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Plant Pathology

Occurrence of *Moniliophthora perniciosa* on unidentified wood debris in the Southwestern Brazilian Amazon

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ABSTRACT: *Moniliophthora perniciosa*, a basidiomycete fungus, has multiple hosts and has been identified as a significant pathogen affecting cacao and cupuassu trees in Brazil. In this study, *M. perniciosa* was detected on unidentified wood debris in the Parque Estadual Chandless (PEC), located in the state of Acre in northern Brazil. To provide more information about this fungus in Acre, it was characterized both micro and macro-morphologically, and molecularly identified based on two DNA regions: ribosomal internal transcribed spacer (ITS) and ribosomal large subunit (LSU). The phylogenetic and morphological analysis of the collected fungal isolates PEC11 and PECT1 revealed that both belonged to species *M. perniciosa* and shared similarities with fungal isolates from the host *Heteropterys acutifolia*. This constitutes the first report of *M. perniciosa* on unidentified wood debris in Acre, thereby broadening our understanding of the pathogen's distribution and ecological niche. Further studies are necessary to ascertain the geographical distribution of *M. perniciosa* within the country.

Keywords: *Heteropterys acutifolia*, H-biotype, host, witches' broom

Moniliophthora perniciosa (Stahel) Aime and Phillips Mora is the causal agent of witches' broom disease in cacao (*Theobroma cacao* L.) and cupuassu (*Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum.) trees (Mournet et al., 2020). This fungus belongs to the order Agaricales and the family Marasmiaceae (Aime and Phillips-Mora, 2005; Seifert and Gams, 2011). Various biotypes of *M. perniciosa* have been identified and delimited based on their adaptations to different hosts (Lisboa et al., 2020). For instance, the C-biotype infects plants in the Malvaceae family (e.g., cacao) (Evans et al., 2013); S-biotype colonizes plants of the Solanaceae family (e.g., tomato) (Bastos and Evans, 1985; Evans and Barreto, 1996); L-biotype has been found to be associated with members of the Bignoniaceae family (e.g., liana) (Evans, 1978; Griffith and Hedger, 1994) and B-biotype colonizes *Bixa orellana* L. (Bastos and Anderbrhan, 1986). Furthermore, a non-pathogenic biotype has been isolated from *Allophylus edulis* (A.St.-Hil., A.Juss. & Cambess.) Radlk. (Sapindaceae), thereby establishing a new host family record (Lisboa et al., 2020).

The H-biotype corresponds to *M. perniciosa*, a fungus infecting Malpighiaceae plants (Bastos et al., 1998). Initially, this fungus was identified on the host *Mascagnia* cf. *sepium* (A. Juss) Griseb in the Brazilian Amazon (Bastos et al., 1998). Subsequent research identified *M. perniciosa* in *Heteropterys acutifolia* A.Juss., a woody vine commonly found in gallery forests in Southeast Brazil (Resende et al., 2000). Furthermore, *M. perniciosa* basidiospores from brooms of *H. acutifolia* were inoculated into cacao seedlings, inducing witches' broom symptoms (Resende et al., 2000). Recently, in Southeast Brazil, *M. perniciosa* was found on a woody vine from *H. acutifolia* and unidentified wood debris (Lisboa et al., 2020).

The broad array of hosts of *M. perniciosa* underscores its adaptive and specialized lifestyle, intricately linked to its host plants. This fungus can exhibit both a pathogenic and a non-pathogenic lifestyle (Lisboa et al., 2020). Specifically, C-, S- and H-biotypes have been regarded as pathogenic and may potentially employ analogous pathogenicity mechanisms to infect their hosts (Teixeira et al., 2015). In contrast, the L-biotype exhibits a non-pathogenic lifestyle (Lisboa et al., 2020). However, the mechanisms associated with the adaptation of *M. perniciosa* to different hosts are poorly understood (Santos et al., 2023).

In the southwestern Amazon, particularly in the state of Acre in Brazil, only the C-biotype has been detected in cacao and cupuassu trees cultivated in both agroforestry systems and monocultures or under natural conditions (Artero et al., 2017). This is the first report of *M. perniciosa* collected from unknown wood debris in Acre state. The objective of this study was twofold: first, to expand knowledge about the geographic distribution of this species and its hosts, and second, to show a morphological description and molecular characterization of this fungal species.

Basidiomata of two fungi were collected from unidentified wood debris (thin and dry stems) in a primary forest in the Parque Estadual Chandless (PEC), located in the center-south of the Acre state, Brazil (9°55′07″ S, 70°09′27″ W, 320 m altitude) (Figure 1A). The collection of basidiomata was conducted on different days during the rainy season in May 2021, with a distance of approximately 12 km between the two locations. The PEC is an Integral Protection Conservation Unit with 695,303 ha, encompassing parts of the municipalities of Sena Madureira and Santa Rosa

do Purus (Cabral et al., 2021). The Park is the largest conservation unit in the state, and its location is part of the border between Brazil and Peru, with an open rainforest as the dominant vegetation (Cabral et al., 2021). Bamboo is the predominant species in this forest ecosystem, and the climate at PEC is characterized by high temperatures and humidity levels, with average temperatures between 24 and 25 °C (Cabral et al., 2021).

The DNA extraction process of the basidiomata was performed using the method established by Doyle and Doyle (1987). The integrity of the extracted DNA was subsequently assessed through 1 % agarose gel electrophoresis, a technique that allows for the visualization of DNA fragments in an electric field. The purity and quantity of the extracted total DNA were determined using a NanoDrop instrument (Thermo Scientific). This device quantifies the concentration of nucleic acids based on their absorption spectra. The sequences obtained were focused on two genetic regions: the ribosomal internal transcribed spacer (ITS) and the ribosomal large subunit (LSU). The sets of primers ITS1 and ITS4 (White et al., 1990); LR0R (Rehner and Samuels, 1994) and LR5 (Vilgalys and Hester, 1990) were used for amplification and sequencing for ITS and LSU regions, respectively. The reactions involved the utilization of 2 µL of DNA template (10 ng), 1.5 mM $MgCl_2$, 0.4 µM of each primer, 2 mM dNTPs, 5 µL of *Taq* buffer, and 1.25 U of *Taq* DNA polymerase (Ludwig). Polymerase Chain Reaction (PCR) amplification was initiated with an initial denaturation at 95 °C for 3 min, followed by 35 amplification cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min), concluding with an extension step at 72 °C for 5 min. The PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen) and quantified with a NanoDrop (Thermo Scientific). Subsequently, the forward and reverse sequencing of this ITS and LSU region was conducted by ACT Gene Molecular Analyses Ltd., using the AB 3500 Genetic Analyzer (Applied Biosystems).

Nucleotide sequences were manipulated using the Chromas software (http://www.technelysium.com.au/chromas.html, version 2.6.6). The sequences were thoroughly reviewed, and nucleotides with uncertain positions were manually edited. The consensus sequences were then assembled using the Bioedit software (version 7.2). The newly generated sequences were submitted to the NCBI GenBank database, while sequences from previous studies on *M. perniciosa* and closely related species were obtained from the same database (Table 1).

The results of BLAST analyses of the sequences PEC11 and PECT1, obtained from the two basidiomata collected, indicated that the fungi belong to *M. perniciosa*. The sequences were deposited in GenBank with the following accession numbers: OP937176 (ITS) and OP937981 (LSU) for PEC11, and OP937177 (ITS) and OP937982 (LSU) for PECT1 (Table 1). Subsequently, a phylogenetic tree was constructed to compare the nucleotide sequences of this fungus with *M. perniciosa* isolated from other hosts and with fungi from other species and families. These sequences were selected from diverse hosts, species, and genera of *M. perniciosa* to represent the taxonomic diversity. The dataset for each locus was aligned using MUSCLE (Edgar, 2004), and the alignments were concatenated in the software MEGA X (version 11) (Kumar et al., 2018). The phylogenetic tree was performed using Bayesian inference analysis based on the Markov chain Monte Carlo method. The MrMODELTEST v. 3.04 software (Posada and Buckley, 2004) was employed to determine the appropriate nucleotide substitution model for Bayesian inference analysis (Posada and Buckley, 2004). The models for phylogenetic analyses were selected independently for each locus (ITS and LSU). Likelihood values were then calculated, and the model was chosen based on the Akaike information criterion. The evolutionary models selected for each gene region were GTR + I + G for ITS and HKY + G for LSU. The Bayesian inference analysis used MrBayes version 3.1.1 (Ronquist and Huelsenbeck, 2003). The consensus tree was derived following 10 million generations of a Markov chain, implemented across two independent runs, each with four chains, and discarding the initial 25 % as burnin. The convergence of likelihood logs was assessed using TRACER version 1.4.1 software, and the tree was displayed using FigTree version 1.4.4. *Chaetocalathus liliputianus* (Mont.) Singer (MCA485) was employed as the outgroup taxon.

Basidiomata were photographed *in situ* and subsequently removed from the substrate with the assistance of a penknife. They were then placed in a paper bag and sent to the laboratory for further analysis. The macromorphological examination of the basidiomata included the following characteristics: lamellae color, stipe color and size, color and diameter, and pileus shape). For microscopic analysis, small portions of the hymenium were freehanded sectioned with a steel blade, and the glass slide was mounted with 3 % potassium hydroxide and 1 % phloxine dye. Spores were visualized and measured using TCapture software (version 3.9) (NOVEL BM2100 microscope). Width and length measurements of at least 20 individual basidiospore structures were carried out for PEC11 and PECT1. The material studied is deposited at the Laboratório de Botânica Aplicada of the Universidade Federal do Acre (UFAC), Rio Branco, Acre, Brazil.

In the present study, the fungi PEC11 and PECT1 were identified on unidentified wood debris in an area of primary forest in Acre state, Brazil (Figure 1A). The DNA sequences of both fungi were generated, with the PEC11 isolate having amplified regions of 867 base pairs (bp) at the ITS locus and 699 bp at the LSU locus. The amplified regions for the fungal isolate PECT1 were 868 bp (ITS) and 698 bp (LSU). Based on the ITS locus, the PEC11 and PECT1 isolates shared 99.15 and 100 % identity, respectively, with *M. perniciosa* isolated from *H. acutifolia* collected in Minas Gerais state, Brazil (Lisboa et al., 2020). Furthermore, the LSU sequences

demonstrated that the fungi from Acre state, PEC11 and PECT1, exhibited 100 and 99.8 % identity, respectively, with *M. perniciosa* isolated from unidentified wood debris, which was also collected in Minas Gerais state, Brazil (Lisboa et al., 2020).

The phylogenetic analysis results demonstrated that both fungi PEC11 and PECT1 were closely related to *M. perniciosa* isolates, exhibiting a high posterior probability value (pp) = 1 (Figure 1B). Furthermore, the results indicated the emergence of a subclade comprising PEC11 and PECT1, along with fungi isolated from *H. acutifolia* (COAD2614, COAD2615, RWB1267 and UB2553), exhibiting a high value of pp = 0.99 (Figure 1B). According to the phylogenetic analysis and the original host, the ITS and LSU sequences are grouped according to the host. In this case, our results indicate that PEC11 and PECT1 isolates were potentially growing in *H. acutifolia* debris.

A variety of biotypes of *M. perniciosa*, including both pathogenic and non-pathogenic forms, have been identified in a range of hosts distributed across various biomes in Brazil (Andebrhan and Furtek, 1994; Arruda et al., 2003; Rincones et al., 2006; Lisboa et al., 2020). For instance, L- and H-biotypes were identified in plants from the Cerrado, Atlantic Forest, and Amazon biomes, while S-biotype was recovered from hosts present in all

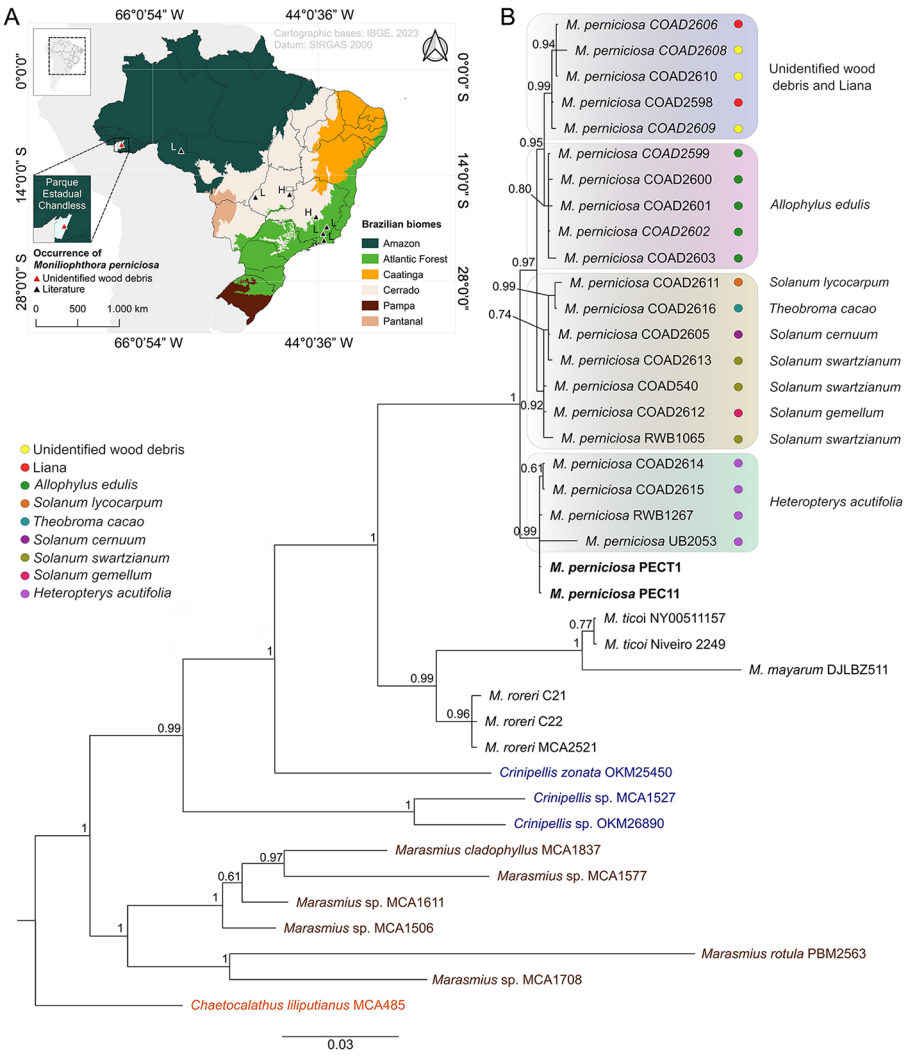

Figure 1 – Geographical distribution and phylogenetic tree of *Moniliophthora perniciosa* isolates PEC11 and PECT1. A) Collection site of *M. perniciosa* isolates PEC11 and PECT1 in Acre state, Brazil (Parque Estadual Chandless = PEC), indicated by the red triangle. Black triangles represent *M. perniciosa* isolates belonging to the L and H-biotypes, according to the data from Lisboa et al. (2020). L-biotype corresponds to fungal isolates from plants of the Bignoniaceae family, while H-biotype refers to isolates from plants of the Malpighiaceae family. B) Phylogenetic tree based on the Bayesian Inference using two combined sequences: ribosomal internal transcribed spacer and ribosomal large subunit of *M. perniciosa* isolates associated with different hosts. The posterior probability is indicated near the branch nodes. The tree was rooted with *Chaetocalathus liliputianus* (MCA485). The fungi PEC11 and PECT1 were recovered from unidentified wood debris.

Table 1 – Collection details and GenBank accession numbers of isolates included in the present study. The accession numbers correspond to sequences from two loci: the ribosomal internal transcribed spacer (ITS) and the ribosomal large subunit (LSU).

Taxon	Fungi ID*	Locality/Country	Host	ITS	LSU	References
Chaetocalathus liliputianus	MCA 485	Puerto Rico	Saprotroph	AY916682	AY916680	Aime and Phillips-Mora, 2005
Marasmius sp.	MCA1708	Guyana	Saprotroph	AY916720	AY916718	
Marasmius rotula	PBM2563	USA	-	DQ182506	DQ457686	Matheny et al. (2006a) (ITS); Matheny et al. (2006b) (LSU)
Marasmius sp.	MCA1506	Guyana	Saprotroph	AY916733	AY916731	Aime and Phillips-Mora, 2005
Marasmius sp.	MCA1611	Guyana	Saprotroph	AY916725	AY916723	
Marasmius sp.	MCA1577	Guyana	Saprotroph	AY916711	AY91679	
Marasmius cladophyllus	MCA1837	Guyana	Saprotroph	AY916705	AY916704	
Crinipellis sp.	OKM26890	Thailand	Saprotroph	AY916698	AY916696	
Crinipellis sp.	MCA1527	Guyana	Saprotroph	AY916701	AY916699	
Crinipellis zonata	OKM25450	USA		AY916692	AY916690	
Moniliophthora roreri	MCA2521	Ecuador	*Theobroma cacao*		AY916750	
M. roreri	C22	Costa Rica	*Theobroma cacao*		AY916749	
M. roreri	C21	Costa Rica	*Theobroma cacao*	AY916746	AY916744	
Moniliophthora mayarum	DJLBZ511	Belize	*Ceiba pentandra*	MT162718	MT162714	Niveiro et al., 2020
Moniliophthora ticoi	Niveiro 2249	Argentina	*Myrcianthes pungens*	MT162720	MT162716	
M. ticoi	NY00511157	Bolivia		MT162721	MT162717	
Moniliophtora perniciosa	PEC11	PEC - Acre, Brazil	Unknown host	OP937176	OP937981	This study
M. perniciosa	PECT1	PEC - Acre, Brazil	Unknown host	OP937177	OP937982	
M. perniciosa	UB2053	Itumirim - Mato Grosso, Brazil	*Heteropterys acutifolia*	AY317137		Arruda et al., 2005
M. perniciosa	RWB1267	Itumirim - Mato Grosso, Brazil	*H. acutifolia*	MK785161		Lisboa et al., 2020
M. perniciosa	COAD2615	Itumirim - Mato Grosso, Brazil	*H. acutifolia*	MK785157	MK785253	
M. perniciosa	COAD2614	Itumirim - Mato Grosso, Brazil	*H.acutifolia*	MK785156	MK785252	
M. perniciosa	RWB1065	Rio de Janeiro - Rio de Janeiro, Brazil	*Solanum swartzianum*	MK785159		
M. perniciosa	COAD2612	Ponta Grossa - Paraná, Brazil	*Solanum gemellum*	MK785154	MK785250	
M. perniciosa	COAD540	Rio de Janeiro - Rio de Janeiro, Brazil	*S. swartzianum*	MK785139		
M. perniciosa	COAD2613	Rio de Janeiro - Rio de Janeiro, Brazil	*S. swartzianum*	MK785155	MK785251	
M. perniciosa	COAD2605	Viçosa - Minas Gerais, Brazil	*Solanum cernuum*	MK785147	MK785243	
M. perniciosa	COAD2616	Linhares - Espírito Santo, Brazil	*Theobroma cacao*	MK785158	MK785254	
M. perniciosa	COAD2611	Viçosa - Minas Gerais, Brazil	*Solanum lycocarpum*	MK785153	MK785249	
M. perniciosa	COAD2603	Viçosa - Minas Gerais, Brazil	*Allophyllus edulis*	MK785145	MK785241	
M. perniciosa	COAD2602	Viçosa - Minas Gerais, Brazil	*A. edulis*	MK785144	MK785240	
M. perniciosa	COAD2601	Viçosa - Minas Gerais, Brazil	*A. edulis*	MK785143	MK785239	
M. perniciosa	COAD2600	Viçosa - Minas Gerais, Brazil	*A. edulis*	MK785142	MK785238	
M. perniciosa	COAD2599	Viçosa - Minas Gerais, Brazil	*A. edulis*	MK785141	MK785237	
M. perniciosa	COAD2609	Viçosa - Minas Gerais, Brazil	Unknown host	MK785151	MK785247	
M. perniciosa	COAD2598	Viçosa - Minas Gerais, Brazil	Liana	MK785140	MK785236	
M. perniciosa	COAD2610	Viçosa - Minas Gerais, Brazil	Unknown host	MK785152	MK785248	
M. perniciosa	COAD2608	Viçosa - Minas Gerais, Brazil	Unknown host	MK785150	MK785246	
M. perniciosa	COAD2606	Viçosa - Minas Gerais, Brazil	Liana (Bignoniaceae)	MK785148	MK785244	

*Identification code of fungal isolate

biomes (Arruda et al., 2005; Rincones et al., 2006; Lisboa et al., 2020). In Acre state, only the C-biotype had been previously reported in cacao and cupuassu (Artero et al., 2017). Notably, populations of *M. perniciosa* obtained from cacao hosts in the states of Acre and Amazonas (located in the southwestern and western Brazilian Amazon, respectively) exhibited the most extensive genotypic diversity compared to populations from other regions of Brazil. This finding suggests that the states of Acre and Amazonas may serve as a center of diversity for this fungus (Artero et al., 2017). The observed diversity across biomes underscores the capacity of

environmental conditions to influence the adaptive traits of *M. perniciosa* biotypes, thereby impacting both their pathogenicity and interactions with diverse hosts. Notably, this study documents the first report of *M. perniciosa* on unidentified wood debris in the Acre state (Figure 1A and B). This finding suggests the potential for the fungus to persist in forest debris, functioning as a latent reservoir of inoculum in natural environments.

Regarding morphology, PEC11 and PECT1 were classified within the Marasmiaceae Singer's system (Singer, 1976). The PEC11 basidiomata resembled *M. perniciosa* isolated from *H. acutifolia*, as described by Lisboa et al. (2020). The pileus measured 13 mm diameter × 1 mm height, exhibiting a pink center and clear margins, convex, with a morphologically depressed center, a rough surface, striate furrowed, membranous consistency, and an irregular margin (Figure 2A). The fungus in question had lamellae free, wide apart, with lamellulae (1-3 lamellulae), membranose, concolor to pileus, stipe 7-8 × 1 mm, central, cylindrical, slender, fragile, wine color with the apex concolor to the lamellae, rigid texture, smooth surface, no ring, volva, and basal mycelium. The basidiospores size ranged from 9-11 × 5-6 µm, ellipsoid, hyaline, with slightly thickened walls (Figure 2B). Cheilocystidia had 22-34 × 5-6-8 µm, clavate, hyaline, thin-walled (Figure 2C and Table 2).

In contrast to fungus PEC11, fungus PECT1 exhibited a distinct coloration of the pileus, a characteristic that differentiates it from *M. perniciosa* isolates derived from *H. acutifolia* found in Minas Gerais state, Brazil (Lisboa et al., 2020). Furthermore, the cream color of the pileus in this fungus is different from the crimson shades exhibited by other *M. perniciosa* isolates (Figure 2D) (Lisboa et al., 2020), suggesting a potential for distinguishing this species based on the coloration of the pileus. However, the other morphological characteristics are similar to *M. perniciosa* from *H. acutifolia*. The fungus PECT1 showed the following characteristics: pileus 6-10-20 mm diameter × 1 mm height, pale cream, plano-convex, with umboned center, a rough surface, striate furrowed, membranous consistency, and an irregular margin. Lamellae exhibited a subdecurrent orientation, wide apart, with lamellulae (3-5 lamellulae), membranose, concolor to pileus. Stipe 5-6-9 × 1 mm, central, cylindrical, equal, fragile, brownish, with the apex concolor to the lamellae, rigid texture, ribbed surface, no ring, volva and basal mycelium. The basidiospores exhibited a size range of

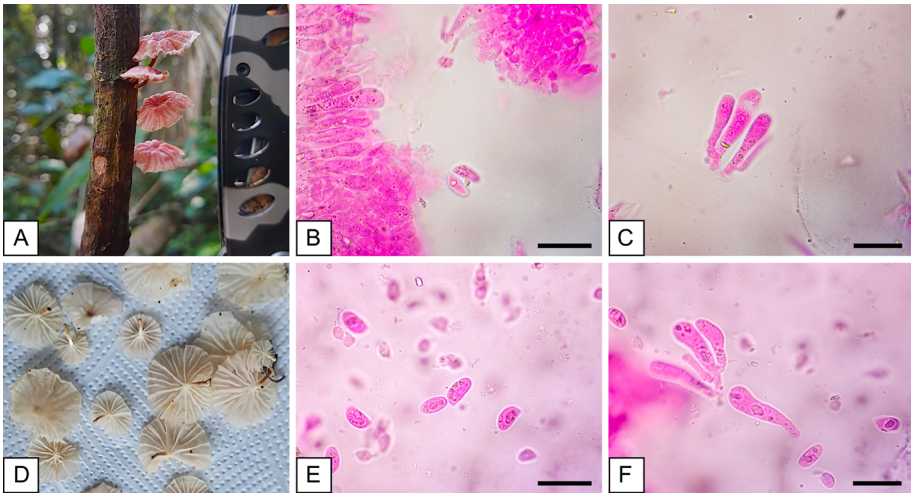

Figure 2 – *Moniliophthora perniciosa* isolates PEC11 and PECT1 on unidentified wood debris. A) Basidiomata from *M. perniciosa* (PEC11). B) Elliptical and apiculate basidiospores (PEC11). C) Clavate cheylocystidia (PEC11). D) Basidiomata from *M. perniciosa* (PECT1). E) Elliptical and apiculate basidiospores (PECT1). F) Clavate cheylocystidia (PECT1). Panels B, C, E, and F are micrographs obtained with an optical microscope (NOVEL BM2100) at 400× magnification and stained with 1 % phloxine dye. Scale bar: 20 µm.

Table 2 – Morphology of Moniliophthora perniciosa isolates PEC11 and PECT1 identified on unidentified wood debris collected at the Parque Estadual Chandless (PEC), Acre state, Brazil.

Code Isolate	PEC11	PECT1
Morphology of cheilocystidia	Clavate	Clavate
Basidiospores shape and size	Elliptical, apiculate/9-11 × 5-6 µm	Elliptical, apiculate/ 9-11 × 5-6 µm
Pileus shape, color and diameter	Convex, with depressed center/pink center and clear margins/13 mm	Convex, with umboned center/pale cream/6-10-20 mm
Stipe color and size	crimson with the apex concolor with the lamellae/7 mm	Brownish/apex concolor with lamellae/6-9 mm
Lamellae color and size	Light pink/ 4-6 (5) mm	Cream/ 5-10 (7) mm

9-11 × 5-6 µm, with an ellipsoid shape and a hyaline appearance, featuring slightly thickened walls (Figure 2E). the cheilocystidia demonstrated dimensions of 24-30 × 6-9 µm, exhibiting a clavate morphology, a hyaline appearance, and thin-walled characteristics (Figure 2F) (Table 2).

The analysis of cheilocystidia morphology has also facilitated the classification of *M. perniciosa* isolates from *H. acutifolia*. A previous study indicated that these isolates from *H. acutifolia* produced lageniform cheilocystidia (Arruda et al., 2005). However, more recent studies have reported variations in morphology, describing the cheilocystidia as clavate to pyriform (Lisboa et al., 2020). In the present study, PEC11 and PECT1 produced clavate cheilocystidia. Therefore, based on the combined analysis of morphology and molecular data, as well as the findings of Lisboa et al. (2020), it is proposed that the PEC11 basidiomata is analogous to *M. perniciosa* isolates from *H. acutifolia* (H-biotype). The results obtained for the PECT1 fungus also suggest that it shares similarities with the H-biotype (*H. acutifolia*). Furthermore, the presence of *H. acutifolia* in the Acre state has been documented (Anderson, 1981).

The H-biotype of *M. perniciosa* has been identified as a pathogenic fungus due to the presence of basidiospores in plants exhibiting symptoms of witches' broom disease in cacao seedlings (Resende et al., 2000). Consequently, it is hypothesized that the presence of this fungus in wood debris may serve as a potential inoculum for cacao and cupuassu plants, contributing to the dissemination of *M. perniciosa* with a pathogenic lifestyle. However, further research is necessary to broaden our understanding of the geographical distribution of hosts in the Amazon and further characterize *M. perniciosa* from various host plants. This will facilitate the development of hypotheses concerning the evolution and pathogenicity of this species.

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Authors' Contributions

Conceptualization: Peters LP, Santos JS. **Data curation:** Peters LP, Santos JS. **Formal analysis:** Peters LP, Santos JS, Prado LS, Teixeira-Silva MA, Carvalho CM, Silveira M. **Funding acquisition:** Peters LP, Carvalho CM. **Investigation:** Peters LP, Santos JS, Teixeira-Silva MA, Carvalho CM, Silveira M. **Writing– original draft:** Peters LP, Santos JS, Carvalho CM, Prado LS. **Writing – review & editing:** Peters LP, Santos JS, Prado LS, Teixeira-Silva MA, Carvalho CM, Silveira M.

Conflict of interest

The authors declare that no conflicts of interest and adhere to *Scientia Agricola*'s data and material sharing policies.

Data availability statement

The ITS and LSU locus sequences of *Moniliophthora perniciosa* isolates PEC11 and PECT1 are deposited in GenBank under the accession numbers: OP937176 (ITS) and OP937981 (LSU) for PEC11, and OP937177 (ITS) and OP937982 (LSU) for PECT1.

Declaration of use of AI Technologies

The authors declare the use of AI tools to assist in editing the English language.

References

Aime MC, Phillips-Mora W. 2005. The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae. Mycologia 97: 1012-1022. https://doi.org/10.3852/mycologia.97.5.1012

Andebrhan T, Furtek DB. 1994. Random amplified polymorphic DNA (RAPD) analysis of *Crinipellis perniciosa* isolates from different hosts. Plant Pathology 43: 1020-1027. https://doi.org/10.1111/j.1365-3059.1994.tb01651.x

Anderson WR. 1981. *Malpighiaceae*. p. 21-305. In: Maguire B. ed. The botany of the guayana highland. Part XI. Memoirs of the New York Botanical Garden, New York, NY, EUA.

Arruda MCC, Ferreira MASV, Miller RNG, Resende MLV, Felipe MSS. 2003. Nuclear and mitochondrial rDNA variability in *Crinipellis perniciosa* from different geographic origins and hosts. Mycological Research 107: 25-37. https://doi.org/10.1017/S0953756202006895

Arruda MCC, Sepulveda GF, Miller RNG, Ferreira MASV, Santiago DVR, Resende MLV, et al. 2005. *Crinipellis brasiliensis*, a new species based on morphological and molecular data. Mycologia 97: 1348-1361. https://doi.org/10.1080/15572536.2006.11832741

Artero AS, Silva JQ, Albuquerque PSB, Bressan EA, Leal Junior GA, Sebbenn AM, et al. 2017. Spatial genetic structure and dispersal of the cacao pathogen *Moniliophthora perniciosa* in the Brazilian Amazon. Plant Pathology 66: 912-923. https://doi.org/10.1111/ppa.12644

Bastos CN, Evans HC. 1985. A new pathotype of *Crinipellis perniciosa* (witches'broom disease) on solanaceous hosts. Plant Pathology 34: 306-312. https://doi.org/10.1111/j.1365-3059.1985.tb01366.x

Bastos CN, Anderbrhan T. 1986. Urucu (*Bixa orellana*): nova espécie hospedeira da vassoura-de-bruxa (*Crinipellis perniciosa*) do cacaueiro. Fitopatologia Brasileira 11: 963-965 (in Portuguese).

Bastos CN, Fonseca SEA, Melo WF. 1998. *Mascagnia* cf. *sepium*, native liana of the Brazilian Amazon, as host of *Crinipellis perniciosa*. Fitopatologia Brasileira 23: 504 (in Portuguese, with abstract in English).

Cabral GS, Silva RS, Araujo RMG, Ghidini AR. 2021. Composition and diversity of phytophilous cladocerans of oxbow lakes of Southwest Amazonian, Acre state, Brazil. Biota Neotropica 21: e20201079. https://doi.org/10.1590/1676-0611-BN-2020-1079

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797. https://doi.org/10.1093/nar/gkh340

Evans HC. 1978. Witches' broom disease of cocoa (*Crinipellis perniciosa*) in Ecuador. Annals of Applied Biology 89: 185-192. https://doi.org/10.1111/j.1744-7348.1978.tb07689.x

Evans HC, Barreto RW. 1996. *Crinipellis perniciosa*: a much investigated but little understood fungus. Mycologist 10: 58-61. https://doi.org/10.1016/S0269-915X(96)80097-8

Evans HC, Bezerra JL, Barreto RW. 2013. Of mushrooms and chocolate trees: aetiology and phylogeny of witches' broom and frosty pod diseases of cacao. Plant Pathology 62: 728-740. https://doi.org/10.1111/ppa.12010

Griffith GW, Hedger JN. 1994. Spatial distribution of mycelia of the liana (L-) biotype of the agaric *Crinipellis perniciosa* (Stahel) Singer in tropical forest. New Phytologist 127: 243-259. https://doi.org/10.1111/j.1469-8137.1994.tb04276.x

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549. https://doi.org/doi:10.1093/molbev/msy096

Lisboa DO, Evans HC, Araújo JPM, Elias SG, Barreto RW. 2020. *Moniliophthora perniciosa*, the mushroom causing witches' broom disease of cacao: insights into its taxonomy, ecology and host range in Brazil. Fungal Biology 124: 983-1003. https://doi.org/10.1016/j.funbio.2020.09.001

Matheny PB, Curtis JM, Hibbett DS. 2006a. Assembling the Fungal Tree of life: NCBI GenBank. NCBI, Bethesda, MD, USA. Available at: https://www.ncbi.nlm.nih.gov/genbank/ [Accessed Aug 10, 2023]

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, et al. 2006b. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98: 982-995. https://doi.org/10.1080/15572536.2006.11832627

Mournet P, Albuquerque PSB, Alves RM, Silva-Werneck JO, Rivallan R, Marcellino LH, et al. 2020. A reference high-density genetic map of *Theobroma grandiflorum* (Willd. ex Spreng) and QTL detection for resistance to witches' broom disease (*Moniliophthora perniciosa*). Tree Genetics & Genomes 16: 89. https://doi.org/10.1007/s11295-020-01479-3

Niveiro N, Ramírez NA, Michlig A, Lodge DJ, Aime MC. 2020. Studies of neotropical tree pathogens in *Moniliophthora*: a new species, *M. mayarum*, and new combinations for *Crinipellis ticoi* and *C. brasiliensis*. MycoKeys 66: 39-54. https://doi.org/10.3897/mycokeys.66.48711

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793-808. https://doi.org/10.1080/10635150490522304

Rehner SA, Samuels GJ. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625-634. https://doi.org/10.1016/S0953-7562(09)80409-7

Resende MLV, Nojasa GBA, Silva LHCP, Niella GR, Carvalho GA, Santiago DVR, et al. 2000. *Crinipellis perniciosa* proveniente de um novo hospedeiro, *Heteropterys acutifolia*, é patogênico ao cacaueiro. Fitopatologia Brasileira 25: 88-91 (in Portuguese).

Rincones J, Mazotti GD, Griffith GW, Pomela A, Figueira A, Leal Junior GA, et al. 2006. Genetic variability and chromosome-length polymorphisms of the witches' broom pathogen *Crinipellis perniciosa* from various plant hosts in South America. Mycological Research 110: 821-832. https://doi.org/10.1016/j.mycres.2006.05.002

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Santos AS, Mora-Ocampo IY, Novais DPS, Aguiar ERGR, Pirovani CP. 2023. State of the art of the molecular biology of the interaction between cocoa and witches' broom disease: a systematic review. International Journal of Molecular Sciences 24: 5684. https://doi.org/10.3390/ijms24065684

Seifert KA, Gams W. 2011. The genera of Hyphomycetes: 2011 update. Persoonia - Molecular Phylogeny and Evolution of Fungi 27: 119-129. https://doi.org/doi:10.3767/003158511X617435

Singer R. 1976. *Marasmiaceae* (Basidiomycetes, Tricholomataceae). Flora Neotropica 17: 1-347.

Teixeira PJPL, Thomazella DPT, Pereira GAG. 2015. Time for chocolate: current understanding and new perspectives on cacao witches' broom disease research. PLoS Pathogens 11: e1005130. https://doi.org/doi:10.1371/journal.ppat.1005130

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315-322. In: PCR protocols: a guide to methods and applications. Academic Press, London, UK.