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FULL PAPER

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Constant association of ophiostomatoid fungi with the bark beetle *Ips subelongatus* invading Japanese larch logs

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Abstract *Ceratocystiopsis*, *Ceratocystis*, *Grosmannia*, and *Ophiostoma* species were isolated from *Ips subelongatus* and beetle-infested Japanese larch logs collected at several areas in central and northern Honshu Island, Japan, to determine constant associates of *I. subelongatus*. *Ceratocystiopsis minuta*, two species of *Ceratocystis*, three species of *Grosmannia*, and four species of *Ophiostoma* were isolated. Of the fungi isolated in the present study, *G. laricis*, *O. brunneociliatum*, and *O. piceae* were constant associates of the beetles. *Ceratocystis fujiensis*, *Ceratocystiopsis minuta*, and *Ophiostoma* sp. F were occasionally isolated with high frequencies of occurrence but were not consistent associates. *Ceratocystis fujiensis* was most often isolated as the leading fungal invasion in the sapwood of Japanese larch logs invaded by *I. subelongatus*, confirming that the fungus acts as a primary invader of sapwood in beetle-attacked logs.

Key words *Ceratocystis* species · *Grosmannia* species · *Ips subelongatus* · *Larix kaempferi* · *Ophiostoma* species

Introduction

Ips cembrae (Heer) infests trees and recently cut logs of European larch (*Larix decidua* Miller.) in Europe and also damages plantations in the United Kingdom (Crooke and Bevan 1957; Redfern et al. 1987). The eight-spined *Ips* beetle infesting Japanese larch (*L. kaempferi* [Lamb.] Carr.) in Japan has been considered to be *I. cembrae* (Nobuchi 1974; Koizumi 1990, 1994). However, Stauffer et al. (2001) recognized the beetle in Japan as a distinct species, *I. subelongatus* Motschulsky, based on morphological and molecular phylogenetic studies. In the present article, we use the name *I. subelongatus* for the eight-spined *Ips* beetle infesting Japanese larch.

Many ophiostomatoid fungi belonging to *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Ceratocystis* Ellis & Halst., *Grosmannia* Goid., and *Ophiostoma* Syd. & P. Syd. are associated with bark beetles infesting conifer trees (Francke-Grosmann 1963; Whitney 1982; Harrington 1993; Zipfel et al. 2006); these include fungi pathogenic to conifer trees (Harrington 1993; Kirisits 2004).

Ips subelongatus also associates with these groups of fungi. Aoshima (1965) first demonstrated the association of ophiostomatoid fungi with *I. subelongatus* (as *I. cembrae*) from samples collected in Hokkaido and Central to Northern Honshu, Japan. The associated fungi were *Ceratocystis coerulescens* (Münch) Bakshi, *C. jezoensis* nom. nud., *C. macrospora* nom. nud., *Grosmannia olivacea* (Mathiesen) Zipfel, Z.W. de Beer & M.J. Wingf. (as *Ophiostoma olivaceum* Mathiesen), *Ophiostoma brunneociliatum* Mathiesen-Käärik, and *O. piceae* (Münch) H. & P. Sydow. Yamaoka et al. (1998) also isolated six species of fungi from the galleries of *I. subelongatus* (*I. cembrae*) on Japanese larch logs in Central Honshu, Japan: they were *Ceratocystiopsis minuta* (Siem.) H.P. Upadhyay & W.B. Kendr. (abbreviated as *Cop. minuta*; = *O. minutum* Siem.), *Ceratocystis laricicola* Redfern & Minter, *O. brunneociliatum*, *O. piceae*, *Grosmannia laricis* (K. van der Westh., Yamaoka & M.J. Wingf.) Zipfel, Z.W. de Beer & M.J. Wingf. (= *O. laricis* K. van der Westh., Yamaoka & M.J. Wingf.), and *Ophiostoma* sp., which is referred to as *Grosmannia* sp. L1 in the present article. *Ceratocystis jezoensis* and *C. macrospora* reported by Aoshima (1965) were considered by Yamaoka et al. (1998) as *C. laricicola* and *G. laricis*, respectively. Marin et al. (2005) reported that Japanese isolates of *C. laricicola* were distinguishable based on DNA sequence comparisons

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from European isolates of *C. laricicola* and *C. polonica*, which are morphologically indistinguishable. The Japanese isolates of *C. laricicola* were treated as a distinct taxon, i.e., *C. fujiensis* M.J. Wingf., Yamaoka & Marin. Chung et al. (2006) added another species as an associate of *I. subelongatus*, *O. breviusculum* Chung, Yamaoka, Uzunovic & Kim, a new species of the *O. piceae* complex.

Some differences in species composition can be seen between the data presented by Aoshima (1965) and by Yamaoka et al. (1998). Aoshima (1965) indicated that the dominant species differed depending on the area, but *O. brunneociliatum* and *O. piceae* were isolated at relatively high frequencies. Yamaoka et al. (1998) and Chung et al. (2006) did not present data on the frequencies of occurrence of each fungus, so it is unclear which fungi were constant associates. Particularly, it is very important to know whether *C. fujiensis* is a constant associate of *I. subelongatus* because this fungus is considered as the most virulent against Japanese larch (Yamaoka et al. 1998).

Some bark beetles are known to possess mycangia to carry constant associates of fungi (Francke-Grosmann 1967; Whitney and Farris 1970). However, there was no report showing the presence of mycangia in *I. subelongatus*.

In the present study, we conducted isolation studies from *I. subelongatus* and beetle-infested Japanese larch logs collected in several areas in Japan and compared frequencies of occurrence of each fungus to determine the constant associates of *I. subelongatus*.

Materials and methods

Eight samples of Japanese larch logs or fallen trees invaded by *I. subelongatus* were collected from the field between 1999 and 2001 (Table 1). Logs left in the Japanese larch plantation, those piled in a log depot, and trees felled by strong winds were used. Most samples were collected in central Honshu (Nagano, Yamanashi, and Tochigi Prefectures) but one was from northern Honshu (Iwate Prefecture). Two to three logs (about 90 cm long) collected at the same time from the same stand were regarded as one sample and brought to the laboratory.

After the bark of the logs was peeled off, adult beetles in egg galleries were removed with sterile fine forceps and placed on the surface of 1% malt extract agar (1% MA; 10 g malt extract, 15 g agar/1000 ml distilled water) in 9-cm Petri dishes. Two small pieces of bark ($2 \times 2 \times 2$ mm) were taken from each egg gallery and pupal chamber and placed on the surface of the 1% MA. The Petri dishes were incubated in the dark at 17°C. Fungi growing on the plates were then purified by transferring small pieces of mycelium, conidium masses, or ascospore masses to fresh 2% MA plates. The process of purifying cultures was the same as described by Yamaoka et al. (1997).

To isolate fungi from sapwood under galleries, disks 10–20 cm thick were cut from the logs. The disks were cut with a surface-sterilized ax along the radius between a bark beetle gallery and the center of the heartwood. Small pieces of wood ($2 \times 2 \times 2$ mm) were taken from the freshly opened longitudinal section along the radius at several points from just underneath the gallery to the border between the sapwood and heartwood at about 5 mm intervals. Small pieces of wood were placed on the surface of the 1% MA in Petri dishes, and then the dishes were treated in the same manner as described above.

Cultures used for identification were grown on 2% MA, 2% malt extract Ebios agar [2% MEBA: 20 g malt extract, 1 g Ebios (Brewer's yeast preparation; Tanabe), 15 g agar/1000 ml distilled water], and 1% Pablum agar (PA: 10 g

Table 1. Beetle-infested Japanese larch logs used for isolation of ophiostomatoid fungi

Sample no.	Locality of collection (altitude, latitude, longitude)	Date of collection	Condition of samples	Growth stage of bark beetles
1	Experimental forests at Yatsugatake,[a] Kawakami, Nagano Pref. (1550 m, 35°55′00 N, 138°29′58 E)	May 12, 1999	Logs left in plantations after thinning in autumn 1998	Nuptial chambers and egg galleries with eggs
2	Sanada, Nagano Pref. (1130 m, 36°29′39 N, 138°21′19 E)	June 26, 1999	Logs left in plantations after thinning	Nuptial chambers and egg galleries with eggs
3	Sumida, Iwate Pref. (680 m, 39°13′ N, 141°36′E)	July 15, 1999	Logs placed in a larch forest in 1999	Pupal stage or immature beetles
4	Sudama, Yamanashi Pref. (1650 m, 35°49′50 N, 138°34′04 E)	Sept. 30, 1999	Logs collected from a log depot	Constructing galleries for overwintering
5	Experimental forests at Yatsugatake, Nagano Pref. (1550 m, 35°55′00 N, 138°29′58 E)	June 28, 2000	Logs placed in a larch forest on May 12, 2000	Larval stage
6	Experimental forests at Yatsugatake, Nagano Pref. (1525 m, 35°55′07 N, 138°29′59 E)	June 28, 2000	Logs left in plantations after thinning in autumn 1999	Pupal stage
7	Experimental forests at Nikko,[b] Nikko, Tochigi Pref. (1500 m, 36°47′29 N, 139°28′18 E)	July 5, 2001	Fallen tree with green needles	Pupal stage
8	Yumihari Pass, Nikko, Tochigi Pref. (1450 m, 36°46′32 N, 139°24′56 E)	July 5, 2001	Logs placed in a larch forest on November 1, 2000[c]	Pupal stage

[a] Experimental Forests at Yatsugatake, Agricultural and Forestry Research Center, University of Tsukuba
[b] Experimental Forests at Nikko, Utsunomiya University
[c] The logs were also invaded by *Dryocoetes baikalicus*

Table 2. Frequencies of occurrence (%) of ophiostomatoid fungi isolated from adult beetles and galleries of *Ips subelongatus*

Species	Sample number															
	No. 1	No. 2		No. 3			No. 4		No. 5		No. 6		No. 7		No. 8	
	AB* ($n = 40$)	AB ($n = 20$)	EG ($n = 10$)	AB ($n = 41$)	EG ($n = 24$)	PC ($n = 22$)	AB ($n = 22$)	EG ($n = 20$)	AB ($n = 10$)	EG ($n = 10$)	EG ($n = 10$)	PC ($n = 10$)	AB ($n = 22$)	EG ($n = 41$)	PC ($n = 12$)	EG ($n = 15$)	PC ($n = 38$)
Ceratocystiopsis minuta	27.5	20.0		7.3	4.2								40.9	7.3			
Ceratocystis fujiensis		10.0	40.0						10.0	10.0	30.0			9.8			2.6
Grosmannia laricis	52.5	20.0	70.0	61.0	29.1	59.1	40.9	45.0	80.0	50.0	80.0	70.0	13.6	63.4	66.7	53.3	57.9
Grosmannia sp. L2	17.5															6.7	10.5
Ophiostoma brunneociliatum	45.0	80.0	40.0	12.2	8.3	13.6	22.7	25.0	40.0	90.0	50.0	50.0	4.5	41.5	41.7	66.7	23.7
O. floccosum	2.5												13.6	2.4			2.6
O. piceae	72.5	100	90.0	80.5	20.8	45.5	27.3	35.0	80.0	90.0	80.0	50.0	95.5	90.2	75.0	60.0	65.8
Ophiostoma sp. F	32.5	10.0		7.3	4.2	9.1	50.0	55.0				10.0	9.1	7.3	33.3	46.7	18.4
Ophiostoma cf. *breviusculum*	10.0															6.7	10.5

AB, adult beetles in egg galleries; EG, walls of egg galleries; PC, wall of pupal chambers
F = (NF/NT) × 100 where F represents the frequency of occurrence (%) of the fungus, NT represents the total number of galleries from which isolations were made, and NF represents the number of galleries from which a particular fungus was isolated

Pablum mixed cereal, 15 g agar/1000 ml distilled water). Small (about 1 cm × 5 mm × 3 mm) pieces of autoclaved bark from Japanese larch were later added to the plates to stimulate ascocarp production. Ascocarps and conidiophores were mounted on glass slides in 1% lacto-fuchsin or polyvinyl alcohol (Omar et al. 1979) and observed with an Olympus BHS-N Nomarski interference contrast microscope.

Frequency of occurrence of each species of fungi from beetles in galleries, egg galleries, and pupal chambers was 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. Frequencies of occurrence of each fungal species from sapwood under the galleries were also determined; NT represents the total number of radii from which isolations were made and NF represents the number of radii from which a particular fungus was isolated.

To indicate constant association of a certain fungus with a beetle, presence was calculated using the following formula:

$$\text{Presence (\%)} = (NFS/NTS) \times 100$$

where NTS represents the total number of samples from which isolates were obtained and NFS represents the number of samples from which a particular fungus was isolated.

Results

Ten species belonging to four genera of ophiostomatoid fungi were isolated from eight samples of *I. subelongatus* and Japanese larch invaded by the beetle. The species isolated were *Ceratocystiopsis minuta*, *Ceratocystis coerulescens*, *C. fujiensis*, *Grosmannia laricis*, *Grosmannia* sp. L2, *Ophiostoma* cf. *breviusculum*, *O. brunneociliatum*, *O. piceae*, *O. floccosum* Mathiesen, and *Ophiostoma* sp. F.

Fungi isolated from adult beetles in egg galleries and from the walls of egg galleries and pupal chambers were almost the same (Table 2). Three species, *G. laricis*, *O. brunneociliatum*, and *O. piceae*, were isolated from all eight samples at relatively high frequencies. *Ophiostoma* sp. F was isolated from seven of eight samples, but frequencies of occurrence of this fungus were relatively low except for samples no. 4 and no. 8. *Ceratocystis fujiensis* was isolated from four of eight samples at low frequencies except for sample no. 4. *Ceratocystiopsis minuta* was also isolated from four of eight samples at low frequencies except for sample no. 7. *Ophiostoma floccosum*, *Ophiostoma* cf. *breviusculum*, and *Grosmannia* sp. L2 were isolated from only one or two samples at low frequencies.

From the sapwood of all four samples, three ophiostomatoid fungi, *G. laricis O. brunneociliatum*, and *O. piceae*,

Table 3. Frequencies of occurrence (%) of ophiostomatoid fungi isolated from the sapwood under galleries of *Ips subelongatus*

Species	Sample number			
	No. 5 (n = 33)	No. 6 (n = 29)	No. 7 (n = 38)	No. 8 (n = 20)
Ceratocystis coerulescens			10.5	20.0
C. fujiensis	75.8	62.1	57.9	
Grosmannia laricis	33.3	31.0	7.9	50.0
Grosmannia sp. L2				5.0
Ophiostoma brunneociliatum	42.4	27.6	31.6	30.0
O. floccosum				5.0
O. piceae	60.6	55.2	89.5	100
Ophiostoma sp. F		10.3	5.3	10.0
Ophiostoma cf. *breviusculum*				10.0

F = (NF/NT) × 100 where F represents the frequency of occurrence (%) of the fungus, NT represents the total number of radii from which isolations were made, and NF represents the number of radii from which a particular fungus was isolated

Table 4. Frequencies of occurrence (%) of ophiostomatoid fungi isolated at the deepest sampling point in each radius of sapwood under galleries of *Ips subelongatus*

Species	Sample number			
	No. 5 (n = 24)	No. 6 (n = 13)	No. 7 (n = 22)	No. 8 (n = 13)
Ceratocystis coerulescens			13.6	30.8
C. fujiensis	70.8	76.9	59.1	
Grosmannia laricis	8.3			7.7
Ophiostoma brunneociliatum	16.7	15.4	9.1	23.1
O. floccosum				7.7
O. piceae	20.8	53.8	31.8	84.6
Ophiostoma sp. F			9.1	
Ophiostoma cf. *breviusculum*				15.4

were again isolated at relatively high frequencies (Table 3). In contrast to isolations from adult beetles and galleries, *Ceratocystis fujiensis* occurred on sapwood in three of four samples at high frequencies. *Ceratocystis coerulescens* was isolated from two samples, but frequencies of occurrence were low. The remaining species, *Grosmannia* sp. L2, *O. floccosum*, *Ophiostoma* sp. F, and *Ophiostoma* cf. *breviusculum*, were isolated at low frequencies. *Ceratocystiopsis minuta* was not isolated from the sapwood.

Ceratocystis fujiensis was isolated most frequently at the deepest sampling point in each radius of sapwood under the galleries in all three samples with fungi present (Table 4). *Ophiostoma piceae* showed the second highest frequency. In sample no. 8, from which *C. fujiensis* was not isolated, *O. piceae* was isolated most frequently at the deepest sampling point, followed by *Ceratocystis coerulescens* and *O. brunneociliatum*.

Discussion

Before the present study, one species of *Ceratocystiopsis* (*Cop. minuta*), two species of *Ceratocystis* (*Ceratocystis coerulescens* and *C. fujiensis*), three species of *Grosmannia* (*G. laricis*, *G. olivacea*, and *Grosmannia* sp. L1), and three species of *Ophiostoma* (*O. breviusculum*, *O. brunneociliatum*, and *O. piceae*) were reported to be associated with *I. subelongatus* in Japan (Aoshima 1965; Yamaoka et al. 1998; Chung et al. 2006). In the present study, all these species except *G. olivacea* and *Grosmannia* sp. L1 were isolated. In addition, three more species (*Ophiostoma* sp. F, *O. floccosum*, and *Grosmannia* sp. L2) were isolated in this study.

Table 5 shows the average frequencies of species from each niche and the presence (%) of the species in eight samples. Three species, *G. laricis*, *O. brunneociliatum*, and *O. piceae*, showed 100% presence with relatively high frequencies of occurrence in all the niches used for isolation. These data indicate that the three species are constant associates of *I. subelongatus* in Japan regardless of the sampling sites or growth stage of the bark beetle.

Grosmannia laricis has so far only been recorded from Japan and is not associated with *I. cembrae* in Europe (van der Westhuizen et al. 1995; Stauffer et al. 2001). *Grosmannia laricis* has been isolated from other bark beetles (*Dryocoetes baikalicus* Reitter and *D. hectographus* Reitter) invading Japanese larch (unpublished data). This fungus is considered to have a strong association with Japanese larch and the bark beetles invading Japanese larch.

Ophiostoma brunneociliatum was first described from Sweden, where it is associated with the bark beetle *I. sexdentatus* Boern on pine (Mathiesen-Käärik 1953). Later, the fungus was shown to be associated with *I. cembrae* on larch in Europe (Stauffer et al. 2001). Aoshima (1965) reported that *O. brunneociliatum* was a dominant fungus associated with bark beetles attacking Japanese larch, including *I. subelongatus* (as *I. cembrae*). There is no report on isolation of this fungus from pine trees in Japan, but *O. brunneociliatum*

Table 5. Average of frequency of occurrence and presence (%) of ophiostomatoid fungi isolated from adult beetles of *Ips subelongatus* in egg galleries

Species	Average of frequency of occurrence (%)				Presence (%) $n = 8$
	AB	EG	PC	Sap	
Ophiostoma piceae	76.0	66.6	59.1	76.3	100
Grosmannia laricis	44.7	55.8	63.4	30.5	100
O. brunneociliatum	34.1	45.9	32.3	32.9	100
Ophiostoma sp. F	21.8	28.3	17.7	8.5	87.5
Ceratocystis fujiensis	10.0	22.5	0	65.3	50.0
Ceratocystiopsis minuta	23.9	5.8	0	0	50.0
O. floccosum	8.1	2.4	2.6	5.0	37.5
Grosmannia sp. L2	17.5	6.7	10.5	5.0	25.0
Ophiostoma cf. *breviusculum*	10.0	6.7	10.5	10.0	25.0
C. coerulescens	0	0	0	15.3	25.0

Presence (%) = (NFS/NTS) × 100 where NTS represents the total number of samples from which isolations were obtained and NFS represents the number of samples from which a particular fungus was isolated

seems to have a strong association with conifer trees belonging to the genus *Larix* and the related bark beetles.

In contrast to *G. laricis* and *O brunneociliatum*, *O. piceae* is one of the most common ophiostomatoid fungi associated with conifers in Japan. This fungus has been isolated from Yezo spruce (Aoshima 1965; Tochinai and Sakamoto 1934; Yamaoka et al. 1997), Japanese red pine (*Pinus densiflora* Sieb. & Zucc.) (Nisikado and Yamauti 1935; Aoshima 1965), and various other conifers and hardwoods (Otani 1988). Aoshima (1965) also indicated that the fungus was one of the dominant fungi associated with bark beetles attacking Japanese larch, including *I. subelongatus*. The fungus has a global distribution, occurring on various conifer hosts (Harrington et al. 2001; de Beer et al. 2003).

Ceratocystis fujiensis, *Cop. minuta*, and *Ophiostoma* sp. F were occasionally present in high frequencies on some samples but absent from others. Averages for frequency of occurrence of the fungi were relatively low, and presences of the fungi were between 50.0% and 87.5% (Table 5). The reason why these fungi are not consistently isolated from the beetles remains uncertain. Isolation time after beetle attack might be one of the factors affecting the results. *Ceratocystis fujiensis* seems to be isolated more frequently from sapwood than other sources in the early stages of beetle development. On the other hand, *Ophiostoma* sp. F is more often isolated from samples in the late stages. These two fungi cannot be regarded as constant associates of the beetle based on the present results, but they appear to have some associations with *I. subelongatus*.

Ophiostoma sp. F is morphologically very similar to *O. abieticola* Yamaoka & Masuya isolated from *Dryocoetes* spp. in *Abies mariesii.* Both species possess orange section-shaped ascospores, perithecia lacking ostiolar hyphae, and a *Pesotum* anamorph without *Sporothrix* synanamorph (Yamaoka et al. 2004). However, *Ophiostoma* sp. F generally produces larger perithecia (perithecial base, 143–254 µm in diameter; neck, 438–1715 µm long) and synnemata (318–913 µm long) than *O. abieticola* (Yamaoka et al. 2001), and thus were morphologically distinguishable. However, isolates that produced teleomorphs were rare. Taxonomic treatment of the fungus will be reported in another paper after more detailed studies.

Ophiostoma sp. F was isolated from all the samples except for sample no. 5, but frequencies of the fungus were relatively low. This fungus often produces synnematous anamorphs in culture but rarely produces perithecia. In the present study, this fungus often occurred together with other *Ophiostoma* species having synnematous anamorphs, such as *O. piceae*. We suspect the frequencies of occurrence of the fungus from beetles, galleries, and sapwood in the present study were underestimated because we may have made an oversight.

The remaining fungi, *C. coerulescens*, *O. floccosum*, *Ophiostoma* cf. *breviusculum*, and *Grosmannia* sp. L2, showed low values of presence and averages of frequencies of occurrence (see Table 5). Thus, they are considered opportunistic associates or fungi that may be carried by other beetles infesting the same logs or infected through wounds. Sample no. 8, from which *Ophiostoma* cf. *breviusculum* and *Grosmannia* sp. L2 were isolated, was invaded not only by *I. subelongatus* but also by *Dryocoetes baikalicus*. Frequencies of occurrence of these fungi from adult beetles and egg galleries of *D. baikalicus* were higher than those of *I. subelongatus* (data not shown). *Grosmannia olivacea* also appears to be an opportunistic associate, because the fungus was isolated from only two of seven populations by Aoshima (1965) and was not found in the present study.

Ceratocystis coerulescens was first described by Münch (1907) as *Endoconidiophora coerulescens*. Harrington et al. (1996) found that there were up to five morphological variants of *C. coerulescens* isolates from conifers, and isozyme variations in the isolates supported distinguishing them as distinct taxa. Analysis of the DNA sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Witthuhn et al. 1998) and the *MAT*-2 idiomorph (Witthuhn et al. 2000; Harrington et al. 2002) also supported this separation. Five taxa are now recognized as distinct species: i.e., *C. rufipennis* Wingfield, Harrington & Solheim, *C. douglasii* (Davidson) Wingfield et Harrington (Wingfield

et al. 1997), *C. pinicola* Harrington & Wingfield, *C. resinifera* Harrington & Wingfield, and *C. coerulescens* sensu stricto (Harrington and Wingfield 1998). Morphological characteristics of the fungus *C. coerulescens* in the present study were based on the description of *C. coerulescens* s.s. presented by Harrington and Wingfield (1998), although the perithecial sizes of the Japanese fungus were larger than the description. Harrington (unpublished data) analyzed sequence data of the ITS region of rDNA obtained from the Japanese isolates of *C. coerulescens* and confirmed the fungus was conspecific to *C. coerulescens* s.s. Aoshima (1965) also reported that *C. coerulescens* was isolated from *I. subelongatus*. His morphological description fits well with our data. It has not yet been confirmed whether the other *Ceratocystis* species formerly regarded as *C. coerulescens* sensu lato (s.l.) are present in Japan. *Ceratocystis coerulescens* s.s. is considered a species more common as a blue-stain fungus on dead wood or cut timber than as a wound colonizer of living trees (Harrington et al. 1996; Harrington and Wingfield 1998). This fungus was isolated from the cutting face of Japanese larch logs left in the forest after thinning in Nagano Prefecture, Japan (unpublished data).

Ophiostoma floccosum was first described by Mathiesen (1951). Later, it was considered as a synonym of *O. piceae* (Upadhyay 1981). However, Harrington et al. (2001) demonstrated that *O. floccosum* can be distinguished from the other species of the *O. piceae* complex, including *O. piceae*, by morphological and cultural characteristics and sequencing of rDNA. *Ophiostoma floccosum* has been isolated from Pinaceae in Europe, North America, South Africa, Australia, and New Zealand (Harrington et al. 2001; de Beer et al. 2003). In Japan, this fungus was recorded from *Polygraphus proximus* Blandford attacking *Abies sachalinensis* (Fr. Schmidt) Masters in Hokkaido (Aoshima 1965). This is the first record of isolation of the fungus from Japanese larch.

The *Ophiostoma* cf. *breviusculum* isolates were morphologically indistinguishable from *O. breviusculum* (Chung et al. 2006). *Ophiostoma breviusculum* has been reported from *Dryocoetes baikalicus* Reitter and from *I. subelongatus* in Japanese larch collected in Central Honshu, Japan. *Ophiostoma breviusculum* in this study was also ecologically indistinguishable from the isolates used in the studies by Chung et al. (2006). However, we confirmed with mating studies that these isolates belonged to two distinct mating populations. Details of the mating groups will be reported in a separate paper.

Grosmannia sp. L2 produced black perithecia with a long neck lacking ostiolar hyphae at the tip, cucullate ascospores, and a *Leptographium* anamorph. A similar species, *Grosmannia* sp. L1, was reported in a previous paper (Yamaoka et al. 1998), but *Grosmannia* sp. L2 formed smaller perithecia than the other species, and morphological characteristics of the *Leptographium* state were also different. Perithecial characteristics of *Grosmannia* sp. L2 fit well with descriptions of *G. europhioides* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf. (= *O. europhioides*) (Wright and Cain 1961; Davidson et al. 1967). Both fungi have ascospores surrounded by hyaline walls, and appear cucullate in side view, quadrangular in face view, and triangular in end view. However, ascospores of the present fungus were narrower and had more distinct, sharp brims in side view than those reported for *G. europhioides*. The fungus also produced longer conidiophores than those typical of *G. europhioides*. *Grosmannia europhioides* was treated as a synonym of *G. piceiperda* (Rumbold) Goid. (Upadhyay 1981; Jacobs et al. 2000; Jacobs and Wingfield 2001), but Hausner et al. (2000) showed that they are distinct. *Grosmannia* sp. L2 certainly belongs to the *G. piceiperda* complex, but further examination is required to determine the taxonomic treatment.

Of the fungi associated with *Ips typographus* infesting Norway spruce, *Ceratocystis polonica* is considered to be the most active invader of the sapwood based on inoculation experiments (Solheim 1988). The fungus is always isolated from the leading edge of the fungal invasion in sapwood of Norway spruce infested by *I. typographus* (Solheim 1986, 1992a,b). The ability of fungi to act as a primary invader of sapwood is regarded as an important characteristic related to tree mortality (Solheim 1988, 1992a). Yamaoka et al. (1998) found that *C. fujiensis* (as *C. laricicola*) was able to invade the sapwood and kill Japanese larch trees. *Ceratocystis fujiensis* is suspected to play a significant role in the death of beetle-attacked trees, but behavior of the fungus in sapwood of the beetle-attacked trees or logs is not understood. In the present study, *C. fujiensis* was most often isolated from the leading edge of the fungal invasion of Japanese larch log sapwood invaded by *I. subelongatus*. We confirmed that the fungus acts as a primary invader of sapwood in the beetle-attacked logs. However, *C. fujiensis* may not always play this important role in the trees after a beetle attack because the fungus seems not to be a constant associate of the beetle.

The bark beetles *I. cembrae* and *I. subelongatus* are closely related, based on molecular phylogenetic data (Stauffer et al. 2001). *Ceratocystis laricicola*, two species of *Ceratocystiopsis*, three species of *Ophiostoma*, *Grosmannia* sp. (= *Ophiostoma*), and *Graphium* sp. are known as fungi associated with *I. cembrae* in Europe (Stauffer et al. 2001). Aghayeva et al. (2004) reported that two more species, *O. fusiforme* D.N. Aghayeva & M.J. Wingfield and *O. lunatum* D.N. Aghayeva & M.J. Wingfield, were associated with *I. cembrae* in Europe. *Ceratocystis laricicola*, *O. brunneociliatum*, and *Graphium* sp. are dominant in Europe. *Ceratocystiopsis minuta*, *O. brunneociliatum*, *O. piceae*, and *Grosmannia* sp. are common associates of both European *I. cembrae* and Japanese *I. subelongatus*. *Ophiostoma brunneociliatum* is the only consistent associate of both European *I. cembrae* and Japanese *I. subelongatus*. Thus, the dominant fungi associated with these two *Ips* species appear to be different. This finding contrasts with the fungi associated with *I. typographus* in Europe and *I. typographus japonicus* in Japan, which are almost identical (Solheim 1986; Yamaoka et al. 1997).

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