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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 ×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)
containing molten water agar (15–20ml). 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).

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**Taxonomy**


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
2. Ascospores more than 1-septate 3
3. 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*

4. Ascospores 25.5–40.5 = 4–(6–7) µm, with 3–4 guttules in each cell, without terminal appendages 5
5. Ascospores 26–36(–40.5) × 6–8 µm, 5–7 septate, primary septum mostly median
6. Ascospores with terminal appendages or sheath 4
7. Ascospores usually 7–9-septate, 30–40(–44) µm (mean, 34.8 ± 6.2 µm), both ends relatively rounded
   - *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”
9. Ascospores usually more than 5-septate, L/W more than 4.0
10. Ascospores 8.5–12.5(–13.5) µm, 1–2 µm thick at the base, narrower and more elliptical towards the apex
11. Ascospores 7–9-septate, 30–40(–44) × 6–9 µm (mean, 34.8 × 7.5 µm), both ends relatively rounded
   - *L. caulium* “var. f”
12. Ascospores 7–9-septate, 30–40(–44) × 6–9 µm (mean, 34.8 × 7.5 µm), both ends relatively rounded

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*Pseudoparaphyses* 2.5–4 (9–)10–13(–14) µm, contain 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 mostly 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 ends cells.

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