A new species of *Ophiostoma* from North America, similar to *Ophiostoma penicillatum*

K. Jacobs, M.J. Wingfield, and D.R. Bergdahl

Abstract: Ophiostomatoid fungi that resemble *Ophiostoma penicillatum* were isolated from insect-infested spruce (*Picea* sp.) in Japan as well as *Larix decidua* Mill. (European larch) in North America. Isolates were characterized based on morphology and could be separated into two distinct groups. Those from spruce in Japan represent *O. penicillatum*, and are characterized by allantoid, slightly curved conidia. The North American isolates from *Larix decidua* are of a previously undescribed species, characterized by long, narrow conidia. The latter fungus is described as *Ophiostoma americanum* with an anamorph, *Leptographium americanum*.

Key words: *Ophiostoma penicillatum*, *Ips typographus*, *Ophiostoma americanum*, *Dendroctonus simplex*, *Larix decidua*.

Résumé : Les auteurs ont isolé à partir d'un *Picea* sp. au Japon et du *Larix decidua* en Amérique du Nord, des champignons ophiostomatoïdes qui ressemblent à l'*Ophiostoma penicillatum*. Les isolats ont été caractérisés sur la base de la morphologie et ont pu être séparés en deux groupes distincts. Ceux provenant du *Picea* sp. au Japon correspondent à l'*O. penicillatum* et se caractérisent par des conidies allantoïdes, légèrement courbées. Les isolats nord-américains provenant du *Larix decidua* appartiennent à une espèce jamais décrite, et se caractérisent par de longues et étroites conidies. Les auteurs décrivent ce nouveau champignon et le nomme *Ophiostoma americanum* avec son anamorphe, le *Leptographium americanum*.

Mots clés : *Ophiostoma penicillatum*, *Ips typographus*, *Ophiostoma americanum*, *Dendroctonus simplex*, *Larix decidua*.
[Traduit par la rédaction]

Introduction

Ophiostoma penicillatum (Grosmann) Siemaszko is well known in Europe as an associate of the destructive bark beetle *Ips typographus* L. that infests *Picea abies* (L.) Karst. (Norway spruce) (Mathiesen 1950; Hopping 1963; Solheim 1986, 1992*a*, 1992*b*, 1993*a*). Like many species of *Ophiostoma* and *Ceratocystis*, this fungus has had a confused taxonomic history since the description of its anamorph (Grosmann 1931) and later its teleomorph (Grosmann 1932; Goidànich 1936; Upadhyay 1981). A neotype was established for *O. penicillatum* by Solheim (1986) with allantoid ascospores, which contrasts the hat-shaped ascospores reported by other authors (Hunt 1956).

Many *Ophiostoma* species cause blue stain in timber and some, such as *O. ulmi* (Buisman) Nannfeldt and *O. novo-ulmi* Brasier, cause serious diseases of forest trees (Lagerberg et al. 1927; Gibbs 1978, 1993; Harrington 1988, 1993; Brasier 1991). *Ophiostoma penicillatum* causes blue stain in spruce and, together with *Ceratocystis polonica* (Siemaszko) Moreau, is associated with the spruce bark beetle *I. typographus* in Europe (Solheim 1986, 1992*a*, 1992*b*, 1993*a*; Visser et al. 1995). Both these fungi are responsible for impairing water conduction in infected trees, although *O. penicillatum* has a low level of virulence in comparison with *C. polonica* (Horntvedt et al. 1983). Therefore, it is considered to be a secondary invader of Norway spruce and not a primary pathogen (Horntvedt et al. 1983; Solheim 1992*a*).

Ophiostomatoid fungi from the Northern Hemisphere are very well studied and ecologically similar (Griffin 1966; Olchowecki and Reid 1974; Upadhyay 1981). *Ophiostoma penicillatum* has been reported on species of pine (*Pinus*) and spruce (*Picea*) from various parts of Europe (Reinerfelt 1950; Mathiesen-Käärik 1953; Griffin 1966; Mielke 1981), although some of these reports are of a dubious nature. There have also been reports of this fungus from North America, some of which have been retracted and many of the isolates in question have been described as new species (Davidson 1958, 1966; Davidson et al. 1967; Harrington 1987). The fungus is clearly associated with *I. typographus* and possibly other insects in Europe and appears to be restricted to this niche (Davidson 1958; Horntvedt et al. 1983; Solheim 1986, 1992*a*, 1992*b*, 1993*a*).

A recent study of the fungi associated with the similar and related bark beetle, *I. typographus japonicus* Niijima, in Japan has shown that *O. penicillatum* is a common associate of it (Yamaoka et al. 1997), as is the case with *I. typographus* in Europe. Isolates of a similar fungus have recently been collected from the related bark beetle, *Ips cembrae* Heer infesting *Larix laricina* (Du Roi) K. Koch (tamarack) in Japan, but this is a distinct species and has been described as

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Table 1. *Ophiostoma penicillatum*-like isolates used in this study.

Europe (spruce)	Japan (spruce)[a]	North America (larch)[b]
CBS 140.36[c]	CMW 2209	CBS 497.96
CBS 211.67[c]	CMW 2303	CBS 498.96
CBS 441.69[c]	CMW 2305	CBS 499.96
CBS 210.67[c]	CMW 2646	CBS 500.96
CMW 2642[d]	CMW 2647	
CMW 2643[d]	CMW 2648	
CMW 2644[d]	CMW 2649	
CMW 2645[d]		

[a]Collected by Y. Yamaoka (Department of Plant Pathology, University of Tsukuba, Tennodai, Tsukuba, Ibaraki, 305, Japan).

[b]Collected by M.J.W. and D.R.B.

[c]Supplied by the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

[d]Supplied by H. Solheim (Norwegian Forest Research Institute, Forest Pathology, P.O. Box 61, N-1432, Ås-NLH, Norway).

O. laricis Van der Westhuizen et al. (Van der Westhuizen et al. 1995). *Larix decidua* Mill. (European larch) in eastern North America is commonly infested with the bark beetle *Dendroctonus simplex* Hopkins, but fungi associated with this niche have not been studied. The aim of this study was to compare an *Ophiostoma* species from *D. simplex* infested *L. decidua* in Vermont, U.S.A., which is peripherally similar to *O. penicillatum*, with *O. penicillatum* isolates from Europe and Japan.

Materials and methods

Isolations were made from 20 bark beetles (*D. simplex*) collected individually from galleries in the sapwood of larch (*L. decidua*) in North America. Insects were washed lightly in sterile distilled water and plated on 2% MEA (20 g Biolab malt extract, 20 g Biolab agar, 1000 mL distilled water) amended with 0.01% cycloheximide and 0.01% streptomycin, after cooling to approximately 60°C. Individual beetles were squashed onto the sorface of agar plates using a sterilized pair of tweezers, and insect parts were spread across the surface of the agar.

All isolates used in this study (Table 1) are maintained in the culture collection of M.J.W. (CMW), and representative isolates have been deposited in accredited mycological culture collections.

All measurements were made from cultures after growth at 25°C on 2% MEA in the presence of host tissue. This was later substituted with sapwood of *Pinus patula* Schlecht. & Cham., which also stimulated sporulation. Measurements were taken of fungal structures occurring on the wood tissue and mounted in lactophenol cotton blue. Fifty measurements of each relevant morphological structure were made and ranges and means computed.

All isolates were examined using scanning electron microscopy. Agar from sporulating colonies was 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, coated with gold palladium, and examined using a JSM 6400 scanning electron microscope.

Cardinal temperatures and growth rates of isolates from North America (CBS 498.96; CBS 499.96) were determined. Isolates were grown on 2% MEA in Petri dishes for 5 days in the dark at temperatures ranging from 5 to 40°C at 5°C intervals. Five Petri dishes, each containing 20 mL of MEA, were inoculated with each isolate to be tested at each of the seven different temperatures. Inoculations were with 5-mm discs of agar taken from the actively growing margins of the test isolates. Cycloheximide tolerance was determined for the North American isolates (CBS 498.96; CBS 499.96) by measuring growth at various concentrations (0, 0.05, 0.1, 0.5, 1, 2.5%) of the antibiotic. Five plates were used for each isolate at each cycloheximide concentration, and the growth of colonies was measured after 5 days in the dark at 25°C. Colony diameters were measured twice perpendicular to each other, and growth was computed as an average of 10 readings (five plates with two measurements per plate).

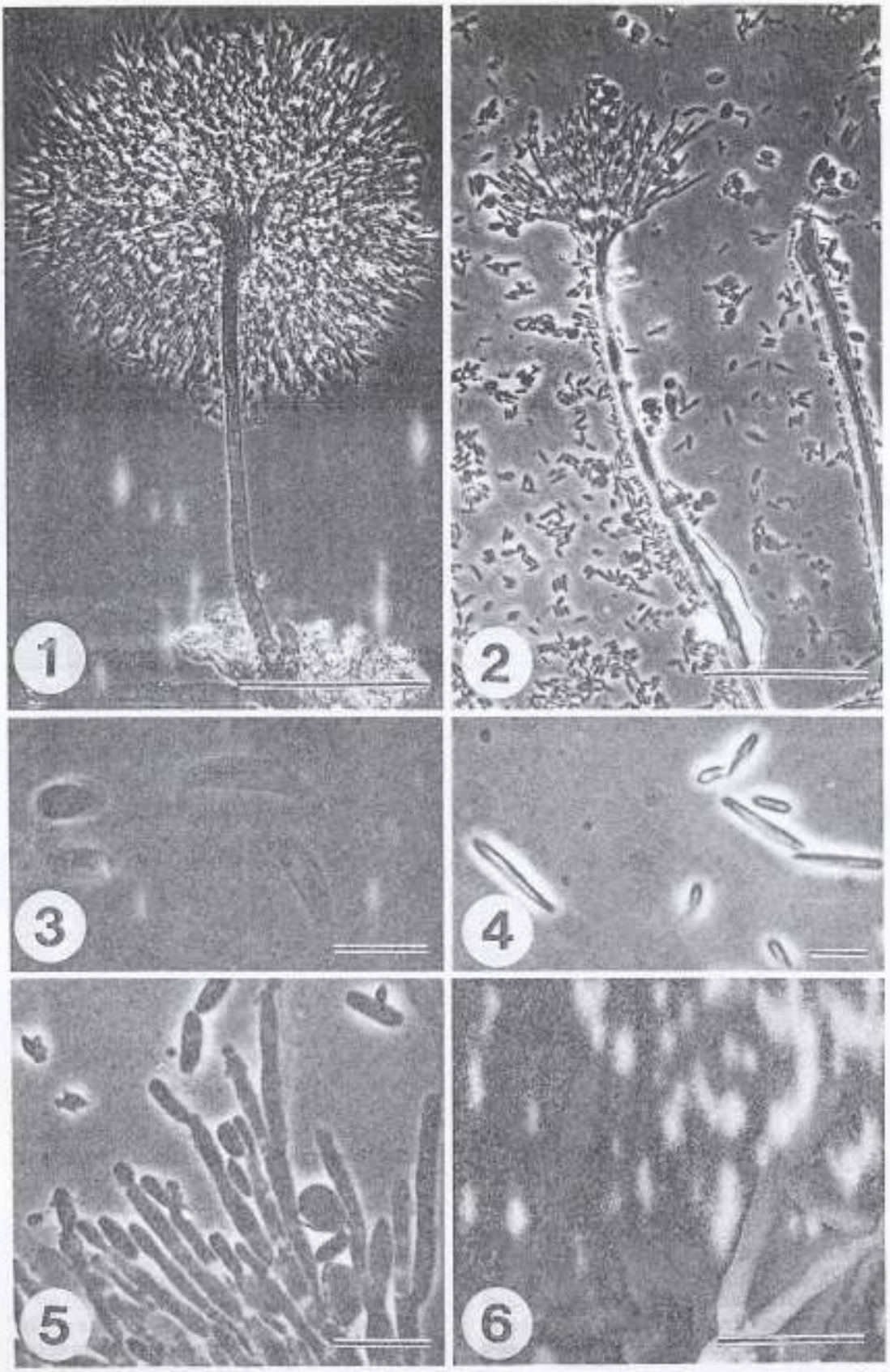
Results

A morphological comparison of the isolates from larch and other related species of *Ophiostoma* are shown in Table 2. Isolates of both *O. penicillatum* and the species from larch had *Leptographium* anamorphs (Figs. 1 and 2) and obovoid conidia (Figs. 3 and 4). The conidiophore lengths of the Japanese isolates from spruce and those of *O. penicillatum* from Europe were in the same size range. In contrast, the North American isolates from larch were nearly twice as long as those of *O. penicillatum* (Table 2). These isolates also had considerably fewer branches than *O. penicillatum* (Figs. 1 and 2). Conidia of isolates from spruce in Japan were identical in size and shape to those of *O. penicillatum* isolates from Europe. In contrast, conidia of the isolates from larch in North America differed markedly from those of *O. penicillatum*. Although the latter conidia were allantoid, they were longer and narrower than those of *O. penicillatum* (Figs. 3–6). These conidia were also considerably more variable in length than those of *O. penicillatum* (Table 2). The length to width ratio of *O. penicillatum* ranged from 2:1 to 3:1, whereas the ratio for the larch isolates ranged from 2:1 to 11:1.

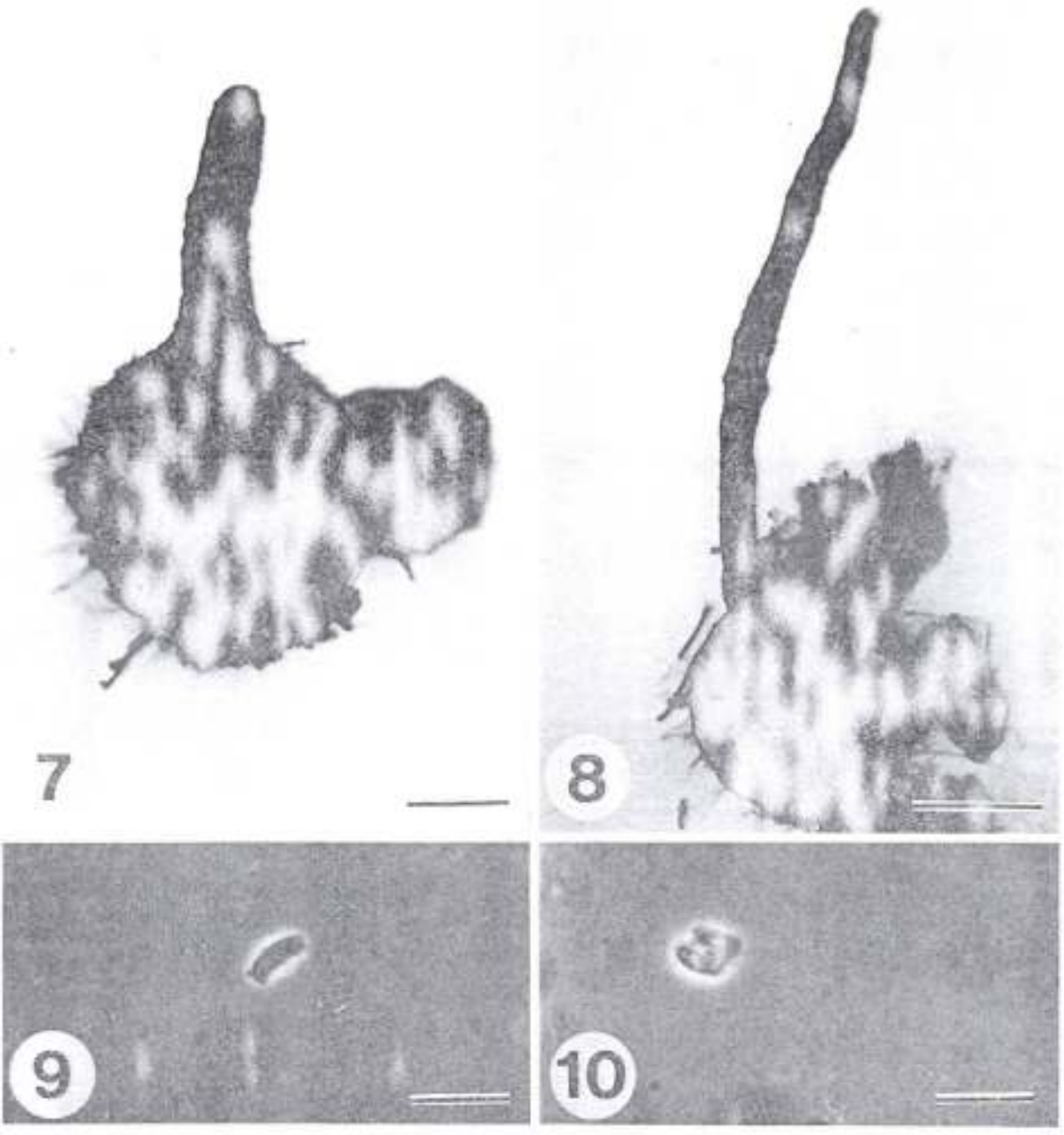
Isolates from *D. simplex* on *L. decidua* failed to produce perithecia in culture and morphological characteristics were obtained from perithecia taken directly from the bark beetle galleries. In contrast, isolates from Japan produced their perithecia readily in culture when supplemented with host material. All isolates had black perithecia with globose bases, characteristic of species of *Ophiostoma*. However, distinct differences were found in the perithecial dimensions and shapes (Figs. 7–10). European isolates of *O. penicillatum* failed to produce perithecia in culture and measurements of these structures were obtained from published descriptions (Grosmann 1932; Solheim 1986). Isolates from spruce in Japan had ascospores of similar shape and size to those characteristic of *O. penicillatum*. In both cases, the spores were curved and surrounded by a hyaline sheath (Figs. 9 and 10). In contrast to the uniform sheaths of *O. penicillatum*, the ascospores of the North American fungus had nonuniform sheaths around them. These ascospores thus had a rectangular appearance (Fig. 10). Based on ascospore and conidial characteristics, host, vectors, and origin, isolates could be placed into two distinct groups. One group includes the *O. penicillatum* isolates from spruce in Europe and Japan and the other included isolates from larch in North America.

Results of this study lead us to conclude that the isolates from spruce in Japan and Europe are similar and generally fit the description of *O. penicillatum*. The isolates from North America could, however, clearly be distinguished from this species. After comparison with other similar species of this genus, we have concluded that these represent a previously undescribed species that is described as follows.

Figs. 1–6. Light and scanning electron micrographs of the anamorphs of *O. penicillatum* and *O. americanum*. Fig. 1. *O. penicillatum* from Japan. Scale bar = 100 μm. Fig. 2. *O. americanum* from larch. Scale bar = 100 μm. Fig. 3. Conidia of *O. penicillatum* from Japan. Scale bar = 10 μm. Fig. 4. Conidia of *O. americanum*. Scale bar = 10 μm. Fig. 5. Conidia and conidiogenous cells of *O. americanum* from larch. Scale bar = 10 μm. Fig. 6. Scanning electron micrograph of the conidiogenous cells of *O. americanum* from larch. Scale bar = 10 μm.



Figs. 7–10. Light micrographs of the teleomorphs of *O. penicillatum* and *O. americanum*. Fig. 7. Perithecium of *O. penicillatum* from Japan. Scale bar = 100 μm. Fig. 8. Perithecium of *O. americanum* from larch. Scale bar = 100 μm. Fig. 9. Ascospores of *O. penicillatum* from Japan. Scale bar = 10 μm. Fig. 10. Ascospores of *O. americanum* from larch. Scale bar = 10 μm.



Taxonomy

Ophiostoma americanum Jacobs & Wingfield sp.nov.

STATUS ANAMORPHIS: *Leptographium americanum* Jacobs & Wingfield

Bases peritheciales atrae, globosae et glabro-tunicatae, inornatae vel sparsissima ornatione, 200–370 ($\bar{x}$ = 284.0) μm diametro, collum fuscum vel atrum, cylindricum exigua apicali angustatione, leve, 690–1300 ($\bar{x}$ = 1027.5) μm longum, 50–70 ($\bar{x}$ = 60.5) μm super basim globosam, 20–40 ($\bar{x}$ = 25.5) μm latum ad apicem, hyphae ostiolares absentes. Asci prototunicati, hyalini, evanescentes. Ascosporae reniformes, aseptatae, hyalinae, curvatae et in vagina investitae, 3.0–5.5 ($\bar{x}$ = 4.5) × 1.0–2.5 ($\bar{x}$ = 1.5) μm (sine vaginis).

Conidiophora evenientia singulatim vel catervatim, exorientia directe ex mycelio, erecta, macronematosa, mononematosa, levia, olivacea vel subbrunnea, 149.0–731.5 ($\bar{x}$ = 327.5) μm longitudine, rhizoidea absentia. Stipes olivaceus vel subbrunneus, levis, cylindricus, simplex, 3–20 septatus, 108.5–691.0 ($\bar{x}$ = 287.5) μm longus, (a primo septo basilari usque infra primarios ramos), 4.5–9.0 ($\bar{x}$ = 5.0) μm latus infra primarios ramos; cellae apicales et basilares non tumidae. Apparatus conidiogenus 25.0–77.5 ($\bar{x}$ = 49) μm longus, massa conidica exclusa, tribus vel quinque seriebus ramorum cylindricorum; duae metulae

Table 2. Comparison of *Ophiostoma* species similar to *O. americanum*.

	O. americanum	*O. penicillatum*[a]	*O. dryocoetidis*[b]	*O. clavigerum*[c]
Anamorph	*Leptographium*	*Leptographium*	*Leptographium*	*Leptographium* and *Graphium*
Conidiophore length (μm)	149–732	185–460	Up to 580	Up to 1444
Conidial shape	Obovoid to allantoid	Allantoid	Obovoid to clavate	Clavate with septa
Conidial length (μm)	3.5–2.2	5.5–13.5	9–23	35–68
Perithecial neck length (μm)	690–1300	300–500	150–560	No necks observed
Ascospore size (μm)	3–5.5 × 1.0–2.5	4.5–6.5 × 1.5–3.5	5.2–7.0 × 2.2–3.2	3.5–5.6 × 2.8–4.2
Ascospore shape	Reniform	Reniform	Like segments of an orange	Cucullate
Ostiolar hyphae	Absent	Absent	Present	Absent

[a]Grosmann (1931, 1932).
[b]Kendrick and Molnar (1965).
[c]Robinson-Jeffrey and Davidson (1968).

primariae. Cellae conidiogenae discretae, 2–3 per ramum, exigue attenuatae a basi ad apicem, cylindricae, rectae, 8–30 ($\bar{x}$ = 14) μm longae et 1.0–3.0 ($\bar{x}$ = 2.0) μm latae. Conidia hyalina, aseptata, obovoidea vel allantoidea, apice rotundato et basibus subtruncatis, 3.5–22 ($\bar{x}$ = 8.0) μm.

HOLOTYPUS: PREM 54866.

Colonies with optimal growth at 20°C on MEA, reaching 31 mm in diameter in 7 days. No growth below 5°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 64% reduction in growth on 2.5% cycloheximide after 6 days at 20°C in the dark.

Perithecia produced in the galleries of the bark-beetle, *Dendroctonus simplex* (Coleoptera: Scolytidae). Perithecial bases black, globose and smooth walled, unornamented or with very sparse ornamentation, 200–370 ($\bar{x}$ = 283.0) μm in diameter, neck dark brown to black, cylindrical with a slight apical taper, smooth, 690–1300 ($\bar{x}$ = 1027.5) μm long, 50–70 ($\bar{x}$ = 60.6) μm above globose base, 20–40 ($\bar{x}$ = 25.5) μm wide at the apex, ostiolar hyphae absent (Figs. 8 and 11*a*). Asci prototunicate, hyaline, evanescent. Ascospores reniform, aseptate, hyaline, curved and invested in a sheath, 3.0–5.5 ($\bar{x}$ = 4.5) × 1.0–2.5 ($\bar{x}$ = 1.5) μm (without sheaths) (Figs. 10 and 11*b*). Sheaths not uniform, giving the ascospores a rectangular appearance.

Hyphae immersed in medium with some aerial mycelia, hyaline to pale brown, smooth, frequently constricted at the septa, 1.5–6.0 ($\bar{x}$ = 3) μm diameter. Conidiophores occurring singly or in groups, arising directly from the mycelium, erect, macronematous, mononematous, smooth, olivaceous to light brown, 149.0–731.5 ($\bar{x}$ = 327.5) μm in length, rhizoids absent. Stipe olivaceous to light brown, smooth, cylindrical, simple, 3–15 septate, 108.5–691.5 ($\bar{x}$ = 287.5) μm long (from first basal septum to below primary branches), 4.5–9.5 ($\bar{x}$ = 5.0) μm wide below primary branches; apical and basal cells not swollen (Figs. 2, 4, and 11*c*). Conidiogenous apparatus 25.0–77.5 ($\bar{x}$ = 49) long, excluding the conidial mass, with three to five series of cylindrical branches; two primary branches, olivaceous, smooth, 0–1 septate, 9.5–20.0 ($\bar{x}$ = 14.0) μm long and 3.0–8.0 ($\bar{x}$ = 4.5) μm wide, secondary branches hyaline to olivaceous, aseptate, 8.0–20.0 ($\bar{x}$ = 10.5) μm long, 3–6 ($\bar{x}$ = 4) μm wide; tertiary branches hyaline to olivaceous, aseptate, 6–15.5 ($\bar{x}$ = 9) μm long, 1.5–6 ($\bar{x}$ = 3.5) μm wide, quaternary branches aseptate, 4–20 ($\bar{x}$ = 9) μm long, 1–5 ($\bar{x}$ = 3) μm wide. Conidiogenous cells discrete, two or three per branch, tapering slightly from the base to the apex, cylindrical, straight, 8–30 ($\bar{x}$ = 14) μm long and 1.0–3.0 ($\bar{x}$ = 2.0) 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 (Minter et al. 1982, 1983; Van Wyk et al. 1987) (Figs. 5 and 6). Conidia hyaline, aseptate, obovoid to allantoid, with a rounded apex and subtruncate bases, 3.5–22 ($\bar{x}$ = 8.0) μm (Figs. 6 and 11*d*). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, becoming pale yellow with age (Fig. 11*e*).

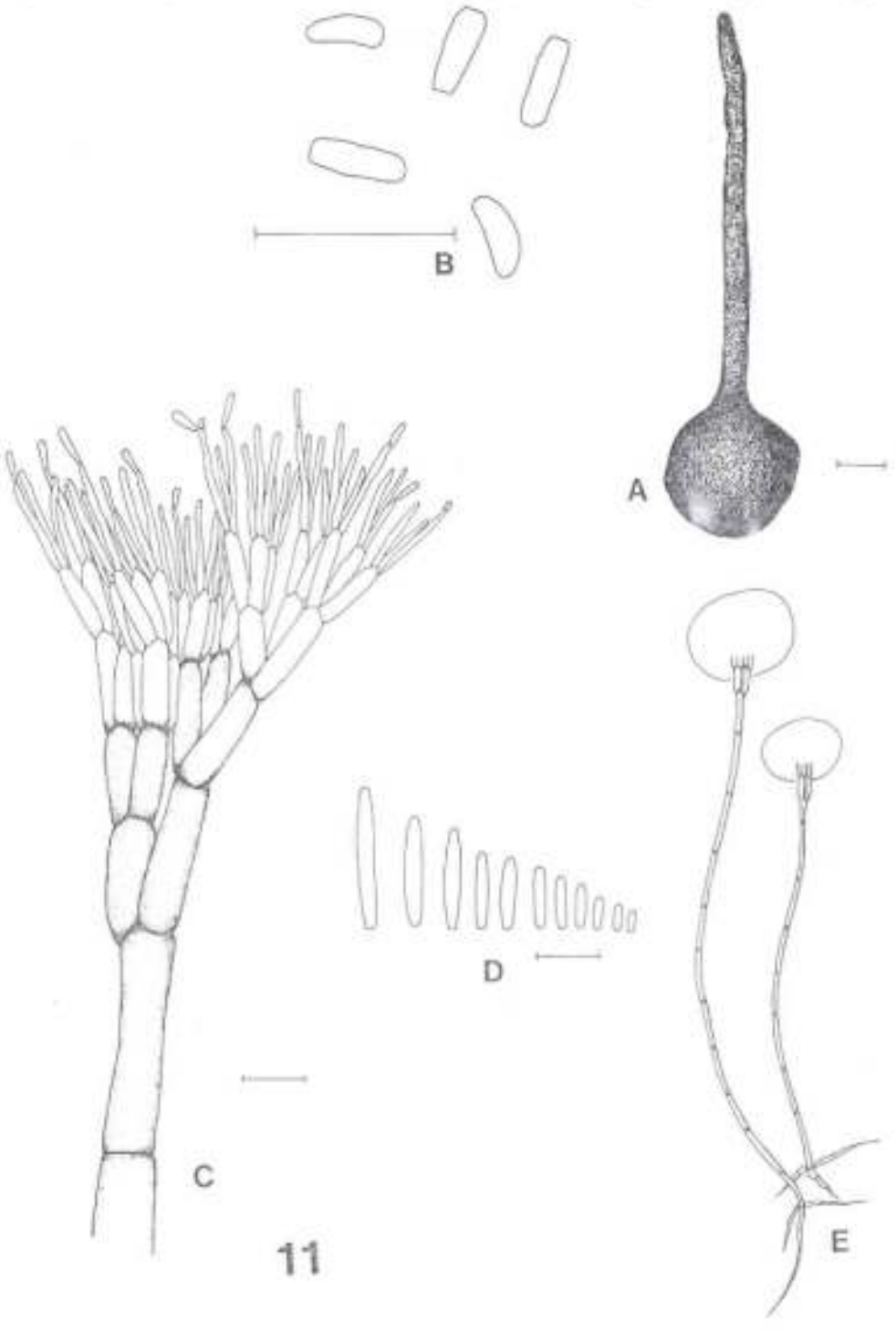
SPECIMENS EXAMINED: Cultures on 2% malt extract agar, isolated from *L. decidua* infested with *D. simplex*, Vermont, USA, May 1994, D.R. Bergdahl and M.J. Wingfield, PREM 54866 (CBS 497.96), holotype. Paratypes: from *L. decidua* infested with *D. simplex*, Vermont, USA, May 1994, D.R. Bergdahl and M.J. Wingfield, PREM 54867 (CBS 498.96); PREM 54868 (CBS 499.96); PREM 54869 (CBS 500.96).

Discussion

The new species of *Ophiostoma from L. decidua* infested with *D. simplex* in North America and described here as *O. americanum* is superficially similar to *O. penicillatum*. However, it can easily be distinguished from the latter species based on morphological characteristics of the conidia and ascospores. Although *O. americanum* has large allantoid conidia similar to those of *O. penicillatum*, they are much longer and narrower than those of the latter species (Table 2). The ascospores of *O. americanum* can also be distinguished from those of *O. penicillatum* based on the shape of the sheaths surrounding the ascospores. Rather than the uniform sheaths of *O. penicillatum*, *O. americanum* has ascospore sheaths that are not uniformly rounded, resulting in a rectangular appearance.

Ophiostoma americanum is one of four species of *Ophiostoma* with conidia that are at least twice and often three or four times longer than they are wide. The other three species with such conidia are *O. penicillatum*, *O. dryocoetidis* W.B. Kendr. & Molnar, and *O. clavigerum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr. The major morphological differences between these species are summarized in Table 2. *Ophiostoma clavigerum* can be distinguished from *O. americanum* based on differences in the anamorphs and differences in

Fig. 11. Line drawings of the anamorph and teleomorph of *O. americanum*. (A) Perithecium. Scale bar = 100 μm. (B) Ascospores surrounded with sheaths. Scale bar = 10 μm. (C) *Leptographium* anamorph. Scale bar = 10 μm. (D) Conidia of different lengths showing the variability in conidial length. Scale bar = 10 μm. (E) *Leptographium* anamorph showing the mucilaginous mass of conidia.



ascospore shapes (Kendrick and Molnar 1965; Robinson-Jeffrey and Davidson 1968; Upadhyay 1981). The only species of *Ophiostoma* with large conidia similar to those of *O. penicillatum* is *O. dryocoetidis* associated with the bark beetle *Dryocoetus confusus* Swaine on *Abies lasiocarpa* (Hook) Nutt. (alpine fir). However, the latter species is distinguished from *O. penicillatum* by the presence of ostiolar hyphae on the perithecia, and the conidia of *O. dryocoetidis* are also considerably larger than those of *O. penicillatum* (Kendrick and Molnar 1965). *Ophiostoma americanum* is distinguished from both *O. dryocoetidis* and *O. penicillatum* based on the difference in length of the conidia.

Ophiostoma penicillatum appears to be an obligate associate of *I. typographus* (Solheim 1986, 1992*a*, 1992*b*, 1993*b*).

Its occurrence in Japan suggests that it occurs across the geographic range of this insect, despite the fact that a distinct insect variety occurs in Japan. This insect occurs on Norway spruce throughout its geographic range, and we might expect that *O. penicillatum* will be recorded in areas such as eastern Europe and northern China in the future.

Our interest in fungi associated with *L. decidua* infested with *D. simplex* from the United States arose from a previous study of fungi associated with larch infested with *I. cembrae* in Japan (Van der Westhuizen et al. 1995). It is perhaps not surprising that the predominant fungus associated with *D. simplex* bears no resemblance to fungi associated with *I. cembrae* in Japan. The two insects in this case are completely unrelated, residing in different genera. Many fungi associated with bark beetles that infest conifers harbour some degree of virulence to the host tree, and the significance of these fungi as pathogens and their role in tree killing remains a matter of debate and contention (Harrington 1993; Solheim 1992*a*, 1992*b*, 1993*a*). Despite this, it would be of interest to acquire knowledge regarding the relative virulence of *O. americanum* on *L. decidua* in North America and we hope to undertake such studies in the future.

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