

The Description and Phylogenetic Placement of Two Putative New Species of *Pythium*

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Introduction

- ▶ *Pythium* is the most diverse genera in the Oomycota with over 120 known species and several new species described each year.
- ▶ There are three large phylogenetic groups within the genus *Pythium* that correspond to sporangia morphology. A group of species referred to as Clade K by Levesque et al. (2004) consists of species with proliferating sporangia which possess papillae similar to *Phytophthora*. There have been several new species identified in this clade, but this clade is not as well understood and has fewer species than the clades which possess species with filamentous and globose sporangia
- ▶ In a previous study in Ohio, 14 different species of *Pythium* were recovered from either corn or soybean, as well as two morphologically distinct groups¹.
- ▶ These two morphologically distinct groups were further evaluated to determine new species status and their phylogenetic placement among other species of *Pythium* and *Phytophthora*.

Material and Methods

- ▶ **Isolate Recovery.** 384 and 382B were recovered from diseased soybean seedlings in Delaware Co. Ohio, and isolates 397 and 399 were recovered from diseased soybean seedling in Woodburn, Indiana.
- ▶ **Morphological Identification.** Isolates were identified by morphological characters using a standard key.³ Hyphal growth pattern was observed on Potato-Carrot Agar (PCA). Oogonia, antheridia, sporangia, and zoospores were observed in the grass blade culture described by van der Plaats-Niterink (1981).
- ▶ **Molecular Identification.** The ITS1, 5.8s, and ITS2 region of ribosomal DNA was amplified using primers ITS1 and ITS4⁴. PCR products were sequenced with the same primers. Sequence data were aligned using Clustal-W, and parsimony analysis was carried out to create a phylogenetic tree with Molecular Evolutionary Genetic Analysis (MEGA) version 3.1. Several sequences were imported from GenBank to compare isolates 382B, 384, 397, and 399 to those which have similar ITS sequences, as well as demonstrate the taxonomic placement of these four isolates in the Genus *Pythium*.

Results

▶ Group 3 - Isolates 382B and 384

Sporangia (Fig. 1 a-d,g) were terminal, globose, proliferating internally, with an average size of 27 x 33 µm. ~30-50 zoospores were produced in each vesicle. Oogonia (Fig. 1 e-f,h) were spherical, terminal, and were on average 28 µm in diameter. Usually one, occasionally two, antheridia per oogonia. Antheridia (Fig. 1 e-f,h) were declivous, making either apical or length-wise contact with the oogonia. Oospores (Fig. 1 e,h) were aplerotic with an average diameter of 24 µm. Hyphal swellings were abundant, ranged in size from 24-40 µm, and were mostly terminal, occasionally intercalary. Both isolates had a radiate growth pattern on PCA, and grew at a rate of 14mm/day.

▶ Group 6 - Isolates 397 and 399

Sporangia (Fig. 2 a-d) were terminal, globose, proliferating internally with an average size of 24 x 28 µm, producing a vesicle containing 15-25 zoospores. No oogonia or antheridia were observed in either the CPA or the grass blade culture. Hyphal swellings (Fig. 2 e-h) were abundant, ranged in size from 22-36 µm, and usually produced 1-3 germination tubes with as many as 5 germ tubes being observed. Both isolates had a radiate growth pattern on PCA and had a mean growth rate of 12mm/day.

Objectives

- ▶ Determine if isolates from Group 3 and Group 6 are distinctive species
- ▶ Evaluate the phylogenetic placement of these isolates in the genus *Pythium*



Fig. 1. The sporangia (A-D,G), vesicles (A,C-D), oogonia (E-F,H), antheridia (E-F,H), and oospores (E) produced by the Group 3 isolates 382B and 384.

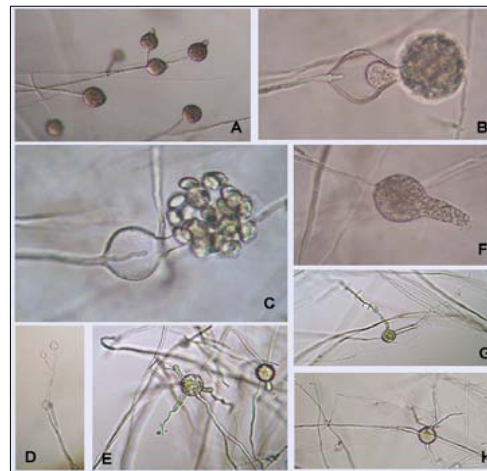


Fig. 2. The sporangia (A-D), vesicles and zoospores (B-C), and germinating hyphal swellings (E-H) of the Group 6 isolates 397 and 399.

Conclusion

- ▶ Based on morphology and sequence data both Group 3 and Group 6 should be classified as distinct species.
- ▶ *Pythium citrinum* had the most sequence similarities with the isolates 382B and 384 but differs in several morphological feature (Fig 3a-b). Most significantly, *P. citrinum* has hypogynous antheridia and plerotic oospores, whereas the Group 3 isolates have declivous paragynous antheridia and aplerotic oospores. In addition, there are 53 nucleotides that are different in the ITS sequence of *P. citrinum* compared to that of the two isolates of Group 3.
- ▶ *Pythium boreale* had the greatest sequence similarity with the isolates of Group 6, but there was only approximately 90% sequence homology between the ITS region of *P. boreale* and Group 6 (Fig 3 a-b). More importantly, sporangia of *P. boreale* have not been observed in culture, but oogonia and oospores have. The contrast is seen for isolates of Group 6 where sporangia and zoospores are observed at room temperature but no oogonia or antheridia are observed. It is uncertain if Group 6 is a heterothallic species or if the sexual structures have yet to be observed.
- ▶ Both Group 3 and Group 6 belong to Clade K according to Levesque et al. (2004). This group of species is not very well understood and there is some debate as to their classification. These two new species should further assist our knowledge of the divergence between *Phytophthora* and *Pythium*, and should allow further evaluation of those species that possess characteristics of both genera.

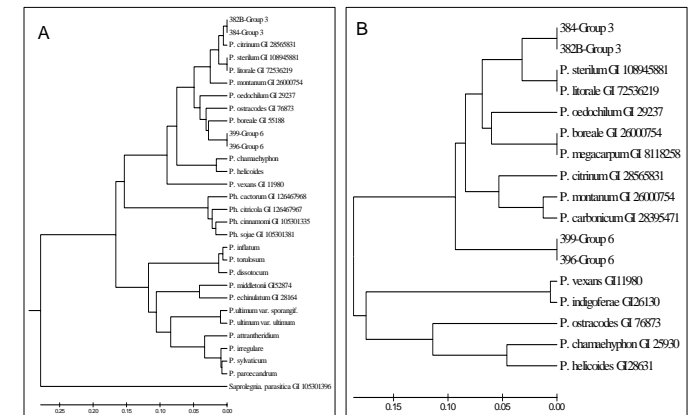


Fig. 3. Phylogeny of (A) *Pythium* and *Phytophthora* species and (B) just species included in Clade K of *Pythium*, conferred from ITS1-5.8S-ITS2 region of the rDNA. Sequence data for species imported from GenBank are followed by their GI numbers.

Literature Cited

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