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SHORT COMMUNICATION

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Monilinia jezoensis sp. nov. in the Sclerotiniaceae, causing leaf blight and mummy fruit disease of *Rhododendron kaempferi* in Hokkaido, northern Japan

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Abstract A new leaf blight and mummy fruit disease caused by a species of *Monilinia* was first found on *Rhododendron kaempferi* at the lakeside of Shikotsu-ko, Hokkaido, northern Japan, in 2002. Studies on morphology, life cycle, cultural characters, and gene analyses of the causal fungus enabled us to conclude that it is a new species of the genus. It is named *M. jezoensis*. *Rhododendron* is a new host genus for *Monilinia* fungi in Japan.

Key words *Monilinia* · *Monilia* · New species · *Rhododendron kaempferi* · Taxonomy

Most species of *Monilinia* Honey (anamorph *Monilia* Bonord.) of the Sclerotiniaceae are parasites on plants of Rosaceae and Ericaceae, causing serious diseases on fruit trees and woody perennials. About 35 species of the genus *Monilinia* are known in the world (Batra 1991; Harada et al. 2004; Honey 1936; Kirk et al. 2001; Whetzel 1945), of which 11 species occur in Japan (Harada 1977; Harada et al. 2004). In 2002, a new disease with leaf blight and mummy fruit symptoms caused by a species of *Monilia* was found on a wild azalea, *Rhododendron kaempferi* Planch., at the lakeside of Shikotsu-ko, Chitose, Hokkaido (Ichihashi et al. 2003). In the following year, we succeeded in producing apothecia from previously collected mummy fruits in the laboratory. Morphologically, the apothecial state of the fungus belonged to the genus *Monilinia*, Sclerotiniaceae, and the ascospores from the apothecia produced *Monilia*-type conidia on cultural media. So far no species of *Monilinia* have been known on *Rhododendron* in Japan (Anonymous 2000). Meanwhile, in Europe, Siberia, and the United States, somewhat similar diseases caused by *Monilinia* have been reported on *Rhododendron* spp.: *M. alpina* L.R. Batra, *M. rhododendri* (E. Fisch. ex Wahrlich) L.R. Batra, and *M. azaleae* Honey (Batra 1991). We performed a series of experiments on pathogenicity, morphology, cultural characters, and gene analyses of the present fungus, with the conclusion that it is distinct from all previously described species of *Monilinia*, and thus newly named the fungus as follows.

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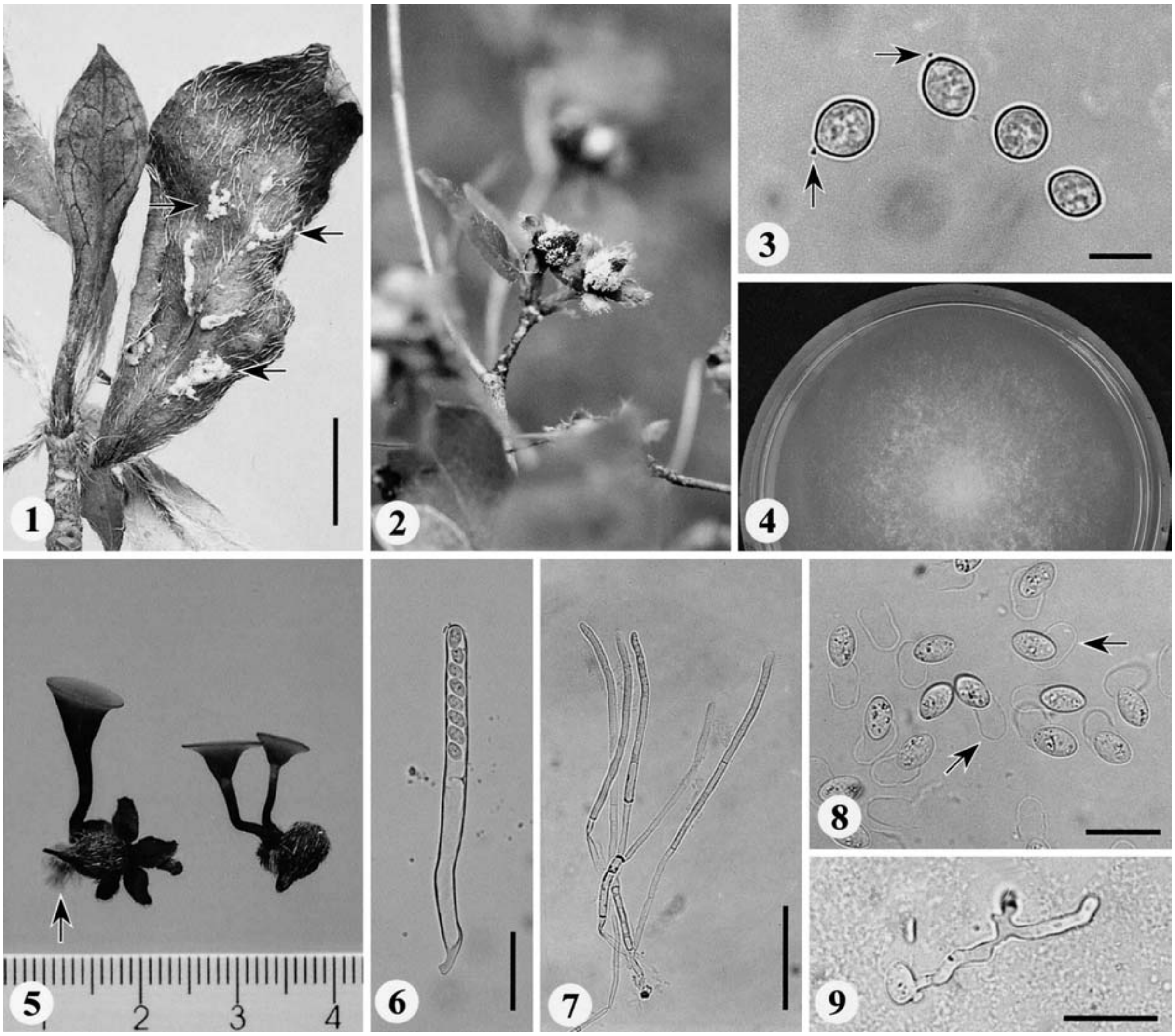
Monilinia jezoensis Yuk. Takahashi, T. Sano & Y. Harada, sp. nov. Figs. 1–9

Apothecia ex pseudosclerotio plerumque 2–4(–6) vel raro 1 orientia, cupuliformia vel infundibuliformia, stipitata. Stipites cylindrici, 1–10 mm longi, 1–2 mm diametro, basi caespite rhizoidei argillaceo praedito. Disci extra pallide brunnei, intra atro-bunnei, 4–8 mm diametro, margine deplanato et fissurato ad maturitatem. Hymenium 130–200 μm crassum; subhymenium 25–90 μm crassum, "textura intricata." Excipulum medullare "textura intricata." Excipulum ectale ex stratosis tribus compositum. Asci cylindro-clavati, 181–244 × 10–16.5 μm, octospori, apice rotundati vel truncatuli, annulo apicali jodo cyanescenti praediti. Paraphyses filiformes, 155–252.5 × 3.5–5.5 μm, 2–3-septatae vel aseptatae. Ascosporae late ellipsoideae, 7.5–17.7 × 4.5–10.5 μm, unicellulares, hyalinae, tunica hyalina gelatinosa circumdantes.

Etymology: *jezoensis*, meaning *jezo* (old Japanese name for Hokkaido, where the fungus was collected) + *ensis* (dwelling in).

Apothecia arising from a pseudosclerotium, usually 2–4(–6) but rarely single, cup-shaped or funnel-shaped, stipitate. Stipe cylindrical, 1–10 mm long, 1–2 mm in diameter. Rhizoidal tuft arising from the base of the stipe, Cray (5D5) (Kornerup and Wanscher 1978). Disc Hair Brown (5E4) at the margin and darker toward the stipe, 4–8 mm in diameter, margin flattened and split at maturity. Hymenium 130–200 μm thick, subhymenium 25–90 μm thick, of "textura intricata." Medullary excipulum of "textura intricata." Ectal excipulum differentiated into three layers; outer layer



Figs. 1–9. *Monilinia jezoensis*. **1** Leaf blight symptom, collected at the lakeside of Shikotsu-ko. *Arrows* indicate masses of conidia (sporodochia). **2** Mummy fruits, collected at the lakeside of Shikotsu-ko. **3** Conidia with disjunctors (*arrows*). **4** Colony on potato sucrose agar (PSA) grown for 7 days at 20°C in the dark. **5** Apothecia, produced on mummy fruit, with rhizoids (*arrow*) at the base of the stipe. **6** Ascus with 8 ascospores. **7** Paraphyses. **8** Ascospores, released from sheath (*arrows*). **9** A germinating ascospore. (**1**, **3** HHUF 28128; **2**, **8**, **9** HHUF 28035; **4** culture 4222; **5–7** HHUF 28061.) *Bars* **1** 5 mm; **3** 10 μm; **6**, **7** 50 μm; **8** 20 μm; **9** 25 μm

of filamentous hyphae, central layer of "textura angularis" to "textura prismatica," inner layer of "textura intricata" to "textura porrecta." Asci cylindric-clavate, 181–244 × 10–16.5 μm (mean, 222 × 14.6 μm; n = 20), 8-spored, rounded or flattened at the apex, with amiloid apical apparatus. Paraphyses filiform, 155–252.5 × 3.5–5.5 μm (mean, 201 × 4.6 μm, n = 20) 2–3-septate or aseptate. Ascospores broadly ellipsoid, 7.5–17.7 × 4.5–10.5 μm (mean, 12.3 × 7.9 μm; n = 300), L/W = 1.2–2.1 (mean, 1.5), one-celled, hyaline, ensheathed with a gelatinous membrane.

Anamorph: ***Monilia jezoensis*** Yuk. Takahashi, Ichihashi & Y. Harada, anam. sp. nov.

Conidia limoniformia, continua, hyalina, 8–13 × 6.5–11 μm. Disjunctores fusiformes, 2 μm longi, 1 μm diametro. Microconidia non observata. Coloniae in agaro decocto tuberorum (PDA) ad 20°C comparate celeriter crescentes, post 14 dies 9 cm diametro attingentes, pannosae, albae; reversum fuscum.

Conidia lemon-shaped, one-celled, hyaline, 8–13 × 6.5–11 μm (mean, 10.1 × 8.4 μm; n = 200), L/W = 1.1–1.5 (mean, 1.2). Disjunctors fusiform, 2 × 1 μm. Microconidia not seen. Colonies grown for 14 days on potato dextrose agar (PDA; Difco, Detroit, ML, USA), plus streptomycin sulfate and penicillin (Batra 1991), at 20°C in the dark, filling the whole agar surface (9 cm in diameter), felty, white, faintly colored, reverse dark brown. In 31 days on PDA under 12-h light : dark cycle (LD), conidial production abundant, sporodochia formed in zones, Orange Gray (5B2), powdery. Microconidia not seen.

The fungus was grown for 30 days on potato sucrose agar (PSA) (Udagawa et al. 1978) at temperatures ranging from

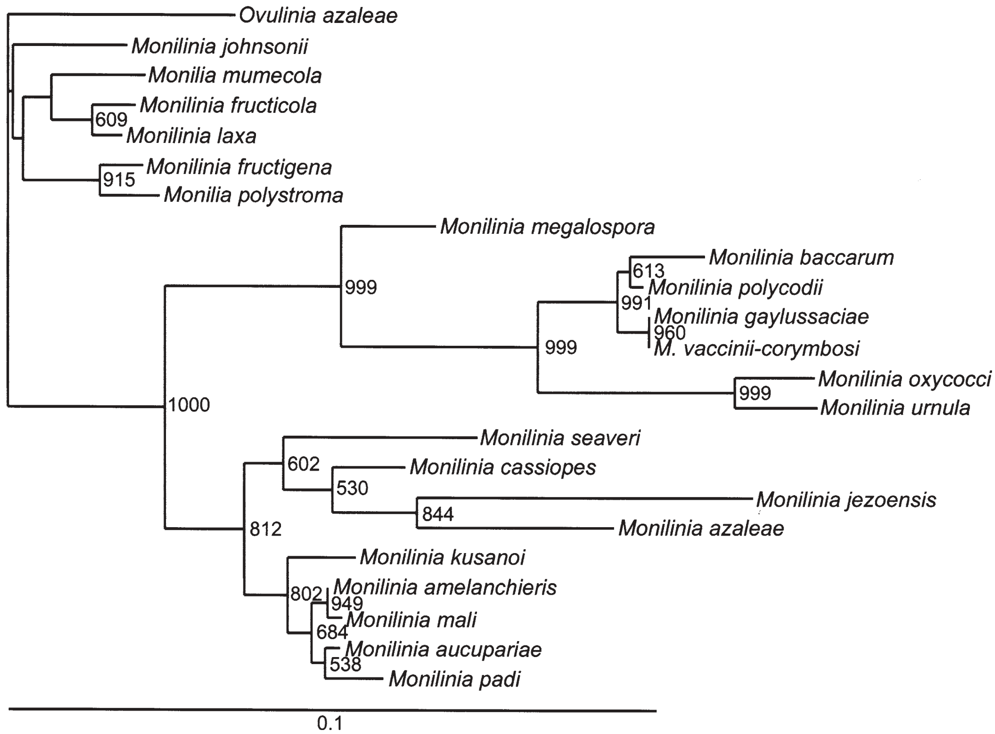


Fig. 10. Phylogenetic tree of *Monilinia* (anamorph: *Monilia*) based on nucleotide sequence of nuclear rDNA internal transcribed spacer (ITS)1/5.8S rDNA/ITS2 region. The tree was constructed by the neighbor-joining method. The values on nodes are the confidence levels from a 1000-replicate bootstrap sampling. DDBJ accession numbers of each isolate are *Ovulinia azaleae* Weiss (Z73797), *M. amelanchieris* (J.M. Reade) Honey (Z73769), *M. aucupariae* (F. Ludw.) Whetzel (Z73771), *M. azaleae* (AB182266), *M. baccarum* (J. Schröt.) Whetzel (Z73773), *M. cassiopes* (Rostr.) L. Holm (Z73776), *M. fructicola* (G. Winter) Honey (Z73777), *M. fructigena* Honey (Z73781), *Monilia polystroma* G. Leeuwen (AB125613), *Monilinia gaylussaciae* L.R. Batra (Z73782), *M. johnsonii* (Ellis & Everh.) Honey (Z73783), *M. kusanoi* (Henn. ex Takah.) W. Yamam. (AB125614), *M. laxa* (Aderh. & Ruhland) Honey (Z73786), *M. mali* (Takah.) Whetzel (AB 125615), *M. megalospora* (Woronin) Whetzel (Z73788), *M. oxycocci* (Woronin) Honey (Z73789), *M. padi* (Woronin) Honey (Z73791), *M. polycodii* (J.M. Reade) Honey (Z73792), *M. seaveri* (Rehm) Honey (Z73793), *M. urnula* (Weinm.) Whetzel (Z73794), *M. vaccinii-corymbosi* (J.M. Reade) Honey (Z73796), *M. jezoensis* (AB182265), and *Monilia mumecola* Y. Harada, Yum. Sasaki & T. Sano (AB125618)

5° to 30°C at 5°C intervals in the dark. The result showed that the optimal temperature for mycelial growth was at 15°–20°C. Little or no growth of the mycelia was seen at 25°–30°C.

Habitat: On leaves and fruits of *R. kaempferi* Planch.

Holotype: HHUF (Herbarium of Hirosaki University, Fungi) 28035. Apothecia arising from pseudosclerotium formed in mummified fruits of *R. kaempferi*, collected at the lakeside of Shikotsu-ko, Chitose, Hokkaido, May 23, 2003, by Y. Harada, dried and kept in the Herbarium (Fungi) of the Faculty of Agriculture and Life Science Hirosaki University.

Fungus materials examined: Sporodochia on leaves of *R. kaempferi*, the lakeside of Shikotsu-ko, Chitose, Hokkaido, May 26, 2002, Y. Harada (HHUF 28128 holotype of anamorphic stage, culture 4222; HHUF 28129); apothecia arising from pseudosclerotia on artificially inoculated *R. indicum* (L.) Sweet fruits, Nishigaoka, Hirosaki, Aomori, June 23, 2002, Y. Harada (HHUF 28061); pseudosclerotia on fruits of *R. kaempferi*, Esan Azalea Park, Esan-cho, Hokkaido, June 30, 2003, Y. Harada et al. (HHUF 28090); pseudosclerotia on fruits of *R. kaempferi*, the lakeside of Shikotsu-ko, Chitose, Hokkaido, Sept. 4, 2003, Y. Harada (HHUF 28121).

Phylogenetic analysis: Nucleotide sequence data of rDNA-internal transcribed spacer (ITS) region (ITS1/5.8S rDNA/ITS2) of *M. jezoensis* and *M. azaleae* (ATCC 58539) were obtained in the same manner as described by Ogata et al. (2000). The sequences were aligned with those of other *Monilinia* species obtained from DDBJ using Clustal X 1.81 (Jeanmougin et al. 1998) and BioEdit v. 5.0.9 (Tom 2001), and phylogenetic analyses were performed using neighbor-joining and maximum-likelihood of PHYLIP systems (Felsenstein 1993). *Monilinia jezoensis* was located in the capsulate group (Holst-Jensen et al. 1997) and most closely related to, but distinct from, *M. azaleae* in 23 nucleotides (15.5%) in ITS1, 2 (1.3%) in 5.8S rDNA, and 12 (8.3%) in ITS2 (Fig. 10). The result supported that *M. jezoensis* is distinct in species level from known *Monilinia* species (van Leeuwen et al. 2002).

Ecology and cultural characters: Three species of *Monilinia* have been known on *Rhododendron* spp. in the world, i.e., *M. alpina* in the Alps, *M. rhododendri* in Eastern Siberia, and *M. azaleae* in the United States (Batra 1991). They seem to be well adapted to cool temperature regions and are all endemic. *Monilinia jezoensis* also was found in a similarly cool temperate area in Hokkaido. The life cycle of *M. jezoensis* was demonstrated by a pathogenicity test.

Table 1. Morphological comparison between *Monilinia jezoensis* and *Monilinia* spp. parasitic for *Rhododendron*

Fungal species	Life cycle	Rhizoids on stipe	Ascospore sheath	Ascospore (μm)	Conidia (μm)	References
M. alpina	Heteroecious	Present	Present	15–20 × 8–10	10–14 × 8–9	Batra 1991
M. azaleae	Autoecious	Present	Absent[a]	9–20 × 5–14	8.5–19 × 5.5–14.5	Honey 1940; Batra 1991
M. rhododendri	Autoecious	Absent	No mention	14.4 × 7.6	None shown	Batra 1991
M. jezoensis	Autoecious	Present	Present	7.5–17.7 × 4.5–10.5	8–13 × 6.5–11	Present paper

[a] Fide Batra (1991)

Conidia of *Monilinia jezoensis* produced in culture were applied onto stigmata of *R. indicum*, one of the most popular and easily available cultivars, using a small cotton ball on a stick, just after pollination with pollen grains from a *Rhododendron* tree of unknown cultivar that was just in bloom. About 1 month after inoculation, capsules of inoculated flowers turned brown with white mycelia and conidial masses on the surface. On the other hands, healthy green capsules developed on pollinated and uninoculated trees. Infected and mummified capsules were sectioned and observed under a light microscope for anatomical features. Five locules of the capsule were filled with stromatic hyphal tissues of "textura intricata," as seen with naturally infected capsules of *R. kaempferi* in Hokkaido. The artificially infected capsules produced apothecia after having been overwintered in the laboratory under controlled temperature conditions simulating a seasonal change in the field. The result shows that the host plant is not specific to *R. kaempferi*, but that other *Rhododendron* spp. also may be infected with the fungus in the field, and that *M. jezoensis* is autoecious, differing from *M. alpina*, the heteroecious species alternating between *Rhododendron* spp. and *Vaccinium myrtillus* L. to complete its life cycle. Two autoecious species of *Monilinia*, *M. rhododendri* and *M. azaleae*, are known to be living on *Rhododendron* spp. (Table 1). *Monilinia jezoensis* differs from *M. rhododendri* in having rhizoidal tufts at the base of the apothecial stipe (see Fig. 5), although the incomplete description for the latter species caused us difficulty in comparing them in more detail (Batra 1991). *Monilinia jezoensis* also differs from *M. azaleae*, another autoecious species, in its ensheathed ascospores, as Honey (1936) did not mention such ascospore envelopes for the latter and Batra (1991) also described it as not having an ascospore sheath in his analytical key. Ascospores and conidia of *M. jezoensis* are both smaller than those of *M. azaleae* (Table 1). In culture, mycelial growth of *M. jezoensis* is apparently faster than that of *M. azaleae*: a colony of *M. azaleae* grown for 14 days on PDA at 20°C in the dark reached 1.5 cm in diameter (Batra 1991), whereas that of *M. jezoensis* covered the whole surface of agar plate (9 cm in diameter) in the same conditions. In addition, colony appearance of *M. azaleae* was described as "water soaked" (Batra 1991), but *M. jezoensis* colony did not show such an appearance, although it looked felty in our observations.

The same diseases caused by *M. jezoensis* have been observed also on *R. kaempferi* trees at the Esan Azalea Park, Esan-cho, Hokkaido (Takahashi and Harada 2004). Prevalence and severity of the disease there have been noteworthy, and further studies are needed to find effective control measures for the disease because rhododendrons are one of the most popular ornamental shrubs of commercial importance.

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