Leptographium, engelmannii, a synonym of Leptographium abietinum, and descrip...
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Leptographium engelmannii, a synonym of *Leptographium abietinum*, and description of *Leptographium hughesii* sp.nov.[1]

K. Jacobs, M.J. Wingfield, P.W. Crous, and T.C. Harrington

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 *Parashorea 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.

Résumé : On trouve le *Leptographium abietinum* en Amérique du nord sur différents membres de la famille des Pinaceae, surtout des épinettes (*Picea* spp.), habituellement en association avec des insectes corticoles (Coleoptera: Scolytidae). Il se caractérise par des conidies claviformes nettement courbées. Tous les isolats étudiés proviennent de Pinaceae de l'Amérique du nord excepté deux isolats examinés par Kendrick, originant du *Parashorea plicata* importé en Angleterre à partir de Bornéo, et du *Melia* sp. importé en Nouvelle-Orléans, aux États-Unis. Après examen des isolats de Bornéo et un isolat comparable du Vietnam, les auteurs concluent que ces deux entitées n'appartiennent pas au *L. abietinum*. Ils les décrivent commme la nouvelle espèce ***Leptographium hughesii***. On ne peut distinguer le *Leptographium engelmannii*, décrit à partir de l'épinette d'Engelmann au Colorado, États-Unis, du *L. abietinum* et conséquemment les auteurs en font un synomyme de cette espèce.

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. 1994*a*, 1994*b*; 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 *Periconia* Tode ex Schweinitz by Saccardo (1886). Hughes (1953) recognized the importance of conidium 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 annelidic with delayed seccession of

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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 *Parashorea 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 *Dendroctonus rufipennis* Kirby (=*Dendroctonus engelmanni* Hopkins) 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 (DAVFP 11869), *Pseudotsuga menziesii*, C. Cottrell, 20 June 1958, McGillivray Lake, British Columbia; DAOM 62102, *Parashorea plicata*, Savary, Borneo, December 1957, Princess Risborough, England, on a ship from Borneo. *Leptographium engelmannii*: USO 422466; *Picea engelmanii*, 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.

Cultures examined included the following. *Leptographium abietinum*: CMW 2817 (=C699), isol. ex *Picea engelmannii*, T.C. Harrington, 1993, Dixie Nature Forest, Utah; CMW 276, isol. ex *Picea engelmannii*, A. Molnar, 1987, Victoria, B.C.; CMW 3083, *Picea* sp. M.J. Wingfield, August 1994, British Columbia; CMW 4052 (=C930), isol. ex the wounds of live *Aquilana* sp., Vietnam, R.A. Blanchette, June 1996, Phu Quoc Island, southern part of Vietnam. *Leptographium engelmannii*: CMW 759 (=C29, C713, CO456, RWD971), collected by R.W. Davidson. The culture collections where these isolates are maintained are as follows: CMW represents the culture collection of the Tree Pathology Co-operative Program and C represent the culture collection of T.C. Harrington.

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% glutaraldehyde 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 JSM 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° 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 *Parashorea 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 obovoid (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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Figs. 1–6. Conidiophores and conidia of *L. hughesii* and *L. abietinum*. Fig. 1. Rhizoids of *L. hughesii* (CMW 4052). Bar = 10 μm. Fig. 2. Foot-cell of *L. abietinum* (CMW 2817). Bar = 10 μm. Fig. 3. Conidiophore of *L. hughesii* (CMW 4052). Bar = 10 μm. Fig. 4. Conidiophore of *L. abietinum* (CMW 2817). Bar = 10 μm. Fig. 5. Conidia of *L. hughesii* (CMW 4052). Bar = 10 μm. Fig. 6. Conidia of *L. abietinum* (CMW 2817). Bar = 10 μm.

Table 1. Comparison between *L. abietinum*, *L. engelmannii*, and *L. hughesii*.

Character	*L. abietinum*	*L. engelmannii*	*L. hughesii*
Host	*Abies grandis, Picea engelmannii, Picea mariana, Picea rubens, Pinus ponderosa, Pseudotsuga mensiezii, Pinus contorta, Pinus* spp.	*Picea engelmannii, Pinus contorta*	*Parashorea plicata, Aquilana* sp.
Associated insect	*Dendroctonus pseudotsugae, Dendroctonus rufipennis, Hylastes longicollis, Hylurgops planirostris*	*Dendroctonus rufipennis*	None reported
Distribution	British Columbia, Canada, eastern and western United States	Colorado	Borneo, Vietnam
Rhizoids	Absent	Absent	Present
Conidiophore length	96–570 μm	100–450 μm	240–1200 μm
Conidium shape	Clavate with curved ends	Clavate with curved ends	Ellipsoid to obovoid, occasionally curved
Conidium size	4.0–7.0 × 1.0–2.5 μm	4.0–6.5 × 1.0–2.5 μm	3.0–6.0 × 1.0–2.5 μm

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 cartridge 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

Leptographium abietinum (Peck) Wingfield, Trans. Br. Mycol. Soc. 85, 92. 1985. Figs. 2, 4, and 6.

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

≡*Periconia abietina* (Peck) Sacc., Sylloge Fungorum, 4, 273. 1886.

≡*Verticicladiella abietina* (Peck) Hughes, Can. J. Bot. 31, 653. 1953.

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

Leptographium hughesii 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 aerio vel ex aerio, erecta, macronematosa, mononematosa, 110–1120 (medius = 650) μm longitudine, structuris rhizoideis praesentibus. Stipites olivaceo-bubalini, leves, cylindracei, simplices, 4–18 septati, 80–1130 (medius = 598) μm. Apparatus conidiogenus 27.0–92.5 (medius = 60.5) μm longus, massa conidiali exclusa, 2–3(–4) seriebus ramorum cylindricorum; 2–3 metulae primariae olivaceo-bubalinae, leves, cylindricae, aseptatae, 11.0–35.5 (medius = 19.0) μm longae et 3.0–6.0 (medius = 4.0) μm latae. Conidiogenesis parietibus compensatis, holoblastice procedit; proliferatione percurrente et secessione retardata impressionem proliferationis sympodialis simulans. Conidia hyalina, aseptata, ellipsoidea vel obovoidea, aliquando exigue 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

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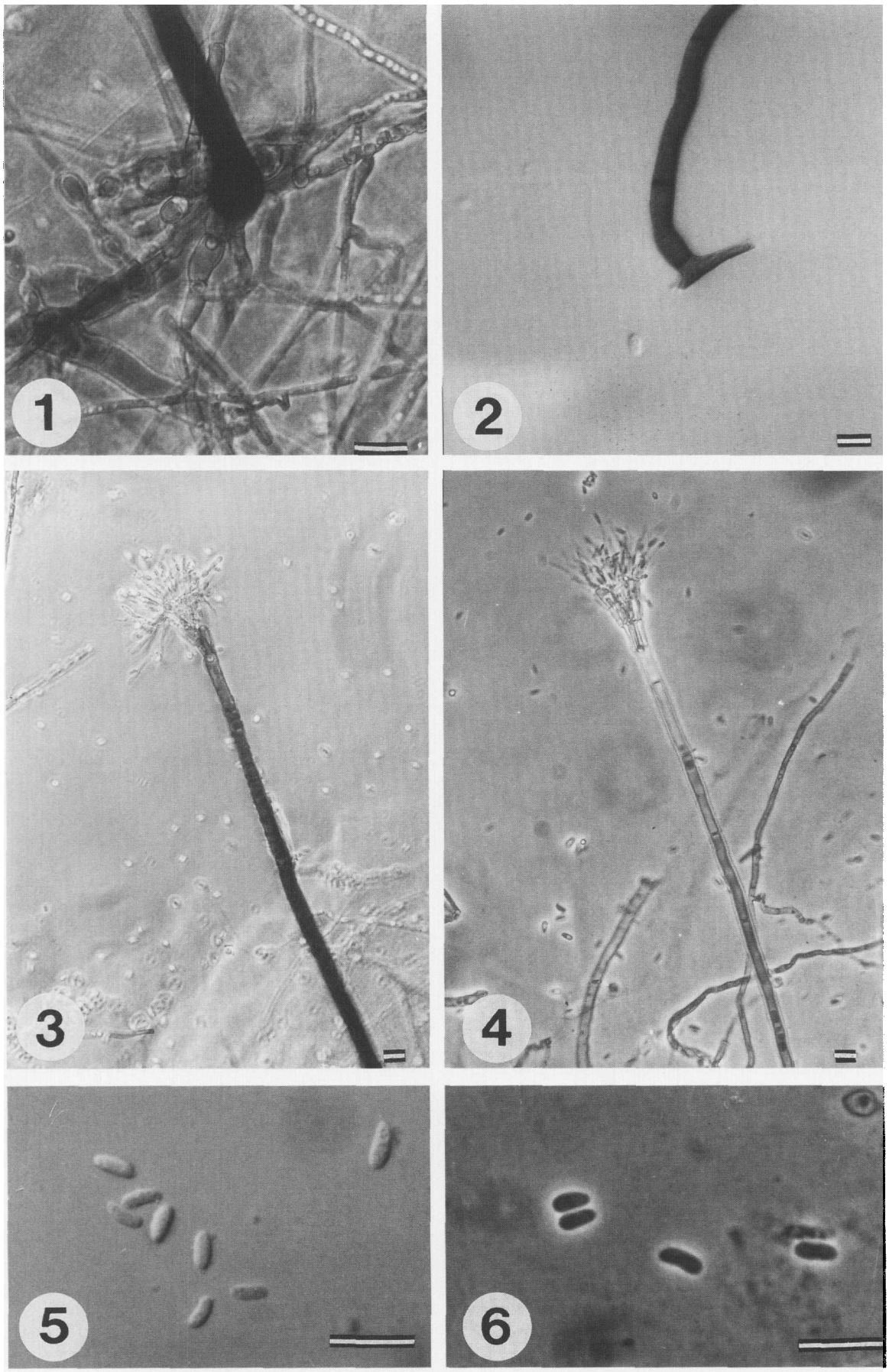

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and no growth at 35°C. Colony olivaceous (21″m) (Rayner 1970), with laciniate margins. Able to withstand high concentrations of cycloheximide with a 60% increase in linear growth on 0.1 mg cycloheximide/mL, with a 63% reduction in growth on 5 mg cycloheximide/mL after 8 days at 20°C in the dark.

Colony 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, macronematous, mononematous, 110–1200 (mean = 650) μm in length, rhizoid-like structures present at the base (Fig. 13A). Stipe olive-buff (21‴b), 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‴b), 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″b), 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 = 8.0) 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. Conidium 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, ellipsoidal 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.

SPECIMENS EXAMINED: Herbarium isolates: holotype: CMW 4052, isolated from the wounds of live Aquilana sp., R.A. Blanchette, June 1996, Phu Quoc Island, southern part of Vietnam; paratype: DAOM 62102, Parashorea plicata, Savary, December 1957, Princess Risborough, England, on a ship from Borneo. Dried specimens and cultures deposited at DAOM and Centraalbureau voor Schimmelcultures (Baarn, The Netherlands), respectively.

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 *Verticicladiella 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 planirostris* (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., *Ceratocystis* spp., and their anamorphs, including *Leptographium* (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 procerum*. 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. procerum* 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. procerum* 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. procerum* 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-

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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.

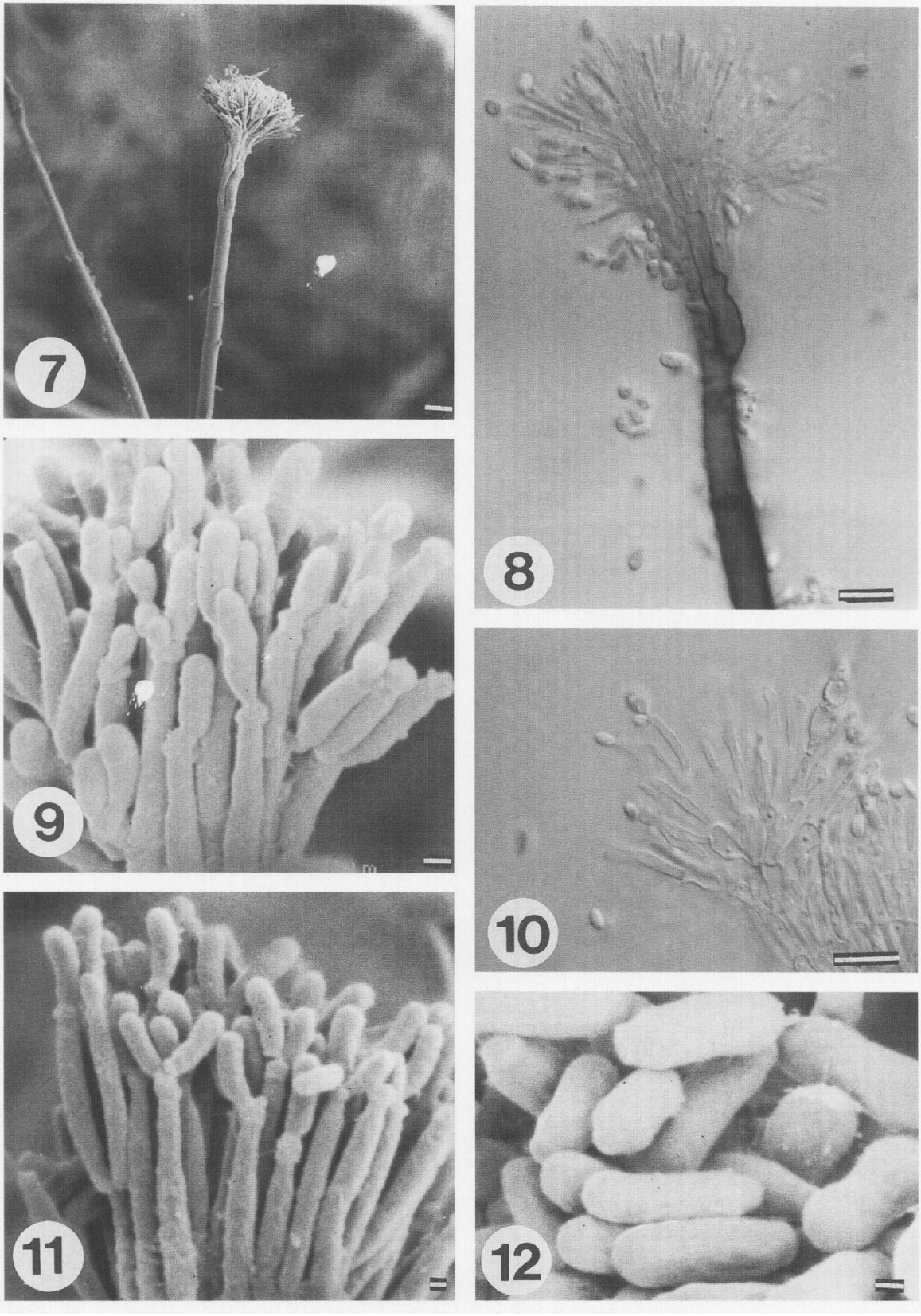

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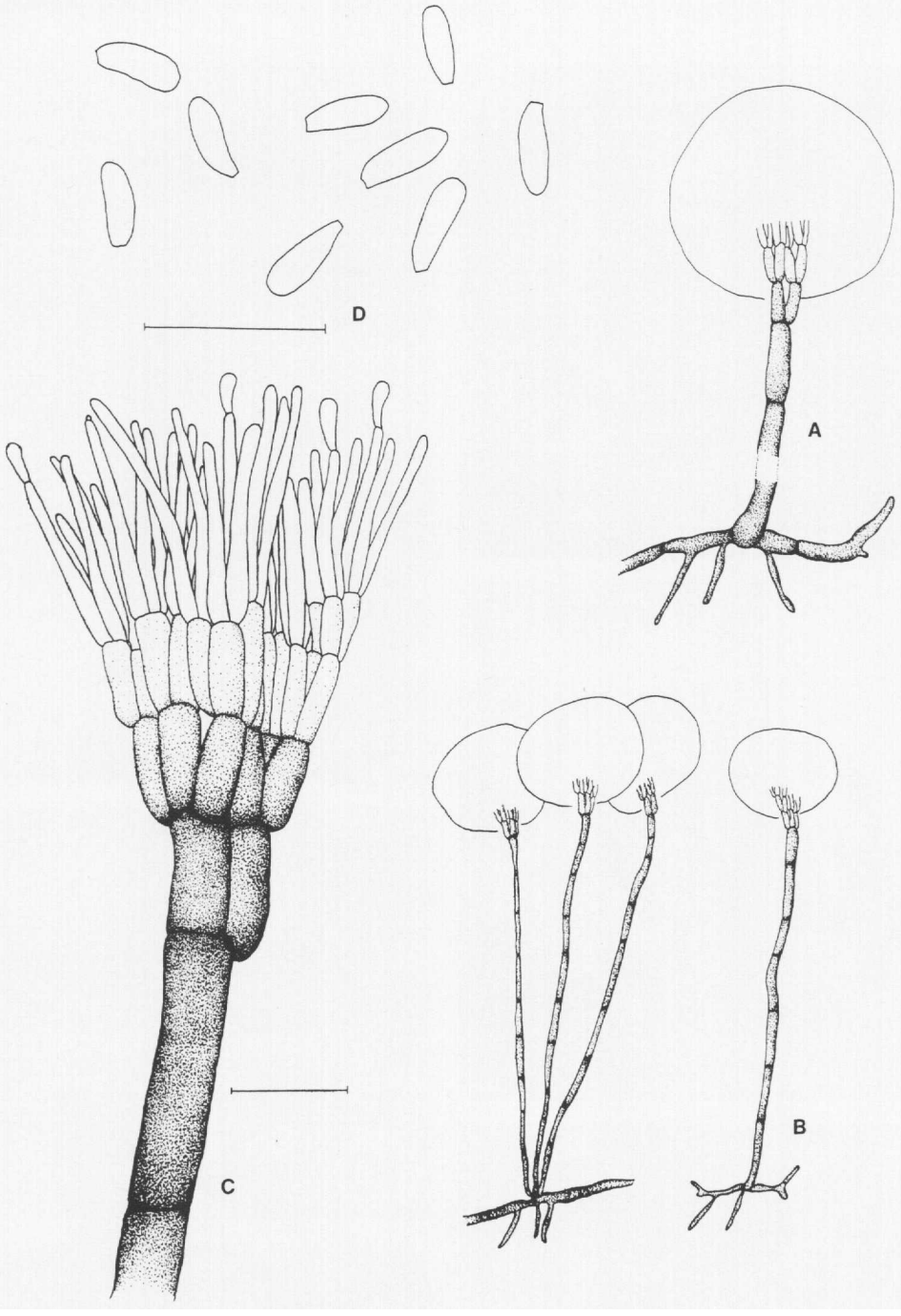
Fig. 13. Conidiophores and conidia of *L. hughesii* (CMW 4052). (A) Conidiophore with rhizoids present. (B) Conidiophores occurring in groups. (C) Conidiogenous apparatus. Bar = 10 μm. (C) Conidia. Bar = 10 μm.



firms the suggestion of Wingfield (1993) that many *Leptographium* spp. remain to be discovered, particularly in poorly studied regions such as Southeast Asia.

Acknowledgments

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