RESEARCH ARTICLE

REVISED Morphological, phylogenetic, and genomic evidence reveals the causal agent of thread blight disease of cacao in Peru is a new species of *Marasmius* in the section *Neosessiles*, *Marasmius infestans* sp. nov. [version 2; peer review: 2 approved with reservations]

Angel Fernando Huamán-Pilco[1], Tito Ademir Ramos-Carrasco[1], Mario Emilio Ernesto Franco[2,3], Daniel Tineo-Flores[1,4], Richard Estrada-Cañari[5], Pedro Eduardo Romero[6], Vilma Aguilar-Rafael[1], Lourdes Adriana Ramírez-Orrego[1], Rosalina Tincopa-Marca[1], Fanny-Rosario Márquez[7], Manuel Oliva-Cruz[1], Jorge Ronny Díaz-Valderrama[1,8]

[1]Grupo de Investigación en Fitopatología y Micología, Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, National University Toribio Rodriguez de Mendoza of Amazonas, Chachapoyas, Amazonas, 01001, Peru
[2]Sustainable Plant Protection Programme, Institute of Agrifood Research and Technology (IRTA), 25198 Lieda, Spain
[3]Department of Soil, Plant and Food Sciences, Universita degli Studi di Bari Aldo Moro, Bari, Apulia, 70126, Italy
[4]Centro Experimental Yanayacu, Dirección de Supervisión y Monitoreo en las Estaciones Experimentales Agrarias, Instituto Nacional de Innovación Agraria, Jaén 06801, Calamarca, Peru
[5]Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria, Lima, Lima, Peru
[6]Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima District, Lima Region, Peru
[7]Escuela Profesional de Ingeniería Agronómica Tropical, Universidad Nacional Intercultural de Quillabamba, Quillabamba, Cusco, Peru
[8]Facultad de Ingeniería y Ciencias Agrarias, National University Toribio Rodriguez de Mendoza of Amazonas, Chachapoyas, Amazonas, 01001, Peru

v2 **First published:** 12 Oct 2023, **12**:1327
https://doi.org/10.12688/f1000research.140405.1
Latest published: 29 Jan 2024, **12**:1327
https://doi.org/10.12688/f1000research.140405.2

Abstract

The thread blight disease (TBD) of cacao (*Theobroma cacao*) in the department of Amazonas, Peru was recently reported to be caused by *Marasmius tenuissimus* (sect. Neosessiles). This same species is known to be the main causal agent of TBD in West Africa. However, some morphological characteristics, such as the presence of rhizomorphs, the almost exclusively white color, and pileus sizes less than 5 mm, among others, differ to the description of *M. tenuissimus.* Therefore, we aimed to conduct a taxonomic revision of the cacao-TBD causal

Open Peer Review

Approval Status ? ?

	1	2
version 2 (revision) 29 Jan 2024		
version 1 12 Oct 2023	? view	? view

1. **Atik Retnowati**, Herbarium Bogoriense,

agent in Peru, by using thorough micro and macro morphological, phylogenetic, and nuclear and mitochondrial genomic approaches. We showed that the causal agent of TBD of cacao in Amazonas, Peru, belongs to a new species, *Marasmius infestans* sp. nov. This study enriches our knowledge of species in the sect. Neosessiles, and strongly suggests that the *M. tenuissimus* species complex is highly diverse.

Keywords
Agaricales, cocoa, Marasmiaceae, tropical phytopathogens

Cibinong, Indonesia

2. **Nopparat Wannathes**, Pibulsongkram Rajabhat University, Phitsanulok, Thailand

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the From genes to genomes: Investigating the population species boundary in non-model Fungi collection.

Corresponding author: Jorge Ronny Díaz-Valderrama (jorge.diaz@untrm.edu.pe)

Author roles: Huamán-Pilco AF: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation; **Ramos-Carrasco TA**: Data Curation, Formal Analysis, Investigation, Validation, Visualization; **Franco MEE**: Formal Analysis, Methodology, Software, Validation, Writing – Review & Editing; **Tineo-Flores D**: Formal Analysis, Investigation, Software, Visualization, Writing – Review & Editing; **Estrada-Cañari R**: Methodology, Software; **Romero PE**: Methodology, Software, Writing – Review & Editing; **Aguilar-Rafael V**: Investigation, Methodology, Writing – Review & Editing; **Ramírez-Orrego LA**: Investigation, Methodology, Writing – Review & Editing; **Tincopa-Marca R**: Formal Analysis, Software, Writing – Review & Editing; **Márquez FR**: Investigation, Methodology, Writing – Review & Editing; **Oliva-Cruz M**: Funding Acquisition, Methodology, Project Administration, Resources, Writing – Review & Editing; **Díaz-Valderrama JR**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This study was financed by PROCIENCIA-CONCYTEC, Peru, under contract 057-2021-FONDECYT; and by project CEINCACAO (CUI N°2315081).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Huamán-Pilco AF, Ramos-Carrasco TA, Franco MEE *et al.* **Morphological, phylogenetic, and genomic evidence reveals the causal agent of thread blight disease of cacao in Peru is a new species of *Marasmius* in the section *Neosessiles*, *Marasmius infestans* sp. nov. [version 2; peer review: 2 approved with reservations]** F1000Research 2024, **12**:1327 https://doi.org/10.12688/f1000research.140405.2

First published: 12 Oct 2023, **12**:1327 https://doi.org/10.12688/f1000research.140405.1

REVISED **Amendments from Version 1**

This new version of the manuscript differs from the original version in several aspects. First, it contains a map where specimens where collected. The description of the new species is much more detailed, including examination of micro structures with Melzer's reagent, and important statistical measurements of basidiospores, such as quotients (length by width), arithmetic mean and standard deviation of dimensions, which were not reported in the previous version. Also, new phylogenetic analyses have been included with ITS, LSU, and ITS-LSU concatenated datasets. Additionally, since this study is a taxonomic study, we have included a paragraph about the current classification of the genus Marasmius in the introduction. In the discussion, we added a comparison of the main differences of the new species with phenetically similar taxa, which was missing in the original version.

Any further responses from the reviewers can be found at the end of the article

Introduction

Marasmius (Marasmiaceae, Agaricales, Agaricomycetes, Basidiomycota) is a hyperdiverse genus of fungi with 1,560 legitimate species names registered in MycoBank, as of Jan. 14th, 2024.[1] It has been traditionally classified into twelve sections, based on morphological characteristics.[2] Thus far, molecular re-examination proved that sections *Globulares*, *Marasmius*, *Sicci*, *Leveilleani* and *Neosessiles* belong to *Marasmius* sensu stricto.[3,4] Other sections from the traditional classification, such as *Androsacei*, *Alliacei*, *Epiphylli*, and *Hygrometrici*, have been accommodated outside *Marasmius* s.s., while sections *Fusicystides*, *Inaequales*, and *Scotophysini* have not been yet molecularly studied.[5–8]

The great majority of species of *Marasmius* are decomposers of leaves and twigs in natural ecosystems, but some species can be pathogenic in agricultural settings, such as the cacao (*Theobroma cacao*) agroecosystem.[9–11] Cacao can get infected by several species of *Marasmius*, including *M. crinis-equi* (sect. *Marasmius*) and some species within the section *Neosessiles*, causing thread-blight disease (TBD).[12] The sect. *Neosessiles* is a paraphyletic group mainly characterized by the pleurotoid habit of growth and the absence or rudimentarity of the stipe.[2,13] Among the TBD-causing *Neosessiles* species, *M. tenuissimus* seems to be the most frequent in West Africa,[11] and in native Awajun and Wampis communities from Northern Peru.[14] *Marasmius tenuissimus* is characterized by pilei between 7 and 22 mm in diameter, with rusty brown, light grayish fusco or greyish orange color; and basidiospore dimensions of about 9-10 × 4-6 µm.[3,9] Even though, nuclear rDNA sequence similarities over 99% point to *M. tenuissimus* reference strains,[3] the morphological characteristics of specimens from West Africa and Northern Peru do not quite match the original description.[10,11] Pileus sizes less than 5 mm, the white color, and the presence of rhizomorphs, as in the West African and Peruvian specimens, were never reported before for *M. tenuissimus,*[10,11] suggesting they may not be of that species. Moreover, the mitochondrial genome of six strains and the genome of one strain of *M. tenuissimus* from West Africa have been recently published,[12,15] which opens up the door to perform mitochondrial and nuclear genomic comparisons between the *Neosessiles* TBD agents from both continents. Therefore, in this study, we aimed to conduct a taxonomic and phylogenetic revision of the status of the cacao TBD-causal agent in Northern Peru, including nuclear and mitogenomic evidence.

Methods

Sampling and morphological analysis

Specimens were obtained during an expedition into the Imaza District (4°47′09.4″S 78°16′51.6″W) in the department of Amazonas, Peru (Figure 1), during August–September 2022 (Table 1). Collections were performed under the authorization N° AUT-IFL-2021-052 granted by the Peruvian National Forestry and Wildlife Service Agency - SERFOR. Morphological studies were conducted in the Plant Health Laboratory of the National University Toribio Rodriguez de Mendoza de Amazonas, Peru (UNTRM-A). The macroscopic characterization was made from fresh pilei. We described the color, shape, and size using a stereomicroscope SMZ18 (NIKON, Tokyo, Japan). The microscopic description was made from fresh and dried material. First, a tiny piece of dried material was carefully sectioned and placed onto a microscope slide. We applied 95% ethanol to the sample for 30 seconds, and then a drop of distilled water was added. The slides were dried out and 5% KOH was applied. Additionally, ethanol-washed sections were mounted on Melzer's reagent as in.[3] We placed a cover slip onto the sample and observed it under an OLYMPUS DP74 (Tokyo, Japan) microscope. Dimensions of microstructures were reported with the ranges between the 5th and 95th percentile, with extreme values in parentheses, as in.[16] Additionally, for basidiospores, the arithmetic mean ± standard deviation (x_m) and the quotient of the length by the width, expressed as range (Q) and arithmetic mean (Q_m), were calculated. The dimensions of basidia and basidiospores were measured with at least thirty individual structures, i.e., $n \geq 30$. Finally, pure cultures were obtained from rhizomorph tissues by the hyphal tip technique. The holotype specimen (TAIM04) was deposited in the KUELAP herbarium of UNTRM-A under voucher number KUELAP-2940.

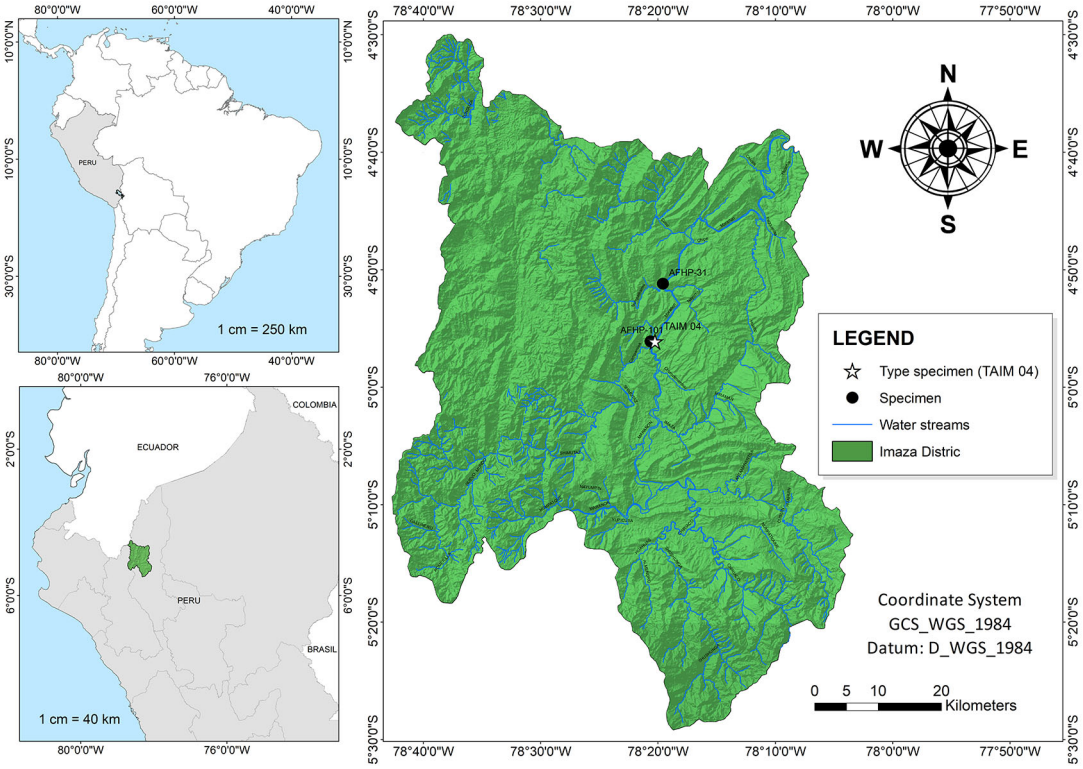

Figure 1. Collection points of specimens in Imaza district, Bagua province, Amazonas department, Peru.

Phylogenetic analysis

DNA extractions were performed from pure cultures using the Wizard® genomic DNA purification kit (Catalogue number A1120; Promega, Wisconsin, USA). DNA was quantified with the BioSpectrometer® Basic (Eppendorf, New Jersey, USA), and diluted to 0.5 ng/µl for PCR reactions. The internal transcribed spacer region (ITS1-5.8S-ITS2, or simply ITS), the large subunit (LSU) rDNA gene, and partial fragments of the genes Elongation Factor 1-α (*EF1α*) and the largest subunit of RNA polymerase II gene (*RPB1*) were amplified following.[17] The amplified PCR products were Sanger sequenced at MACROGEN (Seoul, South Korea). LSU, *EF1α,* and *RPB1* sequences from the West African TBD-causing specimen GHA37 were retrieved from the recently published genome.[15] In addition, we included the mitochondrial cytochrome oxidase I gene (*COX1*) from isolate INDES-AFHP31, which was retrieved from the mitogenome generated in this study, and from other phylogenetic-related species.[12] The introns of *COX1* sequences were removed as in.[18] Most other sequences used in phylogenetic analyses were obtained from other relevant literature.[3,4,11,13,14,19–25] We also included unpublished sequences available in NCBI from *M. tenuissimus*-phylogenetically related isolates C2/06 and C2/33 (accession numbers KM246261 and KM246277 for ITS, and KM246066 and KM246082 for LSU, respectively).

Generated sequences were edited and assembled with Sequencher v.5.4 (Gene Codes, USA). Once all sequences were gathered, they were aligned with MUSCLE[26] implemented in MEGA-X,[27] and concatenated with SeaView 4.7.[28] We used jModelTest v2[29] to identify the most appropriate nucleotide evolution models under the Akaike information criterion. Phylogenetic analysis was performed with IQ-TREE v2[30] which implements the Maximum Likelihood algorithm,[31] in the CIPRES Science Gateway v3.3 portal.[32] The phylogenetic trees were visualized and edited with FigTree.[33]

Genomic analysis

DNA extraction, sequencing, and assembly

Total DNA extraction was performed using the Wizard Purification Kit (Promega Corp., Madison, Wisconsin) following the manufacturer's instructions. For nuclear and mitogenome sequencing, we used the strain INDES-AFHP31 from Northern Peru, previously reported.[14] A paired-end sequencing library was constructed using the TruSeq Nano DNA Kit, according to the manufacturer's instructions. The library was sequenced on an Illumina NovaSeq 6000 platform in paired-end, 2 × 150 format.

Table 1. Taxa used the phylogenetic analysis of this study.

Section	Species	Specimen ID	Origin	Accession number				
				ITS	LSU	*RPB1*	*EF1α*	*COX1*
Neosessiles	***Marasmius infestans***	**TAIM 04***	**Peru**	**OR359411**	**OR364495**	**OR420729**	**OR420730**	–
	Marasmius infestans	**AFHP-31**	**Peru**	**OM720123**	**OM720135**	**KAK1231915§**	**KAK1236186§**	**OQ343345¥**
	Marasmius infestans	**AFHP-101**	**Peru**	**OR359410**	**OR364494**	**–**	**–**	–
	Marasmius neosessiliformis nom. prov.	Buyck 97.615	Madagascar	KX149007	–	–	–	–
	Marasmius tenuissimus	AKD 304/2015	India	MF189066	–	–	–	–
	Marasmius tenuissimus	NW199	Thailand	EU935569	–	–	–	–
	Marasmius tenuissimus	NW192	Thailand	EU935568	–	–	–	–
	Marasmius tenuissimus	SCAU111	China	MF061773	–	–	–	–
	Marasmius sp. 1	RAK 339	Cameroon	MN930548	–	–	–	–
	Marasmius sp. 1	GHA07	Ghana	MN794171	–	–	–	UEX92801
	Marasmius sp. 1	GHA74	Ghana	MN794173	–	–	–	UEX92859
	Marasmius sp. 1	MS2	Ghana	MN794183	MN794074	–	–	UEX92814
	Marasmius sp. 1	GHA37	Ghana	MN794147	–	KAJ8088874	KAJ8084062	UEX92895
	Marasmius sp. 2	GHA64	Ghana	MN794166	–	–	–	UEX92879
	Marasmius sp. 3	GHA63	Ghana	MN794165	MN794071	–	–	UEX92917
	Marasmius sp. 4	GHA79	Ghana	MN794177	MN794073	–	–	UEX92836
	Marasmius sp. 5	C2/33	Brazil	KM246277	KM246082	–	–	–
	Marasmius sp. 5	C2/06	Brazil	KM246261	KM246066	–	–	–
	Marasmius griseoroseus var. *diminutus*	JO390*	Brazil	JX424044	KF742003	–	–	–
	Marasmius conchiformis var. *lenipileatus*	JO287*	Brazil	JX424042	KF742001	–	–	–
	Marasmius conchiformis var. *dispar*	JO290*	Brazil	JX424039	KF742002	–	–	–
	Marasmius conchiformis	JO117*	Brazil	JX424038	KF741998	–	–	–
	Marasmius griseoroseus	JO465	Brazil	KJ173479	KJ173480	–	–	–
	Marasmius conchiformis	JO45	Brazil	KF741996	KF741997	–	–	–

Table 1. *Continued*

Section	Species	Specimen ID	Origin	Accession number				
				ITS	LSU	*RPB1*	*EF1α*	*COX1*
Sicci	*Marasmius nodulocystis*	DED 8278	Madagascar	KX953741	–	–	–	–
	Marasmius nodulocystis	DED 8269	Madagascar	KX953740	–	–	–	–
	Marasmius nodulocystis	DED 8283	Madagascar	KX953742	–	–	–	–
	Marasmius linderioides	JO286*	Brazil	JX424037	KF742000	–	–	–
	Marasmius longisetosus	JO248*	Brazil	JX424040	KF741999	–	–	–
	Marasmius siccus	LE 295985	Russia	KF774132	–	–	–	–
	Marasmius siccus	LE 295984	Russia	KF774131	–	–	–	–
	Marasmius siccus	VA 08.69	Korea	FJ904992	–	–	–	–
	Marasmius siccus	KG 028	Korea	FJ904985	FJ904980	–	–	–
Marasmius	*Marasmius* aff. *curreyi*	JES 135	Madagascar	KX149008	–	–	–	–
	Marasmius curreyi	Buyck 97.374	Madagascar	KX148980	–	–	–	–
	Marasmius ruforotula	TYS438	Malaysia	FJ431271	–	–	–	–
	Marasmius ruforotula	TYS369	Malaysia	FJ431269	–	–	–	–
	Marasmius nigrobrunneus	TYS281	Thailand	EU935575	–	–	–	–
	Marasmius nigrobrunneus	NW223	Thailand	EU935572	–	–	–	–
	Marasmius nigrobrunneus	NW162	Thailand	EU935570	–	–	–	–
	Marasmius gracilichorda	TYS411	Malaysia	FJ431244	–	–	–	–
	Marasmius gracilichorda	TYS396	Malaysia	FJ431242	–	–	–	–
	Marasmius berambutanus	TYS398	Malaysia	FJ431227	–	–	–	–
	Marasmius berambutanus	TYS337	Malaysia	FJ431225	–	–	–	–
	Marasmius graminum	NN005953	Denmark	JN943595	JN941141	–	–	–
	Marasmius graminum	FO 46723	Germany	–	AF291345	–	–	–
	Marasmius crinis-equi	GHA76	Ghana	MN794174	MN794072	–	–	UEX92959
Leveilleani	*Marasmius leveilleanus*	NW268	Thailand	EU935567	–	–	–	–
	Marasmius leveilleanus	NW248	Thailand	EU935566	–	–	–	–
	Marasmius aff. *leveilleanus*	RAK 392	Cameroon	MN930527	–	–	–	–

*Taxa marked with an asterisk are type specimens of the corresponding species.
§These accession numbers correspond to the translated coding sequences of *RPB1* and *EF1α*. The DNA sequence of *RPB1* sequence is located from position 29369 to 32406 of JANHQD010001077.1; *EF1α* sequence from positions 10248 to 12091 of JANHQD010000042.1.
¥This accession number correspond to the accession number of the mitochondrial genome. COX1 sequence is located from position 43,391 to 44,992.

The raw reads were checked by FastQC v.0.11.9. Also, quality trimming (Phred Q > 25) and remotion of adapters were conducted with Trimmomatic v0.36[34] and TrimGalore software,[35] respectively. Jellyfish v.2.[36] was used for k-mer counting, and Genome Scope v1.0.0[37] for assessing genome size, repeat content, and heterozygosity rate. This analysis involved utilizing the output of Jellyfish and the count of 17-mers for k-mer analysis. Additionally, k-depth estimation was performed to identify a predominant single-peak pattern in the frequency distribution analysis of k-mers.

De novo assembly was performed with two assembly algorithms: SOAPdenovo2 v.2.04,[38] and Masurca v.4.0.6.[39] Next, we used QUAST v.5.2.0[40] to evaluate the statistics of assemblies. The assembly validation process employed two distinct methods. First, the filtered paired-end Illumina reads were realigned to identify any errors in the assembly. This was accomplished using Bowtie2 v.2.4.2[41] and SamTools v.1.7[42] software. Second, the completeness of the assembly was evaluated using the Agaricales-specific profile of the BUSCO strategy.[43] To identify vector contamination, we employed VecScreen JCVI (https://github.com/tanghaibao/jcvi), which utilizes the Univecdatabase (https://ftp.ncbi.nlm.nih.gov/pub/UniVec/). We also performed a BLAST v.2.2.26[44] analysis, mapping the scaffolds against the nt/nr NCBI database (found at https://www.ncbi.nlm.nih.gov/). Following this, any contaminated scaffolds and vectors were eliminated before submitting the remaining data to the NCBI database. This assembly has been deposited at DDBJ/ENA/GenBank under the accession: JANHQD01.

Nuclear genome annotation

Genome annotation was performed using Funannotate v1.8.12.[45] Repetitive elements were soft-masked by TANTAN.[46] *Ab initio* gene prediction was carried out using AUGUSTUS v.3.3.3,[47] GlimmerHMM v.3.0.4,[48] and SNAP[49] trained with alignments of the BUSCO agaricales_odb10 dataset,[46] and with GeneMark-ES v.4.69[50] self-trained on the repeat-masked genome sequence. Protein evidence from the UniProt/SwissProt database[51] was mapped to the genome using DIAMOND v.2.0.15[52] and Exonerate v.2.4.0.[53] Finally, EVidenceModeler v.1.1.1[54] was used to generate consensus gene models based on all the above ab initio and evidence-based gene models. The tRNA genes were identified with tRNAscan-SE v.2.0.9.[55] Functional annotations were assigned by similarity to UniProtKB (2021_02),[51] InterPro v89.0,[56] Pfam v.34.0,[57] EggNOG v.5.0,[58] BUSCO (agaricales_odb10 dataset),[43] dbCAN v.10.0,[59] and MEROPS v.12.0.[60] Phobius[61] and SignalP v.5b[62] were used to predict transmembrane topology and signal peptides, respectively. DeepLoc 2.0[63] was used to determine protein localization. Effectors were predicted by EffectorP 3.0[64] based on the subset of extracellular proteins. AntiSMASH v.6.0[65] was used to predict secondary metabolite gene clusters (SMGCs) and secondary metabolite Clusters of Orthologous Groups (smCOGs).

Mitochondrial genome annotation

The mitochondrial genome was confirmed using the default Geneious Prime 2023.1 setup (Biomatters Ltd., Auckland, New Zealand) and GetOrganelle v1.7.6.1.[66] Genes were annotated with MITOS,[67] MFannot[68] and manually confirmed with ORFfinder available in NCBI, and tRNAscan-SE 1.21,[69] finally adjusted in Geneious. A physical map of the mitogenome was created with OGDRAW v 1.2.[70] Our results were compared to the ones reported for another published *M.* aff. *tenuissimus* mitogenomes.[12]

Results

Sampling and morphological analysis

We collected new cacao tissues infected by TBD-bearing fruiting bodies of the pathogen in Imaza province, department of Amazonas, in Northern Peru. Besides AFHP-31 from a previous study,[14] and for which no basidiocarps and only pure agar culture were preserved, two additional specimens were included in this study: TAIM-04 and AFHP-101. The presence of white rhizomorphs, and white pilei no larger than 5 mm in diameter, smooth and non-intervenose are macro morphology hallmarks of the TBD- causing specimens in Amazonas, Peru (Figure 2). In terms of micromorphological features, these specimens produce ellipsoid and smooth basidiospores with dimensions of (6.2-)7.0-8.6(-8.8) × (3.7-)3.8-5.0(-5.2) µm; they also produce *Siccus*-type cheilocystidia and pileipellis broom cells; cheilocystidia are cylindrical slightly narrower at the base, and pileipellis broom cells are ovoid to globose shape (Figure 3). The examination of tissues in Melzer' reagent revealed that most micro structures are inamyloid, except for caulocystidia which reacted in a strongly dextrinoid manner (Figure 4). All these morphological features provided the first point of evidence that the cacao TBD-causal agent in Peru is a new species.

Phylogenetic analysis

We used fifty taxa (type and other reference specimens) of *Maramius* spp. in the *Neosessiles* and other closely related sections. As expected for paraphyletic groups, the phylogenetic analysis grouped sect. *Neosessiles* taxa in two different parts of the trees built with the ITS, LSU, ITS-LSU concatenated dataset, and six-gene-multilocus concatenated dataset

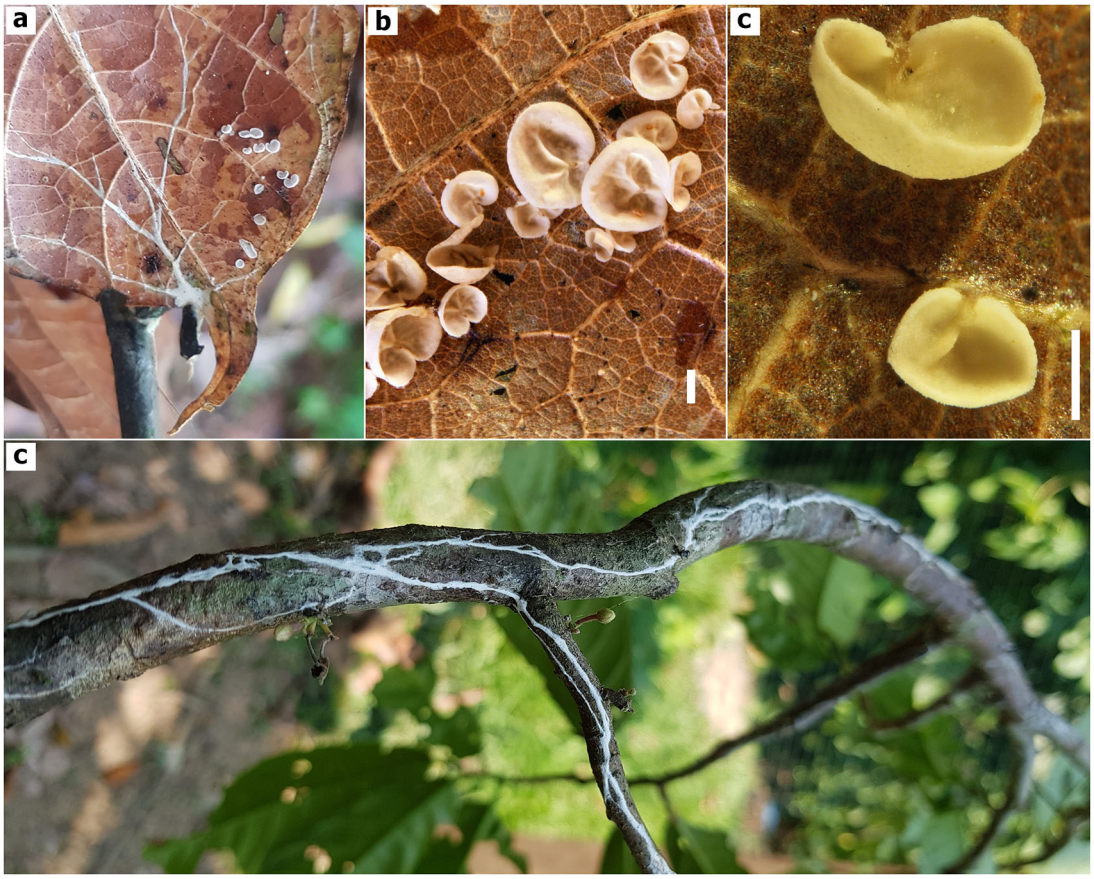

Figure 2. Macroscopic features *Marasmius infestans* sp. nov. (TAIM 04, holotype). a) Small basidiocarps growing on decomposing cacao leaves, b) Mature basidiomata, c) Young basidiomata, d) White-rhizomorphs colonizing cacao stem. Photographed by A. F, Huaman-Pilco. Scale bar in b) and c) = 1 mm.

(Figure 5).[13,24] The Peruvian specimens were grouped together in a highly supported clades in all phylogenetic analyses (85–100% bootstrap support; Figure 5). The closest related clade was composed of the unpublished *Marasmius* isolates (C2/33, C2/06), putatively soybean endophytes according to their NCBI passport information. These two clades were phylogenetically distinct to the *M. tenuissimus* sensu stricto clade conformed by the reference strains NW192, and NW199, and other reference specimens (Figure 5). Therefore, these results, combined with the unique morphological characteristics of the Peruvian specimens, support they belong to a new species within the sect. *Neosessiles*, herein after called *Marasmius infestans* sp. nov.

West African specimens were phylogenetically distinct to both, *M. tenuissimus* sensu stricto, and the Peruvian-specimen clade (Figure 5). They were grouped in at least four phylogenetic clades. The informally described *M. neosessiliformis* nom. prov. was grouped together with isolate GHA64.

Nuclear and mitochondrial genomic analyses

The nuclear genome assembly of *Marasmius infestans* AFH-31 reveals a size of 84.7 Mb, organized in 3,213 contigs (≥1,000 bp) with a GC content of 49.32% and a N50 value of 42,194 kb. We predicted 21,762 protein-coding genes and 656 tRNA genes in the nuclear genome of *M. infestans* strain AFHP31. Functional annotation resulted in the identification of 15,414 Pfam domains; 29,349 InterPro protein families; 31,346 Clusters of Orthologous Groups of proteins (COGs) and EggNog proteins; 606 proteases/protease inhibitors, and 679 carbohydrate-active enzymes (CAZymes). Moreover, we predicted 1,823 signal peptides and 4,010 transmembrane regions. About 8.9% of the proteome (2,152) are extracellular proteins, of which 25.6% (550) and 16.5% (356) were predicted to be apoplastic and cytoplasmic effectors, respectively. The genome contained 11 SMGCs, 18 biosynthetic enzymes, and 27 smCOGs. The draft nuclear genome of *M. infestans* AFHP-31 is larger in size than isolate GHA-37 genome size and, despite having fewer proteins, has more effectors (Table 2).

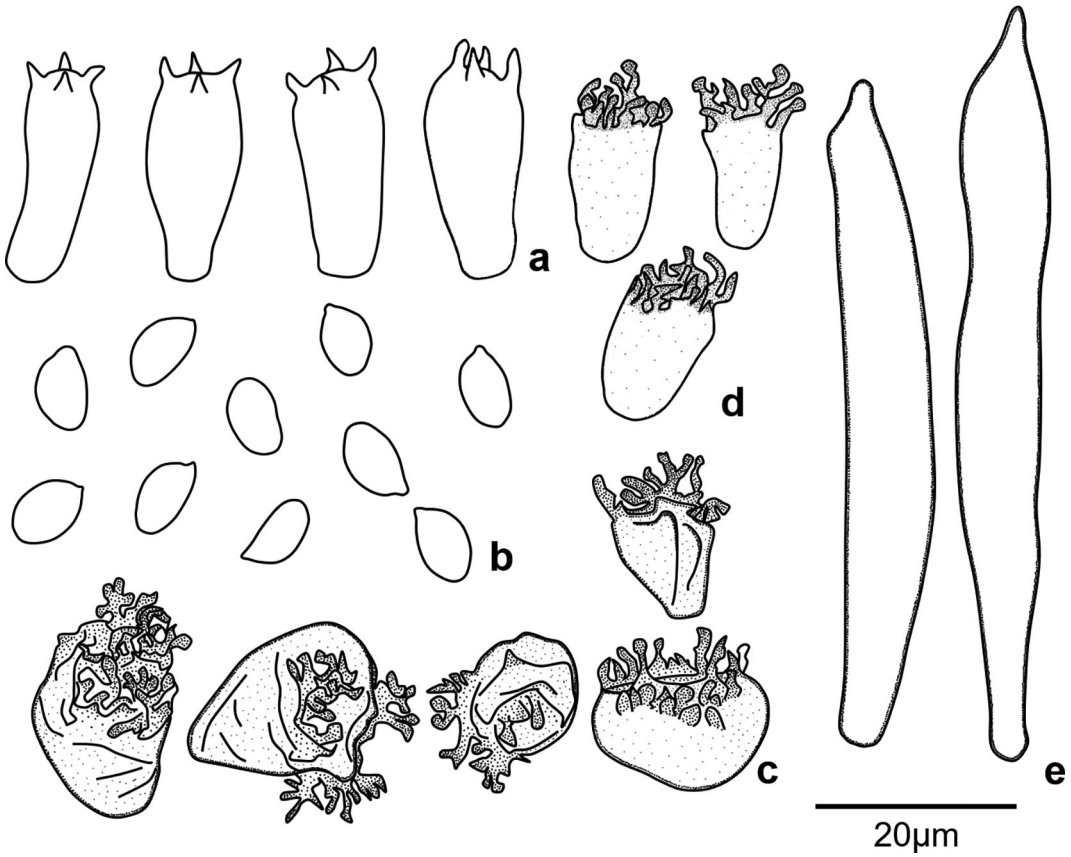

Figure 3. Microscopic features of *Marasmius infestans* sp. nov. (TAIM 04, holotype), a) Basidia, b) Basidiospores, c) *Siccus*-type broom cells of Pileipellis, d) Cheilocystidia of *Siccus*-type broom cells, e) Caulocystidia. Scale bar = 20 µm. Drawings by T. A. Ramos-Carrasco.

The mitochondrial genome of *Marasmius infestans* is circular, 47,389 bp long, and contains 42 genes. It is A + T rich (72.83%) and includes 25 tRNA (trnR, trnL and trnS occur in duplicate, while trnM in triplicate), 14 ribosomal proteins, two rRNA (rnl, rns), and one orf (orf868) (Figure 6). A comparison with the mitochondrial genome of TBD-causal agents from West Africa reveals *M. infestans* differs to them in size, and gene number, and has lost all their introns in *COX1* (Table 3).

Taxonomy:

Marasmius infestans Huamán, Ramos C. & Díaz Val., **sp. nov.** IF 901138 (Figures 1-2).

Etymology: '*infestans*', in reference to the capacity to infect branches and leaves of cacao trees.

Diagnosis: Similar to *Marasmius tenuissimus* and *M. neosessilis* but *M. infestans* has white to white-cream pilei at young and mature stages, lamella non-intervenose, and presence of ovoid to globose pileipellis broom cells. It is also phenetically similar to *M. griseoroseus* but *M. infestans* lacks *Rotalis*- and *Amyloflagellula*-type pileipellis broom cells and pileus-trama is inamyloid.

Type: PERU: Amazonas department, Bagua province, Imaza district, Pumpu native community; -4.785944, -78.281000; leg. Tito Ramos-Carrasco; Sept. 2022. Holotype specimen TAIM-04 (voucher KUELAP-2940).

Other examined specimens: AFHP-101 and dried culture of AFHP-31 (voucher KUELAP-2251).

Pileus: (0.7-)1.1-4.0(-4.9) mm diam., convex, smooth, glabrous, dull to shiny, white to white-cream (n = 84). *Context* white, thin. *Lamellae* adnate, distant (1-5) with 0 series of lamellulae, narrow, smooth, non-intervenose, white,

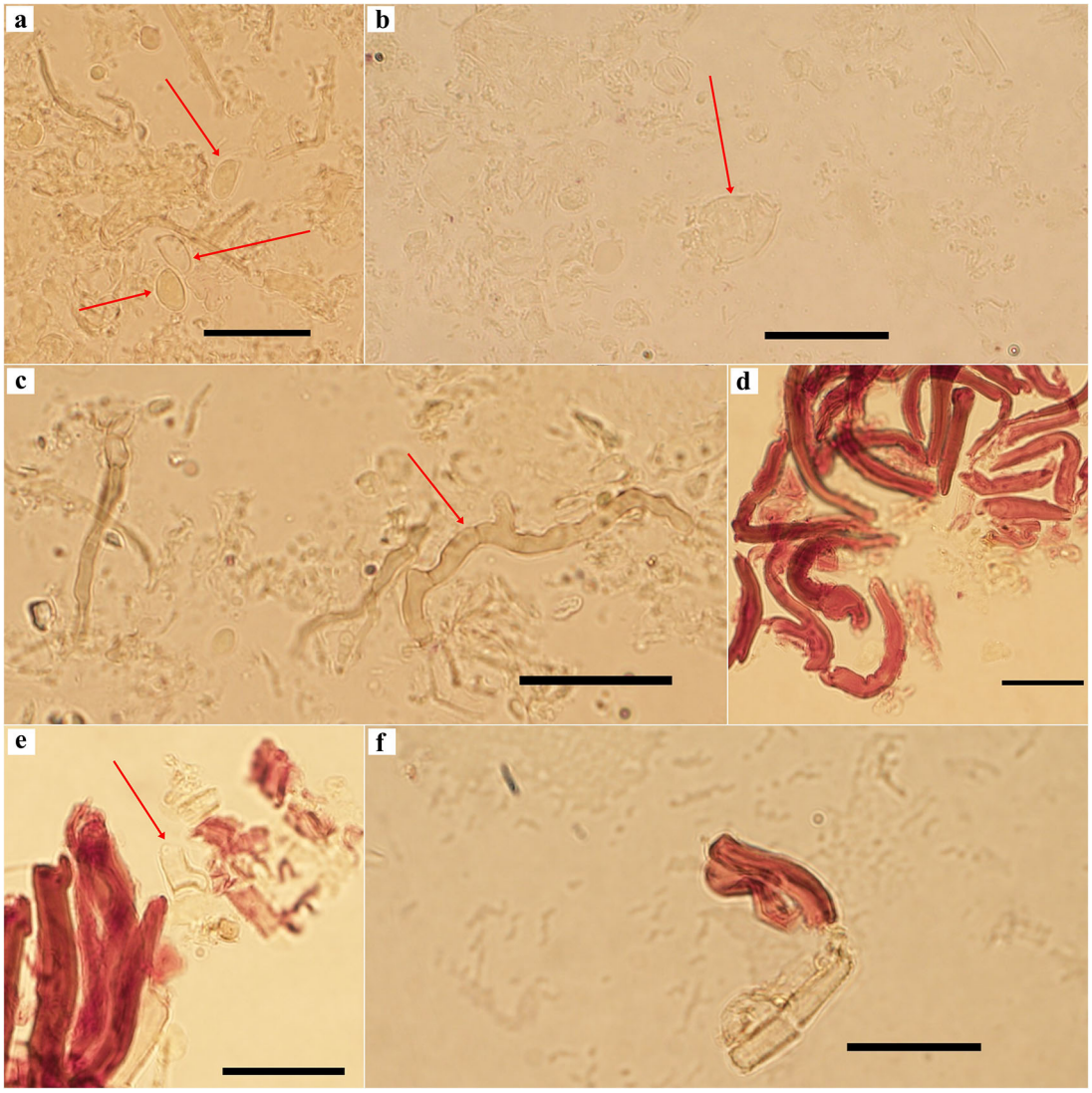

Figure 4. Melzer's reagent examination of *Marasmius infestans* sp. nov. (TAIM 04, holotype), a) Inamyloid basidiospores, b) Inamyloid pileipellis broom cell, c) Inamyloid pileus trama hyphae, d) Strongly dextrinoid caulocystidia, e-f) Inamyloid stipitipellis hyphae and caulocystidia.

non-marginate. ***Stipe:*** absent or extremely rudimentary, eccentric, cylindrical, insititious not arising from rhizomorphs; white rhizomorphs frequently present. *Odor* not distinctive.

Basidiospores: (6.2-)7.0-8.6(-8.8) × (3.7-)3.8-5.0(-5.2) µm [x_m = 7.8± 0.5 × 4.6 ± 0.4 µm, Q = 1.4-2.0, Q_m = 1.7, *n* = 45], hyaline, inamyloid, ellipsoid, smooth, thin-walled (*n* = 44). ***Basidia:*** length (17.3-)17.7-21.5(-22.2) µm, thicker part (7.0-)7.3-10.1(-10.2) µm, thinner part (2.6-)3.1-6.1(-6.3) µm, hyaline, inamyloid, cylindrical to clavate (*n* = 30). ***Cheilocystidia:*** *Siccus*-type broom cells, hyaline, inamyloid; main body 17.8-20.6 × 7.3-9.9, cylindrical, slightly narrower at the base, thin-walled; apical setulae length 2.3-3.8(-4.1) µm, obtuse, thin-walled. ***Caulocystidia:*** setoid, hyaline, strongly dextrinoid, thin- to thick-walled, main body length (41.7-)46.5-85.1(-87.5) µm, thicker part (7.4-)7.6-10.5(-10.9) µm, thinner part 3.0-5.0(-5.1) µm (*n* = 5). ***Pileipellis:*** hymeniderm, mottled, composed of *Siccus*-type broom cells; main body (9.6-)9.8-12.1(-12.2) × (8.5-)8.9-16.8(-17.9) µm, ovoid to globose, light-brown to hyaline, thin- to thick-walled (*n* = 7). ***Pileus trama:*** interwoven, inamyloid. ***Lamellar trama:*** hyphae (2.4-)2.6-3.9(-4.0) µm diam., hyaline, inamyloid, non-gelatinous. ***Stipitipellis:*** hyphae diam 3.4-4.6 µm., hyaline, inamyloid, non-gelatinous. ***Clamp connections:*** present in all tissues.

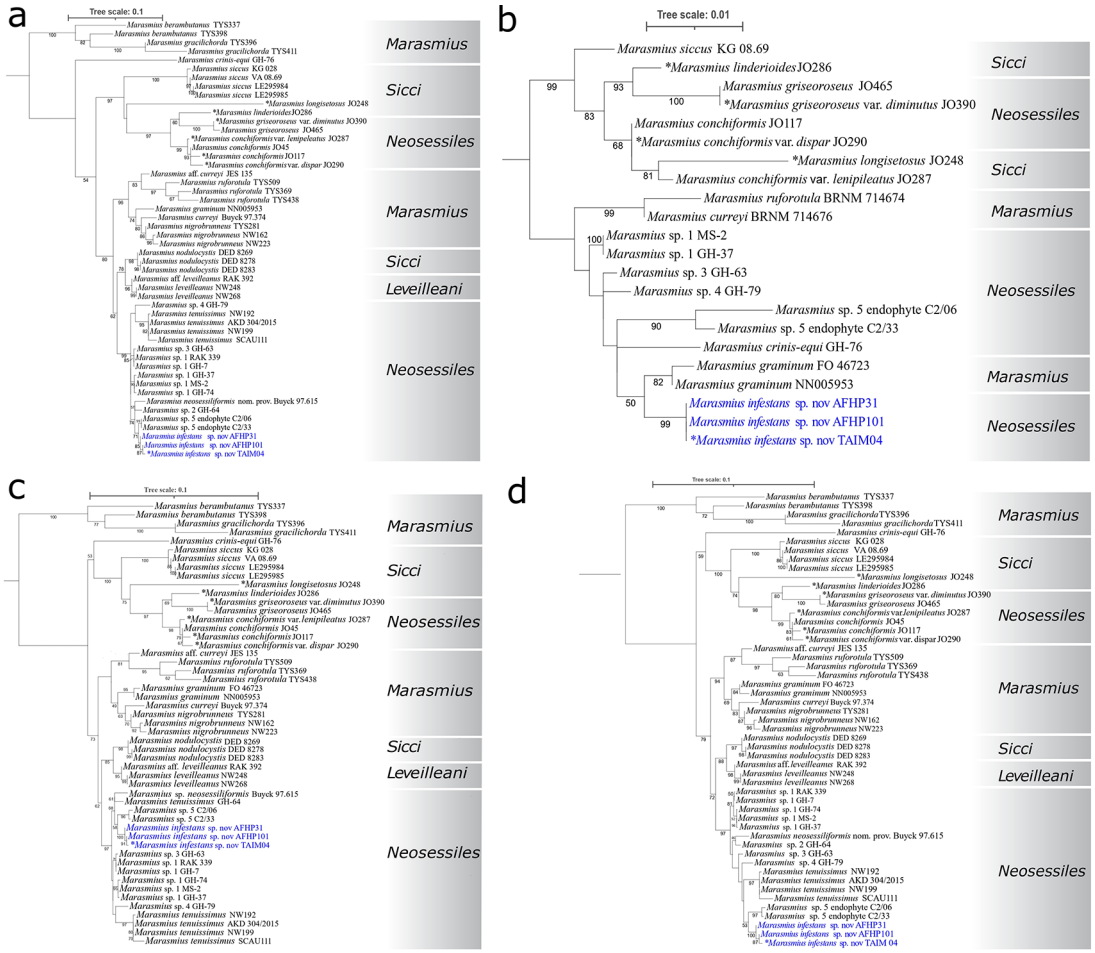

Figure 5. Phylogenetic analyses of *Marasmius* taxa in the *Neosessiles* and other closely related sections conducted in this study. a) Phylogenetic analysis with the internal transcribed spacer (ITS1-5.8S-ITS2, ITS) rDNA region using the Hasegawa-Kishino-Yano nucleotide substitution model with a discrete Gamma model for the rate of heterogeneity (HKY + G). b) Phylogenetic analysis using the large subunit (LSU) rDNA region using the transition model 3 with equal frequency allowing for proportion of invariable sites (TIM3+I). c) Phylogenetic analysis with the ITS-LSU concatenated dataset using the transversion model with unequal base frequency with invariable site plus discrete Gamma model for the rate of heterogeneity (TVM + I +G). d) Phylogenetic analysis with the multi-locus dataset composed by ITS, LSU, and partial sequences of the Translation Elongation Factor 1-α (*EF1α*), the largest subunit of RNA polymerase II gene (*RPB1*), and the mitochondrial cytochrome oxidase I (*COX1*) genes, using the transversion model with unequal base frequency, with a discrete Gamma model for the rate of heterogeneity (TVM + G). All phylogenetic trees were midpoint-rooted and built under the maximum likelihood framework. Models of evolution were determined by jModelTest2.[29] Values above branches = Maximum likelihood bootstrap values. Taxa in blue are the specimens causing thread-blight disease of cacao in Amazonas, Peru, and conform a new species clade, *Marasmius infestans* sp. nov. Type specimens are marked with an asterisk.

Table 2. Comparison of nuclear genomes with the phylogenetic close specimen from West Africa, isolate GHA37.

Genomic characters	*M. infestans* (INDES-AFHP31)	*Marasmius* sp. 1 (GHA37)[15]
Genome Size	84,695,575	71,059,514
Protein-coding genes	21,762	24,991
Number of effectors prediction	906	675
Number of genes with peptide signal	1,823	1,900

Data for isolate GHA37 obtained from.[15]

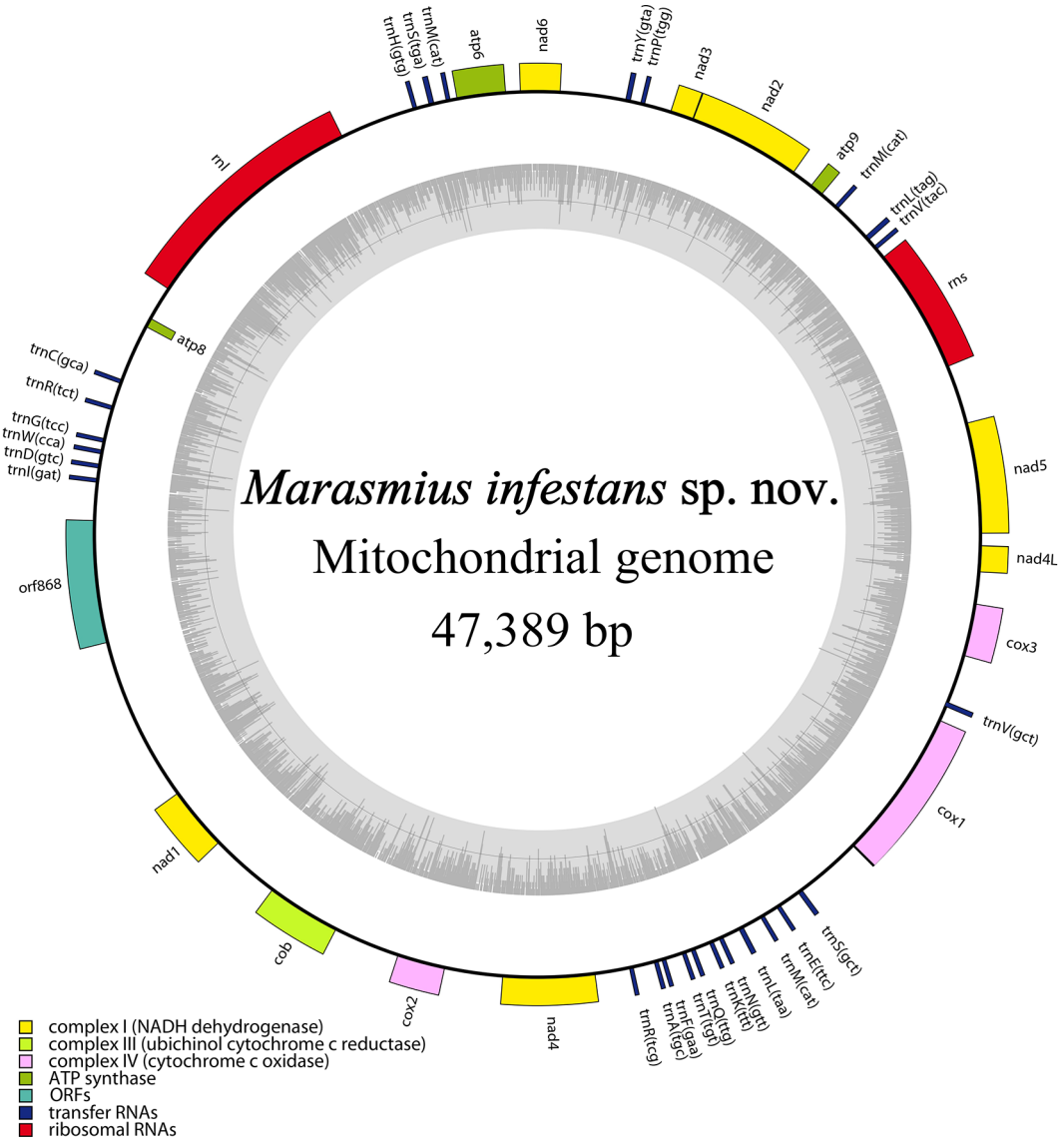

Figure 6. Circular map of *Marasmius infestans* sp. nov. mitochondrial genome. The genes inside and outside the circle are transcribed in clockwise and counterclockwise directions, respectively. Genes belonging to different functional groups are shown in different colors. The dark gray area in the inner circle denotes GC content.

Table 3. Comparison our *M. infestans* with mitochondrial genomes available in GenBank.[12]

Species	Isolate/ Specimen	Length (pb)	% GC	No of tRNAs	No. Protein-coding genes	No. of introns in COX1
Marasmius infestans	AFHP31	47,389	27.17	25	42	0
Marasmius sp. 1	GHA07	44,621	27.04	25	17	1
Marasmius sp. 1	GHA74	44,859	27.01	25	17	1
Marasmius sp. 1	MS2	44,524	27.02	25	18	1
Marasmius sp. 1	GHA37	44,399	26.97	25	18	1
Marasmius sp. 2	GHA64	43,121	26.66	25	19	1
Marasmius sp. 3	GHA63	51,210	27.21	25	21	3
Marasmius sp. 4	GHA79	48,952	26.82	26	21	3

Data for *Marasmius* sp. 1–4 taken from.[12]

Habit and habitat: Rhizomorphic colonizing leaf and twigs of cacao, which subsequently causes thread blight disease. Rhizoids are white; fruiting bodies develop on dead tissue. The fungus occurs on poorly managed cacao farms with excessive shade and high relative humidity, typical characteristics of the tropical rainforest.

Geographic distribution Imaza province, Amazonas department, Peru.

Notes: *Marasmius infestans* differs from *M. tenuissimus* in the color and size of the pilei, and lamellae appearance. *Marasmius infestans* pilei are white to white-cream, while *M. tenuissimus* is between light grayish fuscous to rusty brown when fresh,[9] or greyish or pale orange to golden brown, light brown or orangish white.[3,4,9] Pilei in *M. infestans* can reach up to 5 mm broad, while pilei in *M. tenuissimus* can have diameters typically of 7–40 mm.[3,4] Lamellae in *M. infestans* lacks reticulation while in *M. tenuissimus* lamellae is heavily reticulate. Additionally, West African species within the *M. tenuissimus* species complex can produce pure-white to brown-colored rhizomorphs,[11] while *M. infestans* produces pure-white rhizomorphs. At the genomic level, *M. infestans* is 13,636,061 pb larger than *M.* aff. *tenuissimus* GHA37. *Marasmius infestans* also has 3,229 fewer genes, and 231 more effectors (Table 2). Moreover, *M. infestans* differs from *M. neosessilis* and its varieties (*M. neosessilis* var. *neosessilis* and *M. neosessilis* var. *montepiensis*) in that these taxa can have various pilei colors such as apricot, salmon-flesh, and gray.[9,71] Also, *M. neosessilis* and the other phenetically similar species *M. griseoroseus* have pseudoamyloid (dextrinoid) pileus-trama, while pileus-trama of *M. infestans* is inamyloid.

Discussion

The causal agent of cacao TBD had been previously analyzed based on nuclear rDNA comparison, macro morphology of the fruiting body and rhizomorphic structures, and mycelial culture.[14] However, other important characteristics, such as the micro morphological features of lamella and pileus, were not considered in reports from both, West Africa and Peru.[11,14] In this study, we present morphological, phylogenetic, and genomic evidence that the species of *Marasmius* causing TBD in the Amazonian areas of Northern Peru is a new species in the sect. *Neosessiles*, namely *M. infestans.*

In this study we revealed morphological differences with *M. tenuissimus* s.s. in color and size of pilei, lamellae appearance, and growth habit. *Marasmius infestans* differs from *M. tenuissimus* mainly because of their smaller basidiocarps. With respect to color, *M. infestans* can be easily distinguished by its white to white-cream pilei. Also, *M. tenuissimus* has never been reported to colonize the leaves and stems of plants with abundant white rhizomorphs as *M. infestans.*[9,72] Additionally, other relates species with pleurotoid-habit of growth such as *M. neosessilis, M. griseoroseus, M. conchiformis*, and *M. jasingensis* also differ in the color of pilei, which can go from gray and pale orange to brown and reddish-brown at maturity.[9,13,73] The most macro morphological similar species to *M. infestans* is *M. griseoroseus:* pilei are most frequently no more than 5 mm broad, and lamellae is adnate and distant, just as *M. infestans. Marasmius griseoroseus* can also have white to white-cream pilei.[9,13] However, pilei in *M. griseoroseus* can be pale orange as well. Also, several micro morphological characteristics, such as the presence of both *Rotalis*- and *Amyloflagellula*-type pileipellis broom cells, and some degree of dextrinoidity in pileus trama are marked differences with *M. infestans.*[9,13] Moreover, the informally described species *M. neosessiliformis* nom. prov. is one of the few very closely related taxa for which there is ITS sequence data available in GenBank.[19] The provisional description of this species pointed to several shared characteristics with *M. infestans*, such as the size and the non-intervenose nature of pilei, and the presence of *Siccus*-type broom cells in the pileipellis. However, it differs in that *M. neosessiliformis* nom. prov. has much larger basidiospores (10–11 × 5–6 µm) than *M. infestans*.[19]

In West Africa, four different species were reported to cause TBD of cacao: *M. crinis-equi*, *M.* aff. *tenuissimus*, *M. scandens,* and *Paramarasmius palmivorus* (reported as *Marasmius palmivorus*).[11] Besides the molecular differences, these TBD causal agents presented five rhizomorph morphotypes.[11] The morphotype A of rhizomorphs is characterized by abundant thin, black, "horsehair"-type rhizomorphs, and was only found on *M. crinis-equi.* The morphotype B is characterized by its brown coloration; and the type C, by its intense white color. Both morphotypes B and C, were found on *M.* aff. *tennuisimus.* The morphotype D is characterized by a faint cream or dull white rhizomorph, with the presence of smooth or cream-pruinose pilei, with a diameter up to 8 mm; and the morphotype E is characterized by its aggregation of shiny or silky white hyphae and white or pale yellow basidiocarps, with smooth and convex pilei, with diameter from 10 to 50 mm, observed on *P. palmivorus.*[11] If we followed this classification in our study, we find *M. infestans* has rhizomorphs of morphotype C. Unfortunately, in the West African study, no fruiting bodies from this type of rhizomorphs were reported, so we cannot make a macro morphological comparison.

In the *Marasmius* genus, ITS has been the main locus for phylogenetic studies of species in sect. *Neosessiles.*[3] In this study, we found *M. infestans* is closely related species to *M. tennuisimus* s. s., both species forming individual clades with high bootstrap support (>85%) in all phylogenetic analyses conducted. We also found that the *M. tenuissimus* is a species

complex that has at least five other species that will need proper description, including the previously reported yet not formally described *M. neosessiliformis* nom. prov.,[19] provided the discovery of corresponding basidiocarps. One of these species (*Marasmius* sp. 5) corresponds to the endophytic strains C2/33 and C2/06 from soybean in Brazil forming a distinct phylogenetic clade, with 97% bootstrap support and closely related to *M. infestans* and *M. tennuisimus.* Additionally, TBD-causal strains from West Africa form separate and well-supported clades. Isolate GHA64 groups together with the informally described species *M. neosessiliformis* nom. prov. specimen Buyck 97.615, suggesting it may be another member of this species requiring formal description.

On top of morphological and phylogenetic evidence that *M. infestans* distinguishes from other species within the sect. *Neosessiles*, in this study we present its nuclear and mitochondrial genome sequences. The nuclear genome is about 13 Mb longer than *M.* aff. *tennuisimus* GHA37. *Marasmius infestans* also has 231 more effectors than *M.* aff. *tennuisimus* GHA37. Pathogenic effectors are secreted by pathogenic fungi during infection and play an important role in silencing plant defenses response,[64,74,75] which may help *M. infestans* during cacao infection. Moreover, mitochondrial genomes are known for different sizes and rearrangements despite their similar gene function.[12,76] *Marasmius infestans* mitogenome has 47,389 bp and has undergone the loss of introns in the COX1 gene, as opposed to *M.* cf. *tenuissimus* causing TBD in Africa (Table 3). The gain and loss of introns are common in fungal mitogenomes and are related to their evolution .[76] Moreover, we found that *M.* cf. *tennuisimus* specimens GHA74, GHA37, MS2, and GHA07, which group together in the phylogenetic analyses with the six-gene-multilocus dataset, have very similar mitochondrial genome sizes, ranging from 44,399 to 44,859 bp, supporting they all belong to another species (*Marasmius* sp. 1; Figure 5). Additionally, specimens GHA63, and GHA79, which also formed individual specific lineages, have different mitochondrial genome sizes (51,210 and 48,952 bp, respectively).[11] Therefore, they also represent other *Neosessiles* species in need of formal description (*Marasmius* sp. 3 and *Marasmius* sp. 4, respectively).

Data availability

Underlying data

BioProject: The Genome Shotgun project of specimen AFHP-31. Accession number PRJNA860982; https://identifiers.org/NCBI/bioproject:PRJNA860982

The NCBI accession number of the version of the assembled genome of specimen AFHP-31 described in this paper is JANHQD010000000.

Nucleotide: The mitochondrial genome assembly. Accession number OQ343345; https://www.ncbi.nlm.nih.gov/nuccore/OQ343345.1/

SRA: The raw reads for the nuclear and mitochondrial genome under accession number SRR20354643. https://identifiers.org/insdc.sra:SRR20354643

Sequences obtained through the Sanger method used are deposited at NCBI through accession numbers OR359411, OR364495, OR420729 and OR420730 for specimen TAIM-04; OM720123 and OM720135 for specimen AFHP-31; and OR359410 and OR364494 for specimen AFHP-101.

References

1. Crous PW, Gams W, Stalpers JA, *et al.*: **MycoBank: an online initiative to launch mycology into the 21st century.** *Stud. Mycol.* 2004; **50**(1): 19–22.
2. Singer R: *The Agaricales in Modern Taxonomy.* 4th ed. Koenigstein: Koeltz Scientific Books; 1986; 981.
3. Wannathes N, Desjardin DE, Hyde KD, *et al.*: **A monograph of *Marasmius* (Basidiomycota) from Northern Thailand based on morphological and molecular (ITS sequences) data.** *Fungal Divers.* 2009; **37**: 209–306.
4. Tan YS, Desjardin DE, Perry BA, *et al.*: ***Marasmius* sensu stricto in Peninsular Malaysia.** *Fungal Divers.* 2009; **37**: 9–100.
5. Moncalvo J-M, Vilgalys R, Redhead SA, *et al.*: **One hundred and seventeen clades of euagarics.** *Molec. Phylogen Evol.* 2002; **23**: 357–400.
PubMed Abstract | Publisher Full Text
6. Wilson AW, Desjardin DE: **Phylogenetic relationships in the gymnopoid and marasmioid fungi (Basidiomycetes, euagaric clade).** *Mycologia.* 2005; **97**: 667–679.
Publisher Full Text
7. Jenkinson TS, Perry BA, Schaefer RE, *et al.*: **Cryptomarasmius gen. nov. established in the Physalacriaceae to accommodate members of Marasmius sect. Hygrometrici.** *Mycologia.* 2014; **106**: 86–94.
PubMed Abstract | Publisher Full Text
8. de Oliveira JJ, Moncalvo JM, Margaritescu S, *et al.*: **Phylogenetic and morphological analyses of species of Marasmius sect. Marasmius from the Atlantic Rainforest, Brazil.** *Plant Syst. Evol.* 2020; **306**: 1–46.
Publisher Full Text
9. Singer R: **Marasmieae (Basidiomycetes-Tricholomataceae).** *Flora Neotropica.* New York: Published for Organization for Flora Neotropica by the New York Botanical Garden; 1976; Vol. **17**: 1–347 p.
10. Desjardin DE, Ovrebo CL: **New species and new records of *Marasmius* from Panamá.** *Fungal Divers.* 2006; **21**: 19–39.

11. Amoako-Attah I, Shahin AS, Aime MC, *et al.*: **Identification and characterization of fungi causing thread blight diseases on cacao in Ghana.** *Plant Dis.* 2020 Nov 1; **104**(11): 3033–3042.
PubMed Abstract | Publisher Full Text

12. Ali SS, Amoako-Attah I, Shao J, *et al.*: **Mitochondrial Genomics of Six Cacao Pathogens From the Basidiomycete Family Marasmiaceae.** *Front. Microbiol.* 2021 Oct 28; **12**.
Publisher Full Text

13. de Oliveira JJS, Sanchez-Ramirez S, Capelari M: **Some new species and new varieties of *Marasmius* (Marasmiaceae, Basidiomycota) from Atlantic Rainforest areas of São Paulo State, Brazil.** *Mycol. Prog.* 2014; **13**(3): 923–949.
Publisher Full Text

14. Huaman-Pilco AF, Torres-De la Cruz M, Aime MC, *et al.*: *First Report of Thread Blight Caused by* Marasmius tenuissimus *on Cacao (*Theobroma cacao*) in Peru.* Plant Disease. American Phytopathological Society; 2023; vol. **107**: 219.
Publisher Full Text

15. Leung J, Cohen SP, Baruah IK, *et al.*: **A draft genome resource for *Marasmius tenuissimus*, an emerging causal agent of thread blight disease in cacao.** *PhytoFrontiers.* 2023.
Publisher Full Text

16. Dramani R, Hegbe ADMT, Tabe A, *et al.*: **How are basidiospore size measurements affected by drying?** *Curr. Res. Environ. Appl. Mycol.* 2020 Feb 12; **10**(1): 63–70.
Publisher Full Text

17. Aime MC, Phillips-Mora W: **The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae.** *Mycologia.* 2005; **97**(5): 1012–1022.
PubMed Abstract

18. Dentinger BTM, Didukh MY, Moncalvo JM: **Comparing COI and ITS as DNA barcode markers for mushrooms and allies (Agaricomycotina).** *PLoS One.* 2011 Sep 22; **6**(9): e25081.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Antonín V, Buyck B: **Marasmius (Basidiomycota, Marasmiaceae) in Madagascar and the Mascarenes.** *Fungal Divers.* 2006; **23**: 17–50.

20. Douanla-Meli C, Langer E: **Phylogenetic relationship of *Marasmius mbalmayoensis* sp. nov. to the tropical African *Marasmius bekolacongoli* complex based on nuc-LSU rDNA sequences.** *Mycologia.* 2008; **100**(3): 445–454.
PubMed Abstract | Publisher Full Text

21. Antonín V, Ryoo R, Shin HD: **Marasmioid and gymnopoid fungi of the Republic of Korea. 4. *Marasmius* sect.** *Sicci. Mycol Prog.* 2012 Aug; **11**(3): 615–638.
Publisher Full Text

22. Schoch CL, Seifert KA, Huhndorf S, *et al.*: **Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi.** *Proc. Natl. Acad. Sci. U. S. A.* 2012 Apr 17; **109**(16): 6241–6246.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Kiyashko AA, Malysheva EF, Antonín V, *et al.*: **Fungi of the Russian Far East 2. New species and new records of *Marasmius* and *Cryptomarasmius* (Basidiomycota).** *Phytotaxa.* 2014; **186**(1): 1–28.
Publisher Full Text

24. Shay JE, Desjardin DE, Perry BA, *et al.*: **Biodiversity and phylogeny of *Marasmius* (Agaricales, Basidiomycota) from Madagascar.** *Phytotaxa.* 2017 Jan 25; **292**(2): 101–149.
Publisher Full Text

25. Koch RA, Liu J, Brann M, *et al.*: **Marasmioid rhizomorphs in bird nests: Species diversity, functional specificity, and new species from the tropics.** *Mycologia.* 2020; **112**(6): 1086–1103.
PubMed Abstract | Publisher Full Text

26. Edgar RC: **MUSCLE: Multiple sequence alignment with high accuracy and high throughput.** *Nucleic Acids Res.* 2004; **32**(5): 1792–1797.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Kumar S, Stecher G, Li M, *et al.*: **MEGA X: Molecular evolutionary genetics analysis across computing platforms.** *Mol. Biol. Evol.* 2018 Jun 1; **35**(6): 1547–1549.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Gouy M, Guindon S, Gascuel O: **Sea view version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building.** *Mol. Biol. Evol.* 2010 Feb; **27**(2): 221–224.
PubMed Abstract | Publisher Full Text

29. Darriba D, Taboada GL, Doallo R, *et al.*: **JModelTest 2: More models, new heuristics and parallel computing.** *Nat. Methods.* 2012; **9**: 772.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Minh BQ, Schmidt HA, Chernomor O, *et al.*: **IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era.** *Mol. Biol. Evol.* 2020 May 1; **37**(5): 1530–1534.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Stamatakis A: **RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies.** *Bioinformatics.* 2014 May 1; **30**(9): 1312–1313.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Miller MA, Pfeiffer W, Schwartz T: **The CIPRES Science Gateway: A Community Resource for Phylogenetic Analyses. In: Proceedings of the 2011 TeraGrid Conference: extreme digital discovery.** 2010; p. 1–8.

33. Rambaut A: **Institute of Evolutionary Biology, Univ. of Edinburgh. FigTree.** 2006 [cited 2022 Nov 9].
Reference Source

34. Bolger AM, Lohse M, Usadel B: **Trimmomatic: A flexible trimmer for Illumina sequence data.** *Bioinformatics.* 2014 Aug 1; **30**(15): 2114–2120.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Krueger F: **Babraham Bioinformatics. Trim Galore.** [cited 2023 Jul 6].
Reference Source

36. Marçais G, Kingsford C: **A fast, lock-free approach for efficient parallel counting of occurrences of k-mers.** *Bioinformatics.* 2011 Mar; **27**(6): 764–770.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Vurture GW, Sedlazeck FJ, Nattestad M, *et al.*: **GenomeScope: Fast reference-free genome profiling from short reads.** *Bioinformatics.* Oxford University Press; 2017; **33**: p. 2202–4.
Publisher Full Text

38. Luo R, Liu B, Xie Y, *et al.*: **SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler.** *GigaSciencie.* 2012; **1**(1): 2047–217X.
Publisher Full Text | Reference Source

39. Zimin AV, Marçais G, Puiu D, *et al.*: **The MaSuRCA genome assembler.** *Bioinformatics.* 2013 Nov 1; **29**(21): 2669–2677.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Gurevich A, Saveliev V, Vyahhi N, *et al.*: **QUAST: Quality assessment tool for genome assemblies.** *Bioinformatics.* 2013 Apr 15; **29**(8): 1072–1075.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2.** *Nat. Methods.* 2012 Apr; **9**(4): 357–359.
PubMed Abstract | Publisher Full Text | Free Full Text

42. Li H, Handsaker B, Wysoker A, *et al.*: **The Sequence Alignment/Map format and SAMtools.** *Bioinformatics.* 2009 Aug; **25**(16): 2078–2079.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Simão FA, Waterhouse RM, Ioannidis P, *et al.*: **BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs.** *Bioinformatics.* 2015 Oct 1; **31**(19): 3210–3212.
Publisher Full Text

44. Altschup SF, Gish W, Miller W, *et al.*: **Basic Local Alignment Search Tool.** *J. Mol. Biol.* 1990; **215**: 403–410.
Publisher Full Text

45. Jon P, Jason S: **Zenodo.** 2019 [cited 2022 Jul 9]. nextgenusfs/funannotate: funannotate v1.5.3 (1.5.3).
Publisher Full Text

46. Frith MC: **A new repeat-masking method enables specific detection of homologous sequences.** *Nucleic Acids Res.* 2011 Mar; **39**(4): e23.
Publisher Full Text

47. Stanke M, Keller O, Gunduz I, *et al.*: **AUGUSTUS: A b initio prediction of alternative transcripts.** *Nucleic Acids Res.* 2006 Jul; **34**(WEB. SERV. ISS): W435–W439.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Majoros WH, Pertea M, Salzberg SL: **TigrScan and GlimmerHMM: Two open source ab initio eukaryotic gene-finders.** *Bioinformatics.* 2004 Nov 1; **20**(16): 2878–2879.
PubMed Abstract | Publisher Full Text

49. Korf I: **Gene finding in novel genomes.** 2004.
Reference Source

50. Ter-Hovhannisyan V, Lomsadze A, Chernoff YO, *et al.*: **Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training.** *Genome Res.* 2008 Dec; **18**(12): 1979–1990.
PubMed Abstract | Publisher Full Text | Free Full Text

51. Boutet E, Lieberherr D, Tognolli M, *et al.*: **UniProtKB/Swiss-Prot, the Manually Annotated Section of the UniProt KnowledgeBase: How to Use the Entry View.** Edwards D, editor. *Plant Bioinformatics. Springer Protocols.* New York, NY: Humana Press; 2016; p. 23–54.

52. Buchfink B, Xie C, Huson DH: **Fast and sensitive protein alignment using DIAMOND.** *Nat. Methods.* 2014; **12**: 59–60.

Nature Publishing Group.
Publisher Full Text

53. Slater GSC, Birney E: **Automated generation of heuristics for biological sequence comparison.** *BMC Bioinformatics.* 2005 Feb 15; **6**: 31.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Haas BJ, Salzberg SL, Zhu W, *et al.*: **Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments.** *Genome Biol.* 2008 Jan 11; **9**(1): R7.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Chan PP, Lin BY, Mak AJ, *et al.*: **TRNAscan-SE 2.0: Improved detection and functional classification of transfer RNA genes.** *Nucleic Acids Res.* 2021 Sep 20; **49**(16): 9077–9096.
PubMed Abstract | Publisher Full Text | Free Full Text

56. Jones P, Binns D, Chang HY, *et al.*: **InterProScan 5: Genome-scale protein function classification.** *Bioinformatics.* 2014 May 1; **30**(9): 1236–1240.
PubMed Abstract | Publisher Full Text | Free Full Text

57. Mistry J, Chuguransky S, Williams L, *et al.*: **Pfam: The protein families database in 2021.** *Nucleic Acids Res.* 2021 Jan 8; **49**(1): D412–D419.
Publisher Full Text

58. Huerta-Cepas J, Szklarczyk D, Heller D, *et al.*: **EggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses.** *Nucleic Acids Res.* 2019 Jan 8; **47**(D1): D309–D314.
Publisher Full Text

59. Yin Y, Mao X, Yang J, *et al.*: **DbCAN: A web resource for automated carbohydrate-active enzyme annotation.** *Nucleic Acids Res.* 2012 Jul; **40**(W1): W445–W451.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Rawlings ND, Barrett AJ, Bateman A: **MEROPS: The peptidase database.** *Nucleic Acids Res.* 2009 Nov 5; **38**(SUPPL.1): D227–D233.
Publisher Full Text

61. Käll L, Krogh A, Sonnhammer ELL: **A combined transmembrane topology and signal peptide prediction method.** *J. Mol. Biol.* 2004 May 14; **338**(5): 1027–1036.
PubMed Abstract | Publisher Full Text

62. Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, *et al.*: **SignalP 5.0 improves signal peptide predictions using deep neural networks.** *Nat. Biotechnol.* 2019 Apr 1; **37**(4): 420–423.
PubMed Abstract | Publisher Full Text

63. Thumuluri V, Almagro Armenteros JJ, Johansen AR, *et al.*: **DeepLoc 2.0: multi-label subcellular localization prediction using protein language models.** *Nucleic Acids Res.* 2022 Jul 5; **50**(1): 228–234.

64. Sperschneider J, Dodds PN: **EffectorP 3.0: Prediction of Apoplastic and Cytoplasmic Effectors in Fungi and Oomycetes.** *Mol. Plant-Microbe Interact.* 2022 Feb 1; **35**(2): 146–156.
PubMed Abstract | Publisher Full Text

65. Blin K, Shaw S, Kloosterman AM, *et al.*: **AntiSMASH 6.0: Improving cluster detection and comparison capabilities.** *Nucleic Acids Res.* 2021 Jul 2; **49**(1): W29–W35.
Publisher Full Text

66. Jin JJ, Bin YW, Yang JB, *et al.*: **GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes.** *Genome Biol.* 2020 Sep 10; **21**(1).
Publisher Full Text

67. Bernt M, Donath A, Jühling F, *et al.*: **MITOS: Improved de novo metazoan mitochondrial genome annotation.** *Mol. Phylogenet. Evol.* 2013 Nov; **69**(2): 313–319.
PubMed Abstract | Publisher Full Text

68. Lang BF, Beck N, Prince S, *et al.*: **Mitochondrial genome annotation with MFannot: a critical analysis of gene identification and gene model prediction.** *Front. Plant Sci.* 2023 Jul 4; **14**: 1222186.
PubMed Abstract | Publisher Full Text | Free Full Text

69. Schattner P, Brooks AN, Lowe TM: **The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs.** *Nucleic Acids Res.* 2005 Jul; **33**(SUPPL. 2): W686–W689.
PubMed Abstract | Publisher Full Text | Free Full Text

70. Lohse M, Drechsel O, Bock R: **OrganellarGenomeDRAW (OGDRAW): A tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes.** *Curr. Genet.* 2007 Nov; **52**(5–6): 267–274.
PubMed Abstract | Publisher Full Text

71. Singer R: **New Genera of Fungi. VIII. Notes concerning the sections of the genus Marasmius Fr.** *Mycologia.* 1958; **50**(1): 103–110.

72. Tan YS, Desjardin D, Perry B, *et al.*: ***Marasmius* sensu stricto in Peninsular Malaysia.** *Fungal Divers.* 2009; **37**(January): 9–100.

73. Retnowati A, Seelan JSS: **A new species of Marasmius Sect. Neosessiles (Basidiomycota, Agaricales) from the artificial Dipterocarpaceae forest in Indonesia.** *Taiwania.* 2022; **67**(1): 30–34.

74. De Wit PJGM: **Apoplastic fungal effectors in historic perspective; a personal view.** *New Phytol.* 2016 Dec 1; **212**(4): 805–813.
PubMed Abstract | Publisher Full Text

75. Li Q, Wu P, Li L, *et al.*: **The first eleven mitochondrial genomes from the ectomycorrhizal fungal genus (*Boletus*) reveal intron loss and gene rearrangement.** *Int. J. Biol. Macromol.* 2021 Mar 1; **172**: 560–572.
PubMed Abstract | Publisher Full Text

76. Zubaer A, Wai A, Hausner G: **The mitochondrial genome of *Endoconidiophora resinifera* is intron rich.** *Sci. Rep.* 2018 Dec 1; **8**(1): 17591.
PubMed Abstract | Publisher Full Text | Free Full Text

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 31 October 2023

https://doi.org/10.5256/f1000research.153750.r214959

?

Nopparat Wannathes
[1] Microbiology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, Phitsanulok, Thailand
[2] Microbiology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, Phitsanulok, Thailand

The manuscript entitled "Morphological, phylogenetic, and genomic evidence reveals the causal agent of thread blight disease of cacao in Peru is a new species of *Marasmius* in the section Neosessiles, *Marasmius infestans* sp. nov." described a new species of *Marasmius* from Peru. This manuscript is interesting, with new data and nice photographs and drawings. However, some points need to be considered.

This research reported a new species of *Marasmius*, which is a taxonomic report with some molecular evidence. So a clear morphological description of a new species is needed. The key characteristics of *Marasmius* species have to be informed, such as Melzer's reagent reactions of spores, and the trama tissues. A comparison of a new species with phenetically similar taxa is needed, especially for the species reported from the related geographical area --the Amazon rainforest, not scope to the *Marasmius* species that caused the thread blight disease (TBD) only. A comprehensive dataset for phylogenetics analyses is required, ITS sequences of related species from the Amazon rainforest (Oliveira *et al* 2014--the sixth reference of this manuscript) have to be included. The phylogenetic analyses of a single gene of ITS and LSU and a combined gene of ITS and LSU are required at least as supplemented data. Due to the dataset used in this study being mainly based on both genes, Only a new species and a single specimen of undescribed *Marasmius* sp. have other genes.

There are some suggestions and comments, please look carefully at the following:

1. Introduction: the current classifications of *Marasmius* should be informed. Since, this research aimed to conduct a taxonomic and phylogenetic revision of the fungi in this genus.

2. Methods: The spore Quotient (Q) should be determined, and the statistical analysis of spore

sizes should show the arithmetic mean with standard deviations. The type specimens (holotype, lectotype, or neotype) are designated to a single specimen. So please correct the data in Table 1 and Figure 3. Please correct the abbreviation of the translation elongation factor 1-alpha gene.

3. Results: Please make the scale bar in Figure 1 more clearly. In the Taxonomy subsection, the description that the author provided is not concordant with the specimen photo shown in Figure 1. Please correct the spacing, and number of the lamellae and clarify whether lamellae are intervernose or not. Please indicate the attachment of the stipe base and substrate. The Melzer's reaction is a taxonomic chemical reaction used in Marasmiaceae. Please inform the results of the test in the spores and all kinds of tissue. The color of spores, cells, and hypha are required to be stated in a description. The type of pileipellis, and all kinds of trama have to be informed. In Notes, a comprehensive comparison of related taxa is required especially for the Amazonia species.

4. Discussion: Since a new species reported in this study caused the TBD. The comparison of new species and other TBD causal species is valuable data. However, a species is described based on the sexual stage and the basidiocarp is mainly characterized. Other characters like the rhizomorph or culture morphology could be additional data. So please keep in mind that the TBD causal strains from West Africa are identified without the morphological characteristics of basidiocarp. The author cites that ITS did not provide sufficient support for resolving species in the section *Neosessiles* refers to the work of Wannathes et al 2009. I disagree with this information, please check this carefully. According to the number of available sequences in GenBank and the recent data on the barcode gaps in ITS of *Marasmius* (Wilson *et al.,* 2023[1]), it would be better if the author could investigate the phylogenetic analysis on combined sequences of ITS and LSU.

References

1. Wilson AW, Eberhardt U, Nguyen N, Noffsinger CR, et al.: Does One Size Fit All? Variations in the DNA Barcode Gaps of Macrofungal Genera.*J Fungi (Basel)*. 2023; **9** (8). PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Taxonomy and systematics on agaric

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 31 October 2023

https://doi.org/10.5256/f1000research.153750.r214961

?

Atik Retnowati
[1] Herbarium Bogoriense, Cibinong, Indonesia
[2] Herbarium Bogoriense, Cibinong, Indonesia

This is a study that aims to describe a new species of *Marasmius infestans* from Peru. Combination molecular and morphological study is the best approach to describe these new species. Overall, the work is good, but here are some suggestions:

- A mycobank registration number must be provided for a valid description.
- The previous report of *Marasmius* from Peru must be added to the introduction.
- Map of locality where the species was collected must be added. It will help of the ordinary people to see where the exact type locality.
- Scale bar at the Figure 2 can clearly be seen, and it probably white color scale bar would be better.
- Description: Lamellae adnate with 1-5 series of lamellulae. It should be “Lamellae adnate, distant (1-5) with 0 series of lamellulae”.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Macro-fungal taxonomy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.