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

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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, Tomius yunnanensis, Hylurgops minor, Polygraphus verrucironus and a Pissodes sp. Six of the new species are morphologically and phylogenetically related to species known to occur in Asia such as G. yunnanensis, L. bhutanense, L. bistatum and L. sinoprocessor. 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, Tomius 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 Stereumius (Curculionidae: Molytiniae) 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. yunnanensis 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, Hyalorhinochaletia pinicola, L. leithinum, L. pini-densiflorae, L. procem, L. sinoprocessor 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 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 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).

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 for the ex-type isolates of L. sinoprocerum and L. bhutanense 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. |
|-----------|-------------------------------------------|-------------|---------------|--------|----------------------|
| CBS       | CMW                                       |             |               |        |                      |
| 1         | L. conjunctum (8)                         | 123631      | Pinus yunnanensis / Hylurgops major | Yunnan, Chuxiong | H0406831 H0406879 H0406855 |
|           |                                           | 123632      | Pinus yunnanensis | Yunnan, Chuxiong | H0406832 H0406880 H0406856 |
|           |                                           | 123633      | P. yunnanensis / H. major | Yunnan, Chuxiong | H0406833 H0406881 H0406857 |
| 2         | L. efere (5)                              | 123626      | P. yunnanensis | Yunnan, Chuxiong | H0406834 H0406882 H0406858 |
|           |                                           | 123629      | P. oleosa | Yunnan, Chuxiong | H0406835 H0406883 H0406859 |
|           |                                           | 123630      | Pinus sp. | Yunnan, Chuxiong | H0406836 H0406884 H0406860 |
| 3         | L. manifestum (8)                         | 123604      | Larix olgensis / Ips subelongatus | Jilin, Wangqing | H0406842 H0406885 H0406861 |
|           |                                           | 123606      | P. yunnanensis / Ips fornicatus | Jilin, Wangqing | H0406838 H0406886 H0406862 |
|           |                                           | 123622      | L. olgensis / Ips subelongatus | Jilin, Wangqing | H0406839 H0406887 H0406863 |
| 4         | L. gracile (48)                           | 123623      | Pinus armandi / Pissodes sp. | Jilin, Midu | H0406840 H0406888 H0406864 |
|           |                                           | 123624      | P. armandi / Pissodes sp. | Jilin, Midu | H0406841 H0406889 H0406865 |
|           |                                           | 123625      | P. armandi / Pissodes sp. | Jilin, Lijiang | H0406842 H0406890 H0406866 |
| 5         | L. latens (22)                            | 123615      | P. armandi / Pissodes sp. | Jilin, Lijiang | H0406843 H0406891 H0406867 |
|           |                                           | 123616      | P. armandi / Pissodes sp. | Jilin, Midu | H0406844 H0406892 H0406868 |
|           |                                           | 123623      | Picea koraiensis / Ips typographus | Jilin, Midu | H0406845 H0406893 H0406869 |
| 6         | L. pustulatae (2)                         | 123626      | Pistacia chinensis | Yunnan, Chuxiong | H0406864 H0406894 H0406870 |
|           |                                           | 123627      | P. chinensis | Yunnan, Chuxiong | H0406847 H0406895 H0406871 |
|           |                                           | 123617      | P. koraiensis / P. typographus | Jilin, Wangqing | H0406848 H0406896 H0406872 |
|           |                                           | 123618      | P. koraiensis / P. typographus | Jilin, Wangqing | H0406849 H0406897 H0406873 |
|           |                                           | 123624      | P. koraiensis / P. typographus | Jilin, Wangqing | H0406850 H0406898 H0406874 |
| 7         | L. curviconidium (8)                      | 123612      | L. olgensis / Ips centrae | Jilin, Changchun | H0406852 H0406900 H0406876 |
|           |                                           | 123619      | L. olgensis / Ips centrae | Jilin, Wangqing | H0406851 H0406899 H0406875 |
|           |                                           | 123621      | L. olgensis / Ips centrae | Jilin, Chuxiong | H0406853 H0406901 H0406877 |
| 8         | L. altus (6)                              | 123625      | P. koraiensis | Jilin, Wangqing | H0406854 H0406902 H0406878 |

* CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
* CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.
* 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.

| Taxon 1:  | Taxon 2:  | Taxon 3:  |
|----------|----------|----------|
| L. truncatum | G. yunnanense | G. koreana |
| Conidiodrome (l) | 90–246–409(–685) | 74–227(–233) |
| Conidiogenous apparatus (l) | 35–42–85(–150) | 40–83–88(–127) |
| Rhizoids | Absent | Absent |
| bases, rounded apices | – | – |
| × w) | 3–5 | 3–10 |
| Host: | Pinus spp. | Pinus spp. |
| Distribution: | South Africa, New Zealand, South Africa, New Zealand, China | China |
| References: | Jacobs et al. 2005 | Jacobs et al. 2005 |

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, ß-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 ß-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 4.0b1 (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 MrModeltest 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 &
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 koreana 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 3–4. Taxa 4 and 5 were difficult to distinguish from the ex-type cultures of Leptographium species in four species groups, labelled A to D in the phylogenetic trees (Fig. 1–3).

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 MP 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 within distinct 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 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 h c | Taxon 6: L. pistaciae | G. americana h 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) |
| Conidigenous apparatus (l) | Present | Present            | Absent           | Present        | Present                     | Present             |
| Rhizoids         | Present         | Present             | Absent           | Present        | Present                     | Present             |
| Conidium shape   | Obovoid to ovoid, truncate bases, distinctly curved | Ellipsoidal to obvoid, 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–)14–5(–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 °C   | 39 mm         | 52 mm                      | 44 mm               |
| Teleomorph       | Absent         | Absent              | Present          | Absent        | Absent                     | Absent              |
| Synanamorph      | Sporothrlik-like | Absent              | Absent           | Absent        | Absent                     | Absent              |
| Colony colour    | Umber          | Geseenish olivaceous | –                | Cartridge buff | Sudan-brown                | Cream-buff          |
| Host             | P. radiata     | Pistacia chinensis  | Larix decidua    | Picea, Pseudotsuga spp. | Picea korieiensis, P. koraiensis, L. olgensis |
| Insect           | –              | D. simplex          | Dendroctonus spp. | I. typographus | I. cembrae                |
| Distribution     | Korea           | USA                | USA, Canada      | China         | China                      |
| References       | Kim et al. 2004 | Present study       | Jacobs et al. 1997 | Kendrick 1962, Jacobs & Wingfield 2001 |

Media from which structures were obtained for measurements: *Oatmeal agar; **MEA; †Sterilized wood or agar emended with wood pieces.

*References from which measurements were used in this table.

Table 5  Parameters and statistics for the phylogenetic analyses.

| Dataset          | No. of taxa | No. of char | Outgroup | ¹PIC | No. of trees | Tree length | ¹CI | ¹RI | ¹HI | ⁴Subst. model | ⁴Pinvar | ⁴G | ⁴Nst | ⁴Burn-in |
|------------------|-------------|-------------|----------|------|--------------|-------------|-----|-----|-----|---------------|--------|----|------|---------|
| ITS2 & partial LSU | 66          | 651         | Ophiostoma spp. | 189  | 273          | 745         | 0.660 | 0.886 | 0.339 | TrN+G+I+G   | 0.457  | 0.710 | 6    | GTR+I+G 450 |
| β-tubulin        | 50          | 482         | Ophiostoma spp. | 319  | 123          | 128         | 0.555 | 0.824 | 0.445 | TrN+G      | 0       | –   | 6    | GTR+I+G 350 |
| EF-¹α-1          | 40          | 828         | Ophiostoma spp. | 562  | 6            | 2376        | 0.606 | 0.840 | 0.393 | GTR+I+G+G  | 0.221  | 1.562 | 6    | GTR+I+G 400 |
| β-tubulin, Group A | 23          | 363         | Midpoint rooted | 17   | 1            | 19           | 1    | 1    | 0    | TrN+G      | 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-1, 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-1, 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-1, Group C | 14          | 607         | Midpoint rooted | 182  | 2            | 218          | 0.963 | 0.988 | 0.036 | GTR+G      | 0       | 0.530 | 6    | GTR+I 250 |

¹char = characters
¹PIC = number of parsimony informative characters
¹CI = consistency index
¹RI = retention index
¹HI = homoplasy index
⁴Subst. model = best fit substitution model
⁴Pinvar = proportion of invariable sites
⁴G = gamma shape parameter
⁴Nst = number of substitution rate categories
⁴Burn-in = MCMC burn-in
species such as *L. proserum*. 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. sinoprocera* and *L. bruchiae*. 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 subgroups (Fig. 2, 3).

Taxon 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. pini-densiflorae* (72–)146–349(–485) µm in length (Fig. 4a, d), from the mycelium, erect, macronematous, mononematous, described as *L. sinoprocera* (Fig. 3). The Chinese isolate had an EF-1α sequence identical to *L. pini*, and differed in only 2 bp positions from *L. pini* in the ITS2 and β-tubulin sequences.

**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, including 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, oblong to obovoid, smooth, 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 *Hygrocybe 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 keseyi* 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

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**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%.
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

Conidiophores singulaires vel ad terna aggregatione (120–)239–950(<1365) µm longae, cum structuris rhizoidiformibus. Stipeae cylindracea simplices 1–12-septateae (66–)130–798(<1150) µm longae, infra ramos primarios 3–5 µm latae, cellula apicalis non tumida. Apparatus conidiogenus 58–98(<115) µm longi 3–5 µm latti. Cellulae conidiogenae discrete, 2–5 in quaque 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 olivacea, crescent 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 conidiophores occurring singly or in groups of up to three, arising 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, 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. Conidigenous 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.

Specimens examined. China, Chuxiong, Yunnan, isolated from Pinus kesyia, 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.
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 synanamorph *Hyalorhinocladiella* conidinis 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. CHN, 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,
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, 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.
Notes — *Leptographium gracile* is most closely related to *L. sinoprocerum*, *L. bhutanense* and *L. latens* (Taxon 5, present study). *Leptographium bhutanense* can be distinguished from all three of 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 1.05 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 (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

Conidiophore singulæ vel ad quinque aggregatæ (144–)152–256–(404) µm longae, cum structuris rhizoidiformibus. Stipae cylindricæ 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. Cellulæ conidiogenæ discrete, 2–3 in quoque ramo (11–15–20–23) µm longae, infra ramos primarios 1–3 µm latae. Conidia hyalina non septata oblongo-obovoidae basibus truncatæ 7–10 × 2–4 µm. Coloniae citrines medio annulo olivaceo, postea annulo concentrico citrine, crescunt optime in 25 °C in MEA ad 50 mm diametro in 8 diebus.

Etyymology. 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 (12µm) wide below primary branches, apical cell not swollen, (5–)6–9–10 µm wide at base, basal cell occasionally swollen. Conidiogenus apparatus (29–)35–85–(129) 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 4–5 µm wide, arrangement of the primary branches on the stipe type B (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. Conidiogenus cells discrete, 1–2 per branch, cylindrical, tapering slightly at the apex, (14–)17–22–28 µm long and 1–2 µm wide (Fig. 6e, h). Conidia hyalina, aseptate, ellipsoidae obovoidae, 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 submersed 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. 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

Conidiophore singulæ vel ad senæ aggregatæ (219–)279–630–(1068) µm longae, cum structuris rhizoidiformibus. Stipae cylindricæ simplices 1–11-septatae (143–)198–528–(961) µm longae, infra ramos primarios 5–10 µm latæ. 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. Cellulæ conidiogenæ discrete, 1–2 in quoque ramos ellipsoidae vel obovoidae 3–5 × 2–4 µm. Conidia hyalina non septata subfalcata ellipsoidae vel obovoidae 3–5 × 2–4 µm. Coloniae olivaceae virescentæ, crescunt optime in 25 °C in MEA ad 50 mm diametro in 8 diebus.

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

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 below. Apical cell not swollen, 5–9–12 µm wide at base, basal cell occasionally swollen. Conidiogenus 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 4–5 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, 2–5–6 µm wide. Conidiogenus 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 hyalina, aseptate, ellipsoidae obovoidae, 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 submersed 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 — *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

Conidiophore singulæ vel ad quaternae aggregatæ (126–)175–440–(901) µm longae, cum structuris rhizoidiformibus. Stipae cylindricæ simplices 1–6-septatae (88–)92–351–(799) µm longae, infra ramos primarios (6–)12–14 (14) µm latæ. 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. Cellulæ conidiogenæ discrete, 2–3 in...
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 apices and rounded bases, 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. 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, i, k, l = 1 µm.
**Taxon 8**

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

Conidiophores singulare (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, obvoid, 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 diamin 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 reside 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. yunnanensis, 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 destrucive 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 Hylobitellus chenkupodjirii 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. curvicoroniun (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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