

## Research Article

# Composition and Diversity of Fungal Decomposers of Submerged Wood in Two Lakes in the Brazilian Amazon State of Pará

Eveleise Samira Martins Canto <sup>1,2</sup>, Ana Cláudia Alves Cortez,<sup>3</sup> Josiane Santana Monteiro,<sup>4</sup> Flavia Rodrigues Barbosa,<sup>5</sup> Steven Zelski <sup>6</sup> and João Vicente Braga de Souza<sup>3</sup>

<sup>1</sup>Programa de Pós-Graduação da Rede de Biodiversidade e Biotecnologia da Amazônia Legal-Bionorte, Manaus, Amazonas, Brazil

<sup>2</sup>Universidade Federal do Oeste do Pará, UFOPA, Santarém, Pará, Brazil

<sup>3</sup>Instituto Nacional de Pesquisas da Amazônia, INPA, Laboratório de Micologia, Manaus, Amazonas, Brazil

<sup>4</sup>Museu Paraense Emilio Goeldi-MPEG, Belém, Pará, Brazil

<sup>5</sup>Universidade Federal de Mato Grosso, UFMT, Sinop, Mato Grosso, Brazil

<sup>6</sup>Miami University, Department of Biological Sciences, Middletown, OH, USA

Correspondence should be addressed to Eveleise Samira Martins Canto; [eveleisesamira@hotmail.com](mailto:eveleisesamira@hotmail.com) and Steven Zelski; [zelskise@miamioh.edu](mailto:zelskise@miamioh.edu)

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Aquatic ecosystems in tropical forests have a high diversity of microorganisms, including fungi, which are important decomposers of submerged wood. Despite the importance of their role in decomposition, research concerning the diversity of freshwater fungi from Brazilian Amazonian environments is scarce. The aim of this work was to describe the composition and diversity of fungi present on submerged wood in two lakes of the Brazilian Amazon (State of Pará). Fragments of decaying wood (30 samples per lake) were collected from the Lakes Juá and Maicá. The wood samples were inspected for 6 months in the presence of fungal reproductive structures. Fungi observed in the wood were identified morphologically. Twenty-three taxa were identified in the Lake Juá (10 sexual and 13 asexual) and 26 taxa in the Lake Maicá (17 sexual, 9 asexual). ITS sequences were obtained for 14 taxa to aid in identification. In the Lakes Juá and Maicá, the diversity indices were  $H'$ : 2.6514 and  $H'$ : 2.8174, respectively. The Sorensen index of the fungal communities in the studied lakes was 0.3673. This study is the first to describe the fungal biodiversity of two important aquatic environments in Pará, Brazil.

## 1. Introduction

Freshwater fungi include species that inhabit water for all or part of their life cycle or any species adapted to colonize predominantly aquatic or semiaquatic substrates in nature [1]. These organisms serve as key agents in the decomposition of submerged dead plant material. Fungal biomass is a source of nutrition for other organisms in aquatic ecosystems [2, 3]. Fungal enzymatic activity also modifies plant substrates to make them more palatable to invertebrate shredders and scrapers [4].

Freshwater ascomycetes are a group of fungi known to actively participate in the decomposition of submerged

woody debris [5, 6]. This group is polyphyletic, with the majority of species belonging to the subphylum Pezizomycotina in the classes Leotiomycetes, Sordariomycetes, and Dothideomycetes [2, 7]. Members of the Eurotiomycetes [8] and Orbiliomycetes [9] are less common. Shearer and Raja [10] reported that freshwater ascomycetes are distributed in relatively few orders: Helotiales, Pleosporales, Sordariales, Soryrellales, Microascales, Eurotiales, and Jahnulales. The number of known taxa has increased in recent years due to broader sampling and the use of molecular methods for identification [11, 12].

The Amazon basin is arguably the most complex network of aquatic habitats on the planet [13]. This vast region

contains a variety of aquatic environments that include large rivers, lakes, swamps, and seasonally inundated floodplains [13, 14]. Aquatic ecosystems can be roughly classified into three main types: clear waters [15], white waters [16], and black waters [14, 17]. Water characteristics such as temperature, dissolved oxygen, and amount of organic matter may influence the diversity of freshwater fungal species [18]. The first published studies of freshwater fungi in Amazonian waters (Peru) were conducted by Matsushima in white water rivers [19]. More recent studies have described new species of ascomycetes in the Peruvian Amazon [20–24] and in the Brazilian states of Pará [25, 26] and Amazonas [27, 28]. In general, the microbial community of the Amazon region is still underexplored, and studies describing the diversity and ecology of fungi in the Amazon region are urgently needed. Therefore, we sought to investigate new environments such as the Tapajós River, one of the largest clear water tributaries of the Amazon River [17]. The present work aimed to describe the composition and diversity of fungi present on submerged decomposing wood in two lakes in the Tapajós River basin near Santarém, in the Brazilian state of Pará.

## 2. Materials and Methods

**2.1. Study Sites.** Samples of submerged wood were collected from Lake Juá (2°25'57"S, 54° 46'39"W) and Lake Maicá (2°27'29.0"S, 54° 40'10.8"W) and, essentially, the embayment of the lower Tapajós and Amazon Rivers, respectively. Lake Juá receives water that drains from a stream and the Tapajós. Lake Maicá is a mix of surface drainages and the white water of the Amazon River [14] (Figures 1, 2(a), and 2(b)).

**2.2. Water Characterization.** Physicochemical variables were measured using a ProfiLine 197i WTW multiparameter probe (Wellheim, Germany). Temperature (°C), pH, electrical conductivity ( $\mu$ S), dissolved oxygen concentration (mg/L), and turbidity (NTU) were recorded for each sample collection.

**2.3. Sample Collection.** A single collection was made at each location in Lake Juá in October 2017 and in Lake Maicá in November 2017. At each site, 30 submerged wood fragments that showed signs of active decomposition were collected. The lengths of the samples ranged from 6 to 22 cm and diameters ranged from 6 to 15 cm (Figure 2(c)). These substrates were transported in sealable plastic bags (20 cm  $\times$  10 cm), with a small amount of local water, to the Laboratory of Multidisciplinary Teaching in Applied Biology-Labio, Universidade Federal do Oeste do Pará (UFOPA). In the laboratory, the samples were gently rinsed with running water, placed in moist chambers (plastic boxes with moist paper towels), and incubated at room temperature with 12/12 h light/dark conditions.

**2.4. Isolation and Identification of Fungi Present on Submerged Wood.** Every ten days, for six months, a Stemi DV4 stereomicroscope (Zeiss) was used to search for fungal

structures on the collected wood samples. The structures found were transferred, with dissection needles, to microscope slides containing distilled water. The identification of sexual ascomycetes was performed based on the morphology of ascomata, hamathecium, asci, and ascospores using an Axioskop 40 microscope (Zeiss) as described previously [22, 29, 30]. Aqueous lactophenol cotton blue solution was used to determine the staining reactions of the apical ascus apparatus. Asexual ascomycetes were identified by investigating the types of conidiogenesis, conidiophore, and conidia, using an Axioskop 40 microscope (Zeiss). Isolation of fungal was carried out by transferring the fungal structures to the surface of Antibiotic Water Agar (20 g/L agar and chloramphenicol 250 mg/L), Potato Dextrose Agar (PDA) (Kasvi), and Malt Extract Agar (MEA) (malt extract 30 g/L, mycological peptone 5g/L and 15g/L agar, pH 5.4  $\pm$  0.2). The developed colonies were transferred to PYG agar (1.25 g/L peptone, 1.25 g/L yeast extract, 5 g/L glucose, 250 mg/L chloramphenicol, and 18 g/L agar) [22] (Figures 2(d)–2(g)).

**2.5. DNA Extraction, Amplification, and Sequencing.** DNA extraction was performed according to the protocol of Ferrer et al. [31]. Fungi were grown in test tubes containing 10 mL of potato dextrose broth (120 g potato, 10 g dextrose, and 1000 mL distilled water) for 10 days and transferred to Eppendorf tubes (2 mL) containing 500  $\mu$ L of buffer (2.5 mg SDS, 7.0 mg NaCl, 3.65 mg EDTA, 20 mL of Tris-HCl and 100 mL of DI water, and 5  $\mu$ L of  $\beta$ -mercaptoethanol). The microtubes were subjected to freezing ( $-20^{\circ}\text{C}$ ) and heating ( $65^{\circ}\text{C}$  for 1 h) in order to rupture the cellular structures. Next, 500  $\mu$ L of a phenol/chloroform/isoamyl alcohol solution (v/v/v: 25/24/1) was added to the microtubes and vortexed until a homogeneous suspension was obtained. The suspension was then centrifuged (14,000 rpm, 15 min) and the supernatant was transferred to a new microtube (1.5 mL). Isopropanol was added to the supernatant in equal volume, and the mixture was homogenized and incubated at  $-20^{\circ}\text{C}$  overnight to precipitate the DNA. The precipitated DNA was centrifuged (14,000 rpm for 15 min), washed twice with 70% ethanol, and resuspended in 100  $\mu$ L of sterile water distilled.

Molecular data were gathered by sequencing the ITS region of rDNA according to the procedure of White et al. [32]. The reaction yielded a final volume of 50  $\mu$ L, consisting of PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 1.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M of ITS-1 (5'-TCCGTAGGT-GAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATT-GATATGC-3'), 200  $\mu$ M dNTPs, 1.5 U *Taq* DNA polymerase, and 100 ng of fungal DNA. Thermocycling was performed on an Applied Biosystems ProFlex PCR System with initial denaturation at  $94^{\circ}\text{C}$  for 5 min, followed by 35 cycles of DNA denaturation at  $94^{\circ}\text{C}$  for 1 min, annealing at  $55^{\circ}\text{C}$  for 1 min, extension at  $72^{\circ}\text{C}$  for 2 min, and a final extension at  $72^{\circ}\text{C}$  for 10 min. Two positive controls and one negative control were also tested. Electrophoresis was performed on 1.5% agarose gel in 1x TBE buffer (100 mM Tris base, 100 mM boric acid, and 2 mM EDTA pH 8.0), plus 10  $\mu$ L/100 mL of Siber Green SYBR® Safe. Amplified fragments were visualized under UV

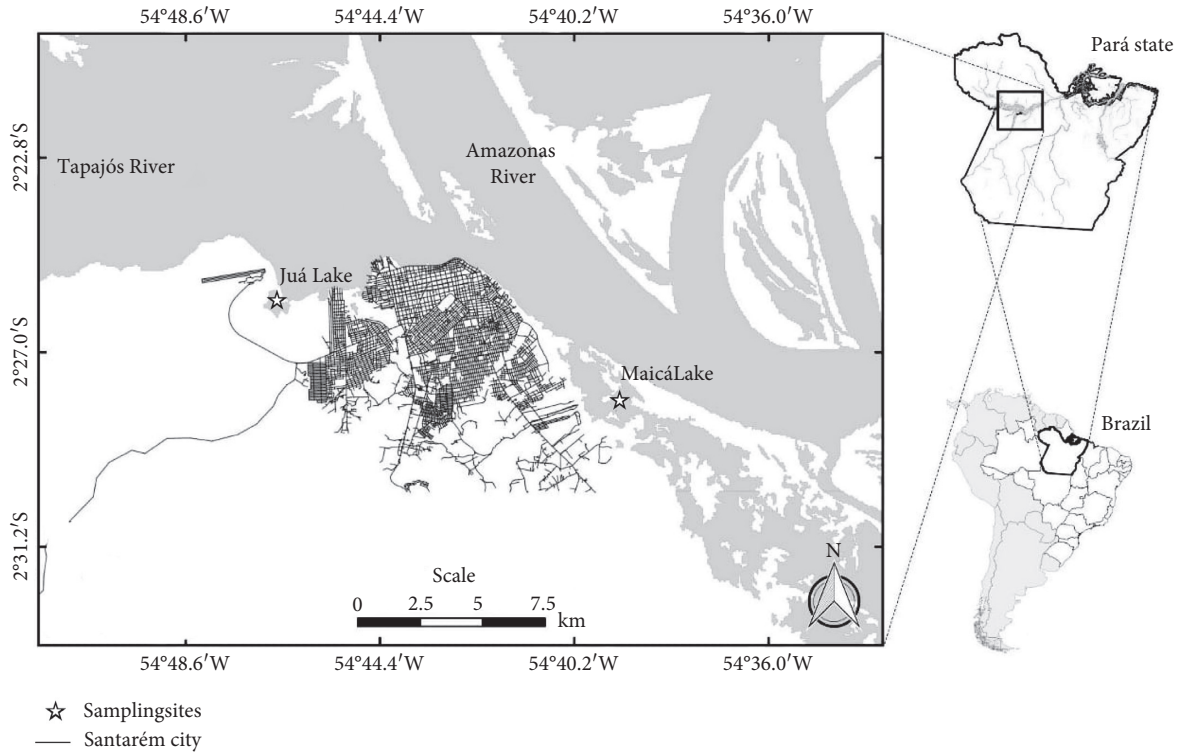


FIGURE 1: Map of the location of the Lakes Juá and Maicá belonging to the basin of the lower Tapajós River, Santarém, Pará, Brazil (source: Lima, J. L).

light. The amplification products were purified using a solution of polyethylene glycol-PEG (10 g polyethylene glycol 800, 7.3 g NaCl, and 35 mL of water) as previously described [33]. Similar volumes of the PEG and amplicon solutions were transferred to microtubes (1.5 mL), homogenized, and incubated (37°C for 15 min). The mixture was then centrifuged (15 min at 6,000 rpm), the supernatant was discarded, and the precipitate was washed twice with 70% ethanol and resuspended in water.

The sequencing reaction was performed using the BigDye® kit (Applied Biosystems). Sequencing was performed on the Seq 3130 Genetic Analyzer (Applied Biosystems). The sequences used in this study were compared and deposited in the GenBank database (Table 1).

**2.6. Richness, Diversity, and Equitability of the Fungal Community.** The number of individuals was determined for each piece of wood by the presence or absence of a species on it. In this study, an individual was quantified on a per-substrate basis. One individual was counted if it was found on a single piece of wood. However, multiple taxa were usually found to colonize individual substrates. Frequency, richness (species numbers), alpha diversity ( $\alpha$ ), and similarity of fungi were calculated for each lake. To characterize the diversity of the fungal community, the Shannon-Weaver diversity index ( $H'$ ) was employed [34]. This index describes diversity with a higher value signifying greater heterogeneity and greater diversity. The following formula was used to perform these calculations:

$$H' = - \sum (pi)^* (\log 2 pi), \quad (1)$$

where  $p_{in} = ni/N$ ,  $ni$  is the individual number of  $i^{\text{th}}$  taxa, and  $N$  is the individual number of all taxa.

In addition, to interpret the Shannon-Weaver index, the evenness index ( $E$ ) was calculated. This index is the uniformity of copy numbers among taxa. The evenness tends toward 0 when one taxon dominates the community and approaches 1 when the taxa have the same abundance. The index is expressed by the following formula:

$$E = \frac{H'}{\ln S}, \quad (2)$$

where  $H'$  is the Shannon-Weaver Index based on the number of individuals and  $S$  is the number of taxa present in the sample.

To evaluate differences in the fungal communities, the similarity among the samples was calculated using the Sørensen index ( $S'$ ) with values ranging from 0 (no similarity) to one (absolute similarity). This was calculated using the following formula:

$$S' = \frac{2c}{a+b}, \quad (3)$$

where  $a$  is the total number of taxa collected in Lake Juá,  $b$  is the total number of taxa collected in Lake Maicá, and  $c$  is the number of common taxa in both lakes. A taxa-area curve was plotted for the two collections to assess the sampling effort. Statistical analyses were performed using the PAST program, version 3.2 [35].

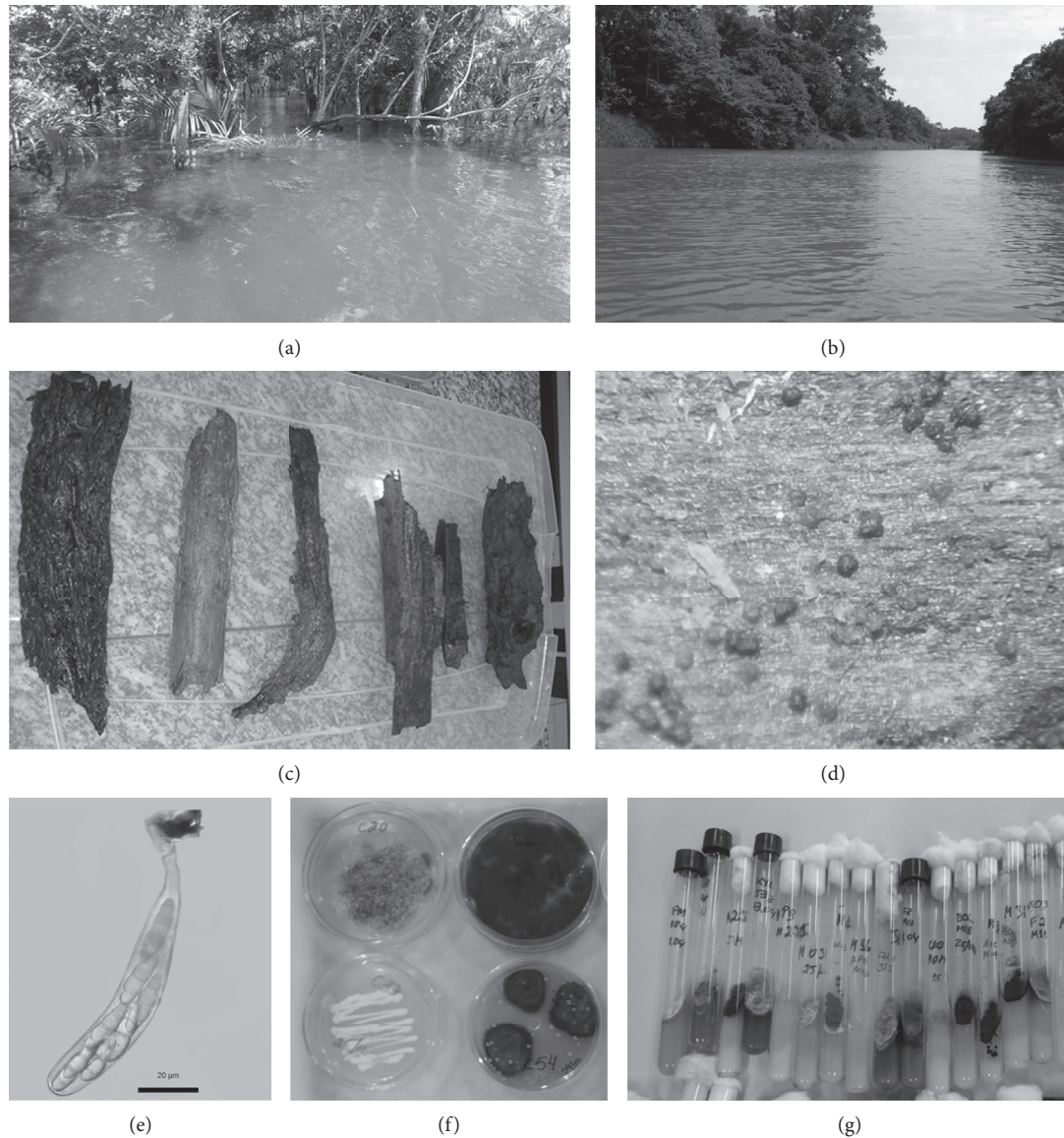


FIGURE 2: Collection site of Lake Juá (a). Collection site of Lake Maicá (b). Wood samples (c). Ascomata on a wood surface (d). Asci in the water of *Dothideomycetes* sp. (e). Colonies on MEA after 14 days at 25°C (f). Culture in PYG agar (g).

TABLE 1: Strain code and similarity (ITS Barcode) of 14 taxa isolated from samples of submerged wood collected in the Lakes Juá and Maicá, Santarém, Pará, Brazil.

Code/GenBank	Taxon	Accession no.	Similarity (%)
MT017512	<i>Pseudallescheria boydii</i>	KJ653823.1	100
MT017513	<i>Curvularia clavata</i>	MF038179.1	99.15
MT017519	<i>Curvularia clavata</i>	MF038179.1	100
MT017518	<i>Curvularia petersonii</i>	NR_158448.1	97.15
MT017514	<i>Cladosporium holotolerans</i>	MN826823.1	97.84
MT017515	<i>Simplicidiella nigra</i>	NR_164383.1	91.3
MT017516	<i>Jattaea</i> sp.	KT823783.1	90.46
MT017517	<i>Jattaea</i> sp.	EU770223.1	90.32
MT017520	<i>Bionectria</i> sp.	GU827483.1	99.41
MT017523	<i>Gonytrichum macroladum</i>	MH857954.1	98.34
MT017524	<i>Fusarium solani</i>	JN882257.1	99.12
MT017525	<i>Helicascus thalassioideus</i>	KP637162.1	90.02
MT017522	<i>Cladophialophora saturnica</i>	NR_111278.1	99.46
MT017521	<i>Calosphaerales</i> sp.	KR817246.1	99.04

In order to determine whether the sample size was sufficient to describe the diversity of the collection sites, the accumulation curve of the taxa found in the samples of submerged wood decomposed in the two lakes under study was analyzed by the ratio between the number of new taxa and the number of wood samples.

### 3. Results

**3.1. Physicochemical Characteristics.** In order to characterize the aquatic environments in this study, physicochemical analyses of the waters of the Lakes Juá and Maicá were performed at the time of sampling of wood samples. Table 2 lists the values of the parameters.

**3.2. Sample Size.** The accumulation curves obtained for the samples from Lakes Juá and Maicá show that the number of taxa does not asymptote, suggesting that we have not adequately captured the freshwater fungal species (Figure 3) and additional collections may be necessary.

**3.3. Identification of Fungi.** Morphologically, 23 different taxa were identified in the Lake Juá, with 43.5% ( $n = 10$ ) being sexual (5 Dothideomycetes and 5 Sordariomycetes) and 56.5% ( $n = 13$ ) being asexual. In the Lake Maicá, 26 taxa were identified morphologically, of which 65.4% ( $n = 17$ ) were sexual (4 Dothideomycetes and 13 Sordariomycetes), and 34.6% ( $n = 9$ ) were asexual. We carried out DNA analysis studies with only fourteen taxa (Table 1). It was not possible to investigate all taxa because most of them were uncultivated fungi that did not allow DNA extraction. ITS sequence data were successfully obtained and used to corroborate morphological identification (Table 3).

**3.4. Richness, Diversity, and Equitability.** In the Lake Juá, the diversity ( $H'$ ) and equitability ( $E$ ) indices reached 2.65 and 0.616255, respectively. In this period, the five most frequent taxa were *Xylomyces giganteus* (18%), *Pseudoxylomyces elegans* (11%), Pleosporaceae MT017518, *Fluviatispora* sp., and Teratosphaeriaceae MT017515 (9%). In the Lake Maicá, the diversity and equitability indices reached 2.82 and 0.643588, respectively. At this site, the most frequent taxa were *Thielavia* sp. (11%), *Longicollum biappendiculatum* (8%), *Submersisphaeria* sp., *Aquaticola* sp., and Morosphaeriaceae MT017515 (7%). The Sørensen index of the fungal communities between the two lakes studied was 0.3673. Eight taxa occurred in both environments. Information on the richness, diversity, and equitability of the taxa observed in the two lakes is described in Table 4.

### 4. Discussion

In the present study, we described the fungal diversity associated with the decomposition of wood in lakes belonging to the Tapajós River (PA, Brazil). This is an important study because it is the first to describe the diversity of these organisms in the lakes of the Tapajós River region. In the

TABLE 2: Characterization of the physicochemical parameters of the water of the Lakes Juá and Maicá, Santarém, Pará, Brazil, during the collections.

Parameters	Lake Juá	Lake Maicá
pH	$4.9 \pm 0.2$	$6.7 \pm 0.2$
Electrical conductivity ( $\mu\text{S}$ )	$9.3 \pm 0.2$	$42 \pm 0.2$
Water temperature ( $^{\circ}\text{C}$ )	$30 \pm 0.1$	$30 \pm 0.1$
Total $\text{O}_2$ (mg/L)	$7.8 \pm 0.1$	$6.5 \pm 0.1$
Turbidity (NTU)	$30 \pm 0.1$	$26.9 \pm 0.1$

experimental conditions, both lakes presented high diversity and few similarities among the taxa.

The Lake Juá had a low pH ( $4.9 \pm 0.2$ ) and low electrical conductivity ( $9.3 \pm 0.2 \mu\text{S}$ ). This was expected because Lake Juá is influenced by the Tapajós River [15, 36]. Its turbidity was  $30 \pm 0.1$  NTU, perhaps due to the direct interference of anthropic actions such as wastewater disposal due to the proximity to the urban area of Santarém. The Lake Maicá had neutral pH ( $6.7 \pm 0.2$ ) and high electrical conductivity ( $42 \pm 0.2 \mu\text{S}$ ). This may be due to the influence of the Amazon River during the flood season [14]. This river has an alkaline pH, high electrical conductivity, and high turbidity due to the large amount of sediment coming from the Andean regions [15, 16]. It is important to know the characteristics of the water in aquatic fungal diversity studies because previous studies have shown that factors such as pH, nutrients, substrate type, river geomorphology, and seasonality affect the fungal communities in aquatic environments [37–39].

The accumulation curve showed approximate stability after the collection of 20 to 25 samples from each lake studied (Juá and Maicá, respectively). Luo et al. [40] collected 100 samples of grass and bamboo from the Lake Dianchi in Thailand during four sampling events over a one-year period and reached asymptote in 50 and 70 samples. Hu et al. [41] collected 100 samples from a dammed lake and 90 samples in a stream, obtaining the stabilization of the curve in 60 and 80 samples, respectively. Cortez [28] collected 60 fragments of submerged wood in a lake in the region of Iranduba (Amazonas, Brazil) in two periods of the seasonal cycle, obtaining a plateau of species in 15 samples collected in the nonrainy period. In this study, 30 samples per lake were collected and appeared sufficient to near a plateau for local diversity.

At the Lakes Juá and Maicá, we observed 23 and 26 taxa, respectively. The number of unique taxa was 15 and 18 in the Lakes Juá and Maicá, respectively (Table 3). These results are similar to those observed by Cortez [28], who identified 25 taxa in a black water lake in Amazonas State. When compared to other works [41–43], the total number of taxa in this study was lower. This may be related to differences in the number of samples per period, the quantity of collections made, and the types of environments studied. In addition, previous studies have stated that physicochemical factors of water play an important role in the richness of ascomycetes present in freshwater and may be a factor in these lakes [44].

The Lake Juá presented greater richness of asexual fungi (56.5%) and Maicá of sexual fungi (65.4%). In the Lake Juá,

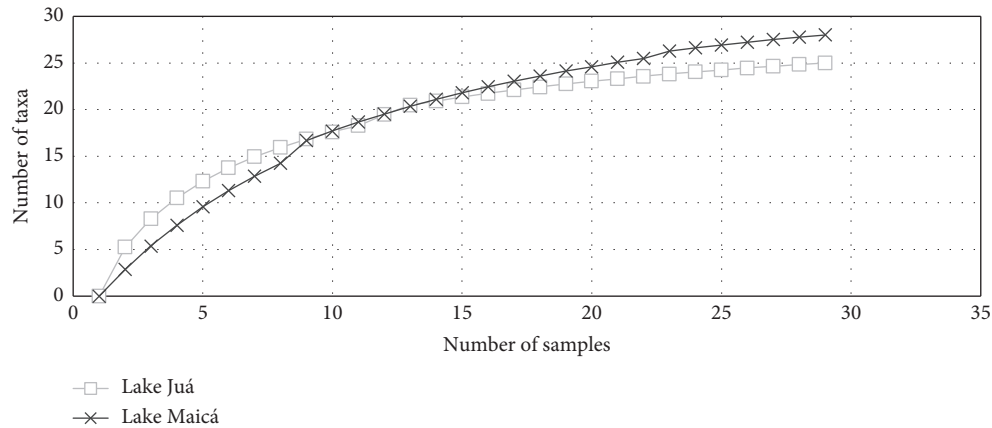


FIGURE 3: Accumulation curve of the number of fungi taxa found in relation to the sampling effort of collecting submerged fragments of decomposed wood in the Lakes Juá and Maicá, Santarém, Pará, Brazil.

TABLE 3: Frequency of taxa observed in samples of submerged wood collected in the Lakes Juá and Maicá, Santarém, Pará, Brazil.

Taxa	Lake Juá		Lake Maicá	
	FAB	FRE (%)	FAB	FRE (%)
<i>Xylomyces giganteus</i> Goh, W. H. Ho, K. D. Hyde & K. M. Tsui	18	15	0	0
<i>Pseudoxylomyces elegans</i> (Goh, W. H. Ho, K. D. Hyde & K. M. Tsui), Kaz. Tanaka & Hiray	13	11	0	0
Teratosphaeriaceae MT017515**	11	9	0	0
Pleosporaceae MT017518**	11	9	0	0
<i>Fluviatispora</i> sp.	11	9	0	0
<i>Cancellidium applanatum</i> Tubaki	9	7	1	1
<i>Cumulospora</i> sp.*	7	6	0	0
<i>Cordana terrestris</i> (Timonin) M. Hern.-Rest., Gené & Guarro	7	6	0	0
<i>Chloridium</i> sp.	5	4	5	6
<i>Lasiosphaeria</i> sp.	4	3	0	0
<i>Jobellisia</i> sp.*	4	3	0	0
<i>Aquaticola</i> sp.	3	2	6	7
<i>Taeniolella</i> sp.*	3	2	2	2
Morosphaeriaceae MT017525**	2	2	6	7
Microascaceae MT017512**	2	2	0	0
Diaporthales sp.1 MT017516; MT017517**	2	2	0	0
<i>Gonytrichum</i> sp. MT017523***	2	2	0	0
<i>Monodictys</i> sp.	2	2	0	0
Unidentified Pleosporales 3	1	1	5	6
<i>Acrogenospora sphaerocephala</i> , (Berk. & Broome) M. B. Ellis	1	1	1	1
<i>Cladophialophora</i> sp. MT017522**	1	1	0	0
<i>Curvularia</i> sp. MT017513; MT017519** B. L. Jain	1	1	1	1
<i>Stilbella</i> sp.*	1	1	0	0
Dothideomycetes sp.	0	0	3	4
Dothideomycetes sp.1	0	0	3	4
<i>Thielavia</i> sp.*	0	0	9	11
<i>Longicollum biappendiculatum</i> Zelski, F. R. Barbosa, Raja, A. N. Mill & Shearer	0	0	7	8
<i>Submersisphaeria</i> sp.	0	0	6	7
<i>Annulatascus</i> -like sp.	0	0	5	6
Savoryellales sp.	0	0	3	4
<i>Aniptodera</i> sp.	0	0	2	2
<i>Pseudohalonectria</i> -like sp.*	0	0	2	2
Annulatascaceae-like sp.	0	0	2	2
<i>Ophioceras</i> -like sp.*	0	0	2	2
Sordariomycetes sp. MT017521**	0	0	2	2
Nectriaceae sp. MT017520**	0	0	2	2
Cladosporiaceae MT017514**	0	0	2	2
Nectriaceae MT017524**	0	0	1	1
<i>Potamomyces armatisporus</i> K. D. Hyde	0	0	2	2
<i>Helicosporium</i> sp.	0	0	1	1
<i>Sporoschisma</i> sp.	0	0	3	4
TOTAL	121	100	84	100

\*First records for the Brazilian Amazon. FAB: absolute frequency; FRE: relative frequency. \*\*ITS Sequence obtained.

TABLE 4: Comparison of diversity (indices:  $H$ ,  $E$ , and  $S$  of the fungi community present in the samples of the Lakes Juá and Maicá, Santarém, Pará, Brazil.

Sampling	Lake Juá October 2017	Lake Maicá November 2017
Sample size	30	30
Number of ascomycetes (sexual)	51	67
Number of ascomycetes (asexual)	70	14
Average number of taxa per sample	4.0	2.8
Singleton taxa	15	18
Overlap in both lakes	8	
Five most common taxa	<i>Xylomyces giganteus</i> , 18 (18%) <i>Pseudoxylomyces elegans</i> 13 (11%)  Pleosporaceae MT017518 11 (9%) <i>Fluviatispora</i> sp., 11 (9%) Teratosphaeriaceae MT017515, 11 (9%)	<i>Thielavia</i> sp., 9 (11%)  <i>Longicollum biappendiculatum</i> , 7 (8%) <i>Submersisphaeria</i> sp. 6 (7%) <i>Aquaticola</i> sp. 6 (7%) Morosphaeriaceae MT017525 6 (7%)
Species richness ( $R$ )	23	26
Shannon-Weaver ( $H'$ )	2.65	2.82
Evenness ( $E$ )	0.616255	0.643588
Sørensen ( $S'$ )	0.3673	

this may be related to the influence of a stream that drains into this environment, supporting the increase of this taxon in the lake [45, 46]. In the Lake Maicá, the sexual species richness was greater. These results resemble those reported by Hu et al. [41], who found greater sexual richness in a lentic environment. Cortez [28] also found similar results and attributed these results to pluviometric action because rainwater can carry fungi from soil or vegetation to the lake.

The five most frequent taxa in the Lake Juá were *Pseudoxylomyces elegans*, *Xylomyces giganteus*, Pleosporaceae MT017518, *Fluviatispora* sp., and Teratosphaeriaceae MT017515. In the Lake Maicá, the five most frequent taxa were *Thielavia* sp., *Longicollum biappendiculatum*, *Submersisphaeria* sp., *Aquaticola* sp., and Morosphaeriaceae MT017525. *Aquaticola* sp. and *Longicollum biappendiculatum* were the most frequent taxa described by Cortez [28]. Abdel-Aziz [43] in a study on submerged stems of *Phragmites australis* (Cav.) Trin. ex Steud listed seven species that were common in this study (*Aniptodera* sp., *Annulatascus*-like, *Aquaticola* sp., *Ophioceras* sp., *Pseudohalonectria* sp., *Thielavia* sp., and *Monodictys*-like). Raja et al. [42], in freshwater environments of the Florida Peninsula, shared five taxa (*Aniptodera* sp., *Ophioceras* sp., *Submersisphaeria aquatica*, *Fluviatispora reticulata*, and *Longicollum biappendiculatum*). It was observed that these taxa occurred in both lentic and lotic environments. According to Shearer et al. [24], the composition of freshwater ascomycetes species is similar between Florida, tropical South America (Peru), and tropical Southeast Asia.

*Cancellidium applanatum*, found in Lake Juá and Maicá, was also reported by Zelski et al. [22] in a survey of ascomycetes in wood submerged in freshwater habitats in Peru. This species is considered a generalist and can occur in herbaceous and woody substrates of lotic and lentic habitats. This species, as well as *Monodictys* sp., can be considered resistant to dry periods [47], and its presence is not surprising considering that the Lake Juá also undergoes considerable periods of drought. In addition, *Acrogenospora sphaerocephala* has recently been reported on leaf material in

rivers and streams in the Caatinga biome as well as in submerged wood in a lake in Manaus, Amazonas [5, 28]. Nine taxa were identified as new records for the Brazilian Amazon, as shown in Table 1.

The Lakes Juá and Maicá presented a diversity of  $H'$  index = 2.65 and 2.82, respectively. Hu et al. [41] in Thailand compared an artificial lake and a stream obtaining a diversity index of 2.34 and 3.68, respectively, indicating that factors such as the composition of riparian forest, damming of rivers, and anthropogenic actions may affect the community and the diversity of fungi in freshwater habitats. Cortez [28] studied the diversity in a lake in the state of Amazonas, making seasonal comparisons, and obtained indices of 2.60 in the rainy season and 2.13 in the nonrainy season. This study inferred that seasonality may affect fungal diversity. In the Nile, Abdel-Aziz [43] also performed seasonal comparisons, with indices of 3.08 and 2.44 for winter and summer, respectively. Hyde et al. [48] emphasized that the number of taxa per wood sample is higher in the tropics than in temperate habitats. According to Graça et al. [49], the variation in low diversity rates in tropical and subtropical areas may be related to ecological and methodological issues. The diversity indexes of the present work are similar to those found in other lentic environments (mean ~ 2.8) and corroborate the assertion that diversity in lotic environments is greater (mean ~ 4.60) [41, 50–52].

Sørensen's similarity index (0.3673) revealed low similarity among the studied lakes. This may be due to the structural and physicochemical differences found during the study that are responsible for the composition of the mycota in aquatic ecosystems [37, 38, 53]. According to Leff [54], the microbial composition is affected in streams and rivers by the unidirectional flow of water (which is reflected in organisms' life strategies), mixing and aeration of water, and the importance of allochthonous materials.

Studies on fungi that decompose submerged wood in aquatic environments are lacking in the Amazon region. Thus, the present work was motivated to contribute to the knowledge of the diversity of aquatic fungi in this region.

The results show that there is a rich and balanced diversity. This study provides more insight into the dynamics of freshwater fungi in clear water habitats and indicates how the community is distributed in the Amazonian lakes. It is noteworthy that further studies are needed to strengthen this line of research and increase available information on biogeography, ecology, and taxonomy of freshwater ascomycetes species in the Brazilian Amazon.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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