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Leptographium guttulatum sp. nov., a new species from spruce and pine in Europe

K. Jacobs[1]
M. J. Wingfield

Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, Republic of South Africa

C. Coetsee

Department of Microbiology and Biochemistry, University of the Orange Free State, PO Box 339, Bloemfontein, 9300, South Africa

T. Kirisits

Institute of Forest Entomology, Forest Pathology and Forest Protection, Universität für Bodenkultur, Hasenauerstrasse 38, A-1190 Vienna, Austria.

B. D. Wingfield

Department of Genetics, University of Pretoria, Pretoria, 0002, Republic of South Africa

Abstract: *Leptographium* spp. are anamorphs of *Ophiostoma* and are characterised by conidiophores with dark mononematous stipes and complex conidiogenous apparatusses with several series of branches. These fungi are also characterised by their distinct tolerance to high concentrations of cycloheximide. Most *Leptographium* spp. cause sapstain in conifer timber and some species are root pathogens of trees. In recent years, an unknown *Leptographium* sp. has been isolated from spruce and pine in Europe. This species is found in association with bark beetles that infest these conifers. These insects most probably act as vectors for this fungus and belong to the genera *Dryocoetes, Hylastes, Hylurgops* and *Tomicus*. Morphological comparisons, as well as partial ribosomal DNA comparisons have shown that this species represents a previously undescribed taxon. In this paper we describe the fungus as new and provide the name *Leptographium guttulatum* sp. nov. for it.

Key Words: *Leptographium, Ophiostoma,* phylogeny, ribosomal DNA

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[1] Corresponding author, Email: kjacobs@fabi.up.ac.za

INTRODUCTION

Species of *Leptographium* Lagerberg & Melin are characterised by dark mononematous conidiophores with complex conidiogenous apparatuses. The conidiogenous cells produce numerous conidia that accumulate in mucilaginous masses at the apex of the conidiophores (Kendrick 1962, Wingfield 1993). *Leptographium* spp. are morphologically adapted for dispersal by insects, especially bark beetles (Coleoptera: Scolytidae) (Lagerberg et al 1927, Leach et al 1934, Upadhyay 1981). Some *Leptographium* spp. are known anamorphs of *Ophiostoma* (Harrington 1987, Wingfield et al 1993). *Leptographium* spp. are best known among plant pathologists for their ability to cause blue-stain in conifer lumber. A few species also cause diseases of trees and the best known of these is black-stain root disease caused by three varieties of *Leptographium wageneri* (Kendrick) Wingfield (Harrington and Cobb 1987, Cobb 1988).

Leptographium spp. can be divided broadly into three groups based on their conidium size. These include species with conidia that are long (7–20 μm), medium (5–8 μm) and short (3–5 μm). Most *Leptographium* spp. can be accommodated in the group with medium sized conidia (Jacobs unpubl). Species in this group include the anamorphs of *O. piceaperdum* (Rumbold) Arx [= *O. europhioides* (Wright and Cain) Solheim], *O. laricis* Van der Westhuizen, Yamaoka & Wingfield and *O. huntii* (Robinson-Jeffrey) De Hoog & Scheffer (Wright and Cain 1961, Robinson-Jeffrey and Davidson 1968, Van der Westhuizen et al 1995). This is in contrast to the anamorphs of species such as *O. penicillatum* Grosmann, *O. americanum* Jacobs & Wingfield and *O. dryocoetidis* Kendrick & Molnar that have conidia that are at least twice or even three times as long as they are wide (Grosmann 1931, 1932, Kendrick and Molnar 1965, Jacobs et al 1997). Other characters that are useful in the identification of *Leptographium* spp. include the length of conidiophores, type of primary branches and the absence or presence of rhizoids.

Recently, an unknown *Leptographium* sp. has been isolated from spruce and pine in Europe. Wingfield and Gibbs (1991) referred to these isolates as *Leptographium* sp. "GG" and postulated that they might be the same as the fungus known as *Ophiostoma penicillatum* f. *palliati* described by Mathiesen (1950),

TABLE I. Isolates used for molecular comparison

Isolate number[a]	GenBank acsession	Species	Conidia	Origin	Host	Insect associate
CMW 2306[b] (YCC107)	AF 224321	*O. penicillatum*	long	Japan	*Picea jezoensis*	*Ips typographus* f.
CMW 2302[b] (YCC070)	AF 224322	*O. penicillatum*	long	Japan	*Picea jezoensis*	*Ips typographus* f.
CMW 2642[c]	AF 224323	*O. penicillatum*	long	Norway	*Picea abies*	*Ips typographus*
CMW 2643[d]	AF 224324	*O. pēnicillatum*	long	Sweden	*Picea abies*	*Ips typographus*
CMW 495 (CBS 497.96)[e]	AF224325	*O. americanum*	long	North America	*Larix decidua*	*Dendroctonus simplex*
CMW 2963[f]	AF 224326	*O. americanum*	long	North America	*Larix decidua*	*Dendroctonus simplex*
CMW 2014[f] (CBS 636.94)	AF 224327	*O. laricis*	short	Japan	*Larix kaempferi*	*Ips cembrae*
CMW 2016[f]	AF 224332	*O. laricis*	short	Japan	*Larix kaempferi*	*Ips cembrae*
CMW 1980[b] (CBS 633.94)	AF 224330	*O. laricis*	short	Japan	*Larix kaempferi*	*Ips cembrae*
CMW 452 (CBS 275.65)[g]	AF 224328	*O. piceaperdum*	short	USA		
CMW 1949[b]	AF 224329	*O. piceaperdum*	short	Japan	*Larix kaempferi*	*Ips cembrae*
CMW 1310[h]	AF 224334	*Leptographium.*	short	England	*Pinus sylvestris*	*Tomicus piniperda*
CMW 742[i]	AF 224335	*Leptographium.*	short	France	*Pinus sylvestris*	*Tomicus piniperda*
CMW 442[j] (CBS 376.66)	AF 224333	*O. dryocoetidis*	long	British	*Abies*	*Dryocoetus confusus*
CMW 662 (CBS 929.69)[k]	AF 224327	*L. procerum*	short	Virginia, USA	Pine forest soil	

[a] CMW—Culture collection of the Tree Pathology Co-operative Programme (TPCP), Forest and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, CBS—Centraalbureau voor Schimmelcultures, Baarn, Netherlands. Collector: [b] Y. Yamaoka, [c] H. Solheim, [d] A. Kāārik, [e] D.R. Bergdahl, [f] M.J. Wingfield, [g] T.C. Harrington, [h] J.N. Gibbs, [i] M. Morelet, [j] A.C. Molnar, [k] C.S. Hod.

but for which no authentic material exists. The aim of this study was to examine a collection of isolates of this fungus in detail at both molecular and morphological levels.

MATERIALS AND METHODS

We collected *Dryocoetus autographus* Ratzeburg, *Hylurgops palliatus* Gyllenhal and *Hylurgops glabratus* Zetterstedt adult beetles from infested Norway spruce (*Picea abies* (L.) Karst.) logs. We isolated fungi by macerating the beetles in a few drops of sterile water and diluting the macerate on the surface of 9 cm plastic Petri dishes with malt extract agar (MEA-strep, 20 g Merck malt extract, 16 g Sigma agar, 100 mg streptomycin sulphate, 1000 mL distilled water). In some cases, 10 mg L^{-1} of cycloheximide was added. Alternatively, we placed entire beetles or parts of the insects on the plates. The dishes were incubated at room temperature in diffuse daylight. Fungi were purified by transferring mycelium or conidial masses to fresh MEA plates. Additionally, isolations were performed from stained sapwood of pine (*Pinus sylvestris* L.) attacked by the bark beetle *Tomicus minor* Hartig.

Isolates used for molecular comparison are listed in TABLE I. All sequences have been submitted to GENBANK. Two isolates of *Leptographium* sp. "GG" were used in the molecular comparison. These were compared with isolates of *L. penicillatum, L. americanum* and the *Leptographium* anamorph of *O. dryocoetidis*. These species were chosen to represent *Leptographium* spp. with long conidia. *Leptographium laricis* and the *Leptographium* anamorph of *O. piceaperdum* were chosen to represent species with short conidia, and an isolate of *Leptographium procerum* was chosen as the outgroup, because it is considered to be a typical *Leptographium* and is characterised by short conidia.

Molecular comparisons.—Cultures used (TABLE I) were grown in 2% liquid malt extract (ME) (2 g/L ME per 100 mL H_2O) in 250 mL Erlenmeyer flasks at 25 C until the surface of the medium was covered with mycelium. A modification of the DNA extraction technique described by Raeder and Broda (1985) was used to extract total genomic DNA. Mycelium was harvested and transferred to sterile Eppendorf tubes with 500 µL extraction buffer (200 mM Tris-HCl, pH 8.5; 250 mM NaCl; 25 mM EDTA, pH 8.0; 0.5 % SDS). The suspension was quick-frozen in liquid nitrogen and ground to a fine paste with a pestle. Proteins were denatured and removed by phenol-chloroform extraction until the interphase was clear. The DNA was precipitated by the addition of 0.54 volume isopropanol and 0.1 volume 3 M NaAc, pH 5.8. After incubation for at least 60 min at −20 C, the DNA was pelleted by centrifugation at 4 C for 30 min at 14 000 rpm (20 800 × g, Eppendorf rotor F45–30–11). The pellets were washed with 70% ethanol, dried and resuspended in 100 µL sterile H_2O. The presence of DNA was determined by agarose gel electrophoresis.

The ITS1 and ITS2 (internal transcribed spacer regions) as well as the 5.8S gene of the rDNA operon were amplified using the Polymerase Chain Reaction (PCR) (Saiki et al 1988). Primers used in the amplification reactions are ITS-1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993), and CS1 (5′-TAGCTGATCCGAGGTCAA-3′) (Strydom et al 1997) for isolates of *L. penicillatum, L. americanum, L. piceaperdum, L. laricis* and *L.* sp. GG. ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), and LR1 (5′-GGTTGGTTTCTTTTCCT-3′) (White et al 1990) were used to amplify the ITS region in *O. dryocoetidis*. Amplifications were per-

formed in a Hybaid Touchdown temperature cycler (Hybaid, Middlesex, UK) for 35 cycles using High Fidelity Expand *Taq* DNA polymerase (Boehringer Mannheim, Germany) with the Expand HF Buffer with $MgCl_2$ supplied by the manufacturer and 2 ng of template DNA. The cycling parameters were: an initial denaturation step at 95 C for 2 min followed by 35 cycles of 95 C for 45 s, 55 C for 45 s, 62 C for 20 s and 72 C for 45 s. The reactions were completed with a final elongation step at 72 C for 8 min. The PCR fragments were visualised on a 1% agarose gel containing ethidium bromide, using an UV light. A single fragment of about 600 bp was obtained. However, small differences in fragment size were observed for the different species.

The PCR products were purified using the Nucleon QC for PCR/oligo clean-up kit (Amersham Life Science, England). The Thermo Sequenase dye terminator cycle sequencing pre-mix kit (Amersham Life Science, USA) on the ABI PRISM 377 automatic sequencer (Perkin Elmer Applied Biosystems) was used for sequencing. A region of 550 bp to 590 bp was sequenced for *O. penicillatum, O. americanum, O. piceaperdum, O. laricis* and *Leptographium* sp. 'GG' using primers ITS-1F and CS1. The ITS2 region for *O. dryocoetidis* was sequenced using primer LR1. The sequence data were edited using Sequence Navigator (PE Applied Biosystems) and manually aligned. Phylogenetic relationships were determined through a branch and bound search using PAUP (Phylogenetic Analysis Using Parsimony) (Swofford 1993) (see FIG. 1). Confidence levels for the various groups were determined using Bootstrap analysis (1000 replicates).

Morphology and growth characteristics.—All measurements were done on fungal structures produced in culture on 2% malt extract agar, MEA, (20 g Biolab malt extract, 20 g Biolab agar and 1000 mL distilled water). Fungal structures for microscopic examination were mounted on slides in lactophenol. Fifty measurements of each relevant morphological structure were made and ranges and averages computed.

For scanning electron microscopy (SEM), small blocks of agar cut from sporulating colonies were fixed in 3% glutaraldehyde and 0.5% OsO_4 in a 0.1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a Joel JSM 840 scanning electron microscope.

The optimal temperature for growth of an isolate (CMW 742) of the *Leptographium* sp. was determined by inoculating eight MEA plates for each temperature with a 0.6 mm diameter agar disk taken from the actively growing margin of a fresh isolate. The plates were incubated at temperatures ranging from 5 to 35 C at 5 C intervals. Colony diameters were measured on the fourth and the eighth day after commencing the experiment, and the diam of colonies were computed as an average of eight readings.

Cycloheximide tolerance of the *Leptographium* sp. was determined by growing CMW 742 on 2% MEA amended with different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1, 2.5 and 5 g/L) in Petri dishes. Dishes were incubated in the dark at 25 C for 8 d and the colony growth was measured. Five replicates were done for each concentration and the growth rate was determined based on an average of ten readings.

RESULTS

Molecular comparisons.—A single DNA fragment of approximately 600 bp was observed in the amplification reactions for all isolates. From these fragments, 550 bp were sequenced for the isolates of *O. penicillatum* (CMW 2306, CMW 2302, CMW 2642, CMW 2643), *O. americanum* (CMW 495, CMW 2963) and *O. dryocoetidis* (CMW 442). A total of 590 bp were sequenced for isolates of *O. laricis* (CMW 2014, CMW 2016, CMW 1980), *O. piceaperdum* (CMW 452, CMW 1949) and *Leptographium* sp. (CMW 1310, CMW 742). Twelve most parsimonious trees were generated with similar topologies using branch and bound PAUP analysis (FIG. 1). *Leptographium procerum* was used as the functional outgroup. The shortest tree length was 496, with a Consistency Index (CI) of 0.823, a Retention Index (RI) of 0.177 and Homoplasy Index (HI) of 0.916.

Isolates of *Ophiostoma penicillatum, O. americanum* and *O. dryocoetidis* clustered to form a single clade (FIG. 1). From the dendrogram it appeared that *O. penicillatum* and *O. americanum* are more closely related to each other than they are to *O. dryocoetidis*. The second clade was comprised of the isolates of *O. piceaperdum* and *O. laricis* (FIG 1). The unknown *Leptographium* sp. (Wingfield and Gibbs 1991), clustered separately to the other groups. The outgroup species *L. procerum* appears to be more closely related to the unknown *Leptographium* sp. than to the other isolates in the study.

Morphological and growth characteristics.—The *Leptographium* sp. considered here, is characterised by conidiophores typical of the genus. The conidiogenous apparatus superficially resembles the brush-like conidiogenous apparatus of the anamorph of *O. piceaperdum*. However, the *Leptographium* sp. is characterised by large guttules (oil-like droplets or vacoules) in its conidia. This characteristic appears to be unique to the species. Wingfield and Gibbs (1991) suggested that this species could be the same that was identified by Mathiesen (1950) as *Ophiostoma penicillatum* f. *palliati*. Harrington (1988) indicated that this fungus was distinct from *O. penicillatum* and probably represents a distinct taxon. From the molecular comparison in this study, it is clear that this species has no relatedness to *O. penicillatum*. The fungus is, therefore, described as a new species in *Leptographium*.

Leptographium guttulatum M. J. Wingf. et K. Jacobs sp. nov.

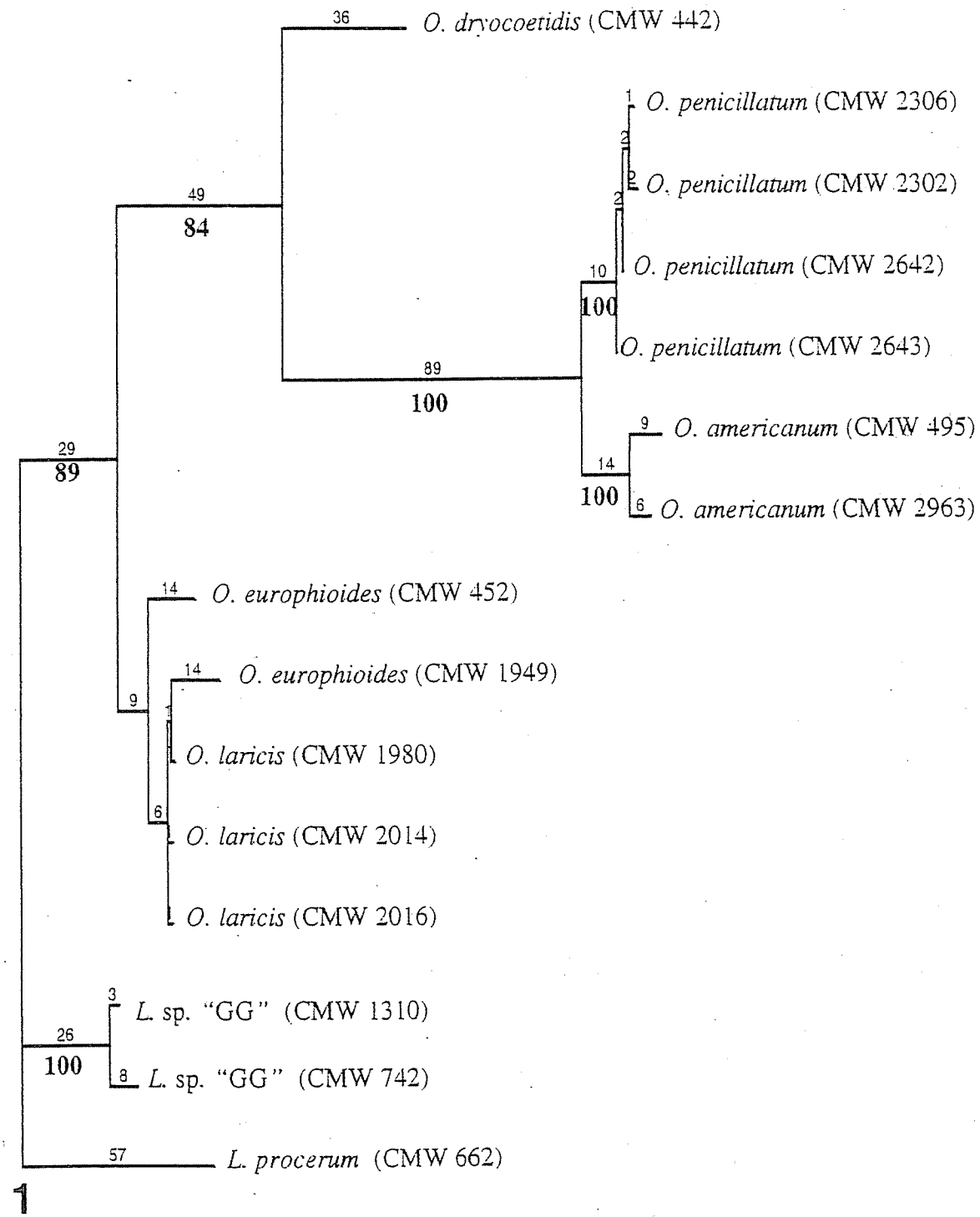


FIG. 1. Phylogram produced by PAUP analysis based on partial sequence of the ITS1 and ITS2 regions, as well as the complete sequence of the 5.8S rRNA gene. *Leptographium procerum* was used as a functional outgroup. The number of base substitutions are indicated above the branches and the bootstrap percentages (100 bootstrap repeats) are indicated below the branches.

Colonia atro-olivacea (21 "m). Coloniae margo levis. Hyphae submersae in medio solido paucis myceliis aeriis, olivaceae, (21 "k), leves, rectae, non constrictae ad septa, (5.0–)9.0(–13) µm diametro. Conidiophora evenientia singulatim, exorientia directe ex mycelio vel aerio mycelio, erecta, macronematosa, mononematosa, (200–)415(–810) µm longitudine, rhizoidaceae structurae absentes. Conidiogenus apparatus (60–)114(–200) µm longus, conidica massa exclusa, 2 usque 4 seriebus ramorum cylindricorum; 2–4 metulae primariae. Conidiogenae cellae discretae, 2–3

per ramum, cylindricae, exigue attenuatae ad apicem, (10–)17(–27) µm longae et 2.0–3.0 µm latae. Conidia hyalina, aseptata, guttulata, oblonga vel exigue obovoidea, (4.0–)7.0(–10.0) × 2.0–3.0 µm.

Colonies with optimal growth at 25 C on 2% MEA, reaching 36 mm diam in 9 d. Little growth at 5 C and no growth above 30 C. Able to withstand high concentrations of cycloheximide with a 5% increase in growth on 0.5 g/L cycloheximide after 9 d at 25 C in the dark. Colony dark olive (21"m). Colony margin smooth. Hyphae submerged on solid medium with little aerial mycelia, olivaceous (21"k), smooth, straight, not constricted at the septa, (5.0–)9.0(–13) µm diam. Conidiophores occurring singly, arising directly from the mycelium or aerial mycelium, erect, macronematous, mononematous, (200–)415(–810) µm long (FIG. 2), rhizoid-like structures absent (FIG. 3, 8a). Stipe olivaceous (21"k), smooth, cylindrical, simple, 2–7 septate, (120–)300(–670) µm long (from first basal septum to below primary branches), (5.0–)8.0(–12) µm wide below primary branches, apical cell not swollen; (5.0–)8.5(–12.0) µm wide at base, basal cell not swollen. Conidiogenous apparatus (60–)114(–200) long, excluding the conidial mass, with 2 to 4 series of cylindrical branches; 2–4 primary branches, light olivaceous (21"k) to olivaceous (21"m), smooth, cylindrical, aseptate, (18–)29(–40) µm long and (5.0–)7.0(–10.0) µm wide, secondary branches light olivaceous (21"k), aseptate, (15–)24.5(–35) µm long, (3.0–)5.5(–8.0) µm wide; tertiary branches hyaline, aseptate, (10–)21(–33) µm long, (3.0–)4.0(–5.0) µm wide, quaternary branches hyaline, aseptate, (9.0–)14.5(–25.0) µm long, (2.0–)3.0(–4.0) µm wide (FIG. 4, 8b). Conidiogenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (10–)17(–27) µm long and 2.0–3.0 µm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving a false impression of sympodial proliferation (Minter et al 1982, 1983, Van Wyk et al 1988) (FIG. 5, 6). Conidia hyaline, aseptate, oblong to slightly obovoid, prominent guttulate, (4.0–)7.0(–10) × 2.0–3.0 µm (FIG.7, 8c). Conidia accumulating in slimy droplets at the apices of conidiogenous apparatus, hyaline at first, becoming cream coloured (19'f) with age (Rayner 1970). Conidial masses cream coloured when wet, remaining the same colour when dry.

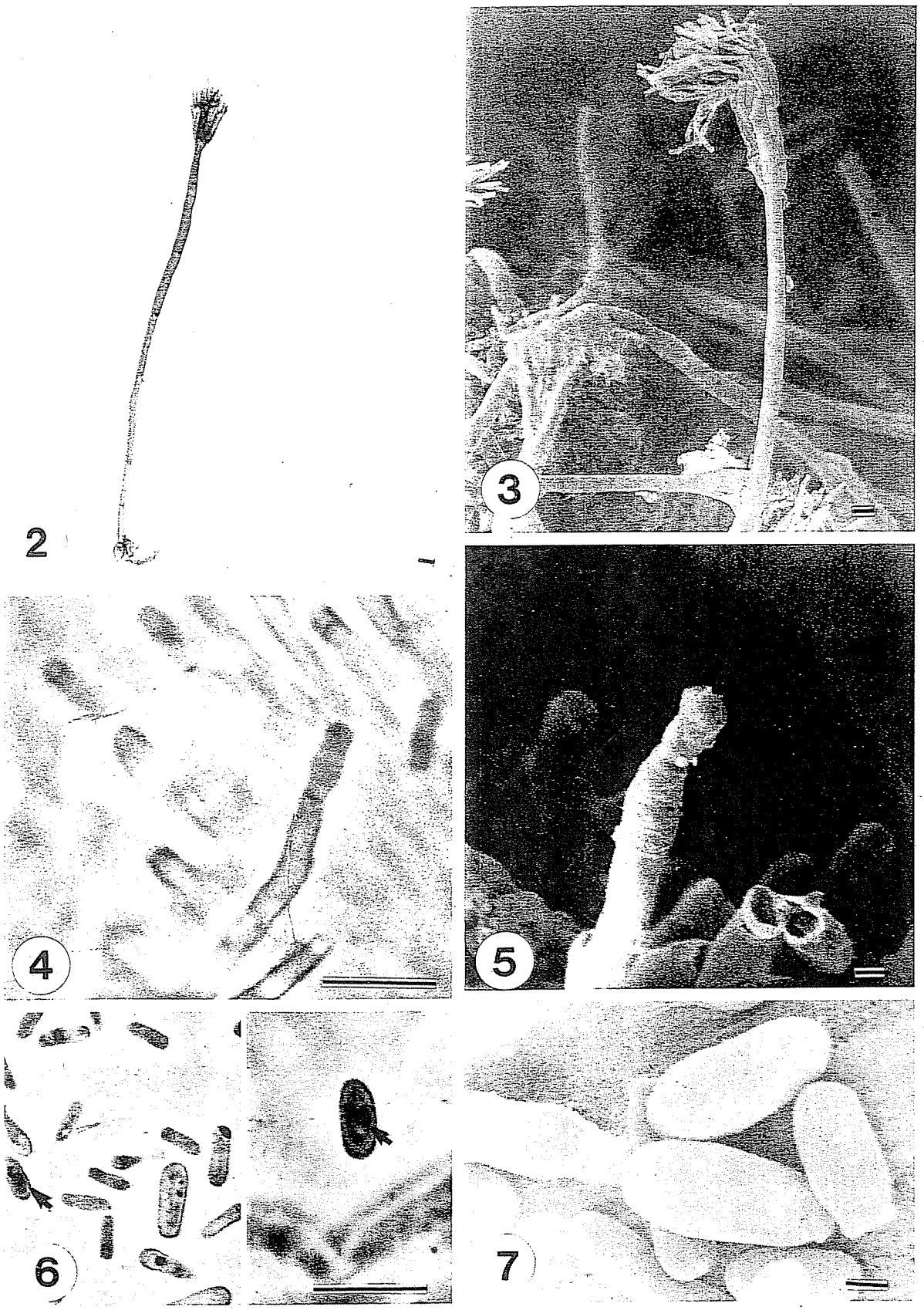
Specimens examined. FRANCE. Orleans, isolated from *Tomicus piniperda* L. from *Pinus sylvestris,* 1984, *M. Morelet, PREM 56389* (HOLOTYPE). ENGLAND. Hampshire, Fresley Cannon Wood, isolated from *Tomicus piniperda* from *Pinus sylvestris,* 1988, *J.N. Gibbs, PREM 56310*; AUSTRIA. Flatz, Lower Austria, isolated from *Hylurgops palliatus* from *Picea abies,* 28 May 1997, *T. Kirisits, PREM 56311*; Glein, Styria, isolated from *Hylurgops glabratus* from *Picea abies,* 3 August 1993, *T. Kirisits, PREM 56309*; Flatz, Lower Niederösterreich, isolated from *Hylurgops palliatus* from *Picea abies,* 28 May 1997, *T. Kirisits. PREM 56308* (PARATYPES).

Insect associates.—Leptographium guttulatum was only rarely isolated from adult beetles of *D. autographus* (2 isolations out of 35 adult beetles), *H. palliatus* (1 isolation out of 7 adult beetles) and *H. glabratus* (1 isolation out of 107 adult beetles). This fungus was also isolated once (1 out of 150 samples) from the sapwood of trees attacked by *Tomicus minor.* Based on the limited material of this study, *L. guttulatum* appears thus to be a minor component of the mycobiota of these four bark beetles. *Ophiostoma piceaperdum* was one of the most frequent ophiostomatoid fungi isolated from *D. autographus, H. palliatus* and *H. glabratus* (data not shown). *Ophiostoma penicillatum* was never isolated from the above mentioned insects.

DISCUSSION

Leptographium guttulatum can easily be distinguished from other *Leptographium* spp. based on the distinct guttules in its conidia, that are unlike those of other *Leptographium* spp. This species is one of several *Leptographium* spp. associated with conifer bark beetles in Europe. Other species include the *Leptographium* anamorph of *O. piceaperdum* and *L. penicillatum* (Solheim 1986). *Leptographium guttulatum* can be distinguished from *O. piceaperdum* primarily based on the absence of a teleomorph in the former species. The anamorph of *O. piceaperdum* can readily be distinguished from *L. guttulatum* based on its considerably smaller conidiophores, although the conidiogenous apparatuses of these two species are similar in their brush-like appearance. The anamorph of *O. penicillatum* is characterised by large allantoid conidia, in contrast to the obovoid conidia of *L. guttulatum.* These two species can, therefore, easily be distinguished. From the molecular data it is also clear that *L. guttulatum* is not related to *O. penicillatum.*

As is true with most *Leptographium* spp., *L. guttulatum, O. penicillatum* and *O. piceaperdum* are associated with bark beetles (Coleoptera: Scolytidae). *Leptographium guttulatum* has been isolated from spruce and pine in Europe and is transmitted by a wide range of bark beetles that belong to the genera *Dryocoetes, Hylastes, Hylurgops* and *Tomicus* (TABLE I, Wingfield and Gibbs 1991). In contrast, *O. penicillatum* is consistently associated with the spruce bark beetles *Ips typographus* (Solheim 1986, Krokene and Solheim 1996), *Ips duplicatus* (Krokene and Solheim 1996) and *Ips amitinus* (Kirisits unpubl). Mathiesen (1950) also reported *O. penicillatum* f. *palliati* to be associated with *H. palliatus,* although this record is



FIGS. 2–7. *Leptographium guttulatum* (PREM 56389). 2. Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus. 3. Conidiophore with complex conidiogenous apparatus. 4, 5. Conidiogenous cells showing false sympodial conidiogenesis. 6. Conidia with prominent guttules. 7. Scanning electron micrograph of conidia. Bars: 2 = 100 μm, 3, 4, 6 = 10 μm, 5, 7 = 1μm.

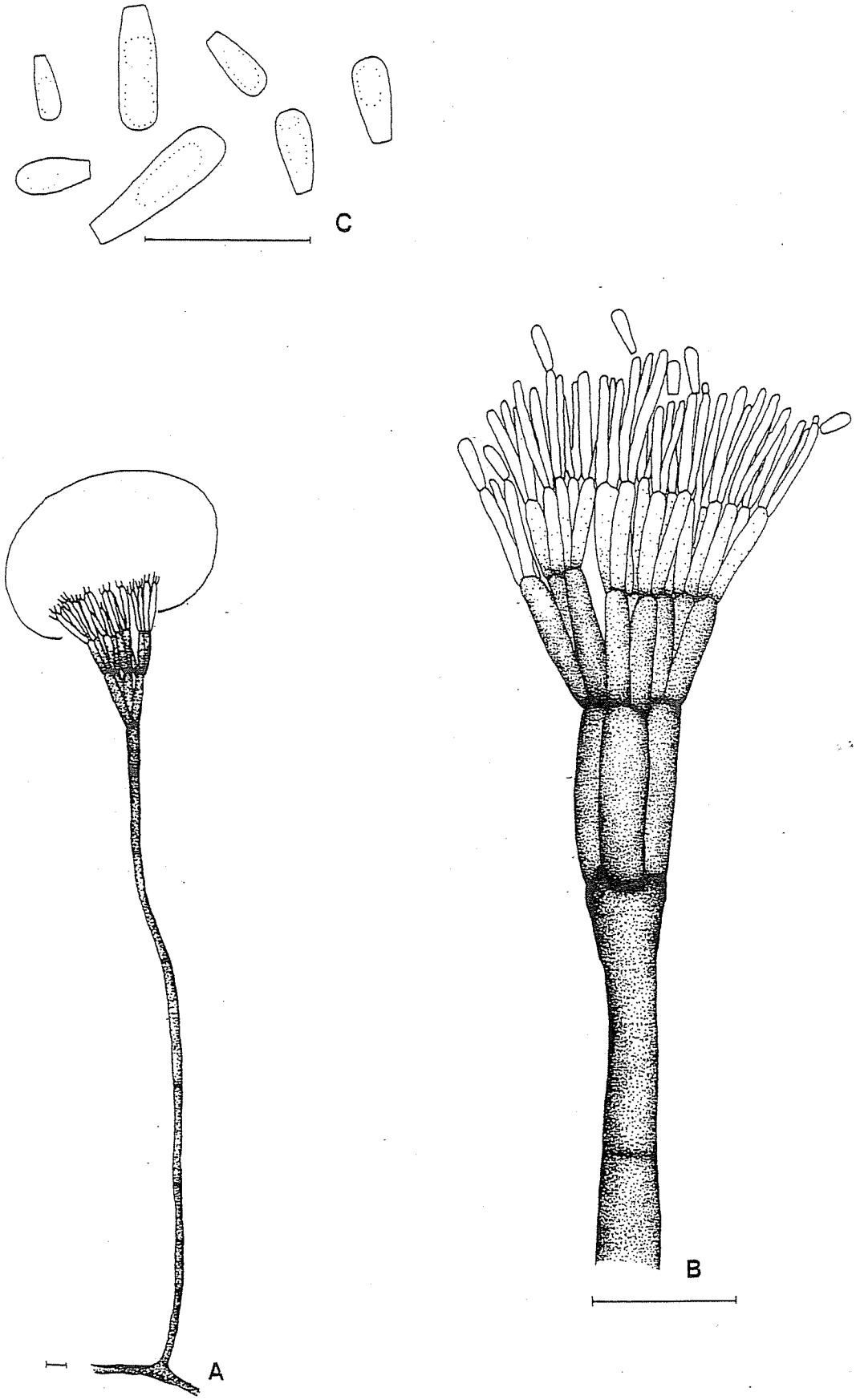


FIG. 8. *Leptographium guttulatum* (PREM 56389). A. Habit sketch of the conidiophore. B. Conidiogenous apparatus. C. Conidia with guttules (dotted lines). Bars = 10 μm.

probably not correct. In the present study, *O. penicillatum* was not isolated from *D. autographus, H. glabratus* or *H. palliatus,* that are vectors of *Leptographium guttulatum.* Furthermore, *O. penicillatum* was not found to be associated with *H. palliatus* in Denmark (Harding 1989) and Norway (Krokene and Solheim 1996). The common insect associates of *L. guttulatum* thus appear to be quite different to those of *O. penicillatum.* Similar to *L. guttulatum, Ophiostoma piceaperdum* is associated with various bark beetles on spruce and pine in Europe. For example, *O. piceaperdum* has been reported to be associated with *Ips typographus* (Solheim 1986), *Ips duplicatus* (Krokene and Solheim 1996), *Ips amitinus* (Kirisits unpublished), *Hylurgops palliatus* (Harding 1989, Krokene and Solheim 1996) on Norway spruce and with *Tomicus piniperda* attacking Scots pine (Solheim and Långström 1991). In this study *O. piceaperdum* was frequently isolated from *D. autographus, H. palliatus* and *H. glabratus.* The literature, as well as the results of the present study, thus clearly show similarities in the habitat of *L. guttulatum* and *O. piceaperdum.*

The taxonomy of *Leptographium* spp. has been in disarray virtually since the establishment of the genus by Lagerberg and Melin (1927). In recent years, efforts have been made to improve this situation. Where species have appeared and have some significant ecological significance, they have been described, although there remain many undescribed species. The description of *L. guttulatum* has lead to a better understanding and taxonomic placement of one species that has been isolated regularly in recent years. What must follow is a comprehensive review of the genus *Leptographium* that is also supported by molecular comparisons. We are currently undertaking such a study and it is hoped that this will resolve many of the remaining taxonomic questions pertaining to species in the genus.

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LITERATURE CITED

Cobb FW Jr. 1988. *Leptographium wageneri,* cause of blackstain root disease: a review of its discovery, occurrence and biology with emphasis on pinyon and ponderosa pine. In: Harrington TC and Cobb FW J,. eds. *Leptographium* root diseases on conifers. St Paul, Minnesota: American Phytopathological Society. p 41–62.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mychorrizae and rusts. Mol Ecol 2:113–118.

Grosmann H. 1931. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. [Contributions to the knowledge concerning the life partnership between bark beetles and fungi.] Z. Parasitenkunde 3:56–102.

———. 1932. Über die systematischen Beziehungen der Gattung *Leptographium* Lagerberg et Melin zur Gattung *Ceratostomella* Sacc. nebst einigen Bemerkungen über *Scopularia venusta* Preuss und *Hantschia phycomyces* Awd. Hedwigia 72:183–194.

Harding S. 1989. Blue stain fungi associated with *Hylurgops palliatus* Gyll. (Coleoptera: Scolytidae). In: The influence of mutualistic blue stain fungi on bark beetle population dynamics. [Ph.D thesis], Copenhagen, Royal Veterinary and Agricultural University, Department of Zoology. p 1–11.

Harrington TC. 1987. New combinations in *Ophiostoma* of *Ceratocystis* species with *Leptographium* anamorphs. Mycotaxon 28:39–43.

———. 1988. *Leptographium* species, their distributions, hosts and insect vectors. In: Harrington TC and Cobb FW Jr, eds. *Leptographium* root diseases on conifers. St Paul, Minnesota: American Phytopathological Society. p 1–39.

———, Cobb FW Jr. 1987. *Leptographium wageneri* var. *pseudotsugae,* var. nov., cause of black stain root disease on Douglas-fir. Mycotaxon 30:501–507.

Jacobs K, Wingfield MJ, Bergdahl D. 1997. A new species of *Ophiostoma* from North America, similar to *Ophiostoma penicillatum.* Can J Bot 75:1315–1322.

Kendrick WB. 1962. The *Leptographium* complex. *Verticicladiella* Hughes. Can J Bot 40:771–797.

———, Molnar, AC. 1965. A new *Ceratocystis* and its *Verticicladiella* imperfect state associated with the bark beetle *Dryocoetus confusus* on *Abies lasiocarpa.* Can J Bot 43:39–43.

Krokene P, Solheim H. 1996. Fungal associates of five bark beetle species colonizing Norway spruce. Can J For Res 26:2115–2122.

Lagerberg T, Lundberg G, Melin E. 1927. Biological and practical researched into blueing in pine and spruce. Svenska Skogsvårdsför Tidskr 25:145–272.

Leach JG, Orr LW, Christensen C. 1934. The interrelationships of bark beetles and blue-staining fungi in felled Norway pine timber. J Agric Res 49:315–341.

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

Minter DW, Kirk PM, Sutton BC. 1982. Holoblastic phialides. Trans Br Mycol Soc 79:75–93.

———, ———, ———. 1983. Thallic phialides. Trans Br Mycol Soc 80:39–66.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Lett Appl Microbiol 1:17–20.

Rayner RW. 1970. A Mycological colour chart. Commonwealth Mycological Institute and British Mycological Society: Kew, Surrey, UK. p 34.

Robinson-Jeffrey RC, Davidson RW. 1968. Three new *Europhium* species with *Verticicladiella* imperfect states on blue-stained pine. Can J Bot 46:1523–1527.

Saiki RJ, Gelfand DA, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Solheim H. 1986. Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. Nordic J Bot 6:199–207.

———, Långström B. 1991. Blue-stain fungi associated with Tomicus piniperda in Sweden and preliminary observations on their pathogenicity. Ann Sci Forg 48:149–156.

Strydom RC, Wingfield BD, Wingfield MJ. 1997. Ribosomal DNA sequence comparison of *Leptographium lundbergii* and *L. truncatum* and neotypification of *L. lundbergii*. Syst Appl Microbiol 20:295–300.

Swofford DL. 1993. PAUP Phylogenetic Analysis Using Parsimony Version 3.1.1. Champaign, Illinois 61820, USA.

Upadhyay H.P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press: Athens. p 176.

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

Van Wyk P, Wingfield MJ, Marasas WFO. 1988. Differences in synchronisation of stages of conidial development in *Leptographium* species. Trans Br Mycol Soc 90:451–456.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis AM, Gelfand DH, Sninsky JJ, WhiteTJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press 315–322.

Wingfield MJ. 1993. *Leptographium* species as anamorphs of *Ophiostoma*: progress in establishing acceptable generic and species concepts. In: Wingfield MJ, Seifert KA, Webber JF, eds. *Ceratocystis* and *Ophiostoma:* taxonomy, ecology and pathogenicity. St. Paul, Minnesota: American Phytopathological Press. p 43–51.

———, Gibbs JN. 1991. *Leptographium* and *Graphium* species associated with pine-infesting bark beetles in England. Mycol Res 95:1257–1260.

———, Seifert KA, Webber JF. 1993. *Ceratocystis* and *Ophiostoma:* taxonomy, ecology and pathogenicity. St Paul, Minnesota: American Phytopathological Society Press. p 293.

Wright EF, Cain RF. 1961. New species of the genus *Ceratocystis*. Can J Bot 39:1215–1230.

Zambino PJ, Harrington TC. 1992. Correspondence of isozyme characterization with morphology in the asexual genus *Leptographium* and taxonomic implications. Mycologia 84:12–25.