

FULL PAPER

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## Three new *Ophiostoma* species with *Pesotum* anamorphs associated with bark beetles infesting *Abies* species in Nikko, Japan

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**Abstract** Three species of *Ophiostoma* possessing *Pesotum* anamorphs isolated from bark beetles and their galleries infesting *Abies* species in Nikko, Japan, are described as new species. *Ophiostoma nikkoense* is characterized by brush-shaped synnemata producing long septate clavate conidia, perithecia with neck, and allantoid ascospores. *Ophiostoma microcarpum* has smaller perithecia with hyphoid ostiolar hyphae on the neck, and the ascospores are cylindrical or ossiform in side and face views. *Ophiostoma abieticola* has perithecia without ostiolar hyphae on the neck and produces orange-section-shaped or reniform ascospores.

**Key words** *Abies* · Bark beetle · New species · *Ophiostoma* · *Pesotum*

### Introduction

Species of *Ophiostoma* Syd. & P. Syd. are commonly associated with bark beetles that infest conifer trees (Francke-Grosmann 1963; Whitney 1982; Harrington 1993). Some

isolation studies have been conducted on *Ophiostoma* species associated with bark beetles in Japan, but a few studies have been done on the fungi associated with bark beetles infesting *Abies* species in Japan (Aoshima 1965; Ohtaka et al. 2002a,b; Yamaoka et al. 2004). In 2000 and 2001, we examined *Ophiostoma* species associated with bark beetles infesting *Abies* species in subalpine and montane forests in Nikko, Central Honshu, Japan. During the survey, many undescribed *Ophiostoma* species were found among the isolates (Yamaoka et al. 2004). In the present articles, we describe three *Ophiostoma* species with *Pesotum* J.L. Crane & Schokn. anamorph sensu Okada et al. (1998).

### Materials and methods

Three *Ophiostoma* species were isolated from *Abies mariesii* Masters, *A. veitchii* Lindley, and *A. homolepis* Siebold et Zucc. infested with bark beetles in Nikko, Tochigi Pref., Central Honshu, Japan. *Abies mariesii* and *A. veitchii* trees were obtained from subalpine coniferous forests more than 2100 m above sea level; *A. homolepis* was obtained from a montane forest at about 1450 m. These isolates were obtained during the isolation study reported by Yamaoka et al. (2004).

Cultures used for observation were grown on 2% malt extract agar (2% MA; 20 g malt extract, 15 g agar/1000 ml distilled water), 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 Pablum mixed cereal, 15 g agar/1000 ml distilled water). Later, small (about 2 cm × 5 mm × 3 mm) pieces of autoclaved bark of *A. veitchii* were added to the cultures to stimulate production of perithecia and conidiophores. Perithecia and conidiophores were mounted on glass slides in polyvinyl alcohol or 1% lacto-fuchsin and studied under an Olympus BHS-N Nomarski interference contrast microscope.

For scanning electron microscopy, small pieces of bark or agar blocks (about 5 mm × 5 mm) with synnemata were

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cut from the cultures and fixed in 3% glutaraldehyde solution overnight. They were dehydrated in a graded ethanol series, passed through ethanol-isoamylacetate, and then dried with a Hitachi critical point drier. After coating with platinum-palladium, the specimens were examined using a Hitachi S-4200 scanning electron microscope operating at 15 kV.

An agar disk 3 mm in diameter cut from an actively growing colony was placed at the center of a 2% MA plate and incubated at 20°C in the dark for 7 days. Three replicate plates were prepared for each isolate. The diameter of each colony was measured twice at right angles. Results are presented as an average of three colonies.

Cycloheximide tolerance was tested by placing an agar disk 3 mm in diameter cut from an actively growing colony at the center of a 2% MA plate containing different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1.0, and 2.5 g/l). Three replicate plates were prepared for each isolate and incubated at 20°C in the dark for 7 days. Measurements were taken in the same manner as described above.

Cultures used in the present study have been deposited in the culture collection of the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan and in the Japan Collection of Microorganisms (JCM). Dried specimens of these cultures have also been deposited with the Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba (TSH). Holotype specimens were deposited in the Herbarium of the National Science Museum (TNS), Tsukuba, Japan.

## Taxonomy

### 1. *Ophiostoma nikkoense* Yamaoka & Masuya, sp. nov.

Figs. 1–6, 18

Perithecia superficialia in medio agari vel in cortice posita in superficie medii; pars basilaris atra, globosa vel subglobosa, 73–165 µm diametro, appendicibus fuscis hyphoideis usque 325 µm longis ornata; colla atra, pallide brunnea vel ad apicem subhyalina, recta vel curvata, cylindrica, hyphis ostiolaribus inclusis 310–570 (–680) µm longa, ad basim 24–49 µm lata, ad apicem 10–17 µm lata; hyphae ostiolaris 10–20, hyalinae, septatae, divergentes, 24–79 µm longae. Ascospores hyalinae, unicellulares, allantoideae a latere visae, ellipsoideae a facie visae, globosae ab apice visae, vagina angusta hyalina cinctae, 3.2–4.8 × 1.4–1.6 µm vagina inclusae, ad apicem colli in guttula alba vel subflavescenti aggregatae.

Conidiophora synnematosae, recta vel flaccida, ad basim angusta, ad apicem scopulata, ad basim atrobrunnea vel atra, ad apicem pallida brunnea vel subhyalina, 357–923 (–1020) µm longa, ad basim 7–17 (–19) µm lata; cellulae conidiogenae integratae, terminales, sympodiales, cylindricae. Conidia hyalina, 0–1 (–2)-septata, clavata, 6.0–38 × 1.0–3.6 (–4.0) µm; conidia parva e conidia magna clavatis saepe enata, hyalina, unicellularia, oblonga vel clavata, (3.6–) 4.0–9.0 × 1.2–3.0 (–3.6) µm.

Holotypus: TNS-F-6687, dried specimen of culture YCC-430 (JCM11728) collected and isolated from an egg gallery of *Polygraphus proximus* Blandford in *Abies mariesii* Masters, west of Mt. Ohmanako, Nikko, Tochigi Pref., July 14, 2000, by Y. Yamaoka.

Etymology: *nikkoense* = latinized from name of the place, referring to the type locality.

Perithecia (Fig. 1) superficial on the agar medium and on the bark placed on the surface of the medium; basal part black, globose to subglobose, 73–165 µm in diameter, ornamented with brown hyphal appendages, up to 325 µm long; necks black, light brown, or subhyaline at the apex, straight or curved, cylindrical, 310–570 (–680) µm long including ostiolar hyphae, 24–49 µm wide at the base, 10–17 µm wide at the tip; ostiolar hyphae (Fig. 2) 10–20 in number, hyaline, septate, divergent, 24–79 µm long. Ascospores (Fig. 3) hyaline, 1-celled, allantoid in side view, ellipsoidal in face view, globose in end view, surrounded by a narrow hyaline sheath, 3.2–4.8 × 1.4–1.6 µm including sheath, aggregating in a white droplet at the tip of the necks.

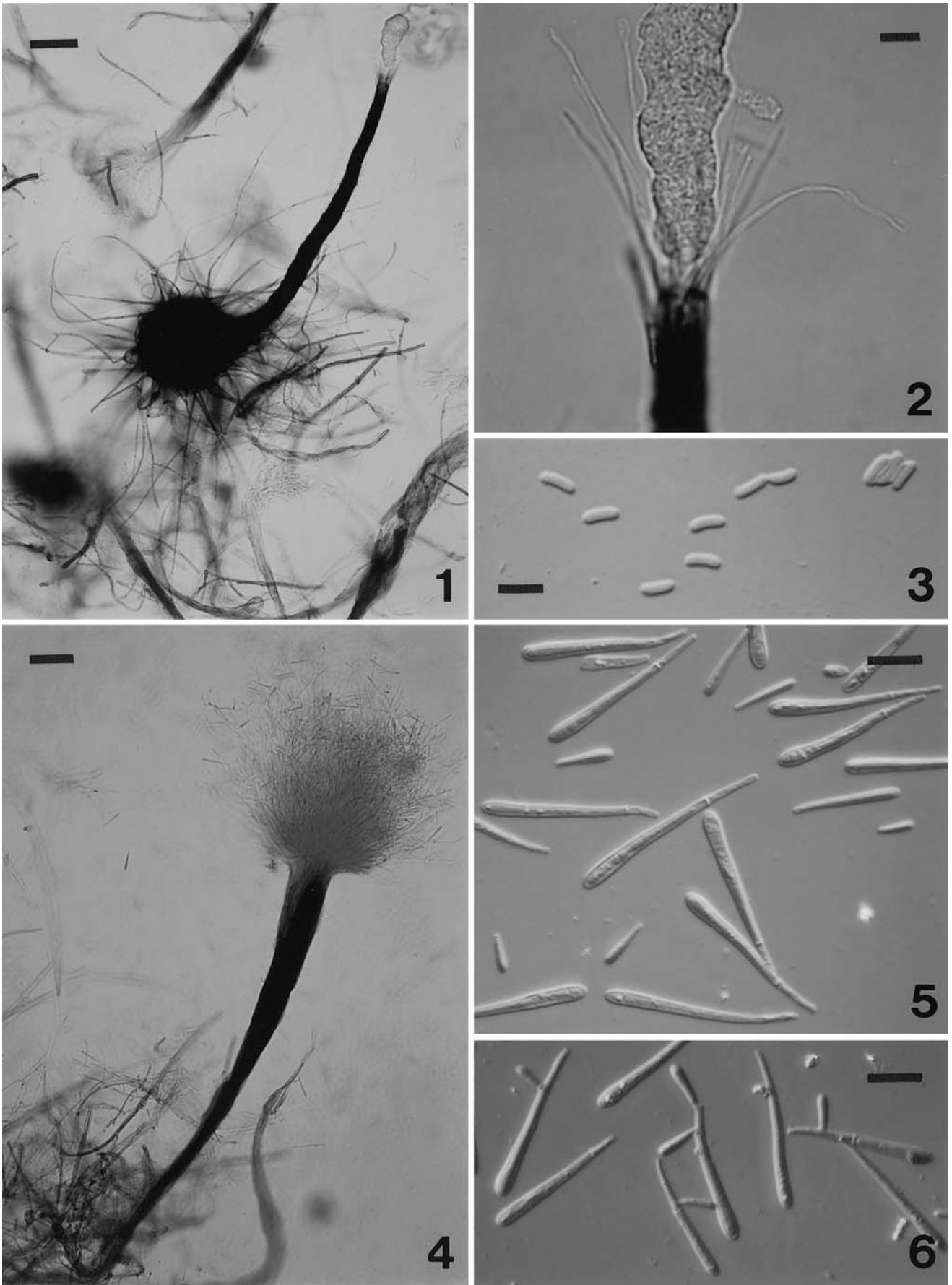
Conidiophores (Fig. 4) synnematosae; synnemata erect or flaccid, narrow at the base, becoming brush- or broom-shaped toward the apex, dark brown to black at the base, becoming light brown or subhyaline at the apex, 357–923 (–1020) µm long including conidiogenous apparatus, 7–17 (–19) µm wide at the base; conidiogenous cells (see Fig. 18) integrated, terminal, sympodial, cylindrical. Conidia (Fig. 5) hyaline 0–1 (–2) septate, clavate; septate conidia 6.0–38 × 1.0–3.6 (–4.0) µm; clavate conidia often forming small conidia (Fig. 6), hyaline, aseptate, oblong, or clavate, (3.6–) 4.0–9.0 × 1.2–3.0 (–3.6) µm.

Colonies on 2% MA appressed, white at first, at least part of the colony becoming yellowish-brown, sometimes with white aerial mycelia. Average diameter of colonies 7 days after incubation at 20°C in the dark was 30.3 mm.

This fungus was able to grow on media containing cycloheximide at all the concentrations tested (0.01–2.5 g/l). Average diameters of colonies after 7 days of incubation at 20°C in the dark were 28.7 mm at 0.05 g/l cycloheximide, 28.5 mm at 0.1 g/l, 25.5 mm at 0.5 g/l, 23.3 mm at 1.0 g/l, and 21.5 mm at 2.5 g/l. This fungus was considered to be cycloheximide tolerant, which is one of the important characteristics of species belonging to genus *Ophiostoma* (Harrington 1981).

Living cultures: YCC-430 (JCM11728), isolate from an egg gallery of *P. proximus* in *A. mariesii*, west of Mt. Ohmanako, Nikko, Tochigi Pref., July 14, 2000, by Y. Yamaoka; YCC-456 (JCM11729), isolate from *P. proximus* in *A. homolepis*, Yumihari Pass, Nikko, Tochigi Pref., August 8, 2001, by Y. Yamaoka; YCC-457, isolate from *Dryocoetes striatus* Eggers in *A. homolepis*, Yumihari Pass, Nikko, Tochigi Pref., August 8, 2001, by Y. Yamaoka.

Dried specimens deposited: TNS-F-6687 (holotype) and TSH-C274, dried culture YCC-430 grown on PA with pieces of autoclaved bark of *A. veitchii* (PAB) at 17°C; TSH-C275, dried culture YCC-430 grown on 2% MEBA with pieces of autoclaved bark of *A. veitchii* (MEBAB) at 17°C; TSH-C293, dried culture YCC-456 grown on PAB at



**Figs. 1–6.** *Ophiostoma nikkoense* (TNS-F-6687, holotype). **1** Ascocarp. **2** Top of neck. **3** Ascospores. **4** *Pesotum* anamorph. **5** Conidia. **6** Budding conidia. Bars **1,4** 50 $\mu$ m; **2,5,6** 10 $\mu$ m; **3** 5 $\mu$ m

**Table 1.** Morphological characteristics of *Ophiostoma nikkoense* and *O. clavigerum*

Characteristics	<i>O. nikkoense</i>	<i>O. clavigerum</i> <sup>b</sup>
Perithecia		
Width of base (µm)	73–165	247–650
Neck	Present	Absent
Ascospores		
Shape	Allantoid	Cucullate
Size (µm) <sup>a</sup>	3.2–4.8 × 1.4–1.6	3.5–5.5 (–6) × 2.2–4.5
Synnemata		
Length (µm)	357–923 (–1020)	500–1150
Larger conidia		
Shape	Clavate	Clavate, to obclavate, cylindrical, broadly fusiform
Size (µm)	6.0–38 × 1.0–3.6 (–4.0)	12.5–62.5 (–85) × (2–) 4–6
No. of septa	0–1 (–2)	0–7
Smaller conidia		
Shape	Oblong or clavate	Cylindrical to clavate or ellipsoidal
Size (µm)	(3.6–) 4.0–9.0 × 1.2–3.0 (–3.6)	2–4 × 1–2.5

<sup>a</sup>Including sheath<sup>b</sup>Data from Upadhyay (1981)

17°C; TSH-C306, dried culture YCC-457 grown on PAB at 17°C.

*Ophiostoma nikkoense* was well characterized by a unique anamorph state, that is, *Pesotum* state producing long septate clavate conidia. There are no records on the species of *Ophiostoma* possessing *Pesotum* anamorphs producing conidia more than 20 µm long except for *Ophiostoma clavigerum* (Rob.-Jeffr. & R.W. Davidson) T.C. Harr., one of the constant associates with mountain pine beetles (*Dendroctonus ponderosae* Hopk.) in North America (Whitney and Farris 1970; Whitney 1971). The anamorph state of *O. clavigerum* is morphologically similar to that of the present fungus. Both species have brush- or broom-shaped synnemata and long septate clavate conidia. However, the present species has shorter conidia (6–38 µm) with fewer septa (0–2, mostly 0–1) than *O. clavigerum* (12.5–62.5 (–85) µm in length, 0–7 septa) (Robinson-Jeffrey and Davidson 1968; Upadhyay 1981) (Table 1). Furthermore, these two species are easily distinguishable based on the morphology of the teleomorph. *Ophiostoma nikkoense* has perithecia with neck and allantoid ascospores, while *O. clavigerum* produces perithecia lacking neck and cucullate ascospores (Robinson-Jeffrey and Davidson 1968; Upadhyay 1981) (Table 1).

Upadhyay (1981) created a new genus *Graphiocladiella* for the anamorph state of *O. clavigerum*. Okada et al. (1998), however, considered synnematos anamorphs of *Ophiostoma* formerly treated under various genera including *Graphiocladiella* as *Pesotum*. Harrington et al. (2001) stated that *Pesotum* should be restricted to synnematos anamorphs with *Sporothrix* Hektoem & C.F. Perkins synanamorphs similar to those of the *O. piceae* complex. Synnematos anamorphs of *Ophiostoma* varied in morphology and conidial ontogeny, and definitions of anamorph genera are still controversial (Upadhyay 1981; Wingfield et al. 1991; Seifert and Okada 1993; Okada et al. 1998; Harrington et al. 2001). We agreed with the opinion that the anamorphs of the *O. piceae* complex were distinguishable from the remaining synnematos anamorphs of

*Ophiostoma* species. However, the remaining synnematos anamorphs are variable in morphology and conidial ontogeny, so that further taxonomic studies are required to determine proper generic names for them. Thus, we chose to follow the definition of Okada et al. (1998) in this article and treat anamorphs of *O. nikkoense* as *Pesotum*. Harrington et al. (2001) also suggested that *Graphiocladiella* has greater affinity with *Leptographium* Lagerb. & Melin than *Graphium* Corda (*Pesotum*). It is uncertain, however, whether the anamorph of *O. nikkoense* has affinity with *Leptographium*.

*Ophiostoma nikkoense* was isolated from adult beetles and galleries of *P. proximus* infesting *A. mariesii* and those of *P. proximus* and *D. striatus* infesting *A. homolepis*. Particularly, this fungus that was referred to as *Ophiostoma* sp. S by Yamaoka et al. (2004) was isolated at a relatively high frequency from *D. striatus* infesting *A. homolepis* (Yamaoka et al. 2004). It has not yet been determined whether *D. striatus* is the major associate of the fungus because the number of beetles used for isolation studies was small (Yamaoka et al. 2004). Aoshima (1965) investigated blue-stain fungi associated with *P. proximus* attacking *A. sachalinensis* (Fr. Schmidt) Masters in Hokkaido, Japan, but did not report fungi similar to *O. nikkoense*.

## 2. *Ophiostoma microcarpum* Yamaoka & Masuya, sp. nov.

Figs. 7–12, 19

Perithecia superficialia in medio agari vel in cortice posita in superficie medii; pars basilaris atra, globosa vel subglobosa, 58–91 µm diametro, appendicibus fuscis hyphoideis usque 280 µm longis ornata; colla atra, recta vel curvata, gradatim attenuata, (56–) 74–170 µm longa praeter hyphas ostiolare, ad basim 20–29 µm lata, ad apicem 7–12 µm lata; hyphae ostiolares 8–24, hyalinae, septatae, hyphoideae, 20–159 µm longae. Ascosporae hyalinae, unicellulares, cylindricae vel ossiformes a latere vel a facie visae, quadratae ab apice visae, (2.8–) 3.2–4.8 × 1.4–2.0 µm vagina inclusae, ad apicem colli in guttula alba vel subflavescenti aggregatae.

Conidiophora synnematoso, recta vel flaccida, ad basim atrobrunnea vel atra, ad apicem pallide brunnea vel subhyalina, 159–423 (–471)  $\mu\text{m}$  longa, ad basim 10–44  $\mu\text{m}$  lata, hyphae basalia conidiophororum interdum laxe aggregatae; etiam sporodochia pulviformia alba vel pallide brunnea formata; cellulae conidiogenae integrae, terminales, annellidicae, cylindricae. Conidia hyalina, unicellularia, oblonga, 3.0–4.0  $\times$  1.6  $\mu\text{m}$ .

Holotypus: TNS-F-6686, dried specimen of culture YCC-439 (JCM11730) collected and isolated from *Cryphalus montanus* Nobuchi in *Abies mariesii* Masters, north of Yusengatake, Nikko, border between Tochigi Pref. and Gunma Pref., August 17, 2000, by Y. Yamaoka.

Etymology: *microcarpum* = small fruit body in Greek, referring to the small perithecia of the species.

Perithecia (Fig. 7) superficial on the agar medium and on the bark placed on the surface of the medium; basal part black, globose to subglobose, 58–91  $\mu\text{m}$  in diameter, ornamented with brown hyphal appendages, up to 280  $\mu\text{m}$  long; necks black, straight or curved, gradually tapering toward the apex, (56–) 74–170  $\mu\text{m}$  long excluding ostiolar hyphae, 20–29  $\mu\text{m}$  wide at the base, 7–12  $\mu\text{m}$  wide at the tip; ostiolar hyphae (Fig. 8) 8–24 in number, hyaline, septate, hyphoid, 20–159  $\mu\text{m}$  long. Ascospores (Fig. 9) hyaline, 1-celled, cylindrical or ovoid in side and face view, quadrangular in end view, (2.8–) 3.2–4.8  $\times$  1.4–2.0  $\mu\text{m}$  including sheath, aggregating in a white droplet at the tip of the necks.

Conidiophores (Fig. 10) synnematoso; synnemata erect or flaccid, dark brown to black at the base, light brown or subhyaline at the apex, 159–423 (–471)  $\mu\text{m}$  long including conidiogenous apparatus, 10–44  $\mu\text{m}$  wide at the base, hyphae composing basal part sometimes loosely aggregated (Fig. 11); also forming white or light brown, cushion-shaped sporodochia; conidiogenous cells (Fig. 19) integrated, terminal, annellidic, cylindrical. Conidia (Fig. 12) hyaline, aseptate, oblong 3.0–4.0  $\times$  1.6  $\mu\text{m}$ .

Colonies on 2% MA appressed, white at first, later may become orange in color, sometimes with white aerial mycelia. Average diameter of colonies 7 days after incubation at 20°C was 17.7 mm

This fungus was able to grow on media containing cycloheximide at all the concentrations tested (0.01–2.5 g/l). Average diameters of colonies after 7 days of incubation at 20°C in the dark were 17.3 mm at 0.05 g/l cycloheximide, 17.5 mm at 0.1 g/l, 17.0 mm at 0.5 g/l, 15.3 mm at 1.0 g/l, and 14.5 mm at 2.5 g/l. This fungus was considered to be cycloheximide tolerant.

Living cultures: YCC-439 (JCM11730), isolate from *C. montanus* in *A. mariesii*, north of Yusengatake, Nikko, border between Tochigi Pref. and Gunma Pref., August 17, 2000, by Y. Yamaoka; YCC-459 (JCM11731), isolate from *P. proximus* in *A. homolepis*, Yumihari Pass, Nikko, Tochigi Pref., August 8, 2001, by Y. Yamaoka; YCC-460, isolate from *P. proximus* in *A. homolepis*, north of Yusengatake, Nikko, border between Tochigi Pref. and Gunma Pref., August 8, 2001, by Y. Yamaoka.

Dried specimens deposited: TNS-F-6686 (holotype) and TSH-C282, dried culture YCC-439 grown on PAB at 17°C; TSH-C283, dried culture YCC-439 grown on MEBAB at

17°C; TSH-C295, dried culture YCC-459 grown on PAB at 17°C; TSH-C296, dried culture YCC-459 grown on MEBAB at 17°C; TSH-C303, dried culture YCC-460 grown on PAB at 17°C.

Four species of *Ophiostoma* with *Pesotum* anamorphs are known to possess ascospores being cylindrical or ovoid in side and face views and quadrangular in end view. They are *O. brunneociliatum* Mathiesen-Käärik, *O. columnare* (Olchow. & J. Reid) Seifert & G. Okada [*columnaris*], *O. ips* (Rumbold) Nannf., and *O. sparsum* (R.W. Davidson) de Hoog & R.J. Scheff. (Table 2). Perithecia of *O. microcarpum* are much smaller than those of these four species (Mathiesen-Käärik 1953; Hunt 1956; Olchowecki and Reid 1974; Davidson 1971; Upadhyay 1981). *Ophiostoma microcarpum* has unique ostiolar hyphae on the perithecial neck, which are hyaline and hyphoid. Ostiolar hyphae of *O. brunneociliatum* are brown and spirally coiled. The other three species do not have ostiolar hyphae on the perithecial neck. Synnematoso anamorphs of *O. ips* and *O. sparsum* are *Graphirubum* type, lacking pigmentation in the synnemata (Upadhyay and Kendrick 1975; Upadhyay 1981), and anamorphs of *O. brunneociliatum* have a brushlike head (Mathiesen-Käärik 1953; Upadhyay 1981). These characteristics did not fit with *O. microcarpum*. Anamorphs of *Ophiostoma columnare* are morphologically similar to the *Pesotum* state of *O. microcarpum*. However, conidia of *O. columnare* are larger than *O. microcarpum* (Olchowecki and Reid 1974; Upadhyay 1981). Thus, we consider the present fungus as a new species.

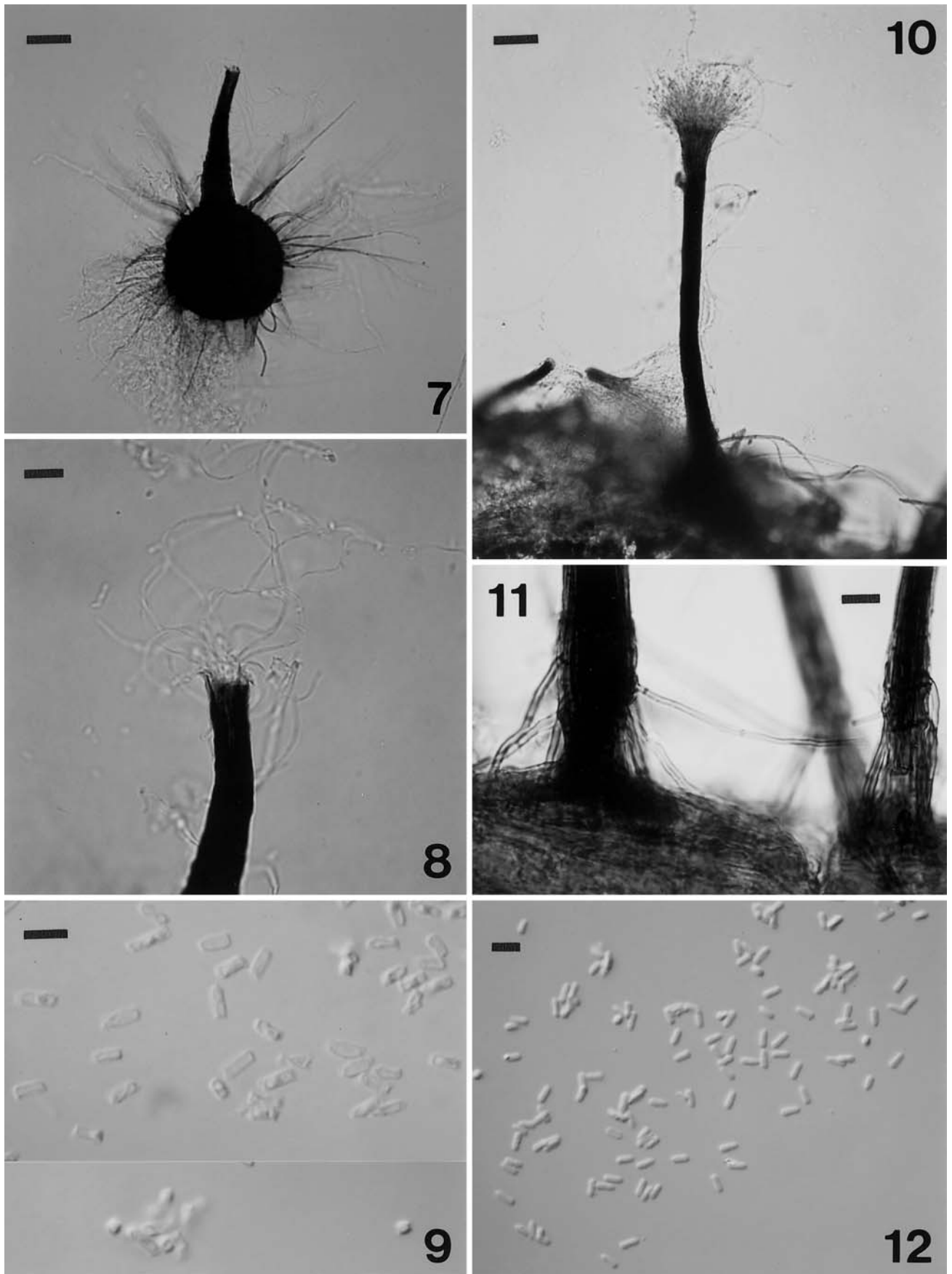
*Ophiostoma microcarpum* was isolated from beetles and galleries of *Cryphalus montanus* and *P. proximus* infesting *A. mariesii* and those of *P. proximus* and *D. striatus* infesting *A. homolepis*. Particularly, this fungus that was referred to as *Ophiostoma* sp. V by Yamaoka et al. (2004) was isolated at a relatively high frequency from *D. striatus* infesting *A. homolepis* (Yamaoka et al. 2004). However, it was not determined whether *D. striatus* is the major associate of the fungus because the number of beetles used for isolation studies was small (Yamaoka et al. 2004).

### 3. *Ophiostoma abieticola* Yamaoka & Masuya, sp. nov.

Figs. 13–17, 20

Perithecia superficialia in medio agari vel in cortice posita in superficie medii; pars basilaris atra, globosa vel subglobosa, 117–175  $\mu\text{m}$  diametro, appendicibus brevibus fuscis hyphoides usque 97  $\mu\text{m}$  longis ornata; colla atra, recta vel curvata, 330–617  $\mu\text{m}$  longa, ad basim 39–58  $\mu\text{m}$  lata, ad apicem (16–) 20–39  $\mu\text{m}$  lata; hyphae ostiolaris absentes. Ascospores hyalinae, unicellulares, in figura segmenti endocarpium fructi citri similes vel reniformae a latere visae, ellipsoideae a facie visae, globosae ab apice visae, vagina angusta hyalina cinctae, (2.8)3.2–4.8  $\times$  1.2–2.0  $\mu\text{m}$  vagina inclusae, ad apicem colli in guttula alba aggregatae.

Conidiophora synnematoso, recta, atrobrunnea vel ad basim atra, ad apicem pallide brunneae vel subhyalinae, 230–427  $\mu\text{m}$  longa, ad basim 22–72  $\mu\text{m}$  lata; cellulae conidiogenae integrae, terminales, annellidicae,



**Figs. 7–12.** *Ophiostoma microcarpum*. **7** Ascocarp. **8** Top of neck. **9** Ascospores. **10** *Pesotum* anamorph. **11** Base of synnemata. **12** Conidia. 7–9 TNS-F-6686, holotype; 10–12 TSH-C283. Bars **7,10** 30 $\mu$ m; **8,11** 10 $\mu$ m; **9,12** 5 $\mu$ m

**Table 2.** Morphological characteristics of *Ophiostoma microcarpum*, *O. brunneociliatum*, *O. columnare*, *O. ips*, and *O. sparsum*

Characteristics	<i>O. microcarpum</i>	<i>O. brunneociliatum</i> <sup>b</sup>	<i>O. columnare</i> <sup>b</sup>	<i>O. ips</i> <sup>c</sup>	<i>O. sparsum</i> <sup>b</sup>
Perithecia					
Width of base (µm)	58–91	(150–) 200–350 (–380)	(45–) 95–230 (–287)	(96–) 120–200 (–320)	(75–) 133–180 (–200)
Length of neck (µm)	(56–) 74–170	(550–) 590–1250 (–1700)	(65–) 350–750 (–900)	Up to 1100	Up to 300
Width of neck at the base (µm)	20–29	30–45 (–57.5)	25–52	27–40	30–40
Width of neck at the tip (µm)	7–12	15–25 (–30)	10–20	10–25	(15–) 18–25
Ostiolar hyphae	Hyaline, hyphoid	Brown to pale brown, spirally curved	Absent	Absent	Absent
Length of ostiolar hyphae (µm)	20–159	22–57.5 (–80)			
Ascospores					
Shape <sup>a</sup>	Cylindrical or ossiform	Ossiform or rectangular	Ossiform	Rectangular	Ossiform
Size (µm)	(2.8–) 3.2–4.8 × 1.4–2.0	3.5–5 × 1.5–2.5 (–3)	2.5–5 × 1.5–2.5	3–5 × 1.5–2.5	3–5.5 × 1.5–2.5
Synnemata					
Color	Dark brown to black at the base	Brown to black at the base	Pale brown to brownish black at the base	Hyaline to pale brown	Creamy-white
Length (µm)	159–423 (–471)	1000–3600 (–4000)	(45–) 70–290 (–560)	Up to 300	55–170 (–250)
Conidia					
Shape	Oblong	Cylindrical to ellipsoidal, oblong	Clavate or cylindrical with or without a medial constriction	Cylindrical to ovoid	Elongate cuneiform to rod-shaped or oblong
Size (µm)	3.0–4.0 × 1.6	(2.5–) 3–6.5 (–8.5) × 1–2 (–2.5)	3–7 × 1–2.5	4–8 × 1.5–3	2.5–5 × 1–2

<sup>a</sup>Including sheath<sup>b</sup>Data from Upadhyay (1981)<sup>c</sup>Data from Hunt (1956)

cylindrica. Conidia hyalina, unicellularia, oblonga, (2.4–) 3.6–6.0 × 1.0–1.6 µm.

Holotypus: TNS-F-6688, dried specimen of culture YCC-424 (JCM11725) collected and isolated from *Dryocoetes hectographus* Reitter in *Abies mariesii*, north of Yusengatake, Nikko, border between Tochigi Pref. and Gunma Pref., August 17, 2000, by Y. Yamaoka.

Etymology: *abieticola* = dweller on *Abies* in Latin, referring to the habitat of the species.

Perithecia (Fig. 13) superficial on the agar medium and the bark placed on the surface of the medium; basal part black, globose to subglobose, 117–175 µm in diameter, ornamented with short brown hyphal appendages, up to 97 µm long; necks black, straight or curved, 330–617 µm long, 39–58 µm wide at the base, (16–) 20–39 µm wide at the tip; ostiolar hyphae absent (Fig. 14). Ascospores (Fig. 15) hyaline, 1-celled, orange-section-shaped or reniform in side view, ellipsoidal in face view, globose in end view, surrounded by a narrow hyaline sheath, (2.8–) 3.2–4.8 × 1.2–2.0 µm including sheath, aggregating in a white droplet at the tip of the necks.

Conidiophores (Fig. 16) synnematus; synnemata erect, dark brown to black at the base, becoming light brown or subhyaline toward the apex, 230–427 µm long including conidiogenous apparatus, 22–72 µm wide at the base; conidiogenous cells (Fig. 20) integrated, terminal, annelidic, cylindrical. Conidia (Fig. 17) hyaline, aseptate, oblong (2.4–) 3.6–6.0 × 1.0–1.6 µm.

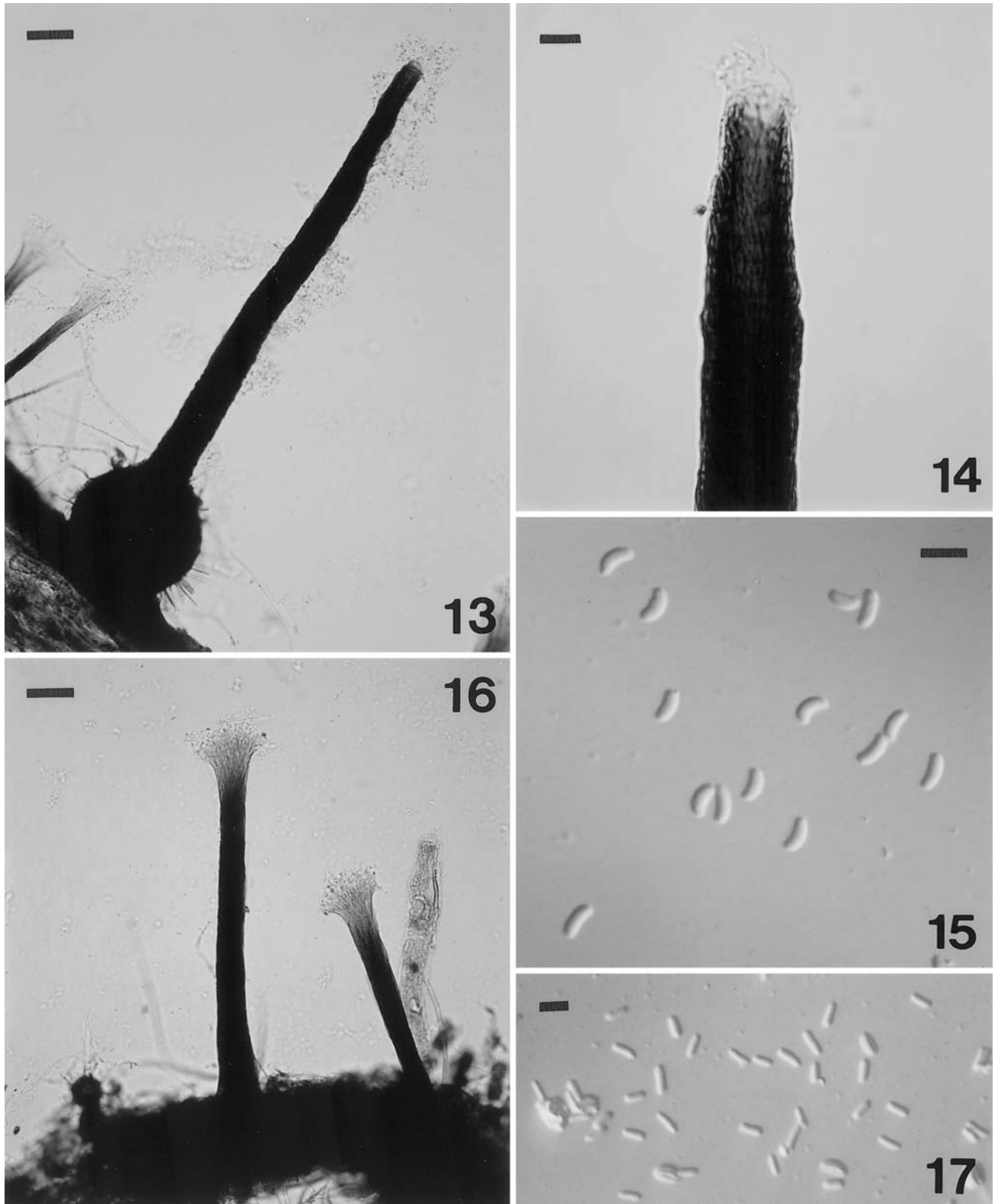
Colonies on 2% MA appressed, white at first, later becoming dark brown in color. When pieces of bark were placed on colonies grown on agar plates, perithecia and synnemata were produced on and around the bark. Average diameter of colonies 7 days after incubation at 20°C was 16.2 mm.

This fungus was able to grow on media containing cycloheximide at all the concentrations tested (0.01–2.5 g/l). Average diameters of colonies after 7 days of incubation at 20°C in the dark were 15.3 mm at 0.05 g/l cycloheximide, 15.3 mm at 0.1 g/l, 16.8 mm at 0.5 g/l, 16.3 mm at 1.0 g/l, and 14.8 mm at 2.5 g/l. This fungus was considered to be cycloheximide tolerant.

Living cultures: YCC-424 (JCM11725), isolate from *Dryocoetes hectographus* in *A. mariesii*, north of Yusengatake, Nikko, border between Tochigi Pref. and Gunma Pref., August 17, 2000, by Y. Yamaoka; YCC-427 (JCM11726), isolate from *D. hectographus* in *A. mariesii*, west of Mt. Ohmanako, Nikko, Tochigi Pref., July 14, 2000, by Y. Yamaoka; YCC-478 (JCM11727), isolate from *D. autographus* (Ratzeburg) in *A. mariesii*, near Goshikinuma, Mt. Shirane, Nikko, Tochigi Pref., July 4, 2001, by Y. Yamaoka.

Dried specimens deposited: TNS-F-6688 (holotype) and TSH-C272, dried culture YCC-424 grown on PAB at 17°C; TSH-C273, dried culture YCC-424 grown on 2% MEBA with pieces of autoclaved bark of *A. veitchii* (MEBAB) at 17°C; TSH-C291, dried culture YCC-427 grown on PAB at 17°C; TSH-C299, dried culture YCC-478 grown on PAB at 17°C.

Two species of *Ophiostoma*, *O. canum* (Münch) Sydow & P. Sydow and *O. introcitrinum* (Olchow. & J. Reid) George Hausner, J. Reid & Klaasen, are known to possess orange-section-shaped ascospores, perithecia lacking ostiolar hyphae, and the *Pesotum* anamorph (Table 3). Morphological characteristics of perithecia were very similar among the three species (Olchow. & Reid 1974; Upadhyay 1981). However, synnematus anamorphs of *O. canum* are *Pachnodium* producing spherical to limoniform holoblastic conidia singly or in chains, and the anamorph of

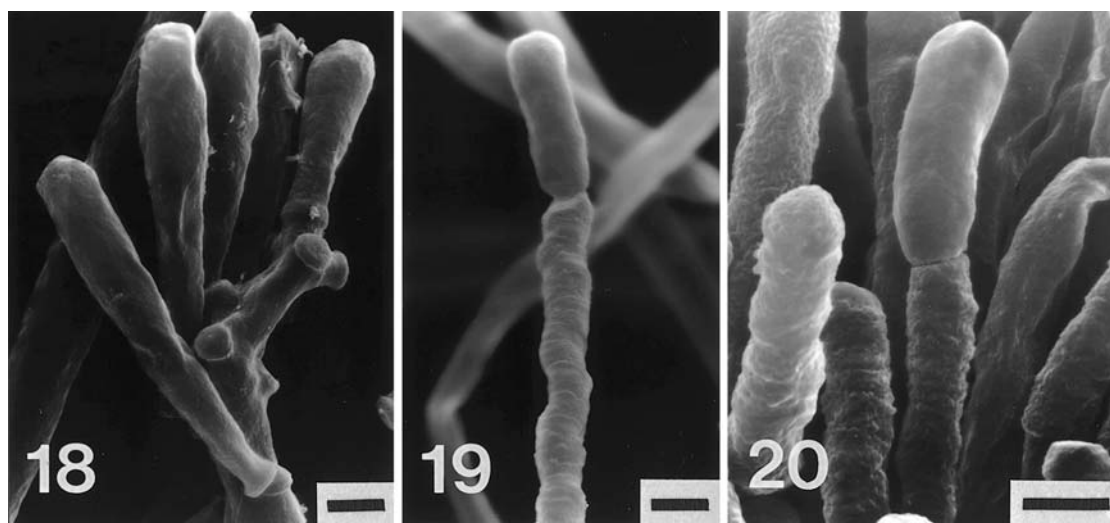


**Figs. 13–17.** *Ophiostoma abieticola* (TNS-F-6688, holotype). **13** Ascocarp. **14** Top of neck. **15** Ascospores. **16** *Pesotum* anamorph. **17** Conidia. Bars **13,16** 50  $\mu\text{m}$ ; **14** 10  $\mu\text{m}$ ; **15,17** 5  $\mu\text{m}$



**Table 3.** Morphological characteristics of *Ophiostoma abieticola*, *O. canum*, *O. introcitrinum*, and *Ophiostoma* sp. F.

Characteristics	<i>O. abieticola</i>	<i>O. canum</i> <sup>a</sup>	<i>O. introcitrinum</i> <sup>a</sup>	<i>Ophiostoma</i> sp. F <sup>b</sup>
<b>Perithecia</b>				
Width of base (µm)	117–175	42–180	117–225 (–260)	175–253
Length of neck (µm)	330–617	Up to 900 (–1100)	(290–) 337–510	438–1293
Width of neck at the base (µm)	39–58	18–25	33–65	54–68
Width of neck at the tip (µm)	(16–) 20–39	(7.5–) 9–15	18–35	19–29
<b>Ascospores</b>				
Shape	Orange-section-shaped or reniform	Orange-section-shaped or curved	Orange-section-shaped	Orange-section-shaped or allantoid
Size (µm)	(2.8–) 3.2–4.8 × 1.2–2.0	4–6.5 × 1.5–3	2–4 (–5) × 1–2	3.6–6 × 1.6–2.5
<b>Synnemata</b>				
Color	Dark brown to black at the base	Brown to black at the base	Creamy-white	Dark brown to black at the base
Length (µm)	230–427	85–800	Up to 850 (–1000)	340–817
<b>Conidia</b>				
Shape	Oblong	Spherical to limoniform	Oblong or clavate	Oblong or clavate
Size (µm)	(2.4–) 3.6–6.0 × 1.0–1.6	2–8	1.5–4 × 1–2	3–6 × 1.6–2

<sup>a</sup>Data from Upadhyay (1981)<sup>b</sup>Data from Yamaoka et al. (2001)**Figs. 18–20.** Scanning electron micrographs of conidiogenous cells. **18** *Ophiostoma nikkoense* (YCC-430). **19** *O. microcarpum* (YCC-439). **20** *O. abieticola* (YCC-424). Bars 1 µm

*O. introcitrinum* was *Hyalopesotum*, lacking pigmentation in the synnemata (Upadhyay and Kendrick 1975; Upadhyay 1981). These species are distinguishable from the *Pesotum* state of *O. abieticola*. *Ophiostoma abieticola* is also similar to *Ophiostoma* sp. F reported by Yamaoka et al. (2001), which was isolated from adult beetles of *Ips cembrae* (Heer) and their egg galleries attacking Japanese larch, *Larix kaempferi* (Kamb.) Carr. *Ophiostoma* sp. F generally produced larger perithecia (perithecial base, 175–253 µm in diameter; neck, 438–1293 µm long) and synnemata (340–817 µm long) than the *O. abieticola*. (Table 3). *Ophiostoma abieticola* appears to be distinguishable from *Ophiostoma* sp. F.

The present fungus was isolated from *C. montanus*, *P. proximus*, *D. hectographus*, and *D. autographus* infesting *A. mariesii* or *A. veitchii*. However, *O. abieticola* that was referred to as *Ophiostoma* sp. J by Yamaoka et al. (2004)

seemed to be closely associated with *D. hectographus* and *D. autographus* (Yamaoka et al. 2004).

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