International Journal of Agricultural Technology 2026 Vol. 22(6):2971-2988
DOI: https://doi.org/10.63369/ijat.2026.22.6.2971-2988
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Phenotypic characterization and molecular identification of leaf litter fungi from mango orchard at Central Luzon, Philippines

Waing, K. G. D. [1, 2, 4, 5*], Espiritu, L. M.[1,3] and Dulay, R. M. R.[1,4, 5]

[1]Department of Biology, College of Science, De La Salle University, Taft Avenue, Manila, Philippines; [2]Department of Science and Technology -Science Education Institute, Taguig City, Philippines; [3]Systems and Computational Biology Research Unit, Center for Natural Sciences and Environmental Research, De La Salle University, Malate, Manila, National Capital Region, Philippines; [4]Flora and Fauna, Analytical and Diagnostic Laboratory, Central Luzon State University, Science City of Munoz, Nueva Ecija, 3120, Philippines; [5]Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Munoz, Nueva Ecija, 3120, Philippines.

Waing, K. G. D., Espiritu, L. M. and Dulay, R. M. R. (2026). Phenotypic characterization and molecular identification of leaf litter fungi from mango orchard at Central Luzon, Philippines. International Journal of Agricultural Technology 22(6):2971-2988.

Abstract Leaf litter is an important component of our ecosystem, providing habitats for various organisms. These serve as reservoirs for various fungal species, which can be explored for their importance in medicine, food processing, and crop enhancement. In this study, ten leaf litter fungal isolates were identified and characterized their cultural and morphological characteristics. The isolates were molecularly identified using the Internal Transcribed Spacer (ITS) region of the leaf litter fungi with ITS1 and ITS4 primers. Results of the BLASTn analysis of the ITS consensus was closely related genera with 98.72% to 100% similarity to *Aspergillus aculeatus* (NR 111412.1), *Aspergillus chinensis* (NR 137441.1), *Aspergillus costaricensis* (NR 103604.1), *Aspergillus flavus* (NR 111041.1), *Aspergillus sydowii* (NR 131259.1), *Mucor irregularis* (NR 172288.1), *Penicillium cataractum* (NR 158822.1), *Sporothrix chilensis* (NR 147568.1), *Talaromyces sparsus* (NR 173912.1), and *Trichoderma rifaii* (NR 137305.1). Phylogenetic analysis using a Neighbor-Joining tree confirmed that the isolated leaf litter fungi belong to the following genera: (i) *Aspergillus,* (ii) *Mucor,* (iii) *Penicillium,* (iv) *Sporothrix,* (v) *Talaromyces*, and (vi) *Trichoderma* species. The isolated leaf litter fungi can be explored further for their applications.

Keywords: *Aspergillus*, Internal Transcribed Spacer, Leaf litter, Phylogenetics

Introduction

Fungi are a large group of microorganisms, classified as either unicellular or multicellular. Multicellular microorganisms, such as filamentous fungi, are composed of hyphae that obtain nutrition by absorption. As heterotrophic microorganisms, they depend on other organisms for their food. Aside from that,

*Corresponding Author: Waing, K. G. D.; **Email:** kgdwaing@clsu.edu.ph

a very important role of fungal organisms in biodiversity is their ability to decompose, returning nutrients to the soil. Therefore, the primary agents responsible for the breakdown of plant litter are fungi. The hyphal networks formed during fungal development at the soil-litter interface facilitate the more efficient distribution of nutrients (Crowther *et al.,* 2012). With this, they carry out the decomposition process with the aid of their various enzymes, which are of importance biotechnologically and industrially (Patel *et al.,* 2013; Naeem *et al.,* 2022). Thus, it is essential to keep identifying specimens, revise taxonomies, assess the functions of fungi in the environment, and provide strains for industrial processes, biological control, and environmental cleanup. Fungi can be found in many different habitats, including water, soil, and other living things. However, only 2% of the world's estimated 5.1 million fungal species are known to exist (Blackwell, 2011; Maia *et al*., 2015). Leaf litter fungal studies in tropical ecosystems were comparatively fewer than in temperate habitats (Sayer, 2006; McGuire *et al*., 2012; Xu *et al*., 2013). Plant debris, including leaf litter, is among the various samples from which fungi can be isolated. Leaf litter, or fallen leaves, naturally piles on the ground and becomes the main source of nutrients and organic matter for the humus layer, offering numerous advantages to the ecosystem. Furthermore, it provides habitat for a variety of creatures and fosters the formation of fungi and fruiting bodies (Johnson and Catley, 2002; Berg and McClaugherty, 2008; Purahong *et al*., 2016; Bani *et al*., 2018) therefore, it can be a valuable source of fungal species. The continuous research on fungal diversity is very significant for discovering new strains and understanding their significance.

Leaf litter, which is primarily made of lignin and cellulose, is primarily broken down by fungi, which are important for the carbon and nitrogen cycles in the forest environment (Johanna, 2009). With this, numerous investigations on the isolation and identification of fungi from diverse leaf litter were carried out. In 2015, Waing *et al.* isolated and identified 30 species of fungi from various leaf litter samples from Central Luzon State University (CLSU), Philippines. Meanwhile, 13 decomposer fungi were isolated and identified from the leaf litter of *Macaranga indica* and *Hibiscus macrophyllus*. *Aspergillus, Penicillium, Mucor, Trichoderma, Cunninghamella,* and *Cladosporium* are represented among the identified fungi (Elfiati and Hasugian, 2019). Moreover, 14 fungal species belonging to six genera have been identified from *Acacia catechu* leaf litter in Madhya Pradesh, Indonesia (Jatav *et al*., 2020). In which Prakash *et al.* (2015) reported that although there were many different kinds of fungi, the variety of fungi in the litter differed according to the kind of tree.

In previous years, mycologists and other fungal enthusiasts relied primarily on observing morphological and cultural characteristics to identify

fungi. Cultural characteristics, such as colony color and growth diameter, and microscopic characters, such as spores and hyphae, are observed. However, fungal identification and taxonomic classification are challenging and easily misleading due to the microscopic nature of many fungi, particularly yeast-forming fungi with little observable differences, and the tendency of convergent evolution to produce homoplasies or identical features across a tree (Stajich, 2017). With the advent of technology, molecular characterization of various fungal organisms using different regions is conducted for identification. In recent years, the internal transcribed spacer (ITS) of nuclear DNA has served as the accepted universal marker for fungal DNA barcoding. It has been used as a target for evaluating the diversity of fungi present in different environmental samples (Bellemain *et al*., 2010). Using ITS1 and ITS4 primers, Malubag *et al.* (2021) were able to isolate and molecularly identify nine fungal endophytes from bananas, eleven fungal endophytes from *Citrofortunella microcarpa* (Reyes *et al.,* 2021), six fungal species associated with potato dry rot (Carreon *et al.,* 2021), and nine species of fungi from *Musa paradisiaca*, infected with anthracnose disease (Bautista *et al.,* 2021).

Mango is one of the most important and valuable fruit crops in the Philippines, particularly at Central Luzon State University, which is an income-generating project of the University Affairs Program through the Nursery Pomology Orchard Project. Thus, mango trees can be found all around the 658-hectare campus of CLSU. Different varieties of mango trees grown at CLSU include *carabao mango, chok anan mango, golden queen mango,* and *peach mango*. However, this does not spare the campus from the excessive leaf litter produced by the mango trees, which contributes to additional waste generated by the community. Numerous studies on leaf litter were conducted at CLSU. Studies such as quantification of the soil nitrogen in its available form in mango orchard as influenced by leaf litter decomposition (De Mesa, 1996), determination of the effect of *Collembola* in mass loss of decomposing leaf litter (Borromeo, 1995), and characterization and testing the pathogenicity of fungi associated with the flowers and fruits of mango (Ruiz, 1986) were conducted. However, this leaf litter can also be a good source of leaf litter fungi, which are ecologically and biotechnologically important. In 2015, Waing *et al.* studied the leaf litter fungi associated with different tree species at CLSU and assessed their ability to degrade cellulose, an important renewable resource. However, no research on fungal isolation from leaf litter, specifically from mango trees, was conducted at Central Luzon State University. The isolation and identification of fungi from various sources, including leaf litter, is still crucial for identifying potential sources of important fungal products and secondary metabolites that can be used industrially, medically, ecologically, and biotechnologically. Thus, the study

aimed to isolate and molecularly identify leaf litter fungi from mango using the Internal Transcribed Spacer region.

Materials and methods

Leaf litter collection and preparation

About 100 grams of mango leaf litter were randomly collected and picked at Mango Orchard near the Flora and Fauna Analytical and Diagnostic Laboratory, Central Luzon State University (Figure 1). Sampling was done on leaf litter found between the freshly fallen leaves and the soil under the stand of the *Mangifera indica* tree in five plots, each measuring 1m x 1m. One hundred grams of leaf litter served as the composite sample. Collected leaf litter was mixed and ground into a fine powder using a miller (Waing *et al.,* 2015).



Figure 1. Collection points of mango leaf litter at Central Luzon State University that are used for fungal isolation

Isolation and purification of fungi

Ten grams of pulverized sample was transferred into a beaker containing 90 mL sterile distilled water and manually shaken vigorously for 5 minutes to obtain a 10^{-1} dilution. One mL of the 10^{-1} dilution was transferred into a test tube containing 9 mL of sterile distilled water to make a 10^{-2} dilution. Then, one mL

of the 10^{-2} was transferred into another test tube with 9 mL sterile distilled water to obtain the 10^{-3} dilution; this process was repeated until the 10^{-7} dilution factor. Finally, one mL aliquots from each dilution were placed in sterile petri plates and poured with previously prepared and sterilized commercially available Potato Dextrose Agar (PDA) (Baiwei) containing 0.01% streptomycin sulfate (Biolab Co., Ltd.). Pour-plated plates were incubated at room temperature for 5-7 days to allow fungal growth (Waing *et al*., 2015; Jatav *et al*., 2020).

Three-point inoculation of the previously isolated leaf litter fungi was performed on previously plated PDA plates, which were incubated for 5 days at room temperature. It will be considered pure if a single colony of the same color is observed growing in the culture medium (Waing *et al.,* 2015; Jatav *et al*., 2020).

Cultural characterization of leaf litter fungi

Isolated leaf litter fungi were inoculated in sterile PDA plates using the three-point inoculation technique. Colony characteristics, such as the color of mycelia on the obverse and reverse sides of the plates, were observed and noted, and mycelial growth diameter was measured using a Vernier caliper after seven days of incubation (Waing *et al*., 2015).

Morphological characteristics of the leaf litter fungi

In morphological characterization, the methods of Prakash and Bhargava (2016) were followed. A loopful of sterilized and solidified PDA media was placed on top of a sterile microscopic slide. Thereafter, fungal mycelia were inoculated, and a sterilized coverslip was placed over them. It was placed on top of a V-shaped foil in a sterile petri plate containing moistened tissue paper and incubated at room temperature for three days. Using a compound microscope (100x), the inoculated slide was examined for spores and hyphae following incubation. The dichotomous keys developed by Gilman (1945), Barnett and Barry (1987), and Williams-Woodward (2001) were used for morphological characterization.

Molecular characterization of the leaf litter fungi

DNA extraction

Plated fungal mycelia were used for DNA extraction using the modified CTAB (cetyltrimethylammonium bromide) method (Perez *et al*., 2012). Fungal mycelia were aseptically scraped from the PDA plate, placed in a 1.5 mL

microcentrifuge tube, and lyophilized. Then, 750 µL of pre-warmed 2X CTAB and 50 µL of 20% sodium dodecyl sulfate (SDS) weew added to the previously ground fungal samples. Then, the mixtures were homogenized using a vortex and incubated in a water bath at 65°C for 30 minutes. Tubes were allowed to cool, then 750 µL chloroform was added. The samples were vortexed again and spun at 9,600 xg for 30 minutes. The aqueous phase was decanted into a new 1.5 mL tube, then 600 µL ice-cold isopropanol was added, and the mixture was incubated overnight. After incubation, the tubes were spun at 9,600 xg for 10 minutes. Isopropanol was decanted, and the pellet was washed with 500 µL 70% ethanol and centrifuged again at 9,600 xg for 3 minutes. After which, excess liquid was drained by inverting the tube in a paper towel. Upon drying, the DNA pellet was dissolved in 25 uL TE buffer containing RNase and incubated for 2 hours or until the pellet dissolved completely. The DNA was stored at -20 °C before use.

PCR amplification using ITS primers

PCR amplification was performed with the following components: genomic DNA (2 µl), Universal ITS1 (5'tccgtaggtgaacctgcgg-3') and ITS4 (5'-tcctccgcttattgatatgc-3') (Reyes *et al*., 2021) primers (0.25 µl), PCR Buffer (1.50 µl), DNA Polymerase (0.06 µl), 5mm dNTP Mix (0.50 µl), 25 mM $MgCl_2$ (0.50 µl) and 3.44 µl of sdH_2O. Cycling parameters on thermal cycler: initial denaturation at 94°C for 5 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, 58°C annealing for 30 seconds, and elongation at 72°C for 70 minutes, with a final extension at 72°C for 5 minutes (Reyes *et al*., 2021). PCR products were sent to Apical Scientific Sdn. Bhd. (Malaysia) for bidirectional Sanger Sequencing services.

Gel electrophoresis

Amplified PCR products were separated on an agarose gel. Two grams of agarose powder and 100 mL of 1x Tris-Borate EDTA (TBE) buffer were used to prepare a 2% agarose gel. One µl of DNA ladder and 1 µl of loading dye were placed in the first well. Subsequently, 1 µl of PCR product and 1 µl of dye were loaded into the remaining wells. The gel was run in an electrophoresis for 30 minutes at 100 volts and visualized in the Gel Doc XR+ Gel Documentation System. The amplified ITS gene was confirmed to be ~700bp in size, as indicated by the DNA ladder.

DNA sequencing and phylogenetic analysis

Internal Transcribed Spacer sequences of published and type strains of closely related species and selected distantly related species were downloaded from NCBI BLAST. Then, the downloaded reference sequences and the fungal

isolate sequences were aligned using CLUSTALW. Phylogenetic analysis using MEGA 7.0 (Kumar *et al.,* 2016) was performed using the Tamura 3-parameter method and bootstrap of 1000 replicates (Tamura, 1992). A Neighbor-Joining phylogenetic tree was constructed using *Gymnoascus* species as an outgroup. The ITS nucleotide sequences of the samples were deposited at NCBI GenBank with the following accession numbers: PV194980, PV194981, PV194982, PV194983, PV194984, PV194985, PV194986, PV194987, PV194988, and PV194989.

Results

Cultural and morphological characterization of leaf litter fungi

In this study, 10 leaf litter fungi were isolated and purified. The isolates grown on PDA were described based on their cultural characteristics on their obverse and reverse sides after 7 days of incubation. Microfungal structures were observed using a compound microscope. Leaf litter fungal code and the presumptive identity based on the observed colony characteristics and morphological characteristics were presented in Table 1 and Figure 2.

Molecular identification of leaf litter fungi

The ITS regions of the isolated leaf litter fungi were successfully amplified using ITS1 and ITS4 primers. The obtained ITS sequences were compared with those of Type strains and published specimens in the NCBI GenBank database using the nucleotide Basic Local Alignment Search Tool (BLASTn). Percentage similarities, e-values, and GenBank accession numbers were recorded. It can be observed that there is 98-100% similarity to the closely related fungal sequence in the NCBI GenBank database. BLAST Analysis revealed that the fungal isolates were identified as *Aspergillus aculeatus*, *Aspergillus chinensis, Aspergillus costaricensis, Aspergillus flavus, Aspergillus sydowii, Mucor irregularis, Penicillium cataractum*, *Sporothrix chilensis, Talaromyces sparsus*, and *Trichoderma rifaii* (Table 2).

Table 1. Observed cultural and morphological characteristics of isolated fungi from mango leaf litter

Leaf Litter Fungal Code	Accession Code	Morphology-based Identity	Colony Diameter (mm)	Colony Characteristics on its Obverse (O) and Reverse (R) Sides	Morphological Characteristics
LLF 001	PV194980	*Aspergillus* sp.	45.20 – 52.94	(O) - lighter to dark brown-colored, almost grayish or whitish on the edge, with velvety growth (R) - light-colored, creamy white to pale yellow	Thin, filamentous hyphae with dark conidia bearing conidiospores
LLF 002	PV194981	*Aspergillus* sp.	28.79 – 34.94	(O) - pale brown color at the center with a greenish granular area encircled with a white fuzzy margin (R) – reddish/purple-colored at the center and creamy white on the outer margin	Columnar conidial heads bearing conidia with hyaline hyphae
LLF 003	PV194982	*Aspergillus* sp.	52.75 – 59.02	(O)- dark brown to grayish-colored, with a yellowish to off-white color of the margin, with feathery and granular texture (R) – creamy white to pale yellow	long, tubular, hyaline hyphae bearing the conidia
LLF 004	PV194983	*Aspergillus* sp.	56.60 – 57.42	(O) - white, cottony at the center, surrounded by yellowish-green mycelia with diffuse, feathery white mycelia (R) – creamy-white colored	Globose-subglobose conidia borne on a conidiophore with hyaline, septated hyphae
LLF 005	PV194984	*Aspergillus* sp.	23.69 – 24.41	(O) - white to off-white at the center with velvety texture (R) – light brown color to off-white towards the margin	hyaline, septated hyphae
LLF 006	PV194985	*Mucor* sp.	62.13 – 66.85	(O) – white to off-white, cottony mycelia (R) – pale yellow to cream colored cottony mycelia	Simple erect sporangiophore bearing globose sporangia with sporangiospores
LLF 007	PV194986	*Penicillium* sp.	15.08 – 23.35	(O) – whitish to grayish color with powdery texture (R) – off-white, cream colored	Conidiophore bearing conidia in phialides
LLF 008	PV194987	*Sporothrix* sp.	24.23 – 25.24	(O) – predominantly white and cottony (R) - predominantly white and cottony	Hyaline septated hyphae bearing ovoid/elongated conidia that are in clusters
LLF 009	PV194988	*Talaromyces* sp.	25.01 – 27.97	(O) - white with a slightly creamy or off-white center with a bluish-green margin (R) – creamy white colored	Septated hyphae with conidia borne on phialides
LLF 010	PV194989	*Trichoderma* sp.	60.94 – 63.24	(O) – white, cottony, and turned green as it aged (R) - predominantly white and cottony	thin, filamentous hyphae with dark, clustered conidial spores

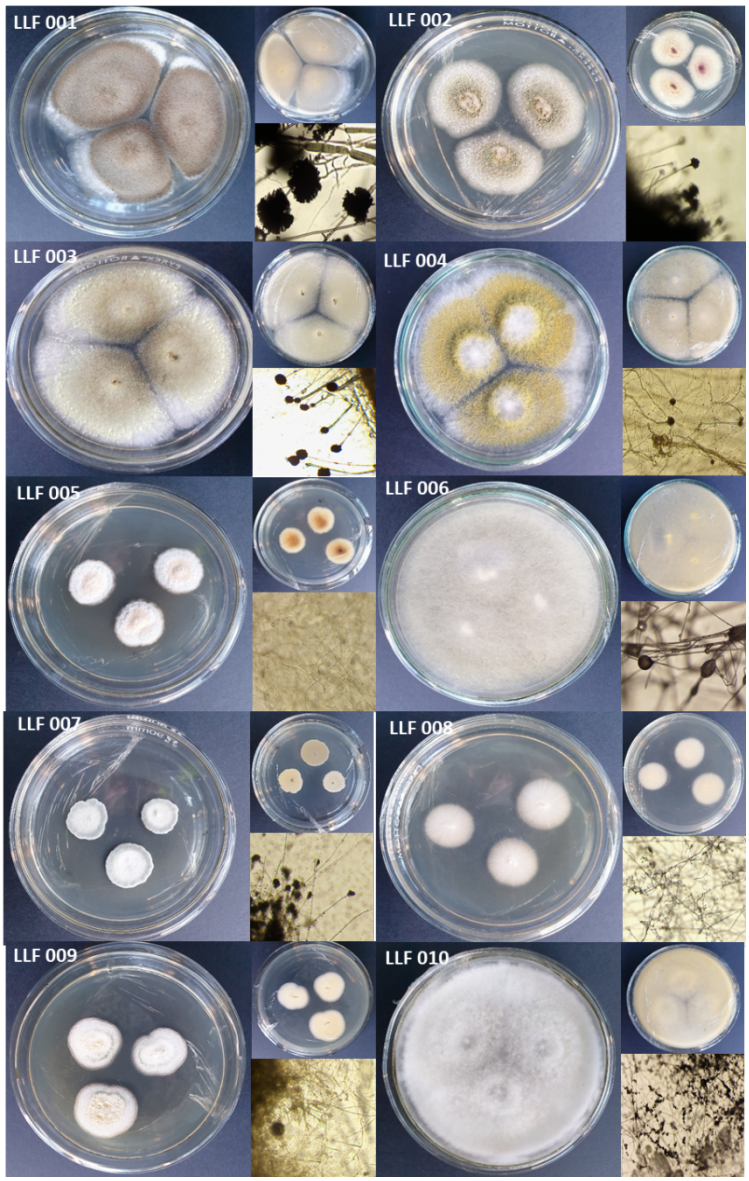


Figure 2. Three-point cultures of various leaf litter fungi, *Aspergillus* (LLF 001, LLF 002, LLF 003, LLF 004, LLF 005), *Mucor* (LLF 006), *Penicillium* (LLF 007), *Sporothrix* (LLF 008), *Talaromyces* (LLF 009), and *Trichoderma* (LLF 010) species, grown on PDA on its obverse and reverse side after 7 days of incubation, as well as the observed microscopic characteristics

Table 2. Identities of the leaf litter fungi based on NCBI BLAST search with Genbank accession number

Leaf Litter Fungal Code	Closely Related Fungal Sequence	E-value	Percentage Similarity	Genbank Accession Number
LLF 001	*Aspergillus aculeatus*	0.00	100.00	NR 111412.1
LLF 002	*Aspergillus chinensis*	0.00	99.28	NR 137441.1
LLF 003	*Aspergillus costaricensis*	0.00	100.00	NR 103604.1
LLF 004	*Aspergillus flavus*	0.00	99.66	NR 111041.1
LLF 005	*Aspergillus sydowii*	0.00	100.00	NR 131259.1
LLF 006	*Mucor irregularis*	0.00	99.66	NR 172288.1
LLF 007	*Penicillium cataractum*	0.00	99.82	NR 158822.1
LLF 008	*Sporothrix chilensis*	0.00	99.22	NR 147568.1
LLF 009	*Talaromyces sparsus*	0.00	98.72	NR 173912.1
LLF 010	*Trichoderma rifaii*	0.00	100.00	NR 137305.1

LLF 001, LLF 002, LLF 003, LLF 004, and LLF 005 have shown ITS sequence percent similarity of 99.28% to 100.00% and phylogenetic affinity to species of *Aspergillus* (strains NR 111412.1, NR 137441.1, NR 103604.1, NR 111041.1, NR 131259.1) with 99 to 100% bootstrap support values. LLF 006, with 99.66% percent similarity with *Mucor irregularis* NR 172288.1, was clustered with *Mucor* species with a 100% bootstrap support value. LLF 007 (99.82% similar to *Penicillium cataractum* NR 158822.1) was grouped with *Penicillium* species with 100% bootstrap support value, thus confirming its identity as *Penicillium* sp. LLF 008, with 99.22% similarity with *Sporothrix chilensis* NR 147568.1, is related to other *Sporothrix* species with a 100% bootstrap support value. LLF 009, which is 98.72% similar to *Talaromyces sparsus* NR 173912.1, has 100% bootstrap support value with other *Talaromyces* species. While LLF, 010 with 100.00% identity to *Trichoderma rifaii* NR 137305.1, has a bootstrap value of 99% with other *Trichoderma* species, confirming its genus identity (Figure 3).

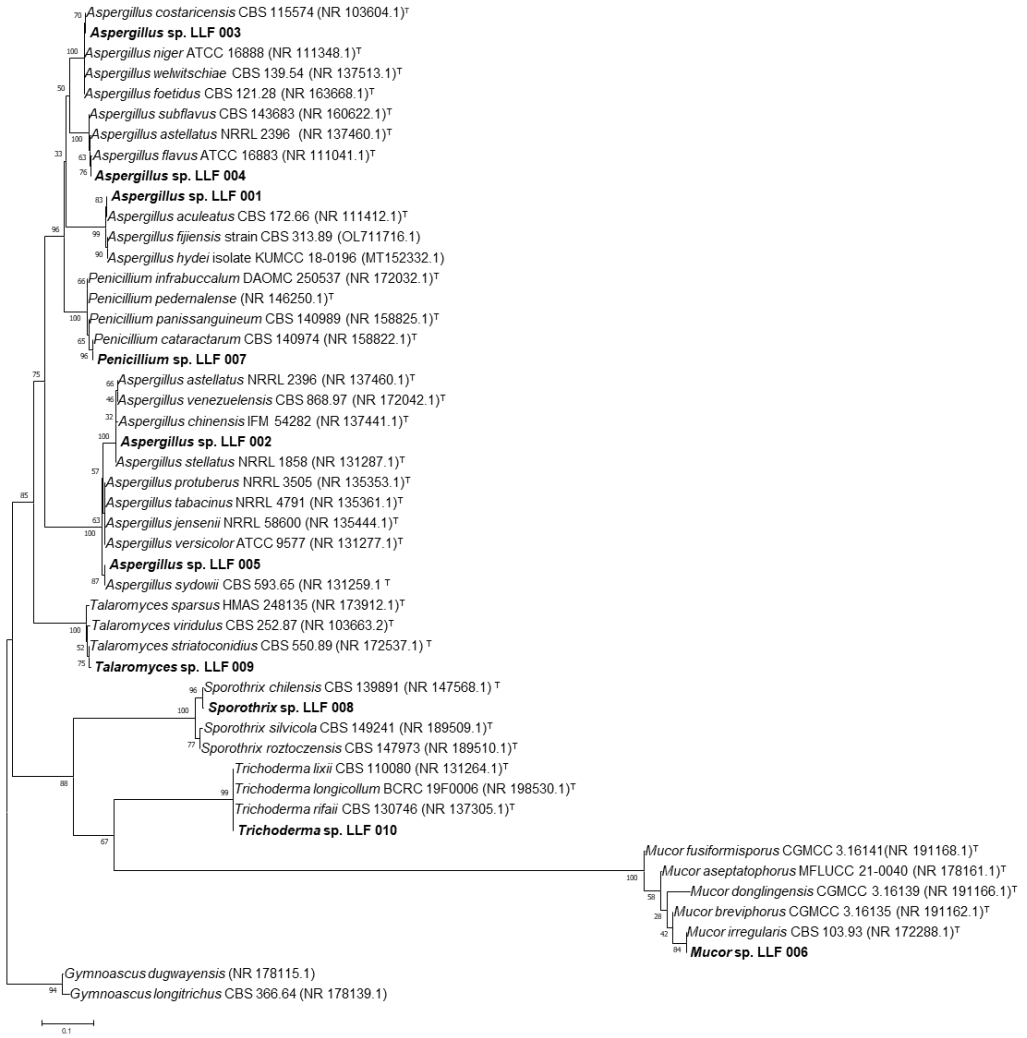


Figure 3. Neighbor-Joining phylogenetic tree of the different leaf litter fungal isolates showing relationships with other related taxa based on Internal Transcribed Spacer sequence analysis using *Gymnoascus* as the outgroup

Discussion

Fungi are a diverse group of organisms that are ubiquitous and can be isolated from the environment. These fungi are studied for their ability to decompose various waste materials and serve as food, as well as for their role in the manufacture of industrial products such as medicines, enzymes, and organic acids. To ensure the availability of important fungal species, it is essential to

continuously search for new strains that can be exploited for research and commercialization (Simões *et al*., 2013). Microbial communities significantly influence the breakdown of plant litter in terrestrial ecosystems, and fungi are among the most important players in the decomposition process (Prakash *et al*., 2015).

All the isolates from the genera *Aspergillus, Penicillium, Mucor, Sporothrix, Talaromyces,* and *Trichoderma* are saprophytic fungi known as degraders. They can be found in a variety of habitats, including plant material. *Aspergillus, Penicillium, Mucor,* and *Trichoderma* were isolated from *Macaranga indica* and *Hibiscus macrophyllus* leaf litter (Elfiati and Hasugian, 2019), in *Acacia catechu* (Jatav *et al.,* 2020), in leaf litter of rain tree, orchid tree, and paper tree (Waing *et al.,* 2015), and in *Quercus serrata* (Sharma *et al.,* 2011). Also, in the study by de Melo *et al*. (2018), *Penicillium, Talaromyces,* and *Trichoderma* species were found in leaf litter samples collected in the Cerrado State Park. While *Talaromyce*s species were isolated and identified from leaf litter in the rain forest of the Colombian Amazon (Yilmaz *et al.,* 2016). Whereas, *Aspergillus, Penicillium,* and *Trichoderma* were found in the degrading leaf litter of *Saccharum officinarum* (Pandey *et al.,* 2020). Meanwhile, species of *Aspergillus* and *Penicillium* were isolated in Salacca zalacca leaf litter (Sari *et al*., 2017). While *Mucor, Trichoderma,* and *Sporothrix* were present in the deciduous leaf litter (Carreiro and Koske, 1992).

The internal transcribed spacer (ITS) region is the universal fungal primary barcode due to its widespread use, ease of amplification, and appropriately large barcode gap for identifying various fungal species when analyzing DNA sequences (Raja *et al*., 2017; Baturo-Cieśniewska *et al*., 2020). Bootstrap values of 70% or higher are often considered indicative of reasonable support (Lemoine *et al*., 218). However, Kiss (2012) stated that the ITS region is not a powerful tool for distinguishing many closely related fungal species but is a good marker only for phylogenetically more distant, albeit congeneric, fungal species. ITS regions are not ideal for highly speciose genera such as in *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma* for species identification; thus, the addition of other protein-coding regions should be considered (Raja *et al.,* 2017). Therefore, it is recommended to use a comparative sequencing-based approach that targets the ribosomal ITS region and protein-coding loci, such as the β-tubulin, and calmodulin regions, to identify species within the *Aspergillus* sp. complex (Tam *et al.,* 2014). In the study of Alshehri and Palanisamy (2020), they show the effectiveness of using ITS regions, β-tubulin and calmodulin genes to assess the genetic diversity among *Aspergillus* species (Alshehri and Palanisamy, 2020). Moreso, the use of ITS alone for *Penicillium* and many ascomycete species is not variable enough for distinguishing all closely related species.

Because of this, a secondary barcode is needed to identify isolates to the species level (Visagie *et al*., 2014). Moreso, the Harzianum Complex Clade includes 95 cryptic species, with only one named *Trichoderma harzianum*. Using additional regions, such as tef1α, rpb2, and ITS loci, *Trichoderm*a species were correctly identified (Ismaiel *et al*., 2024).

In conclusion, ten fungi from mango leaf litter were successfully identified based on their cultural and morphological characteristics. The identities of the leaf litter fungi were confirmed using ITS regions as *Aspergillus aculeatus*, *Aspergillus chinensis, Aspergillus costaricensis*, *Aspergillus flavus*, *Aspergillus sydowii, Mucor irregularis, Penicillium cataractum*, *Sporothrix chilensis, Talaromyces sparsus,* and *Trichoderma rifaii.* Phylogenetic analysis was performed using a Neighbor-Joining tree with the Tamura-Nei parameter method, and the identity of the initially identified fungal species was confirmed. However, due to the species complex, it is recommended to use additional regions to fully identify and verify the isolated fungal species from leaf litter, namely *Aspergillus, Penicillium, Talaromyces, Mucor, Sporothrix,* and *Trichoderma.* The isolated and identified species of leaf litter fungi could be evaluated for their enzymatic capabilities to degrade large amounts of leaf litter as a waste management strategy.

Acknowledgements

The authors would like to acknowledge the Department of Science and Technology-Science Education Institute for the scholarship grant of Ms. Kristine Grace D. Waing and the Flora and Fauna Analytical Diagnostic Laboratory, Biotechnology and Analytical Laboratory, and the Department of Biological Sciences, College of Science, Central Luzon State University for their kind assistance and support to this study.

Conflicts of interest

The authors declare no conflict of interest.

References

Alshehri, B. and Palanisamy, M. (2020). Evaluation of molecular identification of Aspergillus species causing fungal keratitis. Saudi Journal of Biological Sciences, 27:751-756.

Bani, A., Pioli, S., Ventura, M., Panzacchi, P. et al. (2018). The role of microbial community in the decomposition of leaf litter and deadwood. Applied Soil Ecology, 126:75-84.

Barnett, H. L. and Barry, H. (1987). Illustrated genera of imperfect fungi 4th ed. New York: Mac Millan Publishing co.

Baturo-Cieśniewska, A., Pusz, W. and Patejuk, K. (2020). Problems, limitations, and challenges in species identification of Ascomycota members on the basis of ITS Regions. Acta Mycologica, 55:1.

Bautista, M. A. C., Gandalera, E. E. and Waing, K. G. D. (2021). *In-vitro* interactions of fungi associated with anthracnose disease of *Musa paradisiaca*. Plant Pathology & Quarantine, 11:69-82.

Bellemain, E., Carlsen, T., Brochmann, C. et al. (2010). ITS as an environmental DNA barcode for fungi: an *in-silico* approach reveals potential PCR biases. BMC Microbiology, 10:189.

Blackwell, M. (2011). The Fungi: 1, 2, 3 ... 5.1 million species? American Journal of Botany, 98:426-438.

Berg, B. and McClaugherty, C. (2008). Plant litter decomposition, humus formation, carbon sequestration. 2nd Ed, Berlin-Heidelberg: Springer.

Borromeo, H. E. S. (1995). Leaf Litter Decomposition and Population Density of Soil *Collembola* in Mango Orchard. (Undergraduate Thesis). Central Luzon State University, Philippines.

Carreiro, M. M. and Koske, R. E. (1992). Effect of temperature on decomposition and development of microfungal communities in leaf litter microcosms. Canadian Journal of Botany, 70:2177-2183.

Carreon, G. A. D., Gandalera, E. E. and Waing, K. G. D. 2021. Molecular identification and *in vitro* interaction of molds associated with dry rot of potato (*Solanum tuberosum* L.) collected in La Trinidad, Benguet, Philippines. Studies in Fungi, 6:315-326.

Crowther, T., Boddy, L. and Hefin Jones, T. (2012). Functional and ecological consequences of saprotrophic fungus–grazer interactions. The ISME Journal, 6:1992-2001.

de Melo, M., V Araujo, A. C., N Chogi, M. A. and S Duarte, I. C. (2018). Cellulolytic and lipolytic fungi isolated from soil and leaf litter samples from the Cerrado (Brazilian Savanna). Revista de Biología Tropical, 66:237-245.

De Mesa, E. M. (1996). Changes in Soil Nitrogen Availability as Influenced by Leaf Litter decomposition in a Mango Orchard of the College of Agriculture, CLSU. (Undergraduate Thesis). Central Luzon State University, Philippines.

Elfiati, D. and Hasugian, F. A. (2019). Isolation and identification of decomposer fungi from *Macaranga indica* and *Hibiscus macrophyllus* leaf litter from restoration area of Gunung Leuser National Park. IOP Conference Series: Earth and Environmental Science, 374:012018.

Gilman, J C. (1945). A manual of soil fungi. The Iowa State University Press. USA ISBN 8176220116, 9788176220118

Ismaiel, A., Jambhulkar, P. P., Sinha, P. and Lakshman, D. K. (2024). *Trichoderma*: Harzianum Complex Clade species distribution in soils of Central and South America. Preprints, 2024:2024071786.

Jatav, B. K., Sharma, T. K. and Dassani, S. (2020). Succession of microfungi on leaf litter of *Acacia catechu* in Datia, Madhya Pradesh, India. Journal of Pure and Applied Microbiology, 14:581-590.

Johanna, B. (2009). Litter Decomposing Fungi in Boreal forests. Their Function in Carbon and Nitrogen Circulation. (Doctor Thesis). Swedish University of Agricultural Science. Uppsala.

Johnson, E. A. and Catley, K. M. (2002). Life in the leaf litter. American Museum of Natural History, New York.

Kiss, L. (2012). Limits of nuclear ribosomal DNA internal transcribed spacer (ITS) sequences as species barcodes for fungi. Proceedings of the National Academy of Sciences, 109: E1811-E1811.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasents. Molecular Biology and Evolution, 33:1870-1874.

Lemoine, F., Domelevo Entfellner, J. B., Wilkinson, E., Correia, D., Dávila Felipe, M., De Oliveira, T. and Gascuel, O. (2018). Renewing Felsenstein's phylogenetic bootstrap in the era of big data. Nature, 556:452-456.

Maia, L. C., Carvalho Jr, A. A., Cavalcanti, L. H., Gugliotta, A. M., Drechsler-Santos, E. R., Santiago, A. L. M. A., Cáceres, M. E. and da Silva, V. F. (2015). Diversity of Brazilian fungi. Rodriguésia, 66:1033-1045.

Malubag, A. G., Parayao, A. M. and Waing, K. G. D. (2021). Identification of endophytic fungi associated in banana (*Musa paradisiaca* L.) and evaluation of its enzymatic abilities. International Journal of Agricultural Technology, 17:941-958.

McGuire, K. L., Fierer, N., Bateman, C., Treseder, K. K. and Turner, B. L. (2012). Fungal community composition in Neotropical rain forests: the influence of tree diversity and precipitation. Microbial Ecology, 63:804e812.

Naeem, M., Manzoor, S., Abid, M.-U.-H., Tareen, M. B. K., Asad, M., Mushtaq, S., Ehsan, N., Amna, D., Xu, B. and Hazafa, A. (2022). Fungal proteases as emerging biocatalysts to meet the current challenges and recent developments in biomedical therapies: an updated review. Journal of Fungi, 8:109.

Pandey, S., Sharma, T. K. and Dassani, S. (2020). Isolation and screening of cellulolytic fungi from degrading leaf litter of *Saccharum officinarum* L. Plant Archives, 20:7013-7020.

Patel, C., Yadav, S., Rahi, S. and Anuradaha, D. (2013). Studies on biodiversity of fungal endophytes of indigenous Monocotaceous and Dicotaceous plants and evaluation of their enzymatic potentialities. International Journal of Scientific and Research Publications, 3:2250-3153.

Perez, L. M., Pastor, H. M., Domingo, J. M., Tabanao, D. A. and Manigbas, N. L. (2012). DNA fingerprinting in hybrid rice: Its applications in varietal purity testing. Philippine Rice Research Institute, Science City of Munoz, Nueva Ecija, Philippines. ISBN 978-971-9081-75-3

Prakash, P. and Bhargava, K. (2016). A modified micro chamber agar spot slide culture technique for microscopic examination of filamentous fungi. Journal of Microbiological Methods, 123:126-129.

Prakash, C. P., Thirumalai, E. and Rajulu, M. G., Thirunavukkarasu, N. and Suryanarayanan, T. S. (2015). Ecology and diversity of leaf litter fungi during early-stage decomposition in a seasonally dry tropical forest. Fungal Ecology, 17:103-113.

Purahong, W., Wubet, T., Lentendu, G., Schloter, M. et al. (2016). Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Molecular Ecology, 25:4059-4074.

Raja, H. A., Miller, A. N., Pearce, C. J. and Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. Journal of Natural Products, 80:756-770.

Reyes, R. D. C., Parayao, A. M. and Waing, K. G. D. (2021). Identification and evaluation of enzymatic ability of fungal endophytes from *Citrofortunella microcarpa* (Bunge) Wijnands. Studies in Fungi, 6:460-468.

Ruiz, V. M. R. (1986). Characterization and Pathogenicity of Fungi Associated with Mango (*Mangifera indica* Linn.) Flowers and Fruits. (Undergraduate Thesis). Central Luzon State University, Philippines.

Sari, S. L. A., Setyaningsih, R. and Wibowo, N. F. A. (2017). Isolation and screening of cellulolytic fungi from *Salacca zalacca* leaf litter. Biodiversitas Journal of Biological Diversity, 18:1282-1288.

Sayer, E. J. (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biological Reviews of the Cambridge Philosophical Society, 81:1e31.

Sharma, G., Pandey, R. R. and Singh, M. S. (2011). Microfungi associated with surface soil and decaying leaf litter of *Quercus serrata* in a subtropical natural oak forest and managed plantation in Northeastern India. African Journal of Microbiology Research, 5:777-787.

Simões, M. F., Pereira, L., Santos, C. and Lima, N. (2013). Polyphasic identification and preservation of fungal diversity: concepts and applications. In: Malik, A., Grohmann, E., Alves, M. (eds) Management of Microbial Resources in the Environment. Springer, Dordrecht.

Stajich, J. E. (2017). Fungal genomes and insights into the evolution of the kingdom. Microbiology Spectrum, 5:25.

Tam, E. W., Chen, J. H., Lau, E. C., Ngan, A. H., Fung, K. S., Lee, K. C. and Woo, P. C. (2014). Misidentification of *Aspergillus nomius* and *Aspergillus tamarii* as *Aspergillus flavus*: characterization by internal transcribed spacer, β-tubulin, and calmodulin gene sequencing, metabolic fingerprinting, and matrix-assisted laser desorption ionization–time of flight mass spectrometry. Journal of Clinical Microbiology, 52:1153-1160.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Molecular Biology and Evolution, 9:678-687.

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G. and Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium*. Studies in Mycology, 78:343-371.

Waing, K. G. D., Abella, E. A., Kalaw, S. P., Waing, F. P. and Galvez, C. T. (2015). Studies on biodiversity of leaf litter fungi of Central Luzon State University and evaluation of their

enzyme producing ability. Current Research in Environmental & Applied Mycology, 5:273-280.

Williams-Woodward, J. (2001). Simplified fungi identification key. Special Bulletin 37, The University of Georgia. Retrived from http://plantpath.caes.uga.edu/extension/documents/fungikey.pdf

Yilmaz, N., López-Quintero, C. A., Vasco-Palacios, A. M. et al. (2016). Four novel *Talaromyces* species isolated from leaf litter from Colombian Amazon rain forests. Mycology Progress, 15:1041-1056.

Xu, W., Shi, L., Chan, O., Li, J., Casper, P. and Zou, X. (2013). Assessing the effect of litter species on the dynamic of bacterial and fungal communities during leaf decomposition in microcosm by molecular techniques. PLoS ONE, 8:e84613.

(Received: 12 March 2025, Revised: 24 September 2026, Accepted: 14 November 2026)