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FULL PAPER

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Three new freshwater ascomycetes from rivers in Akkeshi, Hokkaido, northern Japan

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Abstract Three lignicolous freshwater ascomycetes from rivers in Akkeshi, Hokkaido, northern Japan are reported. All of these are new species belonging to the Lophiostomataceae and described as *Lophiostoma breviappendiculatum*, *Massarina clionina*, and *Massariosphaeria maxima*. Morphological differences between each species and its similar taxa are noted. All three species have been observed to produce only ascomatal states in artificial culture.

Key words Aquatic fungi · Lophiostomataceae · Pleosporales · Taxonomy

Introduction

In the past 20 years, discoveries of fungal species from freshwater habitats have increased dramatically. In addition, inventory information is rapidly increasing. More than 1000 species of the higher fungi have been reported up to this time (Ho et al. 2001; Tsui and Hyde 2003). Of this group, about 50% are ascomycetes, and 497 species are listed by Shearer (2004). Knowledge of freshwater ascomycetes in Japan, however, is very incomplete, and only about 60 species are reported (Ho et al. 1997; Minoura and Muroi 1978; Tanaka and Harada 2003b; Tsui et al. 2003; Tubaki 1966; Udagawa and Ueda 1979; Ueda 1980, 1994). Further taxonomic studies are needed to resolve this situation.

We obtained an opportunity to investigate the freshwater ascomycetes on submerged wood in Akkeshi located in the far east in Hokkaido, northern Japan. In this area, the Bekanbeushi Marsh and many rivers exist with diverse riparian plants (e.g., *Alnus, Prunus, Rhododendron*, and *Salix*). We report here three lignicolous freshwater fungi collected from four rivers in Akkeshi. They belong to Lophiostomataceae in Pleosporales (sensu Kirk et al. 2001), and are described as new.

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Materials and methods

Decomposing woody substrata submerged in rivers and considered as having been soaked in water for a long time were collected by K. Tanaka (KT) and S. Hatakeyama (SH) in June and September 2003. The collecting sites are shown with their abbreviations below.

R1 – Bekanbeushi River, Oota, Akkeshi, Hokkaido, 144°51.3′ E, 43°07.5′ N
R2 – Small stream, Ariake, Akkeshi, Hokkaido, 144°52.0′ E, 43°01.2′ N
R3 – Sattebetsu River, Ootakita, Akkeshi, Hokkaido, 144°49.03′ E, 43°08.13′ N
R4 – Toraibetsu River, Oota, Akkeshi, Hokkaido, 144°51.08′ E, 43°09.36′ N

The substrata were examined for the presence of ascomata using a hand lens, and samples with ascomata were taken into the laboratory. Methods of microscopic observation, single ascospore isolation, and cultivation were as described in Tanaka and Harada (2003a). Incubation in moist chambers to promote fructification (Shearer 1993) was not used. Specimens cited in this paper were deposited mainly in the Herbarium of Hirosaki University, Fungi (HHUF), and some isotype or paratype materials are preserved at herbaria of IMI and HKU(M). All culture strains were deposited at MAFF (National Institute of Agrobiological Science, MAFF Genebank), and JCM (Japan Collection of Microorganisms, Riken).

Taxonomy

Lophiostoma breviappendiculatum Kaz. Tanaka, Sat. Hatak. & Y. Harada, sp. nov. Figs. 1–12, 35A

Ascomata 220–400 μm alta, 300–550 μm lata, sparsa vel 2–3 gregaria, immersa vel erumpentia, globosa, cum ostiolo rotundato. Rostrum 50–115 μm longum, 85–100(–150) μm latum, breviter papillatum, centrale. Paries ascomatis "textura angularis," uniformiter 25–38(–50) μm crassus, ex cellulis 4–6(–7)-stratis polygonis pallide brunneis 5–18 × 3–10 μm compositus, ad latus cum hyphis sparsis brunneis. Pseudoparaphyses copiosae, 1.5–2 μm crassae, septatae, ramificantes. Asci (120–)127.5–165(–185) × 23.5–31 (–32.5) μm, clavati, basales et leviter laterales, breviter stipitati [7–15(–20) μm longi], octospori. Ascosporae (40–)44–60(–63.5) × (9.5–)11–17.5 μm, fusiformes, submedio 1-septatae, hyalinae, laeves, tunica gelatinosa ut in appendice praeditae.

Holotypus: HHUF 28194.

Etymology: From Latin *brevi* meaning short and *appendiculatus* meaning appendaged, referring to the short appendage-like structures formed by the ascospore sheath.

Ascomata 220–400 μm high, 300–550 μm diameter, scattered or 2–3 crowded, immersed to erumpent, globose, with a round ostiole. Beak 50–115 μm long, 85–100(–150) μm wide, short papillate, central. Ascomal wall textura angularis, 25–38(–50) μm thick at sides and base, composed of 4–6(–7) layers of polygonal, pale brown, slightly thick-walled cells of 5–18 × 3–10 μm, with sparse brown hyphae at sides. Pseudoparaphyses numerous, 1.5–2 μm thick, septate, brunched. Asci (120–)127.5–165(–185) × 23.5–31(–32.5) μm (mean = 145.9 × 28.3 μm, *n* = 85), fissitunicate, clavate, with an apical chamber, basal and somewhat lateral, with a short stipe 7–15(–20) μm long, with 8 ascospores biseriate above and uniseriate below. Ascospores (40–)44–60(–63.5) × (9.5–)11–17.5 μm (mean = 53.7 × 13.5 μm, *n* = 90), L/W = 3.2–5.0 (mean = 4.1, *n* = 79), fusiform, slightly acute at the apex, narrowly rounded at the base, slightly curved, with a submedian (0.51–0.56; mean = 0.53, *n* = 78) septum, strongly constricted at the septum, hyaline, smooth, with an appendage-like narrow sheath 1–3 μm long at both ends. Senescent ascospores brown, echinulate, with 1–5 darkened septa.

Cultural characteristics: Colonies on potato dextrose agar (PDA; Difco, Detroit, MI, USA) 30 mm in diameter after 4 weeks at 20°C in the dark, Dark-Green (30F6; Kornerup and Wanscher 1978), with entire margin of Bluish-Grey (23D2); reverse similar; no pigment produced. On rice straw agar (RSA; Tanaka and Harada 2003a), ascomata formed on the surface of rice straws within 2 months. Ascospores similar in shape and size to those found in nature, measuring (48–)51–60.5(–62.5) × 12–17 μm (mean = 55.5 × 15.4 μm, *n* = 50), L/W = 3.2–4.0 (mean = 3.6, *n* = 50). No anamorph was observed.

Specimens examined: On submerged wood: R3, June 3, 2003, KT and SH 1214 (HHUF 28192), KT and SH 1215 (HHUF 28193; single ascospore isolate MAFF 239291 = JCM 12702), Sept. 7, 2003, KT and SH 1399 [HHUF 28194 holotype; isotypes IMI 391847 and HKU(M) 17479; single ascospore isolate MAFF 239292 = JCM 12701], KT and SH 1400–1403 (HHUF 28195–28198).

Notes: This fungus fits within the broad generic concept of *Massarina* Sacc. provided by Aptroot (1998). However, we assign the species to the genus *Lophiostoma* Ces. & De Not. based on ascospore morphology. The fusiform ascospores in *L. breviappendiculatum*, which have acute ends and an appendage-like sheath, are similar to those of *L. macrostomum* (Tode: Fr.) Ces. & De Not. (type species of the genus), *L. winteri* (Sacc.) G. Winter, and *L. appendiculatum* Fuckel, rather than *M. eburnea* (Tul. & C. Tul.) Sacc. (type of *Massarina*), which has broadly ellipsoidal ascospores with rounded ends. Recent evidence from a molecular study on both genera (Liew et al. 2002) suggests that the slitlike ostiole and "lophiostomatoid" peridium structure, which traditionally have been regarded as important taxonomic features, are unacceptable criteria for *Lophiostoma*, and that *Massarina* species with narrowly fusiform ascospores are more closely related to *Lophiostoma*.

The large ascospores of *L. breviappendiculatum* somewhat resemble those of *L. purpurascens* (K.D. Hyde & Aptroot) Aptroot & K.D. Hyde, which also occurs in freshwater habitats (Hyde and Aptroot 1998; Hyde et al. 2002). It is, however, distinct from the latter in slender asci and a narrow ascospore sheath that extends slightly to form apical appendage-like structures (in *L. purpurascens*, the asci are 120–180 × 40–50 μm and the ascospores are surrounded by an entire sheath 2.5 μm wide; Hyde and Aptroot 1998).

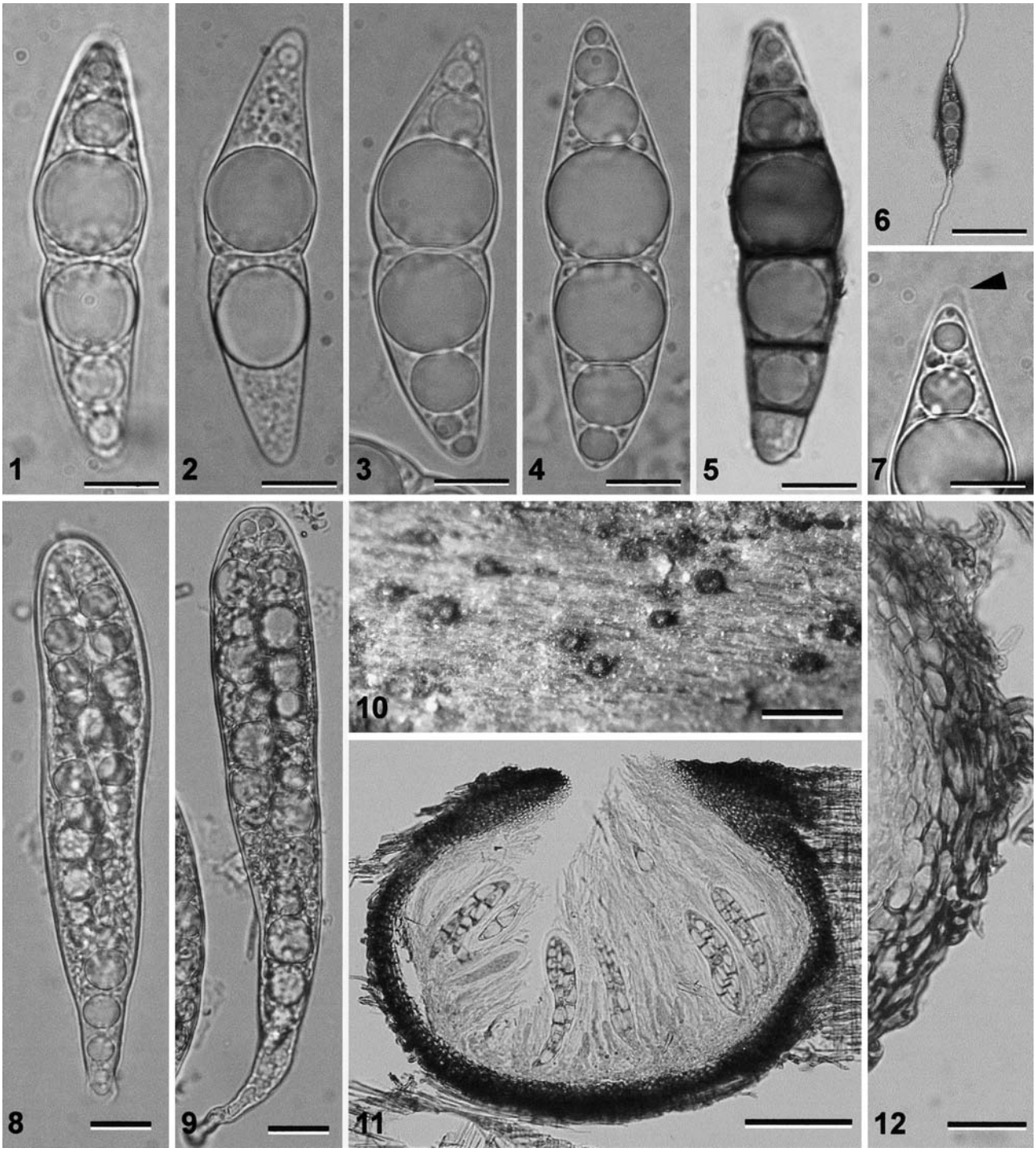
Massarina clionina Kaz. Tanaka, Sat. Hatak. & Y. Harada, sp. nov. Figs. 13–24, 35B

Ascomata 210–280 μm alta 330–430 μm lata, sparsa, immersa, globosa, cum ostiolo rotundato. Rostrum 50–75 μm longum, 75–125 μm latum, breviter papillatum. Paries ascomatis ad latus 15–23 μm crassus, ex cellulis polygonis 4–5-stratis 4–12.5 × 2.5–7.5 μm compositus. Pseudoparaphyses copiosae, 2 μm crassus, septatae, anastomosantes. Asci (81.5–)86–118(–128) × 15–19(–21) μm, clavati, stipitati (5–23 μm longi), octospori. Ascosporae (26–)27.5–34.5(–37) × 7–10(–11) μm, fusiformes, apice utrinque rotundatae, medio 1-septatae, constrictae, hyalinae, laeves, ad septum tunica aliformi gelatinosa ad 3 μm diametro praeditae.

Holotypus: HHUF 28199.

Etymology: From the marine pteropod *Clione* (butterfly-shell), in reference to the winglike sheath of ascospores.

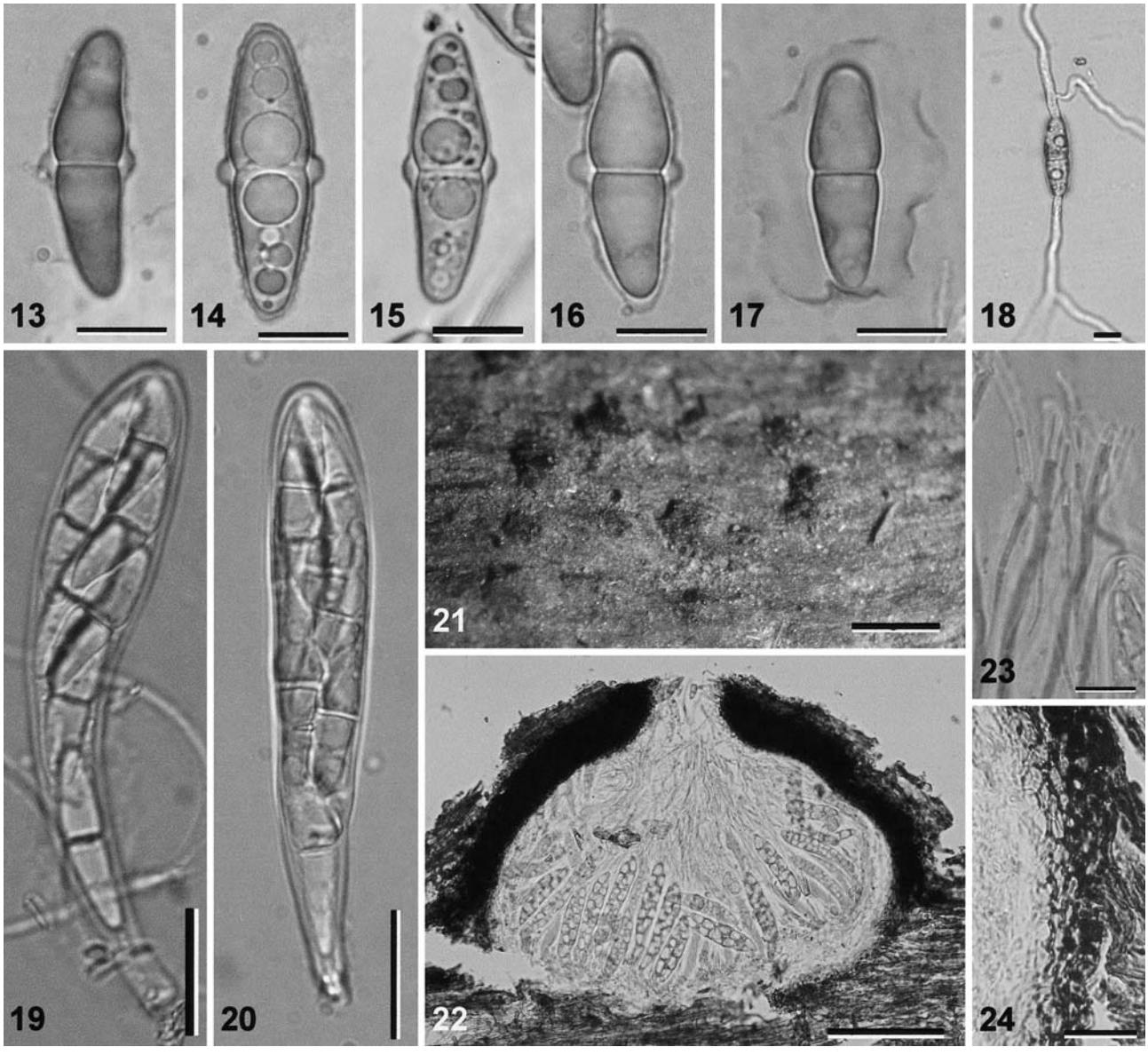
Ascomata 210–280 μm high, 330–430 μm in diameter, scattered, immersed, globose, with a round ostiole. Beak 50–75 μm long, 75–125 μm wide, short papillate, composed of polygonal to globose compressed small cells. Ascomal wall 15–23 μm thick at sides, composed of 4–5 layers of polygonal 4–12.5 × 2.5–7.5 μm cells, poorly developed at the base. Pseudoparaphyses numerous, 2 μm thick, with septa at 8- to 15-μm intervals, anastomosed. Asci (81.5–)86–118(–128) × 15–19(–21) μm (mean = 100.1 × 17.2 μm, *n* = 70), fissitunicate, clavate, with a shallow apical chamber, short-



Figs. 1–12. Micrographs of *Lophiostoma breviappendiculatum*. **1–5** Ascospores (**5**, senescent ascospore). **6** Germinating ascospore. **7** Short appendage-like structure of ascospore (*arrowhead*). **8, 9** Asci. **10** Ascomata on host surface. **11** Ascoma in longitudinal section. **12** Peridium. (**1, 2, 5, 6, 8, 10–12** from HHUF 28194 holotype; **3, 4, 9** from culture MAFF 239292 = JCM 12701; **7** from culture MAFF 239291 = JCM 12702). *Bars* **1–7** 10 µm; **8, 9, 12** 20 µm; **10** 500 µm; **11** 100 µm

stalked (5–23 µm long), with 8 biseriate ascospores. Ascospores (26–)27.5–34.5(–37) × 7–10(–11) µm (mean = 31.0 × 8.7 µm, n = 70), L/W = 3.2–4.1 (mean = 3.6, n = 70), fusiform, round at both ends, with a septum mostly median (0.48–0.52; mean = 0.50, n = 64), constricted at the septum, hyaline, smooth, guttulate, with a winglike sheath. Sheath entire, clearly staining with blue ink, enlarged up to 3 µm thick at both sides of the septum, 1 µm thick at both ends, later swelling up to 7 µm thick and with irregular margin.

Cultural characteristics: Colonies on PDA 20 mm in diameter after 4 weeks at 20°C in the dark, Olive-Brown (4D3) in the center, Greyish-Yellow (4B3) in other parts, with entire margin white; reverse similar; no pigment pro-



Figs. 13–24. Micrographs of *Massarina clionina*. **13–17** Ascospores. **18** Germinating ascospore. **19, 20** Asci. **21** Ascomata on host surface. **22** Ascoma in longitudinal section. **23** Pseudoparaphyses. **24** Peridium. (**13, 17–24** from HHUF 28199 holotype; **14, 16** from culture MAFF 239293 = JCM 12703; **15** from HHUF 28204). *Bars* **13–18, 23** 10 µm; **19, 20, 24** 20 µm; **21** 500 µm; **22** 100 µm

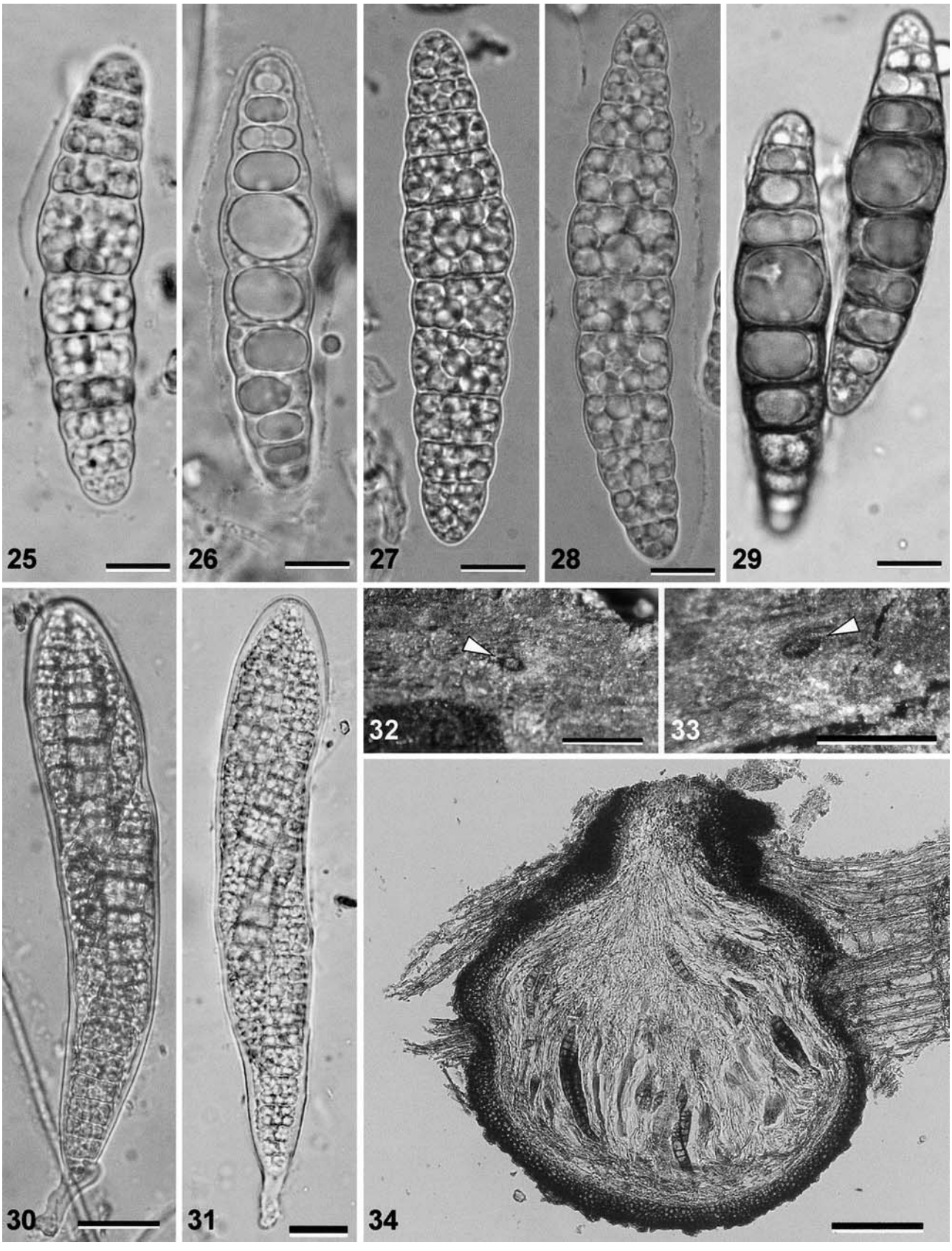
duced. On RSA, ascomata formed on the surface of rice straws within 2 months. Ascospores similar in shape and size to those found in nature, measuring 27–35 × 8–11 µm (mean = 31.0 × 9.3 µm, $n = 50$). No anamorph was observed.

Specimens examined: On submerged wood: R1, June 2, 2003, KT and SH 1149A [HHUF 28199 holotype; isotypes IMI 391846 and HKU(M) 17477; single ascospore isolate MAFF 239293 = JCM 12703], KT and SH 1150–1152 (HHUF 28200–28202); R2, June 3, 2003, KT and SH 1169 (HHUF 28203), Sept. 7, 2003, KT and SH 1384–1386 (HHUF 28217–28219); R3, June 3, 2003, KT and SH 1205–1213 (HHUF 28204–28212), Sept. 7, 2003, KT and SH 1404–1407 (HHUF 28220–28223); R4, June 3, 2003, KT and SH 1220–1223 (HHUF 28213–28216).

Notes: This species is close to *Massarina thalassioidea* K.D. Hyde & Aptroot in having similar-sized asci and ascospores and by its freshwater habitat. *Massarina clionina*, however, differs from *M. thalassioidea* (Aptroot 1998; Hyde and Aptroot 1998) in the following: (1) consistently smaller (330–430 µm in diameter) and globose ascomata (vs. 500–750 µm in diameter and oblate to cylindrical), (2) the thin peridium (15–23 µm vs. up to 70 µm thick), (3) the short ascomal beak (50–75 µm vs. up to 800 µm long), and (4) the winglike ascospore sheath (sheath absent in *M. thalassioidea*).

Massariosphaeria maxima Kaz. Tanaka, Sat. Hatak. & Y. Harada, sp. nov. Figs. 25–34, 35C

Ascomata 300–500 µm alta, 450–520 µm lata, globosa, sparsa, immersa, ad apicem erumpentia, cum rima perforata. Rostrum 80–135 µm longum, 155–220 µm latum, breviter cylindricum. Paries ascomatis uniformiter



Figs. 25–34. Micrographs of *Massariosphaeria maxima*. **25–29** Ascospores (**29,** senescent ascospores). **30, 31** Asci. **32, 33** Ascomata on host surface (*arrowheads*, slitlike ostioles). **34** Ascoma in longitudinal section. (**25, 26, 29, 31–34** from HHUF 28224 holotype; **27, 28, 30** from culture MAFF 239295 = JCM 12704). *Bars* **25–29** 10 µm; **30, 31** 20 µm; **32, 33** 500 µm; **34** 100 µm

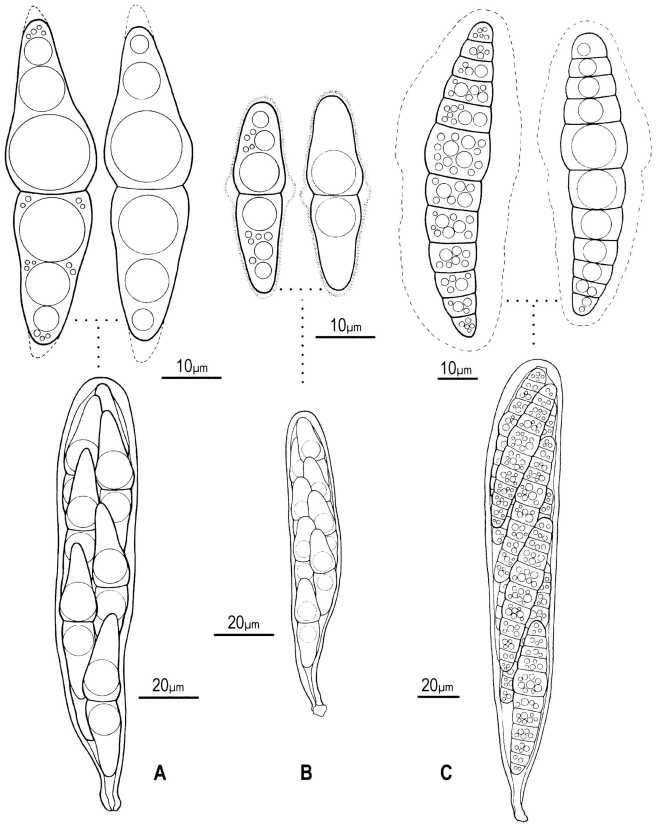


Fig. 35. Line drawings of *Lophiostoma breviappendiculatum* (**A**), *Massarina clionina* (**B**), and *Massariosphaeria maxima* (**C**): ascospores (*upper*) and asci (*lower*)

20–43 µm crassus, ex cellulis 4–6-stratis globosis vel rectangularibus brunneis 3.5–9 µm compositus. Pseudoparaphyses 1.5–2.5 µm crassus, septatae, ramificantes et anastomosantes. Asci (152.5–)175–237.5(–250) × (28–)30–39.5(–42.5) µm, clavati, stipitati (20–35 µm longi), octospori. Ascosporae (53–)58–73(–78.5) × 12–17 µm, fusiformes, leviter curvatae, 9- vel 11-septatae, cum septo primum supramedio (0.46–0.50), hyalinae, laeves, strato mucoso 3–9 µm lato circumdatae.

Holotypus: HHUF 28224.

Etymology: In reference to its ascospores, largest among species of this genus.

Ascomata 300–500 µm high, 450–520 µm diameter, globose, scattered, immersed, erumpent at the slitlike ostiole. Beak 80–135 µm long, 155–220 µm wide, short cylindrical. Ascomal wall uniformly 20–43 µm thick, composed of 4–6 layers of globose to rectangular brown cells 3.5–9 µm in diameter. Pseudoparaphyses 1.5–2.5 µm thick, septate, branched and anastomosed. Asci (152.5–)175–237.5(–250) × (28–)30–39.5(–42.5) µm (mean = 209.4 × 35.2 µm, n = 50), fissitunicate, clavate, with an apical chamber, short stalked (20–35 µm long), with 8 ascospores biseriate above and uniseriate below. Ascospores (53–)58–73(–78.5) × 12–17 µm (mean = 65.9 × 14.1 µm, n = 61), L/W = 4.1–5.3 (mean = 4.7, n = 61), fusiform, slightly curved, with supramedian primary septum (0.46–0.50; mean = 0.48, n = 61), strongly constricted at primary septum, less constricted at additional septa, 9–11-septate (the number of septa in upper hemisphere + the primary septum + the number of septa in lower hemisphere = mostly 4 + 1 + 5, sometimes 4 + 1 + 4 or 5 + 1 + 5), the fifth or sixth cell from the apex enlarged, hyaline, smooth, with numerous small guttules when fresh, surrounded by a gelatinous sheath, 3–9 µm thick. Senescent ascospores brown, echinulate.

Cultural characteristics: Colonies on PDA 18 mm in diameter after 4 weeks at 20°C in the dark, Dark-Green (30F6), with entire margin white; reverse similar to almost black; no pigment produced. On RSA, ascomata formed on the surface of rice straws within 2 months. Ascospores similar to those found in nature in L/W ratio, position of the primary septum and number of septa, but slightly larger, measuring (70–)72–88 × 15–18 µm (mean = 80.1 × 17.1 µm, n = 50). No anamorph was observed.

Materials examined: On submerged wood: R1, June 2, 2003, KT and SH 1157 (HHUF 28224 holotype; single ascospore isolate MAFF 239295 = JCM 12704), KT and SH 1158 (HHUF 28225). Dried culture specimens (from MAFF 239295 = JCM 12704) grown on autoclaved twigs of *Morus australis* Poir.: IMI 391848, HKU(M) 17478, HHUF 28279; grown on autoclaved culms of *Oryza sativa* L.: HHUF 28280.

Notes: There are about 19 species in the genus *Massariosphaeria* (Tanaka and Harada 2004). This fungus is distinguished from others in having the largest ascospores. The ascospore septation of *M. maxima* is identical to that of *M. megaspora* Kaz. Tanaka & Y. Harada but the position of the primary septum is different, 0.46–0.50 in the former and 0.41–0.45 in the latter (Tanaka and Harada 2004).

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