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Finding Spalting Fungi in the Peruvian Tropical Premontane Cloud Forest on Peruvian Native Wood Species

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Abstract: Tropical montane and premontane forests are diverse, including fungi. However, little is known about spalting fungi (decay fungi that change the color of wood) in tropical regions despite the economic importance they could bring by enhancing wood esthetics. To increase the knowledge of the diversity of spalting fungi, a sampling of fallen logs, branches (exposing xylem to identify potential pigmenting and zone line-producing fungi), and fruiting bodies (on wood) was conducted in the premontane moist forest in the district of San Ramon, Junín, Peru. The fungi were collected, cultured, isolated, and sequenced. Also, the identified species were used in a novel test to confirm they were producing spalting on *Guazuma crinita*. The species found belong to the Ascomycota orders Xylariales and Diaporthales and the Basidiomycota orders Agaricales, Polyporales, and Russulales. The fungi collected produced bleaching, different colors of zone lines, and pigmentation in laboratory conditions. The results increase the database of spalted fungi in Peru, and the test used in this research could be the basis for a quick test to identify spalting fungi.

Keywords: pigmenting; spalting; decay fungi; Peru; tropical premontane moist forest

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1. Introduction

Tropical forests are the most diverse ecosystems on earth [1]. The montane and premontane tropical forests are characterized by high precipitation levels, cloud persistence, and altitude variations along mountain ranges that influence species dominance and vegetation structure [2]. These types of forests belong to life zones of the same name classified by Holdridge [3,4] and are recognized as areas of high species richness and endemism [2]. In Peru, the premontane forest shares species with lowland Amazonian forests; both types of forests are characterized by dense evergreen and an even, closed canopy, the main difference being the greater biomass of epiphytes in the premontane forest [5,6]. In the premontane zone of the Chanchamayo Valley (Junín, Peru), located in the Andean region, the Leguminosae, Moraceae, and Lauraceae are the predominant botanical families. The forests situated at an altitude lower than 2000–2500 m above sea level (masl) are characterized by a lower tree diversity due to the higher humidity and rainfall [7] than the higher part of the valley, which is a cloud forest. Some studies show macrofungal diversity in tropical premontane and montane forests [8–10]. However, the diversity of spalting fungi in this region still needs to be explored.

Spalting fungi are a group of wood-decay organisms that modify the natural color of the wood [11]. Spalting can be classified into three types: bleaching, pigmenting, and zone lines.

Bleaching is mainly caused by white rot fungi, such as *Trametes versicolor* (L.) Lloyd, which initially metabolizes the lignin and leaves the cellulose almost intact, creating light-

ened areas and giving this white appearance using class II peroxidases [12,13]. This does not occur in brown rot fungi (e.g., *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss, and Schigel) since they possess only a few nonligninolytic, low-redox potential generic peroxidases and use non-enzymatic strategies by Fenton reaction [13–15] to primarily degrade carbohydrates of the cell wall and leave only a slightly modified lignin [16,17]. Another possibility for the lightening color is the accumulation of white mycelia in wood [18].

Pigmenting could be caused by hyphae or the diffusion of fungal pigments into the substrate [12,19], where they assimilate available nutrients, mainly the nonstructural components of the wood [20], and cause soft rot [21,22]. This pigmentation is not uniformly distributed where fungi grow. It is found in ray parenchyma cells, vessels, and fibers adjacent to ray parenchyma [23]. Fungi produce a range of pigments, such as carotenoids, melanins, flavins, phenazines, quinones, monascins, violacein, and indigo [24,25]. Compounds produced by pigmenting fungi are primarily naphthoquinones [26], including various derivatives of xylindein [23] and the red and orange crystals called "dramada" [27].

Zone lines are created as a mechanism to protect and/or maintain their resources within a sector [28], as well as a response to changes in moisture content [29] and atmospheric conditions [30]. Another cause for the development of zone lines is the presence of decay fungi with different genetic makeup [31], which includes somatic antagonism [32]. Zone lines consist of densely packed hyphae in the lumina of tracheids and fibers [33]. Melanin is the most common pigment present in these lines, giving them their characteristic black/brown color. Still, other secondary metabolites may also be present, giving them different colors, such as orange and red [31,34]. Zone lines can be produced by wood decay fungi belonging to Basidiomycetes and Ascomycetes, differentiating by the mechanism of melanin production. Basidiomycetes go through the DOPA-pathway, which includes cysteinyl-DOPA, resulting in eumelanin and pheomelanin, respectively, whereas ascomycetes use the DHN-pathway, resulting in allomelanin and pyomelanin [31,35,36].

Spalted wood dates to the 14th century in Europe, when artists used this material in marquetry and intarsia [23,37]. These wood artworks have maintained their esthetic characteristics (zone lines, pigmentation, and bleaching) up to this date, indicating the capability of fungal-based wood color modification to be highly stable over the centuries [38]. Spalting in woodwork has been pointed out as a desirable appearance feature [39] used by woodturners because of its unique appeal [40] and potential to add value to some secondary products, such as decorative wood pieces, for which consumers are willing to pay premium prices [41,42]. The use of spalted wood in South America has yet to be documented in detail compared to existing information in the Northern Hemisphere. Still, one study points to the presence of spalted wood in European furniture found in Chile and Peru [42]. In Peru, natural forests are the suppliers of the wood industry, with 70% of the market volume coming from just ten species [43]. In this context, spalting represents an option to add value to species discarded in sawmills.

Currently, there are two previous studies related to spalting fungi in Peru. A study on *Simarouba amara* Aublet, *Brosimum alicastrum* Swartz, and *Matisia cordata* Bonpland, which are Peruvian commercial woods, proved that these wood species could be spalted in laboratory conditions by pigmenting fungi *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl., *Cladosporium herbarum* (Pers.) Link, and *Nigrospora sphaerica* (Sacc.) E.W. Mason [44]. The second study identified spalting fungi reported in the southern Peruvian Amazon forest, which included the orders Botryosphaeriales, Helotiales, Hypocreales, Polyporales, Russulales, and Xylariales [34].

This research aims to increase the knowledge of spalting in the Peruvian territory by identifying fungal species collected in a premontane forest. The collection was carried out in the premontane moist forest of the Chanchamayo Valley. The collected fungi were used in a visual test with *Guazuma crinita* Martius, a fast-growing hardwood species characterized by moderate durability and diverse applications [45,46], to determine their suitability for future research.

2. Materials and Methods

2.1. Location

The fungi were collected from a tropical premontane moist forest [47] at elevations between 800 and 1200 masl, specifically from the Génova station field of the Universidad Nacional Agraria La Molina (UNALM) located in the San Ramon district, Chanchamayo province, Junín region, Peru. The climatic condition of the Génova is characterized by an annual average temperature of 23.1 °C, the highest average temperature between October and November corresponding to 30.1 °C and the lowest in July at 16.7 °C [48]. The average annual total precipitation is approximately 2000 mm, with two clear seasons: the low precipitation season from June to August and an abundant season from December to May [48,49]. The collection took place on 21 and 22 November 2020.

2.2. Collection

The collection was carried out by following a methodology previously applied to spalting fungi sampling in Peru [50]. The collection was made in two phases. The first started from the nearest trail to the field station, and the second was conducted by following the creek near the field station, starting from the place with better accessibility. Both were made within 8 m perpendicular to the road due to safety concerns. Fallen logs and branches with a diameter greater than 5 cm and fruiting bodies were collected. After removing debris (moss, dirt, etc.) and the bark, the wood samples were cut with a machete at approximately 45 degrees until reaching the xylem, as seen in Figure 1. The type of spalting and characteristics of each sample (including color, size, and origin) were recorded. These samples were then placed into brown paper bags labeled with a unique ID code. Samples from the same log were marked with the same letter but differentiated by a number. The labeled bags were subsequently packed in a plastic bag for transport back to the field station. GPS waypoints were recorded for each sample using a Garmin eTrex 10 (Garmin Ltd, Olathe, KS, USA).

Figure 1. Spalted wood sample collected in the Génova, Junín, Peru. (A) Undecayed wood, (B) bleached wood, (C) black zone lines, (D) orange zone lines.

2.3. Media Preparation

Potato dextrose agar (PDA) and malt extract agar (MEA) were prepared based on the UNALM wood preservation lab standards. PDA was made with 250 g boiled potato infusion, 20 g dextrose anhydrous AR (CDH, Delhi, India), 15 g agar powder bacteriological (CDH, Delhi, India), 1 L distilled water, and 50 mg chloramphenicol. MEA was made with 20 g malt extract (IDSA, Lima, Peru), 15 g agar powder bacteriological (CDH, Delhi, India), 1 L distilled water, and 50 mg chloramphenicol. A total of 15 mL screw cap culture tubes with phenolic caps (PYREX, Charleroi, PA, USA) filled with 10 mL of PDA slant were taken to the collection.

2.4. Isolation

The samples were processed on the same day they were collected at the field station. The station did not have a laminar flow hood, so the area where the isolation was performed was cleaned and prepared to avoid contamination of the cultures. The samples were soaked in bleach and distilled water (2%) for two minutes and then rinsed in sterile distilled water for ten minutes. The scalpel blade and forceps were sterilized with 96% ethanol and flame from an alcohol lamp. Using sterilized tools, tissue isolations were obtained from the inner part of each sample (wood or fruiting bodies). The following process was cutting with a scalpel blade and removing the tissue with forceps. For samples with pigments, isolations were taken exclusively from the colored areas, while for samples with zone lines, the tissue was taken from each side of the zone line. The extracted pieces from the newly exposed area were introduced into PDA culture tubes.

Samples were transported to the Wood Protection Laboratory at UNALM in Lima, Peru. The cultures were transferred onto sterile 60 × 15 mm Petri dishes (SPL Life Sciences, Gyeonggi-do, Republic of Korea) containing PDA. This process was repeated until the cultures were free of contaminants such as *Penicillium* spp., *Trichoderma* spp., and *Aspergillus* spp. Once the cultures were deemed pure, a visual evaluation was performed on each plate. This evaluation consisted of observing if the fungi developed zone lines or pigments in the culture media and comparing them to the samples where they were collected in the field [34,50]. Based on previous papers [51,52], the fungi were then moved to 2% malt extract agar (MEA).

2.5. Identification

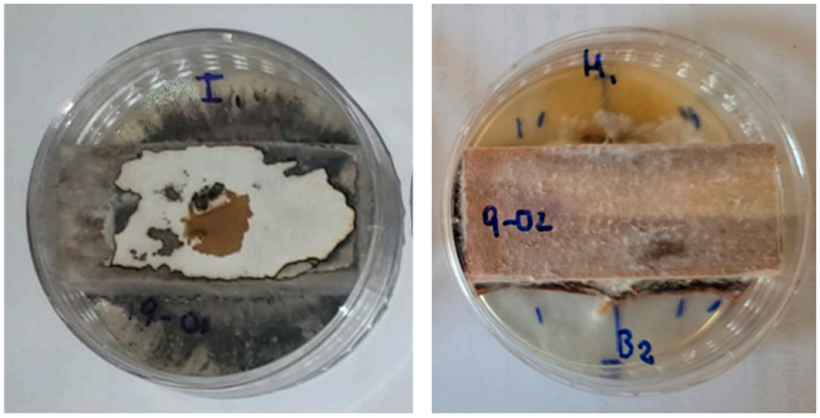
The cultures were sent for sequencing to the Laboratory of Mycology and Biotechnology at UNALM, where Sanger sequencing was performed, and the Internal Transcribed Spacer (ITS) region was amplified using the Macrogen ITS1-F and ITS4 primers. The results were analyzed using SnapGene Viewer software (version 4.1.9), which facilitated the selection of the sequences. Then, they were compared to the database on the BLAST® (basic local alignment search tool) webpage [53] to identify the samples. The identification was based on the highest similarity sequence in the 90%–100% range, a query cover of 90%–100%, and the geographical location of the compared sequence. Additionally, the morphological description was considered when the fruiting body was used for isolation.

2.6. Spalting Potential Test

Using the isolated cultures, a test was conducted to determine if the fungi could produce spalted wood. The species "Bolaina blanca" (*Guazuma crinita* Martius, specific gravity = 0.4), a Peruvian wood species, was used for the testing. The wood was harvested from an 8-year-old forest plantation in a lower tropical wet forest [54] located in the Palcazu district, Oxapampa province, Pasco region, approximately 330 m above sea level, and cut into feeding strips measuring 0.8 × 2 × 5 cm.

The wood strips were soaked in distilled water for 72 h and then sterilized in an autoclave for 30 min at 121 °C. The sterilized wood was placed in the middle of Petri dishes containing MEA. The inoculated Petri dishes were placed in an incubator set to 28 ± 2 °C and at an average RH of 60% in dark conditions for six weeks. Two tests were conducted

per culture: a "single" test and a "paired" test, each with two replicates. For the "single" test, the inoculum was placed in the center of the wood, as seen in Figure 2a. For the "paired" test, different inocula were placed on each side of the wood, ensuring they were in contact with the media, as seen in Figure 2b.



(**a**) (**b**)

Figure 2. (**a**) Culture plate *of Xylaria apiculata* growing on *Guazuma crinita* feeding strip during the test; (**b**) culture plate of *Stereum sanguinolentum* vs. *Polyporus tricholoma* growing on *Guazuma crinita* feeding strip during the test.

After six weeks, the feeding strips were removed from the Petri dishes, cleaned, dried at 45 ± 2 °C for 24 h, and visually inspected to observe any surface changes in the natural color of the wood.

3. Results

3.1. Isolation

A total of 34 samples were collected from the field. Due to the isolation conditions, a low rate of successful isolations was expected. Twenty-six tubes containing growing mycelium arrived at the UNALM. After further purification verifying that the isolate attempts were not contaminated with bacteria and other environmental mold fungi, only thirteen fungal species were successfully isolated. The collected fruiting bodies were morphologically identified and compared with existing UNALM Wood Protection Laboratory samples. Only *Pycnoporus sanguineus* (L.) Murrill was identified this way and was not sent for sequencing as it was not used in the test.

3.2. Identification

Twelve cultures that showed spalting potential were sent for sequencing, the results of which are shown in Table 1.

Table 1. Fungi identified from La Genova.

Spalting Type Observed	Culture Source	Order	Genus	Species	GenBank Accession Number	GenBank Accession Number-Compare	ID % Similarity
Bleaching	wood	Agaricales	*Gymnopilus*	*Gymnopilus* sp.	PQ344248	MH267957.1	100%
Orange zone line	wood	Polyporales	*Polyporus*	*Polyporus tricholoma* Mont.	PQ344249	HQ248223.1	99.14%
Bleaching, black zone line	wood	Agaricales	*Oudemansiella*	*Oudemansiella canarii* (Jungh.) Höhn.	PQ344255	ON426446.1	99.58%

Table 1. *Cont.*

Spalting Type Observed	Culture Source	Order	Genus	Species	GenBank Accession Number	GenBank Accession Number-Compare	ID % Similarity
Bleaching, orange zone line	wood	Agaricales	*Oudemansiella*	*Oudemansiella canarii* (Jungh.) Höhn.	PQ344256	ON426446.1	98.69%
Black zone line, dark yellow pigment	wood	Russulales	*Stereum*	*Stereum sanguinolentum* (Alb. and Schwein.) Fr.	PQ344257	PP102352.1	100%
Black zone line, dark yellow pigment	wood	Xylariales	*Annulohypoxylon*	*Annulohypoxylon stygium* (Lév.) Y.M. Ju, J.D. Rogers and H.M. Hsieh	PQ344258	EU272517.1	99.88%
Black zone line	fruiting body	Xylariales	*Xylaria*	*Xylaria apiculata* Cooke	PQ344260	KP133330.1	100%
Black zone line	wood	Xylariales	*Nemania*	*Nemania* sp.	PQ344263	MH268005.1	100%
Black zone line	fruiting body	Xylariales	*Xylaria*	*Xylaria tuberoides* Rehm	PQ344264	OQ872047.1	100%
Bleaching, black zone line, dark yellow pigment	wood	Diaporthales	*Diaporthe*	*Diaporthe ampelina* (Berk. and M.A. Curtis) R.R. Gomes, Glienke and Crous	PQ344266	KC343016.1	94.29%
Black zone line	wood	Diaporthales	*Diaporthe*	*Diaporthe* sp.	PQ344251	MW775485.1	99.62%
Black zone line, red pigment	fruiting body	Xylariales	*Xylaria*	*Xylaria guianensis* (Mont.) Fr.	PQ344253	MW340809.1	100%

3.3. Spalting Potential Test

The results describing the type of spalting observed in *Guazuma crinita*, after six weeks of incubation, caused by each fungus in the "single" test, are shown in Table 2. The images showing the "single" spalting potential test results can be seen in Figure 3, with Figure 3A showing a control.

The results of the potential spalting tests in *Guazuma crinita* after six weeks of incubation, which show the type of spalting observed caused by the fungus paired against two other fungi, are shown in Table 3. The pairing of fungal species was random. The test was shortened because of the limited availability of plantation wood strips and mainly because the objective was to observe when the fungus was in the "single" test. Also, the "paired" test was carried out without the *Xylaria* genus because it is known to produce zone lines without pairing, as described in Table 2. The images of the "paired" spalting potential test results are shown in Figure 4.

Table 2. Performance of fungi in *Guazuma crinita* after six weeks.

Species	Spalting Type Observed
Xylaria apiculata	Bleaching, black zone lines, and pigmentation (Figure 3B)
Oudemansiella canarii	Bleaching, brown to orange zone lines (Figure 3C)
Polyporus tricholoma	Bleaching, brown to orange pigmentation (Figure 3D)
Diaporthe sp.	Bleaching, black zone lines, black pigmentation (Figure 3E)
Annulohypoxylon stygium	Bleaching, black zone lines, and pigmentation (Figure 3F)
Stereum sanguinolentum	Brown to orange pigmentation (Figure 3G)
Gymnopilus sp.	Bleaching (Figure 3H)
Diaporthe ampelina	Bleaching, black zone lines, and pigmentation (Figure 3I)
Nemania sp.	Black pigmentation (Figure 3J)
Xylaria tuberoides	Bleaching, black zone lines, and pigmentation (Figure 3K)
Xylaria guianensis	Bleaching, black zone lines (Figure 3L)

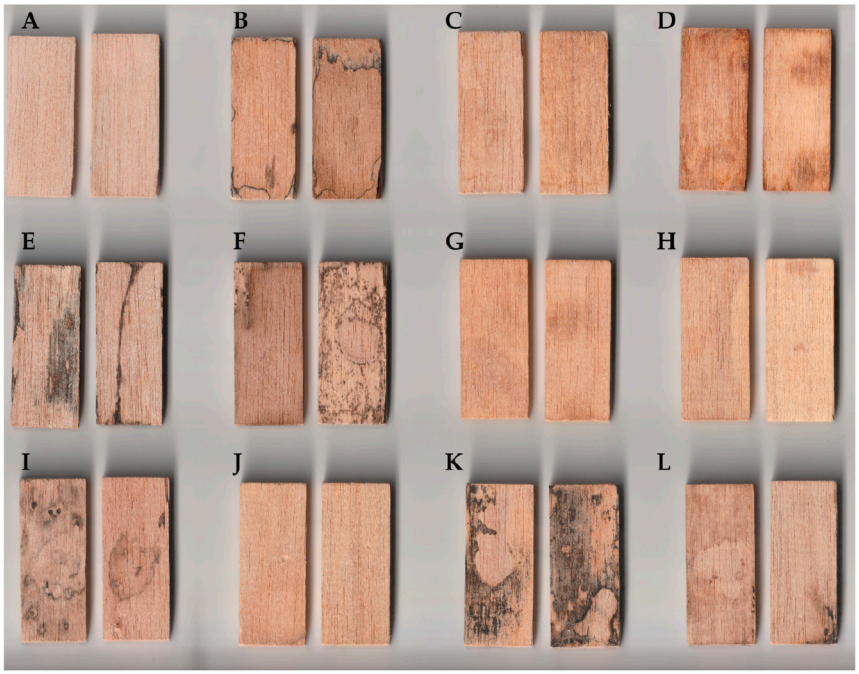


Figure 3. Spalting type observed caused by each fungus. (**A**) Feeding strip without inoculation, (**B**) *Xylaria apiculata*, (**C**) *Oudemansiella canarii*, (**D**) *Polyporus tricholoma*, (**E**) *Diaporthe* sp., (**F**) *Annulohypoxylon stygium*, (**G**) *Stereum sanguinolentum*, (**H**) *Gymnopilus* sp., (**I**) *Diaporthe ampelina*, (**J**) *Nemania* sp., (**K**) *Xylaria tuberoides*, and (**L**) *Xylaria guianensis*.

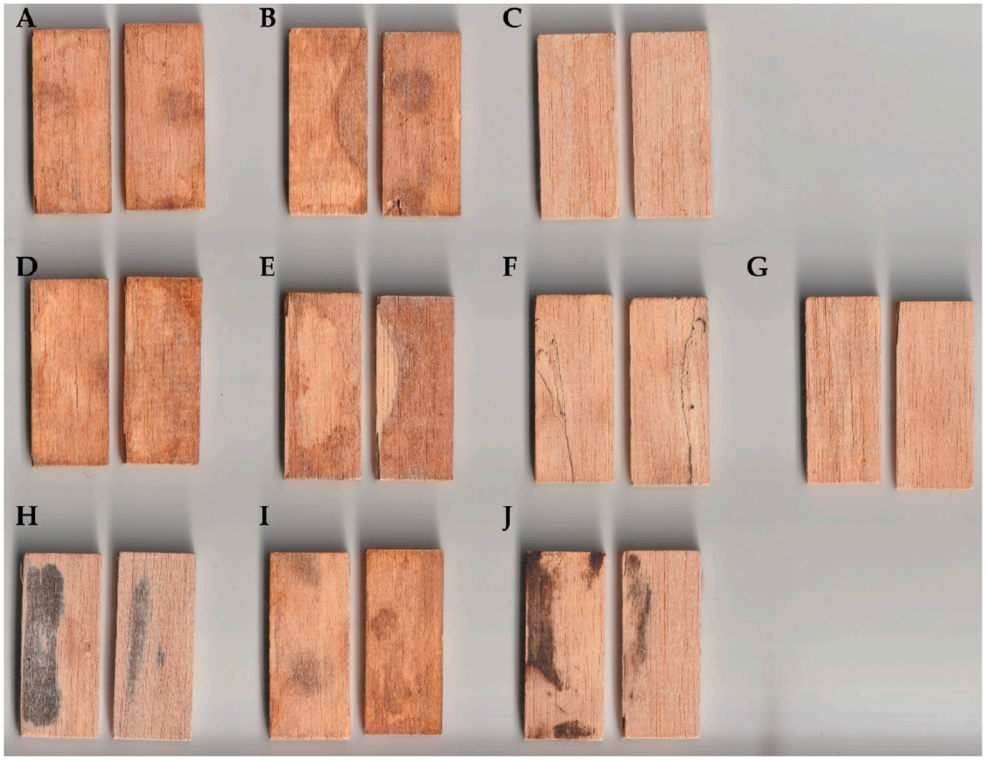


Figure 4. Spalting type observed caused by the pairing of different fungi. (**A**) *D. ampelina* vs. *P. tricholoma*, (**B**) *P. tricholoma* vs. *S. sanguinolentum*, (**C**) *O. canarii* vs. *S. sanguinolentum*, (**D**) *P. tricholoma* vs. *Nemania* sp., (**E**) *Gymnopilus* sp. vs. *P. tricholoma*, (**F**) *Gymnopilus* sp. vs. *Nemania* sp., (**G**) *D. ampelina* vs. *S. sanguinolentum*, (**H**) *S. sanguinolentum* vs. *Diaporthe* sp., (**I**) *O. canarii* vs. *P. tricholoma*, and (**J**) *S. sanguinolentum* vs. *A. stygium*.

Table 3. Performance of pairing fungi in *Guazuma crinita* after 6 weeks.

Species	Spalting Type Observed
D. ampelina vs. *P. tricholoma*	Brown to orange zone lines and pigmentation, black pigmentation (Figure 4A)
P. tricholoma vs. *S. sanguinolentum*	Bleaching, brown to orange zone lines, and pigmentation (Figure 4B)
O. canarii vs. *S. sanguinolentum*	Bleaching, brown to orange zone lines (Figure 4C)
P. tricholoma vs. *Nemania* sp.	Bleaching, black zone lines, brown to orange zone lines, and pigmentation (Figure 4D)
Gymnopilus sp. vs. *P. tricholoma*	Bleaching, brown to orange zone lines, and pigmentation (Figure 4E)
Gymnopilus sp. vs. *Nemania* sp.	Bleaching, black zone lines (Figure 4F)
D. ampelina vs. *S. sanguinolentum*	Bleaching, brown to orange pigmentation (Figure 4G)
S. sanguinolentum vs. *Diaporthe* sp.	Bleaching, black pigmentation (Figure 4H)
O. canarii vs. *P. tricholoma*	Bleaching, brown to orange zone lines, and pigmentation (Figure 4I)
S. sanguinolentum vs. *A. stygium*	Black zone lines, black pigmentation (Figure 4J)

The most common order found was Xylariales, which included the genera *Xylaria*, *Nemania*, and *Annulohypoxylon*. Agaricales includes the genera *Gymnopilus* and *Oudemansiella*, which were isolated twice. The other orders identified were Diaporthales, Polyporales, and Russulales, with a single identified genus.

4. Discussion

4.1. Isolation and Identification

The predominant order found was Xylariales. Their ability to produce melanin was observed throughout the genera. The samples collected and their cultures in laboratory conditions increase confidence in *Xylaria* to produce zone lines [33,55,56].

Xylaria is the most representative genus of Ascomycota found in a macrofungal community evaluation in a lowland tropical/subtropical moist forest in southern Peru [57], and their presence in spalted wood pieces in the Peruvian territory was previously reported [34]. *Xylaria tuberoides* have been collected and sequenced from tropical lowland forests in Costa Rica and Peru [57,58]. *Xylaria guianensis* is also present in the southern Peruvian Amazon rainforest [34,59]. The presence of *Xylaria apiculata* has been reported in Ecuador [60,61] and Colombia [62], and the DNA sequences obtained in the present study also report their presence in the Peruvian territory. The results obtained in this study show bleaching and black zone line formation in the wood when *Xylaria* grows alone due to its inherent somatic incompatibility [32].

Nemania primarily inhabits decaying wood of angiosperms and was closely related to *Xylaria* [63]. Recent phylogenetic studies group species of *Entoleuca*, *Euepixylon*, *Nemania*, and *Rosellinia* into the clade NR [64]. *Nemania* is a plurivorous genus hosted by different tree families, and species found in the Brazilian and Peruvian Amazon rainforests have been sequenced [65,66]. The results show this species needs pairing with other fungi for zone line production, as research shows the creation of zone lines is promoted when a certain fungus is paired [19,51,67].

Annulohypoxylon stygium is a pantropical species commonly found on dying branches in the canopy, and it can also thrive on dead wood after the initial colonization [68]. This genus is often found in collections in the Brazilian Amazon [69]. Results show that *A. stygium* can produce black zone line formation and stain regardless of whether it is paired.

In the order Agaricales, all the samples produced bleaching on wood. *Gymnopilus* is found in the Brazilian and Peruvian Amazon and is related to decayed wood [57,66,70]. The results suggest *Gymnopilus* sp. could be used as a promoter of spalting for other fungi that need pairing. *Oudemansiella canarii* is a wood-decomposing edible mushroom [71,72] that has been reported in the lowland tropical/subtropical moist forest in southern Peru, and the genus can be found in the southern Amazon basin in Brazil [57,59,70]. This species was found in a wood sample with orange zone lines. The evaluation showed that the brown to orange zone lines and pigmentation of the wood with *O. canarii* occur regardless of whether it is paired.

Diaporthe has been reported in the Peruvian Amazon as the most abundant endophytic fungus in the *Hevea* and *Micrandra* genera [66] seeding stems. But these genera have wide host ranges, co-colonizing diseased or dead tissue [73]. *Diaporthe ampelina* causes cane, leaf spots, and infections of pruning wounds of *Vitis* and *Ampelopsidis* spp. [73]. It is the most aggressive in its genus and has been found in several countries [74]. The *Diaporthe* genus shows different spalting behavior depending on the presence of an antagonist; the hypothesis is that *Diaporthe* can colonize in the early stages of decomposition but end up being surpassed by ecological succession in the wood.

Stereum is a common endophyte hosting leaf and sapwood of *Hevea* trees, and some specimens have been reported in the southern Peruvian Amazon rainforest and southern Amazon Basin in Brazil [57,59,70]. *Stereum sanguinolentum* is an important pathogen, a widely distributed, stress-tolerant pioneer, and a combative decomposer that impacts forest management economics [75–78]. It is a white rot fungus that initially produces a reddish-brown, then light brown coloration of the wood [79]. This description is according to the type of spalting observed but has not been previously used for spalting purposes. Based on studies of a species of the same genus, *Stereum hirsutum,* which is easily replaced by other fungi and is related to yellow zone line production [80,81], the results suggest that *S. sanguinolentum* can be used in pairing to enhance the spalting produced by other fungi. For example, pairing *S. sanguinolentum* with *P. tricholoma* produced a more diverse and intense pigmentation. Further testing is required.

Polyporales are primarily white rot fungi [82]. *Polyporus tricholoma* is found on deciduous wood [83] in various parts of the Amazon, from the Andes-Amazon region of southeastern Peru to the southeastern basin in Brazil [57,70,84]. The observation of orange zone lines in the presence of a species of the order Polyporales in the Peruvian territory was previously reported [34], and by following their suggestion of competition testing, results showed *P. tricholoma* caused bleaching, brown to orange zone lines, and pigmentation. The spalting produced by *P. tricholoma* was visually the most evident, thus a future study will focus on this species.

Before placing the fungi in the incubator, an observation made was that the mycelium of *X. tuberoides* was growing very slowly at room temperature (18 + 2 °C). Once in the incubator (28 + 2 °C), it started to grow at a similar rate to the other fungi, indicating that this species has temperature requirements for its proliferation. In contrast, *P. tricholoma* was the fungus with the fastest growth, regardless of temperature and substrate. This growth plasticity could explain its presence in the tropics around the world [85]. Also, the members of the *Diaporthe* genus did not fully colonize the plate, leaving empty spaces outside of where they were inoculated. This observation supports the need to determine the optimal growth temperature and light conditions for tropical spalting fungi [34]. Another interesting result is the back-and-forth between zone lines and pigmentation, consistent among the zone line samples. Zone lines are tridimensional barriers, and the pigmentation could be the boundary or initial stage of formation of the zone line seen from one of the perpendicular axes. Also, surface coloration could be caused by extracellular melanin produced as a response to environmental stress [31].

4.2. Spalting Potential Test

The spalting potential test used in this research was designed as a quick assessment to confirm the ability of the collected fungi to produce spalted wood. Although this test is based on sequencing and visual assessments, it serves as a preliminary guide, highlighting future research directions. Further studies are needed to evaluate the most suitable growth media, like minimal media or vermiculite, as this study used parameters based on previous research in the northern hemisphere [86–89]. Still, they are not specific for tropical fungi. Thus, they could be explored, as there is the possibility that the fungi require more accessible nutrients, given by the media and not from the wood, to develop their spalting capability faster. If applicable, conditions like pH, presence of salts, level, and source of nutrients

such as nitrogen, which have been pointed out as key factors in pigment production in media [90,91], need to be assessed.

Additionally, the duration of the test was based on a previous spalting study on Peruvian woods [44] and needs to be re-evaluated to determine if it can be shortened up to four weeks, as spalting signs have been detected in a previous study with US hardwood species [51]. As this study's goal was to perform a fast-screening method to determine the potential of the fungal species to develop pigmentation in wood, not all of the spalting capabilities of each identified fungi were assessed. Further testing in modified wood decay jars [52] could also be used as a proper evaluation to determine internal spalting.

5. Conclusions

A sampling of spalting fungi from a tropical premontane moist forest between 800 and 1200 m above sea level reveals the predominance of the order Xylariales. Testing with *Guazuma crinita* wood under laboratory conditions shows external spalting similar to those observed in the collected samples from which the fungi were isolated. The test shows the ability of five species from the order Xylariales to produce zone lines. Additionally, *Stereum sanguinolentum*, *Oudemansiella canarii*, and *Polyporus tricholoma* show promising results with the color of their zone lines and pigmentation. Future studies in modified wood decay jars will be performed on the species found in the present study. This study also serves as the first report on *Xylaria apiculata* in Peru.

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