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Environmental sampling of turfgrass using Oxford Nanopore Technologies reveals two new *Pythium* species and a confirmed report of *Pythiogeton ramosum* confirmed by molecular barcoding

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Abstract: As part of the testing of a new oomycete specific primer set designed to amplify the *cox*2–spacer–*cox*1 region and sequenced using an Oxford Nanopore Technologies (ONT) device, environmental samples were taken from symptomatic turfgrass sites in North Brunswick, NJ and Clayton, NC. Phylogenetic analysis revealed the presence of new Pythium species in the New Jersey samples and the presence of a species of the poorly understood genus *Pythiogeton* in the North Carolina sample. All three taxa were baited out of the turf cores and their colony morphology characterized on corn meal agar, V-8 agar and potato-carrot agar. Isolates were grown on V-8 agar at 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C to determine their optimal growth temperatures. All taxa were inoculated onto autoclaved grass blades in sterile soil extract water and incubated at 5, 10, 15, 20 or 25 °C and observed for sporangia, zoospore release, hyphal swellings, oogonia, antheridia and mature oospores. The *Pythiogeton* isolate matched the description of *P. ramosum*. One of the new species of *Pythium* (*Pythium quipu*) released zoospores from undifferentiated hyphae and had frequent hyphal swellings and infrequent thick-walled oospores, while the other (*P. japamala*) produces swollen, lobed sporangia and pleurotic oospores with mostly monoclinous antheridia. No symptoms of root or crown lesions were seen in pathogenicity tests inoculating these species on tall fescue or creeping bentgrass.

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INTRODUCTION

There are over 16000 golf courses in the United States that cover ~0.49 % of the entire land area of the United States (Weinand 2025). Around 40 plant species are commonly managed as turfgrass (Tredway *et al.* 2022). All turfgrass species are susceptible to *Pythiales* species (*Oomycota*) with over 35 known species cultured from turfgrasses: *Phytopythium vexans*, *Pythium afertile*, *P. aphanidermatum*, *P. aristosporum*, *P. arrhenomanes*, *P. catenulatum*, *P. dissimile*, *P. dissotocum*, *P. graminicola*, *P. myriotylum*, *P. oligandrum*, *P. periilum*, *P. plurisporium*, *P. polycarpum*, *P. pyrilobum*, *P. salpingophorum*, *P. tardicrescens*, *P. torulosum*, *P. vanterpoolii*, *P. violae*, *P. volutum*, *P. zingiberis*, *Globisporangium carolinianum*, *G. intermedium*, *G. irregulare*, *G. iwayamae*, *G. multisporum*, *G. paroecandrum*, *G. pulchrum*, *G. rostratum*, *G. splendens*, *G. sporangiiferum*, *G. sylvaticum*, *G. ultimum* (Abad *et al.* 1994 Tredway *et al.* 2022, Clarke *et al.* 2024). The aggressiveness of different *Pythiales* species ranges from highly aggressive to nonpathogenic (Abad *et al.* 1994). A recent survey of *Pythiales* species in golf course irrigation found at least three undescribed *Pythiales* species (Rushford *et al.* 2022), indicating that golf course *Pythiales* diversity may be an understudied area.

As the technology for assaying biological samples improves, the ability to detect the organisms present in plant tissue (Behrens & Fischer 2022, Suderman *et al.* 2023, Theologidis *et al.* 2023, Stothut *et al.* 2024), air (Reich *et al.* 2023), or soil (Hill *et al.* 2000, Delmont *et al.* 2011, Rossman *et al.* 2021, Ma *et al.* 2023), increases, allowing the microbiome of different substrates to be fully characterized. This will lead to advances in disease diagnosis, as pathogens that are difficult or impossible to culture will be identified directly from substrates. It will also enable the identification of organisms that are not causing disease but are present as part of the microbiome.

An ongoing investigation utilizing Oxford Nanopore Technologies sequencers and a new oomycete specific primer set designed to amplify the mitochondrial *cox*2–spacer–*cox*1 region directly from environmental samples, has been shown to accurately identify oomycetes to species (Groben, personal communication). The objective of this study was to explore the oomycete diversity of turfgrass with chlorotic symptoms associated with *Pythiales*.

In this study, we characterize two novel *Pythium* species from turfgrass in New Jersey. DNA sequences were phylogenetically compared to the other *Pythium* species and morphological comparisons were conducted. Detailed descriptions and

pictures of the novel *Pythium* species are presented. In addition, we cultured *Pythiogeton ramosum* and provide photographs of this enigmatic species.

MATERIALS AND METHODS

Initial environmental sequencing and baiting

Environmental samples were collected from Rutgers University HortFarm 2 on a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) in North Brunswick, New Jersey (40°28'N, 74°25'W) and environmental samples were sent from Clayton, North Carolina. A 2.5-cm-diam. corer was used to collect samples which consisted of turfgrass roots, thatch layer, and foliar tissue. Turfgrass samples exhibiting minor symptoms associated with Pythium root rot or Pythium blight, caused by various *Pythiaceae* species, were selected (Tredway *et al.* 2022). The first set of New Jersey samples were collected on 13 Aug. 2023. Analysis of the New Jersey August samples indicated potential new oomycete species so a follow-up collection was conducted on 26 Sep. 2023 at the same site. The North Carolina samples were collected from fields that had previous *Pythium* epidemics that caused severe damage to the field. The North Carolina samples were collected on 15 Jul. 2024. All core samples were transported under APHIS permit to Bio-safety level 2 containment laboratories at the Foreign Disease Weed Science Research Unit at Frederick MD and refrigerated before DNA extraction and baiting.

Initial molecular testing was conducted using a new primer set that amplifies *cox*2–spacer–*cox*1 of oomycetes directly from environmental samples (Groben, personal communication). Representative turfgrass cores were ground in liquid nitrogen. DNA was isolated from 0.25 g of tissue consisting of foliar, thatch, and roots using the QIAGEN DNeasy PowerLyzer PowerSoil kit (QIAGEN, Gaithersburg MD). PCR was conducted using the new oomycete specific COX1-2 primers which amplified 2000–2300 base pair amplicons directly from environmental samples and confirmed to work from 24 oomycete genera (Groben *et al.* 2026). The primer sequences are COX1-2F (5'-CCTTGGCAATTAGGTTTTCAAGA-3') and| COX1-2R (5'-AATCTGGAATTCTTCTAGGCAT-3') (Groben *et al.* 2026). PCR reaction consisted of 0.5 µL of PrimeSTAR GXL DNA polymerase (Takara Bio Inc., Shiga, Japan), 5 µL of 5x PrimeSTAR GXL buffer, 2 µL dNTP mixture, 400 nM of each barcoded primer, 14.5 µL PCR grade water (Sigma-Aldrich, MO, USA), and 1.0 µL of DNA template for a total volume of 25 µL. The PCR conditions were an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 98 °C for 10 s, 55 °C for 15 s, 68 °C for 2 min, with a final extension at 68 °C for 5 min. PCR amplification was verified via agarose gel electrophoresis. Verified amplicons were pooled together and used for library construction with the Oxford Nanopore Technologies SQK-LSK-114 sequencing kits (Oxford, UK). Approximately 100 ng of barcoded byproduct from each PCR reaction was combined. The pooled amplicons were prepared according to the manufacturer's protocol using the NEBNext® Ultra™ II End Repair/dA-Tailing Module and the NEBNext® Quick Ligation Module (New England Biolabs, MA, USA). Sequencing was performed on Flongle Flow Cells R10.4.1 (Oxford, UK) using the Mk1C sequencer (Oxford, UK). Basecalling was conducted in high-accuracy mode on the Mk1C, with a minimum read length of 1000 bp. Basecalled FASTQ files were concatenated and demultiplexed with the AmpBinner Python script (https://github.com/WGLab/AmpBinner). Consensus sequences were generated using NGSpeciesID (Sahlin *et al.* 2021). Open reading frame protein predictions were generated in GeneiousPrime (2023.0.1; Biomatters Inc., Boston MA USA). The consensus sequences were manually curated to confirm functional protein annotations—specifically deleting additional base pairs causing frameshifts—usually needed in regions with >8 of the same base pair. The *cox*1 and *cox*2 regions were extracted from the resulting curated consensus sequence using GeneiousPrime. The extracted sequences were queried against the NCBI nt database (Sayers *et al.* 2022) using BLAST (Altschul *et al.* 1990). Top BLAST hits are reported for both regions in Table # Blast Summary.

Turfgrass cores that were assayed and found to contain DNA from novel or unusual Oomycetes were baited to isolate these organisms. The core samples were flattened and submerged in sterile distilled water in plastic Petri plates (100 × 15 mm, Cat. No. 25384-303, VWR Avantor, 600 Hamilton Street, Allentown, PA 18101) at 20 °C; on the water's surface, 8–10 autoclaved grass blades were floated (Emerson,1958) and some blades were removed at 24, 48 and 72 h. The blades were incubated on selective PARP media (Jeffers & Martin, 1986) and single colonies of oomycetous organisms were isolated and assayed using the same *cox*2–spacer–*cox*1 approach. Those matching the sequences identified in the initial environmental assay as new or rare were maintained on PARP media. One taxon was represented by two isolates and the other two by one isolate each. Unfortunately, the *Pythium* species in the North Carolina samples was not cultured.

Sanger sequencing

To generate additional data for the suspected new *Pythium* species, PCR amplification of the ITS region with the primer set ITS5/ITS4 (Ward & Adams 1998, White *et al.* 1990) and

Table 1. Summary of the best BLAST alignment to the nt database for the *cox*1 and *cox*2 region of each consensus sequence.

	cytochrome oxidase 1			cytochrome oxidase 2		
Isolate	% ID	Species	Accession	% ID	Species	Accession
Sub2	99.26%	*Pythium vanterpoolii*	HQ708994.1	99.82%	*Pythium vanterpoolii*	AB160858.1
Sub3	99.85%	*Pythium vanterpoolii*	HQ708994.1	99.64%	*Pythium vanterpoolii*	AB160857.1
WA	100.00%	*Pythium* sp. BRIP 75664a	OR900714.1	98.30%	*Pythium* sp. FFM-2010	GU222162.1
5-1	99.71%	*Pythiogeton ramosum*	KM819500.1	85.33%	*Elongisporangium disparispinum*	LC806910.1
NC1	92.85%	*Pythium aphanidermatum*	AY129164.1	92.70%	*Pythium aphanidermatum*	EU265663.1

the nc LSU rDNA region with primer set LROR/LR5 (Vilgalys & Hester 1990) was performed. The PCR conditions were initial denaturation at 95 °C for 5 min, 40 cycles of 95 °C for 30 s, 55 °C for 30 s, 68 °C for 1 min, and a final extension at 68 °C for 5 min. Each PCR reaction consisted of 5 µL of *Taq* 5X Master Mix (New England BioLabs, MA, USA), 400 nM of each primer (1 µL of 10 µM stocks), 17 µL PCR grade water (Sigma-Aldrich, MO, USA), 1.0 µL of DNA template for a total volume of 25 µL. The PCR products were cleaned with ExoSAP-IT (Thermo Fisher Scientific, San Jose, California) following manufacturer's protocol and sequenced using Sanger technology in both forward and reverse directions by Eurofins Genomics Engineering LLC (Louisville, Kentucky, USA). The resultant sequences were trimmed and used to create respective alignments with default settings in GeneiousPrime (2023.0.1; Biomatters Inc., Boston MA USA). After trimming a consensus sequence was generated using the forward and reverse sequence with the De Novo Assembly tool and default settings in GeneiousPrime.

Phylogenetic analysis

Phylogenetic analysis used the ITS, nc LSU rDNA, *cox*1, and *cox*2 sequences. The phylogenetic analysis consisted of available *Pythium* type species, the outgroup CBS120945 *Globisporangium apiculatum*—downloaded from NCBI Genbank—and the suspected new *Pythium* species (n = 84) (Supplemental Table S1). MAFFT was used to align each marker region, the ends were trimmed manually, missing markers and shorter gene fragments were filled with "?" and a concatenated four gene tree of ITS, nc LSU rDNA, *cox*1, and *cox*2 was constructed in GeneiousPrime (Supplemental Fasta 1). The resulting alignment was analyzed with IQ-TREE (Nguyen *et al.* 2015) to generate maximum likelihood trees with automatic model selection (Kalyaanamoorthy *et al.* 2017). The resulting Newick tree files were visualized in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). The tree was rooted using the outgroup CBS120945 *Globisporangium apiculatum* (Fig. 1). The same alignment was also analyzed using Bayesian inference via BEAUti and BEAST (Bouckaert *et al.* 2014). Bayesian posterior support values were incorporated into the maximum likelihood phylogenetic tree (Fig. 1).

Temperature studies

To determine optimum temperature for growth for each taxon, three isolates representing the new taxa were inoculated as 6-mm plugs in the center of plates of V-8 agar and incubated at 5, 10, 15, 20, 25, 30, 35 and 40 °C for 48 h. The incubators used at 5–25 °C were Sanyo cooled incubators (Model MIR-554, Panasonic North America PHC Corporation of North America, 1300 Michael Drive, Suite A, Wood Dale, IL 60191) and for 30–40 °C, UVP minidizers ovens (Analytik Jena US, 2066 W. 11th Street, Upland, CA 91786). Diameter was measured at 24 and 48 h from positions perpendicular to each other, with the diameter of the plug subtracted. Diameter was then averaged and divided by two to get the radius of the colony for each plate. The experiments were run three times. The *Pythiogeton* isolate was inoculated and incubated using the same procedure but in a separate set of three experiments.

Morphological methods

To examine colony morphology on culture plates, isolates were grown on three types of agar media at 5, 10, 15, 20 and 25 °C. A 6-mm diam. plug of each isolate was placed in the center of a 9-cm Petri plates of corn meal media (purchased from Becton, Dickinson and Co., Sparks, MD 21152), potato-carrot media (recipe from Simmons 2007), V-8 agar and PARP media. Two plates of each media were grown at 5, 10, 15, 20 and 25 °C. The 5 °C plates were incubated in a Sanyo Labcool Pharmaceutical Large Capacity Refrigerator Model MPR-1410 (Panasonic North America PHC Corporation of North America, 1300 Michael Drive, Suite A, Wood Dale, IL 60191); the 10, 15, and 25 °C plates were incubated in Benchmark MyTemp mini digital incubators (model H220-AC) (Benchmark Scientific 2600 Main Street Extentions, Sayreville NJ 08872); the 20 °C plates were incubated in a Sanyo cooled incubator (Model MIR-554, Panasonic North America PHC Corporation of North America, 1300 Michael Drive, Suite A, Wood Dale, IL 60191). After 1 wk, colony morphology was recorded for each plate. This procedure was repeated.

Morphological features of each taxon were studied on isolates in grass blade culture. Grass blades were cut from an outdoor lawn or taken from Tall Fescue Mix (*Festuca arundinacea*) (The Scotts Company, Marysville, OH 43040) grown from seed in the greenhouse over winter months. Blades were autoclaved for 30 min in water, then 4-5 blades were transferred to 60 ×15 mm Petri dishes (Falcon 351007, Corning Incorporated, one Riverfront Plaza, Corning NY 27712) containing soil extract water (10 % v:v). Grass blade cultures were inoculated with a plug of infested agar and incubated at 10 °C, 15 °C, 20 °C and 25 °C and observed daily with a dissecting microscope (an Olympus SZX16 with a 10–80× zoom; Olympus America Inc. 3500 Corporate Parkway, P.O. Box 610, Center Valley, PA 18034-0610) to determine the optimum temperature for production of sporangia, oogonia and zoospores. Isolates on CMA media or water agar were inverted in order to examine them for appressoria on the bottom of the plate. Colonized leaf blades and agar cubes were examined microscopically using an Olympus BX40 compound microscope, and photographed using a Nikon DS-Fi3 Camera (Nikon Instruments Inc.1300 Walt Whitman Road, Melville, NY 11747-3064, USA), with NIS-Elements D software which allowed the measurement of morphological features. When examination of grass blade tissue was necessary, plant tissue was immersed in 70 % ethanol until tissue was cleared of chlorophyll, then the tissue was stained in trypan blue 0.5 % solution in Dulbecco's phosphate buffered saline (Himedia Laboratories Pvt. Ltd. Mumbai, India) overnight before being rinsed in water and examined microscopically at 40×. Measurements were made for both isolates of the first taxon, but since the measurements didn't differ significantly between isolates, data were pooled to get averages and standard deviation.

Pathogenicity assays

Aggressiveness of two isolates of *P. japamala* and one of *P. quipu* were assessed in growth chamber trials on Tall Fescue Mix (*Festuca arundinacea*), and Crystal Bluelinks, Creeping

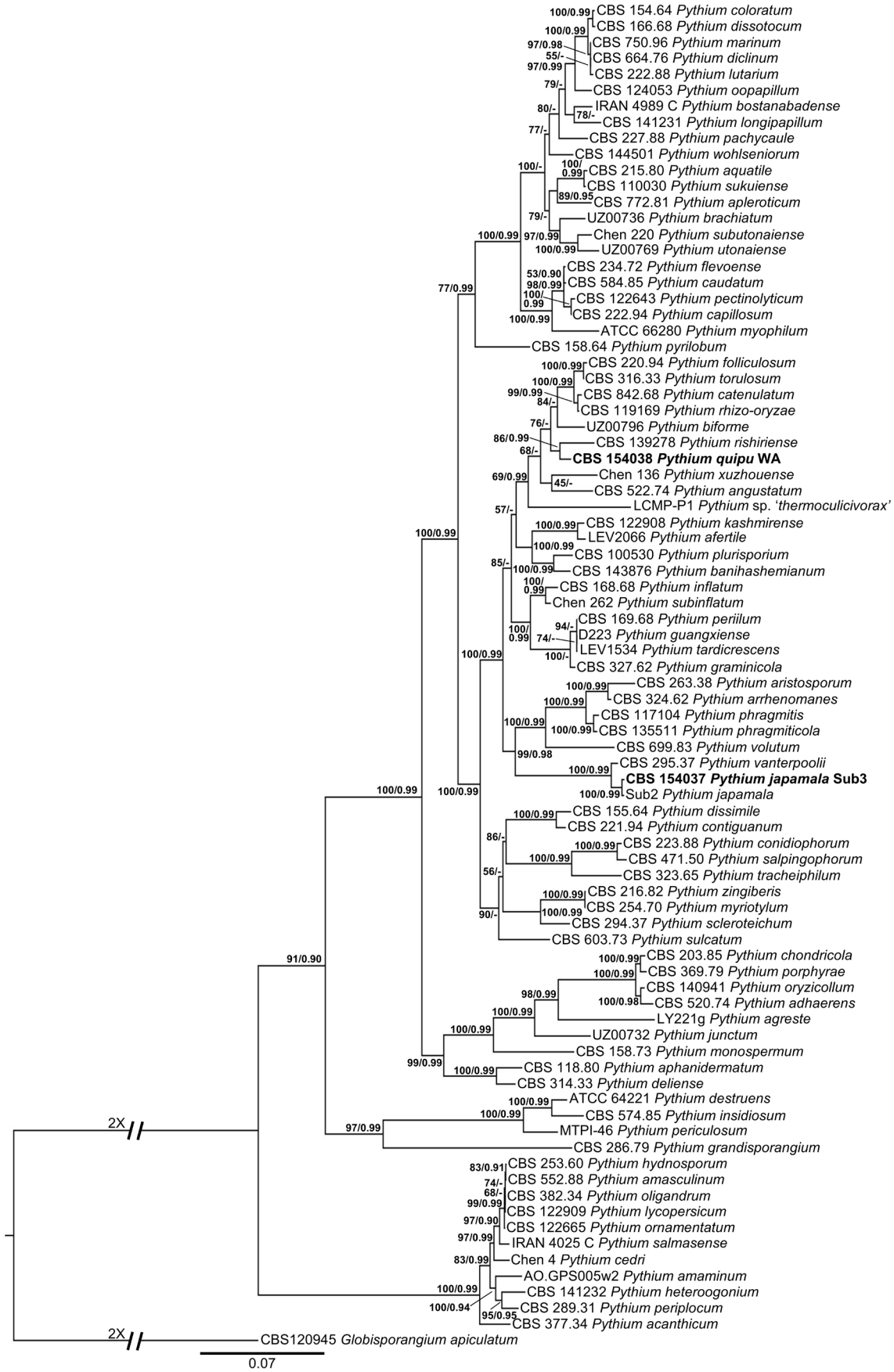

Fig. 1. Concatenated four loci (ITS, nc LSU rDNA, *cox*1, *cox*2) maximum likelihood phylogenetic topology of 83 *Pythium* species and 1 *Globisporangium* species outgroup. New species from this study are in bold (*P. quipu* and *P. japamala*). Taxa are shown in the phylogenetic groupings (depicted in different colored blocks) given by Levesque & De Cock (2004). Numbers at nodes represent Maximum Likelihood Bootstrap support values and Bayesian Posterior Probabilities. The very long outgroup branch lengths were halved indicated by the 2X and //.

Bent Grass (*Agrostis stolonifera*), (Outsidepride Seed Source, LLC,1565 Stryker Rd, Independence, OR 97351). Grass seeds were sown in 6.35 × 6.35 × 8.9 cm plastic pots (T.O. Plastics, 6035 Queens Ave NE, Otsego, MN 55330) that were filled with Sunshine Mix #1 (Sun Gro Horticulture, 770 Silver St, Agawam, MA 01001). Grass was then allowed to grow for 4–5 wk. To prepare inoculum, soil extract solution was autoclaved twice for 1 h and when cool added to 60 × 15 mm Petri plates. Four to five blades of grass (previously autoclaved for 30 min) were transferred to each plate of soil extract. The plates were inoculated with plugs taken from the margins of actively growing colonies of each isolate growing on PARP media and incubated in Benchmark Mytemp mini digital incubators at 15 °C until sporangia formed at 4–5 d and then moved to room temperature to produce zoospores. In the first set of experiments, five pots each of fescue were inoculated with one of the three *Pythium* isolates. Inoculation was done by incorporating a colonized grass blade and soil extract into the upper 5 mm of soil of each pot. Three control pots of uninoculated fescue (water only) were included in each replicate. Inoculated grasses were maintained at 15 °C and 70 % relative humidity (RH) in a Percival Scientific growth chamber (model LT-36VL, 505 Research Dr. Perry, IA 50220) with a 14 : L 10 : D light cycle. After 21 d, grasses were assessed for root health and root samples from each pot were plated onto PARP media. This experiment was repeated. A separate set of two pathogenicity tests were run for *Pythiogeton ramosum* on fescue following the same procedure except inoculated plants were incubated at 30 °C and 70 % RH.

All four isolates were inoculated on creeping bentgrass using the same methodology, except two sets of plants were inoculated with *Pythiogeton ramosum* and one set incubated at 16 °C and one set at 30 °C.

RESULTS

Initial environmental sequencing and baiting

The initial environmental *cox*2–spacer–*cox*1 detected oomycetes from four core samples in the New Jersey collection and all six in the North Carolina collection. The four core samples from New Jersey detected two different *Pythium* species which were both successfully baited out. All six North Carolina samples detected a *Pythium* species, which unfortunately was not baited out. One North Carolina sample 5-1 detected two oomycete signals belonging to *Pythium* and the other to *Pythiogeton* which was successfully baited out (Supplemental Fig. 1).

After baiting, two taxa were isolated that had DNA that matched DNA found in the original assay and appeared to belong to two new species (isolates Sub-2 and Sub-3 making up one taxon, *P. japamala,* and isolate WA making up the other, *P. quipu*). Another isolate baited from North Carolina samples appeared to be a species of *Pythiogeton*.

Initial identification and sequence similarity comparison

The *cox*1 and *cox*2 region were extracted from the consensus sequences and compared to the NCBI nt database, reported in Table 1 Top results. The preliminary results indicated that Sub2 and Sub3 were potentially *Pythium vanterpoolii* but the hits in the nt database were not to the type specimen. Comparison to the type specimen CBS 295.37 *Pythium vanterpoolii* genome (ASM2333453v1) (Nguyen *et al.* 2022) showed lower similarities for both Sub2 — *cox*1 98.21 %, *cox*2 99.49 %, *cox*2–spacer–*cox*1 98.65 % — and Sub3 — *cox*1 98.49 %, *cox*2 99.15 %, *cox*2–spacer–*cox*1 98.74 % — indicating that they are potentially different species. The results for WA were both to undescribed *Pythium* species indicating that it is potentially a new undescribed species. The results from the North Carolina samples had very low similarities (92.7–92.85 %) both to *Pythium aphanidermatum* indicating that it also is an undescribed species. The *cox*1 result for isolate 5-1 was 99.71 % similar to *Pythiogeton ramosum* indicating that 5-1 is *Pythiogeton ramosum*.

Phylogenetic analysis

The concatenated four loci alignment contained 84 species (Supplemental File # Alignment). The alignment was 3383 nucleotides long, of which 1386 were distinct patterns, 893 parsimony informative, 280 singleton sites, and 2210 constant sites. The Maximum likelihood phylogeny is displayed in Fig. 1. Phylogenetic placement with bootstrap supports of 86/0.99 clearly shows that isolate WA is a new species designated as *Pythium quipu*. The placement of Sub2 and Sub3 supports that they are different from the type specimen of *Pythium vanterpoolii* with bootstrap supports of 100/0.99. The two isolates had identical ITS and LSU sequences and are both designated as *Pythium japamala*. Both species belong to *Pythium* clade B.

Morphological results

After 7 d at 15 °C, colonies of the two isolates of *P. japamala* were slightly petaloid on CMA, PCA and PARP and radiate on V-8, with aerial mycelium in tufts on PCA and dense aerial growth on V-8. At 20 and 25 °C, the colonies were more obscurely petaloid on CMA, PCA and PARP.

After 7 d at 15–25 °C, colonies of *P. quipu* were slightly petaloid on CMA and PCA (with aerial mycelium), more densely petaloid on PARP, and cloudy and aerial on V-8.

The *Pythiogeton ramosum* isolate only produced sparse mycelium at 10 °C on CMA, PARP and V-8; it did not grow appreciably on PCA. At 15, 20 and 25 °C, it initially produced sparse mycelium on the surface of agar that grew in wide arcs, but with time produced a densely woven colony on CMA, PARP and V-8 (sometimes with sparse aerial growth consisting of long hyphae reaching the top of the Petri dish), and grew slowly as a dense radial colony on PCA.

In leaf blade culture, *Pythium japamala* produced sporangia at room temperature (22-24 °C), beginning after 4–5 d. Cultures that were incubated at 15 °C until sporangia formed and then moved to room temperature produced zoospores. Oogonia and antheridia began to form within 4–5 d at room temperature. Once oogonia matured into oospores, few antheridia were still visible. Appressoria were visible in CMA 4 d after inoculation of plates. Descriptions and dimensions are given in the *P. japamala* species description (taxonomy section, below).

Pythium quipu cultures grown at 15 °C for 4–5 d showed sparse mycelial growth at the edges of grass blades; when warmed to room temperature zoospores were released from what appeared to be unbranched sporangia that were not morphologically distinguishable from hyphae. When the diameter of nearby branched hyphae and unbranched emptied hyphae were compared, their diameters did not differ (2.82 + 0.57 um for nearby hyphae, 2.82 + 0.61 for emptied hypha, n = 50). Following the emptied hypha back from the terminal vesicle, it was empty of contents as far back as it could be traced, sometimes to its emergence from the grass blade tissue. To make certain such hyphae were not discharge tubes from sporangia submerged in the tissue, grass blades from which numerous vesicles had been observed were cleared in ethanol and stained in trypan blue; hyphae were readily observable, but no swellings of any kind were present at this stage of incubation. Hyphal swellings began to be observed after 6 d at 15 °C. When grass blade cultures were left to incubate for over a week, oogonia, antheridia and oospores could be seen infrequently. Appressoria were visible in CMA 4 d after inoculation of plates. Descriptions and dimensions are given in the *P. quipu* species description (taxonomy section, below).

In grass blade culture *Pythiogeton ramosum* produced long tendril-like hyphae with short side branches (Fig. 2A). The primary hyphae were 5.08 + 0.70 um diam. (n = 50), secondary branches had a diameter of 3.40 + 0.67 um (n = 48), and tertiary branches were 2.86 + 0.61 um (n = 28). From these side branches, appressoria (Fig. 2B) developed in CMA agar where hyphae contacted the plastic bottom of the Petri plate; these were club-shaped to sickle-shaped and measured 18.31 + 2.97 × 8.72 + 0.98 (n = 28). In solid media and in grass culture, large sporangia developed from side branches. These ranged from globose to ovoid to peanut-shaped or rarely tri-lobed (Fig. 2C–E). Average length was 72.45 + 18.09 um and width 39.13 + 4.93 um (n = 59). Cytoplasm in these sporangia was granular and contained regularly spaced vacuoles giving the sporangia a characteristic appearance (Fig. 2C–E). Sporangia in cultures incubated at 15 °C and brought to room temperature developed discharge tubes (Fig. 3A). Discharge tubes were 6.22 + 1.13 diam. and variable in length (97.48 + 53.70 um) (n = 54). Just prior to release of cytoplasm, an evanescent vesicle formed at the end of the discharge tube (Fig. 3B), which burst, leaving a bell-shaped remnant 10.58 + 5.14 um diam. (n = 40). The pool of cytoplasm differentiated into large zoospores with a discernable groove where flagella were set, 19.03 + 2.57 × 13.30 + 1.43 um (n = 44). Encysted zoospores were 15.55 + 1.45 um in diameter (n = 52). The morphology of the isolate closely matched the descriptions of *P. ramosum* given by Le *et al.* (2015) and Huang *et al.* (2013).

Temperature growth rate studies

For the two isolates of *P. japamala*, optimum temperature for growth was at 25 °C, with little or no growth at 30 °C and none at 35 °C (Fig. 4), even if cultures were subsequently moved to room temperature. For *P. quipu*, optimum growth occurred at 30 °C, declining at 35 °C and no growth at 40 °C (Fig. 4), even when plates were moved to room temperature. For *Pythiogeton ramosum*, little or no growth was seen at 5 °C, with a broad range of optimum growth at 25–35 °C, and little or no growth at 40 °C, largely consistent with Le *et al.* (2015), who reported an optimum between 30 and 35 °C, and Huang *et al.* (2013), who reported an optimum at 32 °C.

Pathogenicity tests

No symptoms of root or crown rot were seen on grass roots or crowns for either grass species inoculated with the two isolates of *Pythium japamala*, or *Pythium quipu* and incubated at 15 °C; *Pythiogeton ramosum* inoculated on grasses and incubated at 15 or 30 °C did not cause symptoms. However, root density might have been sparser in plants inoculated with the *Pythium* species. Oomycetous colonies could be recovered from inoculated roots, and representative colonies matched the morphology of the species which was used for inoculation. *P. quipu* was easily recovered from the grasses; *P. ramosum* was only recovered three times after the 3-wk incubation period.

Taxonomy

Based on the results obtained from the phylogenetic analyses and morphological studies, we describe two new species of *Pythium* clade B.

Pythium japamala N. Shishkoff & G. Groben, ***sp. nov.*** MB 860824. Figs 5, 6.

Etymology: From japamala, a loop of prayer beads used in Eastern religions. The epithet is treated as a noun in apposition and is invariable.
Typus: **USA**, New Jersey, North Brunswick, 40°28’ N, 74°25’ W, from *Agrostis stolonifera* (creeping bentgrass) roots maintained as putting green turf, 2024, coll. *G. Groben* (**holotype** = CBS 154037, preserved in metabolically inactive state) ex-type culture Sub3 = CBS 154037. Barcodes: ITS sequence GenBank PX314632, *cox*1 sequence GenBank PQ567643, *cox*2 sequence GenBank PQ567643, LSU sequence GenBank PX314629.

Colonies on cornmeal agar and potato-carrot agar after seven days were slightly petaloid at 10–15 °C (with tufts of aerial mycelium on PCA at 15 °C) and cloudy at 20 °C; on V-8 agar aerial mycelium was present and colonies were radial. In grass blade culture, hyphae were 2.73 + 0.65 um diam. (n = 85). *Appressoria* (Fig. 5F) were formed in clusters, globose to club-shaped, with a diameter of 11.07 + 1.4 µm (n = 17); in club-shaped appressoria where length could be measured, length was 24.04 + 4.95 µm. Young sporangia (Fig. 5A) were generally unbranched short chains of torulose swellings. In young grass blade cultures, the sporangia were terminal, comprised of a few indistinct smaller swellings followed by larger, more crowded and more distinct swellings before tapering into a beak with a blunt apex (thicker than the hypha that preceded the sporangium, 3.90 + 0.80 µm, n = 51). The chains initially consist of 3–(7.0 + 2.1)–13 swellings (n = 126). Swellings measured 14.05 + 3.53 µm in diameter (n = 111). Sporangium length was variable, averaging 123.93 + 60 um (n = 43). Under the proper conditions (incubation

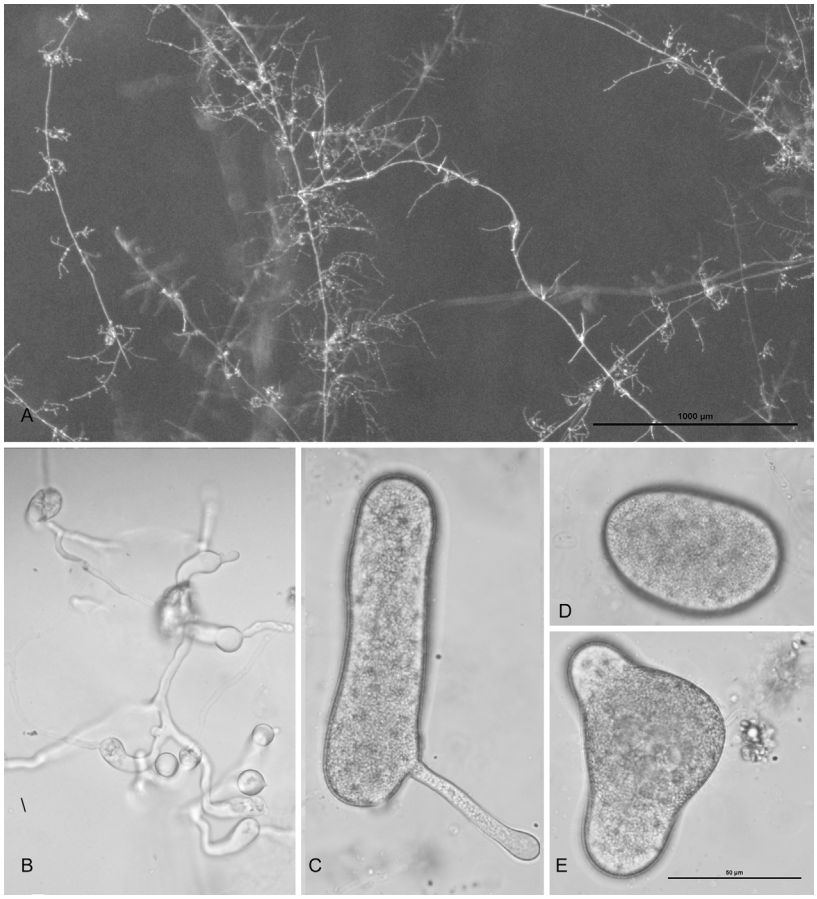

Fig. 2. *Pythiogeton ramosum* isolated from North Carolina turf. **A.** Mycelium in water agar at 10×. **B.** Appressoria at the bottom of a plate of CMA. **C–E.** Sporangia with granular cytoplasm (40×).

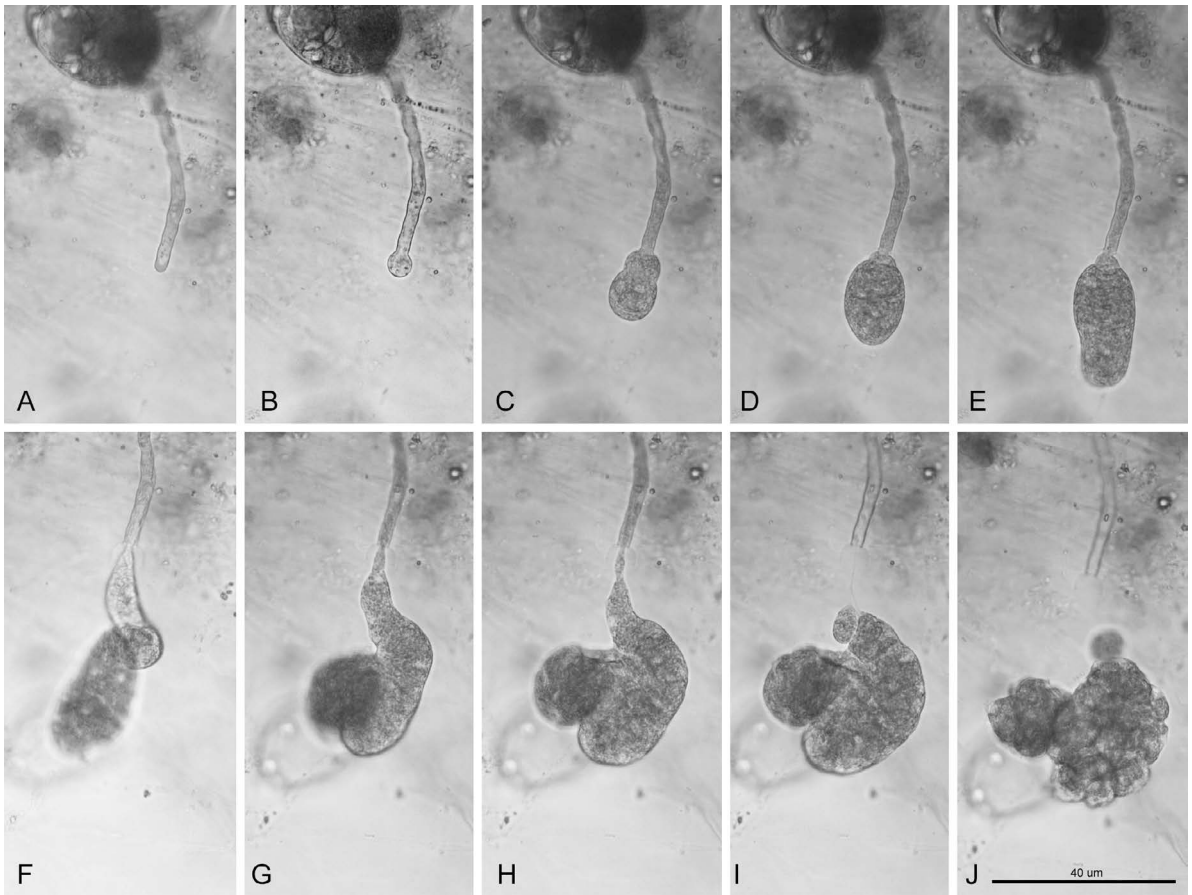

Fig. 3. Sporangium of *Pythiogeton ramosum* releasing cytoplasm that differentiates into zoospores (40×). **A.** Discharge tube growing from a sporangium. **B.** Ephemeral vesicle forming at the end of the discharge tube. **C.** Vesicle bursting to release cytoplasm. **D–H.** Cytoplasm releasing from discharge tube. **I.** Sporangium emptied of cytoplasm; bell-shaped vesicle remnant visible. **J.** Zoospores beginning to differentiate.

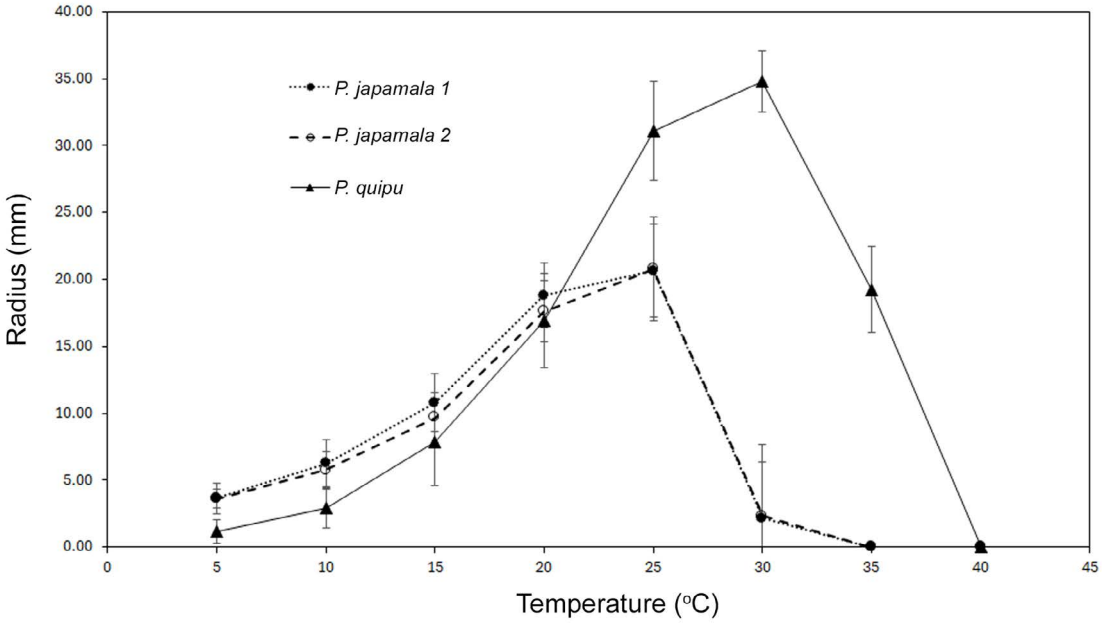

Fig. 4. Growth (mm) of isolates of *P. japamala* (circles) and *P. quipu* (triangles) cultured on V-8 agar for 48 h at different temperatures (5–40 °C). Error bars denote standard deviation.

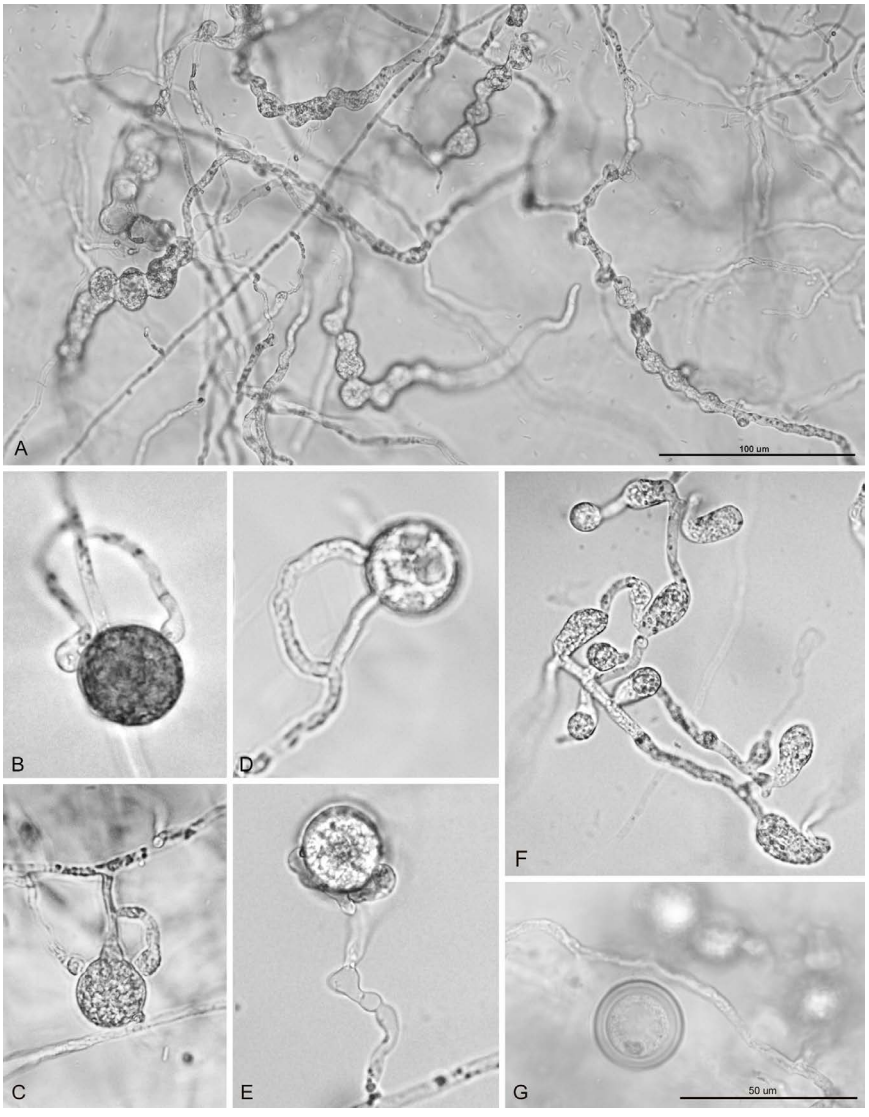

Fig. 5. Morphological features of *P. japamala*. **A.** Young sporangia from grass blade culture (20×). **B–E.** Oogonia and antheridia in grass culture (40×). **F.** Appressoria growing on the bottom of a plate of CMA (40x). G). Oospore (40x).

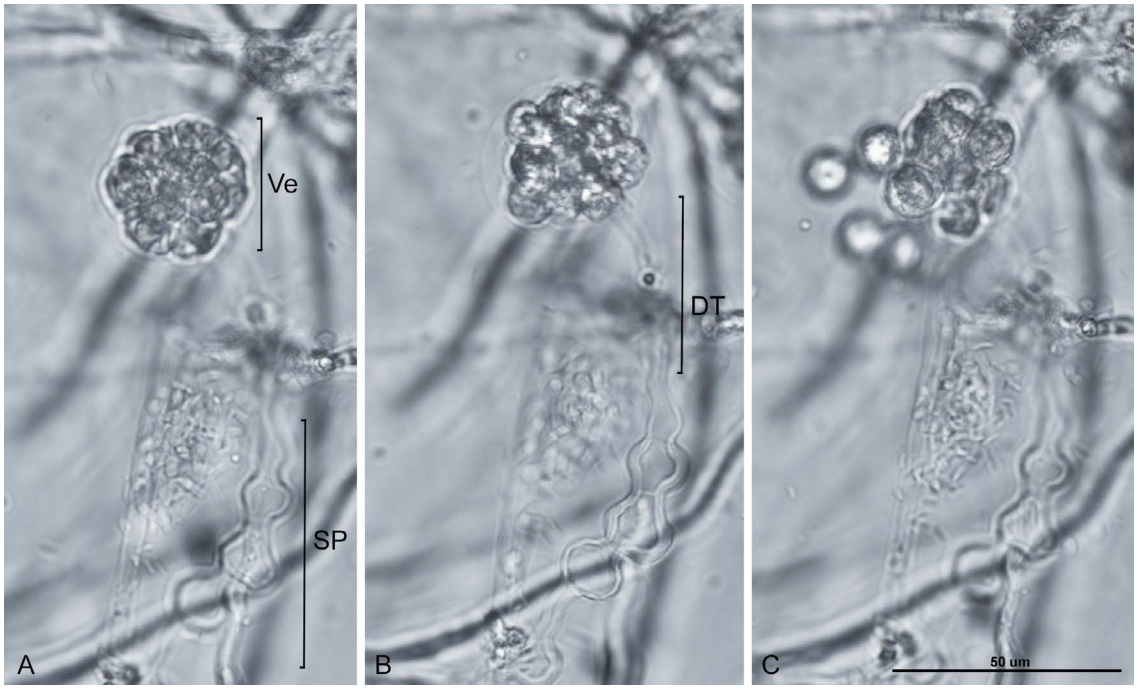

Fig. 6. Sporangia of *P. japamala* releasing zoospores (40×). **A.** Emptied sporangium (SP) with an out-of-focus discharge tube ending in a vesicle (Ve). **B.** Discharge tube (DT) terminating in a vesicle containing developing zoospores. **C.** Zoospores breaking out of vesicle.

at 15 °C in grass blade culture before being brought to room temperature) the apex elongated into a tube of variable length (30–330 um) that discharged zoospores in a vesicle (Fig. 6). Zoospores were 10.3 + 1.02 × 9.01 + 1.51 (n = 19). In older cultures, the sporangium could become complexly branched, or multiple discharge tube-like mycelia could develop from some of the intercalary swellings. Under conditions not conducive to zoospore production these tubes would continue to elongate and produce additional sporangia. Sometimes the cytoplasm in the original sporangium migrated into the discharge tube-like extension when it continued to elongate, leaving the original sporangium empty. Oogonia (21.66 + 2.08 µm diam., n = 100) were readily formed in culture Fig. 5B–E), spherical, with generally one or two (rarely three or four) antheridial branches forming on mycelium below the oogonium, the branches typically forming on the same hypha as the oogonium, 18.63 + 10.19 µm below (n = 50). Antheridia were club-shaped to campanulate (Fig. 5B–E) and attaching apically to the oogonium, 5.45 + 1.22 µm wide and 9.71 + 2.8 µm long (n = 52). They were generally not visible at oospore maturity. Oospores (20.28 + 2.06 µm, n = 100) were nearly or completely pleuritic (Fig. 5G), with a 2-layered wall 2.14 + 0.52 µm thick (n = 100).

Cardinal temperatures: minimum below 5 °C, optimum 25 °C maximum below 35 °C. Daily growth rate at 25 °C 14 mm.

Substrate: A sod core from creeping bentgrass (*Agrostis stolonifera*) in New Jersey.

Pathogenicity: Tests on creeping bentgrass and tall fescue at 15 °C did not result in symptoms of disease.

Habitat: *Agrostis stolonifera* roots maintained as putting green turf.

Geographic distribution: USA: New Jersey.

Notes: *Pythium japamala* closely resembles *P. vanterpoolii* and may not be morphologically distinguishable from it. Sporangia are subglobose and sometimes in chains, often in "catenulate complexes of subglobose or irregularly ellipsoidal unbranched outgrowths of the mycelium, terminal or intercalary" (van der Plaats-Niterink 1981). Oogonia are globose, terminal or intercalary 18–21 µm diam., with 1–2 monoclinous antheridia which disappear after fertilization. Oospores are plerotic, 17–20 µm diam with the wall 2–4 µm thick. Optimum growth is at 25 °C; daily growth 20 mm at °25 °C (van der Plaats-Niterink 1981), which is greater than we observed in our isolates. In the U.S., P. *vanterpoolii* has been found on creeping bentgrass (*Agrostis palustris*) in Ohio (Muse *et al.* 1974) on *Poa annua* in Washington (Stacey *et al.* 2011), rye and wheat (Reeves *et al.* 2021) and maize (Liu 1977).
Pythium arrhenomanes, *P. aristosporum*, and *P. volutum* can be distinguished from *P. japamala* by their larger oogonia (32.5, 32.7, and 30 µm, respectively) and larger number of antheridia per oogonium (3–20, depending on the species) (van der Plaats-Niterink 1981). *Pythium arrhenomanes* has been reported on turf (Hodges & Coleman 1985), on rice (Toda *et al.* 2015), sugarcane (Lee & Hoy 1992), maize (Reyes-Tena *et al.* 2018), wheat and barley (Olsson & Kadir 1994, Waller 2007), and sorghum (Forbes *et al.* 1987), and on many other hosts (van der Plaats-Niterink 1981). *Pythium aristosporum* has been reported from grasses (Hodges & Coleman 1985) and *P. volutum* has been recorded from wheat and oat in Canada and turf grasses in the U.S. (van der Plaats-Niterink 1981).,

Pythium quipu N. Shishkoff & G. Groben, ***sp. nov.*** MB 860841. Figs 7, 8.

Etymology: From quipu, an Incan record-keeping device consisting of knotted string. The epithet is treated as a noun in apposition and is invariable.

Typus: **USA**, New Jersey, North Brunswick, 40°28'N, 74°25'W, from *Agrostis stolonifera* (creeping bentgrass) roots maintained as putting green turf, 2024, coll. *G. Groben* (**holotype** CBS 154038, preserved in metabolically inactive state), ex-type culture WA = CBS 154038. Barcodes: ITS sequence GenBank PX314633, *cox*1 sequence GenBank PQ567644, *cox*2 sequence GenBank PQ567644, LSU sequence GenBank PX314630.

Colonies incubated at 15 °C on CAM, PCA and PARP were densely petaloid after 7 d (with tufts of aerial mycelium on PCA). On V-8, it grew out radially or formed a weak rosette with abundant aerial mycelium. In grass blade culture, hyphae were 2.55 + 0.51 µm diam. (n = 75). *Appressoria* were small and club-shaped (Fig. 7D), attaching to substrate apically, with a width of 6.70 + 1.07 µm (n = 67); in club-shaped appressoria where length could be measured, length was 11.91 + 3.18 µm. After 3–5 d at 15 °C in grass culture and brought to room temperature, zoospores formed from what appeared to be undifferentiated filamentous sporangia (Fig. 8A). Cytoplasm could be observed rapidly emptying from the hypha into the vesicle. In every case where the empty hypha could be traced back, it originated from the grass blade. The empty hypha could be extremely long-from 271–556 µm from vesicle to origin at the surface of the leaf (n = 5). Vesicles (Fig. 8B–F) were 28.60 + 8.56 diam. (n = 17) and differentiated into 6–44 zoospores. Zoospores were 9.39 + 0.70 µm in length and 8.2 + 0.84 µm in width (n = 50). Rarely, lobed structures could be observed that resembled the inflated hyphal sporangia described for *P. xuzhouense* (Chen *et al*. 2020) but these were never observed forming zoospores. After further incubation in grass blade culture, particularly at 15 °C, hyphal swellings formed in chains that could be quite long (Fig. 7A). Swellings were globose or ovoid, 12.75 + 3.08 um diam. (n = 75), but in older cultures could become fingerlike. Oogonia were rarely seen, but could be

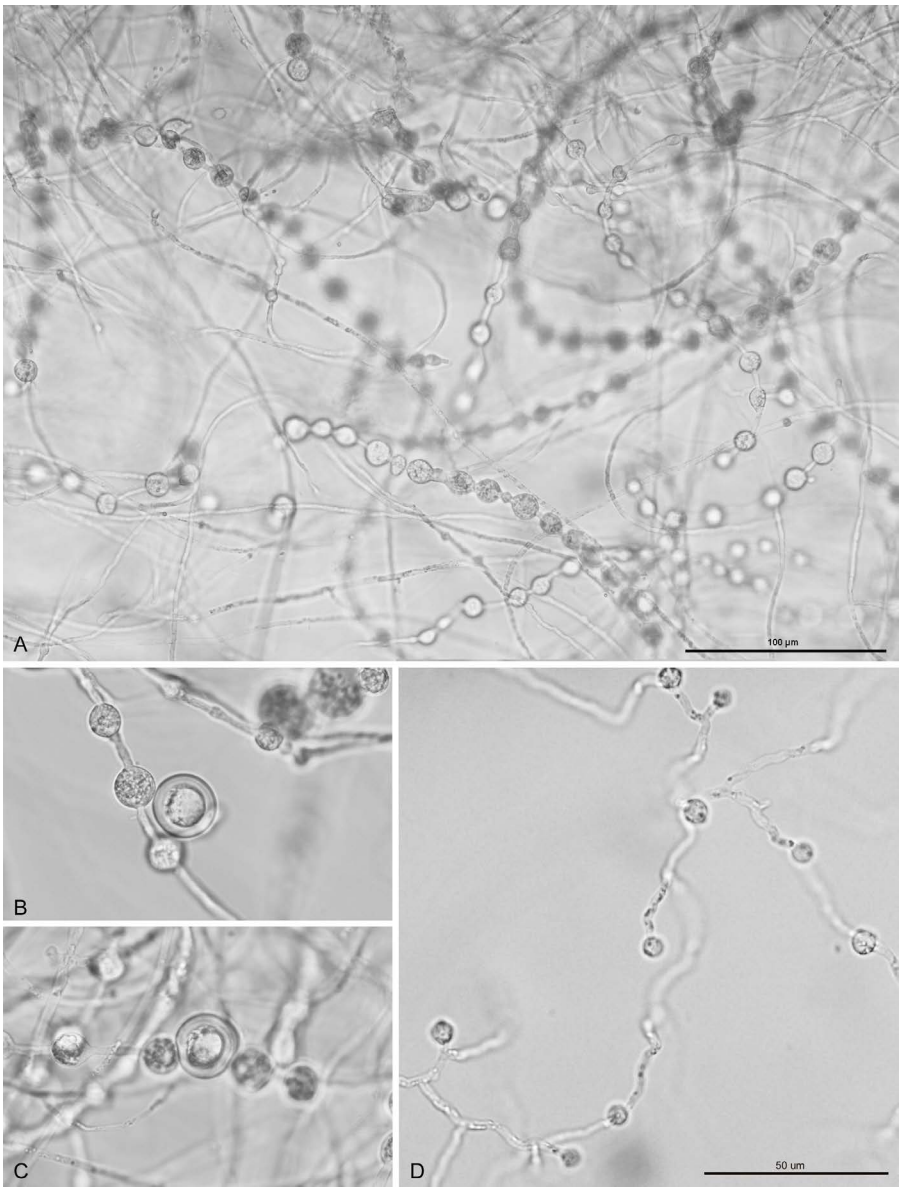

Fig. 7. Morphological features of *P. quipu*. **A.** Chains of hyphal swellings in grass culture (20×). **B, C.** Oospores interspersed with hyphal swellings (40×). **D.** Appressoria forming on the bottom of a plate of CMA (40×).

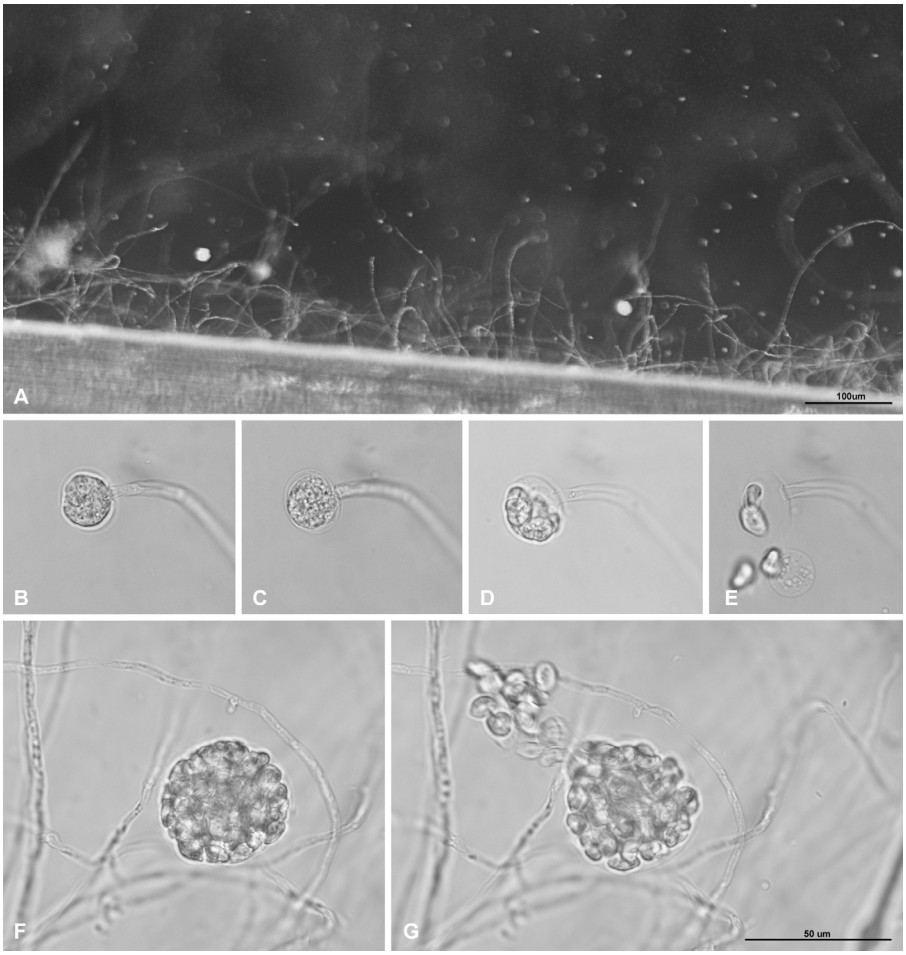

Fig. 8. Sporangia of *P. quipu*. **A.** vesicles and swimming zoospores in grass blade culture at 8×. **B–E.** Small vesicle developing at the end of the hyphal-like sporangium, with zoospores developing and being released (40×). **E, F.** Large vesicle releasing numerous zoospores (40x)

found between hyphal swellings, or sometimes projecting from one on a short stalk. They were spherical, 20.35 + 1.17 µm diam. (n = 13) and small club-shaped antheridia measured 10.42 + 2.76 × 5.97 + 1.79 µm (n= 9). Oospores (Fig. 7B, C) were globose (19.58 + 2.0 µm diam., n = 38) and pleurotic, with a thick 2-layered wall 3.27 + 0.90 µm thick (n = 37).

Cardinal temperatures: Minimum below 5 °C, optimum 30 °C maximum below 40 °C. Daily growth rate at 25 °C 19 mm.

Substrate: Creeping bentgrass (*Agrostis stolonifera*).

Pathogenicity: Tests on creeping bentgrass and tall fescue at 15 °C did not result in symptoms of disease.

Habitat: *Agrostis stolonifera* roots maintained as putting green turf.

Geographic distribution: USA: New Jersey.

Notes: *Pythium catenulatum* is morphologically similar to *P. quipu* and found on turfgrass (Hendrix & Campbell 1969), rice (Balakrishnan 1948), and sugarcane (Hsu 1965). Larger hyphal swellings are present in chains of 3–8, 10–20 µm diam (van der Plaats-Niterink 1981). The species is usually heterothallic, and when present, oogonia are 22.8 µm diam., with 5–12 antheridia per oogonium. Oospores are pleurotic with a wall 1.5 µm thick. Temperature optimum was 30–35 °C, with a daily growth rate of 14 mm at 25 °C (van der Plaats-Niterink 1981). *Pythium rhizo-oryzae* was isolated from soil in rice paddies in India (Bala *et al.* 2006), and its chains of hyphal swellings closely resemble those of *P. quipu*. However, no sporangia or zoospores were observed. Oogonia (19.7 µm diam.) were rare but surrounded by many antheridia forming a tangled mat. Oospores (16.7 µm diam.) were pleurotic or apleurotic, with a thick wall (1–2 µm) (Bala *et al.* 2006). *Pythium torulosum* is also reported from turf (Howard *et al.* 1951), but its sporangia are toruloid complexes. *P. angustatum*, reported from maize (Sparrow 1932) has filamentous non-inflated sporangia, but its oospores are apleurotic. *Pythium rishiriense*, found from water in Japan, has filamentous-inflated sporangia, oogonia (24.3 µm) with 1–5 antheridia and oospores (22 µm) (Rahman *et al.* 2015*). Pythium biforme*, found in lake water in Japan (Uzuhashi *et al.* 2015) and golf course irrigation water in Missouri and Kansas (Rushford *et al.* 2022), has globose or filamentous hyphal bodies, mostly terminal oogonia (av.19.5 µm) with 1–4 monoclinous antheridia and pleurotic oospores (av. 17.7 µm); vesicles and zoospores were not observed (Uzuhashi *et al.* 2015). *Pythium xuzhouense*, from soybean roots in China, has filamentous or inflated sporangia, no hyphal swellings, oogonia (mean 16.5 µm) with 1–3 mostly diclinous antheridia, and pleurotic or nearly pleurotic oospores (mean 15.5 µm) with a thin oospore wall (mean 0.9 µm) thick (Chen, *et al.* 2020).

Sequence similarities with Isolate BRIP 75664a (ITS 99.88 %, *cox*2 100.00 %) indicates that *Pythium quipu* could be present in Australia: Queensland on C4 turfgrass *Zoysia* sp.

DISCUSSION

Pythiales are responsible for three different turfgrass diseases of Pythium blight, Pythium root rot, and Pythium root dysfunction (Tredway *et al.* 2022). With overlapping causal agents and similar morphological features these diseases are difficult to diagnosis to species which provided a good opportunity to test the new *cox*2–spacer–*cox*1 approach. The direct environmental sequencing approach was able to accurately identify oomycete species often producing 100 % identical sequences to the cultures that were eventually isolated from the environmental samples. This approach provides a rapid way to survey environmental samples to identify which had the highest probability of culturing specimen from. The best example from this study was how we cultured *Pythiogeton ramosum*. From the six North Carolina samples, only one produced two signals belonging to a *Pythium* sp. and *Pythiogeton ramosum* which narrowed down our efforts to focus on sample 5-1 and eventually resulted in isolating a pure culture. This approach should be transferable to other oomycete species and could potentially increase the chances of culturing species from the environment.

The variance in aggressiveness between the over 35 known species cultured from turfgrasses varies from highly aggressive (61–100 % diseased) to nonpathogenic (Abad, *et al.* 1994). Research from Abad *et al.* (1994) showed that all *Pythium* species isolates tested had statistically similar aggressiveness except *Pythium vanterpoolii* isolates which ranged from highly aggressive to nonpathogenic. Given that *Pythium japamala* is morphologically very similar to *P. vanterpoolii* and did not cause noticeable disease; it is possible that the non-pathogenic isolates in the Abad *et al.* (1994) study and a nonpathogenic isolate found by Hodges and Coleman (1985) could have been *Pythium japamala*.

Overall, *Pythiales* are some of the most important turfgrass pathogens which often require fungicide applications to control the disease, especially on new seedings (Tredway *et al.* 2022). Different *Pythiales* spp. have different responses to fungicide. For instance, the *Pythiales* spp. that cause Pythium Root Rot are commonly controlled by the fungicides mefenoxam, propamocarb, fosetyl-Al and etridiazole however those same chemistries provide less control on *Pythiales* spp. causing Root Dysfunction (Hampy *et al.* 2022). Understanding which *Pythiales* spp. are infecting turfgrass could lower unnecessary fungicide inputs by reducing preventative applications for low aggressive or nonpathogenic species like *Pythium quipu* and *Pythium japamala*.

Plant pathologists who assay symptomatic grasses generally find more than one *Pythium*. Aoyagi *et al.* (1999), sampling at a number of sites in Japan over time for 5 yr found *Pythium periplocum*, *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* were predominant, along with lower numbers of *P. dissotocum*, *P. iwayamai*, *P. periilum*, *P. spinosum*, *P. sylvaticum*, and unidentified isolates. Abad *et al.* (1994) found 33 *Pythium* spp. from roots and crowns of symptomatic turfgrass species in North Carolina; the predominant species recovered were *P. arrhenomanes*, *P. catenulatum*, *P. intermedium*, *P. oligandrum*, *P. periilum*, *P. torulosum* and *P. vanterpoolii*. Pythium complexes of two or more species from the same tissue sample were common. Kim *et al*. (1999) found 11 species of *Pythium* on symptomatic turfgrasses in golf courses in Korea. Saladini *et al.* (1983), found 17 *Pythium* spp. from healthy and cottony-blighted turf grass samples and concluded that *P. aphanidermatum*, *P. graminicola* and P*. torulosum* were most commonly associated with the disease in Ohio. These investigations suggest that the sod layer is a conducive environment for *Pythium* whether or not disease is triggered, and that many more new species might be found if investigators did systematic assays of healthy and diseased grass environments

The genus *Pythiogeton* was originally described as a saprophytic on dead plant material (Minden 1916), but over time species have been found to cause disease on plants; the first record of disease was of *P. zeae* causing stalk rot of corn in Korea (Jee *et al.* 2000), the second was *P. zizaniae* isolated from bamboo in Taiwan (Ann *et al.* 2006) and the third *P. manoomin* on wild rice in California (Doan & Davis 2019). *Pythiogeton ramosum* was thought to be a saprophyte (Minden 1916, Drechsler 1932) before being found to cause soft rot on ginger in Australia at higher temperatures (Le *et al.* 2015). Isolates identified as *P. ramosum* have been found in Ohio (Drechsler 1932) and New York (Sparrow 1932), and it is likely, given its wide distribution globally, that it is common in the U.S. This, however, is the first molecularly confirmed report of it in the U.S. and a first report for North Carolina.

DATA AVAILABILITY

All supplemental data is available at USDA Ag Data commons https://doi.org/10.15482/USDA.ADC/32348121.

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Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Abad ZG, Shew HD, Lucas LT (1994). Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. *Phytopathology* **84**: 913–921. https://doi.org/10.1094/Phyto-84-91

Ann P-J, Huang J-H, Wang H-T (2006). *Pythiogeton zizaniae*, a new species causing basal stalk rot of water bamboo in Taiwan. *Mycologia* **98**: 116–120. https://doi.org/10.3852/mycologia.98.1.116

Bala K, Gautam N, Paul B (2006). *Pythium rhizo-oryzae sp. nov*. isolated from paddy fields: taxonomy, ITS region of rDNA, and comparison with related species. *Current Microbiology* **52**: 102–107. https://doi.org/10.1007/s00284-005-0116-9

Balakrishnan MS (1948). South Indian Phycomycetes. 2. Some little-known species of *Pythium* occurring in South India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **28**: 27–34. https://www.ias.ac.in/article/fulltext/secb/028/01/0027-0034

Behrens FH, Fischer M (2022). Evaluation of different phyllosphere sample types for parallel metabarcoding of fungi and Oomycetes in *Vitis vinifera*. *Phytobiomes Journal* **6**: 207–213. https://doi.org/10.1094/PBIOMES-11-21-0072-SC

Bouckaert R, Heled J, Kühnert D, *et al.* (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* https://doi.org/10.1371/journal.pcbi.1003537

Chen J-J, Feng H, Song W, *et al.* (2020). Two new *Pythium* species from southern China based on morphological and molecular characters. *Phytotaxa* **432**: 263–273 https://doi.org/10.11646/phytotaxa.432.3.3

Clarke BB, Vincelli P, Koch P, *et al.* (2024). *Chemical Control of Turfgrass Diseases 2024*. University of Kentucky Cooperative Extension, Lexington, KY.

Delmont TO, Robe P, Cecillon S, *et al*. (2011). Accessing the soil metagenome for studies of microbial diversity. *Applied and Environmental Microbiology* **77**: 1315–1324. https://doi.org/10.1128/AEM.01526-10.

Doan HK, Davis RM (2019). *Pythiogeton manoomin*, a new species causing root and basal stalk rot of wild rice in the United States. *Mycology* **10**: 243–249. https://doi.org/10.1080/21501203.2019.1635216

Drechsler C (1932). A species of *Pythiogeton* isolated from decaying leaf-sheaths of the common cat-tail. *Journal of the Washington Academy of Sciences* **22**: 421−449. https://www.biodiversitylibrary.org/part/139411

Emerson R (1958). Mycological organization. *Mycologia* **50**: 589–621. https://doi.org/10.1080/00275514.1958.12024755

Forbes GA, Ziv O, Frederiksen RA (1987). Resistance in sorghum to seedling disease caused by *Pythium arrhenomanes*. *Plant Disease* **71**: 145–148. https://doi.org/10.1094/PD-71-0145

Groben G, Dobbs TD, Batuman O, *et al.* (2026). Oxford nanopore-based *cox*1-spacer-*cox*2 amplicon sequencing enables high-resolution molecular identification of oomycetes from environmental samples. *PhytoFrontiers*: In press.

Hampy HD, Van Ryzin BJ, Butler EL, *et al*. (2022). Etiology and management of Pythium root rot in golf course putting greens. *International Turfgrass Society Research Journal* **14**: 851–860. https://doi.org/10.1002/its2.24

Hendrix FF, Cambell WA (1969). Heterothallism in *Pythium catenulatum*. *Mycologia* **61**: 639–641. https://doi.org/10.1080/00275514.1969.12018778

Hodges CF, Coleman LW (1985). *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. *Plant Disease* **69**: 336–340. https://doi.org/10.1094/PD-69-336.

Hsu SC (1965). A root rot of sugarcane caused by *Pythium catenulatum* in Taiwan. *Phytopathology* **55**: 705–706.

Huang J-H, Chen C-Y, Lin Y-S, *et al*. (2013). Six new species of *Pythiogeton* in Taiwan, with an account of the molecular phylogeny of this genus. *Mycoscience* **54**: 130–147. https://doi.org/10.1016/j.myc.2012.09.007

Jee HJ, Ho HH, Cho WD (2000). *Pythiogeton zeae sp. nov*. causing root and basal stalk rot of corn in Korea. *Mycologia* **92**: 522–527. https://doi.org/10.1080/00275514.2000.12061188

Jeffers SN, Martin SB (1986). Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* **70**: 1038–1043.

Le DP, Smith MK, Aitken EAB (2015). *Pythiogeton ramosum*, a new pathogen of soft rot disease of ginger (Zingiber officinale) at high temperatures in Australia. *Crop Protection* **77**: 9–17. http://dx.doi.org/10.1016/j.cropro.2015.07.012

Lee YS, Hoy JW (1992). Interactions among Pythium species affecting root rot of sugarcane. *Plant Disease* **76**: 735–739. https://doi.org/10.1094/PD-76-0735

Levesque CA, De Cock AW (2004). Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research* **108**: 1363–1383. https://doi.org/10.1017/S0953756204001431

Liu PSW (1977). A supplement to a host list of plant diseases in Sabah, Malaysia. *Phytopathology Paper* **21**: 1–49.

Ma B, Lu C, Wang Y, *et al.* (2023). A genomic catalogue of soil microbiomes boosts mining of biodiversity and genetic resources. *Nature Communications* **14**: 7318. https://doi.org/10.1038/s41467-023-43000-z.

Minden M (1916). Beitrage zur biologie und systematic einheimscher submerser Phycomyceten. *Mykologische Untersuchungen und Berichte von Dr Richard Falck* **1**: 146−255.

Muse RR, Schmitthenner AF, Partyka RE (1974). *Pythium* spp. associated with foliar blighting of creeping bentgrass. *Phytopathology* **64**: 252–253.

Nguyen HDT, Dodge A, Dadej K, *et al.* (2022). Whole genome sequencing and phylogenomic analysis show support for the splitting of genus *Pythium*. *Mycologia* **114**: 501–515. https://doi.org/10.1080/00275514.2022.2045116

Olsson S, Kadir S (1994). Characteristics of a *Pythium arrhenomanes* with high frequency in barley soils. *Journal of Phytopathology* **140**: 335–345. https://doi.org/10.1111/j.1439-0434.1994.tb00616.x

Reeves ER, Kerns JP, Cowger C, *et al.* (2021). *Pythium* spp. associated with root rot and stunting of winter wheat in North Carolina. *Plant Disease* **105**: 986–996.

Rahman MZ, Abdelzaher HMM, Mingzhu L, *et al.* (2015). *Pythium rishiriense sp. nov*. from water and *P. alternatum sp. nov*. from soil, two new species from Japan. *Federation of European Microbiological Societies Microbiology Letters* **362**: 378–1097. https://doi.org/10.1093/femsle/fnv086

Reich J, Chen W, Radford D, *et al.* (2023). Combining air sampling and DNA metabarcoding to monitor plant pathogens. *PhytoFrontiers* **3**: 639–653. https://doi.org/10.1094/PHYTOFR-10-22-0108-R

Reyes-Tena A, Vallejo-González R, Santillán-Mendoza R, *et al.* (2018). *Pythium arrhenomanes* causal agent of root rot on yellow maize in Mexico. *Australasian Plant Disease Notes* **13**: 6. https://doi.org/10.1007/s13314-018-0291-8

Rossmann S, Lysøe E, Skogen M, *et al.* (2021). DNA metabarcoding reveals broad presence of plant pathogenic Oomycetes in soil from internationally traded plants *Frontiers in Microbiology* **12**: 637068. https://doi.org/10.3389/fmicb.2021.637068

Rushford CA, North RL Miller GL (2022). Detection of *Pythium* spp. in golf course irrigation systems. *Plant Disease* **106**: 46–56. https://doi.org/10.1094/PDIS-02-21-0399-RE

Simmons EG (2007). *Alternaria: an identification manual*. CBS Fungal Diversity Center, Utrecht, The Netherlands.

Sparrow FK (1932a). Observations of the aquatic fungi of Cold Spring Harbor. *Mycologia* **24**: 268–303. https://doi.org/10.1080/00275514.1932.12020619

Sparrow FK (1932b). Observations on the parasitic ability of certain species of *Pythium*. *Phytopathology* **22**: 385–390.

Stothut M, Mahla L, Backes L, *et al.* (2024). Recovering plant-associated arthropod communities by eDNA metabarcoding historical herbarium specimens. *Current Biology* **34**: 4318–4324. https://doi.org/10.1016/j.cub.2024.07.100

Sudermann MA, Foster ZSL, Chang JH, *et al.* (2023). Metabarcoding for plant pathologists. *Canadian Journal of Plant Pathology*, **46**: 142–160. https://doi.org/10.1080/07060661.2023.2290041

Theologidis I, Karamitros T, Vichou AE, *et al.* (2023). Nanopore-sequencing metabarcoding for identification of phytopathogenic and endophytic fungi in olive (*Olea europaea*) twigs. *Journal of Fungi* **9**: 1119. https://doi.org/10.3390/jof9111119.

Toda T, Iwasa A, Fuji S, *et al.* (2015). Widespread occurrence of *Pythium arrhenomanes* pathogenic to rice seedlings around Japanese rice fields. *Plant Disease* **99**: 1823–1831. https://doi.org/10.1094/PDIS-01-15-0124-RE

Tredway LP, Tomaso-Peterson M, Kerns JP, *et al.* (2022). *Compendium of turfgrass diseases*, 4th ed. American Phytopathological Society, St. Paul, MN, U.S.A.

Uzuhashi S, Okada G, Ohkuma M (2015). Four new Pythium species from aquatic environments in Japan. *Antonie van Leeuwenhoek* **107**: 375–391. https://doi.org/10.1007/s10482-014-0336-8

van der Plaats-Niterink AJ (1981). Monograph of the genus *Pythium*. *Studies in Mycology* **21**: 1–244. https://www.cabdirect.org/cabdirect/abstract/19821379677

Waller JM (1979). Observations on Pythium root rot of wheat and barley. Plant Pathology 28: 17–24. https://doi.org/10.1111/j.1365-3059.1979.tb02612.x

Weinand JM, Pelser T, Kleinebrahm M, et al. (2025). Countries across the world use more land for golf courses than wind or solar energy. Environmental Research Communications 7: p.021012. https://doi.org/10.1088/2515-7620/adb7bd

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Supplementary material

Fig. S1. An example of an environmental sequencing from One North Carolina (sample 5-1) showing two oomycete signals, one belonging to *Pythium* and the other to the *Pythiogeton* which was successfully baited out.