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

D. Paciura¹, Z.W. de Beer¹, K. Jacobs³, X.D. Zhou¹², H. Ye⁴, M.J. Wingfield¹

Key words
bark beetles
China
conifers
Grosmannia
hardwoods
Ophiostomatales

Abstract


Grosmannia and Leptographium spp. are well-known agents of sapstain of conifer and 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, Tomius and Orthotomicus (Curculionidae: Scolytinae), as well as Hylastes and Hylurgops (Scolytidae: Hylesini) (Kirisits 2004). They have also been reported in association with root weevils in the genera Hylobius, Pachylobius, Pissodes and Stereumius (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, 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 Tomius 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. althenuim, L. pini-densiflorae, L. procercum, L. sinoprocercum 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.
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 squishing the beetles on 2 % malt extract agar amended with 0.05 % cycloheximide (MEA: 20 g Biola 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 malt extract, 20 g Biolab agar and 1 000 mL deionised water). The isolates were then grouped based on the method described by Grobbelaar et al. (2010). Each isolate was also crossed against itself as a control. For light microscopy, 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 the β-tubulin and elongation factor 1 genes. The primers ITS3 (White et al. 1990) and LR3 (Vilgalys & Hester 1990) were used to amplify the ITS2-28S region, Bt2a and EF1F and EF2R (Jacobs et al. 2004) for the EF-1 gene.

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 DNA ronaper, 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, B2α and B2β (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.

<table>
<thead>
<tr>
<th>Taxon no.</th>
<th>Species (total no. of isolates from survey)</th>
<th>Isolate no.</th>
<th>Host / Insect Origin</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS</td>
<td>CMW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>L. conjunctum (5)</td>
<td>123631</td>
<td>Pinus yunnanensis / Hylurgops major</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123632</td>
<td>Pinus yunnanensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123633</td>
<td>P. yunnanensis / H. major</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td>2</td>
<td>L. celere (5)</td>
<td>123626</td>
<td>P. yunnanensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123629</td>
<td>P. yunnanensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123630</td>
<td>Pinus sp.</td>
<td>Jilin, Yanji</td>
</tr>
<tr>
<td>3</td>
<td>L. manifestum (8)</td>
<td>123604</td>
<td>Larix olgensis / Ips subelongatus</td>
<td>Jilin, Wangqing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123606</td>
<td>P. yunnanensis / Polyphagus virulicronis</td>
<td>Yunnan, Lufeng</td>
</tr>
<tr>
<td>4</td>
<td>L. gracile (48)</td>
<td>123623</td>
<td>Pinus armandii / Pissodes sp.</td>
<td>Yunnan, Mudu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123624</td>
<td>P. armandii / Pissodes sp.</td>
<td>Yunnan, Mudu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123625</td>
<td>P. armandii / Pissodes sp.</td>
<td>Yunnan, Lijiang</td>
</tr>
<tr>
<td>5</td>
<td>L. latens (22)</td>
<td>123615</td>
<td>P. armandii / Pissodes sp.</td>
<td>Yunnan, Lijiang</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123616</td>
<td>P. armandii / Pissodes sp.</td>
<td>Yunnan, Mudu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123622</td>
<td>P. yunnanensis / L. glomeratus</td>
<td>Yunnan, Mudu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123623</td>
<td>P. yunnanensis / L. glomeratus</td>
<td>Yunnan, Mudu</td>
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<tr>
<td></td>
<td></td>
<td>123624</td>
<td>P. yunnanensis / L. glomeratus</td>
<td>Yunnan, Mudu</td>
</tr>
<tr>
<td>6</td>
<td>L. pistaciae (2)</td>
<td>123626</td>
<td>Pistacia chinensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123627</td>
<td>P. chinensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
<tr>
<td>7</td>
<td>L. curviconidium (8)</td>
<td>123617</td>
<td>P. koraiensis / L. typographus</td>
<td>Jilin, Wangqing</td>
</tr>
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<td></td>
<td></td>
<td>123618</td>
<td>P. koraiensis / L. typographus</td>
<td>Jilin, Wangqing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123619</td>
<td>P. koraiensis / L. typographus</td>
<td>Jilin, Wangqing</td>
</tr>
<tr>
<td>8</td>
<td>L. altius (6)</td>
<td>123612</td>
<td>L. olgensis / L. cephaloidea</td>
<td>Jilin, Changchun</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123613</td>
<td>L. olgensis / L. cephaloidea</td>
<td>Jilin, Changchun</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123614</td>
<td>L. olgensis / L. cephaloidea</td>
<td>Jilin, Changchun</td>
</tr>
<tr>
<td>9</td>
<td>L. pinetii (1)</td>
<td>123621</td>
<td>P. yunnanensis</td>
<td>Yunnan, Chuxiong</td>
</tr>
</tbody>
</table>

a CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
bc CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
Ex-type isolates.
Table 2  

<table>
<thead>
<tr>
<th>Species</th>
<th>Conidiophore (l)</th>
<th>Conidiogenous apparatus (l)</th>
<th>Rhizoids</th>
<th>Colony colour</th>
<th>Host</th>
<th>Distribution</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. koreana</td>
<td>(74–227)</td>
<td>(40–83–88(–127)</td>
<td>Absent</td>
<td>Dark-olivaceous</td>
<td>Pinus yunnanensis</td>
<td>Japan, Korea, China</td>
<td>Lu et al. 2009a, b</td>
</tr>
<tr>
<td>L. manifestum</td>
<td>(11–)15–32(–48)</td>
<td>(36–50–77(–100)</td>
<td>Present</td>
<td>Umber-brown</td>
<td>Polygraphus verricifrons</td>
<td>China</td>
<td>Lu et al. 2009a, b</td>
</tr>
</tbody>
</table>

| Media from which structures were obtained for measurements: | Oatmeal agar; MEA; Sterilised wood or agar emended with wood pieces. |

PCR reactions of 25 µL, containing 1X PCR buffer, 2.5 mM MgCl₂, 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-recombination (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.zoo.uct.ac.za/software/). Bayesian inference was conducted in MrBayes v3.1 (Huelsenbeck &...
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. pini-densiflorae and L. profanum 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.

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. Phylogenograms 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 yunnanensis, G. koreana, L. truncatum and Hyalorhinocladiella pinicola in Group A for all the gene regions (Fig. 1–3). In both 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 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.

<table>
<thead>
<tr>
<th>Character</th>
<th>L. sinoprocerum*</th>
<th>L. bhutanense*</th>
<th>Taxon 4: L. gracile*</th>
<th>Taxon 5: L. latens*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Olive mycelial, concentric rings</td>
<td>Olive mycelial, concentric ring</td>
<td>Pale olivaceous with wide white concentric ring</td>
<td>Citrine with narrow olivaceous ring in middle, followed by wide, lighter citrine concentric ring</td>
</tr>
<tr>
<td>Host</td>
<td>Pinus spp.</td>
<td>Pinus wallichiana</td>
<td>Pinus amandii</td>
<td>Picea koraiensis, P. amandii</td>
</tr>
<tr>
<td>Insect</td>
<td>Dendroctonus valens</td>
<td>Hylobitelli chenupoderji</td>
<td>Pissodes sp.</td>
<td>Pissodes sp.</td>
</tr>
<tr>
<td>Distribution</td>
<td>China</td>
<td>Bhutan</td>
<td>China</td>
<td>China dorado</td>
</tr>
<tr>
<td>References</td>
<td>Lu et al. 2008, 2009</td>
<td>Zhou et al. 2008</td>
<td>Present study</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Media from which structures were obtained for measurements: * Oatmeal agar.
Table 4  Morphological characteristics of Group C, including Taxa 6, 7 and 8 and their closest relatives. All measurements in µm unless indicated otherwise.

<table>
<thead>
<tr>
<th>L. bistatum</th>
<th>Taxon 6: L. pistaciae</th>
<th>G. americana</th>
<th>L. abietinum</th>
<th>Taxon 7: L. curviconidium</th>
<th>Taxon 8: L. altius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Conidiogenous apparatus (l)</td>
<td>50</td>
<td>50–74–108–119</td>
<td>31 mm at 20 °C</td>
<td>74–535–(–870)</td>
<td></td>
</tr>
<tr>
<td>Conidioma</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Conidium shape</td>
<td>Obovoid to ovoid, truncate bases, distinctly curved</td>
<td>Ellipsoidal to obovoid, slightly curved</td>
<td>Obovoid to allantoid, subtruncated bases</td>
<td>Clavate, truncate bases, curved</td>
<td>Obovoid, elongated with truncated bases</td>
</tr>
<tr>
<td>Conidium size (l × w)</td>
<td>3–6 × 1–2</td>
<td>3–5 × 2–4</td>
<td>3.5–22 × 1–3</td>
<td>3–4 × 5–(7) × 1–2</td>
<td>9–12 × 3–4</td>
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<tr>
<td>Growth on MEA for 8 d at 25 °C</td>
<td>27 mm</td>
<td>50 mm</td>
<td>31 mm at 20 °C</td>
<td>39 mm</td>
<td>44 mm</td>
</tr>
<tr>
<td>Teleomorph</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
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<tr>
<td>Synanamorph</td>
<td>Sporothrix-like</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
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<tr>
<td>Colony colour</td>
<td>Umber</td>
<td>Greenish olivaceous</td>
<td>–</td>
<td>Cartridge buff</td>
<td>Sudan-brown</td>
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<td>Host</td>
<td>P. radiata</td>
<td>Pistacia chinensis</td>
<td>Larix decidua</td>
<td>Picea, Pseudotsuga spp.</td>
<td>Picea koraiensis, P. koraiensis, L. olgensis</td>
</tr>
<tr>
<td>Insect</td>
<td>–</td>
<td>–</td>
<td>D. simplex</td>
<td>Dendroctonus spp.</td>
<td>I. cembrae</td>
</tr>
<tr>
<td>Distribution</td>
<td>Korea</td>
<td>USA</td>
<td>USA, Canada</td>
<td>China</td>
<td>China</td>
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</tbody>
</table>

References from which measurements were used in this table.

Table 5 Parameters and statistics for the phylogenetic analyses.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>No. of taxa</th>
<th>No. of char</th>
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<td>HKY+I</td>
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achar = characters
IPIC = number of parsimony informative characters
CI = consistency index
RI = retention index
HI = homoplasy index
Subst. model = best fit substitution model
Pinvar = proportion of invariable sites
G = gamma shape parameter
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 sub-trees (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_. EF-1α and are closely related to _G. yunnanensis_. Howevet, the two Chinese isolates formed a distinct, well-supported lineage in both the EF-1α and β-tubulin subtrees (Fig. 3, 4). However, the conidiophores of _L. conjunctum_ reach much greater lengths (< 485 µm) than those

** Taxonomic

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


**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. _Conidigenous 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. _Conidigenous 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, oblonga vel obvoideae 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_, infected by _Hydriogona 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. conjunctum_ reach much greater lengths (< 485 µm) than those
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


**Etymology.** Name reflects the colony growth in the fungus that begins as directly from the mycelium, erect, macronematous, mono-nematous, (120–)239–950(–1365) µm in length (Fig. 4g, i), 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...
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, 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). **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.

Notes — *Leptographium celere* has much longer conidio-
phores 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. pini-
cola*.

**Taxon 3**

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

Conidiophores singulare vel ad quaternae aggregatae (83–)103–243(–363) 
µm longae, cum structuris rhizoidiformibus. Stipae cylindraceae 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- 
vell 3-seriatis. Rami primarii 2–3, non septati, (8–)10–18(–22) µm longi 2–6 
µm lati. Cellulae conidiogenae discreteae, 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,

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. co-
nidiogenous cells; i, l. conidia. — Scale bars: a, d, g, j = 20 µm; b, c, h, i = 5 µm; e, f, k, l = 1 µm.

(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, 
ellipsoide, 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 oli-
vaceous to olivaceous, smooth, straight, 4–5(–6) µm wide.

*Specimens examined.* Снята, 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,

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


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 hyalina, aseptate, oblong obvoid 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.
Notes — Leptographium gracile is most closely related to *L. sinoporum*, *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 of *L. sinoporum* and *L. latens* are the shortest, respectively reaching 337 and 404 µm (Table 3). The conidia of *L. latens* tend to be longer (Table 3) 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


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–i


Etyymology. Name relates to the host *Pistacia chinesis*.

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 longae in length, *rhizoid-like* structures present. Stipes pale olivaceous, not constricted, cylindrical, simple, 3–4–septate, (88–)100–198–(320) µm longae, (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. *Conidigenous apparatus* (60–)74–108–(119) µm longae, 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 longae 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 longae, 3–5–(8) µm wide. *Tertiary branches* hyaline to pale olivaceous, aseptate, 12–16–(17) µm longae, 2–6 µm wide. *Conidigenous cells* discrete, 1–2 per branch, cylindrical, tapering slightly at the apex, (14–)17–22–(28) µm longae and 1–2 µm wide (Fig. 6h, k). *Conidia* hyalina, aseptate, ellipsoidal to ovoid, 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.

Notes — Comparisons with other species discussed above, under *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

Conidiophores singulae vel ad quaedam aggregatae (126–)175–444–(901) µm longae, cum structuris rhizoidiformibus. Stigae cylindricae simplices 1–6–septatae (88–)92–351–(799) µm longae, infra ramos primarios (6–)12–14–(17) µm longae. Apparatus conidigenus (46–)95–120–(138) µm longus, 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 conidigenae discrete, 2–3 in
D. Paciura et al.: Eight new Leptographium species

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 µm 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.


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.
**Taxon 8**

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


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.


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

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**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 9


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 *L. 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 as *T. yunnanensis* and *D. valens*, are destructive pests that cause significant losses (Kirkendall et al. 2008, Lu et al. 2009a, b). Although *L. koreana* 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 *Hylobitlus chenkupordjii* 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. piceae* (Taxon 6), *L. curvicordium* (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. piceae* (Taxon 6) that is closely related to *L. bistatum*, isolated from *P. radiata* logs in Korea (Kim et al. 2004). *Leptographium piceae* 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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