, conidiogenous apparatus (bar = 20 μm). E: Conidiogenous cells showing tapering apex with conidia (bar = 10 μm). F: Aggregated conidiophores of *Leptographium*-like anamorph (bar = 20 μm). G: Conidia (bar = 10 μm)

Two months after inoculation, none of the 12 inoculated trees in the two plots showed any visible disease symptoms in the crowns. Trees inoculated with the control exhibited a slight brown discoloration in inner bark, extending up to 1.4 mm long from the border of the hole; this was observed after removal of the outer bark. Lesions produced by the *Leptographium* strains for each species (CXY1552 for *L. zhangii*, CXY1547 for *L. innermongolicum*, and CXY1554 for *L. taigense*) were not significantly longer than those observed in control-inoculated trees (Table 2). Moreover, no significant differences were found between the lesion lengths produced by the *Leptographium* strains in Genhe and Huangganglang. The inoculated fungi were readily reisolated from selected lesions, while the control holes did not yield *Leptographium* spp.

Discussion

The present study reveals that three species of *Leptographium* are associated with *I. subelongatus* infestations of larch forests in northern China, including *L. taigense* and two new species, *L. zhangii* and *L. innermongolicum*. The strains of these three species occurred at a very low frequency, not exceeding 5 % of the total isolates.

According to the Melbourne code 2011 (Hawksworth 2011; McNeill et al. 2012) and the "one fungus, one name" principle, we adopted *Leptographium* as the formal genus name based on priority and usage of the name (Jacobs and Wingfield 2013). Accordingly, the two new species were named under the genus *Leptographium*.

Leptographium taigense has been described on the basis of a few collections made from various bark beetles on pine and spruce in the Karelia forest of north-western Russia (Linnakoski et al. 2012). In three-locus phylogenetic studies, this species formed a standalone taxon distinct from the other known species complexes (Linnakoski et al. 2012).

To our knowledge, the present study is the first to report the occurrence of *L. taigense* on *L. gmelinii* in association with *I. subelongatus* in northern China; our data indicate a broader host range and geographic distribution for the fungal species. In addition, a genetically closely related taxon, *L. innermongolicum*, was found to co-occur in the same environment. According to our analysis, *L. innermongolicum* differs from *L. taigense* only in terms of its EF1-α DNA sequence data (Fig. 3). The partial sequences of ITS-28S and β-tubulin were found to be identical between these two species. Furthermore, these two species are morphologically similar, producing a similar macronematous, synnematous asexual form resulting from the aggregation of mononematous conidiophores, as described by Linnakoski et al. (2012). However, the synnemata of *L. innermongolicum* are much shorter than those of *L. taigense*: 120–272 μm and 287–566 μm, respectively (Linnakoski et al. 2012). *Leptographium innermongolicum* may additionally be distinguished from *L. taigense* based on colony characteristics. The colony color of *L. innermongolicum* is initially white, becoming dark celadon with age, whereas the colony color of *L. taigense* is hyaline, later becoming light brown.

In addition to *L. taigense*, *L. innermongolicum* may be compared with *G. galeiformis* Zipfel et al., which also produces a synnematous form in culture (Zhou et al. 2004). However, the conidia of *L. innermongolicum* are smaller to those of *G. galeiformis*. The conidiophores of *L. innermongolicum* are dark brown, whereas those of *G. galeiformis* are middle brown in color. The colony color of *L. innermongolicum* is white, becoming dark celadon with age. However, the colony color of *G. galeiformis* is light gray, becoming dark brown with age. The sexual forms of *L. innermongolicum* and *L. taigense* are unknown at present. Linnakoski et al. (2012) failed to obtain perithecia in vitro for *L. taigense* via mating studies.

Leptographium was originally characterized by the presence of mononematous conidiophores (Jacobs and Wingfield 2001). However, recent phylogenetic studies have shown that members of the *Leptographium* lineage may exhibit diverse asexual forms, including *Hyalorhinocladiella*, reduced *Leptographium* structures, loose and tight aggregates of *Leptographium*-like structure, or even *Phialographium*-like structure (Zipfel et al. 2006; Paciura et al. 2010; Linnakoski et al. 2012; Huang and Chen 2014).

Table 2 Lesion observed in the inner bark of *Larix* two months after inoculation with *Leptographium* spp. strains at the forest plots

Strain (CXY)	Forest plot in Genhe		Forest plot in Huangganglang	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)
L. zhangii CXY1552	1.72±0.19a	1.09±0.23a	1.71±0.05a	1.11±0.25a
L. innermongolicum CXY1547	1.64±0.05a	1.13±0.14a	1.65±0.13a	1.15±0.19a
L. taigense CXY1554	1.60±0.12a	1.09±0.11a	1.66±0.10a	1.12±0.05a
Control	1.37±0.07a	1.03±0.18a	1.39±0.04a	1.05±0.13a

Treatments that were not statistically different ($p < 0.05$) are indicated by the same letters ("a") within a column

Leptographium zhangii belongs to the *G. piceaperda* clade (Figs. 1 and 2), which differs from *G. piceaperda* and from *G. aenigmatica* in terms of the shape of the ascospores, including the mucilaginous sheath; *L. zhangii* has oblong ascospores which are cucullate in *G. piceaperda* and *G. aenigmatica* (Jacobs et al. 1998; Jacobs et al. 2000). No asexual form was found for *L. zhangii*, despite searching for conidiogenous cells in cultures.

Leptographium zhangii is also similar to *G. laricis*, which has been described in association with several bark beetles and is considered pathogenic to larch trees (Stauffer et al. 2001; Yamaoka et al. 1998, 2009). *Leptographium zhangii* differs in having much shorter perithecial necks, viz. 140–215 μm in length, compared with those of *G. laricis*, which are 400–1320 μm long (Jacobs and Wingfield 2001).

Little is known about the ecology of *L. zhangii* and *L. innermongolicum*. To date, these species have only been described in the context of *L. gmelinii* ecosystems in Inner Mongolia, northern China, in association with *I. subelongatus*. Both species were isolated from the insect itself as well as its breeding galleries, suggesting that *I. subelongatus* may act as a vector. *Leptographium* species are adapted to be carried by bark-infesting beetles or other insects that act as vectors (Harrington and Cobb 1988; Wingfield et al. 1993; Jacobs and Wingfield 2001).

These three species do not seem to be pathogenic to larch in the *L. gmelinii* ecosystems. After 2 months of incubation, stem inoculation with the three *Leptographium* species resulted in non-significant lesions that were visible at the inner bark, with no discernible symptoms in the crowns of mature larch trees.

As *L. innermongolicum* is closely related to *L. taigense*, it should not be surprising that the two species may exhibit the same pathogenicity. The pathogenicity of *L. zhangii* to *L. gmelinii* in northern China is weaker than that of other members of the *G. piceaperda* complex, of which *G. piceaperda* and *G. laricis* are pathogenic to trees (Yamaoka et al. 1998; Sallé et al. 2005).

Leptographium species typically occur in conifer forests, in association with bark beetles. Ten species of *Leptographium* have been isolated from larch worldwide (Bakshi 1950; Mielke 1979; Yamaoka et al. 1998, 2009; McBeath et al. 2004; Paciura et al. 2010). To date, there are few reports of the occurrence of *Leptographium* in association with *I. subelongatus* in China. The present study reports the isolation of *L. taigense*, *L. zhangii*, and *L. innermongolicum* from *L. gmelinii*. Future studies should contribute interesting insights into the ecology, biodiversity, and biogeography of the fungi.

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References

Aoshima K (1965) Studies on wood-staining fungi of Japan. Doctoral thesis, University of Tokyo, Tokyo

Bakshi BK (1950) Fungi associated with ambrosia beetles in Great Britain. Trans Br Mycol Soc 33(1):111–IN11

De Beer ZW, Wingfield MJ (2013) Emerging lineages in the Ophiostomatales. In: Seifert KA, De Beer ZW, Wingfield MJ (eds) The ophiostomatoid fungi: expanding frontiers. CBS, Utrecht, The Netherlands, pp 21–46

Eckhardt LG, Weber AM, Menard RD, Jones JP, Hess NJ (2007) Insect–fungal complex associated with loblolly pine decline in central Alabama. For Sci 53:84–92

Gams W, Hoekstra ES, Aptroot A (1998) CBS course of mycology, 4th edn. Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61(4):1323–1330

Goidánich G (1936) II genere di Ascomiceti '*Grosmannia*' G. Goid. Boll Staz Pat veg Roma 16:26–60

Grosmann H (1931) Contributions to the knowledge concerning the life partnership between bark beetles and fungi. Z Parasitenkd 3:56–102

Harrington TC, Cobb FW (1988) *Leptographium* root diseases on conifers. American Phytopathological Society Press, St. Paul, MN

Hawksworth DL (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. IMA Fungus 2:155–162. This article was first published in MycoKeys, 1:7–20 (2011)

Huang YT, Chen CY (2014) *Leptographium globosum* sp. nov., a new species with globose conidia. Mycol Progress 13:841–848

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Jacobs K, Wingfield MJ (2001) *Leptographium* species: tree pathogens, insect associates, and agents of blue-stain. American Phytopathological Society Press, St. Paul, MN

Jacobs K, Wingfield MJ (2013) An overview of *Leptographium* and *Grosmannia*. In: Seifert KA, De Beer, Wingfield MJ (eds) The ophiostomatoid fungi: expanding frontiers. CBS, Utrecht, The Netherlands, pp 47–56

Jacobs K, Wingfield MJ, Wingfield BD, Yamaoka Y (1998) Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov. Mycol Res 102:289–294

Jacobs K, Wingfield MJ, Crous PW (2000) *Ophiostoma europhioides* and *Ceratocystis pseudoeurophioides*, synonyms of *O. piceaperdum*. Mycol Res 104(2):238–243

Jacobs K, Wingfield MJ, Wingfield BD (2001) Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. Can J Bot 79(6):719–732

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD (2004) *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol Res 108(4):411–418

Jacobs K, Solheim H, Wingfield BD, Wingfield MJ (2005) Taxonomic re-evaluation of *Leptographium lundbergii* based on DNA sequence comparisons and morphology. Mycol Res 109(10):1149–1161

Jacobs K, Eckhardt LG, Wingfield MJ (2006) *Leptographium profanum* sp. nov., a new species from hardwood roots in North America. Can J Bot 84:759–766

Jacobs K, Krokene P, Solheim H, Wingfield MJ (2010) Two new species of *Leptographium* from *Dryocetes authographus* and *Hylastes cunicularius* in Norway. Mycol Prog 9(1):69–78

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acid Res 30:3059–3066

Kendrick WB (1962) The *Leptographium* complex *Verticicladiella* Hughes. Can J Bot 40:772–797

Kim JJ, Lim YW, Breuil C, Wingfield MJ, Zhou XD, Kim GH (2005) A new *Leptographium* species associated with *Tomicus piniperda* infesting pine logs in Korea. Mycol Res 109(03):275–284

Kirisits T (2004) Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF (eds) Bark and wood boring insects in living trees in Europe, a synthesis. Kluwer, Dordrecht, The Netherlands, pp 181–235

Kirkendall LR, Faccoli M, Ye H (2008) Description of the Yunnan shoot borer, *Tomicus yunnanensis* Kirkendall & Faccoli sp. n. (Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of *Tomicus*. Zootaxa 1819:25–39

Lagerberg T, Lundberg G, Melin E (1927) Biological and practical researches into blueing in pine and spruce. Svenska Skogsvårdsfören Tidskr 25:145

Lim YW, Alamouti SM, Kim JJ, Lee S, Breuil C (2004) Multigene phylogenies of *Ophiostoma clavigerum* and closely related species from bark beetle-attacked Pinus in North America. FEMS Microbiol Lett 237(1):89–96

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

Lu Q, Decock C, Zhang XY, Maraite H (2008) *Leptographium sinoprocerum* sp. nov., an undescribed species associated with *Pinus tabuliformis–Dendroctonus valens* in northern China. Mycologia 100:275–290

Lu Q, Decock C, Zhang XY, Maraite H (2009a) Ophiostomatoid fungi (Ascomycota) associated with *Pinus tabuliformis* infested by *Dendroctonus valens* (Coleoptera) in northern China and an assessment of their pathogenicity on mature trees. Antonie Van Leeuwenhoek 96:275–293

Lu M, Zhou XD, De Beer ZW, Wingfield MJ, Sun JH (2009b) Ophiostomatoid fungi associated with the invasive pine-infesting bark beetle, *Dendroctonus valens*, in China. Fungal Divers 38: 133–145

McBeath JH, Cheng M, Gay P, Ma M (2004) First report of *Leptographium abietinum* associated with blue stain on declining western Siberian larch in Alaska. Plant Health Progress (March):1–2

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema J, Turland NJ (eds) (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Regnum vegetabile 154. Koeltz Scientific Books, Königstein

Mielke PW (1979) On asymptotic non-normality of null distributions of MRPP statistics. Commun Stat Theory Methods 8(15):1541–1550

Paciura D, De Beer ZW, Jacobs K, Zhou XD, Ye H, Wingfield MJ (2010) Eight new *Leptographium* species associated with tree-infesting bark beetles in China. Persoonia 25:94–108

Pfeffer A (1995) Zentral- und Westpaläarktische Borken- und Kernkäfer. Naturhistorishes Museum Basel, Basel, 310 pp

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute and the British Mycological Society, Kew, Surrey

Sallé A, Monclus R, Yart A, Garcia J, Romary P, Lieutier F (2005) Fungal flora associated with *Ips typographus*: frequency, virulence, and ability to stimulate the host defence reaction in relation to insect population levels. Can J For Res 35:365–373

Seifert KA, Webber JF, Wingfield MJ (1993) Methods for studying species of *Ophiostoma* and *Ceratocystis*. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. American Phytopathological Society Press, St. Paul, MN, pp 255–259

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stauffer C, Kirisits T, Nussbaumer C, Pavlin R, Wingfield MJ (2001) Phylogenetic relationships between the European and Asian eight spined larch bark beetle populations (Coleoptera, Scolytidae) inferred from DNA sequences and fungal associates. Eur J Entomol 98:99–105

Swofford DL (2001) PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0.b5

Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA

Van der Westhuizen K, Wingfield MJ, Yamaoka Y, Kemp GHJ, Crous PW (1995) A new species of *Ophiostoma* with a *Leptographium* anamorph from larch in Japan. Mycol Res 99(11):1334–1338

Wagener WW, Mielke JL (1961) A staining-fungus root disease of pondersoa, Jeffrey, and pinyon pines. Plant Dis Rep 45:831–835

White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc Guide Methods Appl 18(1):315–322

Wingfield MJ, Capretti P, Mackenzie M (1988) *Leptographium* spp. as root pathogens on conifers. An international perspective. In: Harrington TC, Cobb FW Jr (eds) *Leptographium* root diseases on conifers. American Phytopathological Society Press, St. Paul, MN, pp 113–128

Wingfield MJ, Seifert KA, Webber JF (1993) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. American Phytopathological Society Press, St. Paul, MN

Wood SL, Bright DE (1992) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index Volume A. Great Basin Naturalist Memoirs, No. 13. Brigham Young University, Provo, UT, 833 pp

Yamaoka Y, Wingfield MJ, Takahashi I, Solheim H (1997) Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. japonicus in Japan. Mycol Res 101:1215–1227

Yamaoka Y, Wingfield MJ, Ohsawa M, Kuroda Y (1998) Ophiostomatoid fungi associated with *Ips cembrae* in Japan and their pathogenicity of Japanese larch. Mycoscience 39:367–378

Yamaoka Y, Masuya H, Chung WH, Goto H, To-Anun C, Tokumasu S, Zhou X, Wingfield MJ (2008) The teleomorph of *Leptographium yunnanense*, discovered in crosses among isolates from Thailand, China, and Japan. Mycoscience 49:233–240

Yamaoka Y, Chung WH, Masuya H, Hizai M (2009) Constant association of ophiostomatoid fungi with the bark beetle *Ips subelongatus* invading Japanese larch logs. Mycoscience 50:165–172

Yang JL, Lin Q, Chen GF (2007) Risk analysis of *Ips subelongatus* Motschulsky. J Northeast For Univ 35(3):60–63

Yin HF, Huang FS, Li ZL (1984) Economic insect fauna of China. Science and Technology Press, Beijing, pp 54–55

Yin M, Duong TA, Wingfield MJ, Zhou X, De Beer ZW (2015) Taxonomy and phylogeny of the *Leptographium procerum* complex, including *Leptographium sinense* sp. nov. and

Leptographium longiconidiophorum sp. nov. Antonie Van Leeuwenhoek 107:547–563

Zhou XD, Jacobs K, Morelet M, Ye H, Lieutier F, Wingfield MJ (2000) A new *Leptographium* species associated with *Tomicus piniperda* in southwestern China. Mycoscience 41:573–578

Zhou XD, De Beer ZW, Harrington TC, McNew D, Kirisits T, Wingfield MJ (2004) Epitypification of *Ophiostoma galeiformis* and phylogeny of species in the *O. galeiformis* complex. Mycologia 96:1306–1315

Zhou XD, Jacobs K, Kirisits T, Chhetri DB, Wingfield MJ (2008) *Leptographium bhutanense* sp. nov., associated with the root collar weevil *Hylobitelus chenkupdorjii* on *Pinus wallichiana* in Bhutan. Persoonia Molecular Phylogeny Evol Fungi 21(1):1–8

Zipfel RD, De Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ (2006) Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Stud Mycol 55:75–97