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Phylogenetic Diversity, Host Specificity, and Distribution of the Wood-Decaying Fungus *Phellinotus teixeirae* in Western Colombia's Seasonally Dry Tropical Forest

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Abstract: *Phellinotus* (Polyporales) is a common genus of wood-decay fungi in tropical and subtropical areas, endemic to the Seasonally Dry Tropical Forest (SDTF) biome. However, *Phellinotus* diversity remains unexplored, despite being a major threat to living trees. Therefore, this study is aimed at confirming and characterizing through morphological and molecular data the first isolates of *Phellinotus teixeirae* in *Pithecellobium dulce* (Fabaceae) trees (locally referred to as '*Chiminango*') from the endangered Colombian SDTF biome. Fifteen fungal specimens were recovered from living *P. dulce* trees, in the urban area and at the Universidad del Valle campus, and classified as *P. teixeirae* based on taxonomical descriptors. Phylogenetic relationships were inferred from a four-loci dataset (ribosomal and gene-coding regions), including 82 taxa covering 3991 nucleotide positions. The analysis recovered seven highly supported (>90% bootstrapping) monophyletic taxa of the 'Phellinotus Clade', and confirmed the new distribution range of *P. teixeirae* (100% bootstrap support), which extends approx. 1000 km north in the Neotropics. Hierarchical stratified Analysis of MOlecular VAriance (AMOVA) provided a clear genetic distinction between species (70% of variation, p-value = 0.001) and low differentiation among country of origin within species (11%, p-value = 0.044). Discriminant Analysis for Principal Components (DAPC) indicated complex clustering including closely related species, probably a signal of recent radiation and weak species boundaries. Median-joining haplotype network analysis identified unique haplotypes, which may correlate with new host colonization and population expansion (Tajima's D ≤ -0.5). In conclusion, this study provides the first assessment of the genetic diversity of *P. teixeirae* in a novel geography (SDTP) and host tree (*P. dulce*). However, increasing the number of isolates remains critical to understand further the genus' distribution patterns and drivers of genetic diversity.

Keywords: genetic diversity; forest pathogens; fungi; Hymenochaetaceae; molecular phylogeny; Seasonally Dry Tropical Forest (SDTP); *Pithecellobium dulce* (Fabaceae); '*Chiminango*' tree; population expansion

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1. Introduction

Some of the most common wood-decay fungi in tropical and subtropical regions belong to the *Phellinotus* (Drechsler-Santos, Robledo and Rajchenb.) genus. *Phellinotus* is a genus of wood-decay fungi distributed in America and Australia's tropical and subtropical climatic zones, growing on living members of various angiosperms, mainly of the Fabaceae family [1,2]. The genus was initially described as comprising two species, *P. neoaridus* Drechsler-Santos and Robledo and *P. piptadeniae* (Teixeira) Drechsler-Santos and Robledo [3]. Currently, *Phellinotus* includes six additional species referred to as *P. magnoporatus* Salvador-Mont. and Drechsler-Santos, *P. teixeirae* Salvador-Mont., Elias and Drechsler-Santos, and *P. xerophyticus* Robledo, Urcelay and Drechsler-Santos, described from phylogenetic analyses; and *P. badius* (Cooke) Salvador-Mont., Popoff and Drechsler-Santos, *P. resinaceus* (Kotl. & Pouzar) Salvador-Mont. and Drechsler-Santos, and *P. scaber* (Berk.) Salvador-Mont., Drechsler-Santos and Popoff, the three of which were included in the genus based on morphological analysis [2].

Species of *Phellinotus* are morphologically characterized by perennial basidiomata with dark lines and a mycelial core, a monomitic hyphal system in the context and dimitic in the tubes, and yellowish basidiospores with a flattened side that becomes chestnut brown in KOH solution [3–5]. *Phellinotus* is phylogenetically related to *Arambarria* Rajchenb and Pildain, *Inocutis* Fiasson and Niemelä, *Fomitiporella* Murrill, *Fulvifomes* Murrill, *Phylloporia* Murrill, and *Rajchenbergia* Salvador-Montoya, Popoff and Drechsler-Santos, as well as other taxonomically unresolved lineages within the 'Phellinotus Clade' [1–3,6–8]. This clade groups poroid Hymenochaetaceae taxa with thick-walled and colored basidiospores and without setae or setal hyphae. Additionally, the adaxially flattened basidiospores seem to be a distinctive taxonomic feature and are probably phylogenetically significant for the clade [2]. The taxonomy of Hymenochaetales comprises the largest species radiation of wood-decaying fungi [9], and its classification has been improved in recent years (e.g., [10–17]). However, specimens in this order remain understudied and under-collected in the Neotropics, especially in the Seasonally Dry Tropical Forest (SDTF) biome [3,4,18]. The SDTF is considered one of the world's most endangered biomes [19,20]. In Colombia, the SDTF matches regions with intense human pressure, been subjected to intense transformation [21].

The characterization of Hymenochaetales biodiversity in the SDTF ecosystem is therefore considered incipient due to the limited studies conducted for the understanding of its taxonomy and phylogenetics, in addition to the fact that some of these efforts remain unpublished. Recent works have described multiple species recorded in the country, reporting approximately 113 species of Hymenochaetales [22,23], but none of them have been phylogenetically corroborated. To bridge this gap, during a survey of poliporoid and corticioid fungi in the SDTF of Valle del Cauca, Colombia, in urban areas and at the Universidad del Valle campus, several specimens were collected from *Pithecellobium dulce* trees and tentatively identified as *Phellinotus* sp. The tree species *P. dulce*, classified in the subfamily Mimosoideae–Fabaceae, is widely distributed in Colombia's endangered SDTP biome. The tree is native to the Pacific coast (locally known as '*Chiminango*'), and the foothills of the highlands in Mexico, Central America and northern South America, yet it is present too in India, the Philippines, and East Africa. Despite the wide distribution of *P. dulce*, wood-decay fungal pathogens associated with living trees of the species have been poorly characterized in Neotropical areas, partly because of the complexity in the antagonistic biotic interaction. Therefore, the goal of this study was to comparatively assess the morphological and phylogenetic diversity of such fungal collections and discuss their taxonomical classification, geographic distribution, and host specialization on Fabaceae trees. This effort will inform knowledge of fungal diversity in the SDTF, which so far has been understudied despite being a major threat for living trees, including the iconic '*Chiminango*' (*P. dulce*).

2. Materials and Methods

2.1. Morphological Analyses

A total of 15 specimens were collected in the SDTF biome at the Universidad del Valle campus and the urban perimeter vegetation of Cali, Colombia, in a time-lapse from 2017 to 2022. Basidiomes growing on living trees of *Pithecellobium dulce* were photographed, removed from different heights in the trees, placed in paper bags, and taken to the laboratory. The macro-morphological features included the size, shape, pileal surface, context, tubes, pores, and dissepiments, as well as the number of pores per millimeter and color of basidioma. Culture isolation included small portions of contextual tissue of basidioma and/or small portions of the associated wood rot in a substrate of 2% malt extract agar medium.

The isolates were preserved in distilled water [24] and deposited in the in vitro tissue collection of the Universidad del Valle herbarium following standardized CUVC coding. For microscopic analysis, free-hand sections of basidiomes were mounted on microscope slides and examined. All microscopical structures were measured with the aid of an eyepiece micrometer, with a subjective accuracy of 0.1 µm, using Cotton Blue (abbreviated CB, Merck 1275; Kenilworth, New Jersey, USA) prepared in lactic acid, following Miettinen et al. [25], with ×1000 magnification and phase contrast illumination. Melzer's reagent (IKI) was used to determine the presence/absence of amyloid or dextrinoid or a negative reaction. Additionally, a 3% KOH solution was used for microscopy. Observations were then marked as CB+ for cyanophilic, CB− for acyanophilic, or IKI− meaning neither amyloid nor dextrinoid reaction.

Further morphological confirmation required at least 20 hyphae from the subiculum and hymenophoral trama and five basidia, and at least 30 basidiospores were measured per specimen. When variation of hyphal width and basidiospore size were observed, the 20% and 5% extreme tails were respectively reported (different thresholds are advised when hyphal width variation is larger than spore size variation [26]). Sections of the tramal and context of the basidioma were incubated in 3% (*v/w*) NaOH solution for 48 h at 40 °C, and carefully dissected under a stereomicroscope to accurately describe the hyphal system [3]. Microscopic descriptions were abbreviated as follows: L for basidiospore length, W for basidiospore width, Q' for length/width ratio of individual spores, and Q—L/W for number (*n*) of basidiospores measured per specimen.

2.2. DNA Extraction, Amplification and Sequencing of Barcodes

Total DNA was extracted from dried herbarium samples of basidiomata or mycelia growing in agar cultures. Sample tissue was cut out with a scalpel and then homogenized with a pestle in a 1.5 mL microcentrifuge tube. Further steps were performed according to the kit manufacturer's protocol of the E.Z.N.A. Forensic DNA kit (Omega Bio-tek, Norcross, GA, USA).

The primers ITS5 and ITS4 were used for amplifying and sequencing the nuclear ribosomal DNA Internal Transcribed Spacer (ITS1–5.8S–ITS2) [27]. The nuclear ribosomal Large SUbunit (nLSU) region was amplified with the primers CTB6 (as UNI in Haynes et al. [28]) and LR7, and sequenced with the same primers or combining CTB6–LR5 [29]. The primers 983F and 1567R were used for amplifying and sequencing the translation elongation factor 1-α (TEF1-α) [30].

Polymerase chain reactions (PCRs) used BlastTaq™ 2X MasterMix (abm) in a 25 µL volume reaction and were carried out on a thermal cycler (C1000 Touch™ Thermal Cycler Bio-Rad; Hercules, CA, USA). For ITS and nLSU regions, the cycling parameters described by Oghenekaro et al. [31] were followed, while for TEF1-α, the Miettinen et al. [26] protocol was followed. PCR products were visualized in 1.5% agarose gel via electrophoresis. Amplified products were purified and sequenced in both directions on an Applied Biosystem 3730xl DNA analyzer (MacroGen Ltd.; Seoul, Republic of Korea).

The electropherograms of forward and reverse sequences were assembled, visualized, and edited using the Consed/PhredPhrap package v.20 [32–34] The electropherograms were visually inspected to ensure good sequence quality, and ambiguous sequence reads

were discarded. Double peaks were interpreted as true base ambiguities when they were detected in both forward and reverse sequencing electropherograms. Consensus sequences were queried against the entire GenBank database using BLAST+ 2.15.0 algorithm (http://blast.ncbi.nlm.nih.gov/ accessed on 30 June 2023), and hits' pairwise identity was recorded. All newly generated consensus sequences were made available in GenBank.

2.3. Phylogenetic Analysis

The consensus sequences generated in this study and the sequences chosen based on Salvador-Montoya et al. [2] were downloaded from GenBank (www.ncbi.nih.gov/genbank/, accessed on June 30 2023 Table 1).

Table 1. Taxa of wood-decay fungi sampled in this study and used in phylogenetic analyses. For each collected sampled, the species name, voucher, and GenBank accession number are provided. Passport data include both the fungi scientific name, as well as the scientific name of the corresponding tree host species. The reference collection keeping the fungi voucher is also indicated. Missing information is indicated with an *n*-dash (–). The GenBank accession numbers of sequences generated in this study are highlighted in bold, as compared to already available data download from the GenBank repository. Finally, (T) corresponds to type specimens and (*) mark the generic types.

	Passport Data				GenBank Accession Number			
Genera	**Fungi Species**	**Country (ISO)**	**Reference Collection**	**Tree Host**	**ITS**	**nLSU**	***tef1***	***rpb2***
Arambarria Rajchenb. and Pildain	*A. destruens* Rajchenb. and Pildain *	AR	CIEFAPcc 347	*Diostea juncea*	KP347538	KP347523	KY907666	–
	A. cognata (Bres.) Rajchnb. Pildain	UY	CGP473	*Dodonaea viscosa*	KY907683	KY907687	KY907675	–
	A. cognata	UY	CGP474	*Dodonaea viscosa*	KY907682	KY907692	KY907676	–
Fomitiporella Murrill	*F. austroasiana* Y.C. Dai, X.H. Ji and J. Vlasák **(T)**	CN	Dai 16244	On fallen angiosperm trunk	MG657328	MG657320	–	–
	F. caryophylli (Racib.) T. Wagner and M. Fisch.	IN	CBS 448.76	*Shorea robusta*	AY558611	AY059021	–	–
	F. chinensis (Pilát) Y.C. Dai, X.H. Ji and J. Vlasák	CN	Cui 11230	*Quercus* sp.	KX181309	KY693759	KY693958	–
	F. inermis (Ellis & Everh.) Murrill	US	JV 1009/56	*Ilex mucronata*	KX181306	KX181347	–	–
	F. subinermis Y.C. Dai, X.H. Ji and Vlasák	CN	Dai 15114	On root of angispoerm tree	KX181308	KX181344	–	–
	F. umbrinella (Bres.) Murrill *	US	JV 0509/114	Unknown	KX181314	KX181336	–	–
	F. umbrinella (Bres.) Murrill	BR	FLOR 51648		MK802943	MK802941	–	–
Fulvifomes Murrill	*F. elaeodendri* Tchotet, M.P.A. Coetzee, Rajchenb. and Jol. Roux	ZA	CMW 47825	*Elaeodendron croceum*	MH599094	MH599134	MT108964	–
	F. centroamericanus Y.C. Dai, X.H. Ji and J. Vlasák **(T)**	GT	JV 0611_III	On living angiosperm tree	KX960763	KX960764	–	–
	F. fastuosus (Lév.) Bondartseva and S. Herrera	PH	CBS 213.36	*Gliricidia sepium*	AY558615	AY059057	–	–
	F. nilgheriensis (Mont.) Bondartseva and S. Herrera	US	CBS 209.36	On dead deciduous wood	AY558633	AY059023	–	–
	F. robiniae (Murrill) Murrill	US	CBS 211.36	*Robinia pseudoacacia*	AY558646	AY059038	–	–
	F. squamosus Salvador-Montoya and Drechsler-Santos	PE	USM258361	*Acacia macracantha*	MF479267	MF479266	–	–
Inocutis Fiasson and Niemelä	*I. dryophilus* (Berk.) Fiasson and Niemelä	US	DLL 2012-001	*Quercus alba*	KU139186	KU139255	–	KU139317
	I. jamaicensis (Murrill) A.M. Gottlieb, J.E. Wright and Moncalvo	US	RLG 15819	*Quercus arizonica*	KY907684	KY907703	–	–
	I. rheades (Pers.) Fiasson and Niemelä *	RU	CBS	–	–	MH866581	–	–
Inonotus P. Karst.	*I. cuticularis* (Bull.) P. Karst. *	–	JV 1109/89-J	–	KF446595	–	KF446610	–
	I. griseus L.W. Zhou	CN	Dai 13436	–	KX674583	KX832925	KY693959	KX364919
	I. vitis A.A. Brown, D.P, Lawr. And K. Baumgartner **(T)**	US	OC1/CBS 1453555	*Vitis vinifera*	MN108118	MN113944	MN114509	MN104164

Table 1. *Cont.*

Genera	Fungi Species	Country (ISO)	Reference Collection	Tree Host	ITS	nLSU	*tef1*	*rpb2*
		Passport Data			**GenBank Accession Number**			
Phellinotus Drechsler-Santos, Robledo and Rajchenb.	*P. magnoporatus* Salvador-Mont. And Drechsler-Santos	PE	USM 250523	*Ocotea aurantiodora*	MZ954859	MZ964981	OK000625	–
	P. neoaridus Drechsler-Santos and Robledo * **(T)**	BR	URM 80362	*Caesalpinia* sp.	KM211294	KM211286	–	–
	P. neoaridus	BR	URM77673	–	MZ954857	–	–	–
	P. neoaridus	BR	URM83203	–	MZ954858	MZ964977	–	–
	P. neoaridus	BR	HUEFS 122186	*Cenostigma pyramidale*	–	MZ964976	–	–
	P. neoaridus	BR	URM 80579	*Caesalpinia* sp.	–	MZ964978	–	–
	P.piptadeniae (Teixeira) Drechsler-Santos and Robledo	BR	URM 80345	*Senegalia* sp.	KM211291	KM211283	–	–
	P. piptadeniae	BR	URM 80322	*Mimosa* sp.	KM211290	KM211282	–	–
	P. piptadeniae **(T)**	BR	URM 80361	*Mimosa* sp.	KM211288	KM211280	–	–
	P. piptadeniae	BR	URM 80768	*Piptadenia stipulaceae*	KM211289	KM211281	–	–
	P. piptadeniae	BR	URM 80766	*Mimosa* sp.	KM211293	KM211285	–	–
	P. piptadeniae	BR	URM 80360	*Mimosa* sp.	KM211292	KM211284	–	–
	P. piptadeniae	BR	FLOR 51451	*Piptadenia gonoacantha*	MZ954839	MZ964964	–	–
	P. piptadeniae	BR	FLOR 63105	*Piptadenia gonoacantha*	MZ954847	MZ964971	OK000617	–
	P. piptadeniae	BR	FLOR 63111	*Piptadenia gonoacantha*	MZ954845	MZ964969	OK000618	–
	P. piptadeniae	BR	FLOR 62129	–	MZ954840	MZ964965	–	–
	P. piptadeniae	BR	FLOR 62132	–	MZ954841	MZ964966	–	–
	P. piptadeniae	BR	FLOR 63627	*Piptadenia gonoacantha*	KP412305	KP412282	–	–
	P. piptadeniae	BR	FLOR 63101	*Piptadenia gonoacantha*	MZ954846	MZ964970	OK000619	–
	P. piptadeniae	UY	MVHC 5756	*Calliandra tweediei*	MZ954842	MZ964968	–	–
	P. piptadeniae	UY	MVHC 5754	*Calliandra tweediei*	MZ954843	MZ964967	–	–
	P. piptadeniae	UY	MVHC 5561	*Calliandra tweediei*	MZ954844	KT266877	–	–
	P. piptadeniae	UY	MVHC 5562	*Calliandra tweediei*	KT266876	KT266878	–	–
	P. piptadeniae	BR	FLOR 39572	*Piptadenia gonoacantha*	MZ954848	–	–	–
	P. teixeirae Salvador-Mont., Elias and Drechsler-Santos (**T:** corresponds to type specimens)	PE	USM 250528	*Pithecellobium excelsum*	MZ954853	MZ964972	–	–
Phellinotus Drechsler-Santos, Robledo and Rajchenb.	*P. teixeirae*	PE	USM 258362	*Libidibia glabrata*	MZ954854	MZ964975	OK000621	OK000626
	P. teixeirae	PE	USM 278225	*Libidibia glabrata*	MZ954855	MZ964974	OK000622	OK000627
	P. teixeirae	BR	URM 80889	*Pytyrocarpa moniliformis*	MZ954852	–	–	–
	P. teixeirae	BR	URM 80403	*Piptadenia sp.*	MZ054849	–	–	–
	P. teixeirae	BR	URM 80636	*Pytyrocarpa moniliformis*	MZ954850	–	–	–
	P. teixeirae	PE	USM 258366	*Libidibia glabrata*	MZ954856	MZ964973	–	–
	P. teixeirae	AR	CTES 515266	–	MZ954851	–	–	–
	P. teixeirae	**CO**	**ACB 431**	***Pithecellobium dulce***	**OR205894**	**OR186199**	**OR209163**	–
	P. teixeirae	**CO**	**ACB 432**	***Pithecellobium dulce***	**OR205895**	**OR186200**	**OR209164**	**OR204685**
	P. teixeirae	**CO**	**ACB 433**	***Pithecellobium dulce***	**OR205896**	–	**OR209165**	**OR204686**
	P. teixeirae	**CO**	**ACB 460**	***Pithecellobium dulce***	**OR205897**	–	**OR209166**	**OR204687**
	P. teixeirae	**CO**	**ACB 463**	***Pithecellobium dulce***	**OR205898**	–	**OR209167**	**OR204688**
	P. teixeirae	**CO**	**ACB 553**	***Pithecellobium dulce***	**OR205899**	**OR186201**	**OR209168**	–
	P. teixeirae	**CO**	**ACB 848**	***Pithecellobium dulce***	**OR205900**	–	–	–
	P. xerophyticus Robledo, Urcelay and Drechsler-Santos **(T)**	AR	CORD 3551	*Prosopis* sp.	–	MZ964979	OK000624	OK000629
	P. xerophyticus	AR	CORD 3552	*Prosopis* sp.	–	MZ964980	OK000623	OK000628
Phylloporia Murrill	*P. crataegi* L.W. Zhou and Y.C. Dai	CN	Dai 18133	*Crataegus* spp.	MH151191	MH165865	MH167431	MH161224
	P. elegans Ferreira-Lopes, Robledo and Drechsler-Santos	BR	FLOR 51178	–	KJ639049	KJ631408	–	–
	P. gabonensis Decock and Yombiy.	GA	MUCL 55571	*Dichostema glaucescens*	KU198355	KU198353	–	–
	P. nodostipitata Ferreira-Lopes, Robledo and Drechsler-Santos	BR	FLOR 51153	On living roots	KJ639057	KJ631414	–	–
	P. parasitica Murrill *	AR	Leif Ryvarden 19843	–	KU198361	–	–	–
	P. pectinata (Klotzsch) Ryvarden	AU	Voucher 113	–	MH151181	MH165867	MH167421	MH161213
	P. pseudopectinata Yuan Y. Chen and B.K. Cui	CN	Cui 13749	Angiosperm	MF410323	KX242356	MH167429	MH161222

Table 1. *Cont.*

Passport Data					GenBank Accession Number			
Genera	**Fungi Species**	**Country (ISO)**	**Reference Collection**	**Tree Host**	**ITS**	**nLSU**	***tef1***	***rpb2***
Rajchenbergia Salvador-Montoya, Popoff & Drechsler-Santos	*R. mangrovei* (Y.C. Dai, X.H. Ji and J. Vlasák) Salvador-Mont. Drechsler-Santos and Popoff	FR	JV1612/25-J	*Conocarpus erectus*	MG657325	MG657331	–	–
	R. pertenuis (Xavier de Lima and Oliveira-Filho) Salvador-Mont. Popoff and Drechsler-Santos	BR	PPT111	On dead wood	MG806100	MG806101	–	–
	R. tenuissima (H.Y. Yu, C.L. Zhao and Y.C. Dai) Salvador-Mont. Drechsler-Santos and Popoff	CN	Dai12245	Angiosperm	KC999902	KC456242	–	–
Sanghuangporus Sheng H. Wu, L.W. Zhou and Y.C. Dai	*S. baumii* (Pilát) L.W. Zhou and Y.C. Dai	CN	Dai 16900	–	MF772785	MF772802	MF977782	MF973476
	S. sanghuang (Sheng H. Wu, T. Hatt and Y.C. Dai) Sheng H. Wu, L.W. Zhouand Y.C. Dai *	CN	Cui 14419	–	MF772789	MF772810	MF977790	MF973483
	S. vaninii (Ljub) L.W. Zhou and Y.C. Dai	US	DMR-95-1-T	*Populus tremuloides*	KU139198	KU139258	KU139380	KU139318
	S. zonatus (Y.C. Dai and X.M. Tian) L.W. Zhou and Y.C. Dai	CN	Dai 10841	Living angiosperm tree	JQ860306	KP030775	MF977797	MF973490
Tropicoporus L.W. Zhou, Y.C. Dai and Sheng H. Wu	*T. dependens* (Murrill) L.W. Zhou, Y.C. Dai and Vlasák	US	JV 0409/12-J	Angiosperm wood	KC778777	MF772818	MF977799	MF973492
	T. drechsleri Salvador-Mont. and Popoff	AR	CTES 570140	*Patagonula americana*	MG242439	MG242444	–	–
	T. excentrodendri L.W. Zhou and Y.C. Dai * **(T)**	CN	Yuan 6232	*Excentrodendron tonkinense*	KP030790	–	–	–
	T. texanus A.A. Brown, D.P. Lawr. and K. Baumgartner **(T)**	US	TX9-CBS 145.357	*Vitis vinifera*	MN108124	MN113950	MN114515	MN104178
Root	*P. igniarius* (L.) Quél. *	CZ	JV 9411/5	–	KR013061	–	KR013092	–

Sequences were aligned using MAFFT v.7.299 [35]. The ITS and nLSU regions were aligned using the L-INS-I strategy (command line: mafft—localpair-maxiterate 1000). The coding regions were aligned using the E-INS-I strategy with no cost for opening gaps and equal cost for transformations (command line: mafft -genafpair-maxiterate 1000). After alignment, sequences were translated and checked for stop codons using Aliview v.1.18 [36]. The combined dataset had 77 sequences of ITS, 68 of nLSU, 32 of TEF1-α, and 20 of RPB2. The dataset was subdivided into nine data partitions (ITS, nLSU, TEF1-α-1st, -2nd, and -3rd codon positions, TEF1-α-introns, and RPB2-1st, -2nd and -3rd codon positions).

Phylogenetic relationships were inferred in a maximum likelihood as implemented in IQTREE v.2.0 [37]. *Phellinus igniarius* (L.) Quél. was used as outgroup. ModelFinder [38] was used to select the optimal partition scheme and Markovian substitution models. The ultrafast Bootstrap [39] and the Shimodaira–Hasegawa approximate likelihood-ratio test support (SH aLRT, [40]) were subsequently carried out with the command: iqtree -s concat.nex -spp partition.nex. best_scheme.nex -B 1000 -alrt 1000 -pers 0.2 -nstop 1000.

2.4. Data Analysis

The analyses of the patterns of nucleotide diversity were conducted from curated datasets aligned in Bioedit® v.7.1.11 [41], including DNA sequences previously reported (Table 1), recovering 993 bp of ITS, 1016 bp of nLSU, 1241 bp of RPB2, and 1120 bp of TEF1-α-1 gene regions. Summary statistics were conducted in R Studio with the packages *ape* and *pegas* (R Core Team). The concatenated dataset (ITS and nLSU) covered 2011 nucleotide sites. Downstream analyses included a boxplot and clustered heatmap for pairwise F_{ST}, with 1000 bootstrap replicates for each case, using the R packages *poppr*, and *adegenet* (R Core Team). An additional concatenated dataset was considered, including only the *Phellinotus* species to identify intra-genus relationships.

In terms of clustering, Analysis of MOlecular VAriance (AMOVA) was performed in R packages *poppr* and *adegenet* [42] using hierarchical and stratified data corresponding to the species levels and country of origin. Discriminant Analysis for Principal Components (DAPC) was carried out for each dataset independently with 1000 bootstrap replicates using the R packages *poppr*, *pegas–ape*, and *adegenet* [42]. Finally, to identify unique and

shared alleles, Venn diagrams were drawn using the web-based tool InteractiVenn [43], available at http://www.interactivenn.net/ accessed on 30 June 2023. Median-joining haplotype networks were built for ITS and nLSU in the R package *pegas–ape* (R Core Team).

3. Results

3.1. Taxonomy–Record Description for Phellinotus Teixeirae Salvador-Montoya, Elias and Drechsler-Santos [MycoBank MB840997]

Basidioma was mostly perennial, pileate, up to 105 mm long, 74 mm wide, 50 mm thick at the base, sessile, applanate to triquetrous, sometimes with a basal umbo, woody hard, solitary, or in groups with many basidiomata widespread along the substrate. Margins were convex, entire, thick, pubescent, always distinct from older parts of the basidioma, yellowish when young, then turning brown with age, or when beaten.

Pileus fulvous was brown to black greyish, dull, azonate, sulcate, and strongly cracked. The context was zonate, with black lines below the upper surface of the pilei, a granular core variably present, pale to dark yellowish, becoming black with KOH, and a thick black crust above the context, up to 8 mm thick in mature specimens. Tubes were stratified, with/without contextual tissue layer between them, dark reddish brown, up to 42 mm long. The hymenophore was poroid, with round regular pores, (3-)4-6 per mm, fulvous brown to dark brown, and dissepiments were entire.

The hyphal structure was pseudo-dimitic, with skeletal hyphae restricted to the trama of the tube layer; skeletal hyphae were reddish dark brown, thin- to thick-walled, with a visible lumen, almost solid, (3.5-)3.8-5.5(-6.5) µm wide, some with small aborted ramifications. Generative hyphae dominated in the context, with a simple septate, pale yellow to reddish dark brown, thin- to thick-walled, branched, (2-)2.5-4(-6.2) µm wide. Generative hyphae in the trama were thin- to thick-walled, with a simple septate, hyaline to pale yellow, becoming sclerified in some portions. Setae or other sterile elements were absent. Basidia were (9-)10-12.5(-14) × 4.5-5.8(-6.2) µm. Basidiospores were broadly ellipsoid to ellipsoid, adaxially flattened, smooth, thick-walled, yellow in water to chestnut brown in KOH, (4.5-)5-6.8(-7) × 4–5(-5.5) µm, L = 5.7 W = 4.5 µm, Q′ = 1-1.1(-1.5) µm, Q = 1.2 (*n* = 354/14), IKI-, CB+.

Regarding habitat, distribution, and host, the species was previously recorded in the SDTF of the Andean valley in northern Peru and southern Ecuador and on the Pacific coast of Ecuador and northern Peru. In this study, *P. teixeirae* was recorded for the first time in southwest Colombia, growing on living trees of *Pythecelobium dulce*, a new host. Previously, the species was recorded growing on other species of living trees of Fabaceae such as *Pythecelobium excelsum*, *Libidibia glabrata*, and *Pityrocarpa moniliformis*, in addition to *Acacia* and *Piptadenia* species [2].

The material examined, in the Colombian province of Valle del Cauca, Cali (urban perimeter) was from living trees of *Pythecelobium dulce*, 12 November 2017, leg. Bolaños-Rojas, A.C., Chaurra, A. and Aguilar, G., ACB381 (CUVC 75577); from the Universidad del Valle campus Melendez, from living trees of *Pythecelobium dulce*, ACB432 (CUVC 75578), 30 November 2018, leg. Bolaños-Rojas, A.C., ACB448 (CUVC 75579), ACB449 (CUVC 75580), ACB455 (CUVC 75581), ACB458 (CUVC 75582), 4 December 2018, leg. Bolaños-Rojas, A.C. and Ramos, A., ACB460 (CUVC 75583), ACB462 (CUVC 75584), 22 January 2019, leg. Bolaños-Rojas, A.C., Gonzalez, L. and Ramos, A., ACB463 (CUVC 75585), ACB496 (CUVC 75586), ACB497 (CUVC 75587, ACB498 (CUVC 75588), ACB842 (CUVC 75589), and 15 March 2021, leg. Motato-Vásquez, V., MV1109 (CUVC 75590).

Notes (Figure 1)—*Phellinotus teixeirae* was characterized with applanate to ungulate basidiomata with cracked and dark greyish brown pileal surfaces, hymenophore with (3-)4-6 pore per mm, dark lines in the context, a monomitic hyphal system in the context and dimitic in the tubes, absence of setae, and broadly ellipsoid to ellipsoid basidiospores, (4.5-)5-6.8(-7) × 4-5(-5.5) µm, with a flattened side turning darker in KOH solution. The species differs from *P. piptadeniae s.s.*, which has a lobulate and cracked olive grey pileal surface, with deep concentric furrows and wide lobes. Morphological patterns of the pileal surface are useful to split species in Hymenochaetaceae [2]. Phylogenetics corroborate this classification (Figure 2), as detailed in the following section.

Figure 1. Fresh basidiomata and microscopic characteristics of wood-decay *P. teixeirae* (ACB462). (**A**,**B**) Basidiomata of *P. teixeirae* growing in situ on *P. dulce* trees. (**C**) Poroid hymenophore. (**D**) Basidiospores. (**E**) Basidia. Scale bars: (**A**–**C**) = 1 cm, (**D**,**E**) = 5 µm. Photos by Bolaños-Rojas, AC.

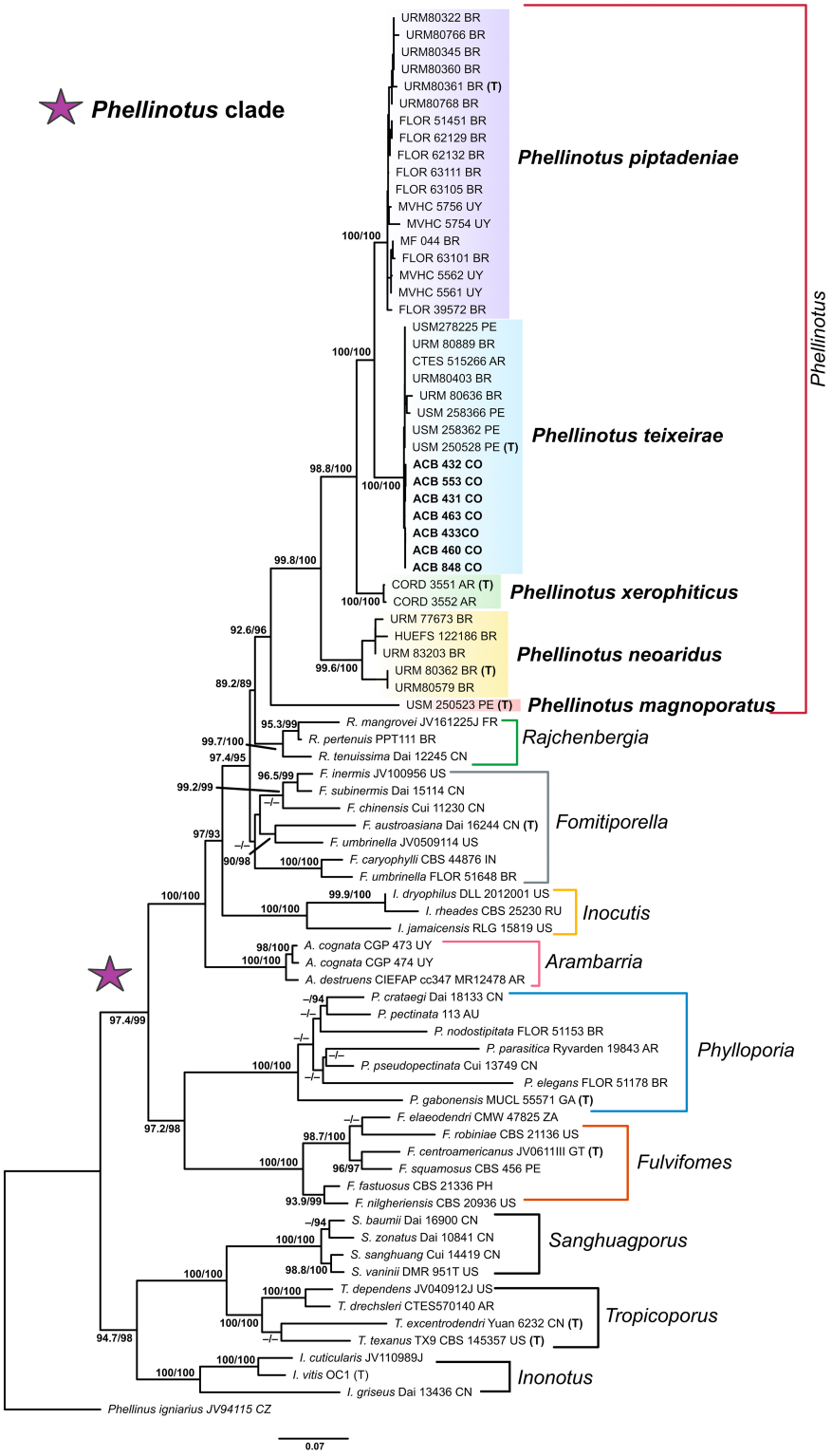

Figure 2. Phylogenetic relationships in wood-decay *Phellinotus* species inferred from a combined dataset of ITS + 28S + TEF + RPB2 created using IQ-TREE optimal tree (log-likelihood = −21,181.5918). All sequences generated in this study are indicated in bold. Values at nodes indicate ultrafast bootstrap (**left**) and the Shimodaira–Haegawa approximate likelihood-ratio test (**right**); (–) indicates bootstrap values lower than 60%. (T) corresponds to type specimens. Two codes after voucher specimens denote country and province of origin. Horizontal lengths indicate the number of expected substitutions per position.

3.2. Phylogenetic Relationships

A total of four loci were sequenced for seven *Phellinotus* isolates including the widely used barcoding ribosomal nuclear regions ITS and nLSU, the coding genes RNA polymerase II second largest subunit (RPB2), and the translation elongation factor (TEF1-α) (Table 1). Twenty, new sequence records were generated comprising seven for ITS, three for nLSU, six for TEF1-α, and four for RPB2. GenBank accession numbers as in Table 1.

The combined dataset with four loci for the 'Phellinotus Clade' (ITS + nLSU + TEF1-α-1st + -2nd + -3rd + TEF1-α-introns + RPB2-1st + -2nd + -3rd) included 82 taxa, resulting in an alignment with 3991 positions, of which 2177 were conserved, 441 varied, and 1373 were parsimony-informative. The best tree inferred in a maximum likelihood framework from ten independent runs had a log-likelihood = −50,925.2117. The selected best-fit models were TN + F + I + G4 for ITS, TIM2 + F + R3 for nLSU, GTR + F + I + G4 for TEF1-α in all codons, HKY + F + R2 for TEF1-α-introns, and TIM2+F + I + G4 for RPB2 in all codon positions. The phylogenetic analysis (Figure 2) was agreed with previous studies [2,3,6,7].

Seven genera were recovered as monophyletic lineages with strong support, all recognized as the 'Phellinotus Clade': *Arambarria* (BS = 100, SH alRT = 100), *Inocutis* (BS = 100, SH alRT = 100), *Fomitiporella* s.s. (BS = 98, SH alRT = 90), *Fulvifomes* (BS = 100, SH alRT = 100), *Phellinotus* (BS = 96, SH alRT = 92.6), *Phylloporia* (BS = 100, SH alRT = 100), and *Rajchenbergia* (BS = 99, SH alRT = 95.3), and the reminiscent groups of *Fomitiporella s.l.*

Both phylogenetic and morphological analyses confirmed that the collected specimens, growing on living trees of *Pithecellobium dulce* (Roxb.) Benth in the SDTF of Valle del Cauca, Colombia, corresponded to the species *Phellinotus teixeirae* (BS = 100, SH alRT = 100). Therefore, this is the first record of the species and the genus in Colombia. This result extends the distribution range of *P. teixeirae* approximately 1000 km north in the Neotropics. The new host, *P. dulce*, classified in the subfamily Mimosoideae–Fabaceae, is a species widely distributed in the SDTF of Colombia [44]. The tree species is native to the Pacific coast, the foothills of the highlands in Mexico, Central America, and northern South America [45], yet, it is present too in India, the Philippines, and East Africa [46–48].

3.3. Patterns of Nucleotide Diversity

Considering the limiting information available in the databases for alternative loci (i.e., TEF1-α, RPB2) in the 'Phellinotus Clade', most of the analyses focused on ITS and nLSU loci. Nucleotide parameters are summarized in Table 2. The calculated value of Tajima's D was significantly negative at ITS for *P. piptadeniae*; *P. neoaridus*, *P. piptadeniae*, and *P. teixeirae* also had highly significant negative values at the LSU locus, indicating possible signals of population expansion. Coding DNA genes *RBP2* and *TEF* exhibited positive and negative Tajima's D values, respectively, although these were non-significant.

Table 2. Summary statistics of the site frequency spectrum for *RBP2* and *TEF*-encoding genes, and ITS and LSU ribosomal DNA regions for the wood-decay 'Phelinotus Clade' species. Abbreviations: S: number of segregating sites; π: nucleotide diversity; and θ_W: Theta of Watterson per site from S.

Gene Name	Taxa	Length	S	π	θ_W	Tajima's D
ITS	All	737	246	0.032	59.32	−2.935 **
ITS	*P. piptadeniae*	457	38	0.002	11.24	−3.663 ***
ITS	*P. teixeirae*	628	13	0.005	4.08	−0.561
LSU	All	992	170	0.028	42.9	−2.424 *
LSU	*P. neoaridus*	746	4	0.0013	2.181	−5.07 ***
LSU	*P. piptadeniae*	557	44	0.0018	13.26	−3.87 ***
LSU	*P. teixeirae*	726	16	0.002	6.53	−3.049 ***
RBP2	All	1247	61	0.022	23.52	0.289
RBP2	*P. teixeirae*	1201	5	0.002	2.189	0.196
TEF	All	504	117	0.06	38.74	−1.134

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.001$.

Fixation indices were computed for each species in the 'Phellinotus Clade' using the total variance detected among the analyzed loci (i.e., ITS, nLSU, and ITS + nLSU). Boxplots for F_{ST} scores summarize pairwise species comparisons against one taxonomic group at a time (Figure 3a–c). Differences among boxplots, that is F_{ST} differentiation of classifications against the rest, were not statistically significant. The ITS locus displayed the highest F_{ST} median values ($\geq$0.04), with *Arambarria* yielding moderate genetic differentiation (median $\geq$ 0.11) and wide variance in allele frequency (Figure 3a). Contrastingly, the nLSU locus contained low variation, both within and between species, with median values ranging from 0.02 to <0.04 (Figure 3b). The concatenated dataset differentiated *Arambarria*, with median values generally between 0.04 and 0.08, indicating small genetic differentiation among groups (Figure 3c).

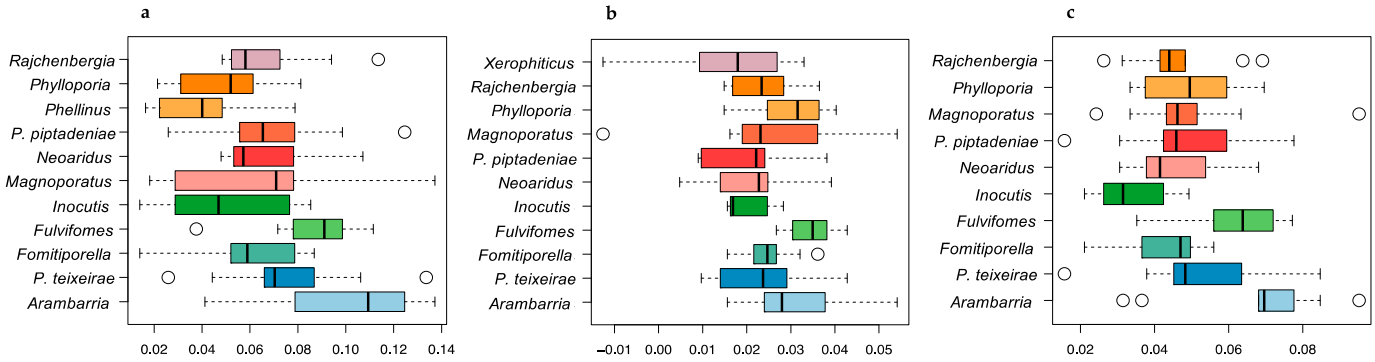

Figure 3. Boxplot of the summarized pairwise F_{ST} distributions (i.e., F_{ST} differentiation of each classification against the rest) in the (**a**) ITS, (**b**) LSU, and (**c**) concatenated (ITS—LSU) datasets analyzed at the genera level for the wood-decay 'Phellinotus Clade'. Boxplots are colored according to taxonomy (genera and species names are indicated in the left axis).

Heatmap analysis, based on F_{ST} values in the ITS, LSU, and concatenated datasets, also clustered all isolates into the *P. teixeirae* clade (Figure 4a–c). *Arambarria* and *Fulvifomes* groups were distant in the 'Phellinotus Clade', whilst *P. teixeirae* and *P. piptadeniae* were genetically similar, based on the ITS (Figure 4a) and concatenated (Figure 4c) datasets, providing similar cluster topologies. LSU-based clustering indicated similar allele frequencies with low nucleotide variation, limiting the discriminatory power of this locus for recent evolutionary events within species (Figure 4b), yet with enough resolution for species discrimination within the 'Phellinotus Clade'.

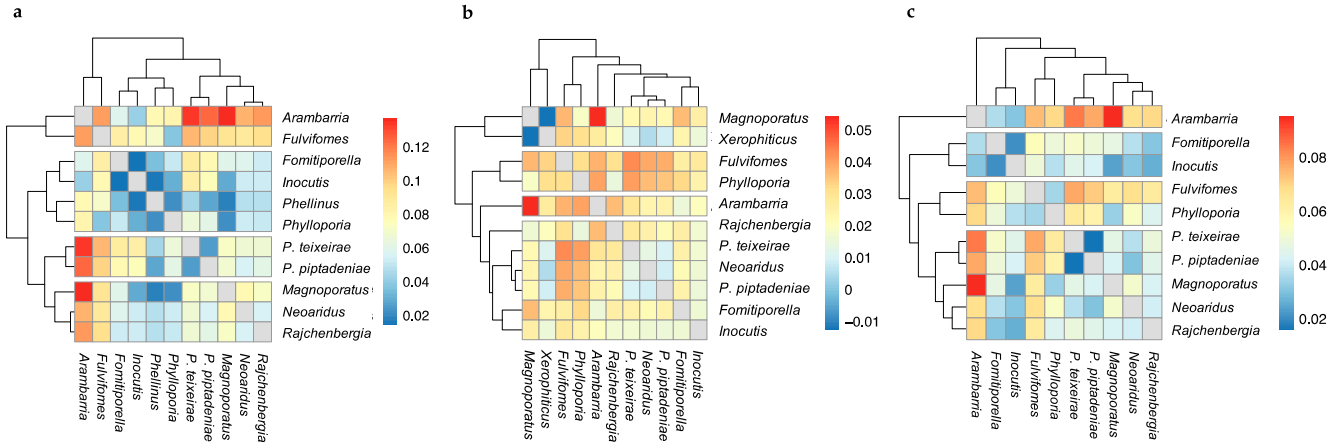

Figure 4. Clustered heatmap of the pairwise F_{ST} distributions in the (**a**) ITS, (**b**) LSU, and (**c**) concatenated (LSU–ITS) datasets analyzed at the wood-decay 'Phellinotus Clade' level.

3.4. Analysis of Molecular Variance

Analysis of MOlecular VAriance (AMOVA) was performed to determine species boundaries, and the possible cluster differentiation was based on the region of origin. Through this process, we prepared a stratified hierarchical AMOVA categorizing species and country-of-origin levels. The final dataset contained four species groups and five localities regarding the country of origin (Argentina, Brazil, Colombia, Peru, and Uruguay). The independent and combinational effects were evaluated to understand the percentage of variation at significant *p*-value thresholds of 0.05 (Table 3). The results showed a high contribution of the species partition, with an explained variance of 70% and a *p*-value of 0.001, higher compared to the country of origin within species that only accounted for 28% of the variation (*p*-value 0.0009). Meanwhile, the locality level explained 11.1% of the within-species variation (*p*-value 0.04). In general, differences between species contributed to most of the distinction. As the variation within species was lower than between species, ribosomal loci offered a clear taxonomic distinction.

Table 3. Analysis of MOlecular VAriance (AMOVA) considering species and country of origin of wood-decay *Phellinotus* species. **Df** = degrees of freedom; **Sum Sq** = sum of squares; **Mean Sq** = mean of squares; **%** = percentage variation. Significant *p*-values are in bold (*p*-value < 0.05) and underlined (*p*-value $\leq$ 0.01).

Species/Locality Level	Df	Sum Sq	Mean Sq	Sigma	%	*p*-Value
Between species	3	1812.04	604.01	79.28	70.09	**0.0019**
Between samples within spp.	4	148.81	37.20	1.11	0.98	0.117
Between localities	28	916.14	32.72	32.72	28.93	**0.0009**
Total	35	2877	82.20	113.11	100	–
Species level						
Between species	3	1812.04	604.01	79.84	70.58	**0.0009**
Between samples	32	1064.96	33.23	33.23	29.42	–
Total	35	2877	82.20	113.12	100	–
Locality level						
Between localities	3	410.58	136.87	9.64	11.11	**0.044**
Between samples	32	2466.42	77.08	77.076	88.89	–
Total	35	2877	82.20	86.71	100	–

3.5. Discriminant Analysis of Principal Components

Discriminant Analysis for Principal Components (DAPC) was carried out separately for each dataset. Genetic variation was partitioned into between- and within-group components. The isolates obtained here were clustered with the *P. teixeirae* group for the three datasets, ITS, LSU, and concatenated, denoting similarity in allele frequencies.

The ITS locus explained > 60% of the genetic diversity by retaining the first 15 components, enabling discrimination of four groups and differentiating *P. teixeirae*, *Fulvifomes* sp., and *Phylloporia* sp. into the 'Phellinotus Clade'. Additionally, the LSU locus explained > 80% of the genetic similarity condensed in the first 15 components. However, it provided lower discriminatory power, displaying only three well-differentiated groups, *Fulvifomes* sp. being the most divergent cluster. For the concatenated dataset, 10 components were retained that explained > 60% of the genetic diversity, discriminating between *Fulvifomes* sp. and *Phylloporia* sp. Most of the *Phellinotus* species were closely related according to the ITS analysis (Figure 5).

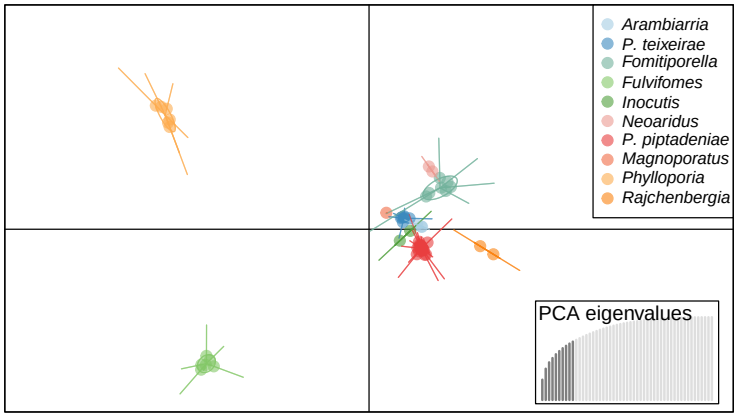

Figure 5. Discriminant Analysis for Principal Components (DAPC [42]) of genetic polymorphism in the ITS–LSU concatenated dataset of wood-decay *Phellinotus* species. The lower right inset corresponds to the principal components retained in the analysis. Dots are colored according to genera.

3.6. Patterns of SNP Allelic Distribution in Phellinotus Species

To investigate the number of SNP loci (unique and shared) in the different species analyzed here, comprising 26 samples distributed in four species, 189 SNP loci were retained at a Minor Allele Frequency (MAF) $\geq$ 0.05, computed in Tassel v5 [49], from 1738 nucleotide sites in the original concatenated dataset (ITS + nLSU). Most of the SNPs were recovered from the ITS locus (118), representing 62.4%. Large variation was observed between species. For instance, *P. neoaridus* contained the highest number of unique alleles (85), followed by *P. magnoporatus* (80), *P. teixeirae*, and *P. piptadeniae* (51) (Figure 6). Overall, the pattern of shared alleles showed large variation among the four *Phellinotus* species. *Phellinotus piptadeniae* and *P. teixeirae* had the most shared alleles (a total of 40), compared to *P. magnoporatus* and *P. piptadeniae* with nine alleles. Between all species, only 15 alleles were shared, corresponding to 7.93%. Overall, there were more shared alleles between *P. teixeirae* and *P. piptadeniae* than between any other compared species, whereas *P. magnoporatus* and *P. neoaridus* showed the lowest frequency of shared alleles across all analyzed species pairs.

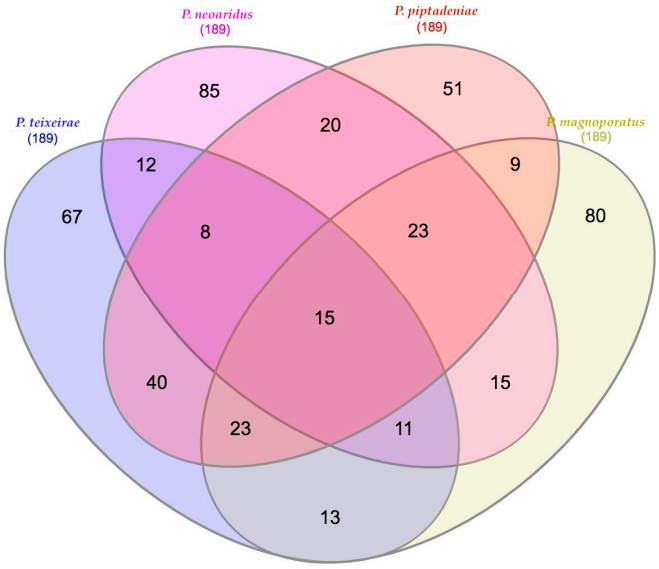

Figure 6. Multi-dimensional Venn diagram (*s.l.* Euler diagram) including four wood-decay *Phellinotus* species analyzed using 189 SNP loci recovered in Tassel v5 [49] from the concatenated dataset (ITS–nLSU) at MAF $\geq$ 0.05. Ellipses are colored according to species. Intersections indicate the number of SNPs shared among groups. Unique SNPs are in the no-intersection zones.

3.7. Network Analysis and Haplotype Distribution of Phellinotus Species

To detect the number of haplotypes per species and their distribution in the region, haplotype network reconstructions were made for ITS (Figure 7a) and nLSU (Figure 7b) separately marking the sampling origin of the four *Phellinotus* species. For ITS and LSU loci, the same species were included, except for LSU in which *P. xerophiticus* was additionally incorporated. A total of twenty haplotypes were identified for the ITS locus, distributed into nine haplotypes for *P. piptadeniae*, seven for *P. teixeirae*, three for *P. neoaridus*, and one for *P. magnoporatus*. For the LSU locus, twenty-two haplotypes were recognized, including nine for *P. piptadeniae*, six for *P. teixeirae*, four for *P. neoaridus*, two for *P. xerophiticus*, and one for *P. magnoporatus*. Haplotype distribution for ITS and LSU indicated a high frequency across countries for *P. piptadeniae* and *P. teixeirae*. Regarding the country of origin, the identified notes displayed a clear distinction between Brazilian and Uruguayan isolates for *P. piptadeniae* at the ITS locus. For *P. teixeirae*, the Colombian isolates were discriminated through the two loci, generating five nodes for ITS and three nodes for LSU. Interestingly, by following the haplotype connections (i.e., mutational events between pairs of nodes) over the geographic regions, it was possible to identify that the isolates of *P. teixeirae* registered for Colombia in the present study were closely related to the haplotypes reported in Peru and Brazil according to the ITS, and to the ones from Peru, based on the LSU.

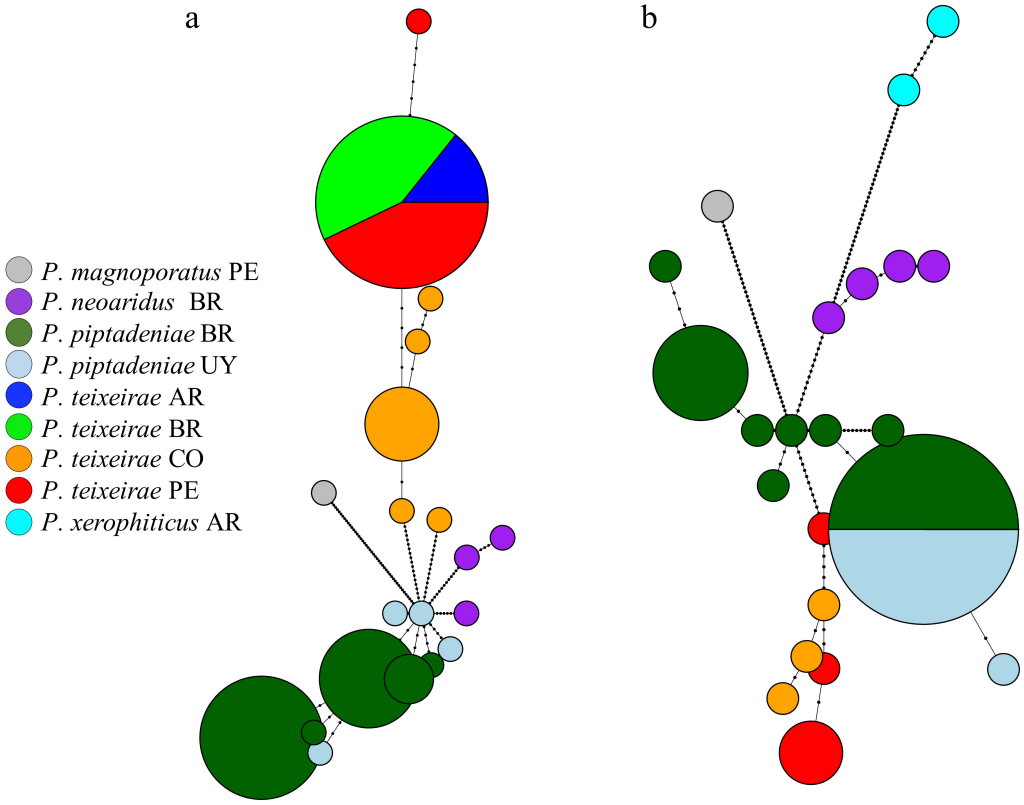

Figure 7. Haplotype networks of genetic polymorphism in the (**a**) ITS and (**b**) LSU ribosomal DNA sequences in the 'Phellinotus Clade'. Four species were included for ITS and five for LSU networks, respectively (insets). Nodes represent haplotypes and their size is relative to their frequency. Small black dots in the connecting lines between pairs of nodes represent mutational events among haplotypes. Nodes are colored according to taxonomy and origin. *P. magnoporatus* (grey), *P. neoaridus* (purple), *P. piptadeniae* (dark green—Brazil, light blue—Uruguay), *P. teixeirae* (blue—Argentina, light green—Brazil, red—Peru, orange—Colombia), and *P. xerophiticus* (cyan).

4. Discussion

Our phylogenetic analysis of the combined dataset across four loci (ITS, nLSU, TEF1-α, and RPB2) recovered the 'Phellinotus Clade' genera (i.e., *Arambarria, Inocutis, Fomitiporella s.s.*, *Fulvifomes, Phylloporia* and *Rajchenbergia*) as a monophyletic group with high support, corroborating the hypothesis proposed by Pildain et al. [6] and Salvador-Montoya et al. [2]. Yet, in the current study the genus *Phellinotus* was discovered to form a sister clade with *Rajchenbergia*, which also presents a dark line and mycelial core in the context, but differs through having a monomitic hyphal structure in the basidiomata [7]. In contrast, Salvador-Montoya et al. [2] showed that *Phellinotus* forms a sister clade with *Rajchenbergia* + *Fomitiporella s.s.*, which indicates that it is still necessary to add different molecular markers for future analysis to further elucidate the underlying phylogenetic relationships of *Phellinotus* within the clade.

The genus *Phellinotus* is considered a pathogenic polypore, commonly living on members of the family Fabaceae. Morphologically speaking, species of *Phellinotus* are classified and can be recognized through the presence of a dark line, a mycelial core, a dimitic hyphal structure with skeletal hyphae only present in the trama of the tube layer and adaxially flattened, ellipsoid, and thick-walled, the absence of setae or setal hyphae, and pale yellow basidiospores that turn chestnut in KOH solution [2]. In Hymenochaetaceae, *Phellinotus* was traditionally considered a genus with a Neotropical distribution based on phylogenetic and phylogeographic analyses [3]. However, Salvador-Montoya et al. [2], based on morphological analysis of *P. badius* (Berk. ex Cooke) Salvador-Montoya, Popoff and Dreschler-Santos, *P. resinaceus* (Kotl. & Pouzar) Salvador-Montoya and Dreschler-Santos, and *P. scaber* (Berk.) Salvador-Montoya, Dreschler-Santos and Popoff, reported the expanded distribution of the genus further into the tropical and subtropical climatic zones of America and Australia.

Species of *Phellinotus* have been widely studied in South America (*i.e.*, [1–3,7], mainly *P. Piptadeniae*). Recently, Salvador-Montoya et al. [2] showed that *P. teixeirae* conforms an entirely different entity from *P. piptadeniae s.s.*, and showed that the former is distributed within the limits of SDTFs in South America, growing on living tree species of the Fabaceae family such as *Pithecellobium excelsum*, *Libidibia glabrata*, and *Pityrocarpa moniliformis*. These three species are distributed within the limits of SDTFs, which makes them ecologically related to some degree [50]. Hence, several authors have considered *P. teixeirae* as an SDTF host specialist. In this study, *P. teixeirae* was recorded growing on *Pithecellobium dulce,* one of the most representative trees in the SDTF biome in the inter-Andean valley of Valle del Cauca, Colombia. *Pithecellobium dulce* is commonly and locally known as '*Chiminango*'. It is a tree that has several uses, such as for living fences, posts, shade, medicine, utensils, firewood [51], edible fruits, construction, fodder [52,53], and in the urban arborization of many cities in Colombia.

Morphologically, *P. teixeirae* differs from *P. piptadeniae s.s.* because the latter presents a lobulated and cracked olive-gray pileal surface with deep concentric furrows and broad lobes. The morphological patterns of the pileal surface have been documented as useful characteristics to distinguish species in the Hymenochaetaceae (i.e., *Fulvifomes* species; [8]). Furthermore, *P. piptadeniae s.s.* is distributed both in dry and wet forests in eastern South America, unlike *P. teixeirae*, considered as an SDTF host habitat specialist (i.e., it grows restrictedly in SDTF habitats [2]). The current results reinforce that specimens and taxa from other unexplored regions of the world should continue being included in phylogenetic analysis to understand in more detail the diversity of the genus and the natural classification of Hymenochaetaceae.

4.1. Patterns and Processes of Genetic Diversity in Phellinotus Species

Due to uncertainties in the species classification within the 'Phellinotus Clade', the application of molecular markers has provided a more precise assessment of species assignation and diversity [6], especially considering recently discovered species [3], and the developing understanding of the ecology and evolution of these complex taxa for which morphological validation is often lacking. The phylogenetic studies on the 'Phellinotus

Clade' [1–3,5,54] demonstrated instability, although the application of several morphological and molecular descriptors has provided more robust alternative inferences. The application of multi-locus molecular markers is a tool that can potentially further unveil fungal phylogenetic patterns [6]. Here, we have reported an alternative perspective on genetic diversity in the 'Phellinotus Clade' by screening the most common loci used for fungal molecular systematic analysis [55].

Multiple molecular approaches have been utilized to understand the diversity of the recently discovered genus *Phellinotus* [3], which is proposed to be part of a species complex including still unresolved taxa (i.e., *Fomitiporella*, *Fulvifomes*, *Inocutis*, and *Phylloporia*). Fortunately, highly supported phylogenetic nodes in our multi-locus reconstructions reinforce the monophyletic hypothesis of the clade [6]. The specimens all coincided in showing high variability at the surveyed loci, as previously reported in other phylogenetic analyses [3]. These molecular markers provide robust species delimitations for *P. piptadeniae* and *P. teixeirae*. To continue unveiling the patterns of genetic diversity and identification of new *P. teixeirae* strains, an alternative analytical approach, not only for *P. teixeirae* but also for the 'Phellinotus Clade', was incorporated, As detailed below.

Interestingly, high nucleotide and haplotype diversity, and inflated Tajima's D scores at the rDNA loci are indicative of divergent selection as part of the population expansion towards the northern Andes while involving colonization and adaptation to new hosts [56]. These levels of nucleotide diversity could, in turn, harness species delimitation between the closely related taxa *P. piptadeniae* and *P. teixeirae*. Similarly, the high nucleotide diversity found in *P. piptadeniae* indicates that clusters of divergent sequences occur within the species, possibly as a consequence of geographic distance, environmental local adaptation (considering its disjunct distribution [1]), divergent host interactions, or ancient intraspecific rDNA polymorphisms [57,58]. For *P. teixeirae*, the nucleotide variation was very low between our isolates and previous reports, despite the new host interactions with *Pithecellobium dulce*. These contrasting outcomes have been identified in eukaryotic genomes [59–63], particularly coupled with considerable variation in gene copy number at rDNA loci in fungal species [57,58,64,65] and intragenomic variation at these loci [58,64,65]. The dynamics of these variations complicate the analysis of rDNA amplicons, being a critical factor for species delimitation, especially for microbial communities in heterogeneous environments [6]. Additionally, the persistence of rDNA variants through lineages that exhibit optimal fitness to spread over new hosts and distant geographic regions has been found to be the result of purifying selection and concerted evolution [60,61,66,67].

Through analyzing the haplotype networks of the most representative species in this study, i.e., *P. piptadeniae* and *P. teixerae*, it was found that *P. piptadeniae* was the basal haplotype. The reticulated network spread outwards from a single *P. piptadeniae* haplotype, involving few mutational points among *P. piptadeniae* isolates, yet considerably increasing from closely (i.e., *P. teixeirae* and *P. neoaridus*) to highly (*P. magnoporatus* and *P. xerophiticus*) divergent sequences. The patterns of the haplotype network found for *P. teixeirae* in Colombia are closely related to the isolates from Peru, exhibiting possible correlations with ecological signatures of new host colonization since the Colombian haplotypes are unique. The newly identified *P. teixeirae* isolates provide clues for the population expansion hypothesis towards the northern Andes following the geographic equivalency [1] associated with the SDTF [5]. The identification of dominant, widespread haplotypes for *P. teixeirae* and *P. piptadeniae* correlates with the assumption of new variant acquisition with potential adaptation to novel hosts and environments [68], especially in highly tree-species-diverse Neotropical ecosystems [69].

Although some of these fungal species have been reported to parasite specific hosts [5,54], with a narrow distribution range [3], this perspective is changing based on the integration of new species into the genus [2], host records [1,5], and the identification of new strains supporting its potential broad distribution. New *Phellinotus* records indicate a broad spectrum of tree species serving as hosts, with a predominant parasitic behavior for species of the Fabaceae family, probably a result of its geographical predominance in the SDTF biome.

4.2. Perspectives

More intense sampling is necessary to support the population expansion hypothesis and the possible genetic adaptation to new hosts. Considering the defense mechanisms of plants driven via secondary metabolites [70], these fungal species are particularly interesting as model organisms to study the metabolic strategies underlying parasitism on novel hosts. Also, mapping the genetic variation responsible for host colonization could provide a more detailed comprehension of the population diversity patterns in *Phellinotus* species, and whether they are congruent with selection pressures acting on *de novo* mutations or recruiting standing adaptive variation. Assessing these scenarios would help build a more cohesive understanding of the mechanisms favoring new host colonization in the face of climate change and the rapid niche variation in the SDTF [71].

The application of novel sequencing technologies further provides an alternative method to study the genetic traits under selection during a co-evolving parasitic biotic interaction [72], with potential detection of species-specific genes, metabolites, and interspecific gene transfer among closely related species [70,73]. Although the application of barcoding genes has been useful for species identification in genetically structured populations [74], more notably for some groups of fungi than others [75], exploration of additional loci may also have potential applications for sub-generic-level identification [76] in intricate taxa, in addition to accounting for [77] and reconstructing [78] the complexity of the underlying demographic histories.

5. Conclusions

Pithecellobium dulce (Fabaceae) as a new host tree of the wood-decay fungus *P. teixeirae* in the northern Andes broadens the perspective of the potential distribution of the recently discovered *Phellinotus* genus in the Neotropics, extending its distribution range 1000 km north. These results suggest that *Phellinotus* species could be undergoing population expansion following the geographic equivalency associated with the SDTF biome, making the group phylogenetically complex, and potentially subject to multiple local selection pressures driving adaptation. The incorporation of new technologies in the study of this taxa would provide new insights for the discovery of new species and their potential adaptation to new environments.

Author Contributions: A.C.B.-R. conceived and designed the project, directed all field sampling, and collected fungal material. A.C.B.-R. and V.M.-V. carried out all the laboratory procedures including strain isolation, morphological characterization, and molecular evaluation. A.C.B.-R., V.M.-V. and J.M.L.-C. analyzed all sequence data, and together with A.J.C., performed the interpretation of the genetic results. A.C.B.-R., V.M.-V., J.M.L.-C., and A.J.C. contributed to the overall discussion of the results, as well as to the writing of the manuscript. A.C.B.-R., in her role as principal investigator (PI), provided all the resources required to accomplish the research, and supervised the execution of the project. A.J.C. also arranged funding resources to cover the articles' APC. All authors have read and agreed to the published version of the manuscript.

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