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Eight new *Leptographium* species associated with tree-infesting bark beetles in China

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Key words

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Abstract *Leptographium* spp. are anamorphs of *Grosmannia* residing in the order *Ophiostomatales*. These fungi are typically associated with bark-beetles and are common causal agents of sapstain in lumber and some are important tree pathogens. In this study, *Leptographium* spp. associated with bark beetles collected during a survey in Jilin and Yunnan provinces of China, were identified. Identifications were achieved using comparisons of morphological characters and DNA sequence data for the ITS2-partial LSU rDNA region, as well as the β-tubulin and EF-1α gene regions. Eight unknown species of *Leptographium* are recognised and described from conifer and hardwood hosts, associated with beetles including *Ips subelongatus*, *Tomicus yunnanensis*, *Hylurgops minor*, *Polygraphus verrucifrons* and a *Pissodes* sp. Six of the new species are morphologically and phylogenetically related to species known to occur in Asia such as *G. yunnanense*, *L. bhutanense*, *L. bistatum* and *L. sinoprocerum*. The remaining two taxa are related to those in a group containing *G. americana* and *L. abietinum*, found in North America. This study also provides the first report of *L. pineti* on *Pinus kesiya* in China.

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INTRODUCTION

Leptographium spp. are anamorphs of the teleomorph genus *Grosmannia* that resides in the *Ophiostomatales* (Zipfel et al. 2006). *Grosmannia* states have been identified for 33 species (Jacobs & Kirisits 2003, Kim et al. 2005b, Masuya et al. 2005, Zipfel et al. 2006, Yamaoka et al. 2008) and the remaining 35 species are known only based on their anamorphs (Jacobs & Wingfield 2001, Kim et al. 2004, Masuya et al. 2004, Lee et al. 2005, Jacobs et al. 2006, Massoumi Alamouti et al. 2006, Lu et al. 2008, Zhou et al. 2008).

Grosmannia and *Leptographium* spp. are well-known agents of sapstain of mainly conifer lumber, and less often on hardwoods (Harrington & Cobb 1988, Wingfield et al. 1993, Jacobs & Wingfield 2001). A few species are saprophytes found in the soil or on decaying plant material, and some are important tree pathogens (Harrington & Cobb 1988). Like most ophiostomatoid fungi, *Leptographium* spp. and their *Grosmannia* teleomorphs are best known as associates of bark beetles (Harrington & Cobb 1988, Kirisits 2004). In this regard, they are morphologically adapted to be carried by these insects, with erect conidiophores or ascomata with long necks and conidia and ascospores produced in slimy masses at the apices of these structures (Six 2003, Kirisits 2004, Cardoza et al. 2008). The fungi gain entrance to the trees through the wounds created by bark beetles, and spores rub off onto the sapwood and inner bark as the beetles burrow and move through their galleries (Six 2003).

The most common insect associates of *Leptographium* spp. are bark beetles residing in the genera *Dendroctonus*, *Ips*, *Tomicus* and *Orthotomicus* (*Curculionidae*: *Scolytinae*), as well as *Hylastes* and *Hylurgops* (*Scolytidae*: *Hylesininae*) (Kirisits 2004). They have also been reported in association with root weevils in the genera *Hylobius*, *Pachylobius*, *Pissodes* and *Steremnius* (*Curculionidae*: *Molytinae*) and with long horn beetles (*Coleoptera*: *Cerambycidae*) including *Monochamus* species (Wingfield 1987, Witcosky et al. 1986, Jacobs et al. 2000b, Eckhardt et al. 2007). Several studies have been conducted on various aspects of the symbiotic relationships between the beetles and fungi (Six 2003, Kirisits 2004, Plattner et al. 2008, Bleiker & Six 2009). However, for the majority of the *Leptographium* species, very little is known regarding their biology or the roles that they play in the life histories of bark beetles, their host trees or their interactions with other closely associated organisms such as mites and bacteria (Harrington 2005).

Much of the literature published on *Leptographium* and *Grosmannia* has focused on the taxonomy and ecology of European and North American species (Harrington & Cobb 1988, Jacobs & Wingfield 2001, Six 2003, Kirisits 2004, Harrington 2005). In the case of East Asia, the best studied examples are those from Japan (Yamaoka et al. 1997, 1998, Masuya et al. 1998). These fungi are virtually unknown in China and presently only eight species of *Leptographium* or *Grosmannia* have been reported from this large country with its large resource of conifers. The species include *G. yunnanense* associated with the native *Tomicus yunnanensis* infesting *Pinus yunnanensis* (Zhou et al. 2000, Kirkendall et al. 2008, Yamaoka et al. 2008). All the other species, including *G. koreana*, *Hyalorhinocladiella pinicola*, *L. alethinum*, *L. pini-densiflorae*, *L. procerum*, *L. sinoprocerum* and *L. truncatum*, have recently been reported from *Dendroctonus valens*, introduced from North America, and now attacking *P. tabuliformis* in China (Lu et al. 2008, 2009a, b).

During the course of a survey of ophiostomatoid fungi associated with bark beetles and weevils in the north-eastern and south-western forestry areas of China, many of the collected isolates superficially resembled *Leptographium* spp. The aim of this study was to identify these fungi by comparing their morphology and DNA sequences to those of known species.

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MATERIALS AND METHODS

Isolates

Field surveys were conducted during 2001 and 2002 in plantations and sawmills in the Jilin and Yunnan provinces, respectively situated in north-eastern and south-western China. Different conifer and hardwood hosts including genera such as *Larix*, *Picea*, *Pinus* and *Pistacia* were examined for the presence of bark beetle and weevil galleries. Beetles were placed individually in Eppendorf tubes, and stored in a cool box or at 4 °C until isolations were made by squashing the beetles on 2 % malt extract agar amended with 0.05 % cycloheximide (MEA; 20 g Biolab malt extract, 20 g Biolab agar and 1 000 mL deionised water). In addition, beetle galleries were incubated in plastic containers or Petri dishes on moist tissue paper until fruiting structures formed. Fungi were then isolated by transferring spore masses from the fruiting structures to the selective medium. Strains were purified on MEA and are stored in the culture collections (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, and at Yunnan University, China. Representative isolates of new taxa described in this study were also deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, herbarium specimens in the National Collection of Fungi (PREM), Pretoria, South Africa, and taxonomic novelties in MycoBank (Crous et al. 2004).

Morphology

Fungal structures for morphological studies were obtained from cultures grown on Oatmeal agar plates for 20 d (OA; 30 g oatmeal, 20 g Biolab agar and 1 000 mL deionised water), on which they sporulate more abundantly than on MEA. All isolates of each taxon were crossed in all possible combinations with each other to induce production of ascomata, following the method described by Grobbelaar et al. (2010). Each isolate was also crossed against itself as a control. For light microscopy, the structures were fixed on glass slides in lactophenol coloured with cotton blue. The isolates were then grouped based on morphology. Fruiting structures for two isolates per group were measured and the averages and ranges were calculated. Some taxa closely resembled known species. In these cases, the ex-type isolates of the known species were obtained and compared with the Chinese cultures. These included the ex-type cultures of *L. sinoprocerum* (CMW 26231 = MUCL 46352) and *L. bhutanense* (CMW 18649 = CBS 122076).

Scanning Electron Microscopy (SEM) was done for the species to be described, using actively growing fungal colonies after 2 wk of growth. Specimens were prepared and examined as described by Paciura et al. (2010).

Growth studies

The optimal growth temperature was determined, using two strains for each morphological group and four replicates per strain. A round plug of 5 mm diam taken from an actively growing fungal colony was placed at the centre of MEA plates. These were incubated at seven different temperatures at 5 °C intervals, ranging from 5 °C to 35 °C, for 8 d. The diameter of each colony was measured after 4 and 8 d. The average of eight readings per strain was calculated. This was also done for the ex-type isolates of *L. sinoprocerum* and *L. bhutanense*. Colony colours were described based on the colour chart of Rayner (1970).

DNA extraction and sequencing

DNA was extracted from 8 d old cultures on PDA, obtained from hyphal tips of representative isolates for each of the morphological groups (Table 1). The PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, CA, USA), was used following the protocol of Linnakoski et al. (2008). The DNA concentration was determined using a NanoDrop-1000 Spectrophotometer v3.2 (NanoDrop Technologies Inc., Wilmington, DE, USA).

DNA sequences were determined for three gene regions, including the internal transcribed spacer region 2 (ITS2) and part of the large subunit (LSU) of the rDNA operon, as well as fragments of the β-tubulin and elongation factor 1α (EF-1α) genes. The primers ITS3 (White et al. 1990) and LR3 (Vilgalys & Hester 1990) were used to amplify the ITS2-28S region, Bt2a and Bt2b (Glass & Donaldson 1995) for the β-tubulin gene, and EF1F and EF2R (Jacobs et al. 2004) for the EF-1α gene.

Table 1 Isolates of *Leptographium* spp. from Yunnan and Jilin provinces in China, sequenced in this study.

Taxon no.	Species (total no. of isolates from survey)	Isolate no.		Host / Insect	Origin	GenBank accession no.		
		[a]CBS	[b]CMW			ITS2-LSU	β-tubulin	EF-1α
1	*L. conjunctum* (8)	123631	12473[c]	*Pinus yunnanensis / Hylurgops major*	Yunnan, Chuxiong	HQ406831	HQ406879	HQ406855
		123632	12449	*Pinus kesiya*	Yunnan, Chuxiong	HQ406832	HQ406880	HQ406856
		123633	12452	*P. yunnanensis / H. major*	Yunnan, Chuxiong	HQ406833	HQ406881	HQ406857
2	*L. celere* (5)	123628	12422[c]	*P. kesiya*	Yunnan, Chuxiong	HQ406834	HQ406882	HQ406858
		123629	12421	*P. kesiya*	Yunnan, Chuxiong	HQ406835	HQ406883	HQ406859
		123630	12483	*Pinus* sp.	Jilin, Yanji	HQ406836	HQ406884	HQ406860
3	*L. manifestum* (8)	123604	12433	*Larix olgensis / Ips subelongatus*	Jilin, Wangqing	HQ406837	HQ406885	HQ406861
		123606	12461	*P. yunnanensis / Polygraphus verrucifrons*	Yunnan, Lufeng	HQ406838	HQ406886	HQ406862
		123622	12436[c]	*L. olgensis / I. subelongatus*	Jilin, Wangqing	HQ406839	HQ406887	HQ406863
4	*L. gracile* (48)	123623	12398[c]	*Pinus armandii / Pissodes* sp.	Yunnan, Midu	HQ406840	HQ406888	HQ406864
		123624	12396	*P. armandii / Pissodes* sp.	Yunnan, Midu	HQ406841	HQ406889	HQ406865
		123625	12316	*P. armandii / Pissodes* sp.	Yunnan, Lijiang	HQ406842	HQ406890	HQ406866
5	*L. latens* (22)	123615	12310	*P. armandii / Pissodes* sp.	Yunnan, Lijiang	HQ406843	HQ406891	HQ406867
		123616	12319	*P. armandii / Pissodes* sp.	Yunnan, Midu	HQ406844	HQ406892	HQ406868
		124023	12438[c]	*Picea koraiensis / Ips typographus*	Yunnan, Midu	HQ406845	HQ406893	HQ406869
6	*L. pistaciae* (2)	123626	12499[c]	*Pistacia chinensis*	Yunnan, Chuxiong	HQ406846	HQ406894	HQ406870
		123627	12500	*P. chinensis*	Yunnan, Chuxiong	HQ406847	HQ406895	HQ406871
7	*L. curviconidium* (8)	123617	12441	*P. koraiensis / I. typographus*	Jilin, Wangqing	HQ406848	HQ406896	HQ406872
		123618	12486	*P. koraiensis / I. typographus*	Jilin, Wangqing	HQ406849	HQ406897	HQ406873
		124024	12425[c]	*P. koraiensis / I. typographus*	Jilin, Wangqing	HQ406850	HQ406898	HQ406874
8	*L. altius* (6)	123612	12426	*L. olgensis / Ips cembrae*	Jilin, Changchun	HQ406852	HQ406900	HQ406876
		123619	12471[c]	*P. koraiensis*	Jilin, Wangqing	HQ406851	HQ406899	HQ406875
		123621	12501	*L. olgensis / I. cembrae*	Jilin, Changchun	HQ406853	HQ406901	HQ406877
9	*L. pineti* (1)	–	12457	*P. kesiya*	Yunnan, Chuxiong	HQ406854	HQ406902	HQ406878

[a] CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[b] CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
[c] Ex-type isolates.

Table 2 Morphological characteristics of Group A, containing Taxa 1, 2 and 3 from China and their closest relatives. All measurements in µm unless indicated otherwise.

	L. truncatum [a, b]	*G. yunnanense* [b]	*G. koreana* [b, c]	*H. pinicola* [a, b]	Taxon 1: *L. conjunctum* [a]	Taxon 2: *L. celere* [a]	Taxon 3: *L. manifestum* [a]
Conidiophore (l)	(90–)246–409(–685)	74–227(–233)	59–306	(11–)15–32(–48)	(72–)146–349(–485)	(120–)239–950(–1365)	(83–)103–243(–363)
Conidiogenous apparatus (l)	(35–)42–85(–150)	(40–)83–88(–127)	24–79	–	(37–)42–85(–100)	58–98(–115)	(36–)50–77(–100)
Rhizoids	Absent	Absent	Absent	–	Absent	Present	Present
Conidium shape	Broadly ellipsoid, truncate bases, rounded apices	Obovoid	Oblong to obovoid	Obtuse to obovoid with broadly truncated ends	Oblong to obovoid	Obovoid, truncate bases	Elongated, pointed ends
Conidium size (l × w)	3–5 × 2–4	4–11 × 2–6	3–10 × 2–4	3–5(–6) × 2–3	4–6 × 2–4	3–4 × 1–3	3–5 × 1–2
Growth on MEA for 8 d at 25 °C	24 mm in 4 d	17 mm	17 mm/d	5 mm in 4 d	50 mm	60 mm	52 mm
Colony colour	Olivaceous	Dark-olivaceous	Olivaceous-brown	–	Umber-brown	Olivaceous	Umber-brown
Teleomorph	Unknown	Present	Present	Unknown	Unknown	Unknown	Unknown
Synanamorph	Absent	Absent	Absent	–	Absent	Absent	*Hyalorhinocladiella*-like
Host	*Pinus* spp.	*Pinus* spp.	*Pinus* spp.	*Pinus* spp.	*P. yunnanensis*, *P. kesiya*	*P. kesiya*, *Pinus* sp.	*Larix olgensis*, *P. yunnanensis*
Insect	*Hylastes* sp., *Dendroctonus valens*	*T. yunnanensis*, *D. valens*	*Tomicus*, *Hylastes*, *Ips*, *Orthotomicus*, *D. valens*	*D. valens*	*Hylurgops major*	Unknown	*Polygraphus verricifrons*, *Ips subelongatus*
Distribution	South Africa, New Zealand, United Kingdom, Canada, China	Thailand, China, Japan	Japan, Korea, China	Canada, Japan, China	China	China	China
References	Wingfield & Marasas 1983, Jacobs et al. 2005 [d], Lu et al. 2009a, b	Zhou et al. 2000 [d], Yamaoka et al. 2007, 2008, Lu et al. 2009a, Masuya et al. 2009	Kim et al. 2005a [d], Masuya et al. 2005, 2009, Lu et al. 2009a	Jacobs et al. 2005 [d], Lu et al. 2009b	Present study	Present study	Present study

Media from which structures were obtained for measurements: [a] Oatmeal agar; [b] MEA; [c] Sterilised wood or agar emended with wood pieces.
[d] References from which measurements were used in this table.

PCR reactions of 25 µL, containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM of each primer, and 2.5 U/µL Taq-polymerase enzyme were performed on a thermal cycler (Mastercycler® Perkin Elmer Corporation, MA, USA). The PCR conditions were the same as those described by Paciura et al. (2010), except that annealing temperatures varied between 54–62 °C, depending on the primers used. The PCR products were visualised under UV light on a 2 % agarose gel stained with Ethidium bromide. The PCR fragments were cleaned using Sephadex® G-50 (Sigma-Aldrich, Amersham Biosciences Limited, Sweden), following the manufacturer's protocols.

The purified PCR fragments were sequenced, using 10 µL volume per sequencing reaction, containing Big Dye™ Terminator v3.0 cycle sequencing premix kit (Applied Biosystems) and the primers listed above for each gene region. The PCR sequencing fragments were purified with Sephadex® G-50 and analyzed using an ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis

The sequences obtained were assembled using MEGA v4.1 (Tamura et al. 2007). Contigs were subjected to BLAST searches on NCBI GenBank, and published sequences of closely related species were downloaded. Datasets were aligned online using the E-INS-i strategy in the online version of MAFFT v6 (Katoh & Toh 2008).

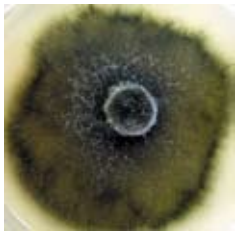
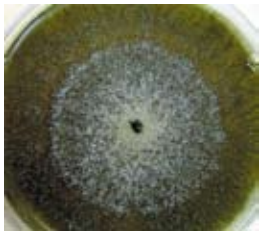
Sequence data for the ITS2-LSU, β-tubulin and EF-1α gene regions are commonly combined for phylogenetic analyses of *Leptographium* species. However, in several instances sequences for all three gene regions of a single reference isolate or species are not available from GenBank. Combining the datasets would have required the exclusion of reference species generated in other studies, from our analyses. This was avoided by analysing the gene regions separately. Furthermore, only one isolate per species and one isolate per unknown taxon were included in the three large datasets of the respective gene regions, to incorporate as many as possible species in the analyses. After analyses of the large datasets had revealed the *Leptographium/Grosmannia* group in which unknown taxa resided, smaller datasets for that specific group were compiled. These included all available sequences for the unknown taxa and those of closely related species in the respective species groups. Using these smaller datasets, more reliable alignments could be achieved for the extremely variable β-tubulin and EF-1α regions. Including all available isolates per species also served to illustrate variability within species, an aspect often overlooked when only one or two isolates per species are included in analyses. All datasets were subjected to maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses.

MP analyses were done in the Windows version of PAUP 4.01b (Swofford 1998). A total of 1 000 heuristic replicates of random sequence addition were performed using the tree-bisection-recognition (TBR) algorithm for branch swapping, and treating gaps as missing data. Branch support was assessed by 1 000 bootstrap replicates.

For ML, the best substitution models were determined independently for each dataset using the Akaike Information Criterion (AIC) in Modeltest v3.7 (Posada & Crandall 1998). The ML analyses were conducted online in the program PhyML v3.0 (Guindon & Gascuel 2003), using 1 000 bootstrap replicates to evaluate branch support.

For Bayesian analyses, the most appropriate substitution models were selected for the respective datasets using the AIC in MrModeltest v2.3 (http://www.abc.se/~nylander/). Bayesian inference was conducted in MrBayes v3.1 (Huelsenbeck &

Table 3 Morphological characteristics of Group B, including *L. sinoprocerum*, *L. bhutanense*, and the newly described Taxa 4 and 5. All measurements, including those for the two previously described species, were done in the present study, and are given in µm unless indicated otherwise.

	L. sinoprocerum [a]	*L. bhutanense* [a]	Taxon 4: *L. gracile* [a]	Taxon 5: *L. latens* [a]
Conidiophore (l)	(140–)189–294(–337)	(232–)303–527(–642)	(380–)473–859(–1050)	(144–)152–256(–404)
Conidiogenous apparatus (l)	(48–)54–77(–91)	(71–)77–105(–132)	(68–)78–157(–292)	(29–)35–85(–129)
Rhizoids	Present	Present	Present	Present
Conidium shape	Oblong to subclavate	Oblong to obovoid	Oblong to obovoid with truncated bases	Broadly ellipsoidal to obovoid
Conidium size (l × w)	3–6 × 2–3	3–6 × 1–3	3–5 × 1–3	7–10 × 2–4
Growth on MEA for 8 d at 25 °C	53 mm	39 mm	50 mm	50 mm
Colony on MEA plates				
Colony colour	Olivaceous with wide aerial, white mycelial, concentric rings	Olivaceous with wide honey concentric ring, followed by a darker olivaceous ring at the edge	Pale olivaceous with wide white concentric ring	Citrine with narrow olivaceous ring in middle, followed by wide, lighter citrine concentric ring
Host	*Pinus* spp.	*Pinus wallichiana*	*Pinus armandii*	*Picea koraiensis*, *P. armandii*
Insect	*Dendroctonus valens*	*Hylobitelus chenupdorjii*	*Pissodes* sp.	*Ips yunnanesis*, *Pissodes* sp.
Distribution	China	Bhutan	China	China
References	Lu et al. 2008, 2009a	Zhou et al. 2008	Present study	Present study

Media from which structures were obtained for measurements: [a] Oatmeal agar.

Ronquist 2001) using the Markov chain Monte Carlo (MCMC) approach with 5 000 000 generations, to estimate posterior probabilities. The burn-in value for each dataset was determined in Tracer v1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/).

RESULTS

Isolates

A total of 108 isolates representing *Leptographium* spp. were collected from bark beetles and their galleries in China (Table 1). The majority of these were from conifers and particularly *Pinus* spp. A relatively small number of isolates were from *Larix olgensis* or *Picea koraiensis* and two isolates were from the hardwood tree *Pistacia chinensis*. All isolates were tolerant to and growing on 0.05 % cycloheximide in the isolation medium.

Morphology

Based on culture characteristics and micromorphology, nine morphological groups of isolates (taxa) could be distinguished. Three isolates from each group were selected for sequencing. However, for taxon 6 only two, and for taxon 9 only one isolate was available (Table 1). All isolates produced *Leptographium*-like anamorphs in culture, and none of the attempted crosses produced ascomata. Morphological characters of all taxa were compared with those published for related species in Tables 2–4. Taxa 4 and 5 were difficult to distinguish from *L. bhutanense* and *L. sinoprocerum* based only on descriptions. These two taxa were thus compared with the ex-type cultures of *L. bhutanense* and *L. sinoprocerum* (Table 3).

DNA sequencing

Amplification of the ITS2-LSU region yielded fragments of ± 1 000 bp. The β-tubulin gene region was ± 500 bp in length and included exons 4, 5, part of exon 6, interspersed with introns 3–5. The EF-1α gene fragments were ± 1 000 bp, and included exon 3, part of exon 4, and introns 2 and 3. The length of the final datasets, after the ends of sequences were trimmed and alignments had been completed, are presented in Table 5 together with other parameters used and statistical values resulting from the analyses. GenBank accession numbers of published sequences are shown in the phylogenetic trees, while accession numbers of sequences obtained in the present study are presented in Table 1.

Phylogenetic analyses

For each of the sequence datasets, MP, ML and Bayesian analyses resulted in trees with similar topologies. Phylograms obtained with ML are presented for all the datasets (Fig. 1–3), with nodal support obtained from ML, MP and Bayesian analyses indicated on the trees. Results of these analyses confirmed that the nine morphological groups in which the Chinese isolates resided, represented nine distinct taxa. These taxa grouped with known *Leptographium* species in four species groups, labelled A to D in the phylogenetic trees (Fig. 1–3).

Taxon 1 of the Chinese isolates was related to *Grosmannia yunnanense*, *G. koreana*, *L. truncatum* and *Hyalorhinocladiella pinicola* in Group A for all the gene regions (Fig. 1–3). In both the β-tubulin and EF-1α subsets (Group A, Fig. 2, 3), taxon 1 represented a distinct, well-supported lineage.

Taxon 2 grouped in a lineage with *L. procerum*, *L. sinoprocerum*, *L. bhutanense*, *L. pini-densiflorae* and *L. profanum* based on ITS2-LSU (Group B, Fig. 1). However, based on β-tubulin and EF-1α, taxon 2 formed part of Group A (Fig. 2, 3), grouping close to *L. truncatum*, *G. koreana* and *H. pinicola.* The lineage formed by isolates of taxon 2 in the EF-1α tree had good statistical support (Fig. 3).

Taxon 3 resided in Group B based on the ITS2-LSU (Fig. 1), closely related to *L. procerum*, *L. sinoprocerum*, *L. bhutanense*, *L. pini-densiflorae* and *L. profanum*. In the β-tubulin (Fig. 2) and EF-1α (Fig. 3) analyses, taxon 3 resided in Group A. The taxon 3 lineage had good bootstrap support in the EF-1α (Fig. 3) analyses. Although the β-tubulin (Fig. 2) does not separate taxa 2 and 3, sequences of the two taxa differed in 10 bp in this gene region.

Taxa 4 and 5 formed part of Group B based on ITS2-LSU, β-tubulin and EF-1α analyses (Fig. 1–3), together with

Table 4 Morphological characteristics of Group C, including Taxa 6, 7 and 8 and their closest relatives. All measurements in µm unless indicated otherwise.

	L. bistatum [b, c]	Taxon 6: *L. pistaciae* [a]	*G. americana* [b, c]	*L. abietinum* [b]	Taxon 7: *L. curviconidium* [a]	Taxon 8: *L. altius* [a]
Conidiophore (l)	200–927	(219–)279–630(–1068)	149–731	74–535(–870)	(126–)175–444(–901)	(173–)188–268(–369)
Conidiogenous apparatus (l)	42–69	(60–)74–108(–119)	25–77	(25–)45–50(–100)	(46–)95–120(–138)	(37–)60–126(–169)
Rhizoids	Present	Present	Absent	Present	Present	Present
Conidium shape	Oblong to ovoid, truncate bases, distinctly curved	Ellipsoidal to obovoid, slightly curved	Obovoid to allantoid, subtruncated bases	Clavate, truncate bases, curved	Allantoid with truncate bases, curved	Obovoid, elongated with truncated bases
Conidium size (l × w)	3–6 × 1–2	3–5 × 2–4	3.5–22 × 1–3	(3–)4–5(–7) × 1–2	9–12 × 3–4	(5–)6–10(–11) × 2–4
Growth on MEA for 8 d at 25 °C	27 mm	50 mm	31 mm at 20 ºC	39 mm	52 mm	44 mm
Teleomorph	Absent	Absent	Present	Absent	Absent	Absent
Synanamorph	*Sporothrix*-like	Absent	Absent	Absent	*Hyalorhinocladiella*-like	Absent
Colony colour	Umber	Greenish olivaceous	–	Cartridge buff	Sudan-brown	Cream-buff
Host	*P. radiata*	*Pistacia chinensis*	*Larix decidua*	*Picea*, *Pseudotsuga* spp.	*Picea koraiensis*	*P. koraiensis*, *L. olgensis*
Insect	–	–	*D. simplex*	*Dendroctonus* spp.	*I. typographus*	*I. cembrae*
Distribution	Korea	China	USA	USA, Canada	China	China
References	Kim et al. 2004 [d]	Present study	Jacobs et al. 1997 [d]	Kendrick 1962; Jacobs & Wingfield 2001 [d]	Present study	Present study

Media from which structures were obtained for measurements: [a] Oatmeal agar; [b] MEA; [c] Sterilized wood or agar emended with wood pieces.
[d] References from which measurements were used in this table.

Table 5 Parameters and statistics for the phylogenetic analyses.

Dataset	No. of taxa	No. of [a]char	Outgroup	MP						ML				MrBayes	
				[b]PIC	No. of trees	Tree length	[c]CI	[d]RI	[e]HI	[f]Subst. model	[g]Pinvar	[h]G	[i]Nst	[f]Subst. model	Burn-in
ITS2 & partial LSU	66	651	*Ophiostoma* spp.	189	273	745	0.660	0.886	0.339	TrN93+I+G	0.457	0.710	6	GTR+I+G	450
β-tubulin	50	482	*Ophiostoma* spp.	319	123	1328	0.555	0.824	0.445	TrN93	0	–	6	GTR+I+G	350
EF-1α	40	828	*Ophiostoma* spp.	562	6	2376	0.606	0.840	0.393	GTR+I+G	0.221	1.562	6	GTR+I+G	400
β-tubulin, Group A	23	363	Midpoint rooted	17	1	19	1	1	0	TrN93	0	–	6	HKY	250
β-tubulin, Group B	16	362	*L. pini-densiflorae*	42	1	46	0.978	0.985	0.022	GTR	0	–	6	GTR	250
β-tubulin, Group C	18	480	Midpoint rooted	315	1	458	0.947	0.985	0.052	GTR+I	0.490	–	6	GTR+I	350
EF-1α, Group A	21	560	Midpoint rooted	47	2	53	0.962	0.989	0.038	HKY+I	0.718	–	2	HKY+I	270
EF-1α, Group B	16	725	*L. pini-densiflorae*	164	3	204	0.960	0.971	0.039	HKY+I	0.394	–	6	HKY	100
EF-1α, Group C	14	607	Midpoint rooted	182	2	218	0.963	0.988	0.036	GTR+G	0	0.530	6	GTR+G	250

[a]char = characters
[b]PIC = number of parsimony informative characters
[c]CI = consistency index
[d]RI = retention index
[e]HI = homoplasy index
[f]Subst. model = best fit substitution model
[g]Pinvar = proportion of invariable sites
[h]G = gamma shape parameter
[i]Nst = number of substitution rate categories

species such as *L. procerum*. The β-tubulin tree (Fig. 2), did not distinguish Taxa 4 and 5 from each other, although there are differences between the two species in 5 bp positions. They formed a strongly supported monophyletic lineage together with *L. sinoprocerum* and *L. bhutanense*. However, in the EF-1α subtree (Group B, Fig. 3), the four species were clearly distinguished from each other.

In trees obtained from all three gene regions, taxon 6 formed part of group C (Fig. 1–3), closely related to *L. bistatum*. However, the two Chinese isolates formed a distinct, well-supported lineage in both the EF-1α and β-tubulin subtrees (Fig. 2, 3).

Taxa 7 and 8 consistently formed part of group C (Fig. 1–3) and are closely related to *G. americana* and *L. abietinum*. EF-1α and β-tubulin data distinguished clearly between these two taxa and the related species with good statistical support (Group C, Fig. 2, 3).

Taxon 9 (represented by only one isolate) formed a distinct, monophyletic lineage (D) together with *L. pineti* in all the trees (Fig. 1–3). The Chinese isolate had an EF-1α sequence identical to *L. pineti*, and differed in only 2 bp positions from *L. pineti* in the ITS2 and β-tubulin sequences.

Taxonomy

Based on the results of the phylogenetic analyses based on DNA sequence data and the morphological comparisons, eight novel *Leptographium* spp. could be distinguished. Furthermore, *L. pineti* was shown to be present in the collection from China. The new species are characterised as follows:

Taxon 1

Leptographium conjunctum Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516733; Fig. 4a–f

Conidiophorae singulae vel ad quaternae aggregatae (72–)146–349(–485) µm longae, sine structuris rhizoidiformibus. Stipae cylindricae simplices 2–7-septatae (35–)104–270(–385) µm longae, infra ramos primarios 2–5 µm latae, cellula apicali non tumida. Apparatus conidiogenus (37–)42–85(–100) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3, 17–20(–22) µm longi 4–5 µm lati. Cellulae conidiogenae discretae, 2–3 in quoque ramo (25–)26–35(–40) µm longae 2–4 µm latae. Conidia hyalina non septata oblonga vel obovoidea basibus truncatis 4–6 × 2–4 µm. Coloniae succineae, crescunt optime in 25 °C in 2 % MEA ad 50 mm diametro in 8 diebus.

Etymology. Name refers to the very small conidiophores that are closely joined together.

Conidiophores occurring in groups up to four, arising directly from the mycelium, erect, macronematous, mononematous, (72–)146–349(–485) µm in length (Fig. 4a, d), *rhizoid*-like structures absent. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 2–7-septate, (35–)104–270(–385) µm long, 2–5 µm wide below primary branches, apical cell not swollen, 2–5 µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (37–)42–85(–100) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2–3, pale olivaceous, smooth, cylindrical, 0–2-septate, 17–20(–22) µm long and 4–5 µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, 0–1-septate, (16–)18–20(–23) µm long, 2–4 µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (25–)26–35(–40) µm long and 2–4 µm wide (Fig. 4b, e). *Conidia* hyaline, aseptate, oblong to obovoid with truncate bases, 4–6 × 2–4 µm (Fig. 4c, f). *Conidial droplet* hyaline at first becomes cream-coloured with age.

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 50 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies amber-brown, colony margin smooth. Hyphae submerged in agar with very little aerial mycelium except in the edges of the colony, greenish olivaceous to olivaceous, smooth, straight, occasionally constricted at the septa, 3–8 µm wide.

Specimens examined. China, Chuxiong, Yunnan, isolated from *Pinus yunnanensis*, infested by *Hylurgops major*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 59987, culture ex-type CMW 12473 = CBS 123631; PREM 59989, paratype, culture ex-paratype CMW 12452 = CBS 123633; and isolated from *Pinus kesiya* PREM 59988, paratype, culture ex-paratype CMW 12449 = CBS 123632.

Notes — *Leptographium conjunctum* is most closely related to *G. yunnanense* (Fig. 3, 4). However, the conidiophores of *L. conjuntum* reach much greater lengths (< 485 µm) than those

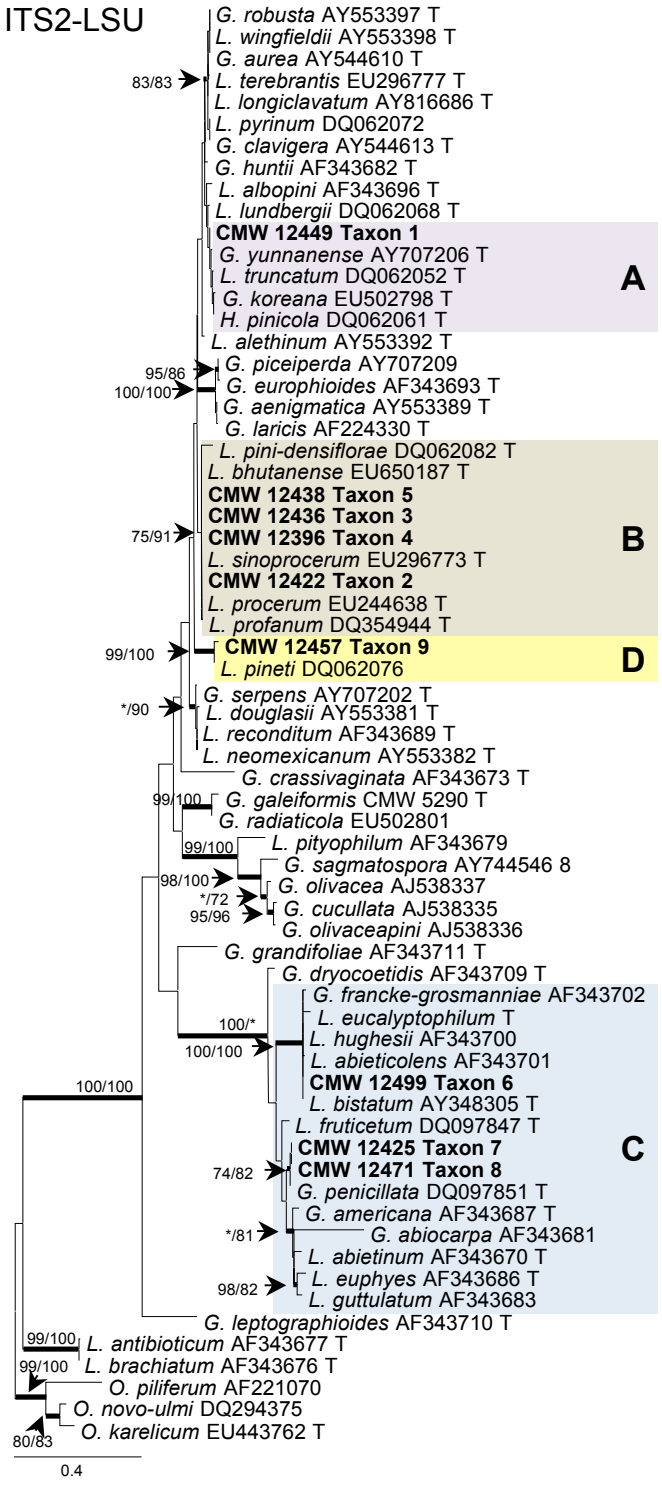


Fig. 1 ML tree obtained from ITS2-LSU sequence data of *Leptographium* isolates from China (**bold** type). Dark branches indicate posterior probabilities > 0.95. Bootstrap values at nodes are for 1 000 replicates (Maximum Likelihood/Maximum Parsimony). * are bootstrap values < 75%.

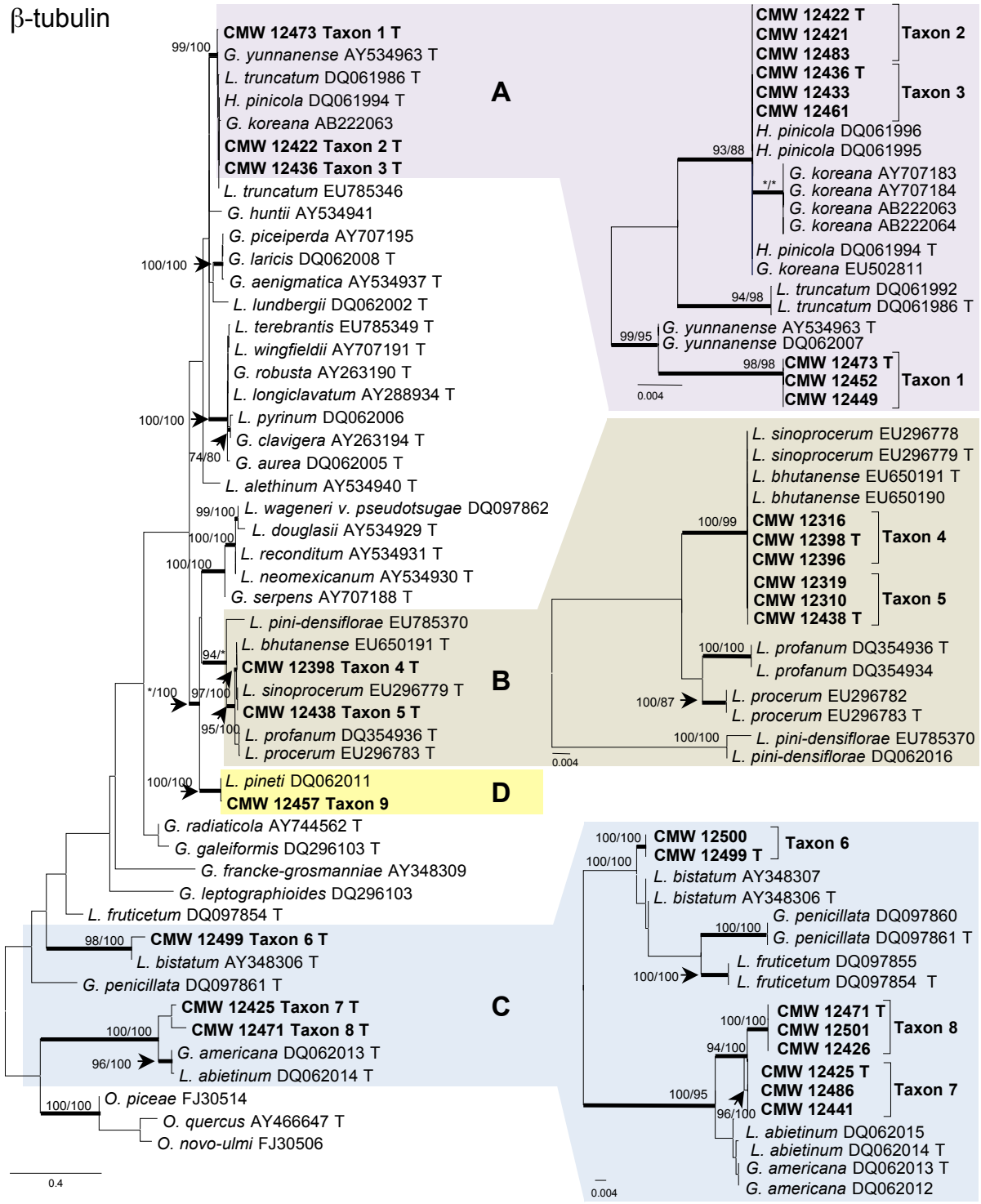


Fig. 2 ML tree obtained from β-tubulin sequence data of *Leptographium* isolates from China (**bold** type). Dark branches indicate posterior probabilities > 0.95. Bootstrap values at nodes are for 1 000 replicates (Maximum Likelihood/Maximum Parsimony). * are bootstrap values < 75%.

of *G. yunnanense* (< 233 µm), and its cultures grow up to 50 mm diam on MEA in 7 d, with those of *G. yunnanense* reaching only 13 mm in the same time (Zhou et al. 2000).

Taxon 2

Leptographium celere Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516734; Fig. 4g–l

Conidiophorae singulae vel ad ternae aggregatae (120–)239–950(–1365) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 1–12-septatae (66–)130–798(–1150) µm longae, infra ramos primarios 3–5 µm latae, cellula apicali non tumida. Apparatus conidiogenus 58–98(–115) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3, 15–20(–25) µm longi 3–5 µm lati. Cellulae conidiogenae discretae, 2–3 in quoque ramo 13–15(–20) µm longae 2–3 µm latae. Conidia hyalina non septata obovoidea basibus truncatis apicibus rotundatis 3–4 × 1–3 µm. Coloniae olivaceae, crescunt optime in 25 °C in 2 % MEA ad 60 mm diametro in 8 diebus.

Etymology. Name reflects the colony growth in the fungus that begins as a rapidly growing white mycelium that darkens with age.

Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (120–)239–950(–1365) µm in length (Fig. 4g, j), *rhizoid*-like structures present. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 1–12-septate, (66–)130–798 (–1150) µm long, 3–5 µm wide below primary branches, apical cell not swollen, 2–5 µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* 58–98(–115) µm, excluding

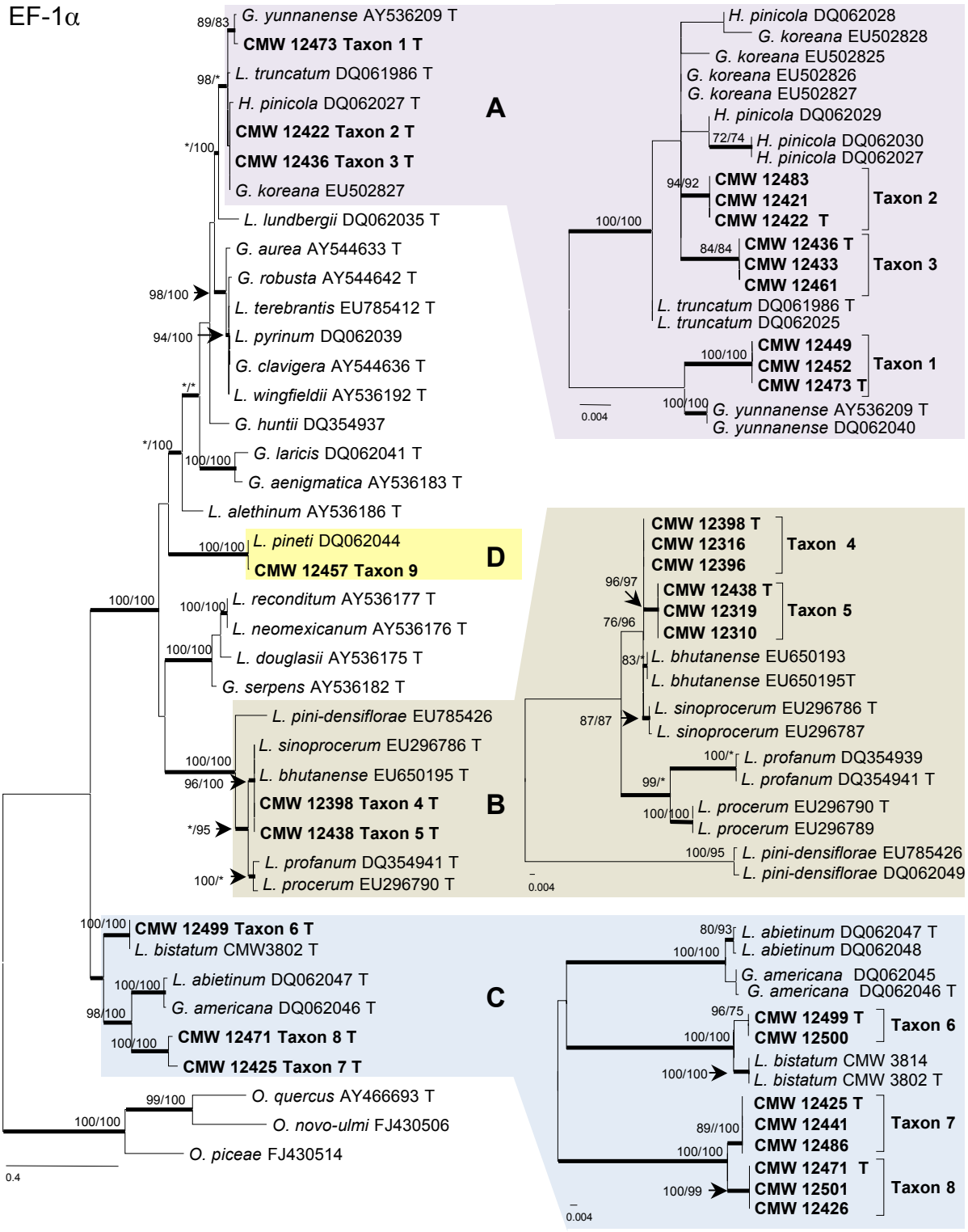


Fig. 3 ML tree obtained from EF-1α sequence data of *Leptographium* isolates from China (**bold** type). Dark branches indicate posterior probabilities > 0.95. Bootstrap values at nodes are for 1 000 replicates (Maximum Likelihood/Maximum Parsimony). * are bootstrap values < 75%.

the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2–3, pale olivaceous, smooth, cylindrical, aseptate, 15–20(–25) µm long and 3–5 µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, 0–1-septate, 16–18(–19) µm long, 2–4 µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, 10–12(–14) µm long, 2–3 µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, 13–15(–20) µm long and 2–3 µm wide (Fig. 4h, k). *Conidia* hyaline, aseptate, obovoid with truncate bases, 3–4 × 1–3 µm (Fig. 4i, l). *Conidial droplet* hyaline at first, becoming cream-coloured with age.

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 60 mm in diam 8 d. No growth below 5 °C or above 35 °C. Colonies olivaceous, colony margin smooth. Hyphae submerged in agar with abundant aerial mycelium, greenish olivaceous to olivaceous, smooth, straight, occasionally constricted at the septa, 3–6 µm wide.

Specimens examined. China, Chuxiong, Yunnan, isolated from *Pinus kesiya*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 59990, culture ex-type CMW 12422 = CBS 123628; PREM 59991, paratype, culture ex-paratype CMW 12421 = CBS 123629; Yanji, Jilin, isolated from *Pinus* sp. July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 59992, paratype, culture ex-paratype CMW 12483 = CBS 123630.

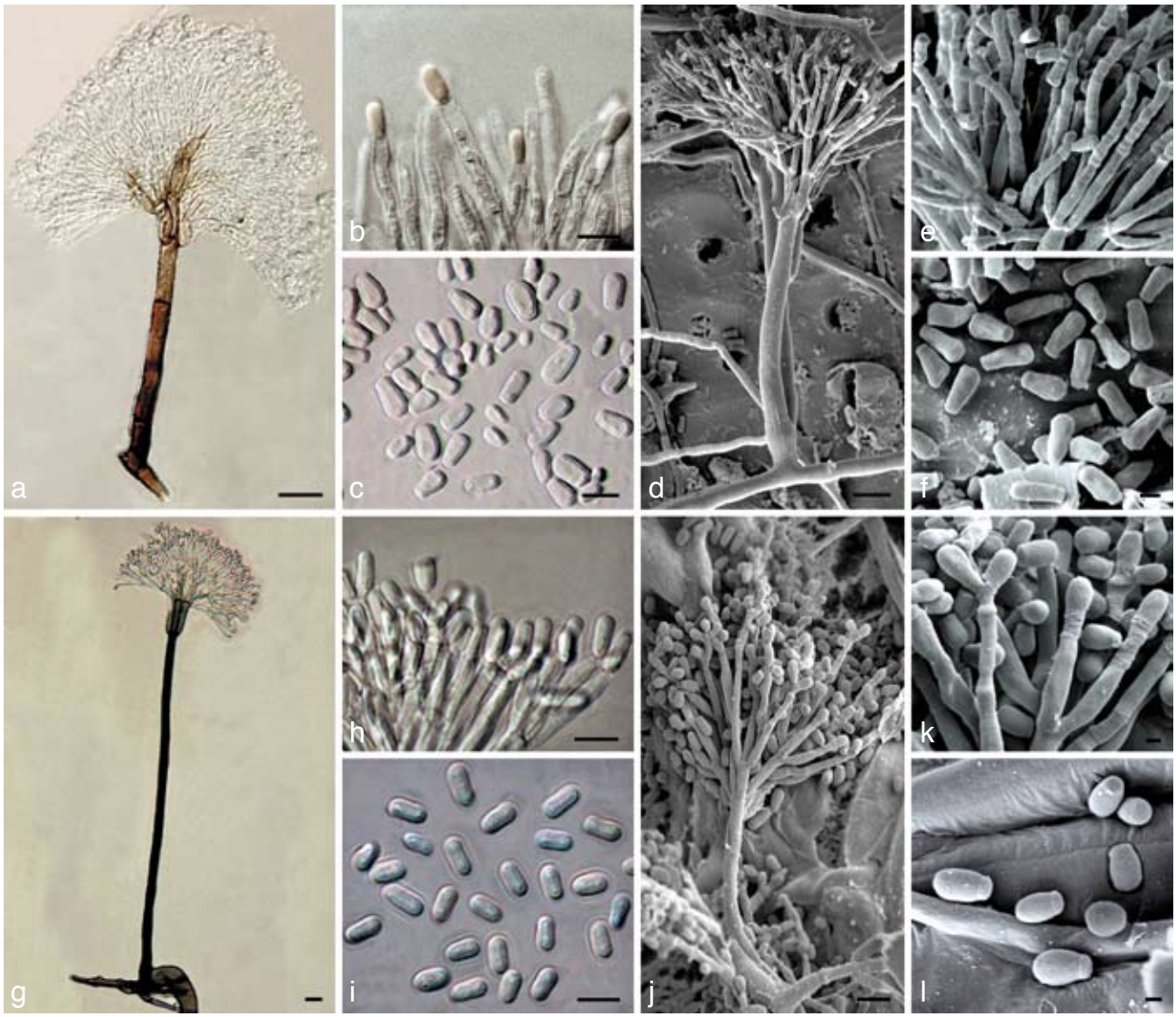


Fig. 4 a–f: *Leptographium conjunctum* sp. nov. a, d. Conidiophore; b, e. conidiogenous cells; c, f. conidia. — g–l: *L. celere* sp. nov. g, j. conidiophore; h, k. conidiogenous cells; i, l. conidia. — Scale bars: a, d, g, j = 20 µm; b, c, h, i = 5 µm; e, f, k, l = 1 µm.

Notes — *Leptographium celere* has much longer conidiophores and slightly shorter conidia in comparison to related species such as *G. koreana*, *H. pinicola* (Jacobs et al. 2005, Kim et al. 2005a, Masuya et al. 2005) and *L. manifestum* (Taxon 3, this study). Furthermore, *L. celere* and *L. manifestum* both form rhizoid-like structures at the bases of their conidiogenous apparatus, which are absent in both *G. koreana* and *H. pinicola.*

Taxon 3

Leptographium manifestum Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516735; Fig. 5a–g

Conidiophorae singulae vel ad quaternae aggregatae (83–)103–243(–363) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 1–3-septatae (33–)49–170(–269) µm longae, infra ramos primarios 3–6(–7) µm latae. Apparatus conidiogenus (36–)50–77(–100) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3, non septati, (8–)10–18(–22) µm longi 2–6 µm lati. Cellulae conidiogenae discretae, 1–2 in quoque ramo 7–8(–11) µm longae 1–2 µm latae. Conidia hyalina non septata elongata extremis acutis 3–5 × 1–2 µm. Adest synanamorpha *Hyalorhinocladiella* conidiis hyalinis non septatis, subfalcatis ellipsoideis 7–8(–12) × 2–3 µm. Coloniae succineae, crescunt optime in 25 °C in 2 % MEA ad 52 mm diametro in 8 diebus.

Etymology. Name reflects the conspicuous production of conidiophores on the medium.

Conidiophores occurring singly or in groups of up to four, arising directly from the mycelium, erect, macronematous, (83–)103–243(–363) µm in length (Fig. 5a, d), *rhizoid*-like structures present. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 1–3-septate, (33–)49–170(–269) µm long, 3–6(–7) µm wide below primary branches, apical cell not swollen, 3–6 µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (36–)50–77(–100) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2–3, pale olivaceous, smooth, cylindrical, aseptate, (8–)10–18(–22) µm long and 2–6 µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, aseptate, 10–13 µm long, 3–4 µm wide. *Conidiogenous cells* discrete, 1–2 per branch, cylindrical, tapering slightly at the apex, 7–8(–11) µm long and 1–2 µm wide (Fig. 5b, e). *Conidia* hyaline, aseptate, elongated with pointed ends, 3–5 × 1–2 µm (Fig. 5c, f). Presence of *Hyalorhinocladiella*-like synanamorph with conidia hyaline, aseptate, slightly curved, ellipsoid, 7–8(–12) × 2–3 µm (Fig. 5g).

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 52 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies umber-brown. Colony margin smooth with abundant aerial mycelium. Hyphae greenish olivaceous to olivaceous, smooth, straight, 4–5(–6) µm wide.

Specimens examined. China, Wangqing, Jilin, isolated from *Larix olgensis* infested by *Ips subelongatus*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 59998, culture ex-type CMW 12436 = CBS 123622; PREM 59999, paratype, culture ex-paratype CMW 12433 = CBS 123604; Lufeng, Yunnan,

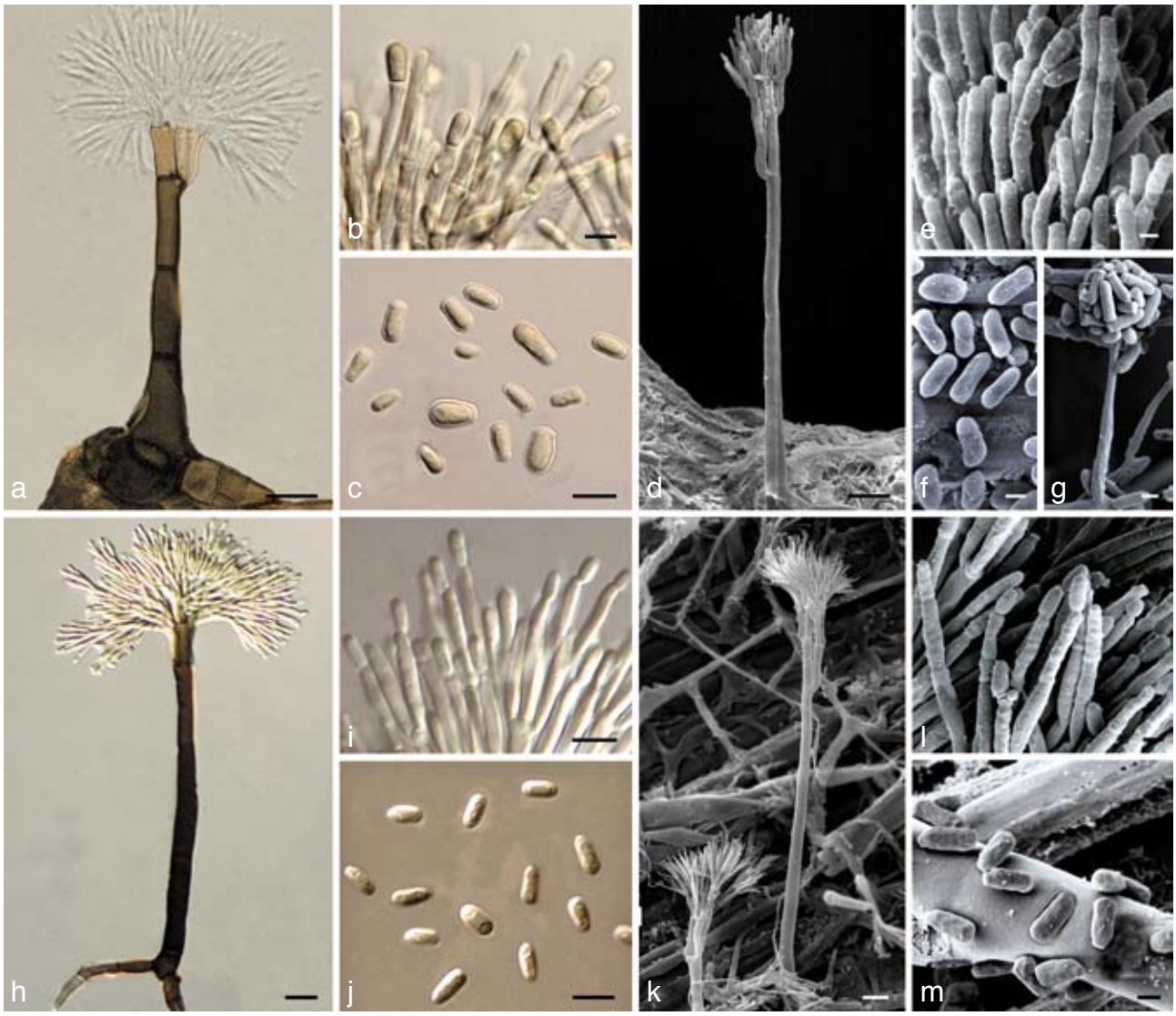


Fig. 5 a–f: *Leptographium manifestum* sp. nov. a, d. Conidiophore; b, e. conidiogenous cells; c, f. conidia. — g. *Hyalorhinocladiella*-like synanamorph. — h–m: *L. gracile* sp. nov. h, k. conidiophore; i, l. conidiogenous cells; j, m. conidia. — Scale bars: a, d, h, k = 10 µm; b, c, i, j = 5 µm; e–g, l, m = 1 µm.

isolated from *Pinus yunnanensis* infested by *Polygraphus verrucifrons*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 60000, paratype, culture ex-paratype CMW 12461 = CBS 123606.

Notes — *Leptographium manifestum* has a distinctive *Hyalorhinocladiella*-like synanamorph with curved conidia, which differ from those in closely related species such as *G. koreana* and *H. pinicola* (Kim et al. 2005a, Masuya et al. 2005, Jacobs et al. 2005). Other distinguishing characteristics of *L. manifestum* are discussed above in the notes for *L. celere*.

Taxon 4

Leptographium gracile Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516736, Fig. 5h–m

Conidiophorae singulae vel ad ternae aggregatae (380–)473–859(–1050) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 3–9-septatae (269–)332–771(–956) µm longae, infra ramos primarios 6–10(–13) µm latae. Apparatus conidiogenus (68–)78–157(–292) µm, ramis cylindricis 2-seriatis. Rami primarii 2–3 non septati, (10–)13–25(–26) µm longi 3–8 µm lati. Cellulae conidiogenae discretae, 2–3 in quoque ramo 7–11(–16) µm longae 1–2 µm latae. Conidia hyalina non septata oblongo-obovoidea basibus truncatis 3–5 × 1–3 µm. Coloniae pallide olivaceae, crescunt optime in 25 °C in 2 % MEA ad 50 mm diametro in 8 diebus.

Etymology. Name reflects the simple and thin conidiophores.

Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (380–)473–859(–1050) µm in length (Fig. 5h, k), *rhizoid*-like structures present. *Stipes* olivaceous, not constricted, cylindrical, simple, 3–9-septate, (269–)332–771(–956) µm long, 6–10(–13) µm wide below primary branches, apical cell not swollen, 5–11(–12) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (68–)78–157(–292) µm, excluding the conidial mass, with 2 series of cylindrical branches. *Primary branches* 2–3, olivaceous, smooth, cylindrical, aseptate, (10–)13–25(–26) µm long and 3–8 µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* pale olivaceous, aseptate, (7–)10–15(–22) µm long, 2–5 µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, (8–)11(–15) µm long, 2–5 µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, 7–11(–16) µm long and 1–2 µm wide (Fig. 5i, l). *Conidia* hyaline, aseptate, oblong obovoid with truncate bases, 3–5 × 1–3 µm (Fig. 5j, m).

Culture characteristics — Conidial droplet hyaline at first, becoming cream-coloured with age. Colonies with optimal growth at 25 °C on MEA, reaching 50 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies pale olivaceous, with a wide white concentric ring, colony margin smooth. Hyphae submerged in agar with very little aerial mycelium except in the edges of the colony, greenish olivaceous to olivaceous, smooth, straight, occasionally constricted at the septa, 4–8 µm wide.

Specimens examined. China, Midu, Yunnan, isolated from *Pinus armandii*, infested by *Pissodes* sp. July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 59995, culture ex-type CMW 12398 = CBS 123623; PREM 59996, paratype, culture ex-paratype CMW 12396 = CBS 123624; Lijiang, Yunnan, isolated from *Pinus armandii*, infested by *Pissodes* sp. July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 59997, paratype, culture ex-paratype CMW 12316 = CBS 123625.

Notes — *Leptographium gracile* is most closely related to *L. sinoprocerum*, *L. bhutanense* (Lu et al. 2008, Zhou et al. 2008) and *L. latens* (Taxon 5, present study). *Leptographium bhutanense* can be distinguished from all three these species by its slower growth in culture. The ranges of conidiophore length for the four species overlap, with those of *L. gracile* reaching the longest lengths (up to 1 050 µm). The conidiophores *L. sinoprocerum* and *L. latens* are the shortest, respectively reaching 337 and 404 µm (Table 3). The conidia of *L. latens* tend to be longer than those of the other three species that have similar sizes (Table 3).

Taxon 5

Leptographium latens Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516737; Fig. 6a–f

Conidiophorae singulae vel ad quinae aggregatae (144–)152–256(–404) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 3–4-septatae (88–)100–198(–320) µm longae. Apparatus conidiogenus (29–)35–85(–129) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3 non vel ad 2-septati, 16–22(–24) µm longi (6–)7–8(–9) µm lati. Cellulae conidiogenae discretae, 2–3 in quoque ramo (11–)15–20(–23) µm longae 1–3 µm latae. Conidia hyalina non septata oblongo-obovoidea basibus truncatis 7–10 × 2–4 µm. Coloniae citrinae medio annulo olivaceo, postea annulo concentrico citrino, crescunt optime in 25 °C in MEA ad 50 mm diametro in 8 diebus.

Etymology. Name chosen to reflect the growth habit of the fungus in culture where the mycelium is typically immersed in the agar.

Conidiophores occurring singly or in groups of up to five, arising directly from the mycelium (Fig. 6a, d), erect, macronematous, mononematous, (144–)152–256(–404) µm in length, *rhizoid-like* structures present. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 3–4-septate, (88–)100–198(–320) µm long, (6–)7–10(–13) µm wide below primary branches, apical cell not swollen, (5–)6–9(–10) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (29–)35–85(–129) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches* 2–3, pale olivaceous, smooth, cylindrical, 0–2 septate, 16–22(–24) µm long and (6–)7–8(–9) µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, aseptate, 14–15(–19) µm long, 4–5(–7) µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, (14–)16–19(–21) µm long, (3–)4–5(–6) µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (11–)15–20(–23) µm long and 1–3 µm wide (Fig. 6b, e). *Conidia* hyaline, aseptate, broadly ellipsoidal to ovoid, 7–10 × 2–4 µm (Fig. 6c, f). *Conidial droplet* hyaline at first, becoming cream-coloured (19 "f) with age.

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 50 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies citrine with an olivaceous, thin ring at the middle, followed by a lighter citrine, wide concentric ring; colony margin smooth. Hyphae submerged in agar with aerial mycelium concentrated in the centre of the colony, greenish olivaceous, smooth, straight, occasionally constricted at the septa, 3–6 µm wide.

Specimens examined. China, Midu, Yunnan, isolated from *Picea koraiensis* infested by *Ips typographus*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 60007, culture ex-type CMW 12438 = CBS 124023; Lijiang, Yunnan, isolated from *Pinus armandii* infested by *Pissodes* sp. July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 60008, paratype, culture ex-paratype CMW 124310 = CBS 123615; Midu, Yunnan, isolated from *Pinus armandii* infested by *Pissodes* sp. July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 60009, paratype, culture ex-paratype CMW 12319 = CBS 123616.

Notes — Comparisons with other species discussed above, under *L. gracile* (Taxon 4) and in Table 3.

Taxon 6

Leptographium pistaciae Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516738; Fig. 6g–l

Conidiophorae singulae vel ad senae aggregatae (219–)279–630(–1068) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 1–11-septatae (143–)198–528(–961) µm longae, infra ramos primarios 5–10 µm latae. Apparatus conidiogenus (60–)74–108(–119) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2 non septati, (17–)19–25(–30) µm longi 4–9 µm lati. Cellulae conidiogenae discretae, 1–2 in quoque ramo ellipsoideae vel obovoideae 3–5 × 2–4 µm. Conidia hyalina non septata subfalcata ellipsoidea vel obovoidea 3–5 × 2–4 µm. Coloniae olivaceae virescentiae, crescunt optime in 25 °C in MEA ad 50 mm diametro in 8 diebus.

Etymology. Name relates to the host *Pistacia chinensis*.

Conidiophores occurring singly or in groups of up to six, arising directly from the mycelium, erect, macronematous, mononematous, (219–)279–630(–1068) µm in length (Fig. 6g, j), *rhizoid-like* structures present. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 1–11-septate, (143–)198–528(–961) µm long, 5–10 µm wide below primary branches, apical cell not swollen, 5–9(–12) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (60–)74–108(–119) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2, pale olivaceous, smooth, cylindrical, aseptate, (17–)19–25(–30) µm long and 4–9 µm wide, arrangement of the primary branches on the stipe – type A (two branches). *Secondary branches* hyaline to pale olivaceous, aseptate, (10–)13–17(–20) µm long, 3–5(–8) µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, 12–16(–17) µm long, 2–6 µm wide. *Conidiogenous cells* discrete, 1–2 per branch, cylindrical, tapering slightly at the apex, (14–)17–22(–28) µm long and 1–2 µm wide (Fig. 6h, k). *Conidia* hyaline, aseptate, ellipsoidal to obovoid, slightly curved, 3–5 × 2–4 µm (Fig. 6i, l). *Conidial droplet* hyaline at first, becoming amber-coloured with age.

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 50 mm diam in 8 d. No growth below 5 °C and growth 2.5 mm at 35 °C. Colonies greenish olivaceous. Colony margin smooth. Hyphae submerged in agar with abundant aerial mycelium except in the edges of the colony, greenish olivaceous to olivaceous, smooth, straight, occasionally constricted at the septa, 4–6 µm wide.

Specimens examined. China, Chuxiong, Yunnan, isolated from *Pistacia chinensis*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 59993, culture ex-type CMW 12499 = CBS 123626; PREM 59994, paratype, culture ex-paratype CMW 12500 = CBS 123627.

Notes — *Leptographium pistaciae* lacks the *Sporothrix* synanamorph that is commonly found in its closest known relative, *L. bistatum* (Kim et al. 2004). The Chinese species also differs from the latter species in having slower growth, slightly curved conidia and based on its hardwood host (Table 4).

Taxon 7

Leptographium curviconidium Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516739; Fig. 7a–g

Conidiophorae singulae vel ad quaternae aggregatae (126–)175–444(–901) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 1–6-septatae (89–)92–351(–799) µm longae, infra ramos primarios (6–)8–12(–14) µm latae. Apparatus conidiogenus (46–)95–120(–138) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3 non septati, (9–)15–22(–27) µm longi (2–)4–7(–8) µm lati. Cellulae conidiogenae discretae, 2–3 in

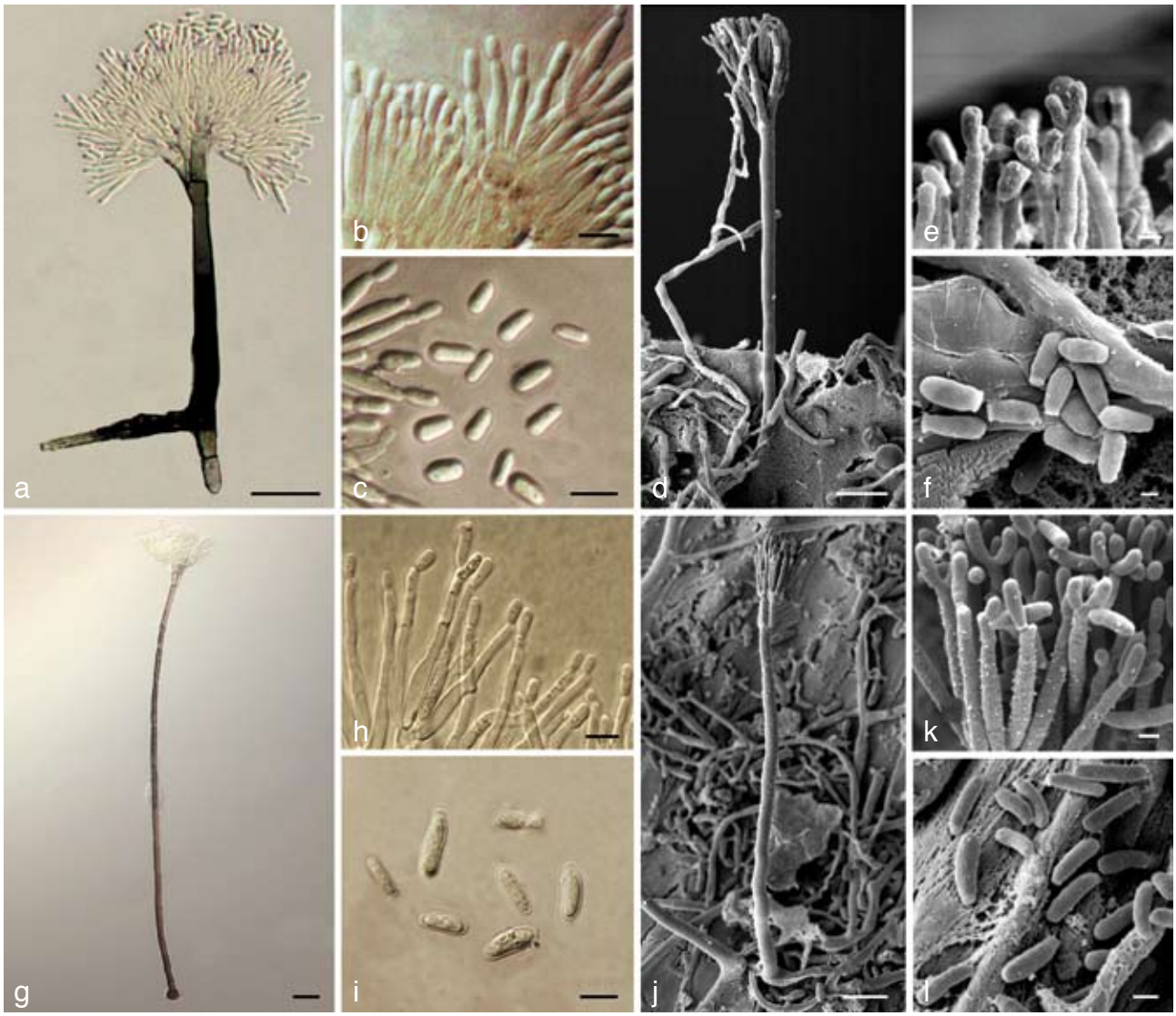


Fig. 6 a–f: *Leptographium latens* sp. nov. a, d. Conidiophore; b, e. conidiogenous cells; c, f. conidia. — g–l: *L. pistaciae* sp. nov. g, j. conidiophore; h, k. conidiogenous cells; i, l. conidia. — Scale bars: a, d, j = 20 µm; b, c, h, i = 5 µm; g = 50 µm; e, f, k, l = 1 µm.

quoque ramo 38–56(–62) µm longae 2–3 µm latae. Conidia hyalina non septata subfalcata ellipsoidea vel obovoidea 9–12 × 3–4 µm. Adest synanamorpha *Hyalorhinocladiella* conidiis oblongis vel obovoideiss 3–4 × 2–3 µm. Coloniae brunnei color 'Sudan' dictus, crescunt optime in 25 °C in MEA ad 52 mm diametro in 8 diebus.

Etymology. The name reflects the curved conidia produced by this species.

Conidiophores occurring singly or in groups of up to four, arising directly from the mycelium, erect, macronematous, mononematous, (126–)175–444(–901) µm in length (Fig. 7a, d). *Rhizoid*-like structures present. *Stipes* pale olivaceous, not constricted, cylindrical, simple, 1–6-septate, (89–)92–351(–799) µm long, (6–)8–12(–14) µm wide below primary branches, apical cell not swollen, (4–)6–10(–12) µm wide at base, basal cell occasionally swollen. *Conidiogenous apparatus* (46–)95–120(–138) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2–3, pale olivaceous, smooth, cylindrical, aseptate, (9–)15–22(–27) µm long and (2–)4–7(–8) µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, aseptate, (9–)13–17(–20) µm long, 3–7 µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, 8–10(–12) µm long, 2–5 µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, 38–56(–62) µm long and 2–3 wide (Fig. 7b, e). *Conidia* hyaline, aseptate, allantoid with truncate bases and rounded apices, slightly curved, 9–12 × 3–4 µm (Fig. 7c, f). Presence of *Hyalorhinocladiella*-like synanamorph with oblong to obovoid conidia, 3–4 × 2–3 µm. *Conidial droplet* hyaline at first, becoming cream-coloured with age (Fig. 7g).

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 52 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies sudan-brown. Colony margin smooth. Hyphae submerged in agar with little aerial mycelium, olivaceous, smooth, straight, occasionally constricted at the septa, 4–6 µm wide.

Specimens examined. China, Wangqing, Jilin, isolated from *Picea koraiensis* infested by *Ips typographus*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 60004, culture ex-type CMW 12425 = CBS 124024; PREM 60005, paratype, culture ex-paratype CMW 124441 = CBS 123617; PREM 60006, paratype, culture ex-paratype CMW 12486 = CBS 123618.

Notes — *Leptographium curviconidium* has longer conidiogenous apparatuses than the closely related *L. abietinum* and *G. americana* (Kendrick 1962, Jacobs et al. 1997). Its conidia are longer than those of *L. abietinum* and *L. altius* (Taxon 8), and it does not exhibit the extreme variability in length of those of *G. americana* (Table 4). Furthermore, *L. curviconidium* produces curved conidia, similar in shape to those of *L. abietinum*, but longer. *Leptographium curviconidium* has a distinctive *Hyalorhinocladiella*-like synanamorph, not present in any of the related species.

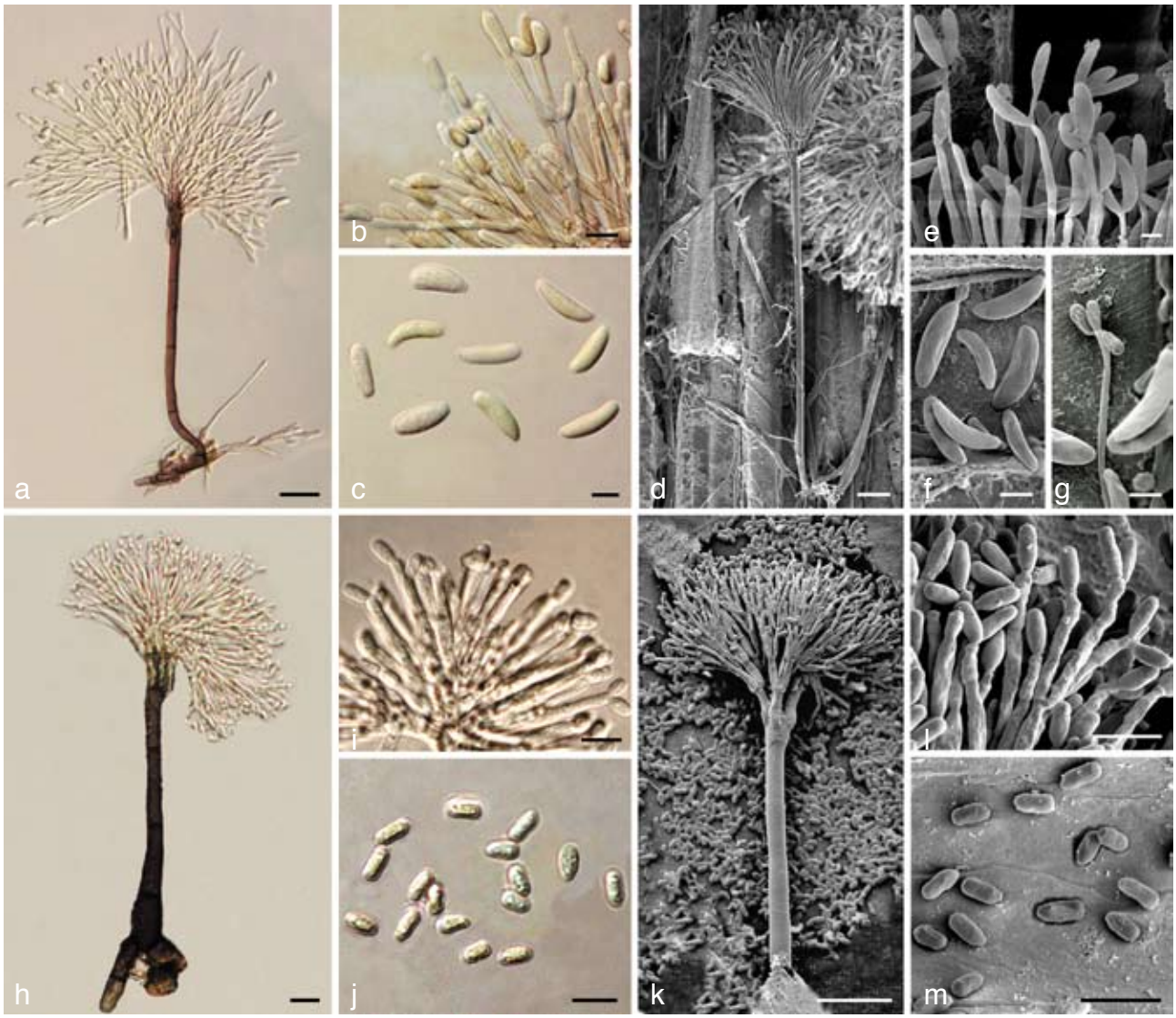


Fig. 7 a–f: *Leptographium curviconidium* sp. nov. a, d. Conidiophore; b, e. conidiogenous cells; c, f. conidia. — g. *Hyalorhinocladiella*-like synanamorph. — h–m: *L. altius* sp. nov. h, k. conidiophore; i, l. conidiogenous cells; j, m. conidia. — Scale bars: a, d, h, k = 20 µm; b, c, e, f, g, i, j, l, m = 5 µm.

Taxon 8

Leptographium altius Paciura, Z.W. de Beer & M.J. Wingf., *sp. nov.* — MycoBank MB516740; Fig. 7h–m

Conidiophorae singulae (173–)188–268(–369) µm longae, cum structuris rhizoidiformibus. Stipae cylindricae simplices 5–8-septatae (113–)137–222(–238) µm longae, infra ramos primarios (5–)7–10(–14) µm latae. Apparatus conidiogenus (37–)60–126(–169) µm, ramis cylindricis 2- vel 3-seriatis. Rami primarii 2–3 non septati, (11–)13–20(–24) µm longi (4–)5–6(–7) µm lati. Cellulae conidiogenae discretae, 2–3 in quoque ramo (14–)18–25(–27) µm longae 2–4 µm latae. Conidia non septata, obovoidea basibus truncatis (5–)6–10(–11) × 2–4 µm. Coloniae cremeo-bubalinae, crescunt optime in 25 °C in MEA ad 44 mm diametro in 8 diebus.

Etymology. Name refers to the rhizoids in this species that are deeply immersed in the agar.

Conidiophores occurring singly, very scarce arising directly from the mycelium, erect, macronematous, mononematous, (173–)188–268(–369) µm in length (Fig. 7h, k). *Rhizoid*-like structures present. *Stipes* pale olivaceous, slightly constricted on the septae, cylindrical, simple, 5–8-septate, (113–)137–222(–238) µm long, (5–)7–10(–14) µm wide below primary branches, apical cell not swollen, (4–)6–9(–11) µm wide at base, basal cell swollen. *Conidiogenous apparatus* (37–)60–126(–169) µm, excluding the conidial mass, with 2 to 3 series of cylindrical branches. *Primary branches*, 2–3, pale olivaceous, smooth, cylindrical, aseptate, (11–)13–20(–24) µm long and (4–)5–6(–7) µm wide, arrangement of the primary branches on the stipe – type B (more than two branches). *Secondary branches* hyaline to pale olivaceous, aseptate, (9–)10–13(–14) µm long, 3–5 µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, (7–)9–10(–11) µm long, 2–4 µm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (14–)18–25(–27) µm long and 2–4 µm wide (Fig. 7i, l). *Conidia* aseptate, obovoid, elongated with truncated bases, (5–)6–10(–11) × 2–4 µm (Fig. 7j, m).

Culture characteristics — Colonies with optimal growth at 25 °C on MEA, reaching 44 mm diam in 8 d. No growth below 5 °C or above 35 °C. Colonies cream-buff. Colony margin smooth. Hyphae submerged in agar with very little aerial mycelium, greenish olivaceous to olivaceous, smooth, straight, occasionally constricted at the septa, 3–5 µm wide.

Specimens examined. China, Wangqing, Jilin, isolated from *Picea koraiensis*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, holotype PREM 60001, culture ex-type CMW 12471 = CBS 123619; PREM 60002, paratype, culture ex-paratype CMW 12426 = CBS 123612; Changchun, Jilin, isolated from *Larix olgensis* infested by *Ips cembrae*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, PREM 60003, paratype, culture ex-paratype CMW 12501 = CBS 123621.

Notes — Comparisons with other species discussed above, under *L. curviconidium* (Taxon 7) and in Table 4.

Taxon 9

Leptographium pineti K. Jacobs & M.J. Wingf., Mycoscience 41: 596. 2000. — MycoBank MB466544

Description — Jacobs et al. (2000a).

Culture characteristics — Colonies dark-olivaceous, with no aerial mycelium. Optimal growth at 25 °C on MEA, reaching 48 mm diam in 8 d.

Specimen examined. China, Chuxiong, Yunnan, isolated from *Pinus kesiya*, July 2001, *X.D. Zhou*, *Z.W. de Beer*, CMW 12457.

Notes — The Chinese isolate was identified as *L. pineti* based on its morphology and its position in the phylogenetic inference (Fig. 1–3).

DISCUSSION

Eight new species of *Leptographium* were identified in this study, collected from conifers and hardwoods infested with bark beetles and weevils. In addition to these eight species, *L. pineti* was found in China for the first time. The phylogenetic analyses of DNA sequences showed that the eight new taxa resided in three main groups and *L. pineti* was in an unrelated fourth group.

Interestingly, two of the major phylogenetic lineages (Groups A & B) in which five of the new species from China occurred, consisted primarily of species described from conifers in Asia. Group A included *G. koreana*, *G. yunnanense*, *H. pinicola* and *L. truncatum*. The first two of these have thus far only been found in countries such as Japan, Korea, Thailand and China (Zhou et al. 2000, Kim et al. 2005a, Masuya et al. 2005, 2009, Yamaoka et al. 2007, 2008, Lu et al. 2009a). *Hyalorhinocladiella pinicola* has been recorded from Canada and Japan (Jacobs et al. 2005) and *L. truncatum* from Africa, North America, Europe and New Zealand (Wingfield & Marasas 1983, Hausner et al. 2005, Jacobs et al. 2005). The latter two species have recently also been reported from China (Lu et al. 2009a, b). All the species in Group A, including *L. conjunctum* (Taxon 1) and *L. celere* (Taxon 2) were exclusively isolated from pine. The only exception is *L. manifestum* (Taxon 3) which also forms part of Group A based on EF-1α, that was isolated from both spruce and pine. Most of the species of Group A were isolated in association with more than one bark beetle species (Table 2), suggesting that they do not have fixed associations with particular beetle species. Some of these beetles, such *T. yunnanensis* and *D. valens*, are destructive pests that cause significant losses (Kirkendall et al. 2008, Lu et al. 2009a, b). Although *L. koreanum* and *L. truncatum* appear to have some level of pathogenicity (Lu et al. 2009a) and *L. truncatum* has been implicated as a contributing factor of pine root disease in South Africa and New Zealand (Wingfield & Marasas 1983), none of the previously described species in Group A are considered serious tree pathogens.

The second major lineage (Group B) in which two of the species discovered in the present study reside, also contains *L. sinoprocerum* associated with *D. valens* in China, and *L. bhutanense*, closely associated with the root collar weevil *Hylobitelus chenkupdorjii* in Bhutan (Lu et al. 2008, Zhou et al. 2008). Both of the latter species have been found only on conifers, which is similar to the case for the newly described Chinese species. *Leptographium gracile* (Taxon 4) and *L. latens* (Taxon 5) were both found associated with *Pissodes* spp. In addition, *L. latens* was also isolated from *Ips yunnanensis* galleries, suggesting that these species are not tightly linked to their vectors. It has previously been shown that *L. sinoprocerum* is mildly pathogenic (Lu et al. 2009a), but nothing is known regarding the pathogenicity of *L. bhutanense* (Zhou et al. 2008) or the two new species described in this study.

Phylogenetic Group C that includes *L. pistaciae* (Taxon 6), *L. curviconidium* (Taxon 7) and *L. altius* (Taxon 8), accommodates known species such as *G. americana* and *L. abietinum*, which have been reported previously only from conifers in North America (Kendrick 1962, Jacobs et al. 1997, Jacobs & Wingfield 2001). The only exception is *L. pistaciae* (Taxon 6) that is closely related to *L. bistatum*, isolated from *P. radiata* logs in Korea (Kim et al. 2004). *Leptographium pistaciae* was found on the native hardwood *Pistacia chinensis*, a host very different to *Pinus* from which *L. bistatum* was isolated.

The fact that *L. pineti* was found in China for the first time is perhaps not surprising. This is because the fungus was first described from a conifer (*Pinus merkusii*) infested by an *Ips* sp. in Sumatra, Indonesia (Jacobs et al. 2000a), which is geographically close to China. The discovery of *L. pineti* on *P. kesiya* in China suggests that it has a relatively wide host range on *Pinus* spp. and it would be interesting to learn more regarding its insect vectors.

Jacobs & Wingfield (2001) emphasized that Asia was an area of the world poorly sampled for the ophiostomatalean fungi. In subsequent years, these fungi have been relatively actively studied in Japan and Korea, but China has been overlooked. The results of this study have shown that many new species in the *Ophiostomatales* await discovery in China. This is a large country with diverse forests including many conifers that are hosts to many species of wood-infesting insects. *Leptographium* spp. and related ophiostomatalean fungi are commonly associated with these insects and this suggests that many unknown species exist in those forests. An increased knowledge of these fungi will provide greater insight into their biology and ecological roles, particularly given the opportunity to compare them with species well known in Europe and North America.

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REFERENCES

Bleiker K, Six D. 2009. Competition and coexistence in a multi-partner mutualism: interactions between two fungal symbionts of the mountain pine beetle in beetle-attacked trees. Microbial Ecology 57: 191–202.

Cardoza YJ, Moser JC, Klepzig KD, Raffa KF. 2008. Multipartite symbioses among fungi, mites, nematodes, and the spruce beetle, Dendroctonus rufipennis. Environmental Entomology 37: 956–963.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

Eckhardt LG, Weber AM, Menard RD, Jones JP, Hess NJ. 2007. Insect-fungal complex associated with loblolly pine decline in Central Alabama. Forest Science 53: 84–92.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied Environmental Microbiology 61: 1323–1330.

Grobbelaar J, Beer ZW de, Bloomer P, Wingfield MJ, Wingfield BD. 2010. Ophiostoma tsotsi sp. nov., a wound-infesting fungus of hardwood trees in Africa. Mycopathologia 169: 413–423.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Harrington TC. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega FE, Blackwell M (eds), Insect-fungal associations: ecology and evolution: 257–291. Oxford University Press, New York.

Harrington TC, Cobb FW. 1988. Leptographium root diseases on conifers. APS Press, St. Paul.

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. Canadian Journal of Botany 83: 1222–1237.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.

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. Mycological Research 108: 411–418.

Jacobs K, Eckhardt LG, Wingfield MJ. 2006. Leptographium profanum sp. nov., a new species from hardwood roots in North America. Canadian Journal of Botany 84: 759–766.

Jacobs K, Kirisits T. 2003. Ophiostoma kryptum sp. nov. from Larix decidua and Picea abies in Europe, similar to O. minus. Mycological Research 107: 1231–1242.

Jacobs K, Solheim H, Wingfield BD, Wingfield MJ. 2005. Taxonomic re-evaluation of Leptographium lundbergii based on DNA sequence comparisons and morphology. Mycological Research 109: 1149–1161.

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. Canadian Journal of Botany 75: 1315–1322.

Jacobs K, Wingfield MJ, Bergdahl DR. 2000a. New Leptographium species from Indonesia and eastern North America. Mycoscience 41: 595–606.

Jacobs K, Wingfield MJ, Pashenova NV, Vetrova VP. 2000b. A new Leptographium species from Russia. Mycological Research 104: 1524–1529.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298.

Kendrick WB. 1962. The Leptographium complex. Verticicladiella Hughes. Canadian Journal of Botany 40: 771–797.

Kim J-J, Lim YW, Breuil C, Wingfield MJ, Zhou XD, Kim G-H. 2005a. A new Leptographium species associated with Tomicus piniperda infesting pine logs in Korea. Mycological Research 109: 275–284.

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

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. Mycological Research 108: 699–706.

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

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

Lee S, Kim J-J, Breuil C. 2005. Leptographium longiclavatum sp. nov., a new species associated with the mountain pine beetle, Dendroctonus ponderosae. Mycological Research 109: 1162–1170.

Linnakoski R, Beer ZW de, Rousi M, Niemelä P, Pappinen A, Wingfeld MJ. 2008. Fungi including Ophiostoma karelicum sp. nov., associated with Scolytus ratzeburgi infesting birch in Finland and Russia. Mycological Research 112: 1475–1488.

Lu M, Zhou XD, Beer ZW de, Wingfield MJ, Sun J-H. 2009a. Leptographium and Ophiostoma spp. associated with an invasive pine-infesting bark beetle, Dendroctonus valens in China. Fungal Diversity 38: 133–145.

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

Lu Q, Decock C, Zhang XY, Maraite H. 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 van Leeuwenhoek 96: 275–293.

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.

Masuya H, Kaneko S, Yamaoka Y. 1998. Blue-stain fungi associated with Tomicus piniperda (Scolytidae: Coleoptera) on Japanese red pine. Journal of Forestry Research 3: 213–219.

Masuya H, Kim J, 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, Wingfield MJ, Kubono T, Ichihara Y. 2004. Leptographium pruni, sp. nov. from bark beetle-infested Prunus jamasakura in Japan. Mycologia 96: 548–557.

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.

Paciura D, Zhou XD, Beer ZW de, Jacobs K, Ye H, Wingfield MJ. 2010. Characterisation of synnematous bark beetle-associated fungi from China, including Graphium carbonarium sp. nov. Fungal Diversity 40: 75–88.

Plattner A, Kim JJ, DiGuistini S, Breuil C. 2008. Variation in pathogenicity of a mountain pine beetle-associated blue-stain fungus, Grosmannia clavigera, on young lodgepole pine in British Columbia. Canadian Journal of Plant Pathology 30: 457–466.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Rayner R. 1970. A mycological color chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.

Six DL. 2003. Bark beetle-fungus symbiosis. In: Bourtzis K, Miller TA (eds), Insect symbiosis: 97–114. CRC Press, Washington, DC.

Swofford DL. 1998. PAUP*. Phylogenetic Analysis using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, MA.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.

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 application: 315–322. Academic Press, San Diego, USA.

Wingfield MJ. 1987. Fungi associated with the pine wood nematode, Bursaphelenchus xylophilus, and cerambycid beetles in Wisconsin. Mycologia 79: 325–328.

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. Transactions of the British Mycological Society 80: 231–236.

Wingfield MJ, Seifert KA, Webber JF. 1993. Ceratocystis and Ophiostoma: Taxonomy, ecology and pathogenicity. APS Press, St Paul.

Witcosky JJ, Schowalter TD, Hansen EM. 1986. Hylastes nigrinus (Coleoptera: Scolytidae), Pissodes fasciatus, and Steremnius carinatus (Coleoptera: Curculionidae) as vectors of black-stain root disease of Douglas-fir. Environmental Entomology 15: 1090–1095.

Yamaoka Y, Masuya H, Chung W-H, Goto H, To-Anun C, Tokumasu S. 2007. Two species of Leptographium isolated from blue-stained sapwood of Pinus khasya and bark beetles in Thailand. Mycoscience 48: 100–103.

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. Mycoscience 49: 233–240.

Yamaoka Y, Wingfield MJ, Oshawa M, Kuroda Y. 1998. Ophiostomatoid fungi associated with Ips cembrae in Japan and pathogenicity to Japanese larch. Mycoscience 39: 367–378.

Yamaoka Y, Wingfield MJ, Takahashi I, Solheim H. 1997. Ophiostomatoid fungi associated with the spruce bark beetle Ips typographus japonicus in Japan. Mycological Research 101: 1215–1227.

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.

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

Zipfel RD, Beer ZW de, Jacobs K, Wingfield BD, Wingfield MJ. 2006. Multigene phylogenies define Ceratocystiopsis and Grosmannia distinct from Ophiostoma. Studies in Mycology 55: 75–97.