A new species of *Ophiostoma* with a *Leptographium* anamorph from Larch in Japan

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Recent surveys of felled *Larix* logs infested with *Ips cembrae* in the Mount Fuji area of Japan have yielded numerous ophiostomatoid fungi. One of these *Ophiostoma* species superficially resembles *Ophiostoma penicillatum* in having allantoid ascospores with sheaths. However, the conidia of the *Leptographium* anamorph are small obovoid, and distinct from those of *O. penicillatum*, which are characteristically large, and cylindrical to allantoid. On the basis of the morphologically distinct anamorphs, we conclude that this collection from *Larix* represents a new *Ophiostoma* holomorph. It is consequently described as *Ophiostoma laricis*, anamorph, *Leptographium laricis*.

Ophiostoma Syd. & P. Syd. is perhaps best known as a member of the *Ceratocystis sensu lato* complex. Considerable contemporary evidence exists, showing that *Ceratocystis sensu stricto* and *Ophiostoma* are unrelated (Weijman & De Hoog, 1975; Harrington, 1981; De Hoog & Scheffer, 1984). Their similar morphological structures thus being a consequence of convergence. Various taxonomic studies based on interpretation of nucleic acid sequences have provided additional support for this view (Hausner, Reid & Klassen, 1992*a*, *b*; Spatafora & Blackwell, 1994). In this paper, *Ophiostoma* is therefore treated as a distinct taxon with little or no relatedness to *Ceratocystis s.s.*

Most *Ophiostoma* spp. are associated with insects and particularly bark beetles (Coleoptera: Scolytidae) that infest trees (Lagerberg, Lundberg & Melin, 1927; Mathiesen-Käärik, 1960; Mathre, 1964; Dowding, 1984). Some of these fungi are important plant pathogens and the best known of these are *O. ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier that cause Dutch elm disease (Gibbs, 1978; Brasier, 1991). Other species have varying degrees of virulence and their role in tree death is unknown. They may contribute to tree death or alternatively be accidental associates of the insects that are disadvantageous rather than advantageous to their vectors (Harrington & Cobb, 1988).

Ophiostoma spp. have anamorphs in a number of genera. The taxonomy of these anamorph states has been confused and controversial (Kendrick, 1963; Upadhyay & Kendrick, 1974; Upadhyay & Kendrick, 1975; Upadhyay, 1981). Recent studies have led to a consolidation of these genera of which the most common are *Sporothrix* Hektoen & C. F. Perkins, *Hyalorhinocladiella* H. P. Upadhyay & W. B. Kendr., *Graphium* Corda and *Leptographium* Lagerb & Melin (Wingfield, 1985; Wingfield, Kendrick & Van Wyk, 1991; Mouton, Wingfield & Van Wyk, 1994). Some of these fungi, such as *Graphium* and *Leptographium*, are characterized by erect conidiophores with conidia produced at their apices in gloeoid masses that are produced in niches visited by insects and thus contribute to dispersal (Harrington & Cobb, 1988).

Species of *Ophiostoma* are best known from the northern hemisphere and particularly from Europe and North America (Upadhyay, 1981). Given the fact that tree species and insects that infest them are similar in many parts of Asia, North America and Europe, it is likely that the lack of reports of these fungi from Asia are due to inadequate collections from that area. Additional collections of *Ophiostoma* spp. from Asia are likely to increase our knowledge of this group considerably.

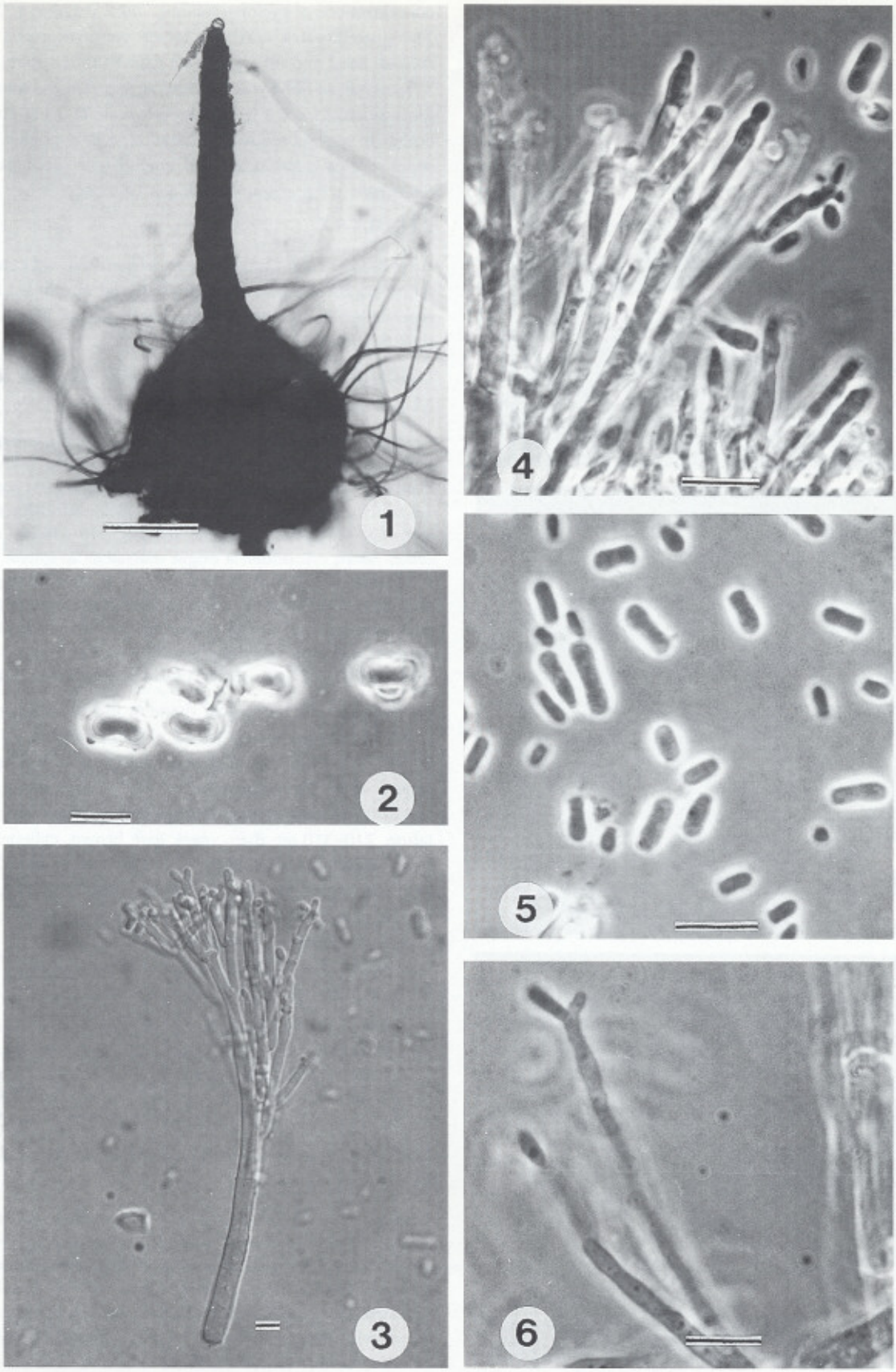
This study was part of a broader project by the second and third authors to characterize ophiostomatoid fungi from Japan. Here we describe a new species of *Ophiostoma* with a *Leptographium* anamorph from bark-bettle infested Larch (*Larix laricina*) in Japan.

MATERIALS AND METHODS

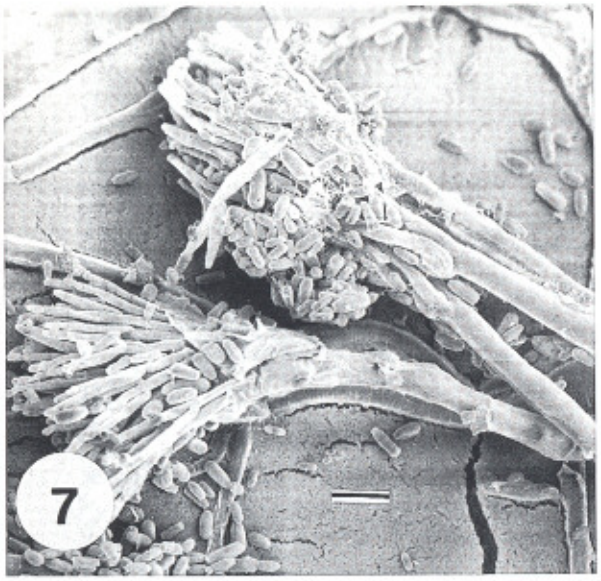
Larch infested with *Ips cembrae* Heer (Coleoptera: Scolytidae) and growing on the slopes of Mt Fuji, Japan, were visited during August 1992. The galleries of the beetles were obviously dark-stained and these were collected and taken to the laboratory for further study. Perithecia of *Ophiostoma* spp. were abundant in the galleries and it was possible easily to remove ascospore masses from their apices. Cultures were established on 2% malt extract agar (MEA) and incubated at 25 °C until the onset of sporulation. In some cases it was necessary to add freshly autoclaved pine wood to cultures to promote the production of perithecia and conidiophores.

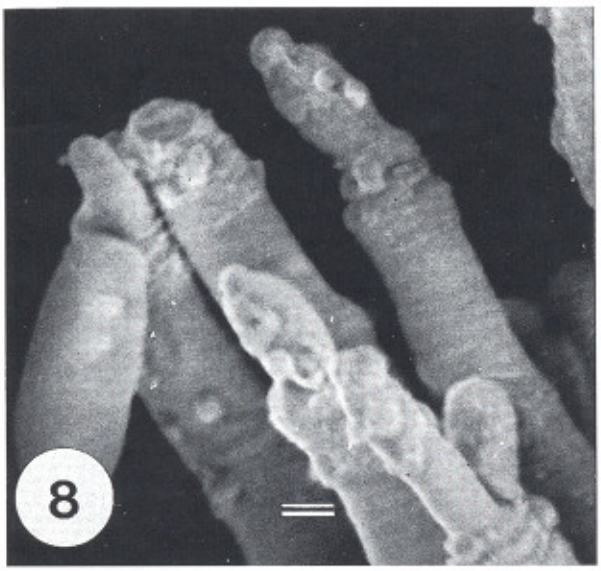
Single ascospore cultures were made by loosening ascospores in sterile distilled water and allowing these to germinate. Germlings, with single germ tubes, were then transferred to MEA plates and incubated.

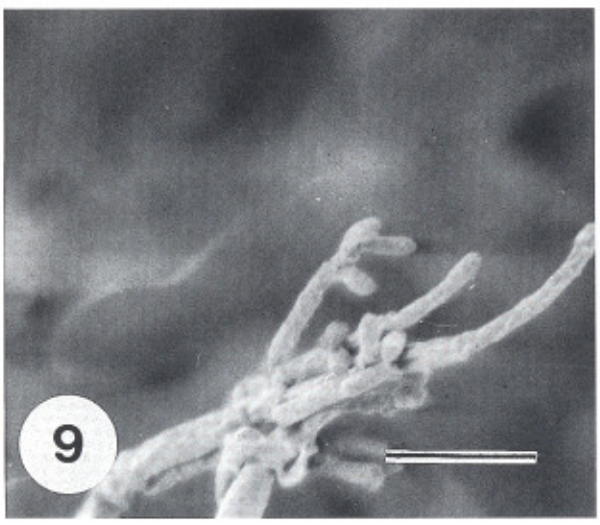
Cycloheximide tolerance, a characteristic of *Ophiostoma* but not *Ceratocystis* (Harrington, 1981), was tested at different



Figs 1–6. Teleomorph and anamorph characteristics of *Ophiostoma laricis*. **Fig. 1.** Perithecium (bar, 100 µm). **Fig. 2.** Allantoid ascospores with sheaths (bar, 10 µm). **Fig. 3.** *Leptographium* conidiophore (bar, 10 µm). **Fig. 4.** Conidiogenous cells showing percurrent proliferation (bar, 10 µm). **Fig. 5.** Small obovoid conidia with rounded apex and subtruncate base (bar, 10 µm). **Fig. 6.** *Hyalorhinocladiella* synanamorph (bar, 10 µm).







Figs 7–9. SEM of anamorph characteristics of *Ophiostoma laricis* (Scale bars = 10 μm). **Fig. 7.** *Leptographium* anamorph. **Fig. 8.** Conidiogenous cell showing percurrent conidium development. **Fig. 9.** *Hyalorhinocladiella* anamorph.

concentrations of cycloheximide (0, 0·05, 0·1, 0·5, 1, 2·5, 5 %). The growth rates of the colonies were measured on the second, third and fifth day after growth in the dark at 25°.

Material for SEM was cut from agar plates and fixed in 3 % glutaraldehyde and 1 % osmium tetroxide in 0·1 M phosphate buffer. It was subsequently dehydrated in a graded acetone series, critical-point dried and coated with gold palladium. Specimens were examined using a JSM 6400 SEM.

RESULTS

After study of the *Ophiostoma* sp. in question and comparison with various other similar species, it was concluded that this represented a previously undescribed taxon. The fungus is thus described as follows:

Ophiostoma laricis K. Van der Westh., Yamaoka & M. J. Wingf. sp. nov.

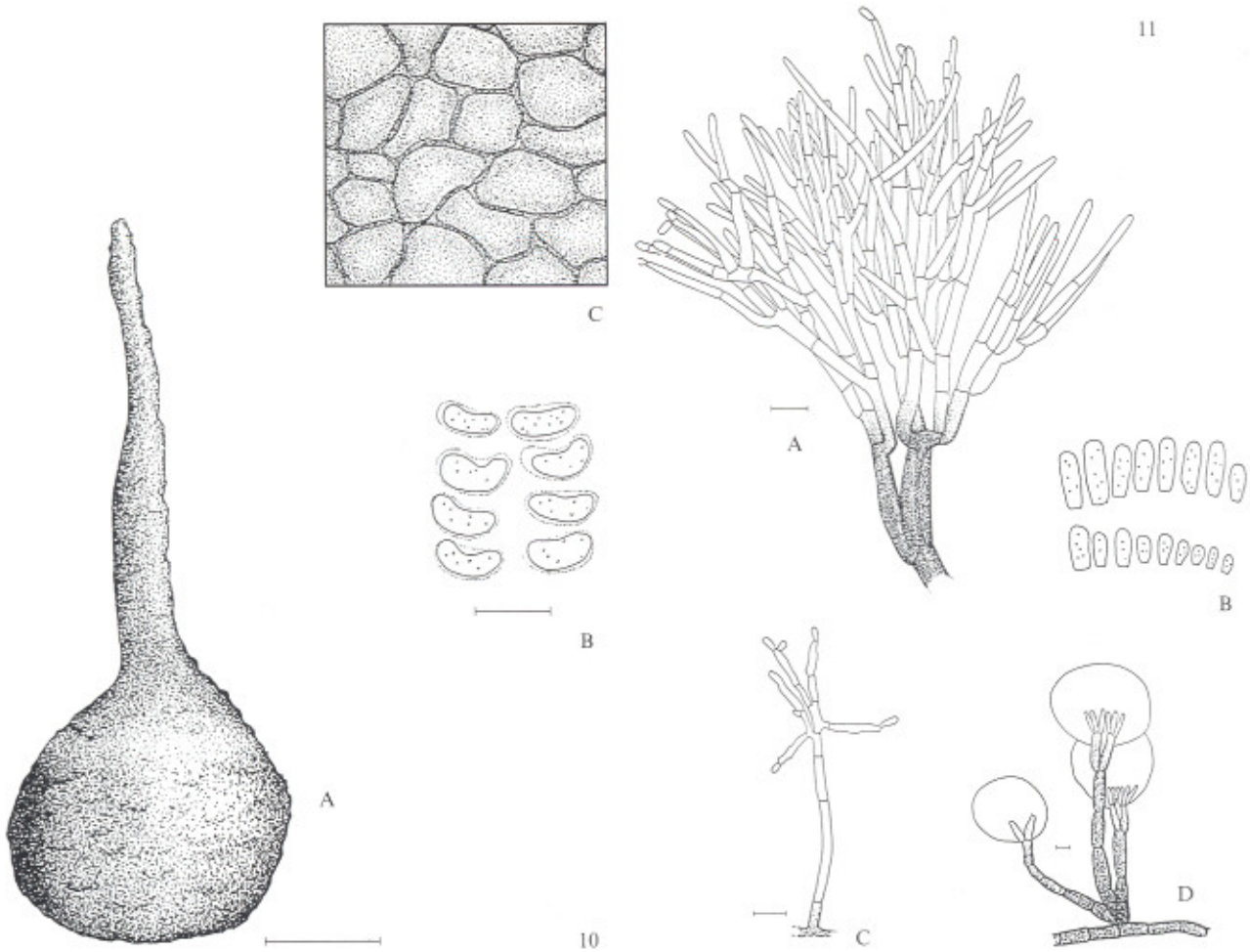
Perithecia genita in hospitis textu vel textu hospitis praesente et in fragmentis vapore sterilifactis *Pinus patulae* in 2% MEA patellis. Perithecia eveniunt singulatim vel usque sena aggregata, superficialia vel basibus semi-immersis, bases atrae, globosae, glabro-tunicatae, inornatae vel sparsa ornatione hyphali, 210–310 μm diametro, collum fusum, cylindricum et exigue attenuatum ad apicem, glabrum, 400–1320 μm longum, 50–70 μm latum super basim globosam, 20–50 μm latum ad apicem, hyphae ostiolares absunt (Figs 1, 10). Asci prototunicati, hyalini, evanescentes. Ascosporae aseptatae, hyalinae, guttulatae, curvatae, investitae in vagina, 5–9 × 3–4 μm (sine vaginis), 6–11 × 4—5 μm (vaginis comprehensis) (Figs 2, 10).

Perithecia produced on host tissue or in the presence of host tissue and on autoclaved pieces of *Pinus patula* on 2 % MEA plates. Perithecia occurring singly, or in groups of up to six, superficial or with semi-immersed bases, bases black, globose, smooth walled, unornamented, or with sparse hyphal ornamentation, 210–310 μm diam., neck dark brown, cylindrical with slight apical taper, smooth, 400–1320 μm long, 50–70 μm wide above globose base, 20–50 μm wide at the apex, ostiolar hyphae absent (Figs 1, 10). Asci prototunicate, hyaline, evanescent. Ascospores aseptate, hyaline, guttulate, curved, invested in a sheath, 5–9 × 3–4 μm (without sheaths), 6–11 × 4–5 μm (sheaths included) (Figs 2, 10).

Specimens examined: Cultures on 2 % malt extract agar, isolated from *Larix laricina* infested with *Ips cembrae*, Mt Fuji, Japan, August 1990, Y. Yamaoka and M. J. Wingfield, PREM 51810, holotype. Paratypes: from *Larix laricina* Mt Fuji, Japan, August 1990, Y. Yamaoka and M. J. Wingfield (PREM 51811, PREM 51812, PREM 51813).

Leptographium laricis K. Van der Westh, Yamaoka & M. J. Wingf. sp. nov.

Coloniae creverunt optime ad 25° in MEA perveneruntque ad 44 mm diametro in quinque diebus. Nullum incrementum infra 5° vel super 35°. Coloniae initio hyalinae, bruneolescentes a centro dum senescunt in MEA. Fungus potuit resistere dilutis fortibus cycloheximide, incremento 50 % deminuto in 2·5 % cycloheximide post 5 dies ad 25° in tenebris. Hyphae immersae in medio paucis vel nullis aeriis myceliis, hyalinae vel pallido-brunneae, laeves, 2–5 μm diametro. Conidiophora solitaria, vel usque quaterna aggregata, exorientia recte ex mycelio, erecta, macronematosa, mononematosa, laevia, olivacea vel bruneola, saepe constricta ad septa, 110–350 μm longitudine, rhizoideis absentibus. Stipes olivaceus vel bruneolus, laevis, cylindricus, simplex, 2–5 septatus, 50–170 μm longus (a primo septo basilari usque subter primarios ramos), 5–10 μm latus sub ramis



Figs 10–11. Teleomorph and anamorph characteristics of *Ophiostoma laricis*. **Fig. 10.** A, Perithecium (bar, 100 µm); B, ascospores surrounded with sheaths (bar, 10 µm); C, cells of the perithecial wall. **Fig. 11.** A, D, *Leptographium* anamorph (bar, 10 µm); B, obovoid conidia; C, *Hyalorhinocladiella* synanamorph (bar, 10 µm).

primariis; cellae apicales et basilares non tumidae. Apparatus conidiogenus 50–60 µm longus, massa conidica exclusa, tribus vel quinque seriebus ramorum cylindricorum; metulae primariae duae vel tres, olivaceae, laeves, 0–1 septatae, 20–80 µm longae et 3–8 µm latae, rami secundarii hyalini vel olivacei, 0–1 septati, 10–30 µm longi, 3–6 µm lati, rami tertiani, hyalini, 0–1 septati, 10–25 µm longi, 2·5–5 µm lati; rami quartani, 0–1 septati, 10–15 µm longi, 2·5–4 µm lati (Figs 3, 7, 11). Cellae conidiogenae discretae, 1–3 in quoque ramo, parum attenuatae a basi ad apicem, cylindricae, rectae, 10–30 µm longae et 1·5–3 µm latae (Figs 4, 8). Conidii auctus evenit muris ad restituendum struendis cum ontogenie holoblastica et proliferatione percurrenti secessione retardata, id quod false videtur proliferatio sympodialis. Conidia hyalina, aseptata, guttulata obovoidea vel oblongo-ellipsoidea, apice rotundato et base subtruncata, 2–14 × 2–5 µm (Figs 5, 11). Conidia accumulant in apparato conidiogeno in massa sub-flava, albescentia cum sicca sunt. Hyphae nonnullae hyalescunt 2–4 µm diametro, qoud parit synanamorpham *Hyalorhinocladiellae*. Conidiophora hyalina, simplicia, erecta, 2–4 septata, 90–130 µm longa, 3–4 µm lata ad basim. Cellae conidiogenae terminales lateralesque, cylindricae, rectae vel geniculato-sinuosae, 6–20 × 2–3 µm; conidia formantur enteroblastice et percurrenter in cellis conidiogenis (Figs 6, 9).

Colonies grew optimally at 25° on MEA, reaching 44 mm colony diam. in 5 d. No growth occurred below 5° or above 35°. Colonies were hyaline at first becoming light brown from the centre with age on MEA. The fungus could withstand high concentrations of cycloheximide with a 50% reduction in growth on 2·5% cycloheximide after 5 d at 25° in the dark. Hyphae immersed in medium with little or no aerial mycelia, hyaline to pale brown, smooth, 2–5 µm diam. Conidiophores single, or in groups of up to four, arising directly from mycelium, erect, macronematous, mononematous, smooth, olivaceous to light brown, frequently constricted at septa, 110–350 µm long, rhizoids absent. Stipe olivaceous to light brown, smooth, cylindrical, simple, 2–5 septate, 50–170 µm long (from first basal septum to below primary branches), 5–10 µm wide below primary branches; apical and basal cells unswollen. Conidiogenous apparatus 50–60 µm long, excluding the conidial mass, with three to five series of cylindrical branches; two to three primary metulae, olivaceous, smooth, 0–1 septate, 20–80 µm long and 3–8 µm wide, secondary branches hyaline to olivaceous, 0–1 septate, 10–30 µm long, 3–6 µm wide; tertiary branches hyaline, 0–1 septate, 10–25 µm long, 2·5–5 µm wide; quaternary branches 0–1 septate, 10–15 µm long, 2·5–4 µm wide (Figs 3, 7, 11). Conidiogenous cells discrete, 1–3 per branch, tapering slightly from base to apex, cylindrical, straight, 10–30 µm long and 1·5–3 µm wide (Figs 4, 8). Conidium development occurs through replacement wall building with holoblastic ontogeny and percurrent proliferation with delayed secession, giving the false impression of sympodial proliferation (Minter *et al.*,

1982, 1983; Van Wyk, Wingfield & Marasas, 1988). Conidia hyaline, aseptate, guttulate, obovoid to oblong-ellipsoidal, with a rounded apex and subtruncate base, 2–14 × 2–5 µm (Figs 5, 11). Conidia accumulating on the conidiogenous apparatus in a pale yellow mass, becoming white when dry. Several hyphae becoming hyaline 2–4 µm diam., giving rise to a *Hyalorhinocladiella* synanamorph. Conidiophores hyaline, simple, erect, 2–4 septate, 90–130 µm long, 3–4 µm wide at base. Conidiogenous cells terminal and lateral, cylindrical, straight or geniculate- sinous, 6–20 × 2–3 µm; conidia forming enteroblastically and percurrently on conidiogenous cells (Figs 6, 9).

Specimens examined: Cultures on 2% malt extract agar, isolated from *Larix laricina* infested with *Ips cembrae*, Mt Fuji, Japan, August 1990, Y. Yamaoka & M. J. Wingfield, PREM 51810, holotype. Paratypes: from *Larix laricina*, Mt Fuji, Japan, August 1990, Y. Yamaoka & M. J. Wingfield, (PREM 51811, PREM 51812, PREM 51813).

Dried cultures of the holotype and paratypes have been deposited in the National Collection of Fungi, Plant Protection Research Institute, South Africa (PREM). Subcultures of the type strain have been deposited in CBS and CMI.

DISCUSSION

Ophiostoma laricis superficially resembles *O. penicillatum* (Grosmann) Moreau and *O. europhiodes* E. F. Wright & Cain but can be distinguished from the latter species based on various anamorph and teleomorph characteristics. The ascospores of *O. laricis* are curved and surrounded by a uniform sheath. In this feature, they are similar to those of *O. penicillatum* (Solheim, 1986). However, conidia of this fungus are obovoid and thus very different from the typical large cylindrical, slightly curved conidia of *O. penicillatum* (Kendrick, 1961; Davidson, Francke-Grossman & Käärik, 1967; Upadhyay, 1981). Conidia and conidiophores of *O. laricis* resemble those of *O. europhiodes*. The latter fungus, however, has ascospores that are distinctly hat-shaped and thus very different from those of *O. laricis* (Wright & Cain, 1961; Upadhyay, 1981).

Ophiostoma penicillatum and *O. europhiodes* commonly occur together in Europe associated with the bark beetle *Ips typographus* L. on spruce (Solheim, 1986; Solheim, 1992). The fact that they occur in close proximity with each other has evidently given rise to considerable confusion in the taxonomy of *O. penicillatum*. This fungus has, in the past, been described as having ascospores with uniform sheaths or hat-shaped sheaths. Solheim (1986) has recently clarified this confusion showing that uniform sheaths are typical of *O. penicillatum* and he has also provided an appropriate neotype for the fungus. Clarification of this confusion thus made it possible for us to define the identity of *O. laricis*.

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