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Short Communication

Amylostereum laevigatum associated with a horntail, *Urocerus antennatus*

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A fungus associated with a horntail, *Urocerus antennatus*, in Ibaraki, Kochi, and Nagasaki Prefectures, was studied. Cultures isolated from the mycangia of 12 adult females of *U. antennatus* showed the same cultural characteristics as those of *Amylostereum laevigatum*. One mycangial isolate produced basidiocarps on the stem segments of *Cryptomeria japonica* by artificial inoculation and was identified as *A. laevigatum*. These results indicate that only *A. laevigatum* is carried in the mycangia of *U. antennatus* in Ibaraki, Kochi, and Nagasaki Prefectures.

Key Words——*Amylostereum laevigatum*; horntail; symbiont; *Urocerus antennatus*; wood discoloration.

The horntail *Urocerus antennatus* Marlatt (Hymenoptera: Siricidae) is widely distributed from Kyushu to Hokkaido in Japan and attacks *Abies firma* Sieb. et Zucc., *A. homolepis* Sieb. et Zucc., *Cryptomeria japonica* (L. f.) D. Don, and *Picea jezoensis* (Sieb. et Zucc.) Carrière (Takeuchi, 1962; Kanamitsu, 1978; Sano, 1992). When the female of the horntail oviposits in the wood of *Cr. japonica*, a species of *Amylostereum* is inoculated into the wood together with eggs, and wood discoloration subsequently occurs (Sano et al., 1995). The discoloration of the wood of *Cr. japonica* caused by *U. antennatus* and *Amylostereum* sp. causes problems in plantations in the Kinki and Shikoku regions of Japan (Sano, 1992; Miyata, 1999).

Kanamitsu (1978) reported that *Amylostereum chailletii* (Pers.: Fr.) Boidin was carried in the mycangia of females of *U. antennatus*. Sano et al. (1995) also identified the fungus isolated from horntails in Mie Prefecture as *A. chailletii* by cultural characteristics. However, there have been no reports of the fungus based on basidiocarp morphology (teleomorph) induced by inoculation of cultures isolated from the mycangia of *U. antennatus*. Studies are required to identify the symbionts exactly by teleomorph, as fungal species other than *A. chailletii* may be associated with horntails. For this reason, we studied the fungus in the mycangia of *U. antennatus* that emerged from the felled logs of *Cr. japonica* in Ibaraki and Kochi Prefectures, and horntails captured in a plantation of *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl. in Nagasaki Prefecture. The objective of this paper is to determine the fungal species associated with *U. antennatus* by teleomorph and cultural characteristics. We also confirmed the pathogenicity of isolates from both horntail and basidiocarp by means of inoculation experiments.

Materials and methods

Approximately 100 logs of *Cr. japonica* (10–17 cm in diam, 1–2 m long), which appeared to have been attacked in the previous year by horntails, were collected from two plantations in Kochi and Ibaraki Prefectures. The logs were brought into outdoor cages at the Shikoku Research Center of the Forestry and Forest Products Research Institute, and the Ibaraki Prefectural Forestry Research Institute in April 1995 and May 1998. Newly emerged adult horntails were caught in cages. Adult females of *U. antennatus* were also captured in traps by use of the attractant Hodoron (Hodogaya Chemical Co.) (Kanasugi et al., 1995) in May 1998 in a plantation of *Ch. obtusa* in Nagasaki Prefecture. The main ingredients of Hodoron are benzonic acid and eugenol. A plastic bottle containing the attractant was covered with sticky paper and hung between the trees. Three bottles per plantation were used. The horntails that were trapped on the sticky paper were collected twice a month between May and August. The symbiotic fungus was isolated from the mycangia following the method of Tabata and Abe (1997). The fungus taken from the mycangia was mounted in lactophenol and observed under a light microscope.

Basidiocarps of *Amylostereum* species were found in May and July 1996 in the *Cr. japonica* plantation at Motoyama Town, Kochi Prefecture, where the females of *U. antennatus* were captured by the attractant trap. The basidiocarps were examined morphologically and cul-

tures were isolated from basidiospores.

The cultures isolated from the mycangia of individual female horntails (mycangial isolates) and those isolated from the collected basidiocarps (basidiospore isolates) were grown on potato-dextrose agar (PDA) in Petri plates at 25°C, and their cultural characteristics were recorded and assigned according to the key pattern of Stalpers (1978). Twelve mycangial isolates and four basidiospore isolates were used for cultural study (Table 1).

Inoculation of stem segments and trees was performed following the procedures described by Tabata and Abe (1997). One mycangial isolate (FD-166, Table 1) was cultured on PDA at 25°C in darkness for 3 wk and inoculated into four stem segments of *Cr. japonica* on 20 Feb. 1996 for the production of fruit bodies. Twenty-four- to 25-yr-old trees of *Cr. japonica* (5.5–8.8 m high, 6.0–7.4 cm diam at breast height) at the Shikoku Research Center of the Forestry and Forest Products Research Institute were also inoculated. One mycangial isolate (FD-166) and one basidiospore isolate (FD-120, Table 1) were used for inoculation of trees. Three holes (ca. 2.5 mm in diam and ca. 1 cm long) were drilled at the height of 1.2 m in the stem of each tree. Two toothpicks with each of these isolates (FD-120, 166) and a sterilized toothpick (control) were put into the holes of each tree and covered with plastic film and adhensive tape. Five trees of *Cr. japonica* were inoculated on 31 Aug. 1998. The inoculated trees were felled three months after the inoculation and examined. The maximum extent of discoloration in the wood was measured. Three inoculated trees were used for isolation of the fungus. Four to twenty-five pieces of wood (3×4×3 mm) were taken from the discolored areas. Isolation was performed following the method of Tabata and Abe (1997).

Results and Discussion

A total of eight adult females (Fig. 1) of *U. antennatus* emerged from *Cr. japonica* logs in early June of 1995 and 1998 in Kochi and Ibaraki Prefectures, and six adult females were captured by the attractant trap in late May 1998 in Nagasaki Prefecture. All the adult females of *U. antennatus* were found to have mycangia (Fig. 2) filled with hyphal fragments (Fig. 3). The hyphal fragments were hyaline, 12.0–183 μm long, and 1.5–6.5 μm wide. They consisted of one to five cells and had clamp connections at the septa. A total of 12 mycangial isolates were isolated from the mycangia of individual female horntails (Table 1).

All the mycangial isolates had the same cultural characteristics. Colonies were white at first, later becoming creamy-brown and cottony to felty on PDA (Fig. 5a). Stalpers's key patterns were: 1, 2, 3, 6, 13, 21, (22), 24, 30, (31), (35), (36), (38), 39, 44, 45, 48, (51), 52, 53, (54), (58), (60), 72, (83), 90. There was no difference in the cultural characteristics among the isolates from Ibaraki, Kochi, and Nagasaki Prefectures. However, further studies are required to examine the characteristics of more mycangial isolates from Kochi Prefecture, where only one isolate was obtained.

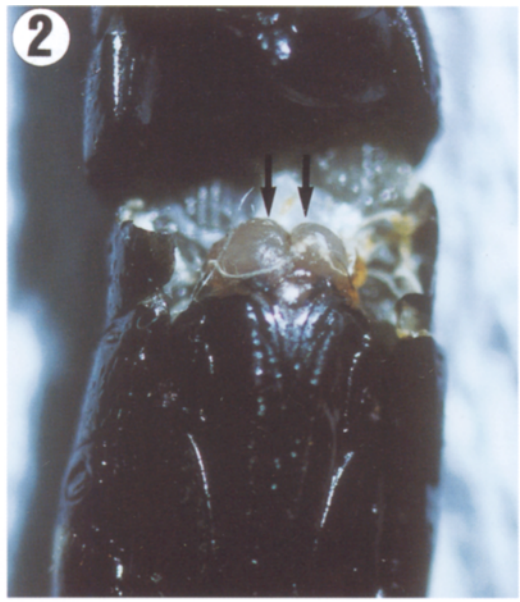
Three basidiocarps of the *Amylostereum* species were collected from the plantation of *Cr. japonica* at Motoyama Town, Kochi Prefecture (Table 1). The basidiocarps were resupinate, 3 to 9 cm in extent, very thin, 140–330 μm thick, and light brown to greyish brown in color. They were identified as *A. laevigatum* (Fr.: Fr.) Boidin (Fig. 4).

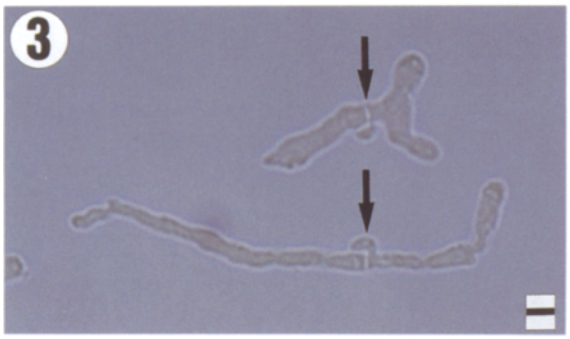
The data of the collected specimens are as follows: SFM5; Motoyama; Kochi; on the bark of felled log of *Cr. japonica*; 28 May 1996. SFM6; Motoyama; Kochi; on

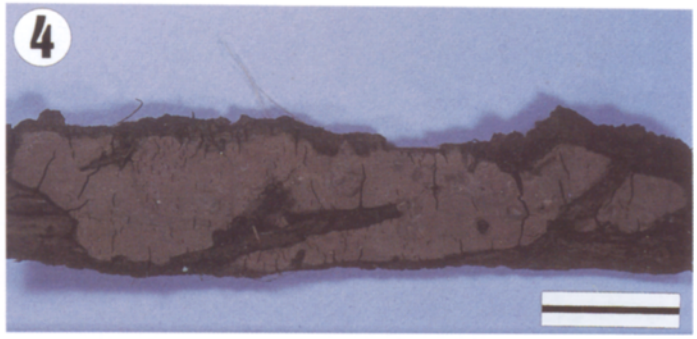
Table 1. Origin of 16 *Amylostereum laevigatum* isolates used for cultural study and inoculation.

Isolate	Locality of collection	Data of isolation	Origin
FD-120	Motoyama[a], Kochi[d]	29 May 1996	Basidiospores of *A. laevigatum*
FD-151	Motoyama[a], Kochi[d]	29 May 1996	Basidiospores of *A. laevigatum*
FD-166	Kitagawa[b], Kochi[d]	10 Jul. 1995	Mycangium of *U. antennatus*
FD-191	Motoyama[a], Kochi[d]	25 Jul. 1996	Basidiospores of *A. laevigatum*
FD-192	Motoyama[a], Kochi[d]	6 Jul. 1996	Basidiospores of *A. laevigatum*
FD-291	Isahaya[c], Nagasaki[d]	25 Jun. 1998	Mycangium of *U. antennatus*
FD-292	Isahaya[c], Nagasaki[d]	25 Jun. 1998	Mycangium of *U. antennatus*
FD-293	Isahaya[c], Nagasaki[d]	25 Jun. 1998	Mycangium of *U. antennatus*
FD-294	Naka[a], Ibaraki[d]	8 Jun. 1998	Mycangium of *U. antennatus*
FD-295	Naka[a], Ibaraki[d]	8 Jun. 1998	Mycangium of *U. antennatus*
FD-296	Naka[a], Ibaraki[d]	8 Jun. 1998	Mycangium of *U. antennatus*
FD-297	Naka[a], Ibaraki[d]	10 Jun. 1998	Mycangium of *U. antennatus*
FD-309	Naka[a], Ibaraki[d]	8 Jun. 1998	Mycangium of *U. antennatus*
FD-310	Isahaya[c], Nagasaki[d]	25 Jun. 1998	Mycangium of *U. antennatus*
FD-312	Naka[a], Ibaraki[d]	10 Jun. 1998	Mycangium of *U. antennatus*
FD-313	Naka[a], Ibaraki[d]	10 Jun. 1998	Mycangium of *U. antennatus*

a) Town; b) Village; c) City; d) Prefecture.







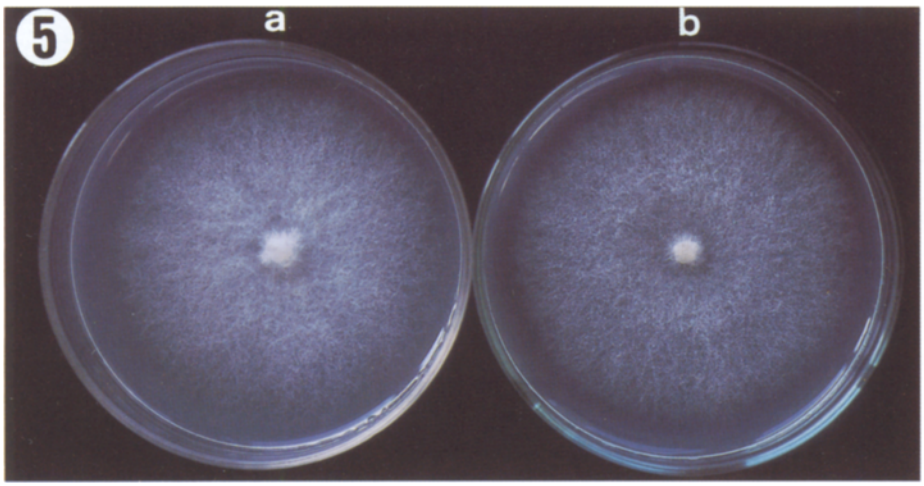


Fig. 1. Adult female of *Urocerus antennatus*. Scale bar = 1 cm.
Fig. 2. Anatomy of abdomen of *U. antennatus*. Arrows show the mycangia.
Fig. 3. Hyphal fragments with clamp connections (arrows) stored in the mycangia of *U. antennatus*. Scale bar = 5 μm.
Fig. 4. Basidiocarp of *Amylostereum laevigatum* on *Cryptomeria japonica* bark collected in the field (specimen SFM5). Scale bar = 1 cm.
Fig. 5. Culture derived from the mycangia of *U. antennatus* (isolate FD-166, a) and culture from *A. laevigatum* basidiocarp collected on a *Cr. japonica* log (isolate FD-120, b). Both cultures were grown on PDA at 25°C in darkness for 1 week.
Fig. 6. Basidiocarp (arrows) produced on the stem segment of *Cr. japonica* artificially inoculated with mycangial isolate (FD-166). Scale bar = 1 cm.

Table 2. Extent of discoloration in wood of *Cryptomeria japonica* inoculated with two *Amylostereum laevigatum* isolates 3 months after inoculation.

Inoculum	Number of inoculated parts	Longitudinal direction (cm)		Tangential direction (cm)		Radial direction (cm)	
		discoloration range	average	discoloration range	average	discoloration range	average
FD-120	5	28.3–92.7	61.4	1.0–1.4	1.1	1.8–3.8	2.7
FD-166	5	12.8–44.6	26.6	0.7–0.9	0.8	1.8–3.8	2.5
Control	5	1.3– 5.2	2.4	0.2–0.3	0.2	0.5–1.5	1.3

the bark of felled log of *Cr. japonica*; 5 Jul. 1996. SFM7; Motoyama; Kochi; on the bark of stump of *Cr. japonica*; 24 Jul. 1996. They have been stored at the Shikoku Research Center of the Forestry and Forest Products Research Institute.

Four basidiospore isolates were isolated from the three collected basidiocarps (Table 1). Their colonies were white at first, later becoming yellowish brown and cottony to felty on PDA (Fig. 5b). The isolates had the same cultural characteristics as the mycangial isolates.

Fruit bodies were produced on three stem segments 6 months after the inoculation of isolate FD-166. They were strictly resupinate, attached tightly to the substrate, formed patches 2–11 cm in extent, 40–150 μm thick, and the hymenial surface was smooth and pale brown (Fig. 6). The basidiocarps have monomitic hyphal system and produced basidiospores 6.5–10 × 3–4 μm in size. The microscopic characteristics of the basidiocarps were identical to those of the specimens (SFM5–7). The basidiospores overlapped in size between the basidiocarps on the felled logs and stump and those produced on the inoculated stem segments. These results indicate that the fungi isolated from the mycangia of *U. antennatus* in Ibaraki, Kochi, and Nagasaki Prefectures are conspecific with *A. laevigatum*.

Of the five recorded species of *Amylostereum* (Chamuris, 1988), *A. areolatum* (Fr.: Fr.) Boidin, *A. chailletii*, and *A. laevigatum* are known as symbionts of both *Sirex* and *Urocerus* (Gaut, 1969, 1970; Sano et al., 1995; Tabata and Abe, 1997). Gaut (1970) found that the same horntail species always associated with the same fungal species. However, our report represents an exception to this rule. Sano et al. (1995) examined the fungus from the mycangia of *U. antennatus* in Mie Prefecture in the Kinki District of Japan and reported it to be *A. chailletii*. So, two different fungal species are associated with the same species of horntail. Therefore, further studies are required to clarify the species of fungus associated with *U. antennatus* in different districts of Japan.

Urocerus antennatus examined in this paper had the same symbiotic fungus, *A. laevigatum*, as *U. japonicus* (Tabata and Abe, 1997). These two horntails have similar geographical distribution pattern and emerge from the felled logs of *Cr. japonica* (Takeuchi, 1962; Sano, 1992). Larvae of Siricinae are known to feed primarily on the sapwood of various coniferous trees (Kanamitsu, 1978). Kukor and Martin (1983) reported that larvae of *S. cyaneus* Fab. acquire the enzymes while ingesting tissue of fungal symbiont that occurs in the wood on which the larvae feed. From these facts, we speculate that the larvae of *U. antennatus* and *U. japonicus* will feed primarily on the sapwood of *Cr. japonica* with the help of the same symbiotic fungus.

Wood discoloration occurred in all trees inoculated with the two isolates. Discoloration was pale brown, spindle-shaped in cross section, and elliptic in longitudinal section. Table 2 shows the extent of discoloration in *Cr. japonica* three months after inoculation. The maximal discoloration in wood inoculated with the mycangial isolate and basidiospore isolate reached 44.6 cm and 92.7 cm, respectively. There was not much difference in the pattern and extent of discoloration between the isolates. The inoculated fungi were reisolated in frequencies of 16.7–83.3% from three discolored areas inoculated with FD-166 and two discolored areas inoculated with FD-120, but not from controls. The results confirmed the pathogenicity of mycangial and basidiospore isolates. In one area of discoloration inoculated with FD-120, the fungus was not isolated, but *Trichoderma* sp. was isolated in a frequency of 43.5%. The results suggest that *Trichoderma* species can easily enter the tree at inoculation points and hinder the inoculated fungus from growing, as indicated by Suto (1994) and Fukuda (1997).

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