

NEW DISEASE REPORT OPEN ACCESS

First Report of *Phytophthora pini* Causing Root and Collar Rot of *Chamaecyparis lawsoniana* and *Taxus baccata* in the Czech Republic

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In April 2021, *Chamaecyparis lawsoniana* with shoot dieback was found in a private garden in Prague (50°02'49.765"N, 14°16'38.415"E). Later, in August 2021, dying yew plants (*Taxus baccata*) were observed in urban greenery in the centre of Prague (50°04'02.5"N, 14°25'18.4"E). Diseased plants showed root rot, collar rot and dieback symptoms (Figure 1).

Segments of lesions of collar tissues of *C. lawsoniana* and *T. baccata*, and leaf pieces from rhododendron used to bait excised roots of diseased plants, were surface-disinfected with 70% ethanol and plated on *Phytophthora*-selective medium NARPH V8 agar (Hüberli et al. 2000). On 20% V8 agar (V8A) and on carrot agar (CA), colonies had a radiate to chrysanthemum-like pattern (Figure 2), the radial growth was approximately 7.7 mm/day on V8A and 3.8 mm/day on CA at 25°C. The cardinal growth temperatures were minimum 3°C, optimum 25°C and maximum 33°C. The isolates were homothallic and produced abundant smooth-walled, hyaline, globose oospores 21.5–33.6 µm in diameter, with cell walls 1.0–2.1 µm thick (on CA, $n = 40$). Antheridia, usually paragynous, measured 5.8–15.1 × 6.9–18.3 µm ($n = 40$). Sporangia were ovoid, single, terminal, noncaducous and semipapillate (Figure 3b), formed on simple or sympodial sporangiophores and measured 29.1–61.2 × 18.2–39.8 µm ($n = 40$). Morphological characteristics resembled those described for

P. pini Leonian 1925 (Hong et al. 2011). Representative isolates were deposited in the Czech Collection of Phytopathogenic Oomycetes (CCPO) under accession numbers 1169.21 and 1230.21. During the CCPO revision, it was found that isolate no. 1105.19 from *Rhododendron yakushimanum* (Přerov n. Labem, Central Bohemia) collected in 2019 also belonged to *P. pini*.

For molecular identification, the partial cytochrome oxidase subunit 1 (*COXI*) gene, internal transcribed spacer (ITS) region and partial translation elongation factor-1 alpha (*TEF-1α*) gene were amplified (Kroon et al. 2004; Robideau et al. 2011), sequenced and deposited in GenBank (Accession Nos. OR778486–OR778491 and OR791504–OR791506 for *COXI*; OR791653–OR791656 for ITS; OR778492–OR778496 for *TEF-1α*). BLASTn analyses showed 99.9% to 100% identity for *COXI* (MH136957 and GQ247650), 99.9% for ITS (FJ392322), and 99.7% for *TEF-1α* (GQ247666) with sequences of the ex-type strain of *P. pini* CBS 181.25 (Hong et al. 2011; Abad et al. 2023).

To fulfil Koch's postulates, *P. pini* isolates (1169.21 and 1230.21) were inoculated on 2-year-old *C. lawsoniana* plants and *T. baccata* twigs (five plants and eight twigs per isolate and control). The inoculation sites were surface disinfected with 70% ethanol and inoculated using 5-mm-diameter V8A mycelial plugs inserted into corresponding holes in the bark and sealed with plastic film.

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FIGURE 1 | Symptoms of *Phytophthora pini*: (a) dieback of *Chamaecyparis lawsoniana* shoots; (b) necrotic collar of *C. lawsoniana*; (c) dying ornamental *Taxus baccata* in Prague; and (d) collar necrosis on *T. baccata*.

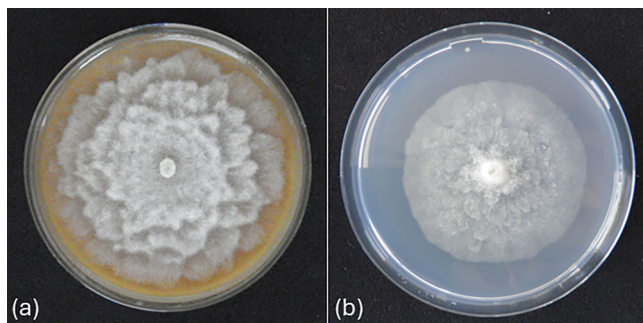


FIGURE 2 | Colony morphology of isolate 1169.21 on (a) V8 agar 7 days/20°C and (b) carrot agar 5 days/20°C.

The plants were kept in a greenhouse at 25°C. All inoculated *C. lawsoniana* plants showed dark brown or black lesions on collars and withered within 28 days, necroses on yew twigs formed within a week (Figure 4). The control plants remained asymptomatic. The pathogen was re-isolated from lesion margins and confirmed by sequencing.

Phytophthora pini is believed to be native to North America but has also been reported in Europe causing disease in Croatia, Germany, Serbia and Slovakia (Jung et al. 2024). To our knowledge, this is the first report of *P. pini* in the Czech Republic. Its

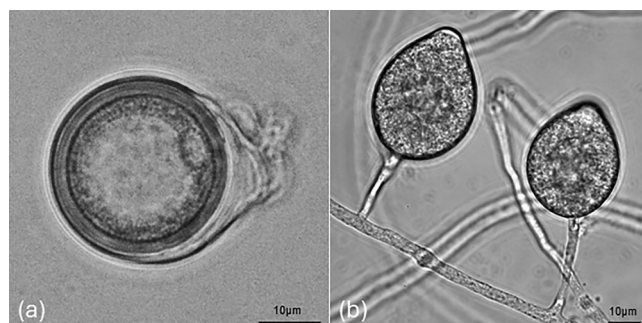


FIGURE 3 | (a) Oogonium of isolate 1230.21 with oospores and (b) semipapillate persistent sporangia of isolate 1169.21, formed on V8 agar in water.

introduction poses a significant risk for the cultivation of many susceptible plant species.

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FIGURE 4 | Pathogenicity of *P. pini* (isolates 1169.21 and 1230.21): (a) inoculated plants of *C. lawsoniana* showing symptoms of infection (left and middle, isolate 1169.23) and control plant (right) 28 days after inoculation; and (b) necrotic lesions on *T. baccata* shoots 7 days after inoculation—control, isolate 1169.21 and 1230.21 (in pairs from the left).

Data Availability Statement

The data that support the findings of this study are openly available in NCBI at <https://www.ncbi.nlm.nih.gov/>, reference number mentioned in the text.

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