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Short Communication

A new *Ophiostoma* species isolated from Japanese oak infested by *Platypus quercivorus**

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A new *Ophiostoma* species was isolated from the sapwood of *Quercus mongolica* var. *grosseserrata* and described as *Ophiostoma longicollum* sp. nov. The species is characterized by a long perithecial neck with a gelatinous cap at the apex and a constricted base, orange-section-shaped ascospores that accumulate in a yellow-orange drop at the apex of the neck, and its *Sporothrix* anamorph.

Key Words——*Ophiostoma*; ophiostomatoid fungi; *Quercus mongolica* var. *grosseserrata*; *Sporothrix*.

During surveys of mortality of *Quercus mongolica* Fisch. ex Turcz. var. *grosseserrata* (Bl.) Rehd. & Wils., which is widespread in several areas along the Sea of Japan proper, several *Ophiostoma* species have been isolated (Kaneko, 1995). We isolated a species of *Ophiostoma*, which was not isolated by Kaneko (1995), from the sapwood of declining *Q. mongolica* var. *grosseserrata* collected in Shiga Pref., western Japan. This paper describes the species as *Ophiostoma longicollum* sp. nov.

Materials and Methods

Logs were collected from declining *Q. mongolica* var. *grosseserrata* infested by *Platypus quercivorus* (Murayama) by Mr. M. Taniguchi in Yogo Town, Shiga Prefecture, Japan on 27 May 1996. Wood blocks (5 × 2 × 1 cm) sampled from the log were kept in humid Petri dishes (9 cm in diam) with a wet filter papar at room temperature. The wood blocks included galleries of the beetles. After 1 mo, ascospore masses were collected from the apices of perithecia produced on the blocks and transferred onto 1% malt extract agar plates (10 g of Difco malt extract, 15 g of agar and 1,000 ml of distilled water) in 9 cm Petri dishes with a sterilized tungsten needle. The plates were incubated at 15°C in the dark for 2 wk. When the cultures were purified, small parts of the colony were transferred onto 1% Pablum agar (10 g of Pablum mixed cereal, 15 g of agar and 1,000 ml of distilled water) and 2% malt extract agar (20 g of Difco malt extract, 15 g of agar and 1,000 ml of distilled water). Small pieces of autoclaved *Q. mongolica* var. *grosseserrata* xylem were sometimes added to the plates in order to stimulate production of perithecia.

Perithecia and conidiophores were mounted on glass slides in 1% lacto-fucsin and observed under a light microscope. For scanning electron microscopy (SEM), agar disks of 8 mm in diam were cut from the colonies and fixed in 3% glutaraldehyde overnight. They were then dehydrated in a graded ethanol series, passed through ethanol-isoamylacetate, and dried with a Hitachi critical point drier. The specimens were examined using a Hitachi S-4200 scanning electron microscope.

The growth rate of isolates was examined at 4, 10, 15, 20, 25 and 30°C. Agar disks of 5 mm in diam were cut from the colonies of each isolate and put on the center of plates with 2% malt extract agar or 1% Pablum agar. Three replicate plates were prepared for each isolate. In addition, cycloheximide tolerance of an isolate (MAFF410859) was also examined at 15°C at different concentrations (0, 0.05, 0.1, 0.5, 1.0 and 2.5 g/L), because cycloheximide tolerance is an important character of *Ophiostoma* species (Harrington, 1981). Colony diam of each plate was measured after incubation for 1 wk, and growth was calculated as mm/d.

Taxonomy

Seventeen ophiostomatoid species have been reported from *Quercus* spp. in Europe and North America (Hunt, 1956; Griffin, 1968; Olchowecki and Reid, 1974; Upadhyay, 1981; Kowalski and Butin, 1989). In Japan, Aoshima (1965) recorded three species, *Ophiostoma pluriannulatum* (Hedgc.) H. & P. Sydow, *O. piceae* (Münch) H. & P. Sydow, and *Ceratocystis moniliformis*

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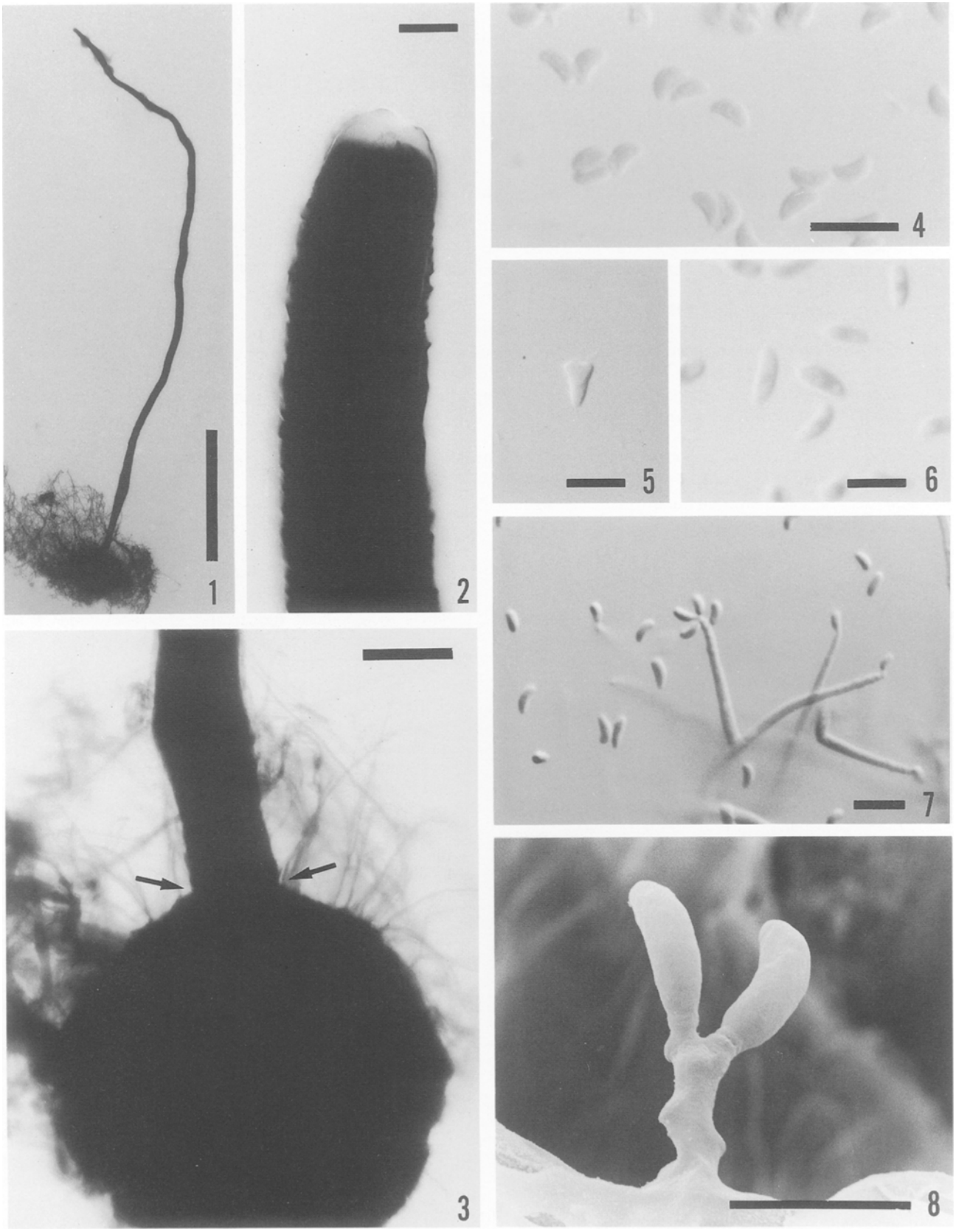
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(Hedgc.) C. Moreau, isolated from *Quercus mongolica* var. *grosseserrata*. Our isolate belonged to *Ophiostoma* and could be distinguished from the species mentioned above by its morphological characteristics. It was also distinct from any other known species in *Ceratocystis* sensu lato. Therefore, we describe the *Ophiostoma* species isolated from *Quercus* as a new species.

Ophiostoma longicollum Masuya, sp. nov. Figs. 1–9

Status anamorphus: *Sporothrix* sp.

Perithecia basi nigra, globosa vel subglobosa, 100–370 μm diam, appendicibus hyphoideis externis brunneolis septatis ornata. Collum cylindraceum, curvatum vel rectum, basi nigrum, pallidiore ad apicem, 1–10 mm longum, ad basim leviter constrictum et 30–60 μm latum, ad apicem 15–50 μm latum, apice obtusum vel truncatum et pileo hyalino tectum, hyphis ostioli non praeditum. Asci non visi. Ascosporae hyalinae, aseptatae, aspectu laterali dimidiato-ellipticae, aspectu frontali ellipsoideae, vagina hyalina circumdantes, 1.9–3.3×0.8–1.6 μm, ad apicem colli in guttula conglobatae. Conidiophora hyalina, septata, micronematosa, mononematosa. Cellulae conidiogenae sympodiales, hyalinae, 0.3–2.0 μm latae, 0.5–5.5 μm longae. Conidia hyalina, aseptata, ellipsoidea, paulo curvata, interdum Y-formia, 2.0–6.0×0.6–2.5 μm, solitaria, dein ad apicem conidiophori in muco aggregata.

Holotype: TFM: FPH7388, dried culture of MAFF410859 (from Yogo town, Ika-gun, Shiga Pref., Japan, on *Quercus mongolica* var. *grosseserrata* infested by *Platypus quercivorus*, isolated by H. Masuya on 27 May 1996, from ascospore)=JCM10198.

Isotype: TSH-C1001

Etymology: *longi*=long in Latin, *collum*=necked in Latin.

Perithecia produced superficially or embedded in 1% Pablum agar medium. Basal part black, globose to subglobose, 100–370 μm in diam, ornamented with brown, unbranched, thin-walled, bent or straight, septate hyphal appendage up to 280×3.0 μm (Figs. 1, 3). Neck black at the base, becoming light color (pale brown) at the apex, nearly cylindrical or tapered, bent or straight, slightly constricted at the base (Figs. 1, 3), 1–10 mm long (mean 6.5 mm), 30–60 μm wide at the base, 15–50 μm at the tip, terminated in an obtuse to truncate apex, and covered by a hyaline gelatinous cap (Fig. 2). Ostiolar hyphae absent. Asci not seen. Ascospores hyaline, 1-celled, orange-section-shaped in side view, ellipsoidal in top view, globose in end view, 1.9–3.3×0.8–1.6 μm, enclosed in an indistinct hyaline sheath (Fig. 4), collecting in a yellow-orange drop at the tip of the neck. Conidiophores arising directly from the surface of agar or from aerial mycelium, hyaline, septate, bearing conidiogenous cells. Conidiogenous cells 0.3–2.0 μm wide and 0.5–5.5 μm long, proliferating sympodially, becoming slightly denticulate (Figs. 7, 8). Conidia sometimes produced directly on the hyphae, holoblastic, hyaline, 1-celled, ellipsoidal, slightly curved (Fig. 6), sometimes Y-shaped (Fig. 5), 2.0–6.0×0.6–2.5 μm, becoming aggregated in slimy masses at the tip of conidiophores.

Colony white to pale brown (Fig. 9). Aerial hyphae particularly abundant on Pablum agar. The growth rate of colonies on 2% malt extract agar 2.4–3.1 (average 2.8) mm/d at 20°C. Growth reduced at temperatures below 20°C and above 30°C, and no growth at 4°C. The fungus tolerated cycloheximide, with growth at 25°C reduced by approximately 60% on 2% malt extract agar containing 2.5 g/L cycloheximide.

Ophiostoma longicollum is characterized by a very long perithecial neck with a gelatinous cap at the apex and orange-section-shaped ascospores which accumulate in an yellow-orange drop at the apex of the perithecial neck. The long perithecial neck without ostiolar hyphae and small orange-section-shaped ascospores are similar to these seen in four other species: *Ceratocystis allantospora* Griffin isolated from several conifers, *Ophiostoma grande* Samuels & Müller reported on stroma of *Diatrype* cfr. *stigma*, *Ophiostoma grandicarpum* (Kowalski & Butin) Rulamort isolated from *Quercus robur* L., and *Ceratocystis magnifica* Griffin, reported on *Abies balsamea* (L.) Mill. However *O. longicollum* can be readily distinguished from *O. grandicarpum* and *O. grande* by its smaller perithecia, presence of a gelatinous cap at the tip of perithecial necks, and its smaller ascospores. *Ceratocystis magnifica* and *C. allantospora* are more similar to *O. longicollum*, but ascospores of *C. allantospora* are allantoid-reniform and distinct from *O. longicollum*. Ascospores of *C. magnifica* resemble those of *O. longicollum*. Therefore, we examined the type specimen of *C. magnifica* (DAOM110149) and found that the perithecial necks of *C. magnifica* are more tapered, wider and shorter than those of *O. longicollum*. Also, the necks of *O. longicollum* are constricted at their bases but those of *C. magnifica* are not.

The anamorph of *O. longicollum* belongs in the genus *Sporothrix* Hektoen & C. F. Perkins and is distinct from the anamorph of *C. allantospora*, which belongs to the genus *Hyalorhinocladiella* H. P. Upadhyay & W. B. Kendr. Although *O. grandicarpum* and *O. grande* have a *Sporothrix* anamorph (Samuels and Müller, 1979; Kowalski and Butin, 1989), they do not have the Y-shaped conidia observed in *O. longicollum*. The anamorph of *C. magnifica* is not known. Griffin (1968) reported that a *Leptographium* state was frequently found near the perithecia of *C. magnifica*. The absence of a known anamorph leaves the correct systematic position of *C. magnifica* in question.

Ophiostoma species are known to be associated with bark beetles or ambrosia beetles (Upadhyay, 1981; Whitney, 1982). Although *O. longicollum* was isolated from oak sapwood attacked by *P. quercivorus*, we could

Figs. 1–8. *Ophiostoma longicollum*.
1. Perithecium. 2. Tip of perithecial neck. 3. Perithecial base. 4. Ascospores. 5. Y-shaped conidia. 6. Lunate conidia. 7. Conidiophore (*Sporothrix* anamorph). 8. Conidiophore (SEM). Scale bars, 1=500 μm; 2=25 μm; 3=50 μm; 4–8=5 μm.

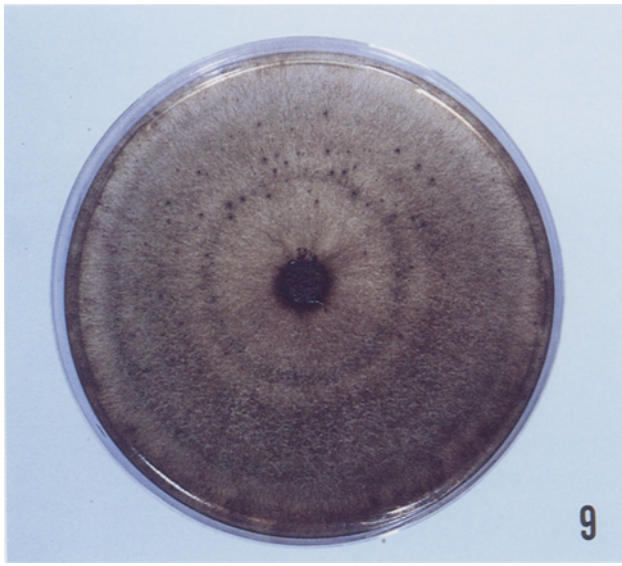

Fig. 9. Colony of *Ophiostoma longicollum* on MA grown for 2 mo. at 15°C.

not confirm a close association of *O. longicollum* with *P. quercivorus*. Further studies are needed to clarify this question. Though many species of *Ophiostoma* and *Ceratocystis* are known to be able to kill their host trees (Bramble and Holst, 1940; Mathre, 1964; Horntvedt et al., 1983; Solheim et al., 1993), the pathogenicity of *O. longicollum* was not confirmed in our preliminary inoculation tests.

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