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ORIGINAL PAPER

Grosmannia and *Leptographium* spp. associated with conifer-infesting bark beetles in Finland and Russia, including *Leptographium taigense* sp. nov.

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Abstract Species of *Grosmannia* with *Leptographium* anamorphs include important forest pathogens and agents of blue stain in timber. They are commonly found in association with forest pests, such as bark beetles. During a survey of ophiostomatoid fungi in eastern parts of Finland and neighboring Russia, species belonging to the genus *Grosmannia* were isolated from 12 different bark beetle species infesting *Picea abies* and *Pinus sylvestris*, the most economically important conifers in the region. Identification of these fungi was based on morphology, DNA sequence comparisons for three gene regions and phylogenetic analyses. A total of ten taxa were identified. These belonged to six different species complexes in *Grosmannia*. The phylogenetic analyses provided an opportunity to redefine the *G. galeiformis*-, *L. procerum*-, *L. lundbergii*-, *G. piceiperda*-, *G. olivacea*- and *G. penicillata*-complexes, and to consider the species emerging from the survey within the context of these complexes. The species included *G. galeiformis*, *G. olivacea*, *L. chlamydatum*, *L. lundbergii*, *L. truncatum* and a novel taxon, described here as *L. taigense* sp. nov. In addition, species closely related to *G. cucullata*, *G. olivaceapini* comb. nov., *G. piceiperda* and *L. procerum* were isolated but their identity could not be resolved. The overall results indicate that the diversity of *Grosmannia* species in the boreal forests remains poorly understood and that further studies are needed to clarify the status of several species or species complexes.

Keywords Bark beetle-associated fungi · Ophiostomatales · Ophiostomatoid fungi · Symbiosis

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Introduction

Species of *Leptographium* Lagerb. & Melin are anamorphs of the Ascomycete genus *Grosmannia* Goid. (Zipfel et al. 2006). These fungi include causal agents of tree diseases such as black stain root disease of conifers in western North America and many are agents of blue stain in timber (Harrington and Cobb 1988; Wingfield et al. 1993). The species are typically

associated with conifer-infesting bark beetles (Coleoptera: Curculionidae, Scolytinae) and other insects (Münch 1907; Rennerfelt 1950; Mathiesen-Käärik 1953). *Leptographium* states are characterized by penicillately branched pigmented conidiophores, which give rise to conidia that accumulate in slimy masses at their apices (Jacobs and Wingfield 2001). *Grosmannia* teleomorph states produce ascomata with globose bases, necks of variable length and ascospores with cucullate sheaths (Jacobs and Wingfield 2001; Zipfel et al. 2006). These anamorph and teleomorph structures typically occur in galleries of bark beetles, and are carried from one tree to another by the beetles or other insects visiting these galleries (Harrington and Cobb 1988; Wingfield et al. 1993).

Species of *Grosmannia* and *Leptographium* in Europe and Scandinavia are mainly known from conifers where they exist in symbiosis with bark beetles (Solheim and Långström 1991; Jacobs and Wingfield 2001; Kirisits 2004). Only one species is known to infest hardwoods in Europe (Davidson 1971). In general, the relationships between these fungi, their insect vectors and host trees remain poorly understood (Six and Wingfield 2011).

In Europe, previous studies have reported numerous *Grosmannia* and *Leptographium* species in association with conifer-infesting bark beetles (Kirisits 2004). However, inventories of species occurring in different niches are incomplete in many countries. Despite the occurrence of several native bark beetle species, reports of these fungi from Finland and Russia are very limited (Table 1). Consequently, an extended survey of bark beetle-associated fungi is currently being undertaken in the boreal forests of Karelia, on both the Finnish and Russian sides of the border (Linnakoski et al. 2010). As part of this survey, fungal collections have been made from the most common bark beetle species infesting the two dominant conifer species, Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) that occur in the boreal forests. The first results of the survey reported 15 species of *Ophiostoma* in association with these beetles (Linnakoski et al. 2010). The aim of the present study was to identify all species of *Grosmannia* and *Leptographium* collected during this survey, but also to discuss the species within the context of their closest relatives. The identifications were based on morphological characteristics and DNA sequence comparisons for the ITS2 and 28S region of the ribosomal RNA, as well as parts of the β-tubulin and elongation factor 1-α (EF 1-α) gene regions.

Materials and methods

Isolation of fungi from bark beetles and galleries

Bark beetles and their galleries were collected from spruce and pine logs and naturally infested trees at four sites in Finland (Ilomantsi, Jouhteninen, Punkaharju, Pyhäselkä) and eight sites in Russia (Kivennapa, Lisino-Corpus, Manga, Nurmoila, Ohtama, Roikonkoski, Uuksujärvi, Volosovo) between 2004 and 2007 (June–July). The latter sites included one with extensive spruce bark beetle (*Ips typographus* L.) damage in the Ohtama region of Russia. At the other sites bark beetles were at an endemic phase. After bark beetles were collected following an opportunistic sampling strategy, they were stored at 4 °C and the fungal isolations were done within 2 weeks.

Fungi were isolated from both bark beetles and their galleries, using the methods described by Linnakoski et al. (2008). The bark beetle galleries were placed in moist chambers and incubated at room temperature for 4–6 weeks to allow fungi to sporulate. During the incubation period, mycelium and/or fungal spore masses that formed in the galleries were transferred to 2 % malt extract agar (MEA; 20 g Difco Bacto™ malt extract, 20 g Difco Bacto™ agar, and 1 L Milli-Q water). Adult male and female beetles were squashed and streaked on to the surface of the same medium, and incubated as described before. Different fungal structures from mixed cultures obtained from the beetles or their galleries were transferred to fresh MEA. Once the resulting fungal isolates had been purified, they were grouped according to culture morphology. Isolates representing each morphological group and those from different sites, associated beetles and host tree species were selected for DNA sequencing. Representative isolates were deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands and the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Isolates of the new taxon found in this study are also maintained in the VTT Culture Collection, VTT Technical Research Centre of Finland, Espoo, and herbarium specimens were deposited in the Kuopio Museum of Natural History (KUO),

Table 1 *Grosmannia* and *Leptographium* spp. previously reported from different beetles and/or host trees in Finland (F) and Russia (R). All identifications in these studies were based on morphology only

Fungus	Beetle	Host tree	Country	Reference
Grosmannia europhioides	*Monochamus urussovi*	*A. sibirica*	R	Pashenova et al. (2004)
	Ips typographus	*P. abies*	F	Viiri (1997)
	I. typographus	*P. obovata*	R	Pashenova et al. (2001)
G. penicillata	*Hylurgops palliatus*	*P. abies*	F	Savonmäki (1990)
	H. palliatus	*P. sylvestris*	F	Savonmäki (1990)
	I. typographus	*P. abies*	F	Savonmäki (1990)
	I. typographus	*P. obovata*	R	Pashenova et al. (2001)
	Pityogenes chalcographus	*P. abies*	F	Savonmäki (1990)
	Tomicus piniperda	*P. abies*	F	Savonmäki (1990)
	Trypodendron lineatum	*P. abies*	F	Savonmäki (1990)
	T. lineatum	*P. sylvestris*	F	Savonmäki (1990)
	I. typographus	*P. obovata*	R	Afanasova (2009)
	–	conifers	R	Pashenova & Polyakova (2009)
Leptographium lundbergii	–	*P. abies*	F	Hallaksela (1977)
	–	*P. abies*	R	Fedorenko (1988)
		P. sylvestris	R	Fedorenko (1988)
L. sibirica	*M. urussovi*	*A. sibirica*	R	Jacobs et al. (2000)
	M. urussovi	*A. sibirica*	R	Pashenova & Polyakova (2009)
	T. lineatum	*A. sibirica*	R	Pashenova et al. (2004)

Kuopio, Finland. Several isolates of known species for which DNA sequences were not available, were also included in the study for comparative purposes (Table 2).

DNA extraction and PCR

Fungal isolates were grown on MEA in 90 mm Petri dishes. DNA was extracted using PrepMan Ultra Sample preparation reagent (Applied Biosystems, Foster City, CA, USA) as described by Linnakoski et al. (2008).

Gene regions sequenced included the internal transcribed spacer (ITS2), part of the large subunit (28S) of the rDNA operon, partial β-tubulin and elongation factor 1-α (EF 1-α) genes as described by Jacobs et al. (2004). The ITS2 and 28S regions were amplified using primers ITS3 and LR3 (White et al. 1990). Part of the β-tubulin gene region was amplified using primers Bt2a and Bt2b (Glass and Donaldson 1995). Primer Bt2b was replaced in some cases with primer T10 (O'Donnel and Cigelnik 1997). The elongation factor 1-α gene region was amplified using primers EF1F and EF2R (Jacobs et al. 2004).

Gene fragments were amplified in 25 µl reaction mixture as described by Linnakoski et al. (2008). The PCR conditions for ITS gene region were: an initial denaturation step at 95 °C for 2 min, followed 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 gene and the elongation factor 1-α (EF 1-α) gene were amplified using denaturation step at 95 °C for 2 min, followed 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. Amplified products were purified using the High Pure PCR Product Purification Kit (Roche Molecular Biochemicals, Indianapolis, USA) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on the ABI Prism 377 Autosequencer (Applied Biosystems, Foster City, CA, USA), using the same primers used for the PCR.

Sequence analyses

For each isolate, sequences obtained using the forward and reverse primers were aligned and consensus sequences determined using Geneious Pro v4.8.4 for

Table 2 Fungal isolates obtained from different bark beetle species infesting pine and spruce and used in this study

Species	Isolate numbers[1]			Herbarium	Origin	Host	Insect vector	Collector	GenBank no.		
	CMW	CBS	VTT	KUO					ITS2-28S	β-tubulin	EF 1-α
Isolates obtained in the present study											
G. cucullata/ *G. olivaceapini*	23123	128299			Ohtama, Russia	*Picea abies*	*Ips typographus*	J Ahtiainen	–	JF280003	JF280042
	23190	128832			Lisino-Corpus, Russia	*P. abies*	*I. typographus*	R Linnakoski	JF279985	JF280005	JF280043
	23192	–			Lisino-Corpus, Russia	*P. abies*	*I. typographus*	R Linnakoski	–	JF280004	JF280044
	23289	128834			Punkaharju, Finland	*P. abies*	*Dryocoetes autographus*	R Linnakoski	–	JF279994	–
	23295	–			Punkaharju, Finland	*P. abies*	*D. autographus*	R Linnakoski	–	JF279993	JF280036
	23300	128833			Jouhteninen, Finland	*P. abies*	*I. typographus*	ZW de Beer	–	JF279996	JF280045
	23307	–			Ilomantsi, Finland	*Pinus sylvestris*	*Hylurgops palliatus*	ZW de Beer	–	–	–
	23312	–			Ilomantsi, Finland	*P. abies*	*Hylastes brunneus*	ZW de Beer	–	JF279991	–
	23313	–			Ilomantsi, Finland	*P. abies*	*D. autographus*	ZW de Beer	–	JF279992	JF280035
	23315	128923			Ilomantsi, Finland	*P. abies*	*D. autographus*	ZW de Beer	JF279982	JF279989	JF280034
	23316	–			Ilomantsi, Finland	*P. abies*	*H. brunneus*	ZW de Beer	JF279986	JF279990	JF280037
G. galeiformis	23282				Ilomantsi, Finland	*P. abies*	*Trypodendron lineatum*	ZW de Beer	JF279981	JF280007	JF280059
G. olivacea	23348	128836			Jouhteninen, Finland	*P. abies*	*I. typographus*	ZW de Beer	–	–	JF280049
	23350	128837			Jouhteninen, Finland	*P. abies*	*I. typographus*	ZW de Beer	–	–	JF280050
	36624	–			Jouhteninen, Finland	*P. abies*	*I. typographus*	ZW de Beer	–	–	JF280048
	36625	128835			Jouhteninen, Finland	*P. abies*	*I. typographus*	ZW de Beer	JF279988	JF279999	JF280051
G. piceiperda C	36628	128925			Pyhäselkä, Finland	*P. abies*	*Pityogenes chalcographus*	ZW de Beer	JF279969	–	JF280072
G. piceiperda D	36626	128838			Ohtama, Russia	*P. abies*	*I. typographus*	J Ahtiainen	JF279968	JF280024	JF280070
	36627	128839			Ohtama, Russia	*P. abies*	*I. typographus*	J Ahtiainen	–	JF280023	JF280071
L. chlamydatum	36631	128840			Pyhäselkä, Finland	*P. abies*	*P. chalcographus*	ZW de Beer	JF279965	JF280028	JF280080
	36632	128924			Punkaharju, Finland	*P. sylvestris*	*P. chalcographus*	R Linnakoski	–	JF280029	JF280081
	36633	–			Punkaharju, Finland	*P. abies*	*P. chalcographus*	R Linnakoski	–	–	–
	36634	128841			Lisino-Corpus, Russia	*P. sylvestris*	*P. chalcographus*	R Linnakoski	–	–	JF280082
	37213	–			Lisino-Corpus, Russia	*P. sylvestris*	*P. chalcographus*	R Linnakoski	JF279966	JF280027	JF280083
L. lundbergii	36635	128843			Lisino-Corpus, Russia	*P. sylvestris*	*P. chalcographus*	R Linnakoski	JF279976	–	JF280066
	36636	128842			Punkaharju, Finland	*P. sylvestris*	*H. palliatus*	R Linnakoski	–	–	JF280067
	36637	–			Lisino-Corpus, Russia	*P. sylvestris*	*H. palliatus*	R Linnakoski	–	JF280022	JF280065
	37212	–			Ilomantsi, Finland	*P. sylvestris*	*H. palliatus*	ZW de Beer	–	–	JF280064
	37211	–			Punkaharju, Finland	*P. sylvestris*	*H. brunneus*	R Linnakoski	JF279975	–	–
L. procerum-like	23285	128844			Lisino-Corpus, Russia	*P. sylvestris*	*H. palliatus*	R Linnakoski	JF279978	JF280018	JF280068
L. taigense sp. nov.	27965[a]	–			Kivennapa, Russia	*P. abies*	*D. autographus*	R Linnakoski	–	–	–
	36629[a,b]	128926	D-101436	022078	Lisino-Corpus, Russia	*P. abies*	*I. typographus*	R Linnakoski	JF279979	JF280016	JF280061
	36630[a,b,T]	128927	D-101435	022077	Lisino-Corpus, Russia	*P. sylvestris*	*H. palliatus*	R Linnakoski	JF279980	JF280017	JF280062
L. truncatum	36638	128845			Punkaharju, Finland	*P. sylvestris*	*D. autographus*	R Linnakoski	JF279974	JF280021	JF280063

Table 2 continued

Species	Isolate numbers[1]			Herbarium	Origin	Host	Insect vector	Collector	GenBank no.		
	CMW	CBS	VTT	KUO					ITS2-28S	β-tubulin	EF 1-α
Isolates of reference species											
G. cucullata	[T]1141	218.83			Norway	*P. abies*	*I. typographus*	H Solheim	AJ538335	JF280000	JF280039
	1871	–			Japan	*Picea jezoensis*	*I. typographus*	Y Yamaoka	JF279983	JF280001	JF280040
	5022	–			Austria	*P. abies*	*I. typographus*	T Kirisits	JF279984	JF280002	JF280041
G. galeiformis	4426	–			UK	*P. sylvestris*	*Tomicus piniperda*	T Kirisits	–	JF280008	–
	[E]5290	115711			UK	*P. sylvestris*	*T. piniperda*	T Kirisits	–	JF280009	JF280060
G. galeiformis A	9490	–			Mexico	*Pinus* sp.		XD Zhou	–	JF280015	JF280053
	12686	–			Austria	*P. abies*	*Hylastes cunicularius*	T Kirisits	–	JF280006	JF280052
G. olivacea	[T]31059	138.51			Sweden	*P. sylvestris*		A Mathiesen	AJ538337	JF279997	JF280046
	31060	152.54			Sweden			A Mathiesen	JF279987	JF279998	JF280047
G. olivaceapini	[A]116	504.86			USA			Hinds	AJ538336	JF279995	JF280038
G. piceiperda B	448 = 479	444.69			USA, Alaska	*Picea glauca*		RW Davidson	JF279973	JF280025	JF280079
	452 = 483	275.65			USA, Washington	*Pseudotsuga menziesii*		RW Davidson	–	JF280033	JF280078
	2811				USA	*Picea rubens*		TC Harrington	AY707209	AY707195	JF280077
G. piceiperda C	446 = 477	229.83			Norway	*P. abies*	*I. typographus*	H Solheim	JF279971	JF280032	JF280076
	3312	–			Austria	*P. abies*	*I. typographus*	T Kirisits	JF279970	JF280026	JF280074
	3313	–			Austria	*P. abies*	*I. typographus*	T Kirisits	JF279972	JF280030	JF280073
	3314	–			Austria	*P. abies*	*I. typographus*	T Kirisits	JF279967	JF280031	JF280075
G. radiaticola	578				South Africa	*Pinus pinaster*		L Strauss	AY649766	JF280010	JF280054
	9478	–			Chile	*Pinus radiata*	*Hylastes ater*	XD Zhou	AY649769	JF280013	JF280057
	9482	–			Chile	*P. radiata*	*Hylurgus ligniperda*	XD Zhou	AY649771	JF280014	JF280058
	9494	–			South Africa	*Pinus elliottii*	*H. ligniperda*	XD Zhou	AY649764	JF280011	JF280055
	9988	150.54			Sweden	*P. abies*	*H. cunicularius*	A Mathiesen	AY649768	JF280012	JF280056
L. procerum	[A]13	516.63			USA	*Pinus resinosa*		B Kendrick	JF279977	EU296783	EU296790
L. sibiricum	4482	–			Russia	*Larix decidua*		VP Vetrova	–	JF280019	JF280068
	4487	120194			Russia	*L. decidua*		VP Vetrova	–	JF280020	–

[1] *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *CMW* Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; *VTT* Culture Collection of the Technical Research Centre of Finland, Espoo, Finland; *KUO* Kuopio Museum of Natural History, Kuopio, Finland

[a] Isolates used in growth studies; [b] Isolates used in morphological descriptions

[T] Ex-type isolates

[A] Authentic isolate from original collection

[E] Epitype

MacIntosh (Biomatters, Auckland, New Zealand). BLAST searches were conducted for preliminary identifications, after which datasets that included published GenBank sequences were compiled in Molecular Evolutionary Genetic Analyses (MEGA) v3.1 (Kumar et al. 2004). Sequences were aligned online with MAFFT v6 (Katoh and Toh 2008), using the FFT-NS-i option with a gap opening penalty of 1.53 and an offset value of 0.00. All sequences of isolates obtained in this study were deposited in GenBank (Table 2). Accession numbers for sequences from reference isolates are presented in the phylogenetic trees (Figs. 1, 2, 3, 4, 5, 6).

Datasets were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). MP analyses were conducted using TNT v1.1 (Goloboff et al. 2008) run on the computer clusters of the CSC, IT Centre for Science, Espoo, Finland. Heuristic searches with 10,000 replicates of random addition sequences (RAS) and tree bisection and reconnection (TBR) branch swapping were carried out. Gaps were treated as a fifth character for all datasets (Odgen and Rosenberg 2007). A Jackknife test (JK) with 10,000 replicates was used to count the support values. ML analyses were performed using RAxML v7.0.4 (Stamatakis et al. 2008) assuming the GTR+G substitution model, run on the CIPRES cluster at the San Diego Supercomputing Center. Support for the nodes was estimated from 1,000 bootstrap replicates (Felsenstein 1985). BI analyses based on a Markov Chain Monte Carlo (MCMC) were carried out with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). The MCMC chains were run for five million generations using the best fitting model selected by the AIC in MrModeltest v2.3 (http://www.abc.se/~nylander/). Trees were sampled every 100 generations resulting in 50,000 trees from both runs, discarding the burn-in of the chain, as calculated for the respective data sets. The remaining trees were used to construct majority rule consensus trees.

Morphological studies

DNA sequence analyses suggested that some of the isolates considered in this study represented an undescribed species of *Grosmannia*. The cultural characteristics of the purportedly unknown taxon were based on the colony description of the representative isolates grown in an incubator at 20 °C. In an attempt to obtain sexual structures and to determine the thallism of the fungus, mating experiments were conducted. Single conidial cultures were prepared from each of the available isolates and these were crossed in all possible combinations as described by Grobbelaar et al. (2010), using sterilized spruce twigs on the agar plates to encourage sporulation. For controls, isolates were paired against themselves. Mating experiments were conducted on three different media including water agar (WA; 15 g Difco Bacto™ agar and 1 L Milli-Q water), MEA and oat meal agar (OA; 15 g oatmeal, 15 g Difco Bacto™ agar and 1 L Milli-Q water). Cultures were inspected regularly for fruiting structures.

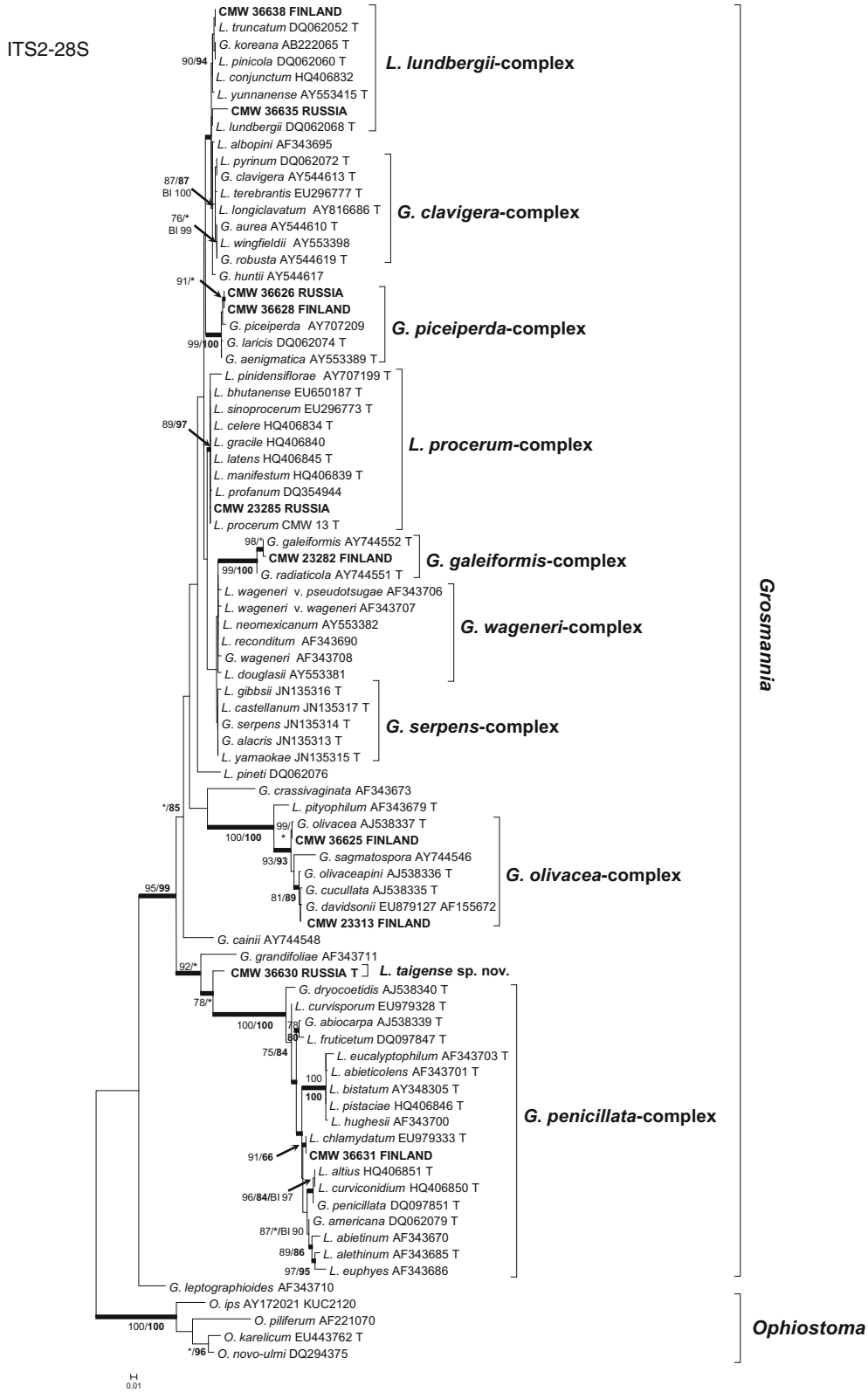
For the species description, anamorph structures were mounted in 85 % lactic acid on glass slides and observed using a Nikon Eclipse 50i phase contrast microscope (Nikon Corporation Tokyo, Japan). A Nikon DS-Fi1 camera system (Nikon Corporation, Tokyo, Japan) was used to capture photographic images. Measurements were made of 50 each of the taxonomically relevant anamorph structures. Averages, ranges and standard deviations were computed for the measurements. The measurements are presented in the format (minimum–) mean minus standard deviation—mean plus standard deviation (–maximum). For scanning electron microscopy (SEM), specimens were prepared and studied as described by Linnakoski et al. (2009). Growth studies were done on three representative isolates (Table 2) of the unknown *Grosmannia* species, also following the method used by Linnakoski et al. (2009). The only difference was that agar plugs used for inoculation were 8 mm and not 5 mm as in the previous study. Mean radial growth rates (mm/day) at 25 °C were calculated as an average of these readings.

Fig. 1 Phylogram obtained from ML analyses of the ITS2 and 28S regions. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75 %. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa ▶

Results

Collections of bark beetles and fungi

Altogether 12 bark beetle species infesting pine and spruce were found in Finland and Russia


ITS2-28S
CMW 36638 FINLAND
L. truncatum DQ062052 T
G. koreana AB222065 T
90/94
L. pinicola DQ062060 T
L. conjunctum HQ406832
L. yunnanense AY553415 T
CMW 36635 RUSSIA
L. lundbergii DQ062068 T
L. lundbergii-complex
L. albopini AF343695
L. pyrinum DQ062072 T
G. clavigera AY544613 T
87/87
BI 100
L. terebrantis EU296777 T
L. longiclavatum AY816686 T
G. clavigera-complex
76/*
BI 99
G. aurea AY544610 T
L. wingfieldii AY553398
G. robusta AY544619 T
G. huntii AY544617
91/*
CMW 36626 RUSSIA
CMW 36628 FINLAND
G. piceiperda AY707209
G. piceiperda-complex
99/100
G. laricis DQ062074 T
G. aenigmatica AY553389 T
L. pinidensiflorae AY707199 T
L. bhutanense EU650187 T
L. sinoprocerum EU296773 T
89/97
L. celere HQ406834 T
L. gracile HQ406840
L. latens HQ406845 T
L. manifestum HQ406839 T
L. profanum DQ354944
CMW 23285 RUSSIA
L. procerum CMW 13 T
L. procerum-complex
98/
G. galeiformis AY744552 T
CMW 23282 FINLAND
99/100
G. radiaticola AY744551 T
G. galeiformis-complex
L. wageneri v. pseudotsugae AF343706
L. wageneri v. wageneri AF343707
L. neomexicanum AY553382
L. reconditum AF343690
G. wageneri AF343708
L. douglasii AY553381
G. wageneri-complex
L. gibbsii JN135316 T
L. castellanum JN135317 T
G. serpens JN135314 T
G. alacris JN135313 T
L. yamaokae JN135315 T
G. serpens-complex
L. pineti DQ062076
G. crassivaginata AF343673
*/85
L. pityophilum AF343679 T
99/
G. olivacea AJ538337 T
100/100
*
CMW 36625 FINLAND
93/93
G. sagmatospora AY744546
G. olivaceapini AJ538336 T
G. cucullata AJ538335 T
81/89
G. davidsonii EU879127 AF155672
CMW 23313 FINLAND
G. olivacea-complex
95/99
G. cainii AY744548
G. grandifoliae AF343711
92/*
CMW 36630 RUSSIA T
L. taigense sp. nov.
78/*
G. dryocoetidis AJ538340 T
L. curvisporum EU979328 T
100/100
78/
80
G. abiocarpa AJ538339 T
L. fruticetum DQ097847 T
75/84
L. eucalyptophilum AF343703 T
100
L. abieticolens AF343701 T
100
L. bistatum AY348305 T
L. pistaciae HQ406846 T
L. hughesii AF343700
G. penicillata-complex
L. chlamydatum EU979333 T
91/66
CMW 36631 FINLAND
L. altius HQ406851 T
96/84/BI 97
L. curviconidium HQ406850 T
G. penicillata DQ097851 T
G. americana DQ062079 T
87/*/BI 90
L. abietinum AF343670
89/86
L. alethinum AF343685 T
97/95
L. euphyes AF343686
G. leptographioides AF343710
Grosmannia
O. ips AY172021 KUC2120
100/100
O. piliferum AF221070
O. karelicum EU443762 T
*/96
O. novo-ulmi DQ294375
Ophiostoma
0.01

during the course of this study (Tables 2, 3). All of these bark beetles were associated with species of *Grosmannia* and/or *Leptographium*. The majority of the bark beetle species infested both pine and spruce. The exceptions were *I. typographus* and an unidentified *Ips* sp. found only on spruce, while *Ips sexdentatus* Boern., *Orthotomicus suturalis* Gyll., *Tomicus minor* L. and *T. piniperda* L. were encountered only on pine. Species that were collected only in Finland included *Hylastes brunneus* Er. and *O. suturalis*, while *I. sexdentatus* and unidentified *Ips* and *Pityogenes* species were found only at Russian collection sites. The isolations from bark beetles and their galleries yielded a total of 263 fungal isolates (Table 3).

DNA sequence analyses

Amplification resulted in fragments of approximately 700 bp for the ITS2 and 28S region, 500 bp for the partial β-tubulin gene, and 900 bp for the partial EF 1-α gene. Phylogenetic analyses were done separately for each gene region. In most cases, the ITS2 and 28S data did not distinguish clearly between closely related species, but was useful to assign isolates to species complexes within *Grosmannia* (Fig. 1). The partial β-tubulin and EF 1-α genes were used to identify isolates to species level. Due to differences in the presence or absence of introns between species complexes in *Grosmannia* (Zipfel et al. 2006), both β-tubulin and EF 1-α datasets for the different complexes were analyzed separately from each other (Figs. 2, 3, 4, 5, 6).

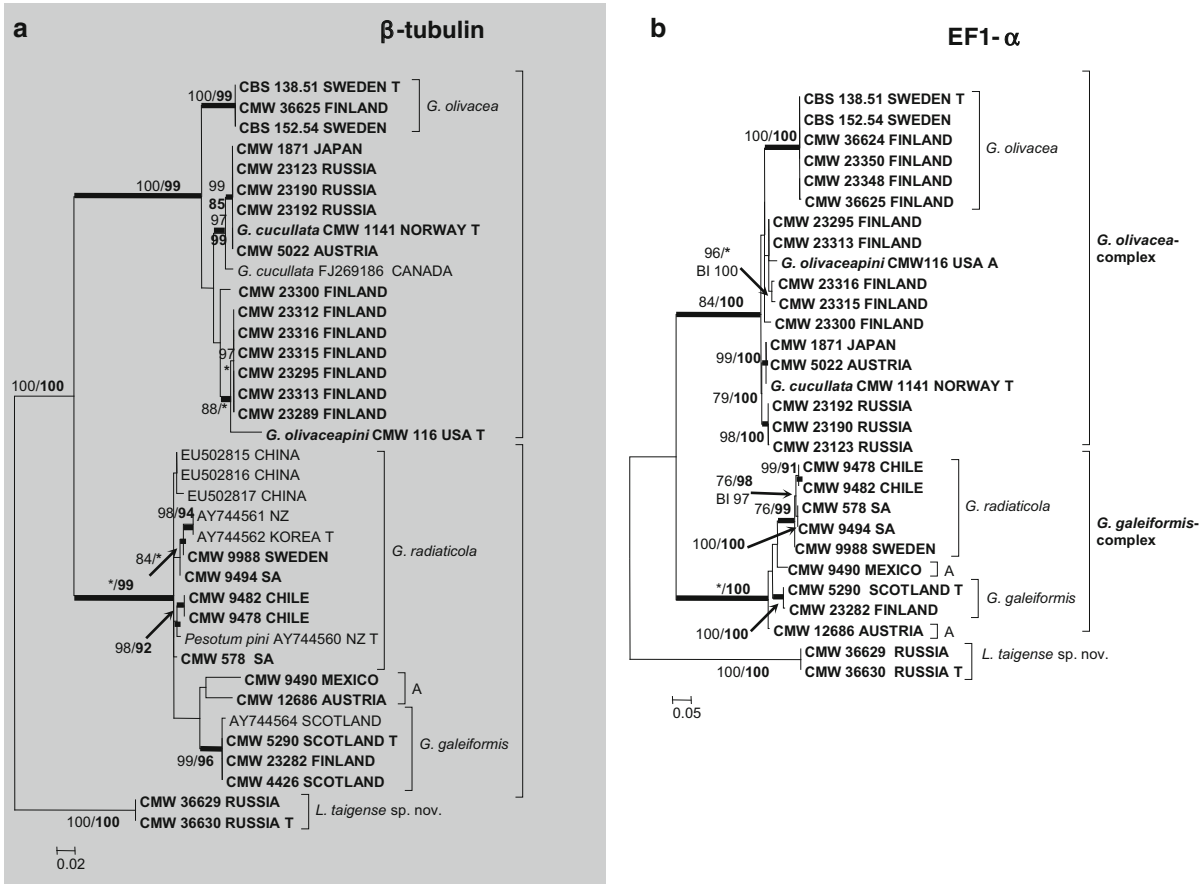


Fig. 2 Phylogram obtained from ML analyses of **a** the partial β-tubulin gene and **b** the partial EF 1-α gene of the *G. olivacea*- and *G. galeiformis*-complexes. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75 %. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa

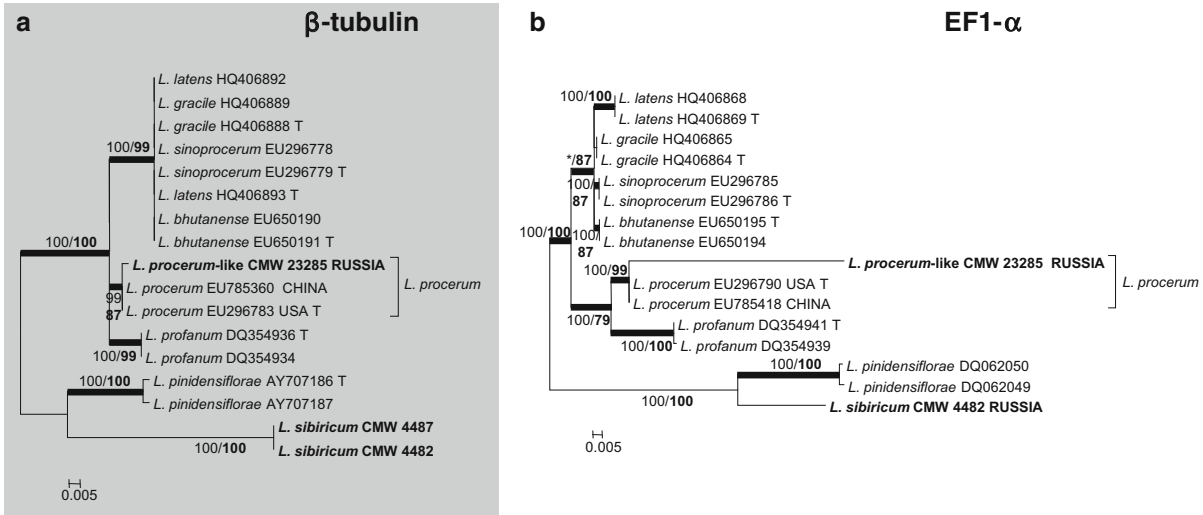


Fig. 3 Phylogram obtained from ML analyses of **a** the partial *β*-tubulin gene and **b** the partial EF 1-*α* gene of the *L. procerum*-complex. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75 %. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa

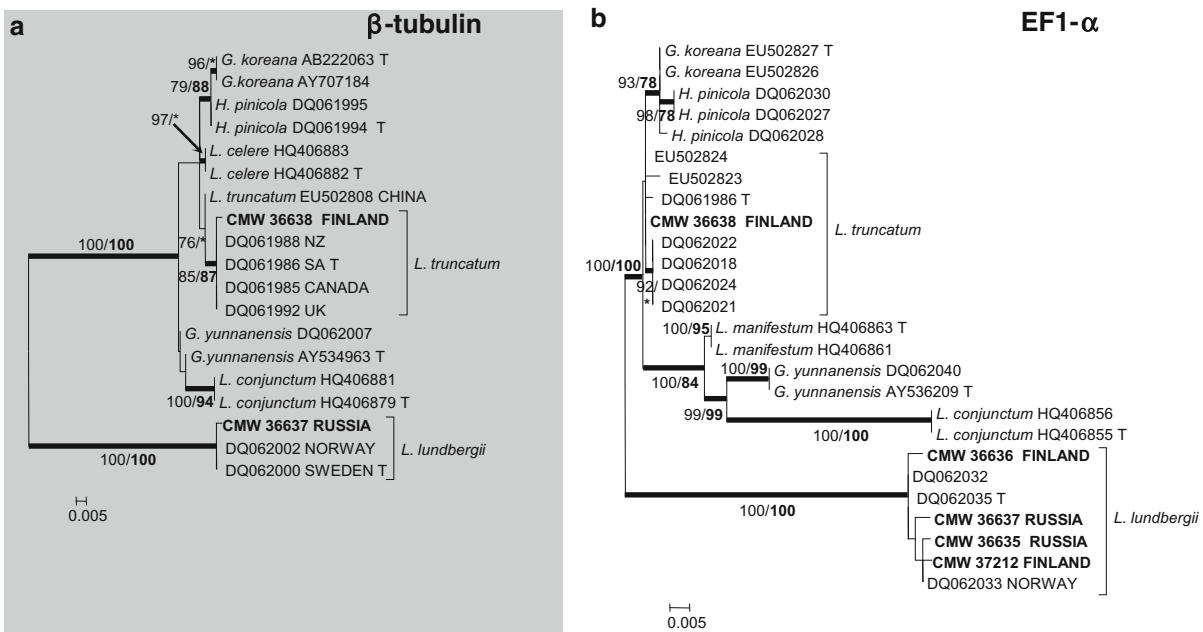


Fig. 4 Phylogram obtained from ML analyses of **a** the partial *β*-tubulin gene and **b** the partial EF 1-*α* gene of the *L. lundbergii*-complex. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75 %. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa

Aligned DNA sequences for the ITS2 and 28S region yielded 640 characters, including gaps (Fig. 1). Alignments of the five *β*-tubulin subsets of sequence data consisted respectively of 381, 375, 373, 366 and 465 characters, including gaps (Figs. 2, 3, 4, 5, 6). Alignments of the five EF 1-*α* subsets consisted

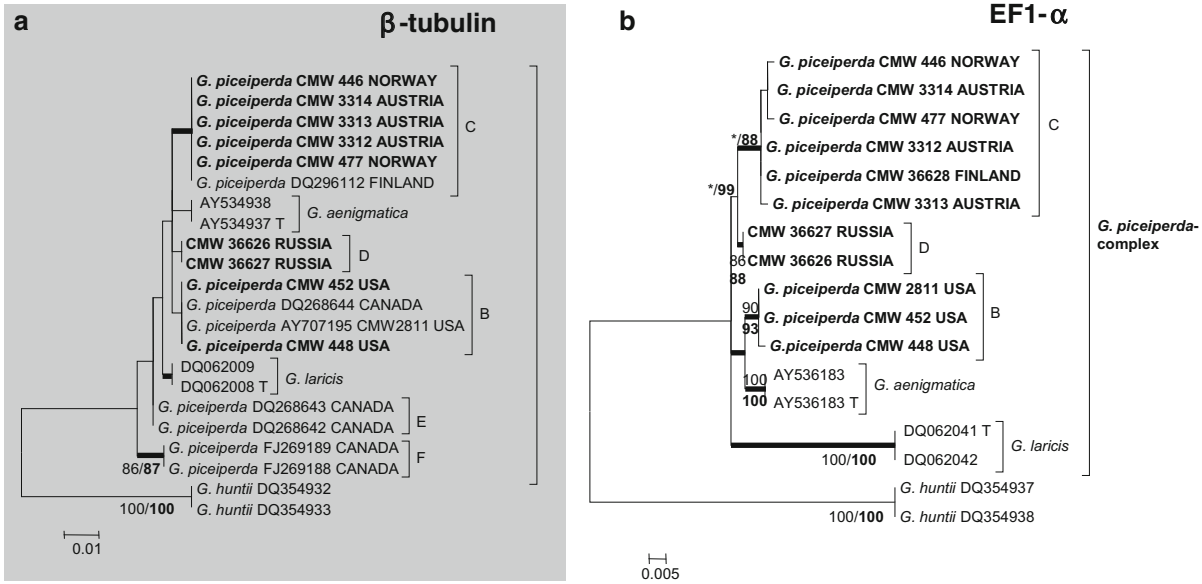


Fig. 5 Phylogram obtained from ML analyses of **a** the partial *β*-tubulin gene and **b** the partial EF 1-α gene of the *G. piceiperda*-complex. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75 %. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa

respectively of 722, 790, 583, 608 and 875 characters, including gaps (Figs. 2, 3, 4, 5, 6). The Bayesian analyses for the ITS2 and 28S region, partial *β*-tubulin and EF 1-α genes produced trees with topologies similar to those of the ML and MP analyses. The best fitting substitution models selected for Bayesian analyses were GTR+I+G (Figs. 1, 2a, 3, 4, 5, 6) and GTR+G (Fig. 2b).

The ITS2 and 28S sequences were used to show the placement of the isolates within *Grosmannia* (Fig. 1). Comparisons of ITS2 and 28S sequences obtained for isolates in this study, with sequences from GenBank and the reference isolates, showed that our isolates resided in six species complexes. These were the *G. galeiformis*-, *L. procerum*-, *L. lundbergii*-, *G. piceiperda*-, *G. olivacea*- and *G. penicillata*-complexes. Isolates in these complexes could not be distinguished from closely related species in the ITS2 and 28S tree. However, some Russian isolates formed a well-resolved lineage closest to the *G. penicillata*-complex, but distinct from any known species for which sequences were available.

Analyses of the *β*-tubulin and EF 1-α data for isolates in the *G. olivacea*- and *G. galeiformis*-complexes (Fig. 2) revealed more variation than was present in the ITS tree. Isolates from the survey resided in four lineages that included reference sequences of known species. The ex-type isolate of *G. olivacea* (Math.-Käärik) Zipfel, Z.W. de Beer & M.J. Wingf. and another isolate from Sweden, had sequences for both gene regions identical to those of some of Finland isolates (Fig. 2). In the *β*-tubulin tree (Fig. 2a), some of the Russian isolates grouped in a well-supported lineage that included the ex-type isolate of *G. cucullata* (H. Solheim) Zipfel, Z.W. de Beer & M.J. Wingf., but in the EF 1-α tree (Fig. 2b), the Russian isolates formed a distinct lineage adjacent to that of the *G. cucullata* isolates. A third group of isolates grouped with an authentic isolate of *Ophiostoma olivaceapini* (R.W. Davidson) Seifert & G. Okada in the *β*-tubulin analyses (Fig. 2b), but differed in several bp from that isolate. These isolates also grouped together in EF 1-α (Fig. 2b), but showed more variability among isolates and had no branch support.

One of the Finnish isolates had identical sequences to the type specimen of *G. galeiformis* (B.K. Bakshi) Zipfel, Z.W. de Beer and M.J. Wingf. from Scotland (Fig. 2). However, the isolates from Mexico and Austria (labelled as a in Fig. 2) previously treated as *G. galeiformis* based on ITS (Zhou et al. 2004c),

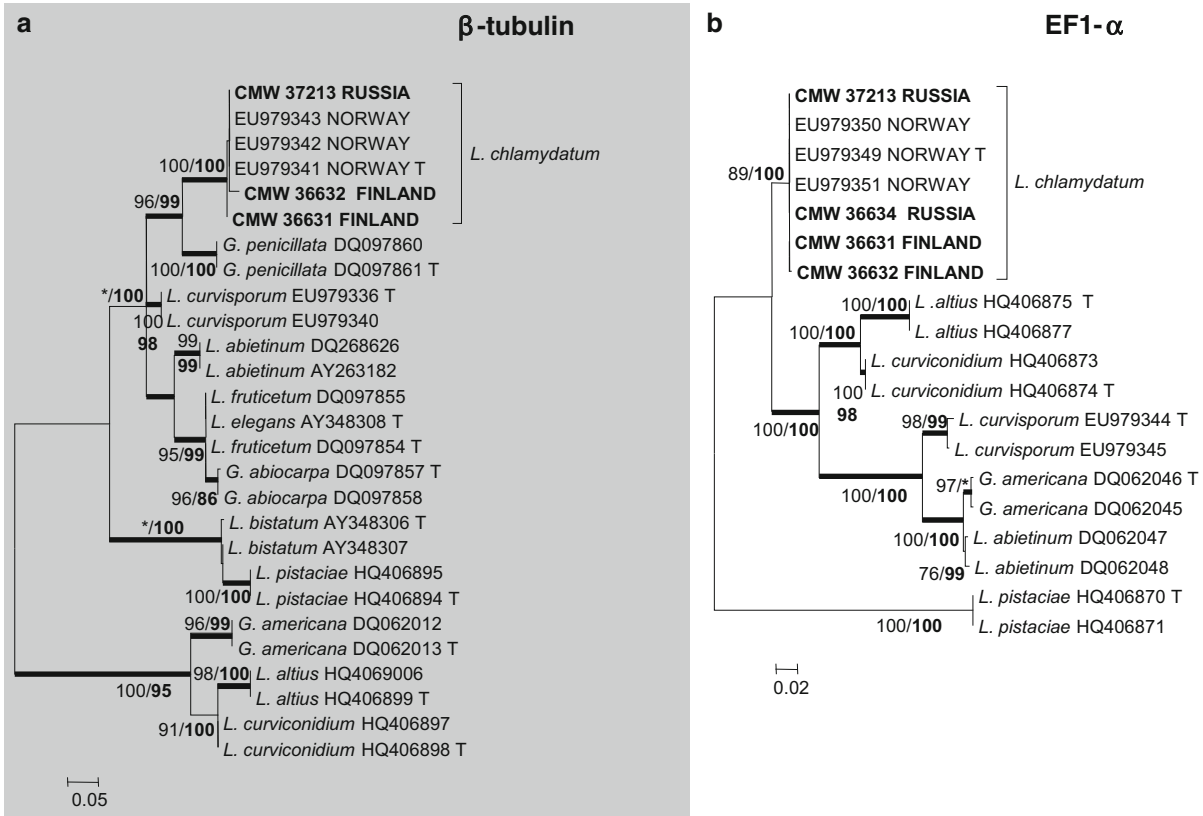


Fig. 6 Phylogram obtained from ML analyses of **a** the partial *β*-tubulin gene and **b** the partial EF 1-*α* gene of the *G. penicillata*-complex. Novel sequences obtained in this study are printed in *bold* type. ML bootstrap support values (1,000) replicates (normal type) and MP Jackknife values (10,000 replicates) (*bold* type) above 75 % are indicated at the nodes. Posterior probabilities (above 90 %) obtained from BI are indicated by *bold lines* at the relevant branching points. *Bootstrap values lower than 75%. *T* ex-type isolates. *Scale bar* total nucleotide difference between taxa

grouped as distinct from the ex-type of the species, and possibly represent undescribed taxa. Isolates from South Africa, Sweden and Chile, also previously considered as *G. galeiformis* (Zhou et al. 2004c), grouped with the ex-type and other isolates of *G. radiaticola* (J.J. Kim, Seifert & G.H. Kim) Zipfel, Z.W. de Beer & M.J. Wingf. The Russian isolates that grouped outside the *G. penicillata*-complex in ITS2-28S (Fig. 1), were analysed with the *G. olivacea*- or *G. galeiformis*-complexes, but were very distinct from all the species complexes treated, forming a strongly supported lineage distinct from all other species.

One isolate collected in this study resided in the *L. procerum*-complex (Fig. 3). Comparisons of the sequences for the partial *β*-tubulin gene (Fig. 3a) showed that this single isolate grouped in a well-supported lineage with *L. procerum* (W.B. Kendr.) M.J. Wingf. However, the EF 1-*α* gene region showed more variation, and this isolate could not be identified with certainty (Fig. 3b). The *L. procerum*-complex also included sequences for isolates representing *L. bhutanense* X.D. Zhou, K. Jacobs & M.J. Wingf., *L. gracile* Paciura, Z.W. de Beer & M.J. Wingf., *L. latens* Paciura, Z.W. de Beer & M.J. Wingf., *L. pini-densiflorae* Masuya & M.J. Wingf., *L. profanum* K. Jacobs, Eckhardt & M.J. Wingf., *L. sibiricum* K. Jacobs & M.J. Wingf., & *L. sinoprocerum* Quan Lu, Decock & Maraite.

Analyses of the *β*-tubulin and EF 1-*α* data for isolates in the *L. lundbergii*-complex confirmed the identities of two known species amongst the isolates obtained in this study (Fig. 4). These included *L. truncatum* (M.J. Wingf. & Marasas) M.J. Wingf. & *L. lundbergii* Lagerb. & Melin. Based on the *β*-tubulin data, the isolates representing *L. truncatum* and *L. lundbergii* grouped in two lineages with good

Table 3 Number of *Grosmannia* and *Leptographium* isolates obtained from 12 bark beetle species and their galleries during the course of this study

	Finland																		
	Picea abies (spruce)																		
			Pinus sylvestris (pine)																
Beetle species →	1		3		4		5		6		8		10		11		12		Total
Fungus species ↓	b	g	b	g	b	g	b	g	b	g	b	g	b	g	b	g	b	g	
G. cucullata/G. olivaceapini	4	0	37	7	14	3	14	0	0	0	15	0	4	0	1	0	2	0	99
G. galeiformis	0	0	0	0	0	2	0	1	0	0	0	5	0	0	0	0	0	0	8
G. olivacea	9	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26
G. piceiperda-complex	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
L. taigense sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. chlamydatum	8	0	2	0	1	0	5	0	18	0	0	0	0	0	0	0	1	0	34
L. lundbergii	0	0	4	0	6	0	5	0	1	0	0	0	0	0	0	0	0	0	16
L. procerum-like	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. truncatum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total isolates	21	17	44	7	21	5	24	1	19	1	15	5	4	0	1	0	3	0	188

	Russia																
	Picea abies (spruce)																
					Pinus sylvestris (pine)												
Beetle species →	1		2		3		5		6		7		9		11		Total
Fungus species ↓	b	g	b	g	b	g	b	g	b	g	b	g	b	g	b	g	
G. cucullata/G. olivaceapini	4	6	0	0	4	1	0	0	0	0	0	1	0	0	1	0	19
G. galeiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G. olivacea	2	0	6	4	1	0	0	0	0	0	0	0	1	0	0	0	14
G. piceiperda-complex	4	0	0	0	0	0	0	3	1	1	0	0	0	0	0	0	9
L. taigense sp. nov.	3	0	0	0	1	1	1	1	4	2	0	1	0	0	0	0	14
L. chlamydatum	1	1	0	0	1	0	0	0	2	9	0	0	0	0	0	0	15
L. lundbergii	0	0	0	0	1	0	3	0	2	0	0	0	0	0	0	0	6
L. procerum-like	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
L. truncatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total isolates	14	7	6	4	8	2	5	4	9	12	0	2	1	0	1	0	75

Bark beetle species: *1 Ips typographus*; *2 Ips* sp.; *3 Dryocoetes autographus*; *4 Hylastes brunneus*; *5 Hylurgops palliatus*; *6 Pityogenes chalcographus*; *7 Pityogenes* sp.; *8 Trypodendron lineatum*; *9 Ips sexdentatus*; *10 Tomicus piniperda*; *11 Tomicus minor*; *12 Orthotomicus suturalis*

b beetles, *g* galleries

statistical support (Fig. 4a). However, in the EF 1-α tree (Fig. 4b) the monophyly of *L. truncatum* was not supported. Other species included in the complex were *G. koreana* J.J. Kim & G.H. Kim, *G. yunnanensis* Yamaoka, Masuya & M.J. Wingf., *L. celere* Paciura, Z.W. de Beer & M.J. Wingf., *L. conjunctum* Paciura, Z.W. de Beer & M.J. Wingf., *L. manifestum* Paciura, Z.W. de Beer & M.J. Wingf., and *Hyalorhinocladiella pinicola* K. Jacobs & M.J. Wingf.

Comparisons of the β-tubulin and EF 1-α sequences for isolates in the *G. piceiperda*-complex (Fig. 5) revealed that isolates labelled as *G. piceiperda* (Rumbold) Goid. from GenBank and our reference collection formed four distinct lineages (B, C, E, F).

Three of these (B, E, F) included only North American isolates, while isolates from Finland, Norway and Austria formed a distinct lineage (C), and those from Russia another lineage (D). Isolates of *G. aenigmatica* (K. Jacobs, M.J. Wingf. & Yamaoka) Zipfel, Z.W. de Beer & M.J. Wingf. & *G. laricis* (K. van der Westhuizen, Yamaoka & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf., also formed distinct lineages in the complex. All isolates in these five lineages originated from *Picea*, except one isolate (CMW 452) that came from *Pseudotsuga* Carr. in the USA.

Based on the analyses of the three gene regions (Figs. 1, 6), the following species were shown to form part of the *G. penicillata*-complex: *G. penicillata* (Grosmann) Goid., *G. abiocarpa* (R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., *G. americana* (K. Jacobs & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf., *L. abietinum* (Peck) M.J. Wingf., *L. altius* Paciura, Z.W. de Beer & M.J. Wingf., *L. bistatum* J.J. Kim & G.H. Kim, *L. curviconidium* Paciura, Z.W. de Beer & M.J. Wingf., *L. curvisporum* K. Jacobs, M.J. Wingf. & H. Solheim, *L. chlamydatum* K. Jacobs, M.J. Wingf. & H. Solheim, *L. fruticetum* Alamouti, J.J. Kim & C. Breuil, and *L. pistaciae* Paciura, Z.W. de Beer & M.J. Wingf. In the β-tubulin trees (Fig. 6a), *L. elegans* M.J. Wingf., Crous & Tzean also grouped in the complex, but based on ITS2-LSU it grouped outside this complex (data not shown). All the Russian and Finnish isolates that formed part of this species complex grouped with *L. chlamydatum* (Fig. 6).

Taxonomy

DNA sequences produced in the present study confirmed that one group of isolates obtained from Russia represented an undescribed taxon that is described below. Sequence data for authentic isolates of *O. olivaceapini* and the ex-type isolate of *H. pinicola* confirmed that these two species have been treated in inappropriate genera. New combinations are provided for these species.

Leptographium taigense Linnakoski, Z.W. de Beer & M.J. Wingf. **sp. nov**. (Fig. 7). MB 564881

Etymology. The epithet *taigense* refers to taiga, also known as the boreal forests and the habitat in which this species was found.

Teleomorph unknown. Synnematous macronematal conidiogenous structures predominant in culture (Fig. 7a–d). Conidiophores single or in groups, dark brown, (120–)287–566(–681) µm long including hyaline capitulum, (24–)37–102(–142) µm wide at base; conidiogenous cells (13–)17–22(–25) × 1–1.5 µm (Fig. 7b); conidia hyaline, aseptate, oblong, (2–)2.5–3(–3.5) × 1–1.5 µm (Fig. 7b–d), aggregating into a mucilaginous spore drop. Rhizoid-like structures present. Mononematous leptographium-like synanamorph present (Fig. 7f), but observed less frequently in culture. Mononematous conidiophores (Fig. 7e) typically soon aggregate to form synnematous structures. Conidiophores with up to 9 septa, (39–)112–223(–257) µm in length, rhizoid-like structures occasionally present. Stipes hyaline to light olivaceous, cylindrical, (18–)66–173(–232) µm long and (1.5–)2–2.5(–3) µm wide, apical cell not swollen, basal cell not swollen. Conidiogenous apparatus (22–)37–65(–94) µm long, excluding the conidial mass, with multiple series of cylindrical branches. Primary branches 2–3, cylindrical, 0–1 septate, (6–)7–20(–37) µm long and (1–)1.5–2.5(–3) µm wide. Secondary branches occasionally swollen, (5–)8–12(–16) µm long and (1–)1.5–3.5(–4.5) µm wide. Tertiary branches sometimes observed, typically swollen, (7.5–)8–11(–12.5) µm long and 3–4(–5) µm wide. Conidiogenous cells discrete, 2–7 per branch, cylindrical, tapering slightly at the apex, (10–)14–22(–28) µm long and 1–1.5(–2) µm wide. Conidia hyaline, oblong, with rounded apices and truncate bases, (2–)2.5–3.5(–4) × 1–1.5(–2) µm; accumulating in hyaline, slimy droplets at the apex of conidiogenous apparatus.

Colonies at first hyaline, later become light brown where synnemata form (Fig. 7g). Mycelium superficial on the agar, small tufts of white aerial mycelium present. Synnematous anamorph dominant in cultures. Optimal temperature for growth 25 °C, no growth observed at 5 and 35 °C. The mean radial growth rate at optimal temperature 5.5 (±0.3) mm/d.

Host range: associated with *Dryocoetes autographus* Ratz., *I. typographus*, *Pityogenes chalcographus* L. and *Pityogenes* sp. on spruce, and *Hylurgops palliatus* Gyll. on pine and spruce. *Distribution*: Presently known from Kivennapa, Lisino-Corpus, and Nurmoila, Russia.

Specimens examined: RUSSIA, Lisino-Corpus, isolated from *I. typographus* infesting *P. abies*, Feb. 2006, R. Linnakoski, holotype KUO 022077, living culture CBS 128927, CMW 36630; RUSSIA, Lisino-Corpus, isolated from *H. palliatus* infesting

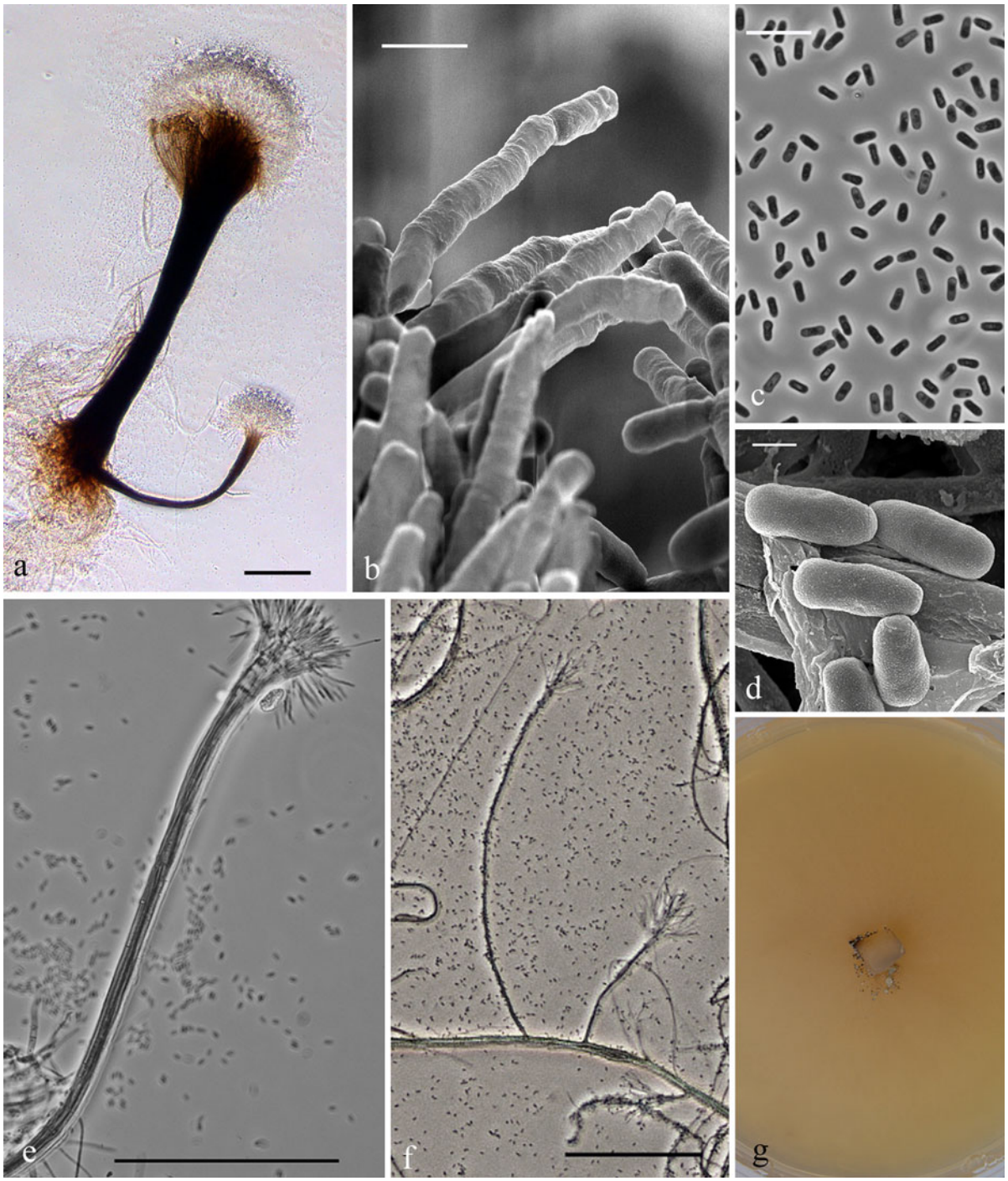


Fig. 7 Morphological characters of *Leptographium taigense* (ex-type isolate) anamorph structures. **a** synnematous anamorph; **b** scanning electron micrograph (SEM) of conidiogenous cells of synnematous anamorph; **c** conidia; **d** SEM of conidia; **e** aggregated conidiophores of leptographium-like anamorph; **f** mononematous conidiophores; **g** fourteen day old culture on MEA. *Scale bars*: **a**, **e**, **f** = 100 μm; **b**, **d** = 2 μm, **c** = 10 μm

P. sylvestris, Feb. 2006, R. Linnakoski, paratype KUO 022078, living culture CBS 128926, CMW 36629.

Note: Attempts to obtain sexual structures for this taxon using mating studies were not successful. The asexual structures superficially resemble pesotum-like synnematous structures. Previously, synnematous anamorphs of *Grosmannia* have been treated in *Pesotum* (Okada et al. 1998; Kim et al. 2005; Zipfel et al. 2006). However, the type species for *Pesotum* is the anamorph of *O. ulmi* (Buisman) Nannf., which forms part of the genus *Ophiostoma* (Harrington et al. 2001; Zipfel et al. 2006). Thus, under emended Code (see discussion) *Pesotum* will be treated as synonym of *Ophiostoma* and is not available for species in other phylogenetically defined genera. Harrington et al. (2001) suggested the use of *Phialographium* for synnematous anamorphs of *Grosmannia*, but the type species for *Phialographium*, *G. sagmatospora* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf., groups in the *G. olivacea* complex, and we would prefer not to apply that genus name at present outside of that complex. On closer inspection it is clear that the synnemata of *L. taigense* are aggregations of typical leptographium-like conidiomata (Fig. 7e). For this reason, and based on phylogenetic inference, this species is treated in *Leptographium.*

Grosmannia olivaceapini (R.W. Davidson) Z.W. de Beer, Linnakoski & M.J. Wingf. **comb. nov**. MB 564882

≡ *Ceratocystis olivaceapini* R.W. Davidson, Mycologia 63: 7. 1971.

≡ *Ophiostoma olivaceapini* (R.W. Davidson) Seifert & G. Okada, in Okada et al., Can. J. Bot. 76: 1504. 1998.

Note: DNA sequences produced for an authentic isolate of this species (CBS 504.86) in the present study, as well as some unpublished ITS sequences (AJ538336) produced earlier by Villarreal et al. (2005) for another authentic isolate (MUCL 18368), confirmed its placement in the genus *Grosmannia*. The sheathed ascospores and synnematous anamorph of this species (Davidson 1971; Upadhyay 1981; Mouton et al. 1992) closely resemble those of species such as *G. olivacea* (Mathiesen 1951), *G. sagmatospora* (Wright and Cain 1961), *G. davidsonii* (Olchowecki and Reid 1974) and *G. cucullata* (Solheim 1986), supporting the new combination provided here.

Leptographium pinicola (K. Jacobs & M.J. Wingf.) Z.W. de Beer, Linnakoski & M.J. Wingf. **comb. nov**. MB 564883

≡ *Hyalorhinocladiella pinicola* K. Jacobs & M.J. Wingf., Mycol. Res. 109: 1157. 2005.

Note: This species was described in the genus *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. based on its sparse conidiogenous apparatuses (Jacobs et al. 2005). However, the type species of *Hyalorhinocladiella*, *H. minuta-bicolor* (R.W. Davidson) H.P. Upadhyay & W.B. Kendr., is the anamorph of *Ceratocystiopsis minuta-bicolor* (R.W. Davidson) H.P. Upadhyay, which groups in *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. (Zipfel et al. 2006). Under the emended Code (see discussion) *Hyalorhinocladiella* will thus be treated as synonym of *Ceratocystiopsis*. Our phylogenetic analyses confirmed that *H. pinicola* forms part of the *L. lundbergii*-complex. The conidiogenous structures of *H. pinicola* (Jacobs et al. 2005) should be interpreted as reduced or degenerative *Leptographium* structures. Furthermore, their conidia resemble those of species such as *L. truncatum* (Jacobs et al. 2005), rather than those of *Ceratocystiopsis* anamorphs. It is thus inappropriate to treat *H. pinicola* in *Hyalorhinocladiella*, despite superficial morphological resemblances, while it clearly belongs in *Leptographium*. *Leptographium pinicolum* should not to be confused with *Ceratocystis pinicola* T.C. Harr. & M.J. Wingf. (Harrington and Wingfield 1998), nor *Ophiostoma pinicola* G.H. Zhao [nom. inval. Art. 36.1, 37.1 & 37.5 ICBN] (Zhao 2005).

Discussion

Ten species of *Grosmannia* and *Leptographium* were detected in this study. They were identified amongst a total of 263 isolates found in association with 12 different bark beetle species, infesting *P. abies* and *P. sylvestris* in the eastern parts of Finland and the Karelia region of Russia. All isolates belonged to one of ten species residing in the following six species complexes in *Grosmannia*, which have been redefined based on DNA sequence data: the *G. galeiformis*-, *L. procerum*-, *L. lundbergii*-, *G. piceiperda*-, *G. olivacea*- and *G. penicillata*-complexes. The species included *G. galeiformis*, *G. olivacea*, *L. chlamydatum*,

L. lundbergii, *L. truncatum* and the novel species described here as *L. taigense*. In addition, isolates closely related to *L. cucullata*, *G. olivaceapini*, *G. piceiperda* and *L. procerum* were found, but these grouped in complexes where all species could not be delineated with certainty. The most commonly encountered species in this study were *L. chlamydatum*, *G. olivacea* and those in the *G. cucullata*-*G. olivaceapini* group. The fungi discovered in this study are all species or relatives of species that have previously been found in Europe (Hallaksela 1977; Viiri 1997; Kirisits 2004; Jacobs et al. 2010).

The definition of species complexes in the genera *Grosmannia* and *Leptographium* in previous studies was obscured due to the lack of teleomorph structures in many species, overlapping morphological features of their anamorphs, and the exclusion of species producing synnematous anamorphs. Analyses of DNA sequence data representing most species in these genera provided the opportunity, not only to identify the species collected, but also to more accurately delineate them and to highlight taxonomic problems in some of these species complexes. The species obtained in this study are thus discussed and interpreted within the context of the species complexes to which they belong. The species complexes have consistently been named based on the oldest known species in each complex.

In view of the recent changes to the International Code of Nomenclature for algae, fungi and plants (ICN) that suggest the discontinuation of the dual nomenclature system for pleomorphic fungi (Hawksworth 2011; Hawksworth et al. 2011; Wingfield et al. 2012), it is necessary to explain the approach followed for naming taxa in the present study. The changes to the Code imply that *Leptographium* should have priority over *Grosmannia* as the older of the two names. However, analyses of ribosomal data in this (Fig. 1) and other recent studies (Harrington et al. 2010; Six et al. 2011; Duong et al. 2012) suggest that *Leptographium* is not a well-supported, monophyletic genus, as had previously been suggested (Zipfel et al. 2006; Massoumi Alamouti et al. 2009). The type species for *Leptographium* forms part of the *L. lundbergii*-complex, while *G. penicillata*, the type species for *Grosmannia*, together with several other species, forms a distinct lineage. Presently available data are not adequate to determine whether these lineages represent distinct genera or not. A comprehensive multigene study including species representing all genera in the Ophiostomatales will be required to confirm or reject the monophyly of these genera. For the purposes of the present study, we have retained the *status quo*, referring to asexually reproducing species as *Leptographium* and to sexually reproducing species as *Grosmannia* (Zipfel et al. 2006). However, our concept of *Leptographium* is based on phylogenetic relatedness, rather than on morphology alone, which means we accept a wider spectrum of morphological types. On one side of this spectrum are species with reduced hyalorhinocladiella-like structures (e.g. *L. pinicolum*). Forming the centre of the spectrum are the majority of species with typical leptographium-like anamorphs, while on the other side of the spectrum we include synnematous aggregates of leptographium-like structures. Examples of the latter include *L. taigense* and the anamorph of *G. clavigera* (R.C. Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf., which had been treated as *L. clavigerum* by Six et al. (2003) and as *P. clavigerum* (H.P. Upadhyay) G. Okada & Seifert by Okada et al. (1998).

The *G. olivacea*-complex

A relatively common species found in the survey had sequences identical to the ex-type isolate of *G. olivacea* (Figs. 1, 2). This species is characterized by a synnematous anamorph (Mathiesen 1951), and has thus been excluded from earlier treatments of the genus *Leptographium* (Jacobs et al. 2001; Jacobs and Wingfield 2001). Kim et al. (2005) showed that it grouped amongst *Leptographium* species and Zipfel et al. (2006) transferred it to *Grosmannia*. Massoumi Alamouti et al. (2007) and Six et al. (2011) showed a close relationship between *G. olivacea, G. cucullata* and *G. olivaceapini*. Not surprisingly, all the species grouping with *G. olivacea* based on our analyses (Figs. 1, 2) have similar teleomorphs and synnematous anamorphs: *G. sagmatospora* (Wright and Cain 1961), *G. olivaceapini* (Davidson 1971), *G. davidsonii* (Olchowecki and Reid 1974), and *G. cucullata* (Solheim 1986).

G. olivacea was originally described from blue-green stained pine in association with the cerambycid beetle, *Acanthocinus aedilis* L., in Sweden (Mathiesen 1951). In this study, the fungus was mainly isolated from an *Ips* sp., with a single isolate from *D. autographus*, which suggest that the fungus does not have a

specific association with a single beetle species. *G. olivacea* has also been reported from *Dendroctonus rufipennis* (Kirby) (Hinds and Buffam 1971; Ohsawa et al. 2000) and *Polygraphus rufipennis* (Kirby) (Ohsawa et al. 2000) infesting spruce in North America, but these reports should be considered with caution since the identifications were based solely on morphology.

By far the largest group of isolates collected in this study and from both Finland and Russia clustered with isolates of *G. cucullata* and *G. olivaceapini* (Fig. 2). Some Russian isolates in this study grouped with *G. cucullata* from Norway, Austria and Japan. An unpublished β-tubulin sequence from GenBank, produced by Bernier et al. (2004) for an isolate from *Dryocoetes affaber* (Mann.) in Canada labeled as *G. cucullata*, grouped close to, but distinct from the ex-type isolate of *G. cucullata* (Fig. 2a). The ex-type isolate of *G. olivaceapini* grouped close to some of our Finnish isolates (Fig. 2), but differed slightly in both β-tubulin and EF 1-α sequences. We were thus not able to assign these isolates to either of the two species with certainty, because the delineation of the species was not optimal. These results suggest that *G. cucullata* and *G. olivaceapini* possibly represent a complex of several cryptic species that should be explored further, including a greater number of isolates and using additional gene regions in the analyses.

G. cucullata was originally described from *I. typographus* infesting spruce in Norway (Solheim 1986), and has subsequently been reported from several bark beetle species in Europe (Kirisits 2004; Jankowiak et al. 2009) as well as from *I. typographus* f. *japonicus* Niij. in Japan (Yamaoka et al. 1997). Apart from the Canadian isolate mentioned in the previous paragraph that could represent a distinct taxon; this species has never been reported form North America. The species thus appears to be associated primarily with insects infesting spruce in Europe.

G. olivaceapini was initially described from *Dendroctonus* and other beetle species on pine in the USA (Davidson 1971), but has to the best of our knowledge not been reported again. In this study, isolates related to these two species were most frequently isolated in association with *D. autographus*, but also from eight other bark beetle species infesting both pine and spruce. Based on currently available data, it appears that the species in this complex are not associates of specific beetle vectors, but beetle-specificity might be revealed once the status of the cryptic species has been resolved.

The *G. galeiformis*-complex

It was not surprising to isolate *G. galeiformis* in this study, as this is a relatively well-known species in Scandinavia and other parts of Europe (Bakshi 1951; Mathiesen-Käärik 1953; Zhou et al. 2004c). Similar to species in the *G. olivacea*-complex, *G. galeiformis* produces synnematous anamorphs and it was not until DNA sequence data became available that its generic placement among *Leptographium* species was revealed (Zhou et al. 2004c; Kim et al. 2005). The second species in the complex, *G. radiaticola*, was described by Kim et al. (2005) as the teleomorph for *Hyalopesotum pini* L.J. Hutchison & J. Reid. Zipfel et al. (2006) subsequently transferred *G. galeiformis* and *G. radiaticola* to the genus *Grosmannia*. Unlike the more tightly structured synnemata of species in the *G. olivacea*-complex, the synnemata of *G. galeiformis* and *G. radiaticola* appear to be loose aggregates of *Leptographium*-like conidiophores.

Only a few isolates of *G. galeiformis* were collected in Finland in this study and they had sequences identical to the epitype for *G. galeiformis* from Scotland (Fig. 2) that was designated by Zhou et al. (2004c). Reference isolates from Austria and Mexico (group A, Fig. 2) did not group with the epitype, nor with *G. radiaticola*, and might represent distinct species. Similarly, ITS sequences of isolates from the USA (Zhou et al. 2004c) grouped apart from either of the two known species (data not shown). One of these (C527) from *P. taeda* L. in Georgia, USA is identical to an unpublished sequence from *Dendroctonus adjunctus* Bland. in Mexico (HM236501).

G. radiaticola isolates, including the ex-type from Korea and some from New Zealand, grouped distinct from *G. galeiformis* (Fig. 2), supporting the separation of the two species by Kim et al. (2005). Several isolates previously treated as *G. galeiformis*, grouped amongst the *G. radiaticola* isolates. These include isolates from South Africa, Chile and Sweden (Fig. 2) from the studies of Zhou et al. (2004b, c). The ITS sequence of an isolate recently obtained from *Hylurgus ligniperda* (Fabr.) infesting *P. halepensis* L. and *P. pinea* L. in California (Kim et al. 2011), and an unpublished ITS sequence from *P. radiata* L. in Spain

(AJ538334), both labelled as *G. galeiformis,* also grouped with *G. radiaticola*.

Three isolates from China, identified as *H. pini* by Lu et al. (2009b), grouped in a sub-clade of their own among the *G. radiaticola* isolates (Fig. 2). Based on ITS sequences, Thwaites et al. (2005) treated *G. radiaticola* as a synonym of *G. galeiformis,* with *H. pini* as its anamorph. However, our results concur with those of Kim et al. (2005) separating *G. galeiformis* and *G. radiaticola,* with *H. pini* as the anamorph of the latter species. We consider the Chinese isolates of Lu et al. (2009b) to represent *G. radiaticola*, but recognize that they could emerge as another cryptic species in the complex.

Given the confused taxonomy in the *G. galeiformis*-complex, interpretation of associations with bark beetles should be made with care. Based on the results this study, the only definitive reports of *G. galeiformis* are those from Scotland, associated with *T. piniperda* on *P. sylvestris* (Zhou et al. 2004c), and from Finland in association with *H. brunneus* and *Trypodendron lineatum* Ol. infesting pine and spruce, and *H. palliatus* from pine. The first report of *G. galeiformis* from Scotland, as an associate of *H. palliatus* and *D. autographus* infesting *Larix kaempferi* (Lamb.) Carr. (Bakshi 1951) remains to be confirmed using DNA sequence data.

Analyses in this study also showed that *G. radiaticola* is associated with a diversity of different beetle species in different countries and continents. Isolates originated from *Hylastes cunicularius* Er. in Sweden (Mathiesen-Käärik 1953), *Hylastes ater* Payk. and *H. ligniperda* on pines in Chile (Zhou et al. 2004a), *H. ligniperda* in South Africa (Zhou et al. 2001) and California (Kim et al. 2011), stained *P. radiata* in Spain, Korea and NZ (Kim et al. 2005; Thwaites et al. 2005), and possibly *D. valens* Le Conte on *P. tabuliformis* Carr. in China (Lu et al. 2009b). These occurrences of the fungus clearly reflect the global movement of this fungus and its vectors, most likely through the timber and wood products.

The isolates of uncertain identity from Austria were from *H. cunicularius* on *P. abies*, those from Mexico from *D. mexicanus* Hopkins infesting *P. pseudostrobus* Lindl. (Zhou et al. 2004b) and *D. adjunctus* on *P. hartwegii* Lindl. (HM236501). The unidentified isolates from the USA were from *P. radiata* and *P. taeda* (Zhou et al. 2004c).

This study represents the first reports of *G. galeiformis* from Scandinavia, from spruce, and from the beetles *H. brunneus* and *T. lineatum*. The authentic *G. galeiformis* has been collected only from conifer-infesting bark beetles in northern Europe, whereas *G. radiaticola* occurs primarily associated with pine-infesting beetles in Europe, Chile, New Zealand, South Africa and Korea.

The *L. procerum*-complex

A species similar but not identical to *L. procerum* was isolated from *P. sylvestris* in Russia, in association with *H. palliatus*. The *L. procerum*-complex presently comprises nine species (listed in the Results section), all known only by their morphologically similar anamorphs, with relatively long conidiophores. Eight of the species have been described since 2000 (Jacobs et al. 2000, 2006; Masuya et al. 2000; Zhou et al. 2008; Lu et al. 2008; Paciura et al. 2010). Six of these species were described from pine and/or pine-infesting beetles in Japan (Masuya et al. 2000), Bhutan (Zhou et al. 2006) and China (Lu et al. 2008; Paciura et al. 2010). The seventh species, *L. sibiricum*, was from *Monochamus urussovi* Fisch. on *Abies* in Russia (Jacobs et al. 2000), with only a single species, *L. profanum*, from hardwoods in the USA (Jacobs et al. 2006). Although *L. procerum* has been reported from Canada, the USA, Europe, New Zealand, South Africa (Jacobs and Wingfield 2001), and Japan (Masuya et al. 2009), the only identifications of this species confirmed using DNA sequence comparisons are those from *T. piniperda* on pine in Canada, pine in New Zealand and Norway (Hausner et al. 2005), *D. valens* on pines in the USA and China (Lu et al. 2009a, b), and *Tetropium* species on *Picea* in Poland (Jankowiak and Kolařík 2010). It thus seems as if Asia is a centre of diversity for this species complex, with eight species reported only from that continent. Then *L. profanum* has been found only in North America, while *L. procerum* appears to have a global distribution in pine-growing areas, many where it has clearly been accidentally introduced.

L. procerum has been associated with a root decline disease known as white pine root decline particularly in the North Central and Eastern United States (Kendrick 1962; Alexander et al. 1988) although its role in tree death has been deeply disputed (Jacobs and Wingfield 2001; Wingfield et al. 1988). Recent outbreaks of *D. valens* in China, have again raised the question as to the role of *L. procerum,* the most

dominant species found with the insect, in tree death (Lu et al. 2008, 2009a, b). While inoculation trials have shown that it is not a virulent pathogen, its association with a novel host and in a new environment appears to influence beetle behavior and its potential to contribute to tree death (Lu et al. 2009b, 2011).

The *L. lundbergii*-complex

Two groups of isolates obtained from Finland and Russia formed part of the *L. lundbergii*-complex (Figs. 1, 4). The complex comprises of *L. lundbergii*, the type species for the genus *Leptographium*, together with seven other species (listed in the Results section). All of these are characterized by relatively short conidiophores producing conidia with broadly truncate bases. Apart from *L. lundbergii* (Lagerberg et al. 1927) and *L. truncatum* (Wingfield and Marasas 1983), all the species in this complex have been described since 2000 from conifers in Asia (Zhou et al. 2000; Jacobs et al. 2005; Kim et al. 2004; Masuya et al. 2005; Yamaoka et al. 2008; Paciura et al. 2010). Of these species, only *L. pinicola* has also been found outside of East Asia, on pine in Canada (Jacobs et al. 2005).

Some Russian and Finnish isolates collected in this study had sequences identical or very similar to those of *L. lundbergii* (Fig. 4), which has often been found in association with pine and spruce bark beetles in Europe (Dowding 1973; Mathiesen 1950; Mathiesen-Käärik 1953; Hallaksela 1977; Harding 1989; Gibbs and Inman 1991; Wingfield and Gibbs 1991; Kirisits 2004; Jacobs et al. 2005; Jankowiak 2006; Jankowiak et al. 2009), as well as with non-native bark beetle species infesting pines in South Africa (Zhou et al. 2001). Earlier reports of this fungus from the USA (Rumbold 1931) and Australia (Webb 1946), were considered to be erroneous (Harrington and Cobb 1988) and a recent report of *L. lundbergii* from pine roots in the USA (Eckhardt et al. 2004) also needs to be substantiated with sequence data. In the present investigation, *L. lundbergii* was isolated from several bark beetle species including *D. autographus*, *H. brunneus*, *H. palliatus*, *I. typographus* and *P. chalcographus*, confirming its association with a wide range of vectors. The fungus is a well-known cause of blue stain (Lagerberg et al. 1927; Gibbs and Inman 1991).

The single *L. truncatum* isolate found during this study in association with *D. autographus* infesting pine in Finland, represents the first report of the species from Scandinavia. The fungus was originally described from roots of non-native *Pinus* species in South Africa, infested by *Hylastes angustatus* Herbst, presumably introduced from Europe (Wingfield and Marasas 1983; Wingfield 1985). Similarly, *L. truncatum* has repeatedly been found on non-native pines in New Zealand (Jacobs et al. 2005; Reay et al. 2005; Thwaites et al. 2005; Kim et al. 2011), and with *T. piniperda* introduced into Canada from Europe (Hausner et al. 2005). However, *L. truncatum* does not appear to be a common associate of beetles in Europe, as it has only been reported from the UK (Jacobs et al. 2005) and Spain (Romón et al. 2007) on this continent. Yet its presence in Europe might have been obscured by the fact that it was thought to be a synonym of *L. lundbergii* (Wingfield and Gibbs 1991) and treated as such (Strydom et al. 1997; Jacobs and Wingfield 2001) until 2005, when the species were shown to be distinct (Hausner et al. 2005; Jacobs et al. 2005).

A report of *L. truncatum* from pine root-infesting beetles in the USA was based only on morphological characters (Eckhardt et al. 2007), and needs to be confirmed with sequence data. Interestingly, the authentic *L. truncatum* (based on the type specimen) has been found in China in low numbers associated with *D. valens*, an insect introduced from North America (Lu et al. 2009a, b). These reports from the USA and China cast doubts regarding a suggested European origin for the fungus, and this requires further investigation. Although *L. truncatum* was linked to pine root disease in South Africa, New Zealand, and the USA, the fungus is not considered a primary pathogen (Wingfield and Marasas 1983; Wingfield et al. 1988; Eckhardt et al. 2007).

The *G. piceiperda*-complex

Phylogenetic analyses in this study showed a clear distinction between European and North American isolates of *G. piceiperda*. Most of the studies conducted in the Northern Europe have reported the occurrence of *G. europhioides* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf. (Solheim 1986; Viiri 1997; Pashenova et al. 2001, 2004). Upadhyay (1981) treated these two species as synonyms, but

several subsequent studies have considered them as distinct (Solheim 1986; Harrington and Cobb 1988; Yamaoka et al. 1997; Jacobs et al. 1998; Hausner et al. 2000). The taxonomy of these fungi has been uncertain, because no ex-type cultures exist for either *G. piceiperda* or *G. europhioides*. To clarify their taxonomic status, epitypification of both species will be necessary, and analyses of a greater number of gene regions. Such studies might reveal that *G. piceiperda* or *G. europhioides* are represented by two of the three North American lineages (b, e, and f in Fig. 5) in our analyses. The implication would be that the third North American, and the European (C) and Russian (D) lineages, represent novel taxa. Other than isolate (CMW 452, group B) that came from *Pseudotsuga* in the USA, all isolates in these five lineages originated from *Picea*. Both of the other species in the complex have been reported only from Japan, with *G. laricis* associated with *I. subelongatus* Motschulsky infesting larch (Van der Westhuizen et al. 1995; Yamaoka et al. 1998, 2009), and *G. aenigmatica* restricted to *I. typographus* f. *japonicus* on spruce (Jacobs et al. 1998; Yamaoka et al. 2000).

The *G. penicillata*-complex

Of 263 isolates collected during the survey in Finland and Russia, more than 18 % were identified as *L. chlamydatum,* a species only recently described (Jacobs et al. 2010) in the *G. penicillata*-complex. *G. penicillata* is the type species for genus *Grosmannia*, which was established in 1936 to accommodate the teleomorphs of four species with *Leptographium*-like anamorphs (Goidànich 1936). Shortly afterwards, the genus was reduced to synonymy with *Ophiostoma* (Siemaszko 1939), and treated until 2006 as synonym of *Ophiostoma* and *Ceratocystis* (Bakshi 1951; Hunt 1956; De Hoog 1974; Upadhyay 1981; Jacobs and Wingfield 2001). Zipfel et al. (2006) then reinstated *Grosmannia* as a genus distinct from *Ophiostoma* s. str. for *Ophiostoma* species with *Leptographium* anamorphs.

The *G. penicillata*-complex presently accommodates three species with known teleomorphs, and eight species known only by their *Leptographium* anamorphs (listed in the Results section). *G. penicillata* has been reported extensively from Europe (Jacobs and Wingfield 2001; Kirisits 2004) where it is a common associate of *I. typographus*. A single report of this species from *Dendroctonus ponderosae* in the USA was based on 97 % similarity of only ITS sequences, and it is probably not a reliable identification. Similarly, a report of *G. penicillata* with *I. typographus* in Japan (Yamaoka et al. 1997) needs to be corroborated using multigene sequences. Four species in the complex are known only from North America: *G. abiocarpa* (Davidson 1966; Cardoza et al. 2006b); *G. americanum* (K. Jacobs & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf. (Jacobs et al. 1997); *L. abietinum* (Ohsawa et al. 2000; Jacobs and Wingfield 2001; Haberkern et al. 2002; Massoumi Alamouti et al. 2006, 2007; Cardoza et al. 2006a); and *L. fruticetum* (Massoumi Alamouti et al. 2006, 2007). Four additional species have been reported only from East Asia: *L. bistatum* from Korea (Kim et al. 2004, 2005) and Taiwan (Li et al. 2009); and *L. altius*, *L. curviconidium* and *L. pistaciae* from China (Paciura et al. 2010). Together with *G. penicillata*, two species have been reported only from Europe, specifically Norway, namely *L. curvisporum* and *L. chlamydatum* (Jacobs et al. 2010).

With the exception of *L. pistaciae* (Paciura et al. 2010) from *Pistacia* L., all the species in the complex are associated with conifer-infesting bark beetles, especially from species of pine and spruce (Davidson 1966; Jacobs and Wingfield 2001; Kim et al. 2004, 2005; Massoumi Alamouti 2006, 2007; Jacobs et al. 2010; Paciura et al. 2010). Two species have also been reported from hardwoods in addition to their conifer hosts: *L. abietinum* from *Pseudotsuga* (Cardoza et al. 2006a) and *L. bistatum* from various hardwood species imported into Taiwan (Li et al. 2009).

It was surprising that all the isolates in this study were those of *L. chlamydatum,* and that neither of the other two European species in the *G. penicillata*-complex, *G. penicillata* or *L. curvisporum*, were collected. To date, *L. chlamydatum* has been reported only from the root-infesting bark beetle *H. cunicularius*, infesting spruce in Norway (Jacobs et al. 2010). Data from this study (Table 3) suggest that the fungus has a much wider range of bark beetle associates, especially *P. chalcographus* and *I. typographus*.

It has been suggested that species such as *G. penicillata* are serious blue stain agents (Grosmann 1930; Siemaszko 1939; Mathiesen 1950). Inoculations with *G. penicillata* (Horntvedt et al. 1983; Solheim 1988; Christiansen and Solheim 1994) did not cause substantial damage, neither did they kill trees.

Leptographium taigense

DNA sequences for three gene regions provided clear evidence that a suite of isolates forming a well-resolved phylogenetic group, distinct from any of the other species complexes, represents an undescribed taxon that was provided with the name *L. taigense*. Morphologically, *L. taigense* most closely resembles *G. galeiformis*, which also produces both synnematous and mononematous anamorphs in culture (Zhou et al. 2004c). The new species was only found in Russia in association with five different bark beetle species on pine and spruce.

Concluding remarks

Numerous studies on the ophiostomatoid fungi associated with bark beetles have been conducted in Europe. Therefore, the discovery of several previously unrecognized *Grosmannia* and *Leptographium* species, associated with common bark beetle species in Finland and Russia, was unexpected. Of the ten species encountered, only *L. lundbergii* had previously been reported in these countries. Two species are reported from both countries for the first time, while four have been found only in Finland and another three only in Russia. The number of new records encountered in this study, covering a relatively small geographic area, clearly indicates that the inventory of these ecologically and often economically important fungi is incomplete in the boreal forests, and that they deserve further investigation. Because some species in this group are important tree pathogens, the pathogenicity of the less well-known species encountered in this study should be considered. Although the view that bark beetles require these fungi to kill trees has recently been challenged (Six and Wingfield 2011), they are increasingly being moved globally through trade in wood and wood products. The response of naïve hosts in novel environments to new bark beetle-fungal interactions is already raising concern (Hulcr and Dunn 2011; Lu et al. 2011).

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References

Afanasova EN (2009) Blue-stain fungi carried by bark beetles in coniferous forests of Central Siberia. In: Pavlov IN, Kutafieva NP (eds) Boreal zone macromycetes. Proceedings of All-Russian research to practice conference. Siberian State Technological University, Russian Federation, Krasnojarsk, pp 138–144

Alexander SA, Horner WE, Lewis KJ (1988) *Leptographium procerum* as a pathogen of pines. In: Harrington TC, Cobb FW Jr (eds) *Leptographium* root diseases on conifers. APS Press, St Paul, pp 97–112

Bakshi BK (1951) Studies on four species of *Ceratocystis*, with a discussion on fungi causing sapstain in Britain. Mycol Pap 35:1–16

Bernier L, Breuil C, Hintz WE, Horgen PA, Jacobi V, Dufour V, Aoun M, Bouvet GF, Kim SH, Diguistini S, Tanguay P, Eades J, Burgess S, de la Bastide P, Pinchback M, Tadesse Y (2004) The Canadian *Ophiostoma* genome project. Invest Agrar: Sist Recur For 13:105–117

Cardoza YJ, Klepzig KD, Raffa KF (2006a) Bacteria in oral secretions of an endophytic insect inhibit antagonistic fungi. Ecol Entomol 31:636–645. doi:10.1111/j.1365-2311.2006.00829.x

Cardoza YJ, Paskewitz S, Raffa KF (2006b) Travelling through time and space on wings of beetles: a tripartite insect-fungi-nematode association. Symbiosis 41:71–79

Christiansen E, Solheim H (1994) Pathogenicity of five species of *Ophiostoma* fungi to Douglas-fir. Medd Skogforsk 47:1–12

Davidson RW (1966) New species of *Ceratocystis* from conifers. Mycopathol Mycol Appl 28:273–286. doi:10.1007/BF02051237

Davidson RW (1971) New species of *Ceratocystis*. Mycologia 63:5–15

De Hoog GS (1974) The genera *Blastobotrys*, *Sporothrix*, *Calcarisporium* and *Calcarisporiella* gen. nov. Stud Mycol 7:1–84

Dowding P (1973) Effect of felling time and insecticide treatment on the interrelationships of fungi and arthropods in pine logs. Oikos 24:422–429

Duong TA, De Beer ZW, Wingfield BD, Wingfield MJ (2012) Phylogeny and taxonomy of species in the *Grosmannia serpens* complex. Mycologia. doi:10.3852/11-109

Eckhardt LG, Jones JP, Klepzig KD (2004) Pathogenicity of *Leptographium* species associated with loblolly pine

decline. Plant Dis 88:1174–1178. doi:10.1094/PDIS.2004.88.11.1174
Eckhardt LG, Weber AM, Menard R, Jones JP, Hess N (2007) Insect-fungal complex associated with loblolly pine decline in Central Alabama. For Sci 53:84–92
Fedorenko SI (1988) On damage to coniferous logs by insects and fungi at remote shift harvesting areas. Ecol For Prot pp 99–104
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Gibbs JN, Inman A (1991) The pine shoot beetle *Tomicus piniperda* as a vector of blue-stain fungi to windblown pine. Forestry 64:239–249. doi:10.1093/forestry/64.3.239
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 61:1323–1330
Goidànich G (1936) II genere di Ascomiceti *'Grosmannia'* G. Goid. Boll Staz Pat veg Roma 16:26–60
Goloboff PA, Farris J, Nixon K (2008) TNT, a free program for phylogenetic analysis. Cladistics 24:774–786. doi:10.1111/j.1096-0031.2008.00217.x
Grobbelaar J, De Beer ZW, Bloomer P, Wingfield M, Wingfield B (2010) *Ophiostoma tsotsi* sp. nov., a wound-infesting fungus of hardwood trees in Africa. Mycopathologia 169:413–423. doi:10.1007/s11046-009-9267-8
Grosmann H (1930) Beitrage zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Z Parasitenkd 3:56–102
Haberkern KE, Illman BL, Raffa KF (2002) Bark beetles and fungal associates colonizing white spruce in the great lakes region. Can J For Res 32:1137–1150. doi:10.1139/X02-033
Hallaksela A (1977) Microbial flora isolated from Norway spruce stumps. Acta For Fenn 158:5–41
Harding S (1989) The influence of mutualistic blue-stain fungi on bark beetle population dynamics. Dissertation. Royal Veterinary and Agricultural University, Copenhagen
Harrington TC, Cobb FW (1988) *Leptographium* root diseases on conifers. APS Press, St Paul
Harrington TC, Wingfield MJ (1998) The *Ceratocystis* species on conifers. Can J Bot 76:1446–1457. doi:10.1139/b98-145
Harrington TC, Aghayeva DN, Fraedrich SW (2010) New combinations in *Raffaelea, Ambrosiella,* and *Hyalorhinocladiella,* and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. Mycotaxon 111: 337–361
Harrington TC, McNew D, Steimel J, Hofstra D, Farrell R (2001) Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch Elm Disease fungi. Mycologia 93:111–136
Hausner G, Reid J, Klassen GR (2000) On the phylogeny of members of *Ceratocystis* s.s. and *Ophiostoma* that possess different anamorphic states, with emphasis on the anamorph genus *Leptographium*, based on partial ribosomal DNA sequences. Can J Bot 78:903–916. doi:10.1139/b00-068
Hausner G, Iranpour M, Kim J-J, Breuil C, Davis CN, Gibb EA, Reid J, Loewen PC, Hopkin AA (2005) Fungi vectored by the introduced bark beetle *Tomicus piniperda* in Ontario, Canada, and comments on the taxonomy of *Leptographium lundbergii*, *L. terebrantis*, *L. truncatum*, and *L. wingfieldii*. Can J Bot 83:1222–1237. doi:10.1139/b05-095
Hawksworth D (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. MycoKeys 1:7–20. doi:10.3897/mycokeys.1.2062
Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ et al (2011) The Amsterdam declaration on fungal nomenclature. IMA Fungus 2:105–112. doi:10.5598/imafungus.2011.02.01.14
Hinds TE, Buffam PE (1971) Blue stain in Engelmann spruce trap trees treated with cacodylic acid. USDA For Serv Res. Note RM-201
Horntvedt R, Christiansen E, Solheim H, Wang S (1983) Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. Medd Nor Inst Skogforsk 38:1–20
Hulcr J, Dunn RR (2011) The sudden emergence of pathogenicity in insect—fungus symbioses threatens naive forest ecosystems. Proc R Soc B 278:2866–2873. doi:10.1098/rspb.2011.1130
Hunt J (1956) Taxonomy of the genus *Ceratocystis*. Lloydia 19:1–59
Jacobs K, Wingfield MJ (2001) *Leptographium* species: tree pathogens, insect associates and agents of blue-stain. APS Press, St Paul
Jacobs K, Wingfield MJ, Bergdahl DR (1997) A new species of *Ophiostoma* from North America, similar to *Ophiostoma penicillatum*. Can J Bot 75:1315–1322. doi:10.1139/b97-843
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. doi:10.1017/S0953756297004917
Jacobs K, Wingfield MJ, Pashenova NV, Vetrova VP (2000) A new *Leptographium* species from Russia. Mycol Res 104:1524–1529. doi:10.1017/S0953756200002689
Jacobs K, Wingfield MJ, Uzunovic A, Frisullo S (2001) Three new species of *Leptographium* from pine. Mycol Res 105:490–499. doi:10.1017/S0953756201003860
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 108:411–418. doi:10.1017/S0953756204009748
Jacobs K, Solheim H, Wingfield BD, Wingfield MJ (2005) Taxonomic re-evaluation of *Leptographium lundbergii* based on DNA sequence comparisons and morphology. Mycol Res 109:1149–1161. doi:10.1017/S0953756205003618
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. doi:10.1139/b06-030
Jacobs K, Krokene P, Solheim H, Wingfield MJ (2010) Two new species of *Leptographium* from *Dryocoetes autographus* and *Hylastes cunicularius* in Norway. Mycol Prog 9:69–78. doi:10.1007/s11557-009-0620-6
Jankowiak R (2006) Mycobiota associated with *Hylurgops palliatus* (Gyll.) on *Pinus sylvestris* L. in Poland. Acta Societatis Botanicorum 75:333–338

Jankowiak R, Kolařík M (2010) Diversity and pathogenicity of ophiostomatoid fungi associated with *Tetropium* species colonizing *Picea abies* in Poland. Folia Microbiol 55: 145–154

Jankowiak R, Kacprzyk M, Młynarczyk M (2009) Diversity of ophiostomatoid fungi associated with bark beetles (Coleoptera: Scolytidae) colonizing branches of Norway spruce (*Picea abies*) in southern Poland. Biologia 64:1170–1177. doi:10.2478/s11756-009-0188-2

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9:286–298. doi:10.1093/bib/bbn013

Kendrick WB (1962) The *Leptographium* complex. *Verticladiella* Hughes. Can J Bot 40:771–797. doi:10.1139/b62-072

Kim J-J, Lim YW, Wingfield MJ, Breuil C, Kim GH (2004) *Leptographium bistatum* sp. nov., a new species with a *Sporothrix* synanamorph from *Pinus radiata* in Korea. Mycol Res 108:699–706. doi:10.1017/S0953756204000036

Kim JJ, Lim YW, Seifert KA, Kim SH, Breuil C, Kim GH (2005) Taxonomy of *Ophiostoma radiaticola* sp. nov. (Ophiostomatales, Ascomycetes), the teleomorph of *Pesotum pini*, isolated from logs of *Pinus radiata*. Mycotaxon 91:481–496

Kim S, Harrington TC, Lee JC, Seybold SJ (2011) *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle *Hylurgus ligniperda* in California. Mycologia 103:152–163. doi:10.3852/10-096

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

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinf 5:150–163. doi:10.1093/bib/5.2.150

Lagerberg T, Lundberg G, Melin E (1927) Biological and practical researches into blueing in pine and spruce. Sven Skogsvårdsfören Tidskr 25:145–272

Li H-Y, Kao H-W, Chen C-Y (2009) Ophiostomatoid fungi from imported wood in Taiwan. Taiwania 54:343–352

Linnakoski R, De Beer ZW, Rousi M, Niemelä P, Pappinen A, Wingfield MJ (2008) Fungi, including *Ophiostoma karelicum* sp. nov., associated with *Scolytus ratzeburgi* infesting birch in Finland and Russia. Mycol Res 112:1475–1488. doi:10.1016/j.mycres.2008.06.007

Linnakoski R, De Beer ZW, Rousi M, Solheim H, Wingfield MJ (2009) *Ophiostoma denticiliatum* sp. nov. and other *Ophiostoma* species associated with the birch bark beetle in southern Norway. Persoonia 23:9–15. doi:10.3767/003158509X46803

Linnakoski R, De Beer ZW, Ahtiainen J, Sidorov E, Niemelä P, Pappinen A, Wingfield MJ (2010) *Ophiostoma* spp. associated with pine- and spruce-infesting bark beetles in Finland and Russia. Persoonia 25:72–93. doi:10.3767/003158510X550845

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. doi:10.3852/mycologia.100.2.275

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

Lu Q, Decock C, Zhang X, Maraite H (2009b) 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 Leeuwenhoek 96:275–293. doi:10.1007/s10482-009-9343-6

Lu M, Wingfield MJ, Gillette N, Sun J-H (2011) Do novel genotypes drive the success of an invasive bark beetle-fungus complex? Implications for re-invasion. Ecology 29:2013–2015

Massoumi Alamouti S, Kim J-J, Breuil C (2006) A new *Leptographium* species associated with the northern spruce engraver, *Ips perturbatus*, in western Canada. Mycologia 98:149–160. doi:10.3852/mycologia.98.1.149

Massoumi Alamouti S, Kim J-J, Humble L, Uzunovic A, Breuil C (2007) Ophiostomatoid fungi associated with the northern spruce engraver, *Ips perturbatus*, in western Canada. Antonie Leeuwenhoek 91:19–34. doi:10.1007/s10482-006-9092-8

Massoumi Alamouti S, Tsui CKM, Breuil C (2009) Multigene phylogeny of filamentous ambrosia fungi associated with ambrosia and bark beetles. Mycol Res 113:822–835. doi:10.1016/j.mycres.2009.03.003

Masuya H, Wingfield M, Kaneko S, Yamaoka Y (2000) *Leptographium pini-densiflorae* sp. nov. from Japanese red pine. Mycosci 41:425–430. doi:10.1007/bf02461660

Masuya H, Kim JJ, Wingfield MJ, Yamaoka Y, Kaneko S, Breuil C, Kim GH (2005) Discovery and description of a teleomorph for *Leptographium koreanum*. Mycotaxon 94:159–173

Masuya H, Yamaoka Y, Kaneko S, Yamaura Y (2009) Ophiostomatoid fungi isolated from Japanese red pine and their relationships with bark beetles. Mycoscience 50:212–223

Mathiesen A (1950) Über einige mit Borkenkäfern assoziierte Bläuepilze in Schweden. Oikos 2:275–308

Mathiesen A (1951) Einige neue *Ophiostoma*-Arten in Schweden. Sven Bot Tidskr 45:203–232

Mathiesen-Käärik A (1953) Eine Übersicht uber die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläupilze. Medd Statens Skogsforskninginst 43:1–74

Mouton M, Wingfield MJ, van Wyk PS (1992) The anamorph of *Ophiostoma francke-grosmanniae* is a *Leptographium*. Mycologia 84:857–862

Münch E (1907) De Blaufäule des Nadelholzes. I-II. Naturwiss Z. Forst- und Landwirtsch 5:531–573

Odgen TH, Rosenberg MS (2007) How should gaps be treated in parsimony? A comparison of approaches using simulation. Mol Phylogen Evol 42:817–826. doi:10.1016/j.ympev.2006.07.021

O'Donnel K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol Phylogen Evol 7:103–116. doi:10.1006/mpev.1996.0376

Ohsawa M, Langor D, Hiratsuka Y, Yamaoka Y (2000) Fungi associated with *Dendroctonus rufipennis* and *Polygraphus rufipennis*, and white spruce inoculation tests. Can J Plant Pathol 22:254–257. doi:10.1080/07060660009500472

Okada G, Seifert KA, Takematsu A, Yamaoka Y (1998) A molecular phylogenetic reappraisal of the *Graphium* complex based on 18S rDNA sequences. Can J Bot 76:1495–1506. doi:10.1139/b98-089

Olchowecki A, Reid J (1974) Taxonomy of the genus *Ceratocystis* in Manitoba. Can J Bot 52:1675–1711. doi:10.1139/b74-222

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. doi:10.3767/003158510X551097

Pashenova NV, Polyakova GG (2009) The study on blue-staining fungi in coniferous forests of Central Siberia. In: Pavlov IN, Kutafieva NP (eds) Boreal zone macromycetes. Proceedings of All-Russian research to practice conference. Siberian State Technological University, Russian Federation, Krasnojarsk, pp 91–94

Pashenova NV, Vetrova VP, Konstantinov MYu, Aphanasova EN (2001) Ophiostomataceae fungi associated with *Ips typographus* in coniferous forests of Central Siberia. Lesovedenie 4:11–19

Pashenova NV, Vetrova VP, Aphanasova EN, Polyakova GG, Konstantinov MYu (2004) Ophiostomatoid fungi in Middle Siberia. In: Proceedings of the IVth International Symposium of Structure, properties and quality of wood, pp 443–446

Reay SD, Thwaites JM, Farrell RL (2005) A survey of *Ophiostoma* species vectored by *Hylastes ater* to pine seedlings in New Zealand. For Pathol 35:105–113. doi:10.1111/j.1439-0329.2004.00393.x

Rennerfelt E (1950) Über den Zusammenhang zwischen dem Verblauen des Holzes und den Insekten. Oikos 2:120–137

Romón P, Zhou X, Iturrondobeitia JC, Wingfield MJ, Goldarazena A (2007) *Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. Can J Microbiol 53:756–767. doi:10.1139/W07-001

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. doi:10.1093/bioinformatics/btg180

Rumbold CT (1931) Two blue-staining fungi associated with bark-beetle infestation of pines. J Agric Res 43:847–873

Savonmäki S (1990) Tärkeimmät kaarnakuoriaisten mäntyyn ja kuuseen levittämät sinistäjäsienilajit. Masters Thesis, Department of Plant Pathology, University of Helsinki

Siemaszko W (1939) Zespoły grzybów towarzyszących kornikom polskim. Planta Pol 7:1–54

Six DL, Wingfield MJ (2011) The role of phytopathogenicity in bark beetle—fungus symbioses: a challenge to the classic paradigm. Ann Rev Entomol 56:255–272. doi:10.1146/annurev-ento-120709-144839

Six D, De Beer ZW, Duong TA, Carroll A, Wingfield MJ (2011) Fungal associates of the lodgepole pine beetle, *Dendroctonus murrayanae*. Antonie Leeuwenhoek 100:231–244. doi:10.1007/s10482-011-9582-1

Six DL, Harrington TC, Steimel J, McNew D, Paine TD (2003) Genetic relationships among *Leptographium terebrantis* and the mycangial fungi of three Western *Dendroctonus* bark beetles. Mycologia 95:781–792

Solheim H (1986) Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. Nord J Bot 6:199–207. doi:10.1111/j.1756-1051.1986.tb00874.x

Solheim H (1988) Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway spruce. Medd Nor Inst Skogforsk 40:1–11

Solheim H, Långström B (1991) Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity. Ann Sci For 48:149–156. doi:10.1051/forest:19910203

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 57:758–771. doi:10.1080/10635150802429642

Strydom RC, Wingfield BD, Wingfield MJ (1997) Ribosomal DNA sequence comparison of *Leptographium lundbergii* and *L. truncatum* and neotypification of *L. lundbergii*. Syst Appl Microbiol 20:295–300. doi:10.1016/S0723-2020(97)80076-8

Thwaites JM, Farrell RL, Duncan SM, Reay SD, Blanchette RA, Hadar E, Hadar Y, Harrington TC, McNew D (2005) Survey of potential sapstain fungi on *Pinus radiata* in New Zealand. N Z J Bot 43:653–663. doi:0028-825X/05/4303-0653

Upadhyay HP (1981) A monograph of *Ceratocystis* and *Ceratocystiopsis*. The University of Georgia Press, Georgia

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 99:1334–1338. doi:10.1016/S0953-7562(09)81217-3

Viiri H (1997) Fungal associates of the spruce bark beetle *Ips typographus* L. (Col. Scolytidae) in relation to different trapping methods. J Appl Entomol 121:529–533. doi:10.1111/j.1439-0418.1997.tb01444.x

Villarreal M, Rubio V, de Troya MT, Arenal F (2005) A new *Ophiostoma* species isolated from *Pinus pinaster* in the Iberian Peninsula. Mycotaxon 92:259–268

Webb S (1946) Australian ambrosia fungi. (*Leptographium lundbergii* Lagerberg et Melin, and *Endomycopsis* spp. Dekker.). Proc R Soc Vic 57:57–80

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–321

Wingfield MJ (1985) Reclassification of *Verticladiella* based on conidial development. Trans Br Mycol Soc 85:81–93. doi:10.1016/S0007-1536(85)80157-1

Wingfield MJ, Gibbs JN (1991) *Leptographium* and *Graphium* species associated with pine-infesting bark beetles in England. Mycol Res 95:1257–1260. doi:10.1016/S0953-7562(09)80570-4

Wingfield MJ, Marasas WFO (1983) Some *Verticicladiella* species, including *V. truncata* sp. nov., associated with root diseases of pine in New Zealand and South Africa. Trans Br Mycol Soc 80:81–93. doi:10.1016/S0007-1536(83)80005-9

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. APS Press, St Paul, pp 113–128

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

Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW (2012) One fungus, one name promotes progressive plant pathology. Mol Plant Pathol. doi:10.1111/j.1364-3703.2011.00768.x

Wright EF, Cain RF (1961) New species of the genus *Ceratocystis*. Can J Bot 39:1215–1230. doi:10.1139/b61-106

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. doi:10.1017/S0953756297003924

Yamaoka Y, Wingfield MJ, Ohsawa M, Kuroda Y (1998) Ophiostomatoid fungi associated with *Ips cembrae* in Japan and their pathogenicity of Japanese larch. Mycosci 39:367–378. doi:10.1007/BF02460897

Yamaoka Y, Takahashi I, Iguchi K (2000) Virulence of ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. *japonicus* in Yezo spruce. J For Res 5:87–94. doi:10.1007/BF02762525

Yamaoka Y, Masuya H, Chung W-H, Goto H, To-Anun C, Tokumasu S, Zhou X, Wingfield M (2008) The teleomorph of *Leptographium yunnanense*, discovered in crosses among isolates from Thailand, China, and Japan. Mycosci 49:233–240. doi:10.1007/s10267-008-0412-x

Yamaoka Y, Chung W-H, Masuya H, Hizai M (2009) Constant association of ophiostomatoid fungi with the bark beetle *Ips subelongatus* invading Japanese larch logs. Mycosci 50:165–172. doi:10.1007/s10267-008-0468-7

Zhao G (2005) [Two new species of *Ceratocystis* on *Pinus massoniana* staining wood] In Chinese with English abstract. J Nanjing Forestry University (Natural Sciences Edition) 29:115–118

Zhou XD, Jacobs K, Morelet M, Ye H, Lieutier F, Wingfield MJ (2000) A new *Leptographium* species associated with *Tomicus piniperda* in south-western China. Mycosci 41:573–578. doi:10.1007/BF02460923

Zhou XD, De Beer ZW, Wingfield BD, Wingfield MJ (2001) Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. Sydowia 53:290–300

Zhou XD, De Beer ZW, Ahumada R, Wingfield BD, Wingfield MJ (2004a) *Ophiostoma* and *Ceratocystiopsis* spp. associated with two pine-infesting bark beetles in Chile. Fungal Divers 15:261–274

Zhou XD, De Beer ZW, Cibrián D, Winfield BD, Wingfield MJ (2004b) Characterization of *Ophiostoma* species associated with pine bark beetles from Mexico, including *O. pulvinisporum* sp. nov. Mycol Res 108:690–698. doi:10.1017/S0953756204009918

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

Zhou XD, De Beer ZW, Wingfield MJ (2006) DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa. Stud Mycol 55:269–277. doi:10.3114/sim.55.1.75

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 21:1–8. doi:10.3767/003158508X332435

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. doi:10.3114/sim.55.1.133