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Pleosporales in Japan (1): the genus *Lophiostoma*

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Abstract Seven species of the genus *Lophiostoma* were the subject of this study. Among these, *Lophiostoma mucosum* is described and illustrated as a new species. All other species, *L. macrostomum*, *L. semiliberum*, *L. arundinis*, *L. caulium*, *L. caudatum*, and *L. winteri*, are reported for the first time in Japan. A key to the species of *Lophiostoma* in Japan is given.

Key words *Lophiostoma* · Lophiostomataceae · Pleosporales · Taxonomy

Introduction

The order Pleosporales is an enormous group in Ascomycota including 19 families, 179 genera, and more than 1400 species (Kirk et al. 2001). The fungi in the Pleosporales occur on dead leaves, herbaceous stems, tree branches, and wood as well as on many green leaves and stems. Some genera, such as *Pyrenophora* Fr. and *Cochliobolus* Drechsler, are known as important plant pathogens that cause leaf spot and blight disease (Luttrell 1973).

In recent years, several important taxonomic studies on this large and difficult group of Ascomycota have been published for species of North America, Europe, China, and elsewhere (Ahn and Shearer 1995, 1998, 1999; Barr 1990a,b; Crane and Shearer 1991; Holm and Holm 1988; Hyde and Fröhlich 1997; Shoemaker 1984a,b; Shoemaker and Babcock 1985, 1987a,b, 1989, 1992). On the other hand, our knowledge of the Japanese Pleosporales is very scanty, because the studies on these fungi have been done mostly from phytopathological aspects (Otani and Mikawa 1971). For instance, the family Lophiostomataceae in Pleosporales

comprises 15 genera, about 204 species (Kirk et al. 2001), whereas in Japan only 10 species in 5 genera have been recorded (Table 1).

In this article, the genus *Lophiostoma* Ces. & De Not. of Lophiostomataceae is considered, which has not been formally treated hitherto in Japan. *Lophiosphaera orientalis* I. Hino & Katum. (1964) on Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) has been recorded from Kyushu, Japan, but the genus *Lophiosphaera* Trevis. is a synonym of *Lophiostoma* (von Arx and Müller 1975; Holm and Holm 1988).

Materials and methods

Ascomata were crushed in a drop of water on a glass slide and then covered with a coverslip to observe pseudoparaphyses, asci, and ascospores. These structures in a water-mounted preparation were measured using an oil immersion lens at a magnification of $\times 1000$. Sometimes blue ink in distilled water was used to detect gelatinous sheath or appendages on or around ascospores. To observe details of ascomal anatomy, ascomata were boiled in water for about 5 min, and sectioned with a freezing microtome (HM 400R; MICROM, Germany). Sections were mounted in lactophenol (20 g phenol, 20 g lactic acid, and 40 g glycerol in 20 ml distilled water) and sealed with nail varnish for long-term observations. These light microscopy observations were conducted using Olympus microscopes (BH2 and CK40).

Single ascospore cultures were obtained according to the methods of Shearer (1993). Growth rate and colony characteristics were recorded from cultures grown on potato dextrose agar (PDA; Eiken, Tokyo, Japan) at 20°C in the dark. Colors were designated according to Kornerup and Wanscher (1978). Induction of sexual reproduction was attempted by placing a small piece of mycelial culture on rice straw agar (RSA) as follows: pieces (4 cm long) of rice straw were autoclaved in glass vials filled with distilled water, and then three pieces were placed in each Petri dish (60 mm)

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containing molten water agar (15–20 ml). The plates were incubated at room temperature (18–22°C) for about 2 weeks. When the straws were colonized, the plates were transferred to an incubator at 20°C under black light and observed for fructifications.

A herbarium specimen was borrowed from the herbarium of Hokkaido University (SAPA). All other specimens and isolates were deposited in the Herbarium of Hirosaki University, Fungi (HHUF).

Taxonomy

Lophiostoma Ces. & De Not., *Commun. Soc. Critt. Ital.* 1:219, 1863 (nom. cons.).

Type species: *Lophiostoma macrostomum* (Tode: Fr.) Ces. & De Not.

Anamorph: *Pleurophomopsis* Petr.-like (Leuchtmann 1985).

The genus *Lophiostoma* was established by Cesati and De Notaris in 1863. This genus, typified by *L. macrostomum*, is characterized as having immersed to erumpent ascomata with a slitlike ostiole; unequal thickness of peridium, which is broader laterally at the base; mostly clavate, bitunicate asci; 1- to several septate, hyaline to dark brown ascospores with terminal appendages or mucous sheath (Holm and Holm 1988).

Recently, the genus was reviewed by Chesters and Bell (1970, European species), Holm and Holm (1988, Swedish species), Barr (1992, North American species), and Yuan and Zhao (1994, Chinese species). Besides the monographic works of these authors, several species were added to the genus (Hyde and Aptroot 1998; Barr and Mathiassen 1998; Hyde et al. 2000). According to the latest edition of the *Dictionary of the Fungi* (Kirk et al. 2001), this genus comprises about 30 species.

The genus *Platystomum* Trevis. shares many characters with *Lophiostoma* but has muriform ascospores. Holm and Holm (1988) treated *Platystomum* as a synonym of *Lophiostoma*, and their concept of the genus has been followed by some later authors (e.g., Yuan and Zhao 1994; Checa 1997; Kirk et al. 2001). On the other hand, Barr (1990a) distinguished these two genera, and placed *Platystomum* in the Melanommatales based on the trabeculate hamathecium. This concept is accepted by Abdel-Wahab and Jones (2000). We also consider these two genera are not congeneric, and the genus *Platystomum* is not treated in this article.

Key to the species of *Lophiostoma* in Japan

1. Ascospores 1-septate 2
1. Ascospores more than 1-septate 3
2. Ascospores (28–)30–41.5(–45.5) × 4–7 μm, 2–4 guttules in each cell, with terminal appendages 2–10 μm long
L. macrostomum
2. Ascospores 25.5–40.5 = 4–6(–7) μm, with 3–4 guttules in each cell, without terminal appendages *L. semiliberum*
3. Ascospores without terminal appendages or sheath 4

3. Ascospores with terminal appendages or sheath 5
4. Ascospores, fusiform, 26–36(–40.5) × 6–8 μm, 5-septate, primary septum mostly median *L. arundinis*
4. Ascospores caudate, 25–32(–35.5) × 5–7.5 μm, 5(–6)-septate, primary septum suprmedian (mostly 0.46) *L. caudatum*
5. Ascospores with a sheath, (27.5–)30.5–40(–46.5) × (5.5–)7–10.5(–12) μm, hyaline, (1–)3(–5)-septate, on woody plant *L. mucosum*
5. Ascospores with terminal appendages 6
6. Ascospores hyaline, 5-septate, 26–35 × 6.5–9 μm
L. winteri
6. Ascospores brown 7
7. Ascospores 5(–7)-septate, 20–27(–29.5) × 5–7(–8) μm (mean, 23.8 × 6.2 μm), L/W mostly 3.9
L. caulium “var. a”
7. Ascospores usually more than 5-septate, L/W more than 4.0 8
8. Ascospores usually 7-septate, (26–)28–34(–36.5) × 5–7.5 μm (mean, 30.9 × 6.2 μm), both ends tapered
L. caulium “var. d”
8. Ascospores usually 7–9-septate, 30–40(–44) × 6–9 μm (mean, 34.8 × 7.5 μm), both ends relatively rounded
L. caulium “var. f”

1. *Lophiostoma macrostomum* (Tode: Fr.) Ces. & De Not., *Commun. Soc. Critt. Ital.* 1: 219, 1863.

Figs. 1, 10, 11, 31

≡ *Sphaeria macrostoma* Tode: Fr., *Syst. Mycol.* 2:469, 1823.

For other synonyms, see Holm and Holm (1988).

Ascomata 450–500 μm high, 400–450 μm diameter, globose to subglobose. Beak 150–170 μm long, 140–250 μm wide. Ascomal wall 25–43 μm thick at sides, composed of rectangular to polygonal brown cells of 5–18 × 2.5–5 μm. Pseudoparaphyses 2.5–4 μm thick at the base, narrower and 1–2 μm thick at the apical portion. Asci (88–)100–125(–135) × (9–)10–13(–14) μm (mean, 111.4 × 11.4 μm, *n* = 62), cylindrical to clavate, with a short stipe (14–27 μm), containing 8 biseriate ascospores. Ascospores (28–)30–41.5(–45.5) × 4–7 μm (mean, 35.7 × 5.4 μm, *n* = 223), L/W 5.5–7.7 (mostly 6.7), narrowly fusiform with acute ends, with a septum mostly median (0.50), hyaline, 2–4 guttules in each cell, smooth, with terminal appendages 2–10 μm long. Senescent ascospores 1- to 3-septate, hyaline to pale brown. Ascospores become 2- to 6-celled at germination. Germ tube mainly from both end cells.

Cultural characteristics. Colonies on PDA 1.6 cm in diameter after incubation for 4 weeks, Khaki (4D5); reverse Olive-Brown (4F5); no pigment is produced. On RSA, numerous black ascomata were produced on the surface of rice straw within 2 months, but no anamorph was formed.

Materials examined. On twigs of *Morus bombycis* Koidz.: Campus of Hirosaki University, Hirosaki, Aomori, 140°28' E, 40°35' N, Apr. 24, 2001, KT.508 (HHUF 27288, culture 4106). On endocarps of *Prunus* sp.: same locality, Apr. 24, 2001, KT.509 (HHUF 27289). On stems of an unknown herbaceous plant: Sanpinai, Hirosaki, Aomori,

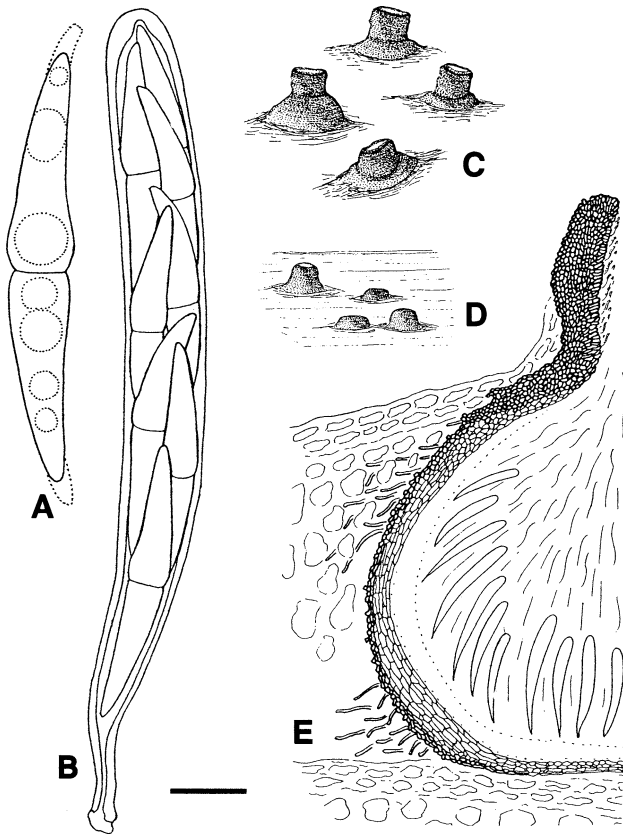


Fig. 1. *Lophiostoma macrostomum*. **A** Ascospore. **B** Ascus. **C,D** Surface view of ascomata. **E** Ascoma in sagittal section. **A** culture 4108; **B** HHUF 27292; **C,E** HHUF 27293; **D** HHUF 27288. Bar **A** 6.7 μm ; **B** 10 μm ; **C,D** 400 μm ; **E** 60 μm

140°30.128' E, 40°34.507' N, Aug. 5, 2001, KT.635 (HHUF 27290, culture 4107); KT.636-1 (HHUF 27291); Aug. 12, 2001, KT.666-1 (HHUF 27292). On stems of an unknown plant: Oowasawa River, river bank, Kadoke, Hirosaki, Aomori, 140°30.532' E, 40°34.276' N, Aug. 25, 2001, KT.709 (HHUF 27293, culture 4108). On stems of *Polygonum* sp.: Nairo River, river bank, Kafuka, Isl. Rebun, Hokkaido, 141°03.223' E, 45°23.196' N, Aug. 30, 2001, KT.730 (HHUF 27294).

Notes. *Lophiostoma macrostomum* is the type species of the genus. It is common on wood and bark of frondose trees and shrubs (Holm and Holm 1988), and also on herbaceous stems, especially on *Agrimonia*, *Rumex*, and *Urtica* (Ellis and Ellis 1985, as *L. angustilabrum*). Recently, Hyde et al. (2000) reported it from palms.

This species is easily distinguishable from other species of *Lophiostoma* by its 1-septate, hyaline, narrowly fusiform ascospores with short terminal appendages. Morphological characters of our specimens agree well with those of *L. macrostomum* in the descriptions provided by Holm and Holm (1988), although the length of ascospores is somewhat greater [(28.2–)30.0–41.5(–45.5) μm vs. 26–33 (–38) μm].

In vitro, this species formed only ascomata in all isolates, as is noted by Leuchtman (1985).

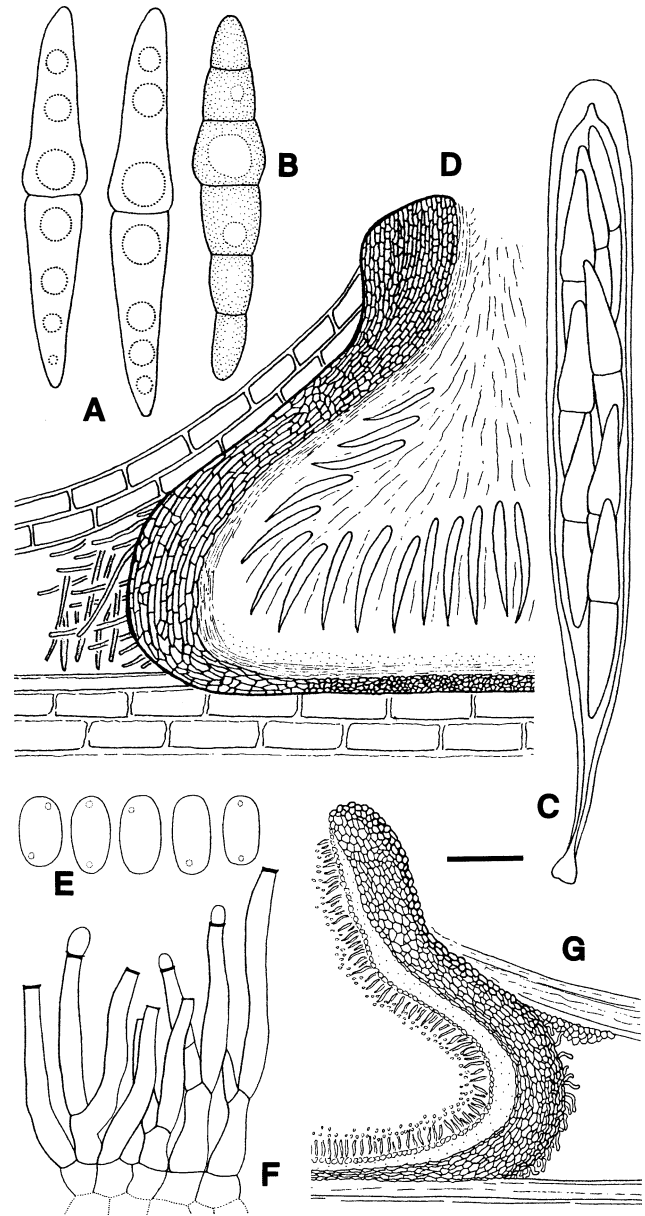


Fig. 2. *Lophiostoma semiliberum*. **A,B** Ascospores (**B** senescent spores). **C** Ascus. **D** Ascoma in frontal section. **E** Conidia. **F** Conidiophores. **G** Conidioma in frontal section. **A** HHUF 27300; **B** HHUF 27295; **C,D** HHUF 27296; **E,F** culture 4111; **G** culture 4109. Bar **A,B** 6.7 μm ; **C** 10 μm ; **D** 60 μm ; **E** 3 μm ; **F** 5 μm ; **G** 30 μm

2. *Lophiostoma semiliberum* (Desm.) Ces. & De Not., Commun. Soc. Critt. Ital. 1:20, 1863.

Figs. 2, 12, 13, 28, 32
 = *Sphaeria semilibera* Desm., Ann. Sci. Nat. Bot. Ser. 3. 6:78, 1846.

= *Lophiotrema semiliberum* (Desm.) Sacc., Michelia 1:338, 1878.

Ascomata 500 μm high, 400–600 μm diameter, subglobose to laterally compressed, flattened at the base. Beak ~150 μm long, 150–250 μm wide. Ascomal wall 40–70 μm thick laterally, 20–30 μm thick at the base. Pseudoparaphyses ~1.5–2.5 μm thick. Asci 92–127(–146) ×

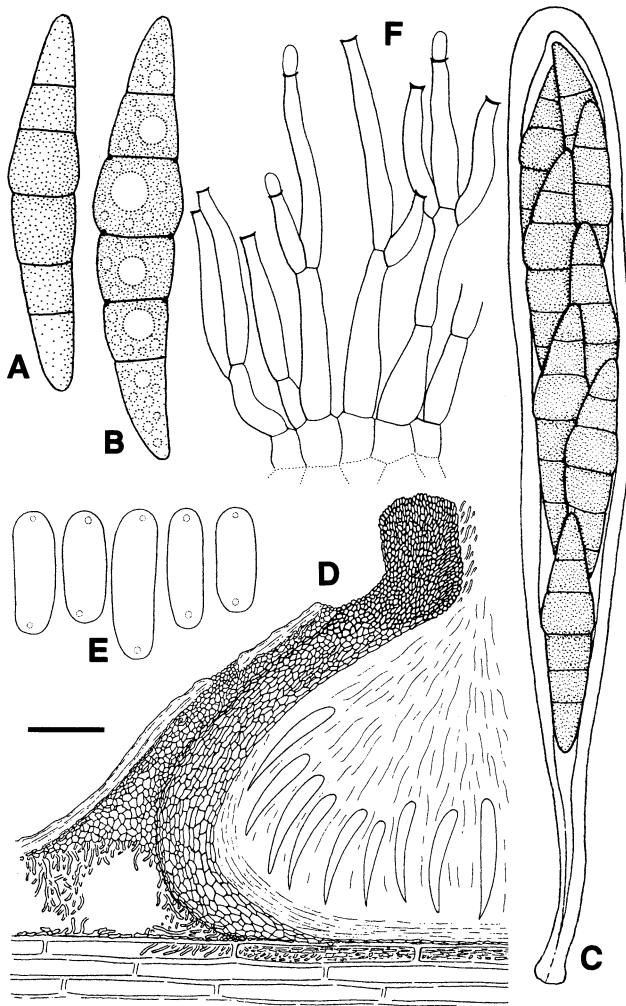


Fig. 3. *Lophiostoma arundinis*. **A,B** Ascospores. **C** Ascus. **D** Ascoma in frontal section. **E** Conidia. **F** Conidiophores. **A** SAPA; **B** culture 4112; **C** HHUF 27303; **D** HHUF 27413; **E,F** culture 4113. Bar **A,B** 6.7 μm ; **C** 10 μm ; **D** 60 μm ; **E** 3 μm ; **F** 5 μm

10–14 μm (mean, $107 \times 11.5 \mu\text{m}$, $n = 32$), cylindrical to clavate, short-stalked, with 8 irregularly biseriata ascospores. Ascospores $25.5\text{--}40.5 \times 4\text{--}6(-7) \mu\text{m}$ (mean, $32.2 \times 5.1 \mu\text{m}$, $n = 124$), L/W 5.1–7.7 (mean, 6.3, $n = 124$), fusiform, with 1 transverse septum, hyaline to pale green, without sheath or appendage, with 3–4 guttules in each cell. Senescent ascospores pale brown, 3- to 5-septate in $1 + 1 + 1$ or $2 + 1 + 2$ (septa of upper hemisphere + the primary septum + septa of lower hemisphere).

Cultural characteristics. Colonies on PDA 2.5 cm in diameter after 4 weeks, Silver-White (2B2), with irregular margin; reverse similar; no pigments formed. On RSA, conidiomata were formed on the surface of rice straw after incubation for 1 month. Conidiomata 100–170(–300) μm high, 150–230 μm diameter, scattered or sometimes in groups, subglobose with a flattened base, covered with brownish septate hyphae 2.5–3 μm wide. Beak 60(–125) μm long, ~75 μm wide, central, papillate to cylindrical, composed of dark brown polygonal to subglobose cells of 2.5–8 \times 2–3 μm . Wall 25 μm thick at the side, slightly thinner at

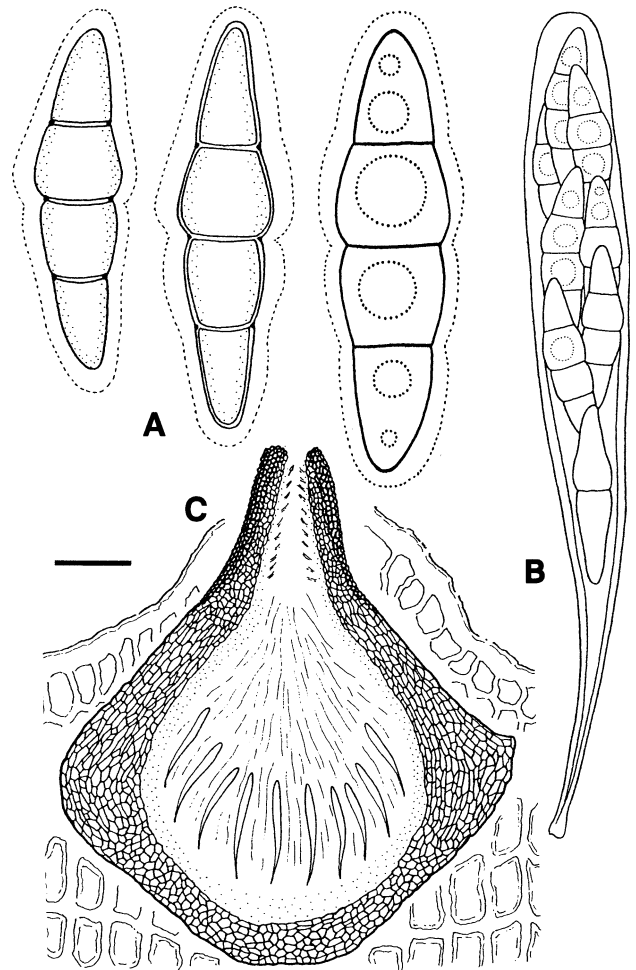


Fig. 4. *Lophiostoma mucosum*. **A** Ascospores. **B** Asci. **C** Ascoma in sagittal section (HHUF 27335 holotype). Bar **A** 6.7 μm ; **B** 15 μm ; **C** 100 μm

the base, composed of subglobose to polygonal cells of 2.5–5 \times 1–2 μm ; inner layer 7–10 μm wide, of hyaline compressed cells. Conidiophores 9–21(–30) \times 2–3 μm , septate, branched, hyaline, and formed all around the locular cavity. Conidiogenous cells phialidic. Conidia 2–3.5 \times 1–2 μm (mean, $2.9 \times 1.6 \mu\text{m}$, $n = 50$), L/W 1.5–2.3 (mean, 1.8, $n = 50$), ellipsoid to obovoid, aseptate, hyaline, smooth. Sometimes immature ascomata were formed, which did not mature within 3 months.

Materials examined. On culms of *Phragmites australis* (Cav.) Trin. ex Steud.: Toyohira River, river bank, Sapporo, Hokkaido, $141^{\circ}21.489' \text{ E}$, $43^{\circ}02.229' \text{ N}$, June 23, 2000, KT.304 (HHUF 27295); July 7, 2000, KT.374 (HHUF 27296); Sanpinai, Hirosaki, Aomori, $140^{\circ}30.128' \text{ E}$, $40^{\circ}34.507' \text{ N}$, May 27, 2001, KT.533 (HHUF 27297); Hirakawa, river bank, Hiraka, Aomori, $140^{\circ}32.030' \text{ E}$, $40^{\circ}34.005' \text{ N}$, Aug. 5, 2001, KT.652 (HHUF 27298, culture 4109). On culms of an unknown Gramineae: Oowasawa River, river bank, Kadoke, Hirosaki, Aomori, $140^{\circ}30.532' \text{ E}$, $40^{\circ}34.276' \text{ N}$, Aug. 4, 2001, KT.622 (HHUF 27299, culture 4110). On culms of an unknown herbaceous plant: Oowasawa River, river bank, Simizumori, Hirosaki,

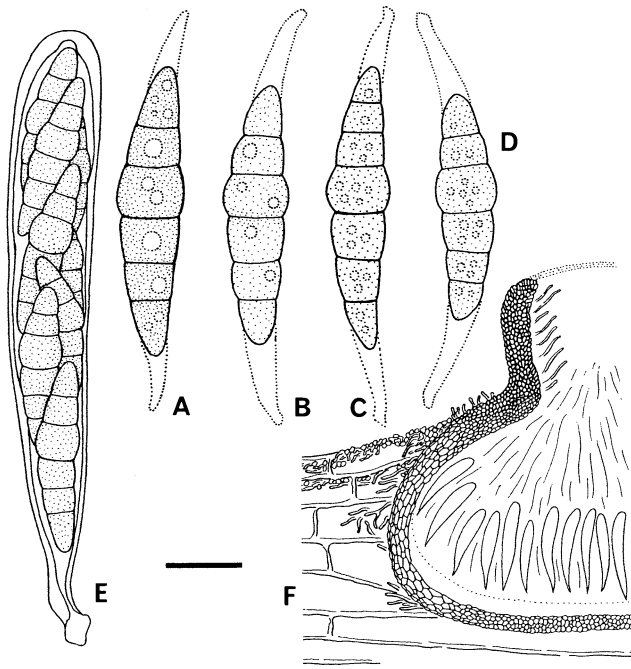


Fig. 5. *Lophiostoma caulium* "var. a". **A–D** Ascospores. **E** Ascus. **F** Ascoma in frontal section. **A** HHUF 27308; **B,E** HHUF 27307; **C** culture 4114; **D** culture 4115; **F** HHUF 27306. Bar **A–D** 6.7 μ m; **E** 10 μ m; **F** 60 μ m

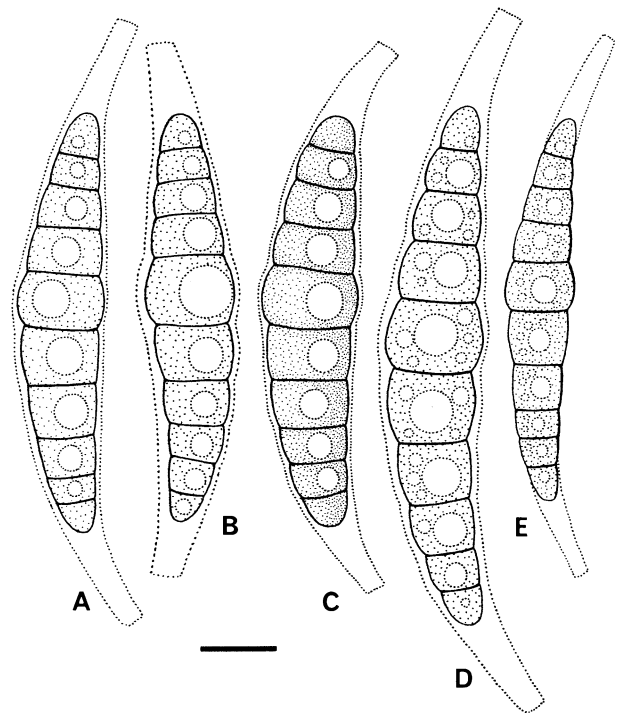


Fig. 7. *Lophiostoma caulium* "var. f". **A–E** Ascospores. **A** HHUF 27315; **B** HHUF 27313; **C** HHUF 27311; **D** culture 4119; **E** culture 4121. Bar 6.7 μ m

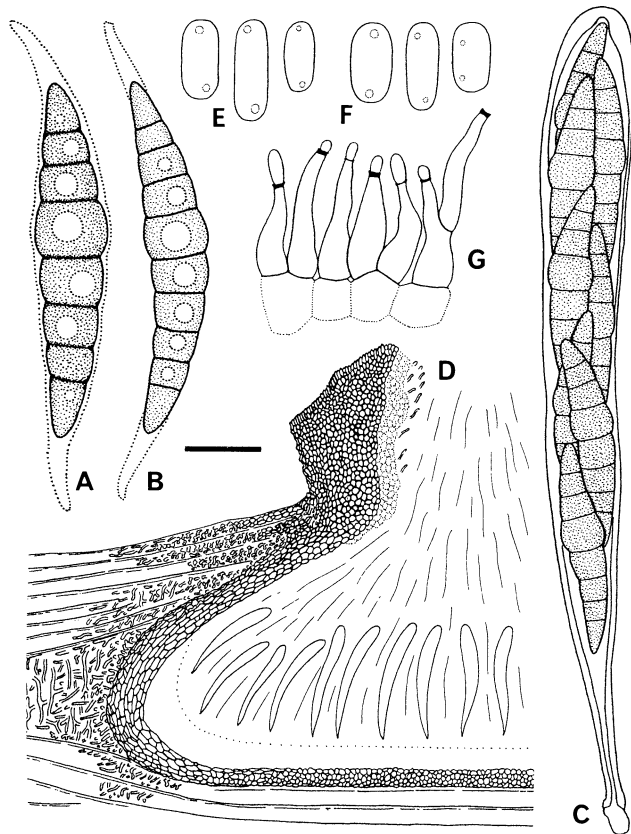


Fig. 6. *Lophiostoma caulium* "var. d". **A,B** Ascospores. **C** Ascus. **D** Ascoma in frontal section. **E,F** Conidia. **G** Conidiophores. **A,C** HHUF 27309; **B,D** HHUF 27310; **E,G** culture 4117; **F** culture 4116. Bar **A,B** 6.7 μ m; **C** 10 μ m; **D** 60 μ m; **E,F** 3 μ m; **G** 5 μ m

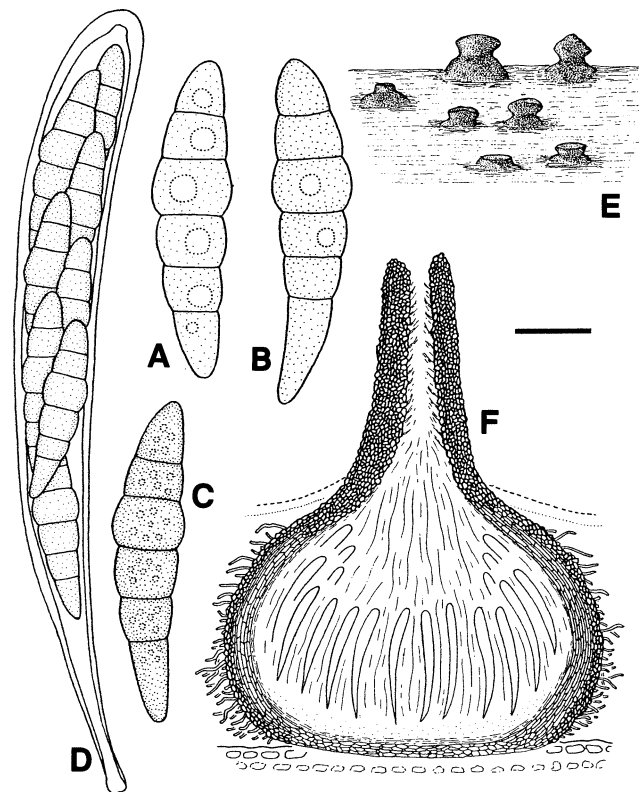


Fig. 8. *Lophiostoma caudatum*. **A–C** Ascospores. **D** Asci. **E** Surface view of ascomata. **F** Ascoma in sagittal section. **A,D–F** HHUF 27318; **B** HHUF 27320; **C** culture 4124. Bar **A–C** 6.7 μ m; **D** 10 μ m; **E** 400 μ m; **F** 60 μ m

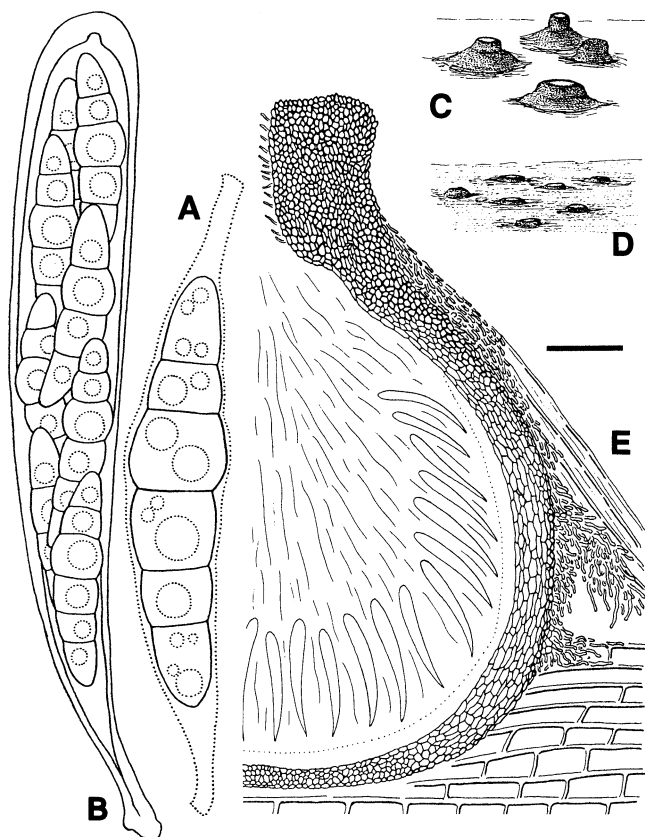


Fig. 9. *Lophiostoma winteri*. **A** Ascospores. **B** Ascus. **C, D** Surface view of ascomata. **E** Ascoma in frontal section. **A** culture 4122; **B, C, E** HHUF 27317; **D** HHUF 27316. Bar **A** 6.7 μm ; **B** 10 μm ; **C, D** 400 μm ; **E** 60 μm

Aomori, 140°30.163' E, 40°34.181' N, Oct. 28, 2001, KT.828 (HHUF 27300, culture 4111).

Notes. *Lophiostoma semiliberum* is most frequently found on graminaceous hosts, especially on *Phragmites* (Chesters and Bell 1970). Holm and Holm (1988) also recorded it on *Brachypodium*, *Deschampsia*, *Elymus*, *Elytrigia*, *Festuca*, *Glyceria*, *Melica*, *Nardus*, and *Secale*. It resembles *L. macrostomum*, but is easily distinguished from the latter by its ascospores without short terminal appendages.

This species had been considered as an immature stage of *L. arundinis* (Munk 1957; Eriksson 1967; Eriksson and Yue 1986). Actually, when these two species occur together on the same sample, it is difficult to distinguish them under a dissecting microscope because they have similar ascomal appearance. The senescent ascospores of *L. semiliberum* somewhat resemble those found in *L. arundinis*. However, the latter species has darker and wider ascospores (L/W 4.5 in *L. arundinis*), even if in an early stage of development. Also, they possess different anamorphic states, and *L. semiliberum* usually forms shorter conidia than *L. arundinis* (2.9 \times 1.6 μm vs. 4.8 \times 1.7 μm on average). Therefore, we regard *L. semiliberum* as a distinct species, as did Chesters and Bell (1970) and Holm and Holm (1988).

3. *Lophiostoma arundinis* (Pers.: Fr.) Ces. & De Not., *Commun. Soc. Critt. Ital.* 1:220, 1863. Figs. 3, 14, 15, 29

\equiv *Sphaeria cristata* β *arundinis* Pers., *Synop. Methods Fungi* 56, 1801; *Sphaeria arundinis* Pers.: Fr., *Syst. Mycol.* 2:510, 1823.

For other synonyms, see Holm and Holm (1988).

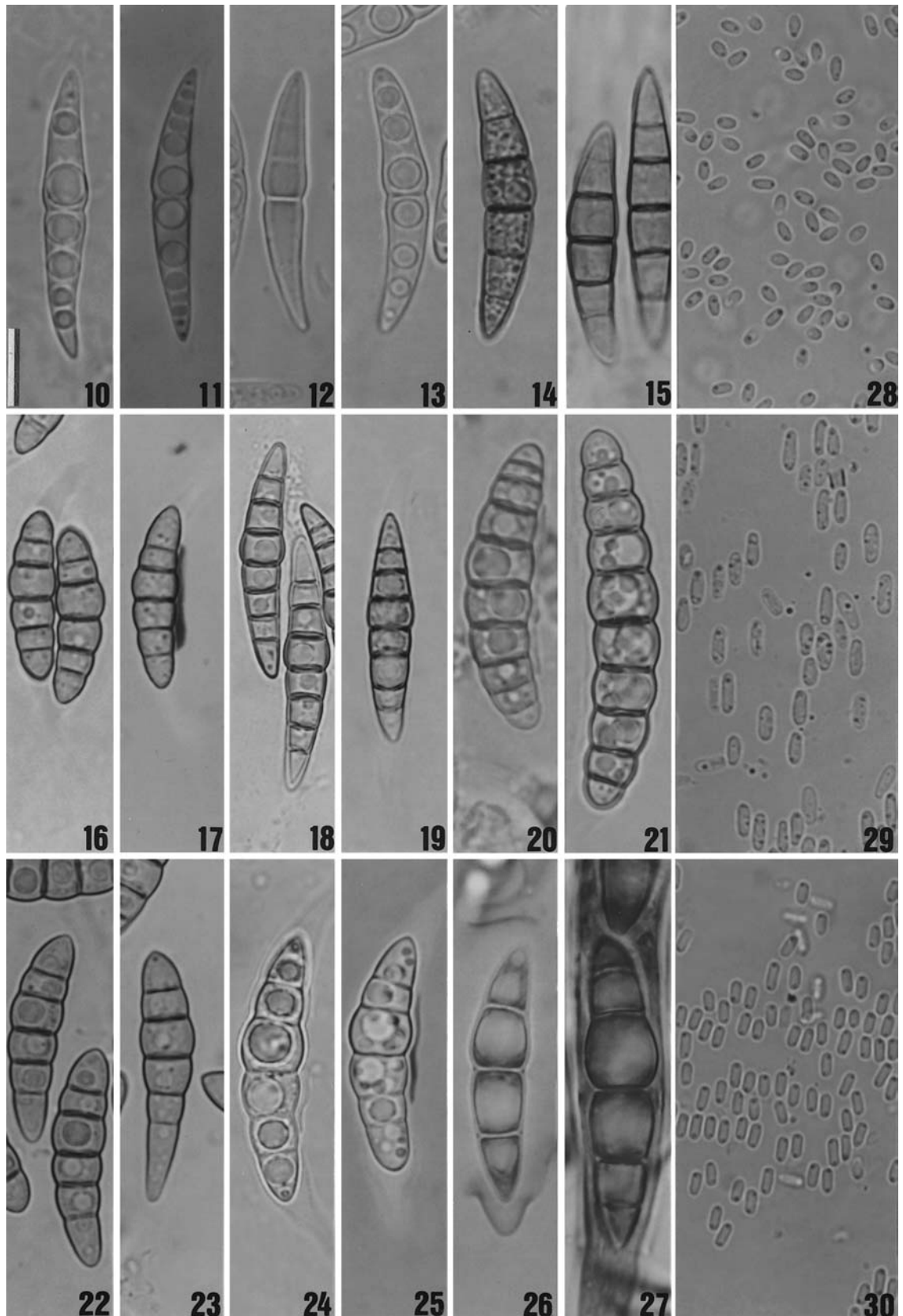
Ascomata 330–450 μm high, 500–650 μm diameter, subglobose and flattened at the base. Beak 100–150 μm long, 140–250 μm wide. Ascomal wall 38–50 μm thick at the side, very thin at the base. Pseudoparaphyses \sim 1–3 μm thick. Asci 100–135(–188) \times 12.5–16(–17) μm , clavate, short-stalked, 15–40 μm long, with 8 irregularly biseriolate ascospores. Ascospores 26–36(–40.5) \times 6–8 μm (mean, 31.9 \times 7.1 μm , $n = 65$), L/W 4.0–5.0(–5.4) (mean, 4.5, $n = 65$), fusiform, 5-septate in 2 + 1 + 2, yellowish-brown to brown with slightly lighter colored end cells, with or without guttules, slightly echinulate, without sheath or appendage.

Cultural characteristics. Colonies on PDA grow extremely slowly, less than 0.5 cm in diameter after 4 weeks, Olive-Brown (4D4); reverse similar. On RSA, ascomata were found within 2 months, resembling those occurring in nature. Black masses of ascospores exude from the ostiole. Ascospores were slightly larger than those found on the host, measuring 32–40(–45) \times 6–9 μm (mean, 35.8 \times 7.4 μm , $n = 60$). Sometimes conidiomata were formed on the surface of rice straw. Conidiomata produce white masses of conidia from the ostiole. Conidiophores 12–30 \times 2–4 μm , septate, branched, hyaline, and formed all around the locular cavity. Conidiogenous cells phialidic. Conidia 4–6 \times 1.5–2 μm (mean, 4.8 \times 1.7 μm , $n = 30$), cylindrical to ellipsoid, aseptate, hyaline, smooth.

Materials examined. On culms of *Phragmites australis* (Cav.) Trin. ex Steud.: Toyohira River, river bank, Sapporo, Hokkaido, 141°21.489' E, 43°02.229' N, June 23, 2000, KT.301-3 (HHUF 27301); July 7, 2000, KT.362 (HHUF 27302); KT.367-2 (HHUF 27303); Oowasawa River, river bank, Kadoke, Hirosaki, Aomori, 140°30.532' E, 40°34.276' N, July 29, 2001, KT.606 (HHUF 27304, culture 4112); Oowasawa River, river bank, Horikoshi, Aomori, 140°31.181' E, 40°34.304' N, Aug. 14, 2001, KT.668 (HHUF 27413); Hirakawa, river bank, Hiraka, Aomori, 140°32.030' E, 40°34.005' N, Aug. 5, 2001, KT.651 (HHUF 27305, culture 4113). On culms of *Sasa palmata* (Lat.-Marl. ex N.E.Br.) Nakai: Sapporo, Hokkaido, May 19, 1928, K. Sasaki (SAPA as *Melanomma* sp.).

Notes. *Lophiostoma arundinis* is found on dead culms of *P. australis* and associated with other pyrenomycetes such as *L. semiliberum* and *Massarina arundinacea* (Sowerby: Fr.) Leuchtm., but it is easily distinguished from the latter two species by its brown 5-septate ascospores.

This species is common in northern Japan, although Holm and Holm (1988) stated, “*L. arundinis* is perhaps a rare species, at least in Scandinavia.” It usually occurs on *P. australis*, and rarely on *Glyceria fluitans* (L.) R.Br. A specimen on *Sasa* (SAPA) differed from *L. arundinis* on *Phragmites* in its erumpent long beak and thin pseudoparaphyses recalling those of *Astrosphaeriella* species. Its other morphological features, particularly of the ascospores, however, agree well with those in the description of *L. arundinis* provided by Holm and Holm (1988).



Figs. 10–30. *Lophiostoma* spp. **10–27** Ascospores. **28–30** Conidia. **10,11** *L. macrostomum* (**10** HHUF 27294; **11** culture 4107). **12,13,28** *L. semiliberum* (**12** HHUF 27298; **13** HHUF 27300; **28** culture 4111). **14,15,29** *L. arundinis* (**14** culture 4112; **15** SAPA; **29** culture 4113). **16,17** *L. caulium* “var. a” (**16** HHUF 27307; **17** culture 4114). **18,19,30** *L. caulium* “var. d” (**18** HHUF 27310; **19** HHUF 27309; **30** culture 4117). **20,21** *L. caulium* “var. f” (**20** HHUF 27311; **21** culture 4119). **22,23** *L. caudatum* (**22** HHUF 27318; **23** HHUF 27320). **24,25** *L. winteri* (**24** HHUF 27316; **25** culture 4123). **26,27** *L. mucosum* (HHUF 27335). Bar 10µm; all same magnification

4. *Lophiostoma mucosum* Kaz. Tanaka & Y. Harada, sp. nov. Figs. 4, 26, 27, 34

Ascomata 400–700 μm alta, 400–750 μm diametro, copiosa, dispersa vel congregata, subimmersa vel dein erumpentia, subglobosa vel aliquantum pyriformia, ad basim interdum villosa. Rostrum 140–230 μm longum, 140–180 μm latum, papillatum vel cylindraceum, ex cellulis pullatis vel nigris subglobosis vel polygonis pachydermicis 7–12 μm compositum. Periphyses hyalinae. Parietis ad latus 35–120 μm crassus, ex cellulis brunneis rectangulatis vel polygonis, 20–28 \times 3–15 μm compositus, ad basim 60–100 μm crassus, ex cellulis subglobosis vel polygonis, 5–13 μm diameter compositus. Pseudoparaphyses cellulosa, 1–1.5 μm latae. Asci 120–175(–192.5) \times 15–20.5 μm , bitunicati, copiosi, clavati, apice rotundati, stipitati, 30–60(–70) μm longi, (4–)8-spori, biseriatati. Ascospores (27.5–)30.5–40(–46.5) \times (5.5–)7–10.5(–12) μm , fusiformes, rectae vel leviter curvatae, (1–)3(–5)-septatae, cum septum primum fere supramedium efferentes, hyalinae vel pallide virides, guttulate vel eguttulate, laeves, strato mucoso 2–5 μm lat circumdatae.

Holotypus: On twigs of an unknown woody plant: Toyohira River, river bank, Sapporo, Hokkaido, 141°21.489' E, 43°02.229' N, Oct. 8, 2000, KT.438 (HHUF 27335).

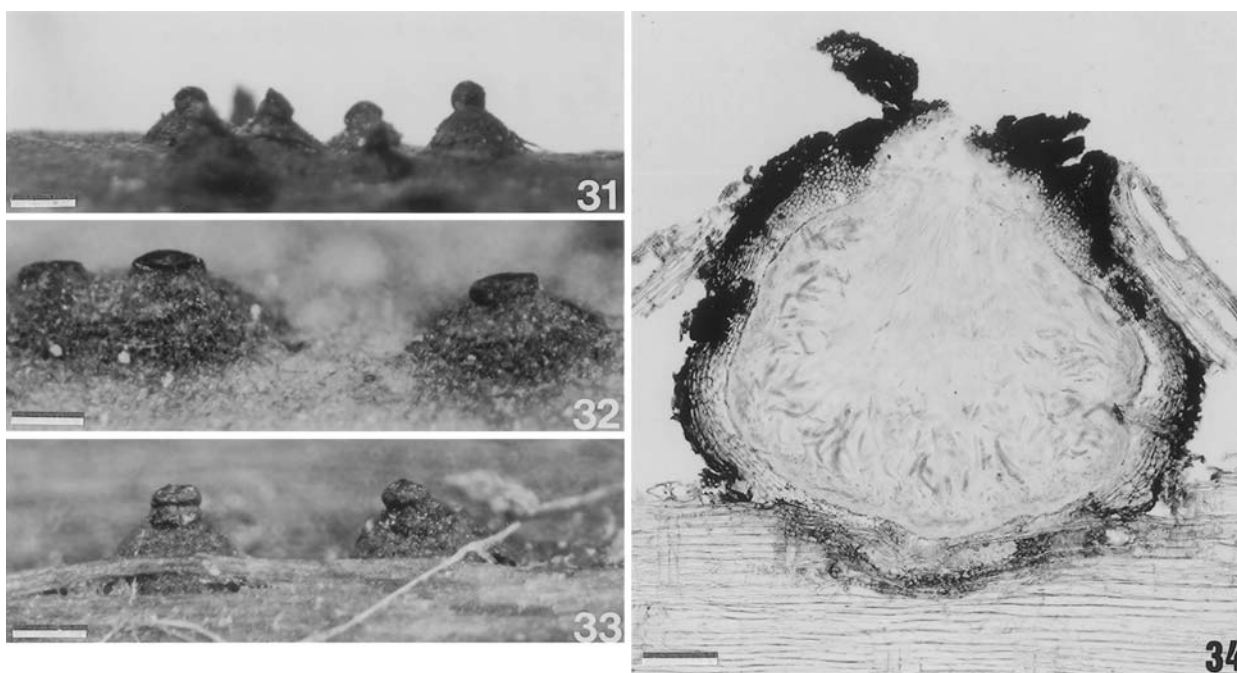
Etymology: From the Latin mucosus, in reference to the mucilaginous sheath of the ascospores.

Ascomata 400–700 μm high, 400–750 μm diameter, numerous, scattered to clustered, semiimmersed or becoming erumpent, subglobosa to somewhat pyriform, sometimes coated with brown short hyphae 3–5 μm wide at the base. Beak 140–230 μm long, 140–180 μm wide, papillate to cylin-

drical, with hyaline periphyses; cells subglobose to polygonal, dark brown to black, thick-walled, 7–12 μm diameter. Ascomal wall at sides 35–120 μm thick, composed of parallel rows of rectangular to polygonal brown cells of 20–28 \times 3–15 μm in 10–13 layers; at the base 60–100 μm thick, composed of subglobose to polygonal cells of 5–13 μm diameter. Pseudoparaphyses numerous, cellular, about 1–1.5 μm thick, septate, branched, anastomosed, with guttules. Asci 120–175(–192.5) \times 15–20.5 μm , bitunicate, numerous, basal and somewhat lateral, clavate, rounded at the apex, with a long stalk of 30–60(–70) μm long, containing (4–)8 biseriate ascospores. Ascospores (27.5–)30.5–40(–46.5) \times (5.5–)7–10.5(–12) μm (mean, 35.0 \times 8.8 μm , $n = 60$), L/W 3.5–4.6(–5.5) (mean, 4.0, $n = 60$), fusiform, straight or at times slightly curved, (1–)3(–5)-septate, constricted at the primary septum, mostly supramedian (0.46–0.52; mean, 0.49, $n = 60$), slightly constricted at other septa, the second cell from the apex enlarged downward, hyaline to pale green, with or without guttules, smooth, with a sheath 2–5 μm wide.

Cultural characteristics. Not examined.

Notes. The compressed neck of this fungus is not conspicuous, unlike most other lophiostomataceous fungi. However, it belongs to the genus *Lophiostoma* because it has an unequally thickened peridium composed of rectangular to polygonal brown cells in parallel rows, papillate to cylindrical beak with hyaline periphyses, clavate asci, and fusiform (1–)3(–5)-septate hyaline ascospores. The presence of a compressed neck and a slitlike ostiole has been used to distinguish the Lophiostomataceae from other groups (Huhndorf 1992). However, these striking features are highly unstable (Holm and Holm 1988).



Figs. 31–34. *Lophiostoma* spp. 31–33 Ascomata on host surface. 34 Ascoma in frontal section. 31 *L. macrostomum* (HHUF 27291). 32 *L. semiliberum* (HHUF 27296). 33 *L. winteri* (HHUF 27317). 34 *L. mucosum* (HHUF 27335). Bars 31–33 250 μm ; 34 100 μm

Within the genus *Lophiostoma*, our collection is most similar to *L. heterosporum* (De Not.) M.E. Barr (basonym: *Sphaeria heterospora* De Not.) in the size of ascospores. *Lophiostoma heterosporum* has been described and illustrated in various genera by some authors; e.g., in *Leptosphaeria* by Müller (1950), in *Trematosphaeria* by Holm (1957), Shoemaker and Babcock (1989), and Ellis and Ellis (1985), in *Phaeosphaeria* by Boise (1985), and in *Lophiostoma* by Barr (1992) and Ahn and Shearer (1999). The fungus under discussion is different from *L. heterosporum* in several ways, when compared with the descriptions of the latter species by these authors. Ascospores of the fungus are considerably larger (400–750 µm diameter) than those reported for *L. heterosporum* by Boise (1985; 240–450 µm diameter). Also, the peridium is thicker in the former than in the latter (35–120 µm vs. 20–50 µm given by Ahn and Shearer 1999). Asci of our collection are 120–175(–192.5) µm long, (4–)8-spored and with a long stalk (30–60(–70) µm long) whereas in the latter, asci are shorter to a considerable extent (up to 136 µm long) (Boise 1985), 8-spored, and with a short stalk (Shoemaker and Babcock 1989). In the fungus, ascospores are (1–)3(–5)-septate, mostly suprasedian (0.49), slender (L/W 4.0), scarcely pigmented, and with a mucilaginous sheath, whereas ascospores of the latter are consistently 3-septate, median (0.50), wider (L/W 3.3), pale brown, and without sheath (Boise 1985; Shoemaker and Babcock 1989). Furthermore, *L. heterosporum* is collected, unlike *L. mucosum*, on herbaceous plants, such as *Iris*, and rarely on *Plantago* and *Typha* (Ahn and Shearer 1999; Barr 1992; Boise 1985).

5. *Lophiostoma caulium* (Fr.) Ces. & De Not., *Commun. Soc. Critt. Ital.* 1:219, 1863.

“var. a” Holm & Holm, *Symb. Bot. Ups.* 28(2):13, 1988.

Figs. 5, 16, 17

Ascospores 200–300 µm high, 230–350 µm diameter, globose to subglobose, flattened at the base. Beak 95–120 µm long, 90–150 µm wide. Ascospore wall 12–15 µm thick at the side, slightly thinner at the basal margin. Pseudoparaphyses 1–3 µm thick. Asci 72.5–90(–100) × 10.5–16 µm (mean, 83.0 × 12.6 µm, $n = 53$), clavate, with a short stipe 10–22(–30) µm long, 8-spored. Ascospores 20–27(–29.5) × 5–7(–8) µm (mean, 23.8 × 6.2 µm, $n = 150$), L/W 3.4–4.4 (mean, 3.9, $n = 150$), fusiform, usually with 5 septa (2 + 1 + 2) or rarely 6–7 septa (3 + 1 + 2, 3 + 1 + 3), with the primary septum mostly submedian (0.49–0.53; mean, 0.51, $n = 129$), brown, with or without guttules, smooth, with terminal appendages of 5–8(–10) µm.

Cultural characteristics. Colonies on PDA 2.5 cm in diameter after 4 weeks, Tobacco-Brown (5F6), with white margin; reverse similar; no pigment is produced. On RSA, numerous ascospores were formed within 2 months, but no conidial state has been found. Ascospores are similar in shape and size to those found in nature, 18–26 × 5–6 µm (mean, 21.3 × 5.6 µm, $n = 60$).

Materials examined. On stems of an unknown herbaceous plant: Oowasawa River, river bank, Kadoke, Hirosaki, Aomori, 140°30.532' E, 40°34.276' N, July 29, 2001, KT.603 (HHUF 27306, culture 4114); Sanpinai,

Hirosaki, Aomori, 140°30.128' E, 40°34.507' N, Aug. 5, 2001, KT.633 (HHUF 27307, culture 4115). On twigs of an unknown woody plant: Hirakawa, river bank, Hiraka, Aomori, 140°32.030' E, 40°34.005' N, Aug. 5, 2001, KT.638-2 (HHUF 27308).

Notes. Several authors have considered *L. caulium* to be a complex of morphologically several slightly different forms, and these forms are lumped together under the broad concept of *L. caulium*. Chesters and Bell (1970) stated, “an extensive study of the herbarium material by us has shown that these ‘species’ are linked by a number of intermediate forms having various spore size and degrees of septation.” To survey the variation, Holm and Holm (1988) divided the “species” examined by them into five “varieties,” without formal status or names, but indicated with letters a–e. They considered that these varieties may correspond to natural taxa in some degree.

Our materials described here agree with “*L. caulium* var. a” on the basis of the description provided by Holm and Holm (1988). The ascospores are fusiform, brown, with terminal appendages, 20–27(–29.5) × 5–7(–8) µm, L/W 3.9 on average, and mostly 5-, rarely 6–7-, -septate.

Single ascospore isolates from “var. a” formed the teleomorph in pure culture and produced ascospores that are identical to those on the herbarium specimens. Also in “var. f” (see 7), which has 7–9-septate large ascospores, those are similar between natural and culture conditions. We believe that these “varieties” should be treated at the species level because it seems that ascospore dimensions and degrees of septation are stable in each “variety.” However, we follow the concept of *L. caulium* proposed by Holm and Holm (1988), until further information from type specimens is made available.

6. *Lophiostoma caulium* (Fr.) Ces. & De Not., *Commun. Soc. Critt. Ital.* 1:219, 1863.

“var. d” Holm & Holm, *Symb. Bot. Ups.* 28(2):14, 1988.

Figs. 6, 18, 19, 30

Ascospores 320–500 µm high, 350–630 µm diameter, ellipsoidal to subglobose, flattened at the base. Beak 100–150 µm long, 200–300 µm wide. Ascospore wall 20–30(–60) µm thick at the side, much thinner at the base. Pseudoparaphyses 1.5–2.5 µm thick. Asci (90–)97.5–120 × 10–13 µm (mean, 104.7 × 11.3 µm, $n = 37$), clavate, with a short stalk 13–25 µm long, containing 8 biseriolate ascospores. Ascospores (26–)28–34(–36.5) × 5–7.5 µm (mean, 30.9 × 6.2 µm, $n = 90$), L/W 4.5–5.4 (mean, 5.0, $n = 90$), narrowly fusiform with acute ends, 7-septate (3 + 1 + 3), very rarely 9-septate (4 + 1 + 4), with the primary septum mostly submedian (0.49–0.53; mean, 0.51, $n = 90$), brown, guttulate, smooth, with terminal appendages 4–10 µm long. Ascospores germinating from each cell.

Cultural characteristics. Colonies on PDA 2.2 cm in diameter after 4 weeks, Blond (4C4) in the center, other part white; reverse similar; no pigment is produced. On RSA, only conidiomata were formed on the surface of rice straw within 6 weeks. Conidiomata ~120–140 µm high, 130–250 µm diameter, numerous, with a short beak. Conidiophores (4–)6–12(–15) × 1.5–3 µm (mean, 9.0 × 2.3 µm, $n =$

39), septate, branched, hyaline, and are formed all around the locular cavity. Conidiogenous cells phialidic. Conidia $3-4 \times 1-2 \mu\text{m}$ (mean, $3.3 \times 1.5 \mu\text{m}$, $n = 60$), L/W 1.8–3.1 (mean, 2.3, $n = 60$), ellipsoid, aseptate, hyaline, smooth.

Materials examined. On stems of an unknown herbaceous plant: Oowasawa River, river bank, Kadoke, Hirosaki, Aomori, $140^{\circ}30.532' \text{ E}$, $40^{\circ}34.276' \text{ N}$, July 29, 2001, KT.604 (HHUF 27309, culture 4116); Sept. 16, 2001, KT.777 (HHUF 27310, culture 4117).

Notes. The specimens examined are close to "*L. caulium* var. d" *sensu* Holm and Holm (1988). This fungus differs from "var. a" in having 7- (rarely 9)-septate and also longer ($30.9 \mu\text{m}$ on average) and more slender (L/W 5.0 on average) ascospores compared with 5- (rarely 7)-septate and shorter ($23.8 \mu\text{m}$ on average) and plump (L/W 3.9 on average) ascospores of "var. a".

We obtained six isolates from two materials, all of which, unlike "var. a", produced only *Pleurophomopsis*-like conidiomata.

7. *Lophiostoma caulium* (Fr.) Ces. & De Not., Commun. Soc. Critt. Ital. 1:219, 1863. "var. f"

Figs. 7, 20, 21

Ascomata 260–320(–450) μm high, 220–300(–530) μm diameter, numerous, scattered to clustered, immersed to erumpent, globose to subglobose, with brown septate hyphae 3–4 μm thick at sides, with slitlike ostiole. Beak 85–125 μm long, central, cylindrical, with hyaline periphyses. Ascomal wall 8–15 μm thick at the side, composed of 5–7 layers of polygonal to rectangular brown pseudoparenchymatic cells of $5-10 \times 2.5-5 \mu\text{m}$. Pseudoparaphyses cellular, numerous, 1.5–2.5 μm thick, branched and anastomosed. Asci (92–)100–120.5 \times (13–)15–21 μm (mean, $108.7 \times 16.8 \mu\text{m}$, $n = 32$), bitunicate, numerous, basal and somewhat lateral, clavate, rounded at the apex, apical chamber

present, short-stalked (12–23 μm long), with 8 overlapping linearly biserial ascospores. Ascospores $30-40(-44) \times 6-9 \mu\text{m}$ (mean, $34.8 \times 7.5 \mu\text{m}$, $n = 165$), L/W 4.0–5.8 (mean, 4.7, $n = 165$), narrowly fusiform, straight or slightly curved, 7–9-septate (3 + 1 + 3, 4 + 1 + 4), with the primary septum mostly submedian (0.48–0.53; mean, 0.51, $n = 148$), strongly constricted at the primary septum, slightly constricted at other septa, the fourth cell from the apex enlarged toward base, pale yellow to brown, with guttules, smooth, with terminal appendages of 7–13 μm long.

Cultural characteristics. Colonies on PDA 3.4 cm in diameter after 4 weeks, Tobacco-Brown (5F6) to Blond (4C4), somewhat arachnoid; reverse similar; no pigment is produced. On RSA, numerous ascomata were formed on the surface of rice straw within 6 weeks. Ascospores (26–)28–45(–55) \times 6–8(–10) μm (mean, $34.0 \times 6.8 \mu\text{m}$, $n = 231$).

Materials examined. On culms of *Dactylis glomerata* L.: Campus of Hirosaki University, Hirosaki, Aomori, Oct. 1, 2001, KT.794-1 (HHUF 27311, culture 4118). On stems of an unknown herbaceous plant: Sanpinai, Hirosaki, Aomori, $140^{\circ}30.128' \text{ E}$, $40^{\circ}34.507' \text{ N}$, Nov. 17, 2000, KT.451 (HHUF 27312); July 17, 2001, KT.573 (HHUF 27313, culture 4119). On twigs of an unknown woody plant: Hirakawa, river bank, Hiraka, Aomori, $140^{\circ}32.030' \text{ E}$, $40^{\circ}34.005' \text{ N}$, Aug. 5, 2001, KT.638-1 (HHUF 27314, culture 4120); Hirakawa, river bank, Matsuzaki, Hiraka, Aomori, $140^{\circ}31.524' \text{ E}$, $40^{\circ}35.266' \text{ N}$, Aug. 18, 2001, KT.686-1 (HHUF 27315, culture 4121).

Notes. Although this fungus is certainly a near relative of the *L. caulium* complex, it does not correspond to any of the five "varieties" proposed by Holm and Holm (1988). This fungus is similar to "*L. caulium* var. d", which also has 7(–9)-septate ascospores, but differs in several characters. The ascospores of our materials are much larger ($30-40(-44) \times 6-9 \mu\text{m}$) than those reported for "var. d"

Table 1. Comparison of numbers of species of Lophiostomataceae reported from the world (Kirk et al. 2001) and from Japan

Genus names	Number of species		References to the Japanese species
	In the world	In Japan	
<i>Byssolophis</i> Clem. (1931)	1	0	
<i>Cilioplea</i> Munk (1953)	4	0	
<i>Entodesmium</i> Riess (1854)	6	0	
<i>Herpotrichia</i> Fuckel (1868)	17	0	
<i>Keissleriella</i> Höhn. (1919)	20	0	
<i>Lophiella</i> Sacc. (1878)	1	0	
<i>Lophionema</i> Sacc. (1883)	2 or 3	0	
<i>Lophiostoma</i> Ces. & De Not. (1863)	30	1	1 (as <i>Lophiosphaera</i>)
<i>Lophiotrema</i> Sacc. (1878)	5	0	
<i>Massarina</i> Sacc. (1883)	125	6	2, 3, 4, 5, 6, 7
<i>Massariosphaeria</i> (E. Müll.) Crivelli (1983)	14	0	
<i>Muroia</i> I. Hino & Katum. (1958)	1	1	8
<i>Quintaria</i> Kohlm. & Volkm.-Kohlm. (1991)	1	1	6
<i>Trichometasphaeria</i> Munk (1953)	4	1	7, 9 (as <i>Keissleriella</i>)
<i>Vaginatipora</i> K.D. Hyde (1995)	1	0	
15 genera	~204 species	10 species	

References: ¹Hino and Katumoto (1964); ²Hino and Katumoto (1955); ³Hino (1961); ⁴Katumoto (1968); ⁵Katumoto and Harada (1979); ⁶Nakagiri (1993); ⁷Otani and Mikawa (1971); ⁸Hino and Katumoto (1958); ⁹Otani (1976)

(26–32 × 6–7 μm) by Holm and Holm (1988). In our materials, the ascospores are mostly 9-septate, whereas “var. d” has mostly 7-septate ascospores. The apex of both end cells of ascospore is relatively rounded in the former, but acute in the latter. Therefore, we provisionally described it as “var. f”.

8. *Lophiostoma caudatum* Fabre, Ann. Sci. Nat. Bot. Ser. 6. 9:103, 1879 (“1878”). Figs. 8, 22, 23

= *Lophiostoma dacryosporum* Fabre, Ann. Sci. Nat. Bot. Ser. 6. 9:103, 1879 (“1878”).

= *Lophiostoma prominens* Nitschke, Syst. Bearb. Pyr. Loph. p. 38, 1886.

Ascomata 350–470 μm high, 250–380 μm diameter, globose to depressed globose, sometimes flattened at the base. Beak 160–230 μm long, 200–250 μm wide. Ascomal wall 15–30 μm thick. Pseudoparaphyses ~1.5–2 μm thick. Asci 90–115 × 11–13 μm, clavate, short-stalked (12–25 μm long). Ascospores 25–32(–35.5) × 5–7.5 μm (mean, 28.7 × 6.6 μm, $n = 82$), L/W 3.8–5.1 (mean, 4.4, $n = 82$), fusiform, rounded at the apex, acute at the base, 5(–6)-septate in 2 + 1 + 2 (2 + 1 + 3), with the primary septum suprmedian (0.42–0.50; mean, 0.46, $n = 82$), yellowish-brown to pale brown, mostly with guttules, smooth to slightly echinulate, without sheath or appendage.

Cultural characteristics. Colonies on PDA 4.7 cm in diameter after incubation for 4 weeks, Olive (3D3) in the center, white at other parts, with more or less irregular margins; reverse similar; no pigments formed. On RSA, ascomata were formed on the surface of rice straw within 6–8 weeks. Ascospores were similar to those found in nature, (24–)27–33 × 6–7.5 μm (mean, 29.7 × 6.7 μm, $n = 30$).

Materials examined. On culms of *Dactylis glomerata* L.: Campus of Hirosaki University, Hirosaki, Aomori, Apr. 6, 2001, KT.476 (HHUF 27318); May 21, 2001, KT.530 (HHUF 27319, culture 4124); June 13, 2001, KT.542 (HHUF 27421); July 20, 2001, KT.575 (HHUF 27422). On stems of an unknown graminaceous plant: Akaiwa, Funadomari, Isl. Rebun, Hokkaido, 141°03.375' E, 45°23.217' N, Sept. 1, 2001, KT.741 (HHUF 27320).

Notes. *Lophiostoma caudatum* is clearly distinguished from other *Lophiostoma* or *Lophiotrema* species by its caudate ascospores. This species has a wide host range, and it is found on the Gramineae (*Poa*, *Festuca*, *Elymus*, *Phragmites*) and other woody plants (*Salix*, *Vitis*, *Paliurus*), although it is uncommon (Ellis and Ellis 1985; Holm and Holm 1988). Similarly, it is not recorded in North America according to the studies by Barr (1992). However, this species was frequently collected during spring to summer in northern Japan.

9. *Lophiostoma winteri* (Sacc.) G. Winter, Krypt.-Fl. Deutsch. Oesterr. Schweiz. 2. Aufl. 1(2):297, 1885.

Figs. 9, 24, 25, 33

= *Lophiotrema winteri* Sacc., Michelia 1:358, 1878.

For other synonyms, see Holm and Holm (1988).

Ascomata 425–550 μm high, 480–580 μm diameter, globose to subglobose, somewhat flattened at the base. Beak ~ 90 μm long, 150–250 μm wide. Ascomal wall 25–45

(–60) μm thick at sides, 15–25 μm thick at the base. Pseudoparaphyses 1.5–2.5 μm thick. Asci (90–)100–123 × (13–)15–17 μm, clavate, short-stalked (13–25 μm long). Ascospores 26–35 × 6.5–9 μm (mean, 30.6 × 7.6 μm, $n = 9$), L/W 3.6–4.3 (mean, 4.0, $n = 91$), fusiform, (3)–5-septate (2 + 1 + 2), with the primary septum mostly median (0.47–0.52; mean, 0.50, $n = 91$), hyaline, with fine guttules, smooth, with terminal appendages 5–8(–12) μm long. Senescent spores brown, echinulate. Ascospores mainly germinating from near both ends.

Cultural characteristics. Colonies on PDA 3.5 cm in diameter after 4 weeks, white, wetty, and with irregular margin; reverse similar; no pigments formed. On RSA, ascomata were formed within 6 weeks on the surface of rice straw or in agar near rice straw. Ascospores were similar to those found in nature, but somewhat larger, measuring 29–40 × 7–9 μm (mean, 33.2 × 8.1 μm, $n = 50$). No anamorph was formed within 3 months.

Materials examined. On stems of *Polygonum* sp.: Toyohira River, river bank, Sapporo, Hokkaido, 141°21.489' E, 43°02.229' N, Sept. 2, 2001, KT.764 (HHUF 27316, culture 4122). On stems of an unknown plant: Akaiwa, Funadomari, Isl. Rebun, Hokkaido, 141°03.375' E, 45°23.217' N, Aug. 31, 2001, KT.740 (HHUF 27317, culture 4123).

Notes. *Lophiostoma winteri* is characterized as having relatively wider ascospores (Barr 1992). Although the ascospores of our collections are narrower (6.5–9 μm vs. (6–)8–11 μm wide as given by Holm and Holm 1985, 1988), the fungus agrees in most other respects with the description of *L. winteri*; thus, the collections are considered to be conspecific.

As is noted by Holm and Holm (1988), this species has close affinities with the *L. caulium* complex, which also has terminal appendage phragmospores. However, it can be distinguished from the latter because it always has hyaline ascospores, even in culture condition.

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