SHORT COMMUNICATION

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Monilinia ssiori sp. nov. in the Sclerotiniaceae, causing leaf blight and young fruit rot of *Prunus ssiori* in Japan

Abstract The leaf blight and mummy fruit disease fungus of *Prunus ssiori* in northern Japan is newly named *M. ssiori*, as a fourth member in the *M. padi* group of section *Disjunctoriae* of the genus *Monilinia* (Sclerotiniaceae). It has been misidentified with *Monilinia kusanoi*, but recent studies show it is different from *M. kusanoi* as well as other related species on prunaceous hosts in respect to host relation, pathogenicity, morphology, and gene analysis.

Key words *Monilia* · *Monilinia* · New species · *Prunus ssiori* · Taxonomy

The leaf blight and young fruit rot disease of *Prunus* subgenus *Padus ssiori* F. Schmidt, caused by a species of *Monilia*, has long been found in Aomori and Hokkaido, northern Japan (Figs. 1–4). The causal fungus of the disease was erroneously identified as *Monilinia kusanoi* (Takah.) W. Yamam. (anamorph *Monilia kusanoi* Henn.) by Harada (1977) because of the gross resemblance of disease symptoms to those on *Prunus grayana* Maxim, which was caused by *M. kusanoi*. Harada (1977) reported its cultural characters under the name of *M. kusanoi*, together with other Japanese species of *Monilinia* known at that time.

In early spring 1989, we first collected apothecia of the fungus on mummified young fruits (pseudosclerotia) of *Prunus ssiori* that had overwintered on the ground in Soma-mura, Aomori Prefecture (Fig. 5). Ascospore isolates from the apothecia yielded colonies quite similar to those of conidial isolates from blighted leaves. Also, it was found that the fungus on *P. ssiori* was quite different in cultural characteristics from *Monilinia kusanoi* on other *Prunus* species than *P. ssiori*. This result led us to compare the fungus on *P. ssiori* with other related species on prunaceous hosts in the world, with the conclusion that it is distinct from all previously known species of *Monilinia*. We describe it here as a new species, based on the following experimental data.

Monilinia ssiori Y. Harada, M. Sasaki & T. Sano, sp. nov. Figs. 5,7–11

Apothecia ex pseudosclerotiis singulariter emittentia, carnosa, brunnea, primo cupulata, dein patelliforme, stipitata, 4–5 mm diametro; stipites 3–8 mm longi; asci cylindracei, apice rotundati, infra attenuati, 150–170 × 7.5–10 μm, 8-spori, poro J+ praediti; ascosporae ovoideae vel late ellipsoideae, continuae, hyalinae, biguttulatae, 12.5–17.5 × 5–7.5 μm.

Misapplied name: *Monilinia kusanoi* auct. non (Takah.) W. Yamam.; Harada, Bull. Fac. Agric. Hirosaki Univ. 27: 34, 1977 (pro parte)

Etymology: *ssiori*, from the specific epithet of the host plant originated in its Japanese name.

Anamorph: *Monilia ssiori* Y. Harada, M. Sasaki & T. Sano, anam. sp. nov. Fig. 1–4, 6

Conidia catenata, limoniformia, late ellipsoidea vel raro globosa, continua, hyalina, 13.5–15 × 10–12.5 μm; disjunctores fusiformes, leniter flexi, bicellulares, 7–8 μm longi.

Apothecia 1 from a single mummy (pseudosclerotium), fleshy, brown, first cup-shaped, later almost plane, stipitate, 4–5 mm in diameter, stipes 3–8 mm long; asci cylindrical, rounded at the apex, tapering below, 150–170 × 7.5–10 μm, 8-spored, pore blueing in Melzer's iodine reagent; ascospores ovoid or broadly ellipsoid, continuous, hyaline, 12.5–17.5 × 5–7.5 μm.

Conidia formed in chain, lemon-shaped to broadly ellipsoid, or rarely globose, countinuos, hyaline, 13.5–15 × 10–12.5 μm; disjunctors fusiform, slightly curved, two-celled, 7–8 μm long.

Habitat: On leaves and young fruits of *Prunus ssiori* Fr. Schm. (Rosaceae).

Distribution: Northern Japan (Aomori and Hokkaido).

Specimen examined: On mummied young fruits of *P. ssiori* of previous season, Soma-mura, Nakatsugaru-gun,

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Figs. 1–5. *Monilinia ssiori* on *Prunus ssiori*. **1** Leaf and shoot blight symptom. **2** Shoot blight, enlarged, showing profuse conidial tufts on the surface. **3** Inflorescence with infected (*i*) and healthy (*h*) young fruits. **4** Infected (*i*) and healthy (*h*) young fruits detached from the inflorescence. **5** Apothecia arising from overwintered mummy fruits (pseudosclerotia)

Aomori Pref., 23 iv 1990, by Y. Harada (HHUF19771, holotype for *Monilinia ssiori*, deposited in the Herbarium of Hirosaki University, Fungi (HHUF). On living leaves of *Prunus ssiori*, the same place as above, 27 v 1988, by Y. Harada (HHUF18334, holotype for *Monilia ssiori*, deposited in the same herbarium).

Phylogenetic analysis: Fungal DNA was extracted as described (Ogata et al. 2000) from mycelia cultured in PD broth (potato 200 g, dextrose 20 g, distilled water 1000 ml) at 20°C for 7–14 days in the dark. The DNA was used as template for polymerase chain reaction (PCR) using the primers ITS1 and ITS4 (White et al. 1990). A PCR product of about 500 bp was recovered, cloned into pGEM-T vector (Promega, Madison, WI, USA), and sequenced by Li-Cor DNA sequencer model 4000L using a Thermo Sequenase fluorescence-labeled primer cycle sequencing kit (Amersham Bioscience, Piscataway, NJ, USA). Sequence data were aligned using Clustal W (Thompson et al. 1994) or Clustal X (version 1.81) (Jeanmougin et al. 1998), and phylogenetic analysis were performed using neighbor-
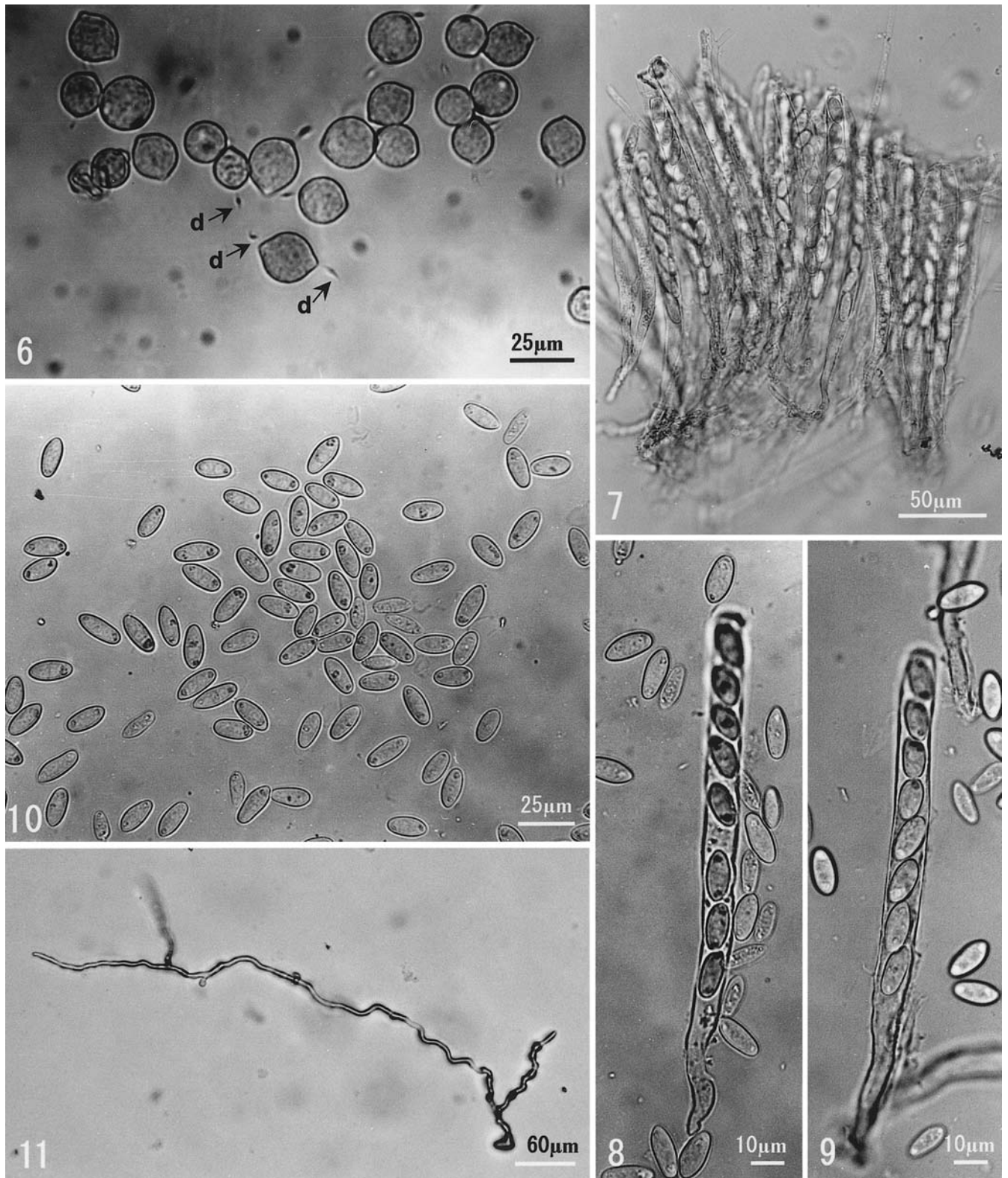
Figs. 6–11. *Monilinia ssiori*. **6** Conidia with disjunctors (*d*). **7** Part of the hymenium comprising asci with ascospores. **8,9** Ascus with 8 ascospores. **10** Ascospores released from asci. **11** An ascospore germinating on potato sucrose agar (PSA).

Table 1. Main features of *Monilinia* section *Disjunctoriae* on prunaceous hosts

	M. padi (Wor.) Honey	M. seaveri (Rehm) Honey	M. demissa (Dana) Honey	M. kusanoi (Tak.) Yamamoto	M. ssiori
Apothecia					
No./sclerotium	1–2 (–3)	1	1 (–2)	1 (–2)	1
Diameter (mm)	3–8	5–10 (Rehm 1905)	2–10	ca. 6	(3–) 5–6 (–8)
Asci (μm)	Avg. 168 × 10	120–140 × 6–8	150–160 × 7–4	125–140 × 7–9	150–170 × 7.5–10
Ascospores (μm)	Avg. 13.2 × 6.6	10–12 × 4.5–5	9–15 × 5–6	10.5–13.5 × 4.5–6	12.5–17.5 × 6–7.5
Conidia (μm)	15.4–17.6 × 11–12.1	(7–) 8–10 (–15) long	7–14 × 3–9	10.5–16.5 × 7–12	10–14 × 9–12
Disjunctors	2–3[a]	3–4	2–4[b]	1.5–3[b]	7–8
Host	Prunus padus L.	Prunus serotina Ehrb.	Prunus demissa Walp.	Prunus yedoensis Matsum., P. avium L.	Prunus ssiori F. Schmidt
References	Woronin (1895)	Rehm (1905) Reade (1908)	Dana (1921)	Hennings (1902) Takahashi (1911)	Present authors

[a] Fide Seaver (1951)
[b] Fide Batra (1991)

joining (NJ) and maximum-likelihood (ML) (PHYLIP 3.5c package) (Felsenstein 1993). The fungus on *Prunus ssiori* (DDBJ accession no. AB220062) was located in a group attacking Rosaceae with dry fruit (Holst-Jensen et al. 1997) and most closely related to, but distinct from, *M. padi* in 3 nucleotides (2.1%) in internal transcribed spacer (ITS)-1, 2 (1.3%) in 5.8S rDNA, and 2 (1.4%) in ITS-2. The number of substitutions in the ITS region was equal to those found between *M. polystroma* and *M. fructigena*, and between *M. fructicola* and *M. laxa* (van Leeuwen et al. 2002), supporting the idea that the fungus is distinct from *M. padi*.

Monilinia ssiori belongs to the section *Disjunctoriae* of the genus *Monilinia* (Honey 1928), on the basis of its disjunctors in the conidial chains, and is further assignable to the *M. padi* group that inhabit leaves and young fruits of species in *Prunus* subgenus *Padus*, comprising so far *M. padi* (Woronin) Honey, *M. demissa* (Dana) Honey, *M. kusanoi* (Takahashi) Yamamoto, and *M. seaveri* (Rehm) Honey (Batra 1991).

In Aomori Prefecture, *M. kusanoi* occurs on several species of *Prunus* subgenus *Cerasus* besides *P. grayana* in the subgenus *Padus*, whereas the known host for *M. ssiori* is confined to *P. ssiori*, even in forest sites where both fungi are sympatric. So far, the range of *M. ssiori* other than Aomori is Hokkaido (M. Akimoto, personal communication). Also, *M. ssiori* and *M. kusanoi* are different in cultural characteristics: when seeded with a single conidial germling, *M. ssiori* grew rather quickly on PSA (potato 200 g, sucrose 20 g, agar 20 g, distilled water 1000 ml) plates in the dark (colony diameter, 63 mm in 20 days) with abundant conidia formed, in contrast to *M. kusanoi*, which grew more slowly (colony diameter, 40 mm in 20 days) with no sporulation.

Morphologically, conidia of *M. ssiori* are broadly lemon-shaped to broadly ellipsoid with acute ends (Fig. 6), or sometimes nearly globose, in contrast to lemon-shaped to broadly lemon-shaped conidia with blunt or papillate ends in *M. kusanoi*. Long and slightly curved disjunctors between the conidia is a striking feature for *M. ssiori*: most species in *Monilinia* section Disjunctoriae have small disjunctors (usually 2–3(–4) μm long), with the exception of *M. urnula* (Weinm.) Whetz. on *Vaccinium vitis-idaea*, which has very long disjunctors (11.2–12.6 μm, fide Woronin 1888). It is also noted that *M. ssiori* and *M. urnula* have a similarity in their broadly lemon-shaped conidia with acute ends, although the size of conidia is far bigger with the latter (30.8–42 × 19.6–25.2 μm, fide Woronin 1888).

Monilinia ssiori is very similar to *M. padi* in that white conidial tufts are profusely formed on blighted shoots as well as petioles and midlimbs of the blighted leaves (see Figs. 1, 2). However, no *Monilinia* (*Monilia*) species has been found on *P. padus* in northern Japan (Hokkaido and Aomori prefectures), where we have native trees of both *Prunus* species in the forest. In April 1996, an inoculation experiment was carried out to see host preference, if any, of *M. ssiori*: ascospores from field-collected apothecia were suspended in distilled water and sprayed onto potted small plants of *P. ssiori* and *P. padus*, which were then kept in a moisture chamber under diffuse sunlight in the laboratory

(room temp. ~15°C). In 2 weeks, all plants (10/10) of *P. ssiori* were infected with the fungus showing profuse conidial production on blighted leaves and shoots, whereas none of the plants (0/3) of *P. padus* were infected. All control plants of both *P. ssiori* (10) and *P. padus* (2) remained healthy. In morphology, *M. ssiori* differs from *M. padi* in conidial size (10–14 × 9–12µm vs. 15.4–17.6 × 11–12.1µm) as well as disjunctor length (7–8µm vs. 2–3µm). *Monilinia seaveri* and *M. demissa* are endemic to North America, occurring, respectively, on *Prunus serotina* and *P. demissa* only (Batra 1991). Morphologically, *M. ssiori* differs from these American species in its broader conidia with much longer disjunctors, in addition to its larger ascospores (150–170 × 7.5–10µm vs. 120–140 × 6–8µm and 150–160 × 7–4µm, respectively).

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