SHORT COMMUNICATION

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Fungi on submerged wood in the Koito River, Japan

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Abstract Woody substrates were collected from the Koito River in Japan, and the biodiversity of fungi on these substrates was investigated. Twenty-eight species were identified, comprising 12 ascomycete and 16 anamorphic taxa. The common fungi included *Chaetosphaeria* sp., *Ophioceras commune, Pseudohalonectria lignicola*, and *Savoryella lignicola*. The occurrence of fungi on submerged wood is discussed, and three interesting taxa – *Pseudohalonectria lignicola*, Bactrodesmium arnaudii, and B. pallidum – are described and illustrated.

Key words Biodiversity · Ecology · Freshwater · Lignicolous · Streams · Wood

Decaying wood is an essential component of stream ecosystems. Branches and twigs regulate stream dynamics by increasing retention of organic matter, and provide habitats for a variety of organisms, including fungi, insects, and fish (Triska and Cromack 1980). Decomposition of these woody substrates is important in nutrient cycling, and the rate of wood decay is determined by both physical and biological factors (Harmon et al. 1986). The role of freshwater fungi in the decomposition of organic matter is vital because they are capable of producing enzymes that break down lignocellulose (Wong et al. 1998). The nutrients and energy locked up within wood are therefore released, and these can be used at higher trophic levels (Graça 1993).

Biodiversity studies of the ascomycetes and anamorphic fungi on submerged wood in river ecosystems have been

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Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan carried out worldwide (Hyde and Goh 1997, 1999; Goh and Hyde 1999; Shearer and von Bodman 1983; Shearer and Crane 1986), and many species are new to science (Tsui et al. 2001, 2002). There are geographic (Shearer 1993), regional (Ho et al. 2001), and longitudinal (Tsui et al. 2001) variations in species composition between different rivers and along a river system. It has been suggested that the variations in fungal communities are related to sampling strategies and environmental factors, such as temperature and riparian vegetation (Wong et al. 1998). As there have been few studies of fungi on submerged wood in Japan (Abdullah et al. 1986; Minoura and Muroi 1978), we carried out a survey to investigate the occurrence of ascomycetes and anamorphic fungi on submerged wood in Japan.

Materials and methods

Forty woody substrates were collected from the Koito River, Chiba, Japan (latitude $35^{\circ}10'$ N, longitude $140^{\circ}0'$ E) on March 4, 2002. The river was characterized by well-oxygenated, soft, and slightly alkaline water (Table 1). Water temperatures ranged between 5° and 18° C during the year, and this range indicated the temperate climate of the river.

The samples ranged from twigs (~1 cm in diameter \times 30 cm long) to branches (6 cm in diameter \times 30 cm long). All samples were placed in plastic bags in the field, taken to the laboratory, and then incubated in plastic boxes (25 \times 10 \times 10 cm) lined with moistened tissue paper at 24°C to promote fruiting of ascomycetes and anamorphic fungi. Woody substrates were examined under a dissecting microscope (50 \times) for fruiting bodies (appearance of ascomata on the wood surface and the conidia from anamorphic fungi) on day 7 and periodically over the next 60 days. Any fungi present were recorded, identified, and isolated following Hyde and Goh (1999). The number of species, the number of occurrence of each species were recorded and calculated. Frequency of occurrence was calculated as the number of wood samples

on which a particular fungal species occurred divided by the number of wood samples examined, expressed as a percentage for each species.

Results and discussion

Of 40 woody substrates, 30 were colonized with fungi. A total of 28 species were recorded on wood in the Koito River, comprising 12 ascomycetes and 16 anamorphic fungi (Table 2). The species most frequently occurring were *Chaetosphaeria* sp., *Pseudohalonectria lignicola*, *Ophioceras commune*, and *Spirosphaera lignicola*, which occurred three to four times within 40 samples. However,

Table 1. Range in physicochemical characteristics of the Koito River,

 Chiba, Japan in 2001

Temperature (°C)	5–18
pH	7.8–8.1
DO (mg/l)	8.8–13
BOD (mg/l)	0.7–1.8
Suspended solid (mg/l)	0.5–2.6
COD Cr (mg/l)	3.7–9.8
NO ₂ -N (mg/l)	<0.01
NO ₃ -N (mg/l)	0.12–0.4
PO₄-P (mg/l)	<0.05
Cl ⁻ (mg/l)	163.3
HCO ₃ ⁻ (mg/l)	290

70% of the species were only recorded once in this investigation (see Table 2).

Several studies have investigated the fungi on submerged wood in temperate freshwater habitats (Hvde and Goh 1999; Lamore and Goos 1978; Révay and Gönczöl 1990; Shearer and von Bodman 1983). Although 25 and 59 species were recorded from the River Coln, England (Hyde and Goh 1999) and Rhode Island (Lamore and Goos 1978), respectively, none of the species overlapped with those reported here from the Koito River, Chiba. Pseudohalonectria lignicola was the only taxon in common with both the Koito River and a stream in Hungary (Révay and Gönczöl 1990). Also, only 2 species, Pseudohalonectria lignicola, and Savoryella lignicola, were in common with the 33 ascomycetes recorded from an Illinois stream (Shearer and von Bodman 1983). These results indicated that the fungal communities on submerged wood in freshwater habitats vary in different temperate countries. Differences in water chemistry and temperature are more important than geographic factors in determining the variations (Bärlocher 1992). Wood-Eggenschwiler and Bärlocher (1983) also found that those streams with contrasting water chemistry had different aquatic hyphomycete communities. Temperature and pH are considered crucial in governing the variations in aquatic hyphomycete communities (Wood-Eggenschwiler and Bärlocher 1985). However, the results were further interpreted cautiously because the species composition in this investigation represented only a single collection of data in a river in Japan. Also, direct compari-

Table 2. Fungi occurring on submerged wood in the Koito River, Japan

Species	Number of occurrences	Frequency of occurrence
Ascomycetes		
Annulatascus velatisporus K.D. Hyde	1	0.025
Chaetosphaeria sp.	3	0.075
Clohiesia corticola K.D. Hyde	1	0.025
Cloheisia sp.	1	0.025
Kirschsteiniothelia elasterascus Shearer	2	0.05
Massarina purpurascens K.D. Hyde	2	0.05
Ophioceras commune Shearer	4	0.1
Ophioceras dolichostomum (Berk. & Curt.) Sacc.	1	0.025
Pseudohalonectria lignicola Minoura & Muroi	3	0.075
Pseudoproboscispora aquatica S.W. Wong & K.D. Hyde	1	0.025
Savoryella lignicola E.B.G. Jones & R.A. Eaton	2	0.05
Unidentified ascomycetes	1	0.025
Anamorphic fungi		
Acrogenospora subprolata Goh, K.D. Hyde & K.M. Tsui	1	0.025
Bactrodesmium arnaudii S. Hughes	1	0.025
Bactrodesmium pallidum M.B. Ellis	2	0.050
Candelabrum brocchiatum Tubaki	1	0.025
Ellisembia opaca (Cooke & Harkn.) Subram.	1	0.025
Helicomyces torquatus Lane & Shearer	1	0.025
Helicosporium abuense Chouhan & Panwar	1	0.025
Helicosporium sp.	1	0.025
<i>Gilmaniella</i> sp.	1	0.025
Selenosporella curvispora G. Arnaud ex MacGarvie	1	0.025
Paradendryphiopsis cambrensis M.B. Ellis	1	0.025
Phaeoisaria clematidis (Fuckel) S. Hughes	1	0.025
Spirosphaera lignicola Abdullah, Gené & Guarro	3	0.075
Sporoschisma saccardoi E.W. Mason & S. Hughes	1	0.025
Unidentified coelomycetes species	1	0.025

son of biodiversity data is difficult because of different sampling strategies.

Interspecific interaction has been considered crucial in determining the distribution of fungi (Yuen et al. 1999b). In this study, woody substrates colonized by P. lignicola had no other fungi recorded (Tsui, unpublished data). This finding may support the inhibitory and competitive activities of P. lignicola reported in other investigations (Asthana and Shearer 1990; Yuen et al. 1999b). Pseudohalonectria spp., particularly P. lignicola, are inhibitory to other freshwater fungi (Asthana and Shearer 1990), with good evidence from both cultural and field competition studies (Asthana and Shearer 1990). In addition, species of Chaetosphaeria, Pseudohalonectria, and Ophioceras were found to be competitive in defending the colonized substrata against invading species when precolonized wood blocks were submerged in a river for 3 months and then retrieved (Fryar et al. 2001). Thus, field and cultural studies may be carried out to investigate if competition accounts for the relatively high occurrence of Chaetosphaeria, Ophioceras, and Pseudohalonectria species in the Koito River.

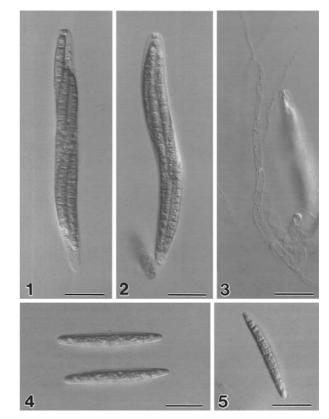
The fungal communities in the Koito River shared some similarities to those tropical and subtropical freshwater habitats (Ho et al. 2001) in the occurrence of Annulatascus velatisporus, Clohiesia corticola, Massarina purpurascens, and Pseudoproboscispora aquatica, which have been rarely collected in other temperate freshwater habitats (Shearer 2001). Thus, these fungi are not restricted to tropical or warm regions but can also occur in temperate habitats. Yuen et al. (1999a) showed that tropical freshwater fungi grew twice as fast as temperate freshwater fungi in vitro, and that there are differences in mycelial growth rates between tropical and temperate strains of the same fungal taxon. Temperate species may be outcompeted by the tropical species in tropical freshwater habitats (Yuen et al. 1999a). If these tropical taxa do not grow well at lower temperatures in the Koito River, they may be outcompeted by the temperate fungi, which can account for their low occurrence.

The occurrence of *Helicomyces torquatus*, *Phaeoisaria clematidis*, *Pseudohalonectria lignicola*, and *Savoryella lignicola* in Japan indicates that these fungi are cosmopolitan in distribution because they have been recorded in places such as the United States (Shearer 1993), Thailand (Sivichai et al. 2000), Australia, and Brunei (Ho et al. 2001). However, further physiological experiments may be necessary to establish the relationship between growth rate and distribution pattern.

The interesting taxa *Pseudohalonectria lignicola*, *Bactrodesmium arnaudii*, and *B. pallidum* are discussed and illustrated next because of their inhibitory ability and rarity, respectively.

Notes on some interesting fungi

Pseudohalonectria lignicola Minoura & Muroi, Trans. Mycol. Soc. Jpn 19:132, 1978 Figs. 1–5



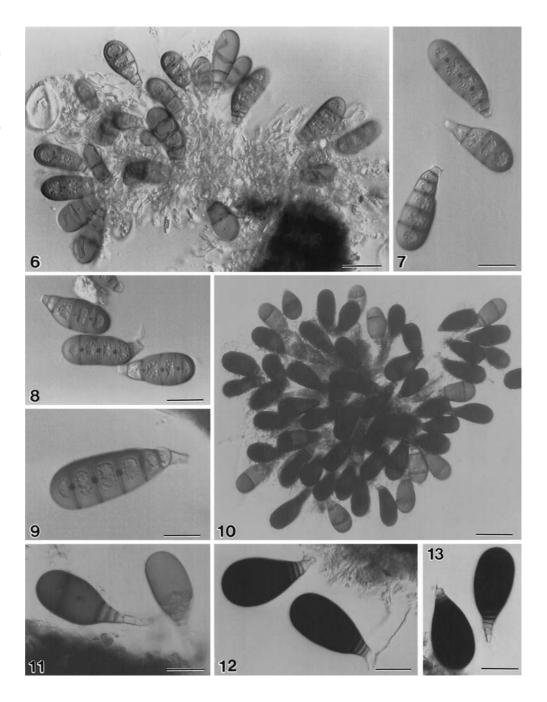
Figs. 1–5. *Pseudohalonectria lignicola.* **1, 2** Asci. **3** Paraphyses. **4, 5** Ascospores. *Bars* **1, 2, 4, 5** 20μm; **3** 15μm

Ascomata immersed, subglobose, gregarious or scattered, yellowish-brown. Paraphyses $2-3\mu m$ wide, more than 200 μm long, numerous, septate, hyaline, tapering distally (Fig. 3). Asci 120–135 × 13–15 μm , cylindrical, 8-spored, short pedicellate, thin-walled, with a J-, refractive apical apparatus, ~1.5 μm high, 2 μm in diameter (Figs. 1, 2). Ascospores 48–57 × 4.5–6 μm , overlapping uniseriate, fusiform, curved cylindrical with tapered ends, 3–5-septate, pale yellow (Figs. 4, 5).

Specimen examined: Japan, Chiba Prefecture, Toyofusa, Kimitsu-shi, Koito River, on submerged wood, March 4, 2002, K.M. Tsui and K. Fukushima, KM 432 (HKU(M) 16139), culture of specimen (HKUCC 9124).

Notes: *Pseudohalonectria lignicola* was firstly described from Lake Biwa, Japan (Minoura and Muroi 1978). It has been reported from many locations such as the United States (Shearer and von Bodman 1983; Shearer 1989), Hong Kong (Tsui et al. 2001), and the Seychelles (Hyde and Goh 1998).

Bactrodesmium pallidum M.B. Ellis, Mycol. Pap. 72:11, 1959 Figs. 6–9 Sporodochia on rotten wood black, aggregated, puntiform, mycelium immersed. Conidiophores micronematous, ~5 μ m wide, simple, pale brown, smooth, septate (Fig. 6). Conidiogenous cells integrated, cylindrical, determinate, pale brown. Conidia acrogenous, 35–50 × 13–18 μ m, 4–6septate, with conspicuous septal pores, broadly ellipsoid, **Figs. 6–9.** Bactrodesmium pallidum. 6 Squash mount of conidia and conidiophores. **7–9** Conidia. Bars 6 28 μm; **7, 8** 20 μm **9** 15 μm. **Figs. 10–13.** Bactrodesmium arnaudii. **10** Squash mount of conidia and conidiophores. **11–13** Conidia. Bars **10** 40 μm; **11, 12** 20 μm; **13** 24 μm



obovoid, pale brown to brown, conidial secession schizolytic (Figs. 7–9).

Specimen examined: Japan, Chiba Prefecture, Toyofusa, Kimitsu-shi, Koito River, on submerged wood, March 4, 2002, K.M. Tsui and K. Fukushima, KM 435 (HKU(M) 16148).

Notes: This species resembles *B. spilomeum* (Berk. & Broome) E.W. Mason & S. Hughes in conidial morphology but differs in having slightly larger conidia with 5–6-septa, whereas the conidia in *B. spilomeum* are 4-septate and pale brown (Ellis 1971).

Bactrodesmium arnaudii S. Hughes, Can. J. Bot. 36:738, 1958 Figs. 10–13

Sporodochia on rotten wood black, aggregated, puntiform, mycelium immersed (Fig. 10). Conidiophores micronematous, subhyaline, smooth, simple, septate. Conidiogenous cells integrated, determinate (Figs. 11, 12). Conidia acrogenous, $40-50 \times 19-24 \mu m$, 4-5-septate, with thick bands at the uppermost and penultimate septa, broadly clavate, ellipsoid, dark brown at upper cells, pale brown in lower cells, conidial secession schizolytic (Figs. 11–13).

Specimen examined: Japan, Chiba Prefecture, Toyofusa, Kimitsu-shi, Koito River, on submerged wood, March 4, 2002, K.M. Tsui and K. Fukushima, KM 436 (HKU(M) 16155).

Notes: This species is most similar to *B. abruptum* (Berk. & Broome) S. Hughes in producing brown conidia with

thick bands at the uppermost and the penultimate septa (Ellis 1971). However the conidia in *B. abruptum* are subcylindrical, 3–7-septate, and $32-70\mu m$ in length.

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