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# First report of *Pythium aphanidermatum* causing *Lagenaria siceraria* root rot in Mexico

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## Abstract

In November 2024, root rot was observed on 30% of 4-month-old Lagenaria siceraria (Mol.) Standley plants in a 3-ha commercial field in Culiacán, Sinaloa, Mexico (24°15'04.7"N 107°11'22.8"W). Diseased plants additionally showed above ground stunting, wilting, leaves yellowing and partial foliage necrosis and defoliation, below ground stem base rot. A total of twenty-five small pieces of rot tissue were generated from cutting roots of ten diseased plants randomly selected, immediately surfaced-disinfected in sodium hypochlorite (1%) and rinsed twice for one minute in sterile distilled water. After drying on sterile absorbent paper, five small pieces were equidistantly plated in Petri dishes containing Potato Dextrose Agar (PDA) medium with streptomycin sulfate (0.3 g L-1) and incubated at 30°C for five days. Among five isolates generated, one per Petri dish, two representative isolates were selected for identification, PYTHCH1CULSIN and PYTHCH2CULSIN. Both isolates were plated on V8 medium (800 ml distilled water, 200 ml v8 juice, 2 g CaCO3 and 15 g agar) and grown for 7 days at 30°C, after which cultures grew white with dense, cottony, aerial and well-branched mycelia, hyphae were hyaline and coenocytic, while sporangia were filamentous, lobulated and irregular in shape. For isolate PYTHCH1CULSIN, widths ranged from 11.5 to 18.1 µm, with an average of 15.5 µm. In contrast, isolate PYTHCH2CULSIN exhibited widths ranging from 14.7 to 19.3 µm, with a mean of 17.1 µm. These values are

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aphanidermatum (Watanabe 2002; Jimenez-Perez et al. 2021). PCR analysis was performed with the internal transcribed spacers ITS1 and ITS4 (White et al. 1990) and the cytochrome oxidase c subunit 1 (COI) (Robideau et al. 2011). The sequences were deposited in GenBank under accession no. PQ670972.1 and PQ670973.1 (ITS) and PX102442 and PX102443 (COI) for isolates PYTHCH1CULSIN and PYTHCH2CULSIN, respectively. BLAST analysis showed that PQ670972.1 and PQ670973.1 shared 99.9 % (816/817 pb) and 100 % (810/810 pb) identity with the reference P. aphanidermatum sequences HQ643438.1 and HQ643439.1, respectively, while PX102442 and PX102443 were 100 % (680/680 pb) identical to HQ708485.1 and HQ708486.1 (Robideau et al. 2011). A phylogenetic analysis based on Maximum Likelihood method grouped the isolates within the P. aphanidermatum clade. To verify the isolates pathogenicity, ten L. siceraria seedlings were independently pipette inoculated to the stem base with 5 mL of inoculum (0.25 g of smashed mycelium + 100 mL of sterile water) five days after emergence. Additionally, ten plants were inoculated with sterile water to serve as controls. All plants were kept in a growth chamber at 28 ± 2 °C, 70 ± 5% RH, and a 12:12 h (L:D) photoperiod for 15 days. After the period, inoculated plants showed similar symptoms with those observed on L. siceraria plants grown in the field. No symptoms were detected on control plants. Pythium aphanidermatum was successfully reisolated and authenticated via morphological and molecular methods from the inoculated roots as previously described, fulfilling Koch's postulates, meanwhile, from control plants was not possible. The pathogenicity test was repeated twice. Pythium aphanidermatum has been previously reported on L. siceraria in China (Tai 1979), India (Misra and Hall 1996) and the United States (Toporek and Keinath 2020). To the best of our knowledge, this is the first report of P. aphanidermatum causing disease on L. siceraria in Mexico. This finding will aid further research on L. siceraria root rot and contribute to the development of management strategies for this disease in the country.

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# First report of *Pythium aphanidermatum* causing *Lagenaria siceraria* root rot in Mexico

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In November 2024, root rot was observed on 30% of 4-month-old *Lagenaria siceraria* (Mol.) Standley plants in a 3-ha commercial field in Culiacán, Sinaloa, Mexico (24°15'04.7"N 107°11'22.8"W). Diseased plants additionally showed above ground stunting, wilting, leaves yellowing and partial foliage necrosis and defoliation, below ground stem base rot. A total of twenty-five small pieces of rot tissue were generated from cutting roots of ten diseased

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pipette inoculated to the stem base with 5 mL of inoculum (0.25 g of smashed mycelium + 100 mL of sterile water) five days after emergence. Additionally, ten plants were inoculated with sterile water to serve as controls. All plants were kept in a growth chamber at 28 ± 2 °C, 70 ± 5% RH, and a 12:12 h (L:D) photoperiod for 15 days. After the period, inoculated plants showed similar symptoms with those observed on *L. siceraria* plants grown in the field. No symptoms were detected on control plants. *Pythium aphanidermatum* was successfully reisolated and authenticated via morphological and molecular methods from the inoculated roots as previously described, fulfilling Koch's postulates, meanwhile, from control plants was not possible. The pathogenicity test was repeated twice. *Pythium aphanidermatum* has been previously reported on *L. siceraria* in China (Tai 1979), India (Misra and Hall 1996) and the United States (Toporek and Keinath 2020). To the best of our knowledge, this is the first report of *P. aphanidermatum* causing disease on *L. siceraria* in Mexico. This finding will aid further research on *L. siceraria* root rot and contribute to the development of management strategies for this disease in the country.

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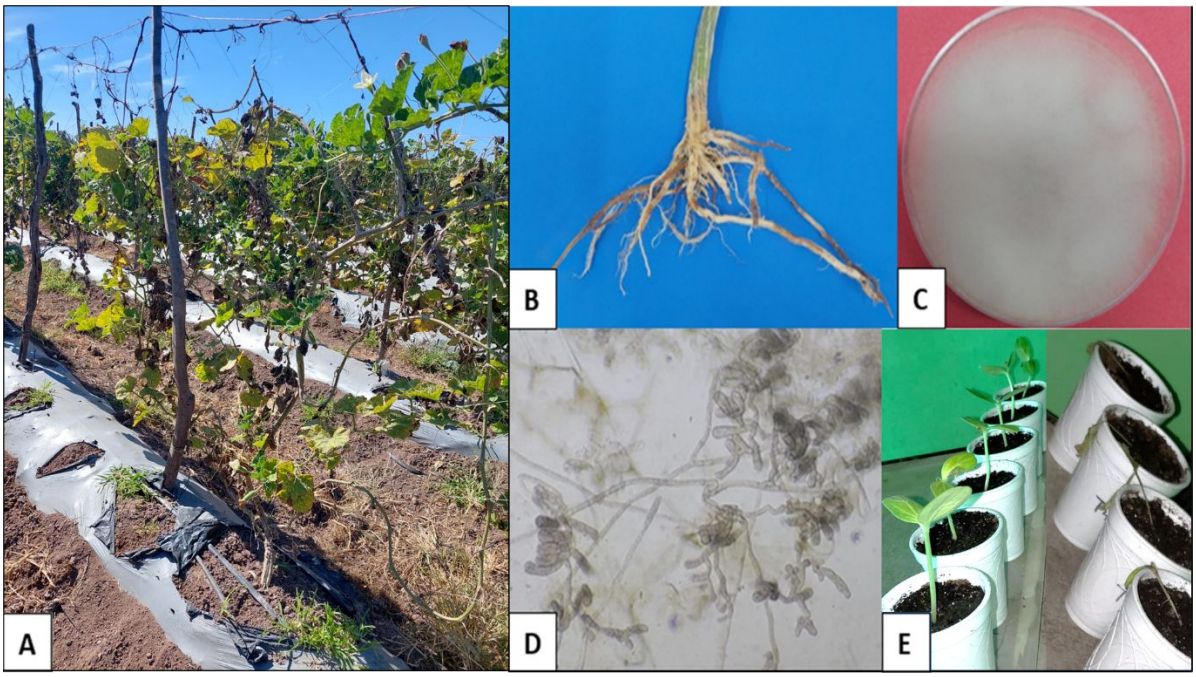
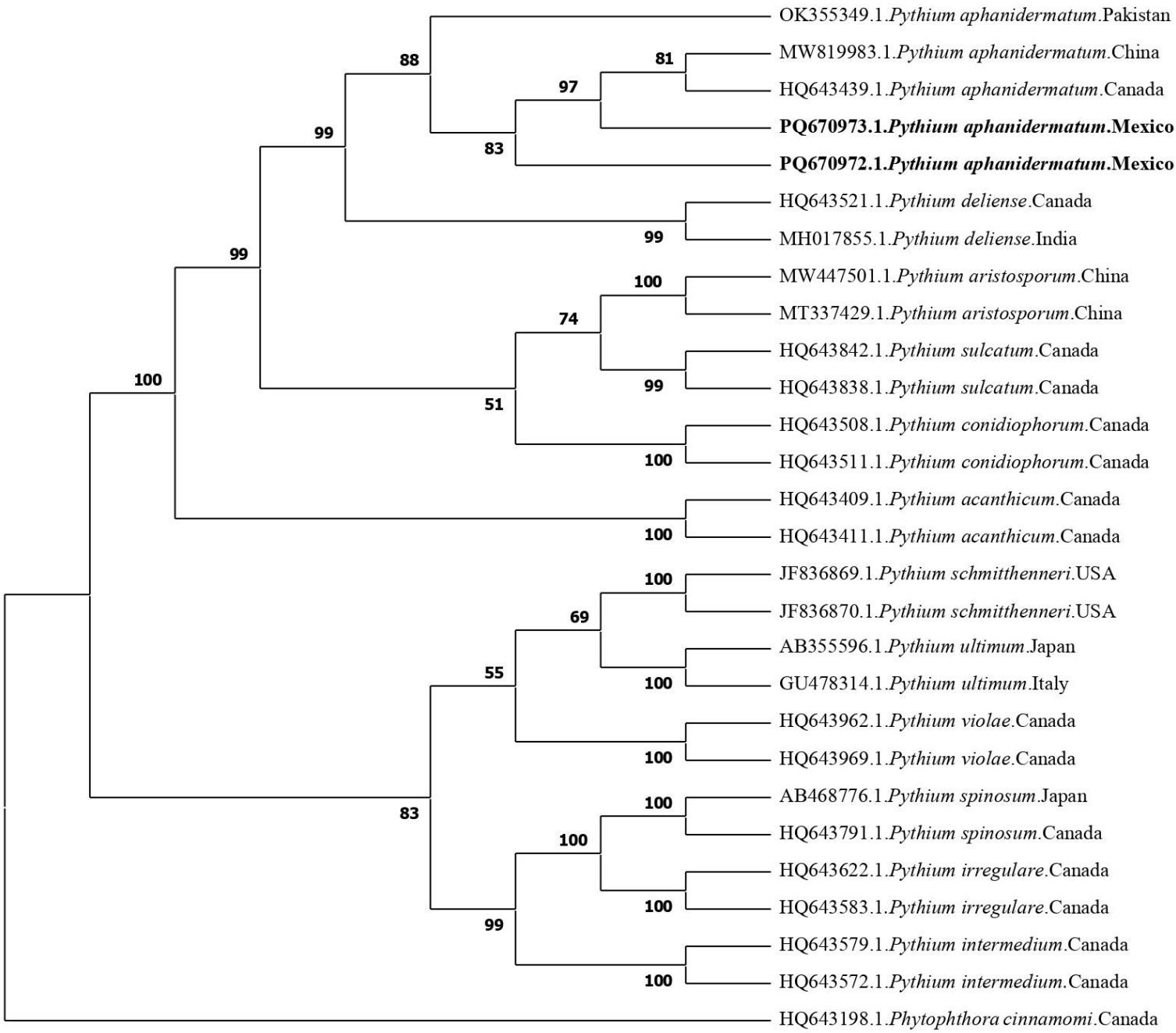



Symptoms and morphological characteristics of *Lagenaria siceraria* root rot associated to *Pythium aphanidermatum.* A = Diseased plants in the commercial field. B = Root rot symptom. C = Top view of *P. aphanidermatum* V8 culture. D = Hyaline and coenocytic hyphae, and filamentous, lobulated and irregular in shape sporangia. E = *Lagenaria siceraria* plants without (left) and with *P. aphanidermatum* inoculation (right).




Phylogenetic dendrogram using Maximum Likelihood method based on the alignment of partial nucleotide sequences of the ITS1 and ITS4 sequences of *Pythium aphanidermatum* PYTHCH1CULSIN (PQ670972.1) and PYTHCH2CULSIN (PQ670973.1) isolates. Values at the nodes represent the percentage bootstrap scores (1000 replicates). *Phytophthora cinnamomi* was used as outgroups.