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Parvodontia relampaga sp. nov.: A Cystostereaceae fungal pathogen that is the causal agent of relampago blight of woody plants in Florida, USA

Claudia A. Paez [a,b,*], Jason A. Smith [c,d], Karen K. Nakasone [e], Sladana Bec [f], Carrie L. Harmon [a], Hector Urbina [b], Jeffrey M. Eickwort [g], Matthew E. Smith [a]

[a] Department of Plant Pathology, University of Florida, Gainesville, FL, 32611, USA
[b] Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Section of Plant Pathology, Gainesville, FL, 32608, USA
[c] School of Forest, Fisheries and Geomatics Sciences, University of Florida, Gainesville, FL, 32611, USA
[d] Department of Biological and Environmental Sciences, College of Natural and Health Sciences, University of Mount Union, Ohio, 44601, USA
[e] Center for Forest Mycology Research, USDA Forest Service, Northern Research Station, One Gifford Pinchot Drive, Madison, WI, 53726, USA
[f] Ball Horticultural Company, 622 Town Rd, West Chicago, Illinois, 60185, USA
[g] Florida Department of Agricultural and Consumer Services, Florida Forest Service, Section of Forest Health, Gainesville, FL, 32608, USA

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ABSTRACT

Starting in the fall of 2019, mortality, blight symptoms, and signs of white fungal mycelia were observed on external host tissues of non-native landscape trees as well as numerous native trees, understory shrubs, and vines throughout northern and central Florida, USA. We determined that the fungus is an undescribed species of Basidiomycota based on morphological characteristics and DNA sequence analysis. Phylogenetic analyses of the internal transcribed spacer (ITS), large subunit (LSU), and translation elongation factor 1-alpha (*tef1*) regions revealed that this novel plant pathogen is an undescribed taxon of the genus *Parvodontia* (Cystostereaceae, Agaricales). We propose the name *Parvodontia relampaga* sp. nov. which describes its unique morphological features and phylogenetic placement. We confirmed the pathogenicity of *P. relampaga* in greenhouse inoculations on host plants from which strains of this novel pathogen were isolated, including the non-native gymnosperm *Afrocarpus falcatus*, the non-native and commercially important *Ligustrum japonicum*, and the native tree *Quercus hemisphaerica*. *P. relampaga* was also detected on a total of 27 different species of woody host plants, including such economically and ecologically important hosts as *Fraxinus, Ilex, Magnolia, Persea, Prunus, Salix, Vitis*, and *Vaccinium*. For this new plant disease, we propose the name "relampago blight," which refers to the lightning-like rhizomorph growth (relámpago means 'lightning' in Spanish). This study presents a newly discovered fungal taxon with a wide host range on both angiosperms and gymnosperms that may be an emerging pathogen of concern in Florida and the Gulf Coast region.

1. Introduction

Thread blight diseases cause significant economic losses worldwide on cacao, citrus, coffee, fig, pome fruits, and tea and are difficult to control (Gasparotto and Silva 1999; Ceresini et al., 2012; Amoako-Attah et al., 2020; Pereira et al., 2000; Benchimol et al., 2001). The first description of a thread blight was reported in coffee plants from India by Cooke in 1876, caused by the fungal pathogen *Corticium koleroga* (Cooke) von Höhnel (Corticiaceae, Corticiales) (Tims et al., 1954). Some examples of well-documented agents of thread blight disease include *Ceratobasidium*-like species on persimmon and tea in Brazil (Ceresini et al., 2012), *C. koleroga* affecting coffee plants in Ethiopia and India (Tims et al., 1954; Dechassa et al., 2020), *Marasmius crinis-equi* F. Muell. Ex Kalchbr (Marasmiaceae, Agaricales) on several tropical tree crops with a wide geographical distribution (Keane and Putter, 1992; Amoako-Attah et al., 2020), *Marasmius pulcher* (Berk. & Broome) Petch on tea in Nigeria (Adedeji 2021), *Marasmius tenuissimus* (Sacc.) Singer causing blight on cocoa in Peru (Huamán-Pilco et al., 2022), and *Marasmiellus scandens* (Massee) Dennis & D.A. Reid (Omphalotaceae, Agaricales) on cocoa in Ghana (Amoako-Attah et al., 2016).

Thread blight pathogens produce aerial mycelial cords (rhizomorphs) that cover twigs, branches, and leaves, eventually causing the leaves to wilt and die (Kusunoki et al., 1997; Amoako-Attah et al., 2016; Dechassa et al., 2020). When rhizomorphs reach a nutrient-rich

* Corresponding author. Department of Plant Pathology, University of Florida, Gainesville, FL, 32611, USA.
E-mail address: claudia.paez@fdacs.gov (C.A. Paez).

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substrate, fine hyphae colonize the target host, causing leaf chlorosis, blight, necrosis, and often senescence (Hedger 1990; Benchimol et al., 2001). In addition, stem tissue can sometimes die, resulting in wilting and crown dieback (Belachew et al., 2015). Thread blight fungi are typically considered opportunistic pathogens because they spend most of their lives as saprobes of dead plant material (Amoako-Attah et al., 2020). Thread blight diseases are most problematic in shaded understory settings or during heavy rainfall and high humidity (Mathew, 1954; Adugna, 1997). These fungi have developed several strategies for dispersal. In some cases, affected leaves remain attached to the host plant by rhizomorphs (e.g., dead, hanging leaves can be seen in the canopy). The mycelium on leaves and branches can serve as a source of inoculum and spread by wind or human activity (Amoako-Attah et al., 2020). Spread by direct contact with other plants by mycelial fans or rhizomorphs on stems, branches, and leaves in the canopy is another method of dispersal (Hu 1984). Kusunoki et al. (1997) showed that thread blight disease caused by *Cylindrobasidium argenteum* (Kobayasi) N. Maek. (Physalacriaceae, Agaricales), was generally not transmissible by spores.

Despite the worldwide distribution of these economically significant pathogens, they are not well characterized phylogenetically. In this study, we determined the phylogenetic placement of a novel thread blight fungal pathogen named here as *Parvodontia relampaga*, based on culture characteristics, macro- and micro-morphology, and molecular data using multi-locus phylogenetic analysis based on three loci (the internal transcribed spacer region (ITS), the large subunit nuclear ribosomal DNA (LSU), and the translation elongation factor 1-alpha (*tef1*)). We also present a list of preliminary host plants for this fungus and evaluated its pathogenicity on *Afrocarpus falcatus* (Thunb.) C.N. Page (Podocarpaceae, Gymnosperms), *Ligustrum japonicum* Thunb (Oleaceae, Angiosperms), and *Quercus hemisphaerica* Bartram ex Willd (Fagaceae, Angiosperms).

2. Materials and methods

2.1. Sample collection

In 2019 fresh samples of host plant tissues (stem and leaf samples) with mycelium and rhizomorphs of the suspected thread blight pathogen were collected from a homeowner and submitted for disease diagnosis to the Institute of Food and Agricultural Sciences (IFAS) Plant Diagnostic Center (PDC) at the University of Florida (UF) (Gainesville, Florida). Additional specimens (including fungal mycelial mats, rhizomorphs, fruiting bodies and plant material) were collected by UF-IFAS extension agents and identified by UF mycologists. In parallel, personnel from the Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), and Florida Forest Service (FFS) in Gainesville, Florida, collected and identified many additional specimens that are included in this work (Supplementary Figs. 1 and 2).

Fresh specimens of the host (leaves and stems) with the mycelium were photographed and placed in paper bags for transport to the laboratory on the same day. A portion of all specimens with the host (5–10 cm of stem and 2 to 4 leaves) were air-dried for 48 h and then placed in a plastic bag with silica gel. Dried specimens were then accessioned into the Fungarium at the Florida Museum of Natural History (FLAS) and the Division of Plant Industry Herbarium Gainesville (PIHG) hosted at the FDACS-DPI headquarters. Fresh samples of mycelium or rhizomorphs were also used for isolation of the fungal pathogen in culture media.

2.2. Isolation of fungal cultures

Fungal isolates were obtained by placing four pieces in different places of the plate of 5–10 mm of rhizomorphs or mycelium growing on the exterior surfaces of host tissues using sterilized forceps on Malt-Extract Yeast-Extract Agar (MEYE) (3 g malt extract, 3 g yeast extract, 5 g peptone, 10 g dextrose, and 1 L distilled water, with the addition of chloramphenicol at 10 mg/L and streptomycin at 100 mg/L) media in 90 mm plates to obtain pure cultures. Plates were incubated at room temperature in the dark for 4 days. Colonies with characteristic white mycelial growth were then subcultured one time onto Difco™ Potato Dextrose Agar (PDA) (Detroit, MI) plates and maintained at room temperature in darkness. For long-term storage, agar plugs of the pure cultures were cut into 5 mm pieces, added to 2 mL Eppendorf tubes containing 10 mm sterile deionized water using sterilized forceps, and stored at room temperature. Three cultures were accessioned into the USDA Agricultural Research Service Culture Collection (NRRL) and at the Fungal Herbarium of the Florida Museum of Natural History at the University of Florida (FLAS-F).

2.3. Morphological characteristics

Morphological and cultures characteristics for taxonomy description were documented with an Olympus BH2 compound microscope with a camera lucida for line drawings of thin, freehand sections or scrapings of basidiomes mounted in 2% (weight/volume) aqueous potassium hydroxide (KOH), 1% (w/v) aqueous phloxine, and Melzer's reagent (Kirk et al., 2008). Cyanophily staining of hyphae and basidiospore walls were observed in 1% (w/v) cotton blue in 60% (w/v) lactic acid. Basidiospores in adaxial view were measured in KOH and phloxine mounts under oil immersion. Q values were calculated from mean spore length divided by mean spore width of at least 30 spores. Color codes and names follow Kornerup and Wanscher (1978), whereas capitalized names are from Ridgway (1912). Cultural studies and species code are described in Nakasone (1990) and Nobles (1948). Phenol oxidase studies follow Davidson et al. (1938).

2.4. Molecular characterization

Total DNA was extracted from a 10 mm square of mycelium scraped from the surface of cultures of each isolate, placed in a 1.5 mL Eppendorf tubes and used Extract–N-Amp rapid extraction kit according to the manufacturer's instructions (Sigma–Aldrich, St. Louis, Missouri, USA). Polymerase chain reaction (PCR) amplification of the nuclear rDNA internal transcribed spacer (ITS) was obtained using primers ITS1F-ITS4B (Gardes and Bruns 1993) and PCR amplification of the nuclear large subunit (LSU) rDNA was obtained using primers LR0R-LR5F (Hopple and Vilgalys 1994; Tedersoo et al., 2008). PCR amplification of the translation elongation factor 1-alpha (*tef1*) gene was performed using primers EF1-983F and EF1-1567R (Rehner and Buckley, 2005).

PCR amplicons were visualized using SYBR Green I stain (Molecular Probes, Eugene, Oregon), separated by electrophoresis on a 1.5% agarose gel in sodium boric acid buffer and photographed under UV light. PCR amplicons were purified using the ExoSAP-IT purification kit (ThermoFisher, Waltham, MA). Sanger sequencing of purified PCR products was performed using forward and reverse primers as above and sequenced bidirectionally by GENEWIZ (https://www.genewiz.com/en, Jupiter, Florida) or by the Molecular Diagnosis Laboratory of FDACS-DPI (Gainesville, Florida). Sequences were then edited and trimmed with Geneious 11.0.6 (Biomatters Inc., San Diego, California) and deposited in NCBI (Table 1).

2.5. Phylogenetic analysis

Taxa in the family Cystostereaceae Jülich and the outgroup were selected based on the results of Li et al. (2022). Sequences from other members of the Cystostereaceae were downloaded from GenBank (21 sequences) or provided by Dr. Shuanghui He, Beijing Forestry University, China (14 sequences) (Li et al., 2022). In addition, we obtained isolates of various Cystostereaceae species from the Center of Forest Mycology Research (CFMR) in Madison, Wisconsin (13 isolates). An alignment of each locus was manually performed in Geneious 11.0.6

Table 1
Parvodontia relampaga observed in this study with Fungarium (ID) numbers and GenBank accession number (ITS).

Host	Common name	Family	Herbarium and Culture collection number[a]	GenBank Acc. Num. ITS	Date	County/City	Collector
Acer floridianum	Florida maple	Sapindaceae	FLAS-F-71001	OP791874	Mar-22	Leon/Bristol	J. Smith
Aesculus pavia	Red buckeye	Sapindaceae	PIHG-17149	OQ437191	Nov-22	Lafayette/Mayo	J. Eickwort
Afrocarpus falcatus	Common yellowwood	Podocarpaceae	FLAS-F-71002 (NRRL 64416)	ON411190	Dec-21	Alachua/ Gainesville	J. Smith
Agarista populifolia	Florida hobblebush	Ericaceae	PIHG-18036	OR822036	Oct-23	Putnam/ Florahome	J. Eickwort
Cleyera sp.	–	Pentaphylacaceae	FLAS-F-71004	OP791876-	Sep-21	Leon/Tallahassee	M. Tancing
Fraxinus caroliniana	Florida ash	Oleaceae	PIHG-17150	OQ437192	Nov-22	Lafayette/Mayo	J. Eickwort
Ilex × *attenuate*	Topal holly	Aquifoliaceae	FLAS-F-71005	OP791878	Dec-21	Leon/Tallahassee	C. Renn
Ilex opaca[2]	American holly	Aquifoliaceae	–	–	Apr-22	Suwannee/ Falmouth	J. Eickwort
Ligustrum japonicum	Japanese privet	Oleaceae	FLAS-F-71011 (NRRL 64415^{Ex-T})	ON303477	Oct-19	Alachua/ Gainesville	PDC
Ligustrum japonicum	Japanese privet	Oleaceae	PIHG-17298	OQ658163	Mar-22	Lake/Sorrento	M. Sellers
Ligustrum japonicum	Japanese privet	Oleaceae	FLAS-F-71006^{T}	OQ645680	Mar-21	Leon/Tallahassee	M. Tancing
Liquidambar styraciflua[2]	American sweetgum	Altingiaceae	PIHG-16401	–	Feb-22	Sumter/Bushnell	J. Eickwort, H. Urbina
Magnolia grandiflora	Southern magnolia	Magnoliaceae	PIHG-16404	OQ658158	Feb-22	Hernando/ Brooksville	J. Eickwort, H. Urbina
Magnolia grandiflora	Southern magnolia	Magnoliaceae	FLAS-F-71007	OP791879	Mar-21	Leon/Tallahassee	C. Paez
Morella cerifera	Southern wax myrtle	Myricaceae	PIHG-17151	OQ658162	Nov-22	Lafayette/Mayo	J. Eickwort
Morus sp.	Mulberry	Moraceae	FLAS-F-71008	OP791880	Mar-21	Leon/Tallahassee	C. Paez
Nekemias arborea[2]	Pepper vine	Vitaceae	PIHG-17148	–	Nov/22	Lafayette/Mayo	J. Eickwort
Persea borbonia	Redbay	Lauraceae	PIHG-16407	OQ437193	Feb-22	Columbia/High Springs	J. Eickwort, H. Urbina
Prunus angustifolia	Florida sand plum	Rosaceae	PIGH-17473	OQ778740	Mar-23	Alachua/ Gainesville	J. Eickwort
Prunus caroniliana	Carolina laurel cherry	Rosaceae	PIHG-17152	OQ658160	Dec-22	Volusia/DeLeon Springs	J. Eickwort
Psychotria nervosa[b]	Wild coffee	Rubiaceae	PIHG-16399	–	Feb-22	Sumter/Bushnell	J. Eickwort, H. Urbina
Quercus hemisphaerica[b]	Darlington oak	Fagaceae	–	–	Jan-23	Orange/Apopka	J. Eickwort
Quercus hemisphaerica	Darlington oak	Fagaceae	FLAS-F-71009	OP791877	Mar-22	Columbia/High Springs	J. Eickwort
Quercus hemisphaerica[b]	Darlington oak	Fagaceae	PIHG-17297	–	Dec-22	Gilchrist/High Springs	J. Eickwort
Quercus hemisphaerica[b]	Darlington oak	Fagaceae	–	–	Mar-23	Levy/Dunnellon	J. Eickwort
Quercus hemisphaerica	Darlington oak	Fagaceae	FLAS-F-70274 (NRRL 64417)	ON411189	Mar-21	Leon/Tallahassee	C. Paez
Quercus hemisphaerica	Darlington oak	Fagaceae	PIHG-18037	OR822037	Oct-23	Putnam/ Florahome	J. Eickwort
Quercus nigra	Water oak	Fagaceae	HHB-6827	OR663960	Jul/72	Alachua/ Gainesville	H.H. Burdsall, Jr.
Quercus nigra	Water oak	Fagaceae	–	OQ437194	Dec-22	Volusia/DeLeon Springs	J. Eickwort
Sideroxylon lanuginosum	Ironwood	Sapotaceae	PIHG-17153	OQ658161	Dec-22	Columbia/High Springs	J. Eickwort
Smilax auriculata	Earleaf greenbrie	Smilacaceae	PIHG-16406	OQ658164	Feb-22	Hernando/ Brooksville	J. Eickwort, H. Urbina
Symplocos tinctoria[b]	Common sweetleaf	Symplocaceae	–	–	Feb-23	Alachua/ Gainesville	J. Eickwort
Taxus floridana	Florida yew	Taxaceae	FLAS-F-71010	OP791881	Mar-22	Leon/Tallahassee	J. Smith
Vaccinium arboreum[b]	Sparkleberry	Ericaceae	PIHG-16402	–	Feb-22	Sumter/Bushnell	J. Eickwort, H. Urbina
Viburnum obovatum[b]	Small-leaf viburnum	Adoxaceae	PIHG-16400	–	Feb-22	Sumter/Bushnell	J. Eickwort, H. Urbina
Vitis rotundifolia	Muscadine	Vitaceae	PIHG-16405	OQ658157	Feb-22	Hernando/ Brooksville	J. Eickwort, H. Urbina

^{T} Isotype specimen is located at the Center for Forest Mycology Research (CFM).
$^{Ex-T}$ Ex-paratype culture located in the USDA Agriculture Research Service Culture Collection (NRRL).
[a] The University of Florida Herbarium-Fungarium (FLAS-F), FDACS-DPI, Herbarium of the Florida Department of Agriculture and Consumer Services (PIHG).
[b] Species identification based on macroscopic (Supplementary 1 and 2) and microscopic characteristics (gloeocystidia and clamp connections).

(Biomatters Inc., San Diego, California). Each locus was analyzed separately to ensure no significant incongruence among loci. For the individual analysis of the regions (ITS, LSU and *tef1*), Maximum Likelihood (ML) analysis was performed using CIPRES 3.3 (Miller et al., 2010) and executed using RAxML 8.2.12 (Stamatakis 2014) under the GTRGAMMA evolutionary model with 1000 bootstraps. Topologies of each of the three loci were congruent, so we proceeded with a concatenated analysis.

A concatenated alignment with all three loci (representing 2286 characters from 57 specimens) was performed using AMAS (Alignment Manipulation And Summary) tool (Borowiec, 2016) on the HiperGator 3.0 (https://www.rc.ufl.edu/about/hipergator/) supercomputer at the University of Florida (Gainesville, FL). The concatenated alignment was partitioned into ITS, LSU and *tef1*. The evolutionary model was chosen for each locus based on Akaike information criterion (AIC) scores by jModelTest 2 2.1.6 (Darriba et al., 2012) in Cyberinfrastructure for Phylogenetic Research Science Gateway (CIPRES) 3.3 (Miller et al., 2010). The GTR + I + G model was selected for all three loci. The concatenated alignment was analyzed with Maximum Likelihood and Bayesian Inference (BI) methods using the HiperGator supercomputer at the University of Florida. Maximum Likelihood analysis was executed by RAxML 8.2.12 (Stamatakis 2014) with 1000 bootstrap replicates. For BI analyses, two runs were performed using MrBayes 3.2.6 (Ronquist et al., 2012) with four chains for 10 million generations with a sampling frequency of 1000, the first 25% of sampled trees were discarded as the burn-in and other parameters were set to default. Phylogenetic trees for ML and BI were visualized in Figtree 1.4.4 (Rambaut et al., 2018). The final tree was edited in Adobe Illustrator version 24.3 (San Jose, California, USA). Nodes were considered strongly supported when the ML bootstrap values were ≥70% and BI posterior probability values were ≥0.95. Alignments were submitted to Open Science Framework (OSF) https://osf.io/4nruz/?view_only=482ddcc2168440f7b766bd24077fd0ed.

2.6. Pathogenicity determination

For the pathogenicity tests of *A. falcatus* and *Q. hemisphaerica*, we used a greenhouse with shade cloth and sprinkler irrigation. In the case of *L. japonicum*, we used a greenhouse with natural light, where we covered the leaves with plastic bags and sprayed them with sterile tap water to maintain high humidity in the dark for seven days after inoculation, greenhouses were maintained at 20–22 °C. We used three different fungal isolates, each tested on the respective host from which they were originally isolated. Isolate FLAS-F-71002 was inoculated *on A. falcatus*, isolate FLAS-F-71011 on *L. japonicum*, and isolate FLAS-F-70274 on *Q. hemisphaerica.*

Koch's Postulates were completed for *A. falcatus* and *Q. hemisphaerica* using a single trial. For *A. falcatus*, inoculations were performed with four nursery-grown seedlings in three-gallon containers. Three leaves on each of the three seedlings were inoculated on the abaxial surface (underside) with a 6-mm plug of seven-day-old mycelium from MEYE media and covered with Parafilm® M (Neenah, WI). Three leaves from an additional seedling were inoculated with sterile agar plugs and served as a mock-inoculated control. Due to the slow growth of the fungus in *A. falcatus*, signs and symptoms were observed at 120 days post-inoculation.

For *Q. hemisphaerica,* ten seedlings (nursery-grown plants in three-gallon containers) were inoculated as described above. Eight seedlings received the fungal treatment, whereas two mock-inoculated controls were inoculated with water agar only. External signs and symptoms were evaluated at 60 days post-inoculation.

For L. *japonicum*, three seedlings (nursery-grown plants in three-gallon containers) were selected and placed in a greenhouse. Inoculation was carried out using the same procedure previously described for *A. falcatus* and *Q. hemisphaerica* except that they were two treatment seedlings and one control seedling. The L. *japonicum* experiment was performed twice (the variation in trials among each host occurred because of insufficient availability of seedlings). External signs and symptoms were evaluated at 30 days post-inoculation.

Pathogen re-isolation was performed for all three trials by fungal threads that developed on the inoculated plants and placed on MEYE media. After fungal growth, DNA was extracted and the ITS rDNA region was sequenced (as mentioned above), to confirm the identity of the fungus.

3. Results

3.1. Taxonomy

Parvodontia relampaga C.A. Paez, Nakasone, J.A. Sm. & M.E. Sm., **sp. nov.** –MycoBank No. 846936 (Fig. 1).

Etymology – the Spanish "relámpago" = lightning, which refers to the appearance of the rhizomorphs, which resemble lightning as they grow across the surface of leaves or stems of the host plants.

Typification – UNITED STATES, FLORIDA, LEON COUNTY: Tallahassee, Inkberry Way, on stem and leaves of *L. japonicum* near swamp/stream, 02 March 2021, legit Mark Tancing, MES 3835, (holotype, FLAS-F-71006; ex-type culture at NRRL; isotype at CFMR) GenBank sequence of holotype: *OQ645680* (ITS) (Figs. 1 and 2).

Morphological description (based on FLAS-F-71006) – Basidiomes resupinate, adnate, widely effused, beginning as circular to oblong patches, coalescing to form linear colonies, up to 10 cm long × 1.2 cm wide, up to 200 μm thick, soft, membranous to crustaceous; rhizomorphs often present, closely appressed, irregularly branched, white, felty; hymenophore smooth with scattered to numerous small tubercules or verruculose to odontoid, up to 7 aculei per mm, pale yellow (4A3), yellowish grey (4B2), greyish yellow (4B3), orange white (5A2), greyish orange (5B3), or Light Buff at first then Tilleul Buff in more mature areas; cracks few to numerous, forming irregular polygons; margin abrupt, adnate, smooth, even, concolorous with hymenophore or thinning out, loosely adnate, bayed, white, fimbriate to arachnoid, up to 1 mm wide.

Hyphal system dimitic with clamped generative hyphae and skeletal hyphae. Rhizomorphs composed of generative hyphae, skeletal hyphae, and gloeocystidia — generative hyphae 1.8–3.5 μm diam, clamped, sparsely branched, walls hyaline, thin, smooth or a few segments lightly encrusted with small, adherent, hyaline crystals; skeletal hyphae dominant, 1.5–2.5 (−3.5) μm diam, aseptate but probably with a basal clamp, unbranched, often solid without lumen, walls hyaline, slightly thick to thick, smooth; gloeocystidia numerous, sphaero-pedunculate to ovoid or fusiform, terminal or intercalary, up to 15 μm diam at widest, with one or more digitate protuberances, empty or with dark yellow, resinous-like, cyanophilous contents that darken in Melzer's, walls hyaline, thin to slightly thickened, smooth. Subiculum up to 30 μm thick, an open to moderately dense, partially agglutinated tissue of empty, collapsed, more or less vertical hyphae, and embedded gloeocystidia; subicular hyphae 1.8–2.2 μm diam, clamped, moderately branched, walls hyaline, thin, smooth; gloeocystidia as described above. Subhymenium thickening, composing mostly of context, a partially agglutinated tissue of numerous, empty, spherical to oblong gloeocystidia, vertically arranged hyphae, and irregular crystal clusters; subhymenial hyphae 2.2–3 μm diam, clamped, moderately branched, walls hyaline, thin, smooth; gloeocystidia as described above. In of gloeocystidia, cystidia, and basidia. Gloeocystidia terminal, sphaero-pedunculate to obclavate, often with one or more constrictions, sometimes with an apical bulb, 20–30 × 7–12 μm, clamped at base, with dark honey yellow, resinous-like, cyanophilous contents that darken in Melzers, walls hyaline, thin, smooth. Cystidia scarce, subfusiform to obclavate, 11–35 × 3–5 μm, clamped at base, enclosed or protruding up to 20 μm, walls hyaline, thin, smooth. Basidia clavate to subfusiform, often with a median constriction, 13–16 × 4–4.5 μm, clamped at base, 4-sterigmate, walls hyaline, thin, smooth. Basidiospores often adherent, broadly

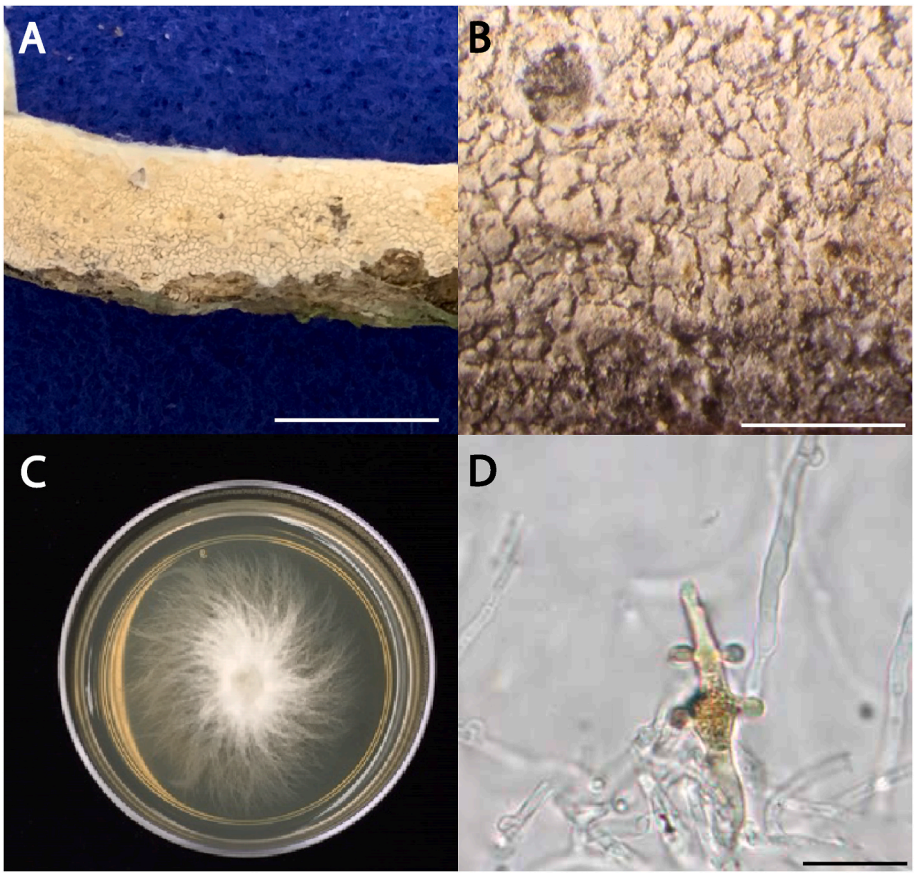

Fig. 1. Morphology of *Parvodontia relampaga*: A–B. Fruiting body, holotype (FLAS-F-71006). C. Colony morphology of a 5-day-old culture on Malt Extract Yeast Extract agar (MEYE). D. Gloeocystidium. Scale bars: A = 10 mm; B = 2 mm; D = 8 μm.

cylindric, (4.5–) 4.7–5.5 (−5.8) × (2.4–) 2.5–3 (−3.2) μm, Q range = 1.6–2 (−2.1), an average of two specimens 4.9–5.1 × 2.8–2.9 μm, Q = 1.8, walls hyaline, thin, smooth, acyanophilous, unresponsive in Melzer's reagent.

Cultural description (based on FLAS-F-71011) – Growth on Difco^{tm} Malt Extract Agar (MEA) moderate, 22 mm radius after 1 week, 75 mm radius after 4 weeks; mats white, moderately thick around inoculum, raised, cottony woolly then slightly thinning toward margin at 3 and 4 weeks; margins appressed, bayed, irregular; no odor or agar discoloration at 2 weeks or 4 weeks. Oxidase reactions positive after 1 week on GAA (gallic acid media) but of light intensity [+++(+)], growth 20 mm diam; on TAA (tannic acid media) positive, but of light intensity [+++(+)], growth 36 mm diam. Advancing zone hyphae 1.8–3.5 μm diam, clamped, long-celled, sparingly branched, walls hyaline, thin, smooth. Submerged hyphae similar to advancing zone hyphae except up to 5 μm diam, frequently branched. Aerial hyphae of two types: (1) generative hyphae 0.5–2 μm diam, clamped, long-celled, walls hyaline, thin, smooth; (2) skeletal 1.8–2 μm diam, aseptate, lumen absent, walls hyaline, thick, smooth. Gloeocystidia or vesicles scattered to abundant in aerial and submerged hyphal mat at 2 and 4 weeks, fusiform to spherical with digitate appendages, up to 12 μm diam., intercalary or terminal, empty or containing dark yellow matter that darkens in Melzer's reagent, walls hyaline, thin, smooth.

Species code: 2.3.8.15.32.36.38.44–45. (50).(51)0.54.(55) (Nakasone, 1990).

Habitat and distribution – Basidiome on dead corticate twigs and small diameter branches of woody angiosperms more often as rhizomorphs and sterile mycelium on stems and leaves of angiosperms, rarely gymnosperms, in shaded environments during the rainy season (Table 1); in central and north Florida, United States (Fig. 3).

Specimens examined – UNITED STATES, FLORIDA, ALACHUA COUNTY: Alachua, on leaves and stem of *L. japonicum*, 19 October 2019, Plant Diagnostic Center (G19-1738, FLAS-F-71011, NRRL 64415) (exparatype); Micanopy, on *A. falcatus* leaves and stem, 13 December 2021, J.A. Smith (FLAS-F-71002); High Springs, on *Q. hemisphaerica,* leaves and stem, 02 March 2022, J.M. Eickwort (FLAS-F-71009); west of Newnans Lake, on *Quercus nigra* corticate twigs, 31 July 1972, H.H. Burdsall, Jr., HHB-6827 (CFMR). LEON COUNTY: Tallahassee, 1502 Inkberry Way on *Q. hemisphaerica* leaves and stem, 17 March 2021, C.A. Paez (FLAS-F-70274), *Magnolia grandiflora* and *Morus* sp. leaves, 17 March 2021, C.A. Paez (FLAS-F-71007, FLAS-F-71008), *ibid*. on leaves and stem of *Cleyera* sp., 20 September 2021, M. Tancing (FLAS-F-71004), on leaves and stem of *Ilex* × *attenuata*, 13 December 2021, C. Renn (AL1073, FLAS-F-71005); LIBERTY COUNTY: Bristol, on leaves and stem of *Acer floridanum*, 02 March 2022, J.A. Smith (FLAS-F-71001), *ibid*. on stem of *Taxus floridana*, 02 March 2022, J.A. Smith (FLAS-F-71010).

Comments – *P. relampaga* is characterized by white rhizomorphs composed of skeletal hyphae, smooth to odontoid, rimose basidiomes with gloeocystidia embedded in the context, and cylindrical basidiospores. Most of the collections were sterile and only produced rhizomorphs, hyphae, and gloeocystidia; the holotype (FLAS-F-71006) and two other specimens (FLAS-F-71009 and HHB-6827) produced fertile basidiomes. Culture comparison with other *Parvodontia* species was impossible due to the lack of available cultures of other species in this genus. The cultures included in this research are the only available cultures for this genus.

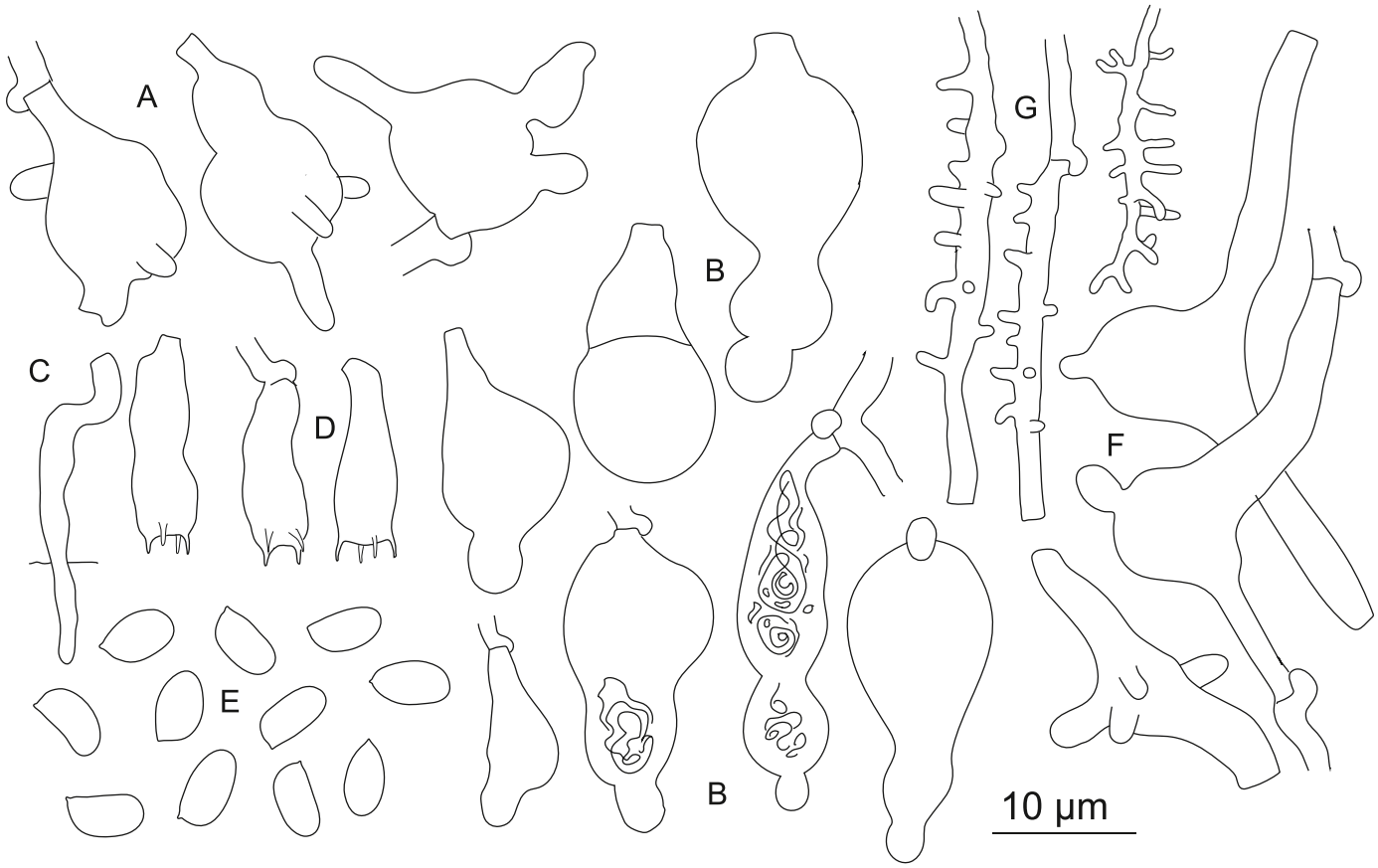

Fig. 2. *Parvodontia relampaga* holotype (FLAS-F-71006): A. Gloeocystidia with digitate protuberances in subiculum. B. Gloeocystidia from subhymenium and hymenium. C. Hymenial cystidium. D. Basidia. E. Basidiospores. F. Gloeocystidia from sterile hyphal mat. G. Hyphal segments with pegs from sterile hyphal mat.

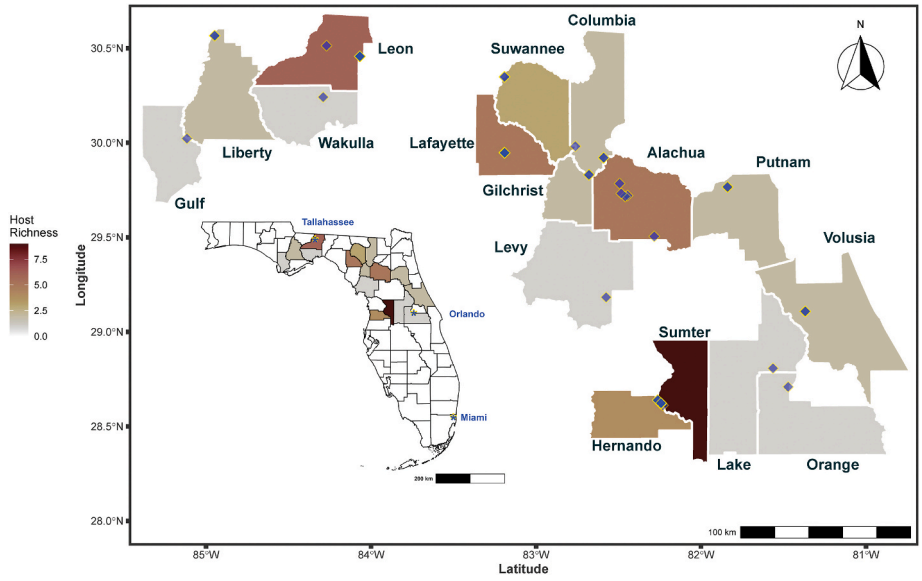

Fig. 3. Current known distribution of *Parvodontia relampaga*. Blue diamond shape represents the location from which we confirmed *P. relampaga* specimens based on ITS sequences and morphological characteristics (Table 1, Supplementary 1 and 2). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Sequencing and phylogenetic analysis

The sequences generated from *P. relampaga* specimens were compared using BLASTn searches against the NCBI nucleotide database (Chen et al., 2015). The results of the ITS sequences (767 bp) produced top BLAST hits to *Cystidiodontia laminifera* (Berk. & M.A. Curtis) Hjortstam with 87% similarity (EU118622 and MH625705). Results from LSU rDNA sequences (899 bp) also produced top BLAST hits to *C. laminifera* (e.g., MT675110) with 95% similarity. However, results from *tef1* (588 bp) produced low BLAST hits to any taxa currently in GenBank. The

highest homology was 83.5% similarity to *Tulostoma* sp. (Agaricaceae, Agaricales) and 83.3% similarity to *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux (Amylocorticiaceae, Amylocorticiales). Prior to this study, *tef1* were absent for any Cystostereaceae in GenBank. Therefore, these initial BLAST hits did not lead to the accurate placement of this novel taxon (*P. relampaga)* so additional loci from cultures in the CFMR collection were sequenced. It is important to note that there was only one variable nucleotide among *P. relampaga* isolates in the ITS and LSU region and no variation in *tef1*. The phylogenetic position of *P. relampaga* was inferred using datasets of ITS, LSU and *tef1*.

The phylogenetic tree based on the ITS region (Supplemental Fig. 3) included 769 characters and was generated from 55 sequences (Table 2). The Maximum Likelihood analysis included species of *Crustomyces*, *Cystostereum*, *Effusomyces*, and *Parvodontia*. The phylogenetic tree had a similar overall topology to the multi-locus tree with *P. relampaga* as a sister taxon to *Parvodontia luteocystidia* Hjortstam & Ryvarden from

Table 2
Collections used in this study with collection (ID) numbers, GenBank accession numbers and references.

Taxon name	GenBank accessions numbers				Reference
	Collection (ID) number	ITS	28S	*tef1*	
Chondrostereum purpureum	He5634	MW557935	MW557942	–	Li et al. (2022)
Chondrostereum purpureum	EL59_97	–	AY586644	–	Larsson et al. (2004)
Chondrostereum purpureum	AFTOL-ID441	DQ200929	–	DQ457632	Matheny et al. (2007)
Crustomyces albidus	CLZhao 6194	MK404325	–	–	Li et al. (2022)
Crustomyces albidus	CLZhao 21168	OM955829	–	–	Li et al. (2022)
Crustomyces albidus	He 6164	ON117191	ON117175	–	Li et al. (2022)
Crustomyces albidus	Z40	JN198404	–	–	Wu et al. (2013)
Crustomyces crassisporum	He3995	–	ON117178	–	Li et al. (2022)
Crustomyces crassisporum	He4740	ON117193	ON117179	–	Li et al. (2022)
Crustomyces isabellinus	Yuan12642	ON117185	ON117170	–	Li et al. (2022)
Crustomyces isabellinus	Yuan12644	ON117186	ON117171	–	Li et al. (2022)
Crustomyces isabellinus	He4766	ON117189	ON117173	–	Li et al. (2022)
Crustomyces isabellinus	He3582	ON117188	ON117172	–	Li et al. (2022)
Crustomyces laminiferus	KHL13057	EU118622	EU118622	–	Larsson, 2007
Crustomyces laminiferus	L-12856	OP935216	OP995722	OP999052	This study
Crustomyces laminiferus	FP-150137	OP935215	OP995725	OP999046	This study
Crustomyces laminiferus	TF10	MH625705	MH625705	–	Li et al. (2022)
Crustomyces pini-canadensis	FP-101900	OP935226	OP995720	OP999040	This study
Crustomyces pini-canadensis	HHB-2136	OP935219	OP995719	OP999047	This study
Crustomyces pini-canadensis	FP-133171	OP935217	OP995726	OP999045	This study
Crustomyces pini-canadensis	HHB-11308	OP935222	OP995715	OP999050	This study
Crustomyces pini-canadensis	HHB-8219	OP935220	OP995718	OP999048	This study
Crustomyces pini-canadensis	HHB-10480	OP935221	OP995717	OP999049	This study
Crustomyces pini-canadensis	HHB-11661	OP935223	OP995716	OP999051	This study
Crustomyces subabruptus	BK323	LR-961824	–	–	Li et al. (2022)
Crustomyces subabruptus	LCP06383	MF183946	–	–	Meistermann et al. (2021)
Crustomyces subabruptus	UC2022949	KP814558	–	–	Unpublished
Crustomyces subabruptus	K(M):249975	MZ159685	–	–	Li et al. (2022)
Crustomyces subabruptus	FP-133011	OP935214	OP995721	OP999044	This study
Crustomyces subabruptus	DLL2011-303	KJ140775	–	–	Li et al. (2022)
Crustomyces subabruptus	SM14-27-5-4	MN905889	–	–	Li et al. (2022)
Crustomyces tephroleucus	Cui13653	MF290418	MF290416	–	Song et al., 2018
Crustomyces tephroleucus	Cui13717	MF290417	MF290415	–	Song et al., 2018
Cystostereum murrayi	357	MZ090565	–	–	Unpublished
Cystostereum murrayi	NIBIO2016-1383/1	MF511075	–	–	Metslaid et al. (2018)
Cystostereum murrayi	G0517	–	MK277917	–	Varga et al. (2019)
Cystostereum murrayi	8952	MH510035	–	–	Unpublished
Cystostereum murrayi	CBS413	MH855588	MH867098	–	Vu et al. (2019)
Cystostereum submurrayi	WEI18-405	OM942664	OM919713	–	Li et al. (2022)
Cystostereum submurrayi	He23301	–	ON117176	–	Li et al. (2022)
Cystostereum submurrayi	He4379	ON117192	ON117177	–	Li et al. (2022)
Cystostereum submurrayi	WEI18-033	OM942662	OM919711	–	Li et al. (2022)
Effusomyces thailandicus	He4126	–	ON117181	–	Li et al. (2022)
Effusomyces thailandicus	He4055	ON117194	ON117180	–	Li et al. (2022)
Gloeostereum incarnatum	3332	AF141637	AF141637	–	Li et al. (2022)
Parvodontia austrosinense	He4339	–	ON117182	–	Li et al. (2022)
Parvodontia austrosinense	He4730	ON117195	ON117183	–	Li et al. (2022)
Parvodontia luteocystidia	FP-102934	OP935225	OP995714	OP999042	This study
Parvodontia luteocystidia	FP-102961	OP935218	OP995723	OP999043	This study
Parvodontia luteocystidia	FP-102806	OP935224	OP995724	OP999041	This study
Parvodontia relampaga	HHB-6827	OR663960	–	–	This study
Parvodontia relampaga	FLAS-F-70274	ON411189	OP784900	–	This study
Parvodontia relampaga	FLAS-F-71001	OP791874	–	–	This study
Parvodontia relampaga	FLAS-F-71002	ON411190	OP784897	OP785744	This study
Parvodontia relampaga	FLAS-F-71003	OP791875	–	–	This study
Parvodontia relampaga	FLAS-F-71004	OP791876	–	OP785745	This study
Parvodontia relampaga	FLAS-F-71005	OP791878	OP784898	OP785746	This study
Parvodontia relampaga	FLAS-F-71011	ON303477	OP784899	–	This study
Parvodontia relampaga	FLAS-F-71007	OP791879	–	–	This study
Parvodontia relampaga	FLAS-F-71008	OP791880	–	OP785747	This study
Parvodontia relampaga	FLAS-F-71009	OP791877	–	–	This study
Parvodontia relampaga	FLAS-F-71010	OP791881	–	–	This study

Puerto Rico (FP-102934, FP-102961, and FP-102806), with a bootstrap support of 86%. *P. relampaga* and *P. luteocystidia* form a sister clade to *Parvodontia austrosinense* Yue Li, Nakasone & S.H. He from China and *Effusomyces thailandicus* Yue Li, Nakasone & S.H with a low bootstrap support (≤70%).

The phylogenetic tree based on the LSU locus (Supplemental Fig. 4), included 899 characters from 36 sequences (Table 2). *P. relampaga* is sister to *P. luteocystidia*, albeit with low bootstrap support (≤70%). However, *P. austrosinense* was resolved as the sister to the clade that included *P. relampaga* and *P. luteocystidia* with a bootstrap support of 98%.

The translation elongation factor (*tef1*) (Supplemental Fig. 5) alignment included 588 characters and was generated from 16 sequences (Table 2). *P. relampaga* is sister to *P. luteocystidia* with a bootstrap support of 100%. We could not compare *P. relampaga* to *P. austrosinense* due to the lack of available *tef1* sequences for *P. austrosinense*.

The concatenated multi-locus alignment based on ITS, LSU, and *tef1* contained 57 specimens (Table 2) with 2266 characters (797 for ITS, 899

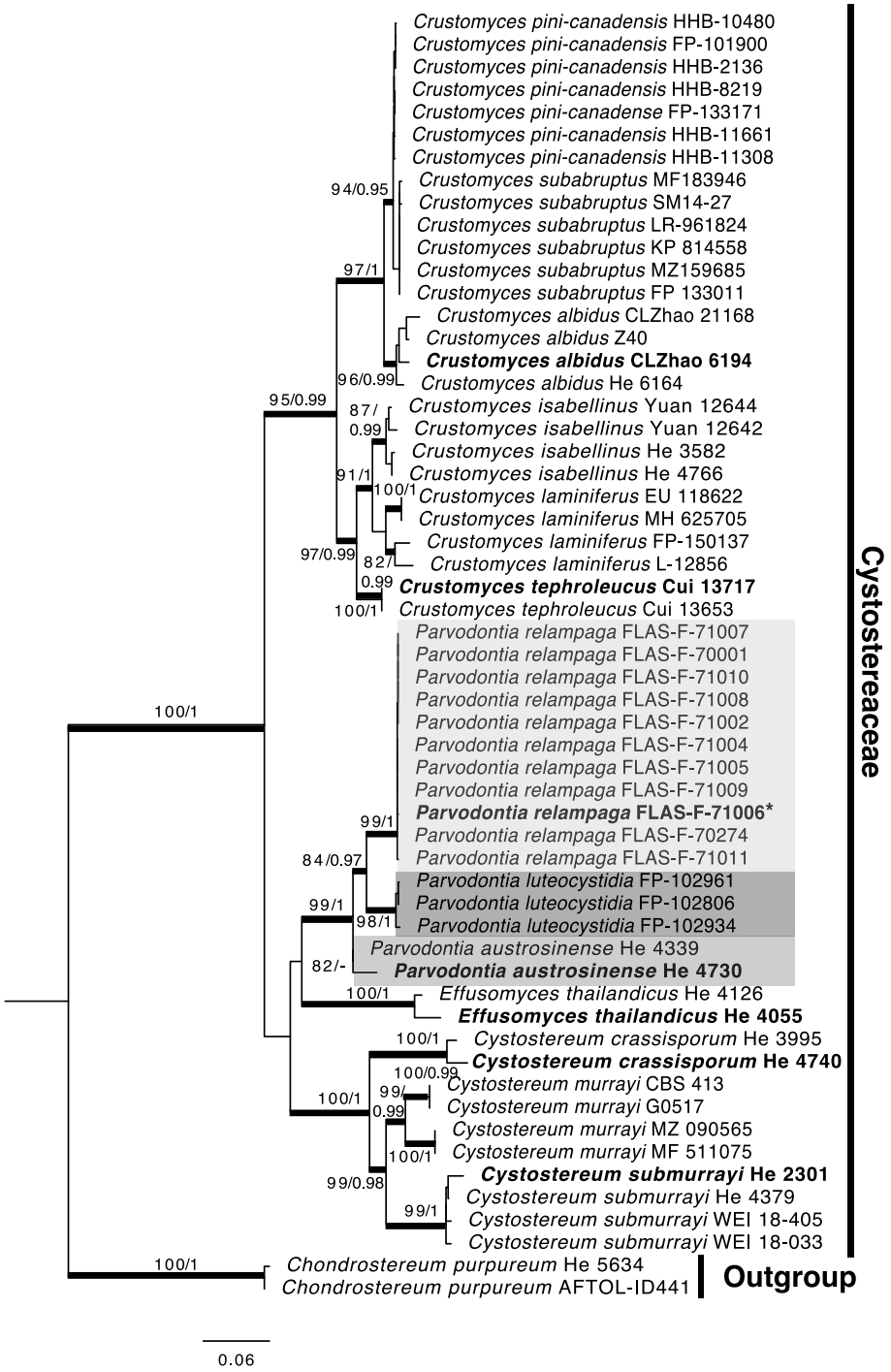

Fig. 4. Phylogenetic tree of members in the family *Cystostereaceae* based on Maximum Likelihood (ML) analysis of three concatenated genes (ITS, LSU, and *tef*1). Numbers next to nodes represent ML bootstrap support values followed by Bayesian posterior probabilities. Bootstrap values ≥ 70% and posterior probabilities ≥0.95 are shown here. Branches that are supported by ML bootstrap support and Bayesian posterior probability are indicated with thickened black branches. Holotypes of genera in Cystostereaceae are in bold. Holotype of the new taxon is marked with an asterisk (*).

for LSU and 588 for *tef1*), where 55 specimens were from the Cystostereaceae and two specimens of *Chondrostereum purpureum* (Pers.) Pouzar (Schizophyllaceae, Agaricales) served as the outgroup. The data matrix contained 24 strains with three loci, 18 with two loci and 15 with one locus. However, at least two loci were available for each species. *P. relampaga* was resolved as the sister taxon to *P. luteocystidia* with 89% bootstrap support and a posterior probability of 0.98. *P. austrosinense* was resolved as the sister group to the clade containing *P. luteocystidia* and *P. relampaga* with 100% bootstrap support and a posterior probability of 1.0 (Fig. 4).

3.3. Pathogenicity determination

To confirm pathogenicity, we conducted greenhouse experiments on three of the most common woody host plants that were initially and repeatedly affected by thread blight: *A. falcatus*, the first gymnosperm recorded as a host; *Q. hemisphaerica*, the most common host of thread blight in natural settings; and *L. japonicum*, the most common landscape ornamental and the first host on which thread blight disease was observed (Fig. 5).

In the pathogenicity experiment for *A. falcatus*, signs (white mycelium that coalesced into mycelial cords/rhizomorphs) and symptoms (leaf blight) were observed on all four plants after 120 days post-

Fig. 5. Mycelial threads of *Parvodontia relampaga* growing on the stem and leaves of *Afrocarpus falcatus*, *Ligustrum japonicum* and *Quercus hemisphaerica*. A – B. Mycelium on stem and leaves of *A. falcatus* with blight symptoms (FLAS-F-71002). C – D. Mycelium growing on the abaxial (underside) and adaxial surface (upperside) of *L. japonicum* leaves with blight symptoms (FLAS-F-71011). E – F. Mycelium growing on the stem and leaves of *Q. hemisphaerica* with blight symptoms (FLAS-F-70274 and FLAS-F-71301).

inoculation (Fig. 6B). The morphology and DNA sequences obtained from the inoculated plants were identical to the original isolate (FLAS-F-71002). No signs or symptoms were observed on mock-inoculated control leaves. For *Q. hemisphaerica*, signs (white mycelial) and symptoms (leaf blight) were observed in all eight inoculated plants after 60 days post-inoculation (Fig. 6C). The pathogen was re-isolated from all inoculated plants. The morphology and DNA sequences obtained from the inoculated plants were identical to the original isolate (FLAS-F-70274). No signs or symptoms were observed on mock-inoculated control leaves (Fig. 6D). For L. *japonicum*, signs (white mycelium) and symptoms (leaf blight and dieback) were observed on all six leaves across both plants after 30 days post-inoculation (Fig. 6F). The pathogen was re-isolated from all inoculated plants. In addition, the morphology and DNA sequences obtained from the inoculated plants were identical to the original isolate (FLAS-F-71011). No signs or symptoms were observed on mock-inoculated control leaves.

The signs in all plants were white mycelial strands (that occasionally coalesced into rhizomorphs) that extended from the inoculation point in

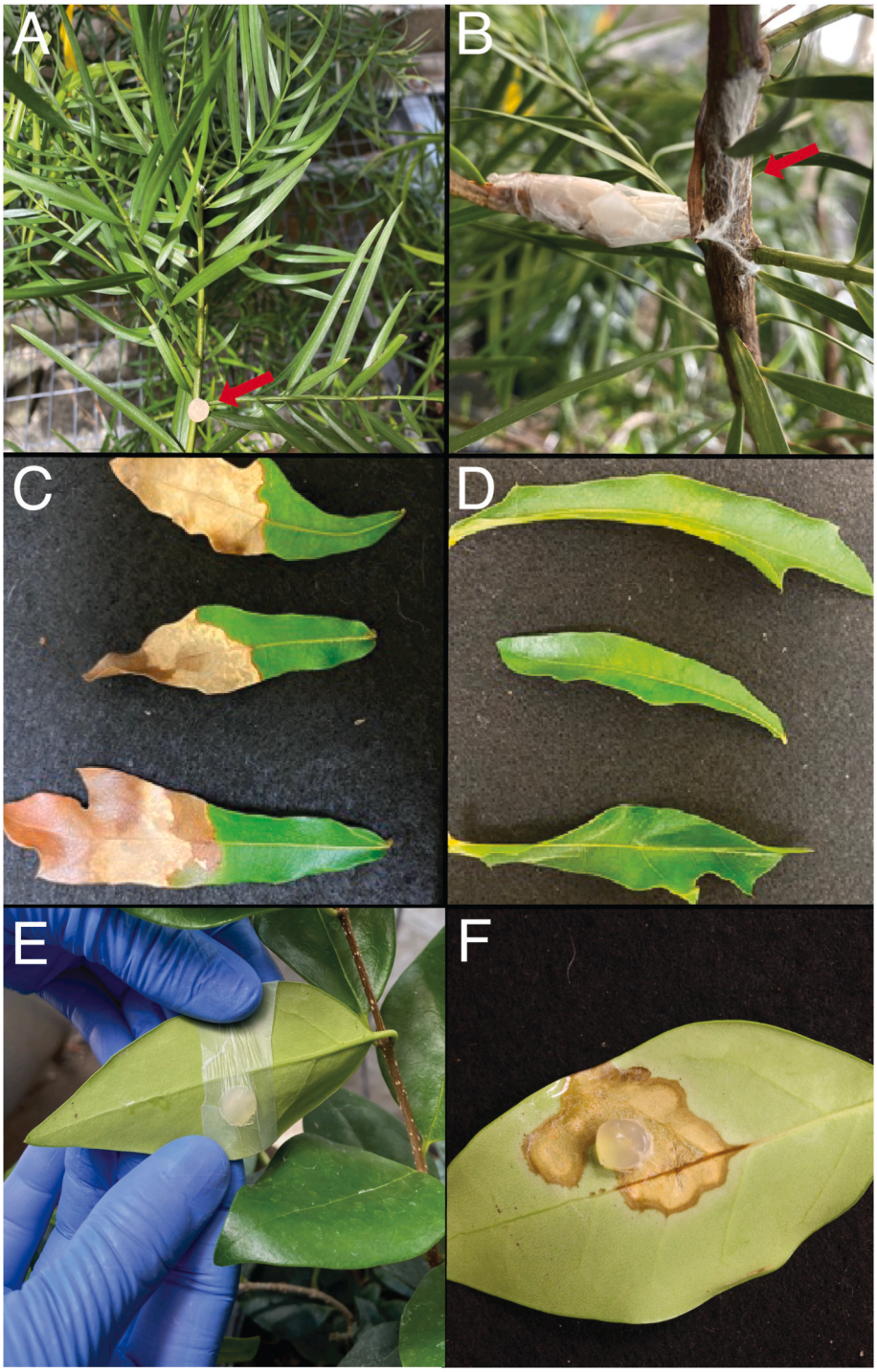

Fig. 6. Pathogenicity test of P*arvodontia relampaga* on *Afrocarpus falcatus, Quercus hemisphaerica* and *Ligustrum japonicum*: A. Inoculation of *L. japonicum* leaves. B. Abaxial side of *L. japonium* leaves 30 days post-inoculation. C. Inoculation site of *A. falcatus* (red arrow). D. White mycelium growing on the stem (red arrow) four months post-inoculation. E. Wilt symptoms of *Q. hemisphaerica* after 60 days post-inoculation. F. Control leaves of *Q. hemisphaerica*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

all directions until reaching other twigs and petioles and they spread onto the abaxial parts of leaves (Fig. 6). *P. relampaga* was only found on the abaxial part of the leaves during the greenhouse inoculation experiments and in field specimens. Necrosis was observed when the abaxial surfaces of the leaves were covered with a layer of mycelium.

4. Discussion

A novel thread blight fungus, *P. relampaga,* was found growing on native *Q. hemisphaerica* and non-native *A. falcatus* and *L. japonicum* in Gainesville, Florida, USA, between 2019 and 2022. The same plant pathogen was subsequently identified on numerous native and non-native hardwood and conifer species at scattered locations from the panhandle to central Florida. This basidiomycete fungal pathogen of woody plants produced characteristic white rhizomorphs and gloeocystidia in all host plant studies. This fungus was most commonly found on understory host plants under wet conditions.

Other species of *Parvodontia* produce similar basidiospores and gloeocystidia but differ in hymenophore color and configuration. *P. luteocystidia* and *Parvodontia albocrustacea* (Rick) Baltazar & Rajchenb, from South America, lack rhizomorphs and develop a mostly smooth hymenophore with well-spaced, tiny aculei but without cracks. *Parvodontia austrosinensis* Yue Li, Nakasone & S.H. He from China also produces a smooth hymenophore but with rounded tubercules and abundant microbinding hyphae in the context. The morphological characteristics and our molecular analyses support the classification of the causal agent of relampago blight within the genus *Parvodontia.*

We propose a life cycle of this fungus in which infected leaves or twigs extend their rhizomorphs to a healthy host and the fungus colonizes tissues by penetrating through cracks or wounds in branches and twigs. The fungus then grows upward along twigs and branches until it reaches the canopy, where it colonizes the leaf abaxial surface and dieback symptoms become noticeable. We hypothesize that colonized plant tissues, rhizomorphs, and thick-walled gloeocystida may act as survival structures. Based on personal observations, *P. relampaga* is also capable of colonizing small seedlings and it may also serve as a litter decomposer in the understory. Based on a previous study (e.g., Kusunoki et al., 1997), we suspect that wind-dispersed basidiospores may act as a secondary mode of infection on healthy hosts, but they are not likely to be the most important method of dispersal and colonization.

The multi-locus phylogenetic tree based on three loci (ITS, LSU and *tef1*) provided strong support for the placement of *P. relampaga* as sister to *P. luteocystidia* strains from Puerto Rico. Originally described from South America, partial sequence of the ITS from the paratype of *P. luteocystidia* from Argentina was identical to sequences from Jamaica and Puerto Rico (pers. comm., K.H. Larsson). However, due to the limited sampling of this fungal group, additional research is essential to reveal potential relationships with other taxa and to determine the geographical distribution of these fungi.

Pathogenicity experiments confirmed that *P. relampaga* causes disease in *A. falcatus*, *L. japonicum* and *Q. hemisphaerica*. The main difference among these hosts was that in *A. falcatus* the fungus was slower to colonize the host. This discrepancy could be attributed to the fact that the leaf area inoculated was smaller than in *Q. hemisphaerica* and *L. japonicum* (e.g., differences in leaf morphology) or be indicative of distinct pathogenicity traits of each fungal isolate specific to its host species. Nevertheless, additional research is necessary to corroborate these hypotheses.

Currently, the number of emerging fungal diseases, including thread blights, appears to be increasing due to several factors, such as increasing introductions of new hosts, invasive fungal species, and the potential impacts of climate change (Ghelardini et al., 2016; Jain et al., 2019). Climate change can increase the chances for fungal pathogens to spread; it can enhance the fitness of pathogens, or it can predispose plants to disease by weakening them (Eastburn et al., 2011; Ghelardini et al., 2016).

Thread blights include emerging fungal diseases that have become problematic on several host plants and in different locations (Belachew et al., 2015; Amoako-Attah et al., 2016; Huamán-Pilco et al., 2022). Important emerging thread blights include white thread blight in cocoa from Peru, one of the most important suppliers of organic and aroma cocoa beans globally (Sánchez Arizo et al., 2019; Huamán-Pilco et al., 2022). Another important white thread blight is caused by *C. koleroga* on coffee in Ethiopia (Belachew et al., 2015). This disease was first reported in 1978 and was considered a minor coffee disease (Merdassa 1986). However, a 2014 outbreak of this disease caused significant losses and it is now considered a much more significant threat to coffee production (Belachew et al., 2015). *M. scandens* is another thread blight disease that threatens cacao production in Ghana, which produces 20% of the world's cocoa (Asante-Poku and Angelucci 2013; Amoako-Attah et al., 2016).

Within Basidiomycota, thread blight fungi have evolved independently in several clades, including Agaricales (*Marasmiellus* and *Marasmius*), Cantharellales (*Ceratobasidium*) and Corticiales (*Corticium*) (Tims et al., 1954; Ceresini et al., 2012; Huamán-Pilco et al., 2022). The Agaricales is one of the largest orders of mushroom-forming fungi and includes 33 families (Kirk et al., 2008). Within the order, the family Cystostereaceae includes seven accepted genera: *Cericium* Hjortstam, *Crustomyces* Jülich, *Cystidiodontia* Hjortstam, *Cystostereum* Pouzar, *Parvobasidium* Jülich, *Parvodontia* Hjortstam & Ryvarden, and *Rigidotubus* J. Song, Y.C. Dai, & B.K. Cui. Members of the Cystostereaceae form resupinate fruiting bodies, which are considered uncommon in Agaricales. This family has a worldwide distribution and species in this family are typically saprotrophic wood-decay fungi associated with a white rot decay of both hardwoods and conifers (Larsson 2007).

Recent molecular phylogenetic studies confirmed the family Cystostereaceae as a monophyletic clade that includes the genera *Crustomyces, Cystidiodontia*, *Cystostereum, Parvodontia* and *Rigidotubus* pro parte (Larsson 2007; Song et al., 2018; Li et al., 2022). Two other genera, *Cericium* and *Parvobasidium*, are also thought to belong to this family based on morphological characteristics, but their placement still needs to be confirmed by molecular data (Larsson 2007; Li et al., 2022). Li et al. (2022) made significant taxonomic changes in Cystostereaceae; they placed *Cystidiodontia* and *Rigidotubus* p.p. in synonymy under the genus *Crustomyces* and described the new, monotypic genus *Effusomyces* Yue Li, Nakasone & S.H. He. In addition, they generated the first molecular data for *Parvodontia* from fresh material of the newly described taxon *P. austrosinensis*.

We identified *P. relampaga* in Florida on a wide range of host plants, including 27 different species that belong to 24 different genera and 20 families. This thread blight pathogen has a wide host range and can affect both angiosperms and gymnosperms. It is important to mention that *P. relampaga* was observed in understory hosts growing in places that were shaded and humid but have not been observed in places with full light or under dry conditions.

We propose the common name of relampago blight for this disease due to a distinctive feature of the white mycelium that is easily recognized in the field. Relampago means "lightning" in Spanish, and the white mycelium looks like lightning when it grows on the bark of affected trees. Relampago blight could pose a serious threat to woody hosts as an emerging pathogen in the Gulf Coast region. It could also be problematic in greenhouses due to higher humidity conditions and the fact that plants are often packed closely under high humidity, which could provide ideal conditions for this fungus.

The origins of the thread blight pathogen *P. relampaga* remain mysterious; with conflicting evidence surrounding its status as a native or introduced species in Florida. The first specimen of *P. relampaga* (HHB-6827) was collected in 1972. It is possible that this fungus was introduced at this time but that environmental conditions were unfavorable for pathogenicity from 1972 until 2019. Alternatively, *P. relampaga* may be a native species that has recently emerged as a problematic pathogen, possibly due to changes in weather patterns (e.g.,

fewer hours of freezing per year) or human alterations in natural ecosystems.

To understand the origins of *P. relampaga* and its potential impact on natural and agricultural systems, further research is clearly needed to better understand the life history of the fungus, elucidate the origins of this new fungal pathogen, and explore how its biology relates to disease development in the field. For example, more studies are needed to clarify the pathogen's dispersal mechanisms, its genetic diversity and population genetic structure. Additionally, it is important to assess the risk this pathogen poses to natural and cultivated hosts and evaluate its potential impact on forest regeneration. Lastly, a high priority should be placed on determining the current geographic distribution to guide monitoring and regulation of plant material and developing effective management strategies to control and mitigate its spread.

5. Conclusions

This study presents a multi-locus phylogeny and morphological description of *P. relampaga*, an undiscovered pathogen within the Cystostereaceae family. This novel pathogen causes thread blight in woody plants and our study includes a pathogenicity test on three different woody hosts, *A. falcatus*, *L. japonicum* and *Q. hemisphaerica*. By providing molecular and morphological information of a newly described pathogenic fungal species in Florida, USA, the study not only enhances our understanding of this pathogen but also contributes valuable knowledge for a broader characterization of Cystostereaceae fungi.

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Declaration of competing interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2024.03.002.

References

Adedeji, A.R., 2021. Thread blight disease of tea [*Camellia sinensis* (L.) O. Kuntze] caused by *Marasmius pulcher* (Berk & Br.) Petch in the South western Nigeria. African Scientist 7 (3), 107–112.

Adugna, G., 1997. Status and economic importance of Fusarium wilt disease of Arabica coffee in Ethiopia. In: Hakiza, G., Birkunzira, B., Musoli, P. (Eds.), Proceedings of the First Regional Workshop on Coffee Wilt Disease. International Conference Centre, Kampala, Uganda, pp. 53–61.

Amoako-Attah, I., Akrofi, A.Y., Rashid, B.H., Mercy, A., Kumi-Asare, E., 2016. White thread blight disease caused by *Marasmiellus scandens* (Massee) Dennis & Reid on cocoa and its control in Ghana. Afr. J. Agric. Res. 11 (50), 5064–5070.

Amoako-Attah, I., Shahin, A.S., Aime, M.C., Odamtten, G.T., Cornelius, E., Nyaku, S.T., Kumi-Asare, E., Yahaya, H.B., Bailey, B.A., 2020. Identification and characterization of fungi causing thread blight diseases on cacao in Ghana. Plant Dis. 104 (11), 3033–3042.

Asante-Poku, A., Angelucci, F., 2013. Analysis of Incentives and Disincentives for Cocoa in Ghana. Technical Notes Series, MAFAP, FAO, Rome, p. 44.

Belachew, K., Teferi, D., Hagos, L., 2015. Coffee thread blight (*Corticium koleroga*): a coming threat for Ethiopian coffee production. J. Plant Pathol. Microbiol. 6 (9), 303–308.

Benchimol, L., Poltronieri, S., Trindade, R., Albuquerque, C., 2001. White thread blight: five new hosts in the State of Pará, Brazil. Fitopatol. Bras. 26, 778-778.

Borowiec, M.L., 2016. AMAS: a fast tool for alignment manipulation and computing of summary statistics. PeerJ 4, e1660.

Ceresini, P.C., Costa-Souza, E., Zala, M., Furtado, E.L., Souza, N.L., 2012. Evidence that the *Ceratobasidium*-like white-thread blight and black rot fungal pathogens from persimmon and tea crops in the Brazilian Atlantic Forest agroecosystem are two distinct phylospecies. Genet. Mol. Biol. 35, 480–497.

Chen, Y., Ye, W., Zhang, Y., Xu, Y., 2015. High speed BLASTN: an accelerated MegaBLAST search tool. Nucleic Acids Res. 43, 7762–7768.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Davidson, R.W., Campbell, W.A., Blaisdell, D.J., 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. 57, 683–695.

Dechassa, N., Chala, A., Belachew, K., Shikur, E., 2020. Morphological characterization of *Corticium koleroga*, cause of thread blight on arabica coffee. Pharm. Sci. Tech. 4 (2), 31.

Eastburn, D.M., McElrone, A.J., Bilgin, D.D., 2011. Influence of atmospheric and climatic change on plant–pathogen interactions. Plant Pathol. 60 (1), 54–69.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Mol. Ecol. 2 (2), 113–118.

Gasparotto, L., Silva, S.E., 1999. New hosts of *Pellicularia koleroga* in the state of Amazonas, Brazil. Fitopatol. Bras. 24 (3), 469.

Ghelardini, L., Pepori, A.L., Luchi, N., Capretti, P., Santini, A., 2016. Drivers of emerging fungal diseases of forest trees. For. Ecol. Manag. 381, 235–246.

Hedger, J., 1990. Fungi in the tropical forest canopy. Mycologist 4, 200–202.

Hopple Jr., J.S., Vilgalys, R., 1994. Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86, 96–107.

Hu, C.C., 1984. Horse-hair blight, new disease of tea bush caused by *Marasmius equicrinis* Muell. in Taiwan. Taiwan Tea Res. Bull. 3, 1–4.

Huamán-Pilco, A.F., Torres-de la Cruz, M., Aime, M.C., Leiva-Espinoza, S.T., Oliva-Cruz, S.M., Díaz-Valderrama, J.R., 2022. First report of thread blight caused by *Marasmius tenuissimus* on Cacao (*Theobroma cacao*) in Peru. Plant Dis. 107 (1), 219.

Jain, A., Sarsaiya, S., Wu, Q., Lu, Y., Shi, J., 2019. A review of plant leaf fungal diseases and its environment speciation. Bioengineered 10 (1), 409–424.

Keane, P.J., Putter, C.A. (Eds.), 1992. Cocoa Pest and Disease Management in Southeast Asia and Australasia. FAO Plant Production Paper 112. Food and Agriculture Organization of the United Nations, p. 223.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. In: Ainsworth & Bisby's Dictionary of the Fungi, tenth ed. CAB International, p. 771.

Kornerup, A., Wanscher, J.H., 1978. Methuen Handbook of Colour. Eyre Methuen, London, p. 252.

Kusunoki, M., Kawabe, Y., Ikeda, T., Aoshima, K., 1997. Role of birds in dissemination of the thread blight disease caused by *Cylindrobasidium argenteum*. Mycoscience 38 (1), 1–5.

Larsson, K.H., 2007. Re-thinking the classification of corticioid fungi. Mycol. Res. 111 (9), 1040–1063.

Larsson, K.H., Larsson, E., Kõljalg, U., 2004. High phylogenetic diversity among corticioid homobasidiomycetes. Mycol. Res. 108 (9), 983–1002.

Li, Y., Nakasone, K.K., Chen, C.C., Zhao, C.L., Cao, T., Yuan, H.S., He, S.H., 2022. Taxonomy and phylogeny of Cystostereaceae (Agaricales, Basidiomycota): a new genus, five new species, and three new combinations. J. Fungi 8 (11), 1229.

Matheny, P.B., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Nilsson, R.H., Hughes, K.W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., Langer, E., 2007. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol. Phylogenet. Evol. 43 (2), 430–451.

Mathew, K.T., 1954. Studies on the black rot of coffee. Proc. Indian Acad. Sci. B 39 (4), 133–170. Springer India.

Meistermann, E., Villaumé, S., Goffette, D., Trarieux, C., Coarer, M., Roulland, C., Dupont, J., 2021. A Basidiomycete fungus responsible for fresh mushroom off-flavour in wines: *Crustomyces subabruptus*, (Bourdot & Galzin) Jülich, 1978. OENO One 55, 283–298.

Merdassa, E., 1986. A review of coffee diseases and their control in Ethiopia. In: Proceedings of the First Ethiopian Crop Protection Symposium. Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia, pp. 187–195.
Metslaid, M., Granhus, A., Scholten, J., Fjeld, D., Solheim, H., 2018. Long-term effects of single-tree selection on the frequency and population structure of root and butt rot in uneven-sized Norway spruce stands. For. Ecol. Manag. 409, 509–517.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), pp. 1–8.
Nakasone, K.K., 1990. Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. Mycol. Mem. 15, 1–412.
Nobles, M.K., 1948. Studies in forest pathology: VI. Identification of cultures of wood-rotting fungi. Can. J. Res. 26 (3), 281–431.
Pereira, C., Ledo, J., Bergo, L., Siviero, A., 2000. Evaluation of arabica and Robusta coffee genotypes in western Amazonia. Acta Amazonica 30, 535–541.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67, 901–904.
Rehner, S.A., Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97, 84–98.
Ridgway, R., 1912. Color Standards and Color Nomenclature, p. 43. Washington, D.C., Published by the author.
Ronquist, F., Teslenko, M., Mark, P. Van Der, Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Sánchez Arizo, V.H., Zambrano Mendoza, J.L., Iglesias Paladines, C., 2019. La cadena de valor del cacao en América Latina y el Caribe.
Song, J., Xing, J., Ji, X., Sun, Y., Cui, B., Dai, Y.C., 2018. *Rigidotubus tephroleucus* gen. et sp. nov. (Cystostereaceae, Agaricales) evidenced by morphological characters and phylogenetic analyses. Phytotaxa 333 (2), 259–266.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Tedersoo, L., Jairus, T., Horton, B.M., Abarenkov, K., Suvi, T., Saar, I., Köljalg, U., 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol. 180, 479–490.
Tims, E.C., Mills, P.J., Exner, B., 1954. Thread-blight (*Pellicularia koleroga*) in Louisiana. Plant Dis. 38, 634–637.
Varga, T., Krizsán, K., Földi, C., Dima, B., Sánchez-García, M., Sánchez-Ramírez, S., Szöllősi, G.J., Szarkándi, J.G., Papp, V., Albert, L., Andreopoulos, W., 2019. Megaphylogeny resolves global patterns of mushroom evolution. Nat. Ecol. Evol. 3 (4), 668–678.
Vu, D., Groenewald, M., De Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., Al-Hatmi, A., Groenewald, J.Z., Cardinali, G., Houbraken, J., Boekhout, T., 2019. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud. Mycol. 92, 135–154.
Wu, L., Han, T., Li, W., Jia, M., Xue, L., Rahman, K., Qin, L., 2013. Geographic and tissue influences on endophytic fungal communities of Taxus chinensis var. mairei in China. Curr. Microbiol. 66, 40–48.