Leptographium engelmannii, a synonym of Leptographium abietinum, and description of Leptographium hughesii sp. nov.¹

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Abstract: Leptographium abietinum occurs in North America on various members of the Pinaceae, especially spruce (Picea spp.), usually in association with bark beetles (Coleoptera: Scolytidae). It is characterized by noticeably curved, clavate conidia. All the isolates were from species of Pinaceae in North America except two isolates examined by Kendrick, originating from Paraskorea plicata imported to England from Borneo and from Melia sp. imported into New Orleans, U.S.A. After examination of the isolate from Borneo and a similar isolate from Vietnam, we have concluded that these do not represent L. abietinum. They are described as a new species, Leptographium hughesii. Leptographium engelmannii, described from Engelmann spruce in Colorado, U.S.A., is indistinguishable from L. abietinum and is considered a synonym of the latter species.

Key words: Hyphomycetes, Ophiostoma, bark beetles.


Mots clés : Hyphomycètes, Ophiostoma, insectes corticoles.

[Traduit par la rédaction]

Introduction

The genus Leptographium Lagerb. & Melin includes a number of economically important species associated with root disease and sapstain of timber (Wagener and Mielke 1961; Harrington 1988, 1993; Wingfield et al. 1988; Wingfield 1993). These fungi are mainly known from conifers, where they are generally associated with bark beetle (Coleoptera: Scolytidae) infestation (Harrington 1988, 1993; Wingfield 1993). Some species have also been isolated from nonconiferous hosts, roots, and soil (Jooste 1978; Webber et al. 1996). Many Leptographium spp. are anamorphs of Ophiostoma, although some species currently included in the genus lack teleomorphs and therefore are of unknown affinity (Jooste 1978; Harrington 1987, 1988; Wingfield 1993; Wingfield et al. 1994a, 1994b; Webber et al. 1996).

Leptographium abietinum (Peck) Wingfield occurs on members of the Pinaceae, especially Picea spp., and is associated with species of Dendroctonus, Hylastes, and Hylurgops that infest these trees (Kendrick 1962; Harrington and Cobb 1983; Harrington 1988; Zambino and Harrington 1992). This species was first described by Peck (1879) as Sporocybe abietina Peck and was later transferred to Perticaria Tode ex Schweinitz by Saccardo (1886). Hughes (1953) recognized the importance of conidia ontogeny as a taxonomic character in anamorphic fungi and established the genus Verticicladiella Hughes based on Sporocybe abietina, which then became known as Verticicladiella abietina (Peck) Hughes.

Verticicladiella was thought to be related to Leptographium but could be distinguished by differences in the proliferation of the conidiogenous cells. In species ascribed to Verticicladiella, proliferation is sympodial whereas in Leptographium species, proliferation is percurrent (Hughes 1953; Kendrick 1962). Wingfield (1985) showed that some species in both of these genera displayed apparently sympodial proliferation, which in fact is anamorphic with delayed secession of

Received February 5, 1998.

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¹ With this paper, we recognize the tremendous contribution that Dr. S.J. Hughes has made to fungal taxonomy, especially to our understanding of the Leptographium complex.

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the conidium, giving a false sympodial appearance (Van Wyk et al. 1988). He thus reduced Verticicladiella to synonymy with Leptographium. This included Verticicladiella abietina, which became known as L. abietinum (Wingfield 1985).

The first complete description of L. abietinum was provided by Kendrick (1962). Two of the isolates he examined were isolated from hosts other than spruce. One of these hardwood isolates, DAOM 62102, which was used to illustrate the protologue of L. abietinum (Kendrick 1962, p. 774), originated from Parasorex plicata imported to England from Borneo. The recent availability of an isolate of Leptographium from Vietnam that resembles L. abietinum prompted us to reexamine the type material of L. abietinum and the specimen from Borneo that was illustrated by Kendrick (1962).

Leptographium engelmannii, which is known from spruce and associated with the bark beetle Dendrocoenos rufipennis (Kirby (=Dendrocoenos engelmanni Hopkips) is also characterized by curved, clavate conidia (Davidson 1955). Harrington (1988) suggested that L. engelmannii and L. abietinum might be synonymous, and isozyme analysis (Zambino and Harrington 1992) supported this synonymy. In the present study, we reexamined L. abietinum and compared it with L. engelmannii to determine whether they could justifiably be maintained as separate species.

Materials and methods

Numerous isolates of L. abietinum as well as herbarium specimens of this and other similar species were included in the study. Herbarium isolates examined were as follows: Leptographium abietinum: slide DAOM 33942, on the bark of spruce, Albany, N.Y.: DAOM 37980, Picea engelmannii, A. Molnar, 20 March 1953, Victoria, B.C.: DAOM 64328 (DAVF 11869), Pseudotsuga menziesii, C. Cottrell, 20 June 1958, McGillivray Lake, British Columbia; DAOM 62102, Parasorex plicata, Savary, Borneo, December 1957, Princess Risborough, England, on a ship from Borneo. Leptographium engelmannii: USO 422466, Picea engelmannii, collected by R. W. Davidson. The herbaria where these isolates are maintained are as follows: DAOM represents the National Mycological Herbarium, Eastern Cereal and Oilseed Research Centre, William Saunders Building, Agriculture and Agri-Food Canada, CEF, Ottawa. ON K1A 0C6, Canada, and BPI indicates the National Fungus Collections, Beltsville, Md.


All measurements were made from fungal structures produced in culture on 2% malt extract agar (MEA). 20 g of Biolab malt extract, 20 g of Biolab agar, and 1000 mL of distilled water in 90-mm-diameter plastic petri dishes containing 20 mL of medium. Fungal structures for microscopic examination were mounted on slides in lactophenol. Fifty measurements of each relevant morphological structure were made and ranges and means computed. Herbarium specimens were examined by placing a drop of 1% KOH on the dried material. After 5 min, small portions of fungal material were removed and mounted in lactophenol on glass slides.

Isolates were also examined using scanning electron microscopy. Small blocks of agar cut from sporulating colonies were fixed in 3% gluteraldehyde and 0.5% osmium tetroxide 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 Jeol 6400 scanning electron microscope.

The cardinal temperatures for growth of the isolates representing L. abietinum (CMW 2817), L. engelmannii (CMW 759), and the isolate from Vietnam (CMW 4052) were determined by inoculating eight MEA plates for each isolate at each temperature with a 6-mm-diameter colonized agar plug taken from the actively growing margin of fresh colonies. The plates were incubated at temperatures ranging from 5 to 35°C at 5°C intervals. Colony diameters were measured after 4 and 8 days, and the size of colonies was computed as an average of eight readings at each respective temperature.

Cycloheximide tolerance of L. abietinum (CMW 2817) and L. engelmannii (CMW 759) was determined after 8 days of growth on 2% MEA amended with 0.5 mg cycloheximide/mL. The plates were incubated at 25°C and colony diameters were measured. Cycloheximide tolerance of the Vietnamese isolate (CMW 4052) was determined on 2% MEA amended with cycloheximide at 0, 0.05, 0.1, 0.5, 1.0, 2.5, and 5.0 mg/mL after 8 days of growth.

Results

The Leptographium sp. from Vietnam occurring on Aquilana sp. was morphologically identical to the fungus isolated from Parasorex plicata from Borneo (DAOM 62102) and illustrated by Kendrick (1962). Another isolate that we examined from hardwood material collected in Malaysia was also morphologically similar to the Borneo material, but this isolate is no longer available. These Southeast Asian isolates have slightly curved conidia and thus resemble the type material and other collections of L. abietinum from Pinaceae in North America. However, these fungi have very different hosts and geographic distributions, and on close examination, they can be distinguished morphologically (Table 1).

Leptographium abietinum is characterized by dark olivaceous colonies on MEA, with conidiophores arising directly from the agar with little aerial mycelium. In contrast, isolates of Leptographium sp. from Vietnam, Malaysia, and Borneo are characterized by having a dense mat of aerial mycelium covering the colony, with conidiophores occurring in groups on the aerial mycelium and agar surface. The Asian isolates produce rhizoids at the bases of the conidiophore stipes whereas these structures are absent or very rarely found in isolates of L. abietinum (Figs. 1 and 2). The conidiophores of the Asian taxon and L. abietinum are similar (Figs. 3 and 4), but those of the Asian taxon are nearly twice as long as those of L. abietinum (Table 1). These two taxa can also be differentiated based on conidial morphology. Although the unnamed Leptographium sp. has curved conidia similar to those of L. abietinum, most of the conidia are ellipsoidal to ovoid (Figs. 5 and 6). The Vietnamese isolate also showed an increase in growth rate on 0.1 mg cycloheximide/mL compared with no cycloheximide, with growth inhibition only at higher concentrations of the antibiotic. In contrast, L. abietinum had a decreased growth rate when grown on 0.1 mg cycloheximide/mL.

From these observations, we conclude that the isolates of the Leptographium sp. from Vietnam and Borneo represent an undescribed taxon, which is described below.

The type specimen of L. engelmannii (USO 422466, BPI)

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was in poor condition, making comparison with the holotype of *L. abietinum* (DAOM 33942) difficult. A culture of *L. engelmannii* from Davidson’s collection, perhaps derived from the holotype, was available for comparison, and the two species appeared morphologically identical in culture. Both have optimum growth temperatures at 25°C and both produce carrot buff to olivaceous (Rayner 1970) colonies. *Leptographium abietinum* and *L. engelmannii* both tolerate high concentrations of cycloheximide, an indication that they are anamorphs of *Ophiostoma* (Hoog and Scheffer 1984; Harrington 1981). Furthermore, *L. engelmannii* was described from spruce infested with *Dendroctonus rufipennis*, a common bark beetle associate of *L. abietinum* (Harrington 1988). They also have similar isozyme electromorphs (Zambino and Harrington 1992). From these data, we conclude that *L. engelmannii* is conspecific with *L. abietinum*, and thus, their synonymy is proposed below.

### Taxonomy


*Sporocybe abietina* Peck, N.Y. State Mus. Rep. 31, 45. 1879


**Leptographium engelmannii** Davidson, Mycologia, 47, 59. 1955.

*Leptographium hughesi* Jacobs, M.J. Wingfield & Harrington sp.nov. Figs. 1, 3, 5, and 7–13.

Conidiophora evenientia singulatim vel usque ad octena aggregata, exorientia directe ex mycelio aero vel ex aeros, erecta, macronematosa, mononomatosa, 110–1120 (medius = 650) μm longitudine, structuris rhizoides praebentibus. Stipites olivaceo-bubalinae, leves, cylindracei, simplices, 4–18 septati, 80–1130 (medius = 598) μm. Apparatus conidiogenus 27.0–92.5 (medius = 60.5) μm longus, massa conidialis exclusa, 2–3(4) seriebus ramarum cylindricorum; 2–3 metulae primariae olivaceo-bubalinae, leves, cylindraceae, aspetatae, 11.0–35.5 (medius = 19.0) μm longae et 3.0–6.0 (medius = 4.0) μm latae. Conidiogenesis partitivus compensatibus, holoblastique procedit; proliferatione percurrentem et secessione retardata impressionem proliferations sympodialis simulans. Conidia hyalina, aseptata, ellipsoidae vel obovoidea, aliquando exigua curvata 1.0–2.5 × 3.0–5.0 (medius = 1.5 × 4.0) μm.

**Holotypus:** CMW 4052, isol. ex *Aquilana* sp., R.A. Blanchette, VI.1996, Phu Quoc Island, southern part of Vietnam. DAOM 225548.

Colonies with optimal growth at 25°C on 2% MEA, reaching 8 mm in diameter after 8 days, with little growth at 5°C.
and no growth at 35°C. Colony olivaceous (21°m) (Rayner 1970), with lacinate margins. Able to withstand high concentrations of cycloheximide with a 60% increase in linear growth on 0.1 mg cycloheximide/mL, with a 63% reduction in growth on 5 mg cycloheximide/mL after 8 days at 20°C in the dark.

Colonies covered in a dense mat of aerial mycelium, hyphae mostly submerged, hyaline, smooth, straight, not constricted at the septa, 1.5-6.0 (mean = 3.0) μm in diameter. Conidiophores occurring singly or in groups of up to eight, arising directly from the agar or aerial mycelium, erect, macroconidia, mononematous, 110-1200 (mean = 650) μm in length, rhizoid-like structures present at the base (Fig. 13A). Stipe olive-buff (21°m), smooth, cylindrical, simple, 4-18 septate, 80-1130 (mean = 598) μm long (from first basal septum to below primary branches), 3.5-7.5 (mean = 5.5) μm wide below primary branches, apical cell not swollen; 5.0-12.0 (mean = 8.0) μm wide at base, basal cell slightly swollen (Figs. 3, 7, and 13B). Conidiogenous apparatus 27.0-92.5 (mean = 60.5) μm long, excluding the conidial mass, with 2-3 (occasionally 4) series of cylindrical branches; 2-3 primary branches, olive-buff (21°m), smooth, cylindrical, aseptate, 7.5-35.5 (mean = 19.0) μm long and 2.0-6.0 (mean = 4.0) μm wide, secondary branches hyaline to olive-buff (21°m), aseptate, 6.0-16.0 (mean = 12.0) μm long, 2.0-4.0 (mean = 3.0) μm wide; tertiary branches hyaline, aseptate, 4.0-13.5 (mean = 8.0) μm long, 1.0-3.0 (mean = 2.0) μm wide, quaternary branches aseptate, 6.0-8.5 (mean = 5.0) μm long, 1.0-2.0 (mean = 1.7) μm wide (Figs. 8 and 13C). Conidiogenous cells discrete, 2-4 per branch, tapering slightly from the base to the apex, 8.0-18.5 (mean = 12.0) μm long and 1.0-2.0 (mean = 1.2) μm wide. Conidiophore development occurring through replacement wall building with holoblastic ontogeny, percurrent proliferation and delayed secession, giving the false impression of sympodial proliferation (Figs. 9-11). Conidia hyaline, aseptate, ellipsoid to obovoid, occasionally slightly curved, 1.0-2.5 × 3.0-5.0 (mean = 1.5 × 4.0) μm. Basal conidium frill absent (Figs. 5, 12, and 13D). Conidia accumulating in white, slimy droplets at the apex of conidiogenous apparatus.


Discussion

Leptographium abietinum is one of the most common fungi occurring on Picea spp. infested with Dendroctonus rufipennis in North America (Kendrick 1962; Harrington 1988). The fungus is characterized by olivaceous colonies, conidiophores ranging in length from 90 to 570 μm, and its distinctive narrow, prominently curved conidia. The latter feature was also recognized as taxonomically significant by Kendrick (1962), who unfortunately chose a culture from Parashorea plicata in Borneo to represent his revised description and illustration of Verticilliadiella abietinum.

At present, we regard L. abietinum as specific to hosts in the Pinaceae, and the species has been isolated from Picea, Abies, Pinus, and Pseudotsuga in North America (Kendrick 1962; Harrington and Cobb 1983; Harrington 1988; Zambino and Harrington 1992). The fungus has been associated with the bark beetles Dendroctonus rufipennis, Dendroctonus pseudotsugae, Hylastes longicollis, and Hylurgops planipennis (Harrington 1988) and appears to be avirulent or weakly virulent to pine and spruce (Harrington and Cobb 1983; Reynolds 1992). Leptographium engelmannii from Engelmann spruce (Picea engelmannii Parry ex Engelm.) in North America is clearly the same fungus, as has been shown in morphological and isozyme comparisons (Zambino and Harrington 1992). In our opinion, the importance of host, geographical distribution, and vectors has been underestimated in the taxonomy of the ophiostomatoid fungi, including Ophiostoma spp., Ceratocysis spp., and their anamorphs, including Lep tographium ( Wingfield 1993).

Leptographium abietinum can easily be distinguished from other species of Leptographium based on morphology, particular by its distinct curved conidia. The slightly curved conidia of L. hughesii are similar to those of L. abietinum, but L. hughesii has longer conidiophores, with basal rhizoids and abundant aerial mycelia. The difference in the geographical distribution and host range of these two taxa is noteworthy. All identified L. hughesii isolates have been from Southeast Asia. It appears that L. abietinum is restricted to North America. An isolate (C172) from spruce in Scotland is morphologically similar to L. abietinum, and it has similar isozyme electromorphs, but the two can be separated by conidiophore morphology and growth rate (Zambino and Harrington 1992).

Leptographium hughesii superficially resembles Leptographium procemum. Both these fungi are characterized by long conidiophores (up to 1250 μm) and rhizoids at the bases of the conidiophores. However, these species can easily be distinguished based on the presence of abundant aerial mycelium in colonies of L. hughesii. Colonies of L. procemum are characterized by submerged mycelia that display concentric zones when grown in culture (Kendrick 1962). Leptographium hughesii is characterized by ellipsoid to obovoid conidia that can be slightly curved in certain cases. In contrast, L. procemum is characterized by small (2.5-5 μm) obovoid conidia that are never curved. A further difference between these fungi is their host preference. Leptographium hughesii is known from nonconiferous hosts whereas L. procemum occurs predominantly on Pinus spp. and exclusively on conifers (Kendrick 1962; Wingfield 1983; Harrington 1988; Wingfield et al. 1988), particularly on white pine (Pinus strobus L.) in association with a disease known as white pine root decline (Wingfield 1983; Alexander et al. 1988; Wingfield et al. 1988). There is no evidence to suggest that L. hughesii is a pathogen.

Many Leptographium spp. have been described from conifers infested with bark beetles (Harrington 1988, 1993), and L. hughesii is unusual in its association with tropical hardwoods. Its vectors have yet to be identified. The cycloheximide tolerance of L. hughesii suggests a relationship to Ophiostoma (Harrington 1981), but no perithecia have been associated with this fungus. Recognition of this species con-
Figs. 7–12. Conidiophores and conidia of *L. hughesii* (CMW 4052). Fig. 7. Scanning electron micrograph of a conidiophore. Bar = 10 μm. Fig. 8. Light micrograph of the conidiogenous apparatus. Bar = 10 μm. Figs. 9–11. Light and scanning electron micrographs showing the conidiogenous cells with percurrent proliferation and annelidic conidiogenesis. Bar = 10 μm. Fig. 12. Scanning electron micrograph of the conidia. Bar = 10 μm.
firms the suggestion of Wingfield (1993) that many Leptographium spp. remain to be discovered, particularly in poorly studied regions such as Southeast Asia.

Acknowledgments

We thank Dr. R.A. Blanchette for cultures of L. hughesi from Vietnam and Malaysia as well as the Foundation for Research Development and members of the Tree Pathology Co-operative Program for financial support.

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