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NOTE

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Polystigma fulvum, a red leaf blotch pathogen on leaves of *Prunus* spp., has the *Polystigmina pallescens* anamorph/andromorph

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Abstract The anamorphic stage of *Polystigma fulvum*, collected as a red leaf blotch on *Prunus ssiori* from Japan, was first confirmed by inoculation tests and field observations in this study. It was identified as *Polystigmina pallescens* based on the characteristic features of sympodial conidiogenous cells and the 80- to 120-μm-long filiform conidia. The description of *P. fulvum* including the *Polystigmina* stage is provided with microscopic illustrations. Presumably the *Polystigmina* stage of this species was considered to be an andromorph rather than an anamorph, possibly involving fertilization of the fungus. A synopsis of the life cycle of *Polystigma fulvum* is briefly noted.

Key words Life cycle · Phyllachorales · Red leaf spot · Spermatia · Teleomorph-anamorph connection

Species of *Polystigma* DC. (Phyllachorales, Sordariomycetes; Lumbsch and Huhndorf 2007) are known as foliicolous pathogens that affect species of *Prunus* sensu lato. They form conspicuous brightly colored stromata on living leaves of host plants and are widely distributed over temperate regions. A red leaf blotch of almonds (*Prunus dulcis*) caused by a species of this genus, namely, *Polystigma amygdalinum* P.F. Cannon, is recognized as a serious disease of almonds (Banihashemi 1990), and the pathogen is often a cause of premature defoliation (Cannon 1996).

In summer, diseased leaves of a Japanese bird cherry (*Prunus ssiori*) showing remarkable stromatal growth, which is considered to be caused by the red blotch pathogen *Polystigma fulvum* Pers. ex DC., were collected (Fig. 1). The stromata, however, contained only the pycnidial stage with filiform conidia instead of the ascomycetous fungus *P. fulvum*. Several species of *Polystigma* have been known to have anamorphs belonging to the genus *Polystigmina* Sacc. (Cannon 1996). No anamorph has been reported for *P. fulvum* thus far (Cannon 1996), but we supposed that the pycnidial stage could be an anamorph of *P. fulvum*.

To confirm the identity between the pycnidial stage and *P. fulvum*, the infected leaves with pycnidial stromata were stored with moistened sphagnum moss in a plastic box at 4°C, to simulate environmental conditions during its overwintering. As a result, an ascomycetous fungus belonging to the genus *Polystigma* was found in 8 weeks or more. It was identified as *P. fulvum* based on the description of the species provided by Cannon (1996), although some small differences such as slender asci [10–12(–14) μm vs. 14.5–16(–18.5) in Cannon 1996] and presence of an ascospore sheath were observed in the materials used in this study. The ascospores produced by the overwintering treatment germinated on water agar (WA), but they did not grow further to form colonies on various culture media such as potato dextrose agar, potato sucrose agar, and WA. However, the ascospores produced the characteristic symptom of red blotch on leaves of *P. ssiori* 5 weeks after woundless inoculation. In 15–24 weeks after inoculation, the pycnidial stage was observed again on the inoculated leaves, and the *P. fulvum* stage was reconfirmed in the stromata of the leaves that had been subjected to the overwintering treatment. Although *P. fulvum* has been defined as lacking anamorphs (Cannon 1996), our observation of the inoculation test revealed that the species has an anamorphic stage with filiform conidia in the conspicuous red stromata on the leaves.

Anamorphs of the *Polystigma* species have been known as *Polystigmina* spp. and are characterized by brightly colored stromata, sympodial conidiogenous cells, and filiform conidia (Cannon 1996; Sutton 1980). The pycnidial stage that we found for *P. fulvum* also has features identical to those of the *Polystigmina* species. According to Cannon (1996), four taxa of *Polystigma* having *Polystigmina*

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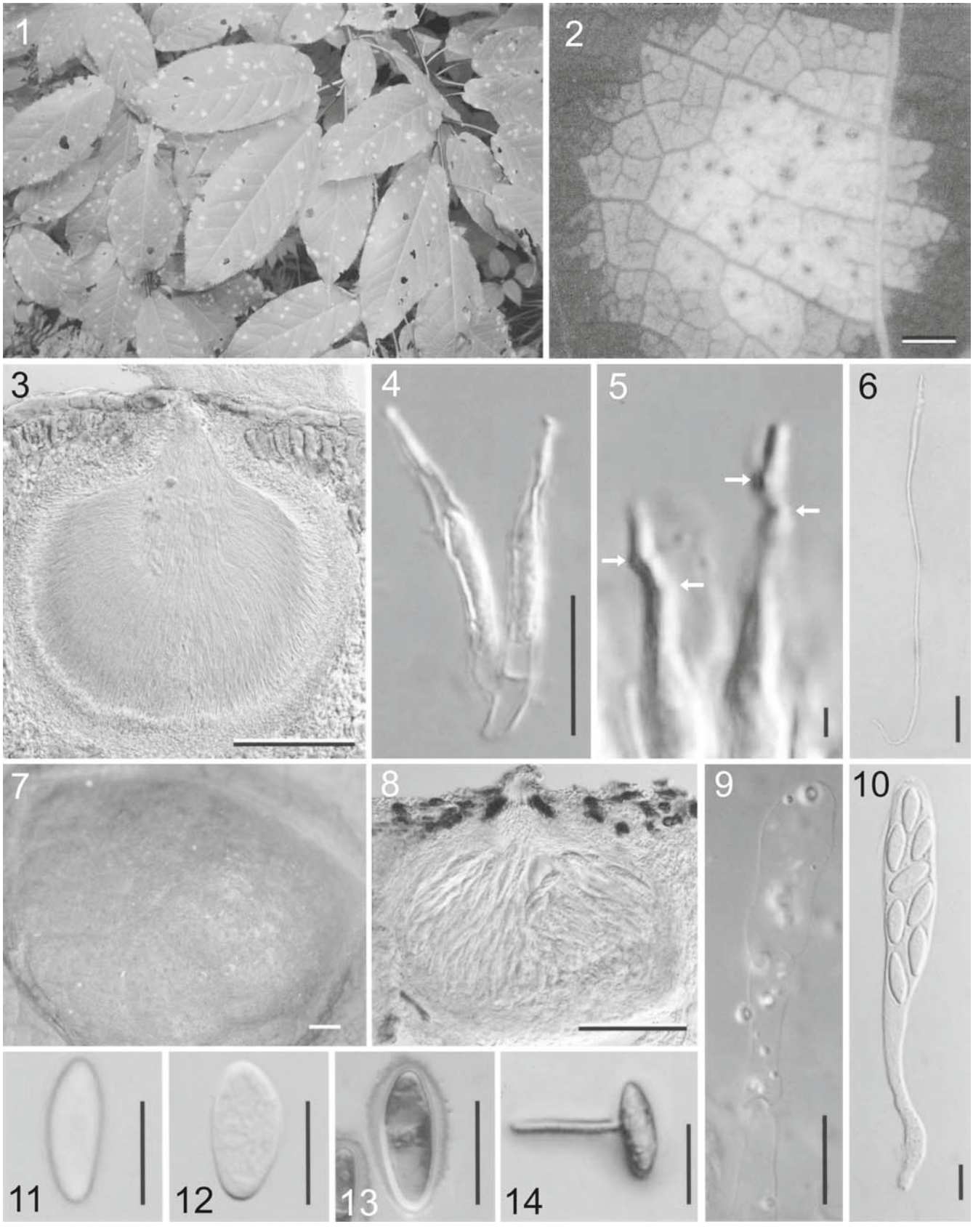

Figs. 1–14. *Polystigma fulvum*. **1** Red leaf blotch of *Prunus ssiori* caused by *P. fulvum*. **2** Pycnidial stroma on host surface. **3** Longitudinal section through pycnidium. **4** Conidiophores and conidiogenous cells. **5** Conidiogenous cells (*arrows* indicate sympodial ontogeny). **6** Conidium. **7** Ascostroma matured by overwintering treatment. **8** Longitudinal section through ascoma. **9** Paraphysis. **10** Ascus. **11–13** Ascospores (note the ascospore sheath in **13**). **14** Germinating ascospore on water agar. **3–6**, **8–12** mounted in distilled water; **13** blue-black ink stain (Pilot Co., Japan). **2–6** from HHUF 29613; **7–14** from HHUF 29616. *Bars* **2**, **7** 1 mm; **3**, **8** 100 µm; **4**, **6**, **9–14** 10 µm; **5** 1 µm

anamorphs and one *Polystigmina* species lacking a teleomorph are known to affect the leaves of *Prunus* spp.: *Polystigma amygdalinum* on almonds (22- to namely 34-µm-long conidia), *Polystigma deformans* Syd. on apricots (38- to 53-µm-long conidia), *Polystigma rubrum* (Pers.) DC. subsp. *rubrum* on plums (22- to 42-µm-long conidia), *Polystigma rubrum* subsp. *ussuriense* (Jacz. & Natalyina) P.F. Cannon on *Prunus* spp. (38- to 63-µm-long conidia), and *Polystigmina pallescens* Petr. on *Prunus* subgen. *Cerasus* and subgen. *Padus* (55- to 120-µm-long conidia). Based on the conidial length (80–120 µm) and the host plant (*Prunus ssiori* belonging to subgen. *Padus*), the anamorphic fungus newly found was identified as *Polystigmina pallescens*, which was originally described from the leaves of the Himalayan bird cherry (*Prunus cornuta* belonging to subgen. *Padus*) in Pakistan (Petrak and Ahmad 1954). Therefore, it was concluded that *Polystigma fulvum* and *Polystigmina pallescens* that grow on the leaves of *Prunus ssiori* are essentially the same species, representing the teleomorphic and anamorphic states, respectively. Although the affinity between *P. pallescens* and *P. fulvum* had been suggested by several authors (e.g., Khan 1961; Cannon 1996), this study is the first to confirm their teleomorph-anamorph connection.

The filiform conidia of the *Polystigmina* stage did not germinate on WA and in sterilized distilled water (SDW). Furthermore, no symptoms were observed on leaves of *Prunus ssiori* inoculation with conidial suspension. Presumably, *Polystigmina pallescens* may not be an anamorph, but it may involve fertilization of the fungus as the spermatia (andromorph), as was reported regarding the scolecosporic stage of species in *Phyllachora* Nitschke ex Fuckel (Parbery 1996).

A detailed description of the species, including the pycnidial stage, is given below. Microscopic measurements were made of material mounted in distilled water.

Polystigma fulvum Pers. ex DC., in de Candolle & Lamarck, Fl. Franç., Edn 3 (Paris) 5/6: 164, 1815. Figs. 1–14

Anamorph/andromorph: ***Polystigmina pallescens*** Petr., Sydowia 8: 183, 1954.

Pycnidial stromata (pseudostromata) on the adaxial surface (sometimes abaxial surface) of the host leaves pale yellow to pale brown at first, later bright orange to orange-brown, thickened, circular with an irregular margin, 5–11 mm, with numerous brownish spots (up to 50 µm) of ostioles on the surface (Fig. 2). Pycnidia 250–290 µm wide, 230–270 µm high, globose to subglobose immersed, scattered to gregarious. Beak epigenous, 50 µm high, 55 µm wide. Wall of pycnidia 5–10 µm thick, composed of 3–5 layers of depressed cells (Fig. 3). Conidiophores formed from the inner layer of the pycnidial wall, 5–20 × 2–3 µm, with verticillated 1–3 conidiogenous cells (Fig. 4). Conidiogenous cells 7–20 × 2–3 µm, holoblastic, sympodial, cylindrical, gradually tapering toward the apex (Fig. 5). Conidia

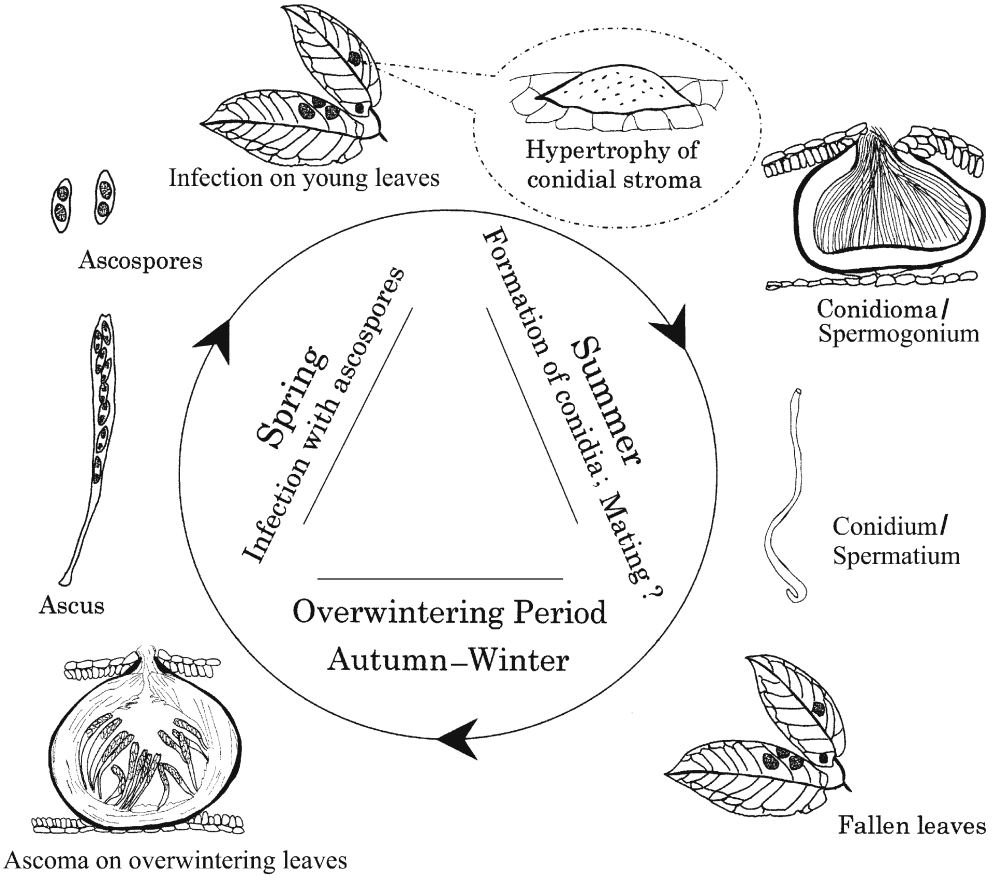

Fig. 15. A synopsis of the life cycle of *Polystigma fulvum* on *Prunus ssiori* assumed by the inoculation test and field observation

80–120 × 0.5–1 µm, filiform, sigmoidally curved, hyaline, aseptate, smooth-walled (Fig. 6).

Ascomatal stromata dark orange-brown to reddish-orange, with dark brown spots of ascomatal ostioles (Fig. 7). Ascomata 200–270 µm high, 200–290 µm wide, globose to subglobose. Wall of ascomata differentiated from stromatal tissue. Beak 50 µm high, 60 µm wide, with black clypeus, periphysate (Fig. 8). Paraphyses up to 80 µm long, 8–13 µm wide at the base, tapering gradually to an acute tip (7–10 µm wide), 2–3-septate, constricted at the septa, sometimes branched, hyaline, very thin-walled (Fig. 9). Asci 95–148 × 10–12(–14) µm (mean 121.0 × 11.3 µm, n = 50), narrowly clavate, long-stalked (25–55 µm), obtuse and with nonamyloid apical apparatus at the apex, 8-spored (Fig. 10). Ascospores 11–15 × 4–6(–7) µm (mean 13.3 × 5.2 µm, n = 150), L/W 2.6, irregularly biseriate, hyaline, ellipsoidal to obovoid, aseptate, often guttulate, with a gelatinous sheath (Figs. 11–13). Ascospores germinate in SDW and on 2% WA but do not grow on agar media (Fig. 14).

Specimens examined: On leaves of *Prunus ssiori*; *Polystigma fulvum* stage formed by overwintering treatment, Owani-kenkyu-hogorin, Owani, Aomori, 11 Oct. 2004, coll. S. Hatakeyama (HHUF 29613), Shikotsuko, Hokkaido, 24 Sept. 2004, coll. Y. Harada (HHUF 29615); *Polystigmina pallescens* stage, Owani-kenkyu-hogorin, Owani, Aomori, 15 Aug. 2005, coll. Y. Suzuki (HHUF 29616), Shinrinkoen, Rishiri, Hokkaido, 29 July 2007, coll. K. Tanaka and G. Sato (HHUF 29758).

Based on the inoculation test and field observation in this study, the life cycle of *P. fulvum* could be considered as shown diagrammatically in Fig. 15: the mature ascospores, which are released from asci in spring (from April to May), infect the young leaves of wild cherry belonging to the *Prunus* subgen. *Cerasus* and *Padus*. The pathogen forms the typical symptom with yellow to pale brown stromata that are approximately 5–10 mm in diameter on the adaxial (rarely abaxial) surface of leaves from late June to July. In summer (late July to August), *Polystigmina pallescens* is formed in the enlarged orange stromata, and this stage may be involved in mating as the spermatia. The diseased leaves containing ascomatal initials turn reddish around the stromata and eventually fall in late autumn (from October to late November). The pathogen in the hypertrophied stromata matures during winter under low-temperature conditions. The life cycle of the pathogen could be fundamentally similar to that of *P. amygdalinum* on almonds, a related species of the genus (Ghazanfari and Banihashemi 1976; Banihashemi 1990; as *Polystigma ochraceum* (Wahlenb.) Sacc.).

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