Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. *aponicus* in Japan

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Fungi were isolated from the beetles, *Ips typographus* f. *japonicus* and Yezo spruce (*Picea jezoensis*) trees infested with the beetles in Hokkaido, Japan. Nine species of ophiostomatoid fungi including one new species were identified. They were *Ceratocystiopsis minuta*, *Ceratocystis polonica*, *Ophiostoma ainoae*, *O. bicolor*, *O. cucullatum*, *O. europhioides*, *O. penicillatum*, *O. piceae*, and a new species described here as *O. japonicum*. Based on frequencies of occurrence, *O. ainoae*, *O. bicolor*, *O. penicillatum*, and *O. piceae* were regarded as dominant associates of *I. typographus japonicus*, and *C. minuta*, *C. polonica*, *O. europhinoides*, and *O. japonicum* were subdominant. The species of ophiostomatoid fungi associated with *I. typographus japonicus* in Japan are almost identical to those associated with *I. typographus* infesting Norway spruce (*P. abies*) in Europe. This study improves our knowledge of the biogeography of the ophiostomatoid fungi and the insects with which they are associated.

The bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae) occurs in Europe and Asia (Christiansen & Bakke, 1988) where it infests various species of spruce (*Picea* spp.). The beetle typically infests stressed or wind-blown trees, but when populations increase to high levels, they may also attack healthy trees. Mass attacks by *I. typographus* of Norway spruce (*Picea abies* (L.) Karst.) are, for example, well known in central and northern parts of Europe (Christiansen & Bakke, 1988).

Fungi, and specifically those in the *Ceratocystis sensu lato* complex, are commonly associated with bark beetles that infest trees, particularly conifers (Francke-Grosmann, 1963; Whitney, 1982; Harrington, 1993). These fungi have been the subject of taxonomic confusion and are currently recognized with two distinct phylogenetic lines including *Ceratocystis sensu stricto* and *Ophiostoma* (Samuels, 1993; Spatafora & Blackwell, 1994). The association of *Ceratocystis* and *Ophiostoma* with insects and bark beetles was first reported at the end of the last century (Hartig, 1878) and has been the subject of considerable study (Wingfield, Seifert & Webber, 1993).

The first record of species of *Ceratocystis s.l.* associated with *I. typographus* was made by Grosmann (1931, 1932). Considerable attention has been given to the fungi associated with *I. typographus* in Europe. Studies have encompassed the taxonomy of the fungi (Siemaszko, 1939; Mathiesen, 1950, 1951; Rennerfelt, 1950; Mathiesen-Käärik, 1953; Kotýnková-Sychrová, 1966; Käärik, 1975; Solheim, 1986; Harding, 1989) as well as their pathogenicity to trees (Horntvedt *et al*., 1983; Christiansen, 1985; Solheim, 1988; Christiansen & Solheim, 1990). In a comprehensive study, Solheim (1986) recorded eleven species of ophiostomatoid fungi associated with *I. typographus* in Norway. Of these, *O. polonicum* Siemaszko has been shown to be the most virulent (Horntvedt *et al*., 1983; Solheim, 1988). Recently, Visser *et al*. (1995) have shown that the fungus known as *O. polonicum* is a species of *Ceratocystis*, which makes it unusual in the *I. typographus*/spruce niche (Harrington *et al*., 1996).

Ips typographus L. f. *japonicus* Niijima is a Japanese form of *I. typographus* known in Europe, and can be separated from the latter based on the presence of an opaque elytral declivity (Kono & Tamanuki, 1939; Nobuchi, 1974). This insect seriously damages Yezo spruce (*Picea jezoensis* (Sieb. & Zucc.) Carr.) and Sachalin spruce (*P. glehnii* (Fr. Schm.) Masters) in Hokkaido, Japan. Outbreaks of the beetles usually occur after trees are damaged by strong winds such as those associated with typhoons (Yamaguchi *et al*., 1963; Furuta, 1989). The fact that fungi are associated with *I. typographus japonicus* has also been recognized in Japan for many years. Sapwood discolouration in spruce trees infested with these insects was noted by Numata (1931). Shortly thereafter, Tochinai & Sakamoto (1934) recorded the presence of *Ophiostoma piceae* (Münch) Syd. & P. Syd. (as *Ceratostomella piceae* Münch) from stained sapwood of Yezo spruce. The first definite association of fungi with *I. typographus japonicus* was made by Aoshima & Hayashi (1956) who consistently isolated *O. bicolor* R. W. Davidson & D. E. Wells from adult beetles as well as from discoloured Yezo spruce. Later Aoshima (1965) undertook a

detailed study of blue stain fungi in logs and windfallen trees of Yezo spruce and Sachalin spruce. In this study, he recognized 10 species of ophiostomatoid fungi including various undescribed species associated with *I. typographus japonicus*. At that time, the taxonomy of ophiostomatoid fungi was in confusion, and many of the names presented by Aoshima (1965) are of uncertain status.

A severe typhoon struck Hokkaido in 1981 and this led to a mass attack of *I. typographus japonicus* and the death of large numbers of spruce trees between 1983 and 1986 (Furuta, 1989). Remaining trees in the area, especially those surrounding log depots, were also killed (Nakayama *et al*., 1991). The aim of this study was to obtain a comprehensive view of the ophiostomatoid fungi associated with *I. typographus japonicus* in Hokkaido, Japan. We also wished to compare this ecologically and biogeographically important mycota with that associated with *I. typographus* in Europe.

MATERIALS AND METHODS

General isolations

Samples of bark and stained sapwood from Yezo spruce (*Picea jezoensis*) infested with *I. typographus japonicus* were collected on five occasions between 1989 and 1991 from a natural mixed forest area (700–900 m above sea level) in the Tokyo University Forest in Hokkaido, situated in central Hokkaido. Yezo spruce is the dominant species in this area. Selective cutting had taken place in this area since 1987, and some of the remaining trees, especially those in the vicinity of log depots, had been killed by beetles (Nakayama *et al*., 1991; Iguchi, Yamamoto & Furuta, 1993).

Strips of bark (20 cm wide × 30 cm long) and sapwood (1–2 cm thick) that included galleries of *I. typographus japonicus* were cut from trees. Galleries in the bark and on the cambium were carefully examined using a dissection microscope. Spore masses accumulating at the tips of perithecia, as well as mononematous and synnematous conidiophores, were carefully lifted from these structures using the tip of a fine tungsten needle and transferred to 1% malt extract agar (1% MA; 10 g malt extract, 15 g agar l^{-1} distilled water) in 9 cm Petri dishes. The dishes were then incubated at 20 °C in the dark and fungi were allowed to sporulate. Where necessary, cultures were purified by transferring small pieces of mycelium or spore masses from individual colonies to fresh malt extract agar plates.

Adult beetles were taken from their galleries in the bark samples using sterile fine forceps and placed on the surface of 2% MA in individual 9 cm plastic Petri dishes. The dishes were then incubated in the dark at 20°. Fungi growing from beetles or from agar over which beetles had walked were then purified by transferring small pieces of mycelium, or conidial or ascospore masses to fresh 2% MA plates.

Frequency of occurrence

A preliminary study of the frequency of occurrence of the various fungi associated with different stages of beetle development was undertaken. Yezo spruce trees infested with *I. typographus japonicus* in the Tokyo University Forest in Hokkaido were felled on 21 June 1990, 31 October 1990 and 31 July 1991. On each date, two trees between 38 and 56 cm diam. at breast height (110–210 yr old) were selected for study. Six discs, approximately 5 cm thick, were cut from the stems of each of these trees at 5 m intervals, between 5 and 20 m above the ground. Using a sharp scalpel, small (6–9 mm^3) pieces of bark were removed from egg galleries, larval galleries, pupal chambers as well as pieces of stained or discoloured sapwood were placed on the surface of 1% MA in Petri dishes. In addition, adult beetles, larvae, pupae and immature beetles were removed from galleries or pupal chambers using fine forceps and placed on the surface of 2% MA. All Petri dishes were incubated at 20° in the dark and cultures were purified as before.

Purified cultures of ophiostomatoid fungi, or their suspected anamorphs, were grown on 2% MA and 1% Pablum agar (1% PA; 10 g Pablum mixed cereal and 15 g agar l^{-1} distilled water) with or without the addition of small (about 2 cm × 5 mm × 3 mm) pieces of autoclaved Yezo spruce bark to stimulate ascocarp production. Ascocarps and conidiophores were mounted on glass slides in 1% lacto-fuchsin and studied using an Olympus BHS-N Nomarski interference contrast microscope.

Frequency of occurrence of each ophiostomatoid fungus from beetles in egg galleries, immature beetles in pupal chambers, walls of egg galleries, walls of larval galleries and stained or discoloured sapwood was calculated for samples from four trees collected on 21 June 1990 and 31 July 1991. Frequencies were computed using the following formula:

$$F = (NF/NT) \times 100,$$

where F represents the frequency of occurrence (%) of the fungus from each niche; NT represents the total number of substrate units from which isolations were made and NF represents the number of substrate units from which a particular fungus was isolated.

Cultures typical of each ophiostomatoid taxon isolated have been deposited in the culture collection of the Laboratory of Plant Pathology and Mycology, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan and in the Japan Collection of Micro-organisms (JCM). Dried specimens of these cultures have also been deposited with the Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba (TSH).

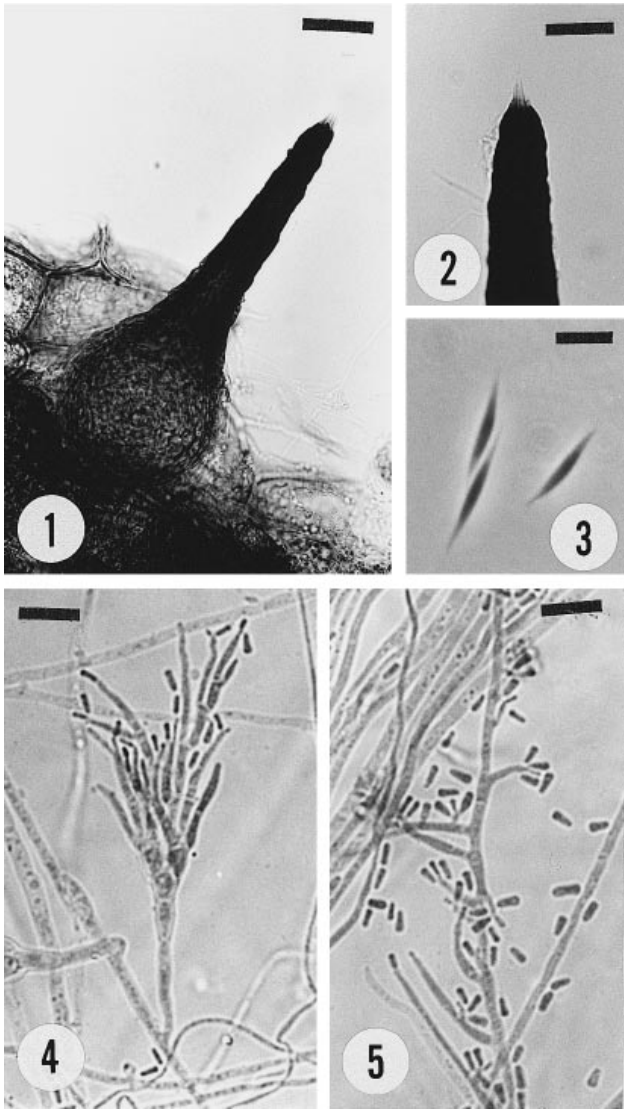
RESULTS AND DISCUSSION

Approximately 1140 isolates of ophiostomatoid fungi were collected from 137 beetles and 570 samples of tree tissue from beetle galleries and stained or discoloured sapwood. Twenty-four isolates of ophiostomatoid fungi were established by directly transferring spores from sporocarps produced in the galleries. These collections resulted in the identification of nine ophiostomatoid taxa.

Ceratocystiopsis minuta (Siemaszko) H. P. Upadhyay & W. B. Kendr., *Mycologia* 67: 800, 1975. (Figs 1–5)

= *Ophiostoma minutum* Siemaszko *Planta Polonica* 7: 23, 1939.

= *Ceratocystis minuta* (Siemaszko) J. Hunt, *Lloydia* 19: 49, 1956.



Figs 1–5. *Ceratocystiopsis minuta*. **Fig. 1.** Ascocarp (bar, 30 μm). **Fig. 2.** Top of neck (bar, 20 μm). **Fig. 3.** Ascospores (bar, 5 μm). **Fig. 4.** Macronematous branched conidiophore (bar, 20 μm). **Fig. 5.** Semi-macronematous conidiophores (bar, 20 μm).

This fungus produced perithecia both on the surface of the 2% MA medium and on the bark placed on the surface of the agar. Perithecia produced on the surface on the agar plates were almost uniformly brown to dark brown in colour. In contrast, perithecia (Fig. 1) on the bark tended to have dark brown necks and bases embedded in the bark were lighter brown in colour. The bases of both perithecial forms were globose to subglobose, 48–87 μm diam. and the necks cylindrical, 67–151 μm long including ostiolar hyphae, 22–34 μm wide at the base, 5–8 μm wide at the tip (at the base of ostiolar hyphae). Ostiolar hyphae (Fig. 2) were aseptate, acicular, converging to form a cone over ostioles, 6–12 μm long. Ascospores (Fig. 3) were hyaline, 1-celled, lunate with rounded ends in side view, fusiform in face view, surrounded by hyaline sheaths appearing falcate with attenuated ends in side view, acicular in face view, 9·6–13·6 × 1·4–2·4 μm including sheaths. Colonies were appressed with sparse aerial mycelium, white, and carried masses of slimy conidia. The anamorph was of the *Hyalorhinocladiella* type. Conidiophores were macro-

Table 1. Frequencies of occurrence of ophiostomatoid fungi from various niches

	Frequencies of occurrence (%)				
	Isolated from*				
	BE	IB	EG	LG	Sap
Ceratocystiopsis minuta	23·7	33·3	15·4	36·4	7·7
Ceratocystis polonica	10·5	4·8	23·1	9·1	16·3
Ophiostoma ainoae	81·6	81·0	61·5	51·5	28·1
O. bicolor	44·8	19·1	55·8	15·2	11·4
O. cucullatum	5·3	0	0	0	0
O. europhioides	15·8	23·8	26·9	21·2	14·2
O. japonicum	21·1	9·5	0	6·1	0·4
O. penicillatum	31·6	76·2	46·2	75·8	30·1
O. piceae	89·5	81·0	75·0	30·3	17·9

* BE, beetles in egg galleries; IB, immature beetles in pupal chambers; EG, walls of egg galleries; LG, walls of larval galleries; Sap, stained or discoloured sapwood.

nematous or semimacronematous (Fig. 5), mononematous, and sometimes branched (Fig. 4). Conidia were hyaline, 1-celled, oblong, cylindrical, or ellipsoidal, 2·4–4 × 1–2 μm, and formed sympodially from the terminal conidiophores.

Our isolates were similar to those of *C. minuta-bicolor* (R. W. Davidson) H. P. Upadhyay & W. B. Kendr. in the sense that they had bi-coloured perithecia. However, the size of ascospores and shape of conidia of this fungus differed from those described for *C. minuta-bicolor* (Davidson, 1966; Upadhyay, 1981), but fitted those for *C. minuta* (Hunt, 1956; Upadhyay, 1981) more closely. This is the first report of *C. minuta* from Japan and also the first report of *Ceratocystiopsis* from the country.

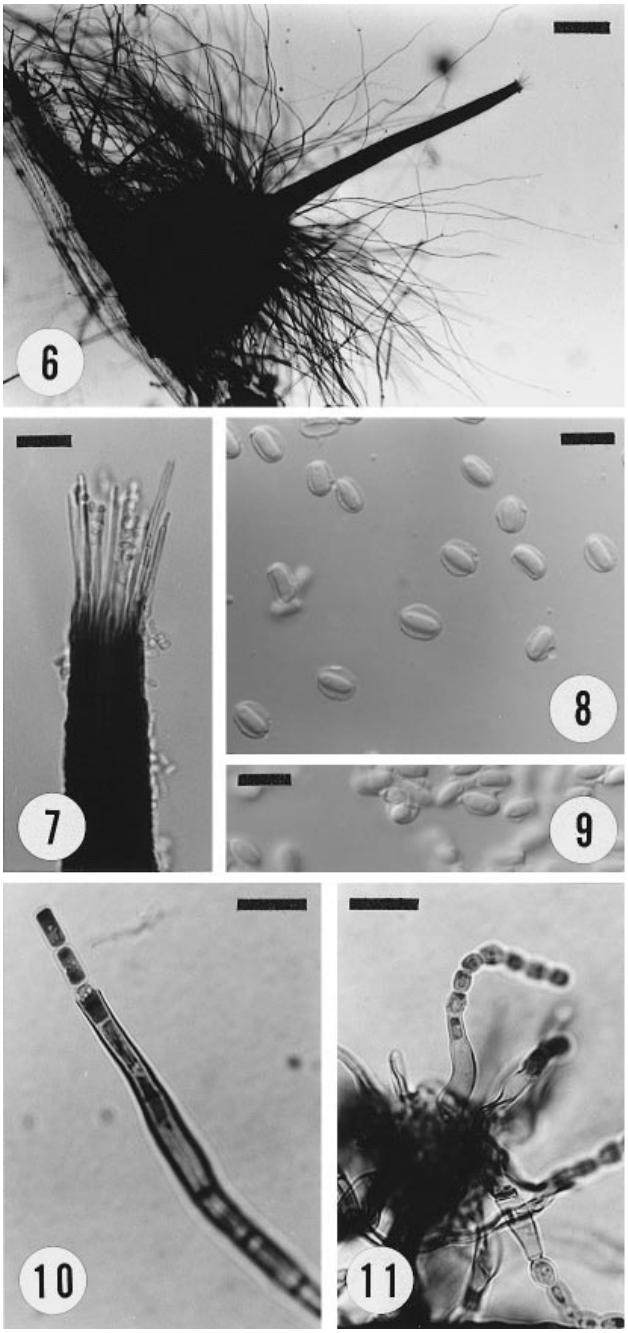
Ceratocystiopsis minuta was common and at least occasionally found in all niches considered. The frequency of occurrence of this fungus ranged between 7·7 and 36·4% (Table 1). *Ceratocystiopsis minuta* is known to be associated with Norway spruce infested by *I. typographus* in Europe (Siemaszko, 1939; Mathiesen, 1950; Kotýnková-Sychrová, 1966; Käärik, 1975; Solheim 1986; Harding, 1989; Solheim, 1993). Solheim (1992*b*) considered this species to be a tertiary invader of sapwood after attack of living Norway spruce trees by I. *typographus*.

Living and dried cultures deposited:
Cultures: YCC-139 (JCM 9367), isolate from an adult *I. typographus japonicus* from *P. jezoensis* at Tokyo University Forest in Hokkaido (TUFH), Yamabe, Hokkaido, Japan. Collected on 21 June 1990 by I. Takahashi(IT), K. Iguchi(KI), and Y. Yamaoka(YY); YCC-251 (JCM 9368), isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C50, dried culture YCC-139 grown on malt extract agar with pieces of autoclaved bark of *P. jezoensis* (MAB) at 20°; TSH-C52, dried culture YCC-251 grown on MAB at 20°.

Ceratocystis polonica (Siemaszko) C. Moreau, *Rev. Mycol. Suppl. Col.* 17: 22, 1952. (Figs 6–11)
= *Ophiostoma polonicum* Siemaszko, *Planta Polonica* 7: 32, 1939.

This fungus produced perithecia superficially both on the surface of the agar medium and on the bark placed on the



Figs 6–11. *Ceratocystis polonica*. **Fig. 6.** Ascocarp (bar, 100 µm). **Fig. 7.** Top of neck (bar, 15 µm). **Figs 8, 9.** Ascospores (bar, 7 µm). **Figs 10, 11.** *Chalara* state (bar, 15 µm).

surface of the agar. The bases of perithecia (Fig. 6) were black, globose to subglobose, 200–390 µm diam., ornamented by dark brown septate hyphae up to 1230 µm long, and the neck slender, black, 600–1150 µm long. Ostiolar hyphae (Fig. 7) aseptate, hyaline, straight, 10–22, slightly divergent, 28–54 µm long. Ascospores (Figs 8, 9) ellipsoid 4·8–5·6 µm × 2·0–2·4 µm surrounded by hyaline sheaths oval in face view and orange section-shaped in side view, 5·2–6·4 × 2·4–3·2 µm. Morphological characteristics of teleomorphs of the isolates in this study were very similar to those in the original description (Siemaszko, 1939). Two conidial states were present. Conidiophores of the first type (Fig. 10) mononematous, cylindrical or slightly tapered, pale brown to brown, composed of 3–6 cells, 74–160 µm long including terminal conidiogenous cell, and 6–8 µm wide at the broadest part. Conidiogenous cells phialidic, cylindrical or lageniform, and 52–88 × 4–8 µm. Conidia hyaline, oblong, barrel-shaped or ellipsoidal, 7–14 × 4–6 µm. Conidiophores of the second type (Fig. 11) mononematous, simple, hyaline to pale brown, aseptate or sometimes septate at the base, 12–38 µm long including terminal conidiogenous cell, and 3·6–6 µm wide. Conidiogenous cells phialidic, lageniform or obclavate, straight or curved, and 12–38 × 3·6–6 µm. Conidia hyaline, oblong, barrel-shaped, cylindrical or ellipsoidal, and 5–10 × 3–4 µm. Both types of conidiophores rarely formed and usually associated with the bases of the perithecia.

Ceratocystis polonica was one of the fungi associated with *I. typographus japonicus*. It was isolated from all niches considered, and frequencies of occurrence ranged from 4·8 to 23·1% (Table 1). This fungus has previously been treated in *Ophiostoma* but has been shown to reside in *Ceratocystis sensu stricto* (Visser *et al*., 1995). *Ceratocystis polonica* is also one of the common species associated with *I. typographus* in Europe (Siemaszko, 1939; Mathiesen, 1950, 1951; Mathiesen-Käärik, 1953, 1960; Käärik, 1975; Solheim, 1986, 1993; Harding, 1989; Furnish, Solheim & Christiansen, 1990; Viiri & von Weissenberg, 1995). In Norway, it is the most active invader of Norway spruce sapwood (Solheim, 1986, 1988, 1992*a*, *b*). *Ceratocystis polonica* has the ability to kill healthy trees of various spruces and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Horntvedt *et al*., 1983; Christiansen, 1985; Christiansen & Solheim, 1990). This fungus was most frequently (35·5%) isolated from the blue-stained and discoloured sapwood of the two trees collected on 21 June 1990, that is approximately 4–6 wk after the beetle infestation. The relatively high frequency of occurrence of the fungus from the sapwood at an early stage after beetle infestation indicates that *C. polonica* is an early invader of sapwood as shown by Solheim (1992*a*, *b*). In Norway, Solheim (1992*a*, *b*) found *C. polonica* at high frequencies in epidemic areas, while the frequencies were lower in endemic areas (Solheim, 1993).

Aoshima (1965) described a *Ceratocystis* species very similar to the fungus that we are now recognizing as *C. polonica* in a study of fungi associated with *I. typographus japonicus*. He provided the name *Ceratocystis jezoensis* for this species but this description was not valid due to the lack of a Latin description. He also noted that this fungus was associated with *Ips cembrae* Heer in Japan.

Recognition of *C. polonica* as a species of *Ceratocystis sensu stricto* has raised numerous interesting and conceptual questions regarding the fungi associated with bark beetles that infest conifers (Visser *et al*., 1995; Harrington *et al*., 1996). Prior to this, *Ceratocystis laricicola* Redfern & Minter, associated with *I. cembrae* attacking introduced European larch (*Larix decidua* Miller) in Scotland (Redfern *et al*., 1987), was the only *Ceratocystis* species known to be associated with a bark beetle that infests conifers. However, Solheim (1995) found that the North American spruce beetle, *Dendroctonus rufipennis* (Kirby) also carries a *Ceratocystis* species, and he considered that the fungus appeared to be a primary invader of sapwood following attack by the beetles. What is interesting regarding *C. polonica*, is the fact that it occurs in association with a large number of

Ophiostoma spp., but belongs to a group that is distantly related in terms not only of taxonomy (de Hoog, 1974; de Hoog & Scheffer, 1984; Harrington, 1987; Wingfield *et al*., 1993; Spatafora & Blackwell, 1994) but also ecology (Harrington *et al*., 1996). Like most *Ceratocystis* species, this fungus is a highly virulent pathogen. However, in its adaptation to the bark beetle habit, it has lost many common features of *Ceratocystis*, such as profuse sporulation in culture and the production of aromatic compounds.

The similarity of *C. laricicola* and *C. polonica* is remarkable and it is possible that these two fungi represent the same species (Visser *et al*., 1995; Harrington *et al*., 1996). The curiosity here relates to the fact that the fungi are associated with two different insects, one associated with spruce and the other infesting larch. The two insects, however, appear to be very closely related based on the analyses of allozymes and sequences of mitochondrial DNA (Christian Stauffer, Institut für Forstentomologie, Univ. f. Bodenkultur, Vienna, Austria, pers. comm.), thus explaining the fact that they share this important fungal associate. It seems likely that *C. laricicola* was introduced into Scotland with *I. cembrae* from Europe, and it will be interesting to consider the fungi associated with this insect in its native range.

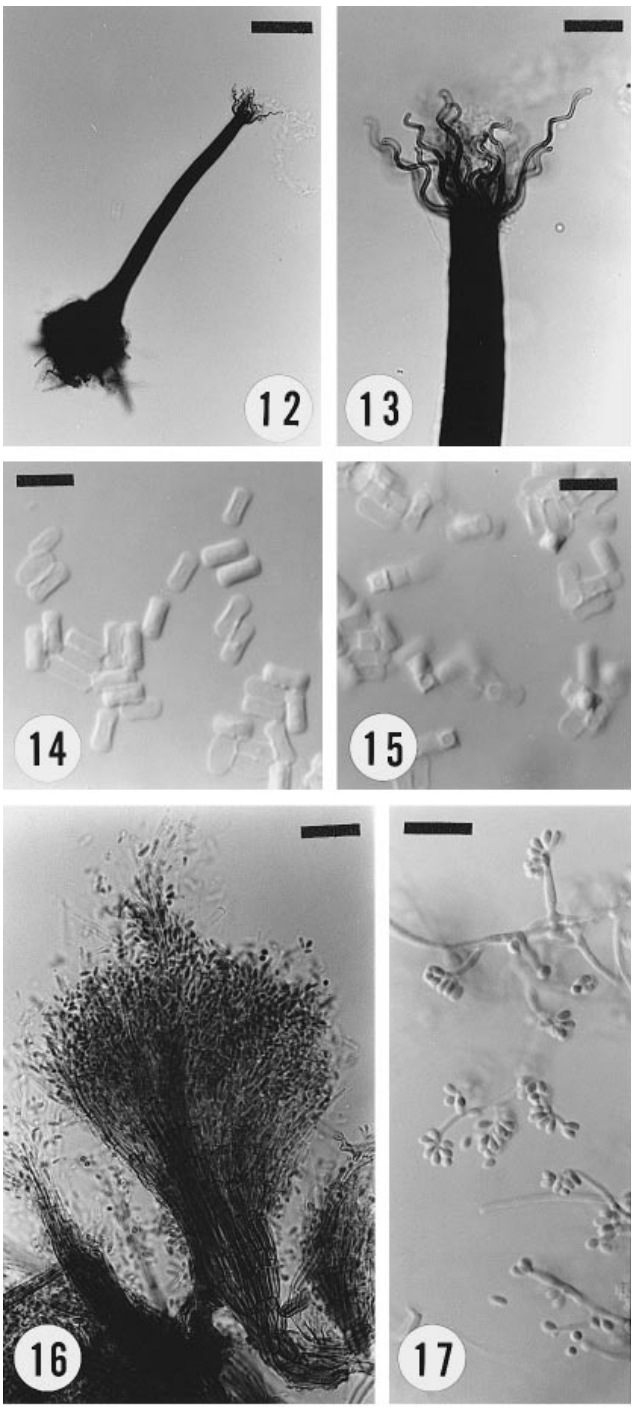
Living and dried cultures deposited:
Cultures: YCC-67 (JCM 9369), isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 10 Aug. 1989 by IT, KI, and YY; YCC-115 (JCM 9370), isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY.

Dried specimens: TSH-C40, dried culture YCC-67 grown on MAB at 20°; TSH-C43, dried culture YCC-115 grown on MAB at 20°.

Ophiostoma ainoae H. Solheim, *Nord. J. Bot*. 6: 201, 1986. (Figs 12–17)

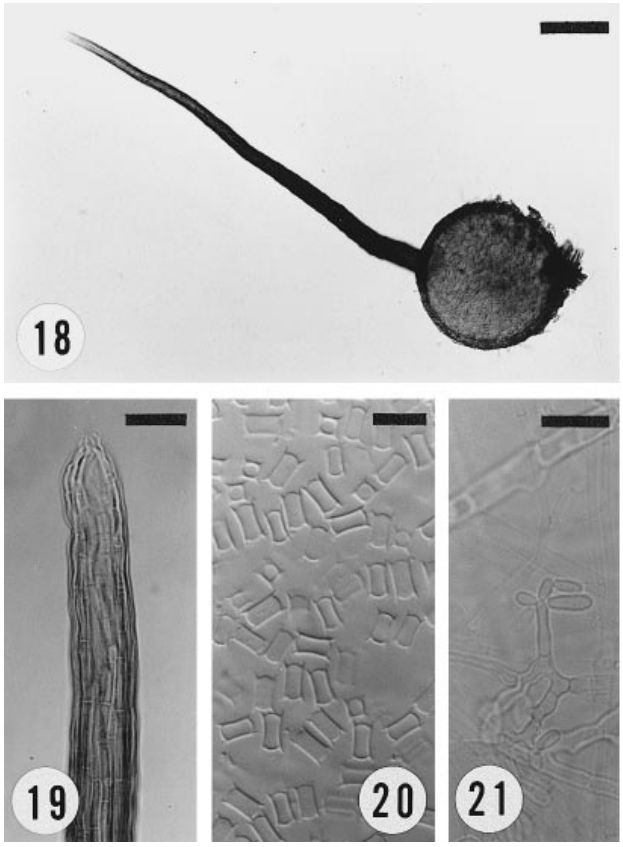
This fungus was first mentioned by Käärik (1975) as *Ceratocystis* gr. *clavata* and was later described by Solheim (1986) as *O. ainoae*. Cultures in this study produced larger perithecia than those in the original description (Solheim, 1986), with bases 79–214 µm in diam. and necks 302–865 µm long. Aoshima (1965) reported that *Ceratocystis clavata* (Math.-Kaarik) J. Hunt (= *O. clavatum* Math.-Kaarik) was one of the most common fungi associated with *I. typographus japonicus*. *Ophiostoma ainoae* resembles *O. clavatum*, since both species have brown, spirally curved ostiolar hyphae at the tip of perithecial necks and *Graphium*-like anamorphs. However, they can be distinguished based on cultural characteristics and ascospore form (Solheim, 1986). The former species has ascospores that are cylindrical in side view whereas *O. clavatum* has ascospores that are orange section-shaped. Aoshima (1965) described ascospores that were elongate ellipsoid or kidney-shaped and he was almost certainly also working with *O. ainoae*.

Ophiostoma ainoae was one of the most commonly isolated taxa in this study. Frequencies of occurrence from adult insects, immature beetles, egg galleries and larval galleries ranged from 51·5 to 81·6% (Table 1). Frequency of occurrence from stained or discoloured sapwood was 28·1%. Although *O. ainoae* was less common in sapwood than in other niches considered, it was the second most common fungus isolated from stained or discoloured sapwood. This fungus was associated with *I. typographus* in Europe by Solheim (1986, 1993), Harding (1989), and Viiri & von Weissenberg (1995). Solheim (1992*a*, *b*) found this species to be a secondary invader of Norway spruce sapwood after infestation trailing behind *C. polonica*.



Figs 12–17. *Ophiostoma ainoae*. **Fig. 12.** Ascocarp (bar, 80 µm). **Fig. 13.** Top of neck (bar, 20 µm). **Figs. 14, 15.** Ascospores (bar, 5 µm). **Fig. 16.** *Graphium* state anamorph (bar, 20 µm). **Fig. 17.** *Hyalorhinocladiella* state (bar, 15 µm).

Living and dried cultures deposited:
Cultures: YCC-126 (JCM 9356), isolate from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY; YCC-234 (JCM 9357), isolate from an adult *I.*



Figs 18–21. *Ophiostoma bicolor*. **Fig. 18.** Ascocarp (bar, 150 µm). **Fig. 19.** Top of neck (bar, 20 µm). **Fig. 20**. Ascospores (bar, 7 µm). **Fig. 21.** *Hyalorhinocladiella* state (bar, 15 µm).

typographus japonicus in *P. jezoensis* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C1, dried culture YCC-126 grown on Pablum agar with pieces of autoclaved *P. jezoensis* bark (PAB) at 20°; TSH-C4, dried culture YCC-234 grown on PAB at 20°.

Ophiostoma bicolor R. W. Davidson & D. E. Wells, *Mycologia* 47: 63, 1955. (Figs 18–21)

= *Ceratocystis bicolor* R. W. Davidson & D. W. Wells) R. W. Davidson, *Mycologia* 50: 665, 1958.

This species was first described by Davidson (1955). Davidson (1964) and Griffin (1968) emphasized variation in colour of perithecia in different specimens. Griffin (1968) treated *O. bicolor* as a complex of three species based on colour and morphology of perithecia. In his monograph of *Ceratocystis* and *Ceratocystiopsis*, Upadhyay (1981), however, treated these fungi as a single species. Aoshima (1965) described Japanese isolates of this fungus in a manner very similar to that of Davidson (1955) and similar to the description of collections from white spruce (*Picea glauca* (Moench) Voss) by Griffin (1968). Morphological characteristics of the isolates of *O. bicolor* obtained in this study were similar to those previously described, other than the fact that the perithecia had larger bases (up to 440 µm) and they sometimes lacked necks when formed on bark in culture. The *Hyalorhinocladiella* anamorph (Fig. 21) rarely formed on MA medium, but sometimes occurred on PA medium.

Ophiostoma bicolor is known to be associated with *I. typographus* in Europe (Kotýnková-Sychrová, 1966; Davidson, Francke-Grosmann, & Käärik, 1967; Käärik, 1975; Solheim, 1986, 1992*a*, *b*, 1993; Harding, 1989; Viiri & von Weissenberg, 1995). Solheim (1992*a*, *b*) found that *O. bicolor* was the species that followed *C. polonica* in sapwood colonization after *I. typographus* attack on living Norway spruce. In this study, it was isolated from all niches considered and frequencies of occurrence ranged from 11·4 to 55·8% (Table 1). Aoshima & Hayashi (1956) and Aoshima (1965) reported that *O. bicolor* was one of the most commonly encountered fungi associated with *I. typographus japonicus*, which is consistent with our results. In terms of frequency of occurrence, it was, however, not the most commonly isolated fungus.

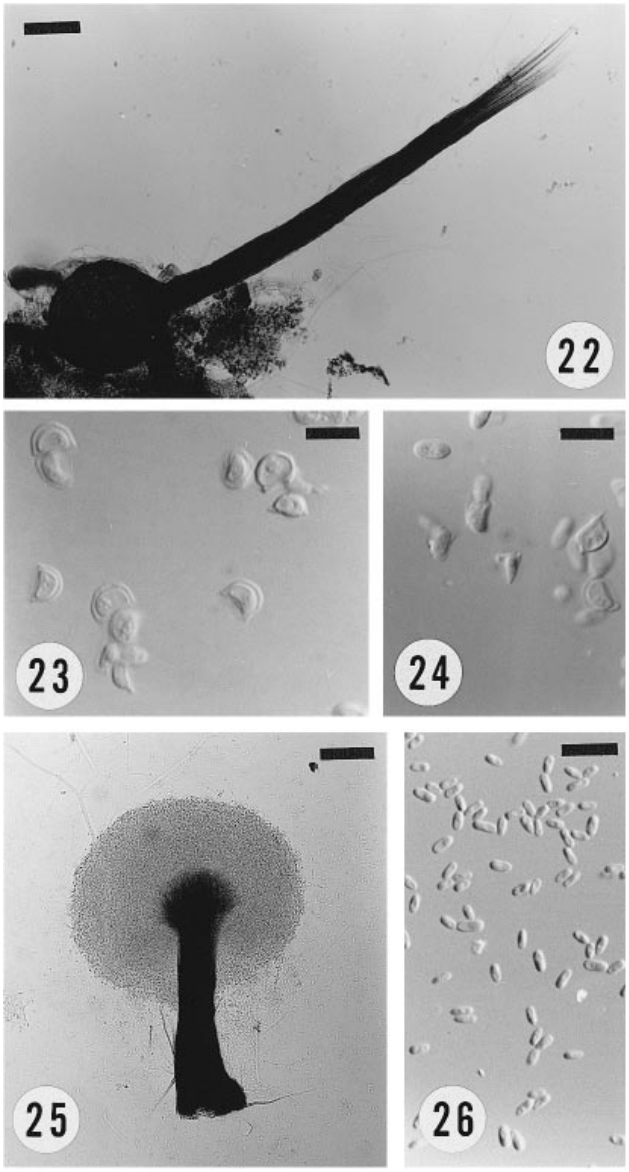
Living and dried cultures deposited:
Cultures: YCC-104 (JCM 9358), isolate from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY; YCC-237 (JCM 9359), isolate from sapwood of *P. jezoensis* infested with *I. typographus japonicus* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C10, dried culture YCC-104 grown on MAB at 20°; TSH-C12, dried culture YCC-237 grown on MAB at 20°.

Ophiostoma cucullatum H. Solheim, *Nord. J. Bot.* 6: 202, 1986 (Figs 22–26)

This species was first described by Solheim (1986) associated with adult *I. typographus* emerging from Norway spruce. In this study, *O. cucullatum* was isolated from only two adult beetles in egg galleries (Table 1).

Ophiostoma cucullatum has perithecia that are morphologically similar to those of *O. brevicolla* (R. W. Davidson) de Hoog & R. J. Scheff., *O. davidsonii* (Olchow. & J. Reid) H. Solheim, *O. sagmatospora* (E. F. Wright & Cain) H. Solheim, and *O. olivacea* Math.-Kaarik. Solheim (1986), however, showed that *O. cucullatum* is distinguishable from the other four species based on morphology of the ascospores. Ascospores of *O. cucullatum* are lunate with obtuse ends, are enclosed in a thick hyaline sheath, and are cucullate in side view. In contrast, ascospores of *O. brevicolla* and *O. davidsonii* are enclosed in uniform sheaths, while those of *O. sagmatospora* and *O. olivacea* are reniform and enclosed in hat-shaped sheaths, with distinct brims. Ultrastructural observations of ascospores of *O. davidsonii* have shown that they have distinct brims and hat-shaped lateral appearance (Van Wyk & Wingfield, 1991*a*). Van Wyk & Wingfield (1991*b*) also compared ascospore ultrastructure and development in *O. davidsonii* and *O. cucullatum* and showed them to be similar but different in the arrangement of ascospores in the asci. *Ophiostoma cucullatum*, *O. davidsonii*, and *O. olivacea* also have morphologically similar *Graphium* anamorphs and can only be clearly distinguished based on ascospore morphology (reniform or lunate) and their patterns of ascospore development. Although further critical comparison of these species is probably required, we feel confident that our isolates represent the fungus known as *O. cucullatum*. The association of this fungus with *I. typographus* in Europe supports this view and this is, therefore, the first record of its occurrence outside Norway.



Figs 22–26. *Ophiostoma cucullatum*. **Fig. 22.** Ascocarp (bar, 50 μm). **Figs 23, 24.** Ascospores (bar, 7 μm). **Fig. 25.** *Phialographium* state (bar, 70 μm). **Fig. 26.** Conidia (bar, 5 μm).

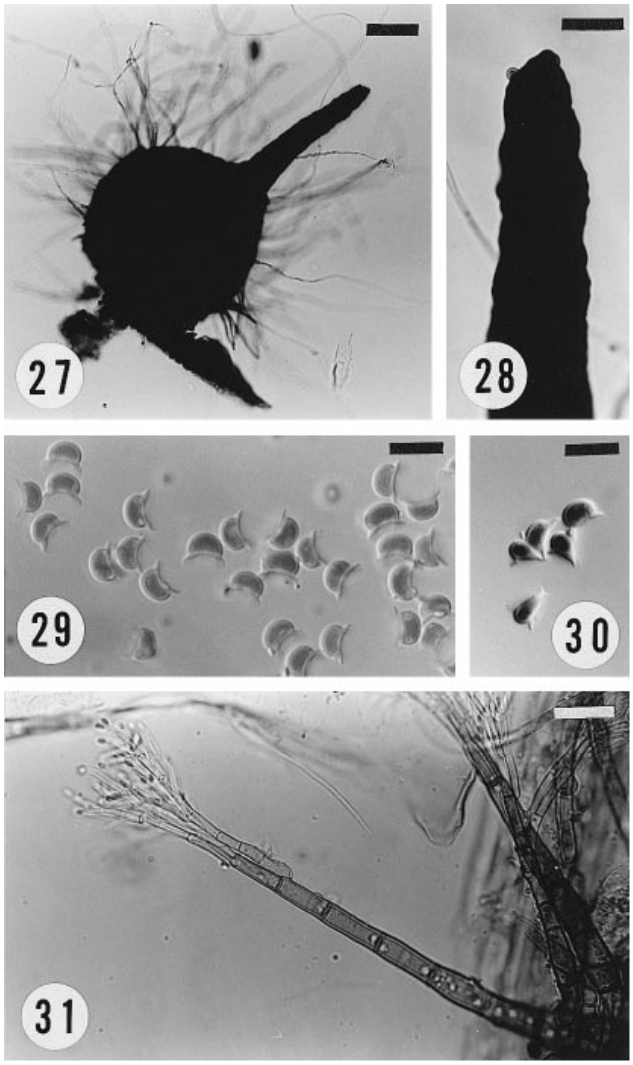
Living and dried cultures deposited:
Cultures: YCC-231 (JCM 8816), isolated from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C56, dried culture YCC-231 grown on MA at 20°.

Ophiostoma europhioides (E. F. Wright & Cain) H. Solheim, *Nord. J. Bot.* 6: 203, 1986. (Figs 27–31)

= *Ceratocystis europhioides* E. F. Wright & Cain, *Can. J. Bot.* 39: 1222, 1961.

Morphological characteristics of the isolates collected in this study were reasonably consistent with those originally described for *O. europhioides* (Wright & Cain, 1961). Perithecial necks were considerably shorter (up to 390 μm) than those described for this fungus (up to 950 μm). They were, however,



Figs 27–31. *Ophiostoma europhioides*. **Fig. 27.** Ascocarp (bar, 70 μm). **Fig. 28.** Top of neck (bar, 20 μm). **Figs 29, 30.** Ascospores (bar, 7 μm). **Fig. 31.** *Leptographium* state (bar, 20 μm).

within the range provided in the description of Davidson *et al.* (1967).

Upadhyay (1981) treated *O. europhioides* as a synonym of *O. piceaperdum* (Rumbold) Arx. *O. piceaperdum* was first described by Rumbold (1936) as *Ceratostomella piceaperda* Rumbold with ellipsoidal ascospores, 3·6–4·7 × 1·0–2·4 μm. She did not describe ascospores of the species as thick-walled nor hat-shaped. Hunt (1956) reported that *O. piceaperdum* had ascospores with a thick sheath, ellipsoidal, often slightly curved, sometimes hat-shaped and 4–6 μm × 2·5–3 μm including sheath. Wright & Cain (1961) described *O. europhioides* with ascospores having sheaths 4·5–6 × 2·4–3·5 μm (excluding sheaths) and sheaths projecting up to 4 μm at each side of the spores. Davidson *et al.* (1967) reported that the ascospores of the fungus were 5·5–6 μm long and without sheaths 3·5–4·5 × 1·6–2·5 μm. These data indicate that ascospores of *O. europhioides* are larger than those of *O. piceaperdum*, although it is difficult and possibly risky to compare dimensions provided by different authors. Upadhyay (1981) showed ascospores of *O. piceaperdum* as (4–)4·6–7·2(–8) × 2–3·5(–4) μm including sheaths, which includes the

range of ascospore dimensions for *O. europhioides sensu* Wright & Cain (1961) and Davidson *et al.* (1967) as well as *O. piceaperdum sensu* Rumbold (1936) and Hunt (1956). It was beyond the scope of this study to establish the validity of the synonymy of *O. europhioides* as *O. piceaperdum*, but we believe that further critical comparison of these species is desired. Since dimensions of ascospores (4·0–5·2 × 2·4–3·2 µm excluding sheath, 4·8–6·4 × 3·2–4·0 µm including sheath) and perithecia in our isolates were most similar to those provided in the original description of *O. europhioides*, we feel most comfortable using this name for them. Aoshima (1965) described a new species *O. shikotsuensis* without a Latin diagnosis in his study. From his description and illustration, it seems likely that this fungus is the same as the one that we have treated as *O. europhioides*.

Ophiostoma europhioides was isolated from all niches considered in this study. The frequencies of occurrence of the fungus ranged from 14·2 to 26·9% (Table 1). It is also well known as an associate of *I. typographus* infesting Norway spruce in Norway (Solheim, 1986, 1993), Denmark (Harding, 1989), and Finland (Viiri & Weissenberg, 1995). Solheim (1992*b*) found *O. europhioides* to be a tertiary invader in his successional study of fungi colonizing Norway spruce sapwood after *I. typographus* attack.

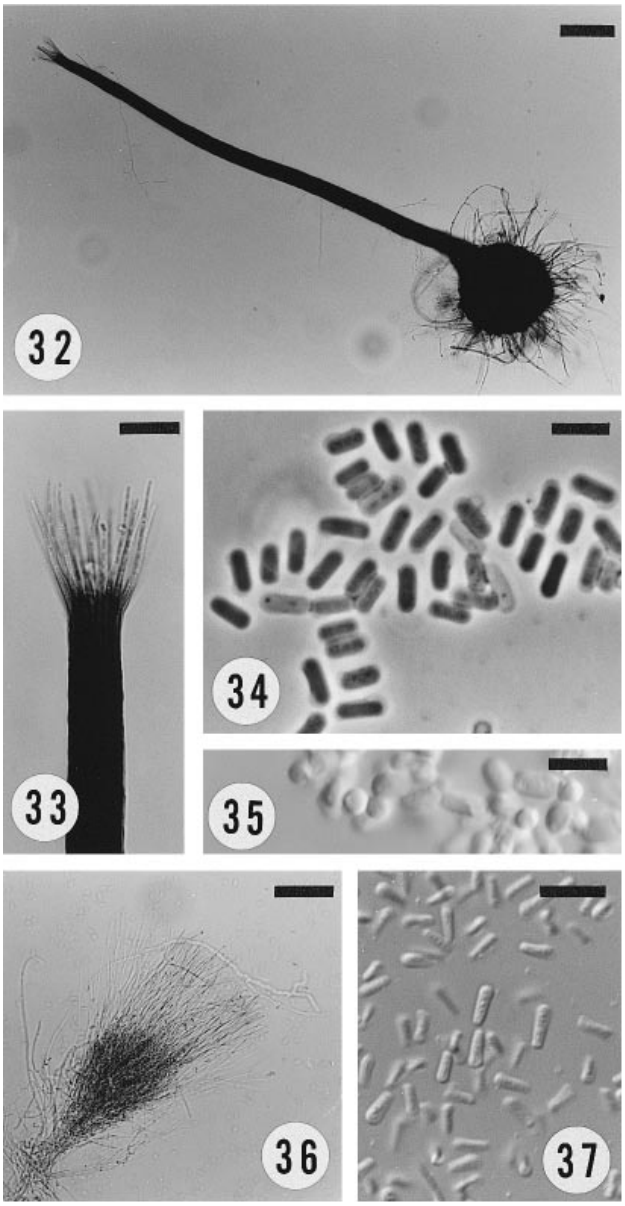
Living and dried cultures deposited:
Cultures: YCC-111 (JCM 9360), isolate from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY; YCC-242 (JCM 9361), isolate from a larval gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C22, dried culture YCC-111 grown on MAB at 20°; TSH-C24, dried culture YCC-242 grown on MAB at 20°.

Ophiostoma japonicum Yamaoka & M. J. Wingf. sp. nov. (Figs 32–37)

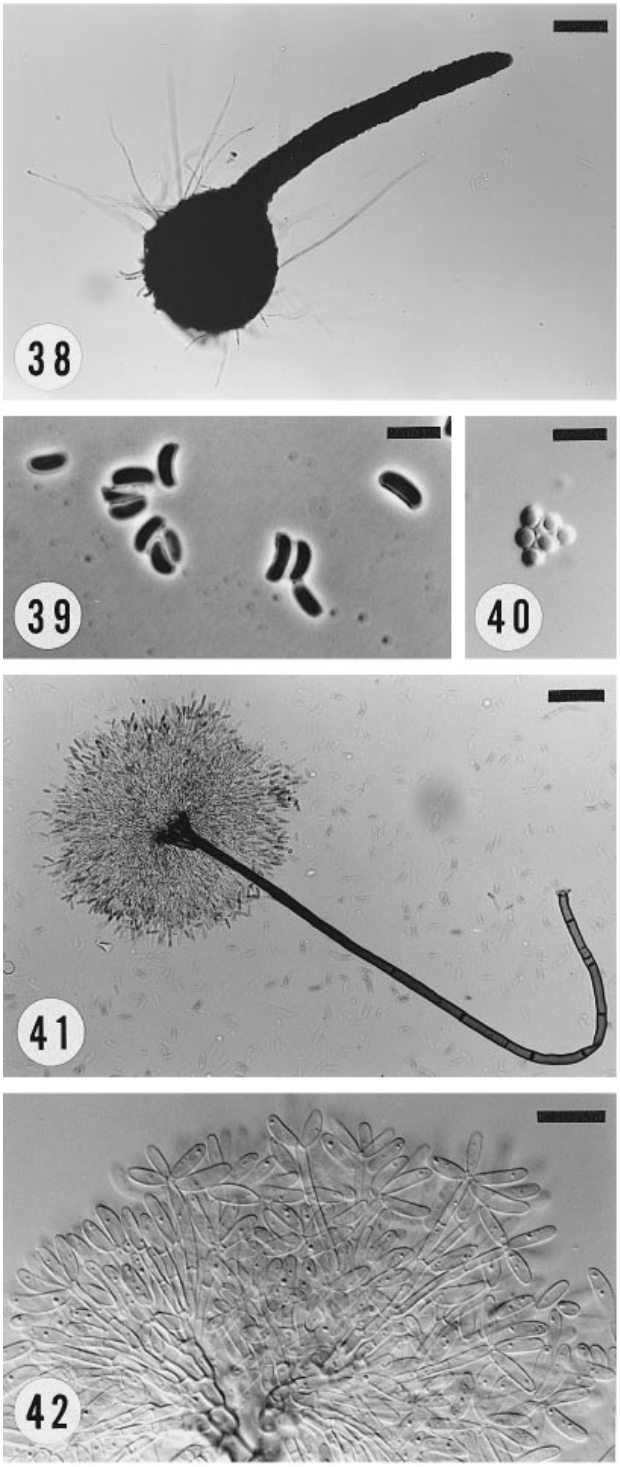
Perithecia superficialia vel partim inclusa in medio agari vel in cortice posita in medii superficie; pars basilaris atra, globosa vel subglobosa, 80–150 µm diametro, ornata appendicibus fuscis hyphalibusque; colla atra, recta vel curvata, gradatim attenuta, 450–800 µm longa hyphis ostiolaribus comprehensis, 25–45 µm lata ad basem, 9–16 µm lata ad apicem; hyphae ostiolares 5–10, hyalinae, septatae, divergentes, attenuatae ad apicem obtusum, 12–34 µm longae. *Ascosporae* hyalinae, unicellulares, oblongae, cylindricae vel ossiformes cum a latere vel a facie videntur, globosae cum ab extremo videntur, cinctae vagina angusta hyalina, 3·2–4·8 × 1·6–2·4 µm vagina comprehensa, se aggregantes in guttulam albam vel subflavescentem ad apicem collorum. *Coloniae* appressae, primum albae, pars quidem coloniae viridifuscens, nonnumquam aspectu nito propter conidia nascentia. Duo typi distincti conidiophororum adsunt. (1) *Conidiophora* synnematosa, alba vel cremicoloria, hyphae penicillate ramosae, vel sporodochis pulviformibus; *conidiogenae cellae* integratae, terminales, sympodicae, cylindricae, rectae. (2) *Conidiophora* macronematosa vel micronematosa, mononematosa, hyalina, septata, simplicia vel ramosa; *conidiogenae cellae* terminales, sympodicae. *Conidia* hyalina, unicellularia, cylindrica vel clavata, 3·5–6(–8) × 1·5–2·5 µm.

Holotypus: TNS-F-180381, dried culture YCC-99 (JCM 9366) grown on malt extract agar with pieces of autoclaved bark of *Picea jezoensis*, isolated from an egg gallery of *I. typographus japonicus* infesting *P. jezoensis* at Tokyo University Forest in Hokkaido. Collected on 10 Aug. 1989 by I. Takahashi, K. Iguchi and Y. Yamaoka. Holotype specimen was deposited in the Herbarium of the National Science Museum (TNS), Tsukuba, Japan.



Figs 32–37. *Ophiostoma japonicum*. **Fig. 32.** Ascocarp (bar, 70 µm). **Fig. 33.** Top of neck (bar, 20 µm). **Figs 34, 35.** Ascospores (bar, 5 µm). **Fig. 36.** *Graphilbum* state (bar, 50 µm). **Fig. 37.** Conidia (bar, 10 µm).

Perithecia (Fig. 32) superficial or partly embedded in the agar medium or in bark placed on the surface of the medium; basal part black, globose to subglobose, 80–150 µm diam., ornamented with brown hyphal appendages; necks black, straight or curved, gradually tapering, 450–800 µm long including ostiolar hyphae, 25–45 µm wide at the base, 9–16 µm wide at the tip; ostiolar hyphae (Fig. 33) 5–10, hyaline, septate, divergent, tapered to a blunt tip, 12–34 µm long. *Ascospores* (Figs 34, 35) hyaline, 1-celled, oblong, cylindrical or ossiform in side and face view, globose in end view, surrounded by a narrow hyaline sheath, 3·2–4·8 × 1·6–2·4 µm including sheath, aggregating in a white or pale yellow droplet at the tip of the necks. *Colonies* appressed,



Figs 38–42. *Ophiostoma penicillatum*. **Fig. 38.** Ascocarp (bar, 70 μm). **Figs 39, 40.** Ascospores (bar, 7 μm). **Fig. 41.** *Leptographium* state (bar, 50 μm). **Fig. 42.** Conidiogenous cells and conidia (bar, 15 μm).

white at first, at least part of the colony becoming greenish brown, sometimes with a glistening appearance due to the production of conidia. Two distinct types of conidiophores present. (1) *Conidiophores* (Fig. 36) synnematous, white to creamy white, hyphae penicillately branched, fitting the description of *Graphilbum* as defined by Upadhyay & Kendrick (1975) or cushion-shaped sporodochia; *conidiogenous cells* integrated, terminal, sympodial, cylindrical, straight. (2) *Conidiophores* macronematous or micronematous, mononematous, hyaline, septate, simple or branched; *conidiogenous cells* terminal, sympodial. *Conidia* (Fig. 37) hyaline 1-celled, cylindrical or clavate, 3·5–6(–8) × 1·5–2·5 μm.

Ophiostoma japonicum is morphologically similar to *O. arborea* (Olchow. & J. Reid) Yamaoka & M. J. Wingf. comb. nov. (= *C. arborea* Olchow. & J. Reid), which was isolated from black spruce (*Picea mariana* (Mill.) B.S.P.) in Canada (Olchowecki & Reid, 1974). Ascospores of this species are, however, surrounded by a narrow hyaline sheath, while those of *O. arborea* are surrounded by a more distinct uniform, hyaline sheath. Furthermore, *O. arborea* has only a *Hyalopesotum* state and the mononematous anamorph appears not to be present. In this study, we compared *O. japonicum* not only with the original description of *O. arborea*, but also with a culture of the type (WIN(M) 69-23) deposited by Olchowecki & Reid (1974). The fungi are clearly very similar to each other and differences in isolate age and cultural conditions might have obscured similarities or differences between them. At this stage we believe that it is possible to distinguish between the species but acknowledge that they deserve critical comparison, preferably at the molecular level.

Ophiostoma japonicum was isolated from adult beetles, immature beetles, larval galleries, and stained or discoloured sapwood. Frequency of occurrence from adult beetles was 21% (Table 1). From the other niches examined, it occurred in lower frequencies ranging from 0·4 to 10%.

Living and dried cultures deposited:
Cultures: YCC-99 (JCM 9366), isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 10 Aug. 1989 by IT, KI, and YY; YCC-102, isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 10 Aug. 1989 by IT, KI, and YY; YCC-134, isolate from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY.

Dried specimens: TNS-F-180381 (holotype) and TSH-C35, dried culture YCC-99 grown on MAB at 20°; TSH-C76, dried culture YCC-99 grown on PAB at 20°; TSH-C36, dried culture YCC-102 grown on MAB at 20°; TSH-C37, dried culture YCC-134 grown on MAB at 20°. TSH-C35, TSH-C36, TSH-C37, and TSH-C76 were deposited in the Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba (TSH) as paratypes.

Ophiostoma penicillatum (Grosmann) Siemaszko, *Planta Polonica* 7: 24, 1939. (Figs 38–42)

= *Ceratostomella penicillata* Grosmann, *Hedwigia* 72: 190, 1932.

= *Grosmannia penicillata* (Grosmann) Goid., *R. Staz. Pat. Veg. Bol., Rome*, n.s., 15: 156, 1935.

= *Ceratocystis penicillata* (Grosmann) C. Moreau, *Rev. Mycol. Suppl. Col.* 17: 22, 1952.

The anamorph and teleomorph states of this fungus were described as *Leptographium penicillatum* Grosmann (Grosmann, 1931) and *Ceratostomella penicillata* (Grosmann, 1932), respectively. The morphology of this species has been a relatively contentious issue in the past. Most of this confusion has been focused on the size and form of the ascospores with various authors reporting various sizes of spore and a lack of

agreement as to whether the ascospores are sheathed or not (Grosmann, 1932; Hunt, 1956; Davidson *et al*., 1967; Upadhyay, 1981). Some of this confusion most probably also resulted from mixtures of cultures of this species and the fungus we now know as *C. polonica* (Visser *et al*., 1995).

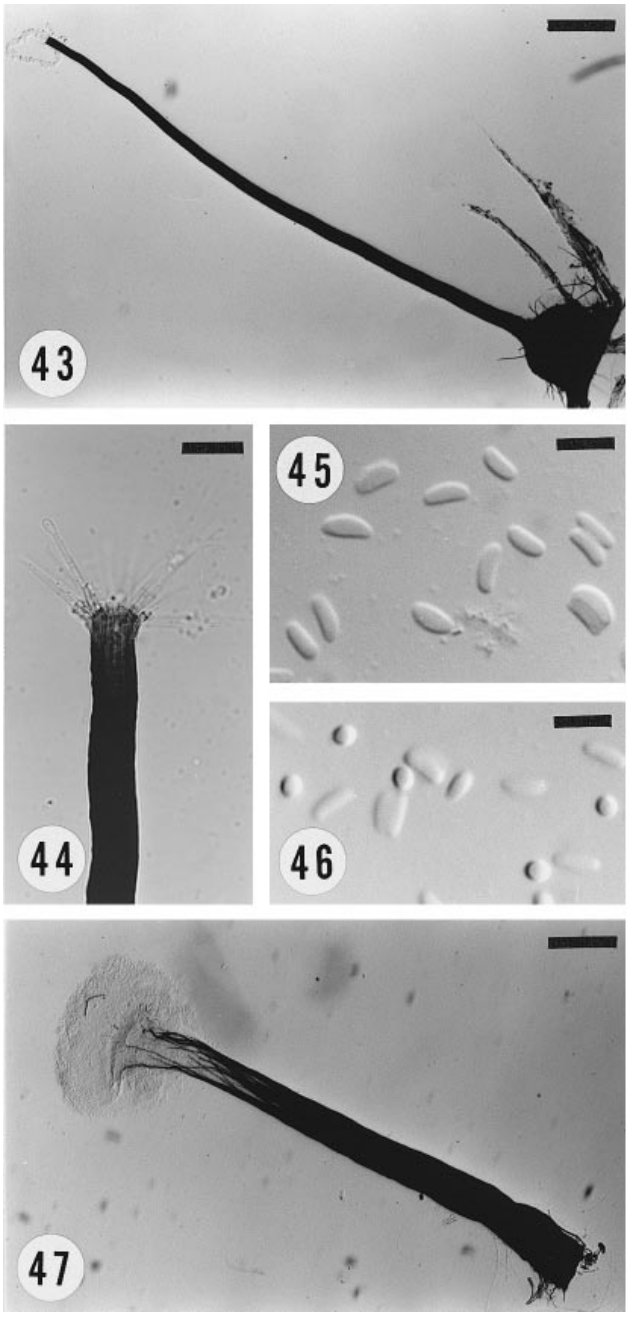
Solheim (1986) intensively studied cultures of *O. penicillatum* from Norway spruce infested with *I. typographus*. From this study he concluded that ascospores were allantoid in side view, cylindrical in face view and globose in end view, 4·5–6·5 × 1·6–2·3 µm and enclosed in distinct sheaths. Based on these observations and past confusion regarding the characteristics of this species, he also designated a neotype for the fungus.

In this study, our isolates of *O. penicillatum* fitted the descriptions of Davidson *et al*. (1967) and Solheim (1986) closely. Our isolates, however, had smaller perithecial bases (150–290 µm) and longer necks (up to 1200 µm) although these differences are not considered to be taxonomically important, and may have arisen due to differences in culture media utilized. This fungus was also reported by Aoshima (1965) with morphological characteristics very similar to ours and those of Davidson *et al*. (1967) and Solheim (1986).

Ophiostoma penicillatum was one of the most common fungi isolated in this study and is also known to be a common associate of *I. typographus* in Europe (Grosmann, 1932; Siemaszko, 1939; Mathiesen, 1950; Rennerfelt, 1950; Solheim, 1986, 1993; Harding, 1989; Furnis *et al*., 1990; Viiri & Weissenberg, 1995). The frequencies with which this fungus occurred ranged from 30·1 to 76·2% (Table 1). This was also the fungus most frequently isolated from stained wood with a frequency of occurrence of 30·1%. *Ophiostoma penicillatum* is a secondary invader of sapwood according to Solheim (1992*b*) trailing *ca* 13 mm behind *C. polonica* in a Norwegian study of sapwood invasion after attack of living Norway spruce (Solheim, 1992*a*).

Living and dried cultures deposited:
Cultures: YCC-107 (JCM 9362), isolate from a stained sapwood of *P. jezoensis* infested with *I. typographus japonicus* at TUFH. Collected on 21 June 1990 by IT, KI, and YY; YCC-240 (JCM 9363), isolate from a sapwood of *P. jezoensis* infested with *I. typographus japonicus* at TUFH. Collected on 31 July 1991 by IT, KI, and YY.

Dried specimens: TSH-C15, dried culture YCC-107 grown on MAB at 20°; TSH-C18, dried culture YCC-240 grown on MAB at 20°.



Figs 43–47. *Ophiostoma piceae*. **Fig. 43.** Ascocarp (bar, 150 µm). **Fig. 44.** Top of neck (bar, 20 µm). **Figs 45, 46.** Ascospores (bar, 5 µm). **Fig. 47.** *Graphium* state (bar, 150 µm).

Ophiostoma piceae (Münch) Syd. & P. Syd., *Ann. Mycol*. 17: 43, 1919. (Figs 43–47)

= *Ceratostomella piceae* Münch, *Naturw. Z. Land- u. Forstw*. 5: 547, 1907.

= *Ceratocystis piceae* (Münch) B. K. Bakshi, *Trans. Br. Mycol. Soc*. 33: 113, 1950.

Morphological characteristics of *O. piceae* isolated in this study are similar to those described by Hunt (1956) and Upadhyay (1981), except that the bases of perithecia were larger (up to 280 µm diam.). Brasier & Kirk (1993) separated *O. piceae* into two intersterile mating groups, one from hardwood sources (OPH) and the other from conifers (OPC). They suggested that isolates from conifers probably represents the original *O. piceae* = *Ceratostomella piceae sensu* Münch (1907), and that those from hardwood are the same as *O. querci* (Georgev.) Nannf. = *Ceratostomella quercus* Georgevitch (1927). In the original description, *O. querci* differed from *O. piceae* only in the colour reaction of the substrate (Georgevitch, 1927) and it was, therefore, treated as a synonym of *O. piceae* by subsequent authors (Hunt, 1956; Griffin, 1968; Olchowecki & Reid, 1974; Upadhyay, 1981). There is reasonable agreement that these two species are distinct, even based on morphology (Halmschlager *et al*., 1994), although mating experiments remain the only means to provide unequivocal identifications (Brasier & Kirk, 1993).

Detailed descriptions of Japanese isolates of *O. piceae* have been presented previously (Nishikado & Yamauti, 1935; Aoshima, 1965). This fungus was one of the most common fungi collected in this study. Frequencies of occurrence from egg galleries, larval galleries, adult beetles and immature beetles ranged from 30·3 to 89·5%, and those from stained or discoloured sapwood were 17·9% (Table 1). Aoshima (1965) also reported that *O. piceae* was one of the most common fungi associated with *I. typographus japonicus* and it is known to be a common associate of *I. typographus* in Europe (Mathiesen, 1950; Käärik, 1975; Solheim, 1986, 1993; Harding 1989; Viiri & Weissenberg, 1995). Solheim (1992*b*) found this fungus to be a tertiary invader of Norway spruce sapwood after *I. typographus* attack.

Living and dried cultures deposited:
Cultures: YCC-69 (JCM 9364), isolate from an egg gallery of *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 10 Aug. 1989 by IT, KI, and YY; YCC-120 (JCM 9365), isolate from an adult *I. typographus japonicus* in *P. jezoensis* at TUFH. Collected on 21 June 1990 by IT, KI, and YY.

Dried specimens: TSH-C29, dried culture YCC-69 grown on PAB at 20°; TSH-C33, dried culture YCC-120 grown on PAB at 20°.

Aoshima (1965) reported on 10 species of ophiostomatoid fungi associated with *I. typographus japonicus* based on isolations from 1679 adult beetles collected in 24 batches at different localities and in different seasons. These fungi included *O. clavatum* (probably the same as *O. ainoae* in this study), *O. bicolor*, *O. piceae*, *O. penicillatum*, *C. shikotsuensis nom. nud.* (probably the same as *O. europhioides* in this study), *C. jezoensis nom. nud.* (probably the same as *C. polonica* in this study), *C. coerulescens* (Münch) B. K. Bakshi, *O. albidum* Math.-Kaarik, *O. pluriannulata* (Hedgc.) Syd. & P. Syd., and *O. truncicola* Davids. The first six of these taxa are believed to have been collected in the present study and were isolated by Aoshima (1965) at frequencies of 28·8, 19·7, 18·2, 12·4, 5·5, and 3·0%, respectively. Frequency of occurrence of the remaining four species was less than 1% and this might explain why we did not collect them in our less intensive study. Aoshima (1965) also reported five species; *O. ainoae*, *O. bicolor*, *O. piceae*, *O. penicillatum*, and *O. europhioides* that were dominant in each batch of samples collected. The results of our study were similar, with *O. ainoae*, *O. bicolor*, *O. penicillatum*, and *O. piceae* as dominant species having frequencies of occurrence of greater than 56%. *Ophiostoma europhioides*, *O. japonicum*, *C. polonica* and *Ceratocystiopsis minuta* were less common with frequencies of occurrence ranging from 21 to 36%. *Ophiostoma cucullatum* was rare and occurred in only 5% of the isolations.

Siemaszko (1939) studied fungi associated with *I. typographus* in Poland, and found *O. penicillatum*, *O. polonicum* and *Graphium pycnocephalum* Grosmann to be the most common, and *O. piceae* and *C. minuta* less common associates of this insect. Mathiesen (1950) and Mathiesen-Käärik (1953) listed eight species of ophiostomatoid fungi; *O. penicillatum* and *O. piceae* were rather common and *O. minus*, *O. floccosum*, *O. albidum*, *O. pluriannulatum* and *O. polonicum* were more occasional. Of interest is the fact that she found *O. albidum* and *O. pluriannulatum* as Aoshima (1965) did in Japan. Käärik (1975) examined fungal succession in spruce killed by *I. typographus* in Sweden, and listed *O. penicillatum* and *O. piceae* in the first stage, and *O. ainoae* (= *C.* gr. *clavata*) (Solheim, 1986), *O. polonicum*, *O. bicolor*, *C. minuta* and *O. tetropii* Math.-Kaarik in the second stage of colonization in the late summer. Solheim (1986) isolated fungi from the sapwood of Norway spruce infested with *I. typographus*, and listed the following 10 species; *C. minuta*, *O. ainoae*, *O. bicolor*, *O. cucullatum*, *O. europhioides*, *O. flexuosum* H. Solheim, *O. penicillatum*, *O. piceae*, *O. polonicum*, and *O. tetropii*. Harding (1989), Solheim (1993) and Viiri & Weissenberg (1995) listed most of the same species associated with *I. typographus* in other studies. The ophiostomatoid fungi associated with *I. typographus japonicus* in Japan are, therefore, almost identical to those associated with *I. typographus* in Europe (Siemaszko, 1939; Käärik, 1975; Solheim, 1986, 1993; Harding, 1989; Viiri & Weissenberg, 1995). This may not be surprising given the fact that *I. typographus* occurring in Europe and *I. typographus japonicus* in Japan are very similar. We might, however, have expected to encounter some obvious differences based on the fact that the Japanese population of *I. typographus* has been isolated from that on the mainland for millennia. The minor differences between the mycobiota of the two insects could easily be explained by differences in sampling procedures and, in any case, these fungi represent the least common fungal associates.

Ceratocystis polonica is considered to be a virulent and important component of the mycobiota of *I. typographus* in Europe (Horntvedt *et al.*, 1983; Christiansen, 1985; Solheim, 1988; Christiansen & Solheim, 1990). It is most likely that the same situation is true in Japan, but pathogenicity tests with fungi associated with *I. typographus japonicus* in Japan have not yet been done. We have already initiated some pathogenicity tests with the fungi associated with this insect against Yezo spruce in Japan.

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