Leptographium profanum sp.nov., a new species from hardwood roots in North America

Karin Jacobs, Lori G. Eckhardt, and Michael J. Wingfield

Abstract: Species of *Leptographium* are anamorphs of *Ophiostoma* and best known as associates of tree-infesting bark beetles. The majority of these fungi and their insect associates are found on conifers where they typically cause sap-stain of lumber. A small number of species are also known as tree pathogens. Relatively few *Leptographium* species are found on angiosperm trees. Species described from these niches have increased in recent years. The objective of this study was to characterize a *Leptographium* species isolated from the roots of various hardwood trees in the southeastern United States. Morphology of this fungus differed from that of other *Leptographium* species. Comparisons of DNA sequences for part of the ITS2–28S ribosomal DNA region, the β-tubulin, and the elongation 1-α gene regions also showed that this fungus represents an undescribed taxon. The fungus is thus described as ***Leptographium profanum*** sp.nov.

Key words: angiosperm host, *Carya* sp., *Nyssa sylvatica*, *Cornus florida*, phylogenetic comparison.

Résumé : Les espèces de *Leptographium* sont des anamorphes des *Ophiostoma* et mieux connus comme associés aux scolytes qui infectent les arbres. La majorité de ces champignons et de leurs insectes associés, se retrouvent chez les conifères où ils causent typiquement la tache colorée de l'aubier. Un petit nombre d'espèces sont également connues comme pathogènes des arbres. On trouve relativement peu de *Leptographium* espèces chez les angiospermes. Les espèces décrites pour ces niches ont augmenté au cours des récentes années. L'objectif de cette étude est de caractériser un *Leptographium* espèce isolé des racines de diverses espèces d'arbres à bois franc, du sud-est des États-Unis. La morphologie de ce champignon diffère de celle des autres *Leptographium* spp. Les comparaisons des séquences d'ADN pour une partie de la région de l'ADN ribosomal ITS2–28S, ainsi que les régions de la β-tubuline et du gène d'élongation 1-α, montrent que ce champignon représente un taxon jamais décrit. On décrit ce champignon comme ***Leptographium profanum*** sp.nov.

Mots clés : *Carya* sp., *Nyssa sylvatica*, *Cornus florida*, comparaison phylogénétique.

[Traduit par la Rédaction]

Introduction

Leptographium is one of several anamorph genera associated with the teleomorph genus *Ophiostoma* (Wingfield et al. 1993). Only half of the approximately 50 described species of *Leptographium* have been linked to a teleomorph (Jacobs and Wingfield 2001). However, species in the genus comprise a monophyletic clade based on DNA sequence comparisons (Jacobs et al. 2001*a*) and, therefore, represent a well-defined natural group.

Species in *Leptographium* are generally characterized by dark mononematous conidiophores with complex conidiogenous apparatuses. Small, single-celled hyaline conidia are produced through percurrent proliferation of the conidiogenous cells. Conidia are produced in sticky slime drops at the apices of the conidiophores (Jacobs and Wingfield 2001).

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Species of *Leptographium* are well adapted for dispersal by insects that pick up their sticky spores, typically produced in bark beetle tunnels. This mutualistic relationship has been well-documented during the course of the last century (Münch 1907; Lagerberg et al. 1927; Grosmann 1931; Mathiesen-Käärik 1960; Harrington and Cobb 1988). Most *Leptographium* species have been described from conifers such as pine, spruce, and larch (Grosmann 1931; Harrington and Cobb 1988; Jacobs and Wingfield 2001). In the wood of these trees, they are known to cause sap-stain, which reduces the economic value of the wood (Münch 1907; Lagerberg et al. 1927; Seifert 1993). In addition, a small number of *Leptographium* species are primary pathogens causing important vascular wilt diseases such as black stain root disease (Wagener and Mielke 1961; Kendrick 1962; Harrington and Cobb 1988) and takamaka disease (Wiehe 1949; Webber et al. 1999).

Relatively few *Leptographium* species are known from non-conifer hosts. Of the approximately 50 described species, 11 have been isolated from deciduous trees or other niches (Jacobs and Wingfield 2001; Masuya et al. 2004). These include *L. costaricense* and *L. reconditum* isolated from the soil environment around the roots of *Talauma sambuensis* and *Triticum aestivum* (wheat), respectively (Jooste 1978; Weber et al. 1996). Most *Leptographium* species including those from conifers, hardwoods, and other niches seem to be habitat specific. The host or environment from which these fungi are isolated appears to be consistent and the habitat

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Table 1. Strains used in the DNA-based comparisons.

Species	Strain No.	ITS[a]	β-tubulin[b]	Elongation 1-α[c]
L. profanum	CMW 10554	DQ354942	DQ354934	DQ354939
	CMW10552	DQ354944	DQ354936	DQ354941
	CMW 10550	DQ354943	DQ354935	DQ354940
L. truncatum	CMW 2402	DQ062051	DQ061985	DQ062018
	CMW 28	DQ062052	DQ061986	DQ062019
L. huntii	CMW 2824	AY553393	DQ354932	DQ354937
	CMW 2868	AY553394	DQ354933	DQ354938
L. lundbergii	CMW 217	DQ062065	DQ061999	DQ062032
	CMW 17264	DQ062068	DQ062002	DQ062035
	CMW 2190	DQ062066	DQ062000	DQ062033
L. douglasii	CMW725	AY553380	AY534928	AY536174
	CMW2078	AY553381	AY534929	AY536175
L. neomexicanum	CMW2079	AY553382	AY534930	AY536176
L. reconditum	CMW15	AY553383	AY534931	AY536177
L. serpens	CMW193	AY553387	AY534935	AY536181
	CMW60	AY553388	AY534936	AY536182
L. aenigmaticum	CMW2199	AY553389	AY534937	AY536183
	CMW2310	AY553390	AY534938	AY536184
O. robustum	CMW2805	AY553396	AY534944	AY536190
	CMW668	AY553397	AY534945	AY536191
L. aureum	CMW709	AY553413	AY534961	AY536207
	CMW714	DQ062071	DQ062005	DQ062038
L. pyrinum	CMW509	AY553414	AY534962	AY536208
	CMW169	DQ062072	DQ062006	DQ062039
L. yunnanensis	CMW5304	AY553415	AY534963	AY536209
	CMW5152	DQ062073	DQ062007	DQ062040
L. wingfieldii	CMW2095	AY553400	AY534948	AY536194
	CMW 2096	AY553398	AY534946	AY536192
	CMW2019	AY553399	AY534947	AY536193
L. pineti	CMW3831	DQ062076	DQ062010	DQ062043
	CMW3837	DQ062077	DQ062011	DQ062044
L. americanum	CMW495	DQ062079	DQ062013	DQ062046
	CMW2929	DQ062078	DQ062012	DQ062045
L. abietinum	CMW2817	DQ062080	DQ062014	DQ062047
	CMW3083	DQ062081	DQ062015	DQ062048
L. laricis	CMW1980	DQ062074	DQ062008	DQ062041
	CMW2014	DQ062075	DQ062009	DQ062042
L. pinidensiflorae	CMW5158	DQ062082	DQ062016	DQ062049
	CMW5162	DQ062083	DQ062017	DQ062050

[a]ITS2 and partial 28S gene.
[b]Partial β-tubulin gene.
[c]Partial elongation 1-α gene.

has, therefore, been effectively used in the identification of *Leptographium* species. (Jacobs and Wingfield 2001).

Species of *Leptographium* from deciduous trees are less well known than those from conifers, and in most cases species are represented by single records and limited numbers of strains. In contrast, species from conifers, many of which have wide distributions, are relatively well known. Another common character of *Leptographium* species not associated with conifers is the fact that very few have been associated with specific insect vectors. Conifer-associated species on the other hand have relatively close associations with specific scolytine bark beetles (Lagerberg et al. 1927; Mathiesen-Käärik 1960; Jacobs et al. 2004).

Isolates of a *Leptographium* sp. have recently been collected from discoloured areas on the roots of three hardwood tree species in central Alabama, USA. These trees included blackgum (*Nyssa sylvatica*), hickory (*Carya* sp.), and dogwood (*Cornus florida*). In common with other species associated with hardwoods, the strains were not associated with insects on the roots from which they were isolated. The objective of this study was to characterize the unknown *Leptographium* sp. from roots of hardwood trees in the southeastern United States.

Materials and methods

Source of strains

Strains used in this study all originated from the roots of

blackgum (*Nyssa sylvatica*), hickory (*Carya* sp.), and dogwood (*Cornus florida*). Roots of these trees were unearthed as part of a study on loblolly decline in Alabama.

The strains were collected using the modified two-root excavation method of Otrosina et al. (1997). Two lateral root segments >3 cm in diameter from each tree were excavated with hand tools from opposite sides of the root collar to the approximate crown drip line. Sections (20 cm long) of each root were cut and kept chilled in ice chests for transport to the laboratory. Roots were stored at 4 °C until they could be processed in the laboratory. They were then examined for lesions and areas of blue or black stain by removing bark with a flamed scalpel. The roots were then cut into pieces, rinsed in tap water, surface sterilized in commercial bleach, EtOH, and distilled H_2O (10:10:80 by volume) for 1 min, rinsed in tap water for 3 min, and blotted dry with sterile tissue. The root pieces were then plated (4 pieces per plate, 20 plates per sample) on 2% malt extract agar (MEA) and CSMA (MEA containing 800 mg·L^{-1} of cycloheximide and 200 mg·L^{-1} of streptomycin sulfate) (Hicks et al. 1980) and incubated at 25 °C under fluorescent light (460 m^{-2}·s^{-1}) in the dark for 2 weeks. The roots were then examined for fungal growth.

Isolates of a *Leptographium* sp. were subcultured by transferring hyphal tips and (or) conidial masses to sterile plates of MEA. Subcultured isolates were placed on filtered V-8 juice agar (CVA, adapted from Jeffers 2000) slants and were stored at 8 °C for subsequent identification.

Phylogenetic analyses

Strains (Table 1) for phylogenetic comparison were grown on commercially produced potato dextrose agar (PDA, Biolab, Midrand, Johannesburg, South Africa) for 10 d at 25 °C. DNA was extracted from pure cultures using the method described by Möller et al. (1992) and modified by Jacobs et al. (2004). The presence of DNA was confirmed on 1% agarose gels stained with ethidium bromide.

The internal transcribed spacer (ITS2), part of the large subunit (28S) of the rDNA operon, β-tubulin, and elongation factor 1-α (EF 1-α) genes were amplified as previously described by Jacobs et al. (2004). PCR reactions were performed in 25 µL volumes (2.5 mmol·L^{-1} $MgCl_2$, 1× PCR buffer, 0.2 mmol·L^{-1} dNTP, 0.2 mmol·L^{-1} of each primer and 2.5 U *Taq*-polymerase enzyme). Primers used in the amplification reactions and for cycle sequencing were ITS3 and LR3 (White et al. 1990) for the ITS2 and 28S region, Bt2a and Bt2b (Glass and Donaldson 1995) for the β-tubulin gene, and EF1F and EF2R for the elongation factor 1-α gene (Jacobs et al. 2004). PCR products were purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using the Big Dye terminator cycle sequencing premix kit (Applied Biosystems, Foster City, California, USA) on an ABI PRISM 3100 automatic sequencer (Perkin Elmer Applied Biosystems, Foster City, California, USA). Sequence contigs were assembled using Sequence Navigator (Applied Biosystems), aligned in ClustalX (Thompson et al. 1997) and manually adjusted in SeAl (Rambaut 1996).

Phylogenetic relationships for the taxa were inferred using distance analysis in PAUP* v.4.0b10 (Swofford 2001). Characters were treated as unweighted in the analysis and gaps were treated as missing data. A single tree for each dataset was obtained using neighbour-joining analysis with an uncorrected P-distance and rooted to midpoint. A bootstrap analysis (1000 replicates using the neighbour-joining option) was performed to determine the confidence levels of the nodes. For all the datasets, ambiguously aligned regions were coded and step matrices to assign different weights to these codes were computed using INAASE 2.3b (Lutzoni et al. 2000). These weighted codes were used in the analysis to replace the ambiguous aligned regions. Both a partition homogeneity test (Farris et al. 1995) and a Templeton Nonparametric Wilcoxon Signed Ranked Test (Kellogg et al. 1996) were performed to determine whether the three data sets could be combined.

A Bayesian Markov Monte Carlo analysis (Larget and Simon 1999) was performed to confirm the confidence levels for the tree nodes in the distance analysis. The analysis was performed in MrBayes 2.01 (Huelsenbeck and Ronquist 2001). The algorithm ran for 500 000 generations, and every tenth tree was sampled. Four cold chains were run simultaneously, and the trees sampled before convergence of the Markov chains were discarded in all the analysis as the burnin period.

Morphological comparisons

All measurements and microscopic observations were made from fungal structures grown on 2% MEA (20 g Biolab malt extract, 20 g agar, 1000 mL distilled water) and oatmeal agar (OA) (Gams et al. 1998) and incubated in incident light at 25 °C. Fungal structures were mounted on slides in 85% lactic acid and examined using phase or differential interference contrast microscopy. Fifty measurements were made for each morphological character and the averages and standard deviations were calculated.

Results

Phylogenetic analyses

Amplification of the ITS2 and 28S region resulted in fragments of approximately 900 base pairs (bp). The aligned data set consisted of 623 characters, of which 507 were constant. Three ambiguously aligned regions (67 bp) were identified and excluded from the analysis. These regions were replaced by weighted, coded characters (Lutzoni et al. 2000). Similar to other studies, the DNA sequences of the ITS2 and 28S region proved to be fairly conserved for the strains examined (Jacobs et al. 2004, 2005)

Amplification of a part of the β-tubulin gene resulted in fragments of approximately 500 bp. The 3′ and 5′ ends of the sequences were trimmed to align them with pre-existing data (Jacobs et al. 2004, 2005; Table 2), and the aligned data set consisted of 476 characters, of which 161 were constant. Four ambiguously aligned regions (232 bp) were identified and excluded from the analysis.

Amplification of a part of the EF 1-α gene resulted in fragments of approximately 900 bp. The aligned data set consisted of 881 characters. of which 306 were constant. Six ambiguously aligned regions were identified (488 bp in total) and excluded from the analysis. Results of both the partition homogeneity test (Farris et al. 1995) and the Templeton Nonparametric Wilcoxon Signed Ranked Test

Table 2. Comparison of *Leptographium profanum* and other *Leptographium* species from non-coniferous habitats.

Characters	*L. profanum*	*O. brevicolle*	*L. calophylli*	*L. costaricense*	*L. elegans*	*L. eucalyptophylum*
Host	*Carya* sp., *Nyssa sylvatica*, *Cornis florida*	*Populus tremuloides*	*Calophyllum inophyllum*	*Talauma sambuensis*	*Chamaecyparis formosensis*	*Eucaluptus*
Conidiophore length (μm)	220–1000	110–265	40–100	150–625	105–430	180–500
Primary branch type	B	A	B	B	B	B
Rhizoids	Absent	Absent	Absent	Absent	Absent	Absent
Conidium shape	Obovoid to oblong	Oblong	Obovoid to oblong	Obovoid	Oblong	Obocoid to clavate
Conidium size (μm)	3–6	3–6	3–7	3–5	3–5	6–9
Synanamorph	Absent	Absent	Absent	Absent	*Sporothrix*	Absent
Teleomorph	Absent	Present	Absent	Absent	Absent	Absent
Ascospore shape	NA	Allantoid	NA	NA	NA	NA
Associated insects	None	*Trypodendron retusus*	*Cryphalus trypanus*	Not known	Not known	Not known

Note: NA, not available.

(Kellogg et al. 1996) showed that the three datasets could be combined.

The aligned combined dataset consisted of 1980 characters, of which 977 were constant. Thirteen ambiguously aligned regions were identified (787 bp in total) and excluded from the analysis. These regions were replaced with weighted codes calculated using INAASE 2.3b (Lutzoni et al. 2000). The tree topologies resulting from analysis of the three separate datasets, as well those of the combined dataset, were similar (data not shown). In all cases, strains of the unknown *Leptographium* sp. from hardwood trees in Alabama resided in a well-resolved clade within the larger *Leptographium* group (Fig. 1). *Leptographium pini-densiflorae* was most closely related to this set of hardwood associated strains (Fig. 1).

Morphology

Morphological examination of the strains from hardwood showed that they were alike, but displayed no apparent similarity to any previously described *Leptographium* species. The strains did not resemble any of the species previously described from hardwoods and they were most similar to *L. peucophilum,* which occurs on conifers. There were, however, clear morphological differences between these two fungi.

The hardwood strains were characterized by long, well-formed conidiophores (Figs. 2, 3), which are typical for species in *Leptographium*. No rhizoids were observed, and the conidiogenous apparatuses were comprised of a complex series of branches (Figs. 4, 5, 8). The conidia of this species were fairly nondistinctive (Figs. 6, 7, 9) and would be considered small compared to other species in *Leptographium* (Jacobs and Wingfield 2001). This combination of characters was not shared by any of the other species in *Leptographium,* and the strains from the roots of blackgum, hickory, and dogwood in Alabama are, therefore, described as a distinct taxon.

Leptographium profanum sp.nov. Jacobs, Eckhardt & Wingfield Figs. 2–9

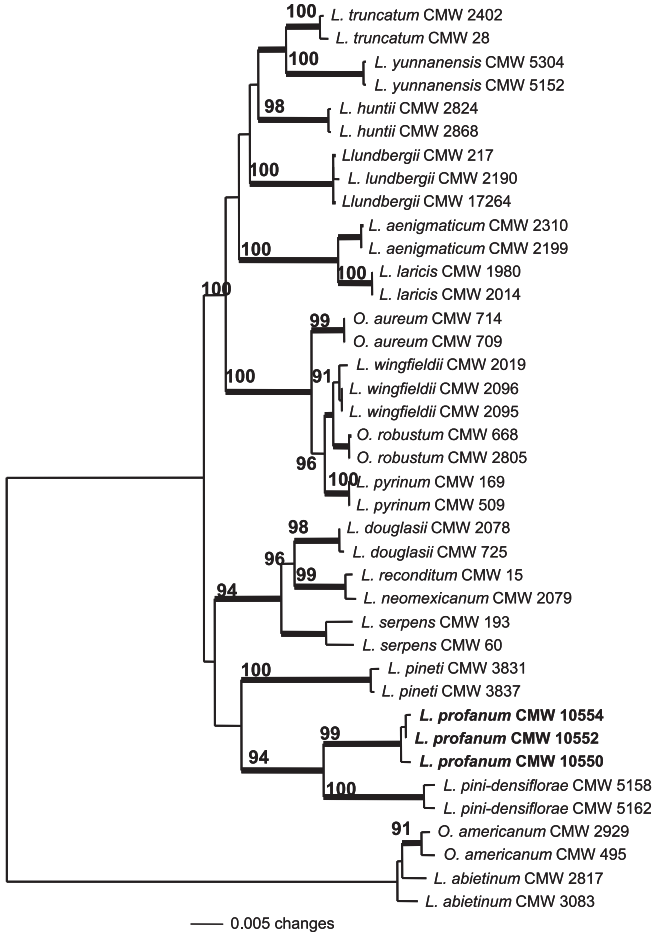
ETYMOLOGY: : Common, ordinary *Leptographium*.

Conidiophorae singulares, e medio recta orientes, (220–)88–758(–1000) μm longae, sine structuris rhizoideis similibus. Apparatus conidiogenus (70–)84–129(–160) μm longus. Rami primarii 2–3, atro-olivacei, non septati, (8–)14–27(–37) μm longi, (3–)4–7 μm lati, secundarii atro-olivacei, non septati, (8–)1–17(–21) μm longi, (3–)4–7 μm lati; tertiarii laete vel atro-olivacei, non septati, (7–)9–14(–18) μm longi, 3–4(–5) μm, quaternarii hyalini vel laete olivacei, non septati, (7–)9–13(–15) μm longi, 2–4 μm lati. Evolutio conidii proliferatione percirrenti. Conidia hyalina, non septata, ellipsoidea vel obovoidea, basibus truncatis, apicibus rotundatis, (3–)4–5(–6) × 2 μm.

Conidiophores occurring singly arising directly from the medium, erect, macronematous, mononematous, (220–)388–758(–1000) μm in length, rhizoid-like structures absent. *Stipes* dark olivaceous, cylindrical, simple, 2–9 septate, (120–)284–644(–850) μm long, apical cell not swollen, (6–)7–9(–10) μm wide at the apex, basal cell not swollen, (8–)9–13(–15) μm wide at the base. *Conidiogenous apparatus* (70–)84–129(–160) μm long, excluding the conidial mass, with multiple series of cylindrical branches. *Primary branches*, 2–3, dark olive, smooth, cylindrical, aseptate, (8–)14–27(–37) μm long and (3–)4–7 μm wide, arrangement of the primary branches on the stipe-type B (more than two branches) (Jacobs and Wingfield 2001), *secondary branches* dark olive, aseptate, (8–)1–17(–21) μm long, (3–)4–7 μm wide, *tertiary branches* dark to light olive, aseptate, (7–)9–14(–18) μm long, 3–4(–5) μm wide, *quaternary branches* hyaline to light olive, aseptate, (7–)9–13(–15) μm long, 2–4 μm wide. *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (7–)10–16(–20) μm long and 2–3 μm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving the false impression of sympodial proliferation (Van Wyk et al. 1988). *Conidia* hyaline, aseptate, ellipsoid to obovoid with truncate bases and rounded apices, (3–)4–5(–6) × 2 μm.

O. francke-grosmanniae	*O. grandifoliae*	*L. hughesii*	*O. leptographioides*	*L. reconditum*	*L. pruni*
Quercus spp.	*Fagus* sp.	*Parashorea plicata*	*Quercus*	*Wheat*	*Prunus* sp.
60–170	80–379	240–1200	77–237	150–725	50–170
B	B	B	B	C	B
Present	Absent	Present	Present	Present	Absent
Broadly ellipsoid	Obovoid	Ellipsoid to obovoid	Obovoid to ellipsoid	Oblong, slightly curved	Subglobose to ellipsoidal
2–5	2–4	3–5	4–9	3–5	2–5
Absent	Absent	Absent	Absent	Absent	Absent
Present	Present	Absent	Present	Absent	Absent
Hat-shaped	Allantoid	NA	Pillow-shaped	NA	NA
Hylecoetus dermestoides	Not known	Not known	Not known	Not known	*Polygraphus* sp.

Fig. 1. Neighbour-joining tree derived from analysis of the combined dataset. Thick branches have bootstrap values above 85. Posterior probabilities for the nodes are indicated above branches.



Colonies reaching 27.5 mm diam. in 8 d at 25 °C on 2% OA and 24.5 mm diam. in 8 d on MEA. *Hyphae* submerged in agar with abundant aerial mycelium, smooth, occasionally constricted at the septa, 2–7(–19) μm wide. Colonies dark olive with laciniate edge.

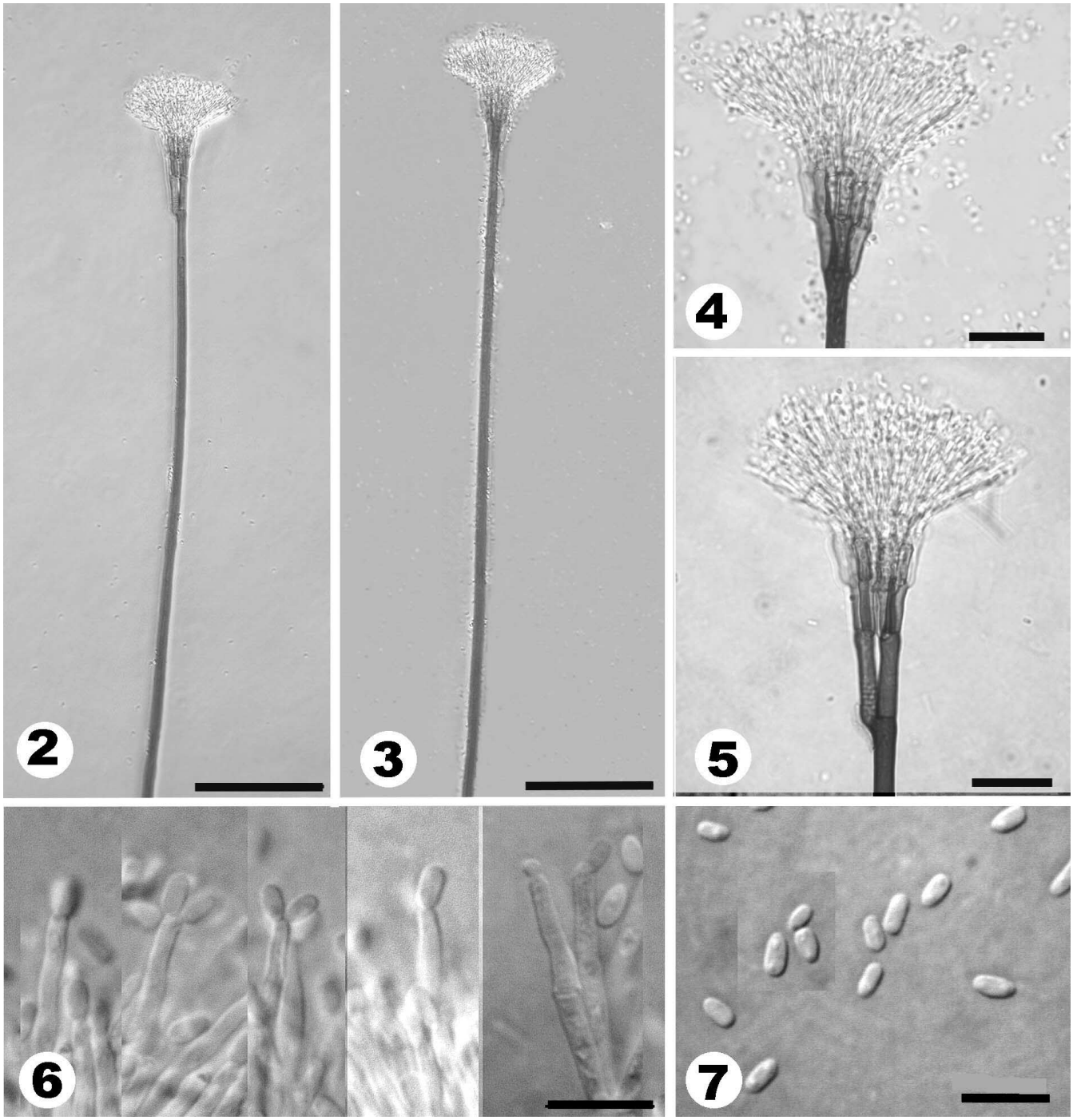
SPECIMENS EXAMINED: USA. CENTRAL ALABAMA: Oakmulgee Ranger District, Talladega Nation Forest, isolated from root material of *Carya* sp., 13 March 2002, *L.G. Eckhardt*, [HOLOTYPE; PREM 58589; ex-type culture CMW 10552 (P21)]. Isolated from root material of *Nyssa sylvatica*, 13 March 2002, *L.G. Eckhardt*, [PARATYPE; PREM 58591; ex-type culture CMW 10554 (P17)]. Isolated from root material of *Cornus florida*, 13 March 2002, *L.G. Eckhardt*, [PARATYPE; PREM 58592; ex-type culture CMW 10550 (P19)]

Discussion

Results of this study have led to the description of a new *Leptographium* species from the roots of *Carya* sp., *Nyssa sylvatica*, and *Cornus florida* in Alabama. The fungus was found associated with lesions on the roots, but no obvious disease symptoms were evident on these roots. It is possible the lesions on the roots from which the isolations were made originated from insect feeding wounds, although signs of insect damage on the roots were not obvious.

Leptographium profanum can be distinguished from other *Leptographium* species from hardwoods and most species from conifers based on its very long conidiophores (up to a 1000 μm). *Leptographium hughesii* is the only other species not associated with conifers that has exceptionally long conidiophores (Jacobs et al. 1998). *Leptographium profanum* can, however, easily be distinguished from *L. hughesii* based on the absence of rhizoids in the former and the presence of these structures in the latter species. *Leptographium profanum* also lacks the slightly curved conidia typical of *L. hughesii* (Jacobs et al. 1998). Living cultures of *L. hughesii* are nonexistent and comparisons of these fungi based on DNA sequence data were not possible. How-

Figs. 2–7. Light micrographs of the morphological characters of *Leptographium profanum* sp.nov. Figs. 2–3. Variation in conidiophore morphology. Scale bar = 100 μm. Figs. 4–5. Conidiogenous apparatus with a complex series of branches. Scale bar = 20 μm. Fig. 6. Conidiogenous cells showing annelidic conidium development and delayed secession of conidia. Scale bar = 10 μm. Fig. 7. Oblong to obovoid conidia with truncated bases. Scale bar = 10 μm.

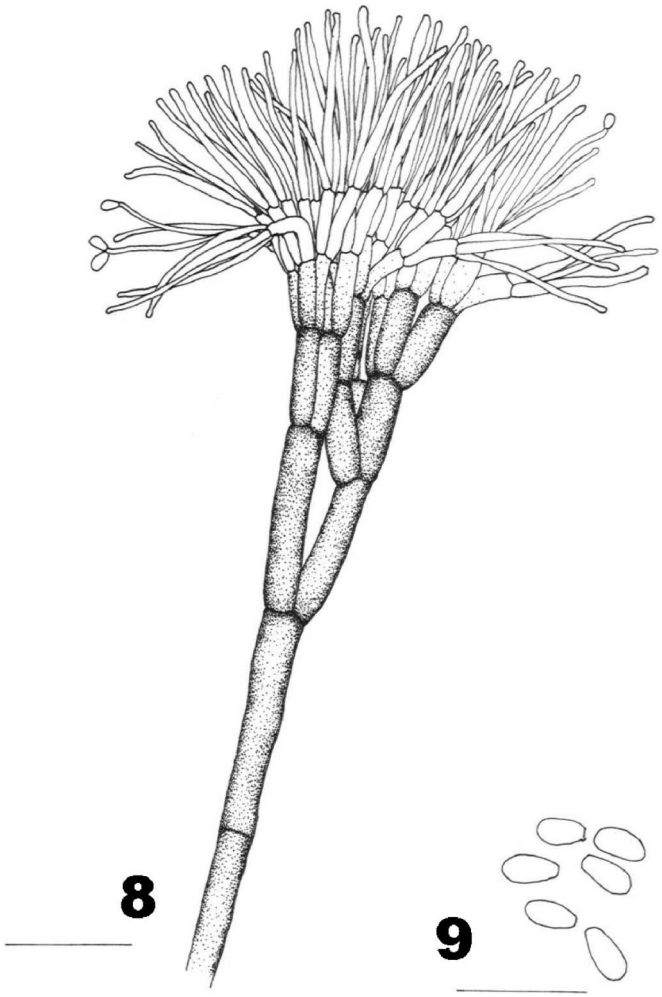


ever, the fungi are morphologically and ecologically very different. *Leptographium profanum* may be common on the roots of hardwood trees in the southeastern United States, whereas *L. hughesii* was isolated from the stained wood of the tropical dipterocarp, *Parashorea plicata*, in Southeast Asia. There is little chance that these fungi could be confused.

Leptographium peucophilum is the species that is morphologically most similar to *L. profanum*. *Leptographium profanum* can easily be distinguished from *L. peucophilum* based on the length of the conidiophores. *Leptographium profanum* has conidiophores that are on average twice as long as those of *L. peucophilum* (Jacobs et al. 2001*b*).

Ecologically, *L. profanum* and *L. peucophilum* share some characteristics (Jacobs et al. 2001*b*). Both species are associated with the roots of trees. *Leptographium peucophilum* is

Figs. 8–9. Line drawings of the morphological characters of of *Leptographium profanum* sp.nov. Fig. 8. Conidiophore morphology. Scale bar = 25 μm. Fig. 9. Oblong conidia with truncated bases. Scale bar = 10 μm.



associated with lesions made by larvae of the swift moth *Korscheltellus gracillus*, but we have no evidence that *L. profanum* has an insect associate. A very distinct difference between the two fungi is that *L. peucophilum* occurs on the roots of spruce (*Picea rubens*) in the northeastern United States, whereas *L. profanum* is associated with hardwood roots in the southeastern United States. The nonoverlapping geographic ranges make it unlikely that they would be mistaken for each other.

Virtually nothing is known regarding the biology of *L. profanum*. It is possible that it is associated with an insect that feeds on the trees from which it was isolated. Another possibility is that it is a soil-inhabiting fungus that colonizes wounds on the roots of trees. Typically, *Leptographium* species are adapted to be carried by insects, although vectors might also include small soil-dwelling animals or even mites that live in this environment (Moser 1985; Moser et al. 1989). Although *L. profanum* was isolated from a few root-feeding beetles, the evidence is circumstantial and needs to be further investigated before any claims can be made. Also, there is no evidence that the fungus is a pathogen. However, this has not been proven, and the ecology and insect associations of *L. profanum* need to be further investigated.

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