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Original Article

Diversity and enzymatic capabilities of fungi associated with the digestive tract of larval stages of a shredder insect in Cerrado and Amazon Forest, Brazil

Diversidade e capacidades enzimáticas de fungos associados ao trato digestivo da fase larval de um inseto triturador em Cerrado e Floresta Amazônica, Brasil

M. F. N. P. Teixeira[a], C. R. Souza[a, b] and P. B. Morais[a*]

[a]Universidade Federal do Tocantins – UFT, Laboratório de Microbiologia e Biotecnologia Ambiental, Palmas, TO, Brasil
[b]Instituto Federal de Educação, Ciência e Tecnologia do Tocantins – IFTO, Gurupi, TO, Brasil

Abstract

Tropical biomes such as Brazilian Cerrado and Amazon Forest have a great diversity of fungi and insects. Interactions between these organisms can be beneficial to both partners. In streams, these interactions contribute to litter decomposition. Studying the digestive tract (DT) of shredder insects as a habitat for fungal microorganisms is an opportunity to obtain fungal strains with biotechnological potential, which may help to understand the symbiotic relationships between these organisms in tropical forests. This study investigated the fungal community in the DT of larvae of *Triplectides* (Trichoptera: Leptoceridae) collected in low-order streams in the Cerrado and Amazon Forest biomes in Brazil. Forty-nine fungal isolates were obtained and identified among 32 species and 12 genera. The genus *Roussoella* was only found in the DT of insects in Amazon Forest streams, while 7 genera only occurred in the DT of insects in Cerrado streams. The genus *Penicillium* (40%) was the most frequent. In the Cerrado, 78% were producers of CMCase, more than two-fold that in the Amazon Forest (35%). And 62% were producers of xylanase, in the Cerrado and 71% in the Amazon Forest. In this context, the fungal community in the DT of *Triplectides* larvae may play an important role in the insect diet by breaking down lignocellulosic material.

Keywords: *Triplectides*, Trichoptera, xylanase, cellulase.

Resumo

Biomas tropicais como o Cerrado brasileiro e a Floresta Amazônica apresentam uma grande diversidade de fungos e insetos. As interações entre esses organismos podem ser benéficas para ambos os parceiros. Em riachos, essas interações contribuem para a decomposição da serapilheira. O estudo do trato digestório (TD) de insetos como um habitat para microrganismos fúngicos é uma oportunidade para obtenção de linhagens fúngicas com potencial biotecnológico, podendo trazer luz para o entendimento das relações simbióticas entre esses organismos em florestas tropicais. Esse estudo investigou a comunidade fúngica do TD de larvas de *Triplectides* (Trichoptera: Leptoceridae) coletados em riachos de baixa ordem nos biomas Cerrado e Floresta Amazônica no Brasil. Foram obtidos 49 isolados fúngicos e identificados entre 32 espécies de 12 gêneros. O gênero *Roussoella* foi encontrado apenas no DT de insetos em riachos da Floresta Amazônica, enquanto sete gêneros ocorreram apenas no DT de insetos em riachos do Cerrado. O gênero *Penicillium* (40%) foi o mais frequente. No Cerrado, 78% foram produtoras de CMCase, mais que o dobro da Floresta Amazônica (35%). E 62% foram produtoras de xilanase, no Cerrado, e 71% na Floresta Amazônica. Nesse contexto, a comunidade fúngica do TD de larvas *Triplectides* pode desempenhar um papel importante na dieta de insetos por quebrar o material lignocelulósico.

Palavras-chave: *Triplectides*, Trichoptera, xilanase, celulase.

Introduction

Brazilian Cerrado and Amazon Forest biomes are considered biodiversity hotspots. This high biodiversity, especially in neotropical regions, represents a great potential for organisms to be discovered (Almeida et al., 2017), as well as their specificities. Thus, these tropical forests hold a large part of the diversity of insects and fungi that coexist in several terrestrial and aquatic habitats, where potential interactions may occur between species of the two groups (Boucias et al., 2012; Douglas, 2015). Insects are colonized by microorganisms, in their body surface, in their digestive tract (DT) and interior of certain tissues. Bacteria and fungi prevail in insect microbiomes and

*e-mail: moraispb@uft.edu.br
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they are essential for survival, maturation and nutritional functions of hosts among honey bees (Kwong and Moran, 2016; Raymann and Moran, 2018) and also in some species of moths (Chen et al., 2016).

The roles of the symbionts in relations between fungi and insects were found to be nutritional, for protection, or even for hormonal maturation of the insect (Mohammed et al., 2018), whereas the microorganisms receive protection and abundant food in the body of the insect. Fungi contribute nutrients to several insect groups (Gibson and Hunter, 2010), as with bark beetles (Six, 2012) and the carmine cochineal that fungi help in the nitrogen recycling process (León et al., 2016).

Fungi have been often found in the DT of several insects that feed on wood or detritus and, possibly, play a role in the digestion of such plant material (Engel and Moran, 2013; León et al., 2016; Santos et al., 2018; Belmont-Montefusco et al., 2020a, Belmont-Montefusco et al., 2020b). Shredder insects feed on senescent plant material in low-order streams (Graça et al., 2001; Jabiol and Chauvet, 2012) where they contribute to the breakdown of organic matter in aquatic ecosystems together with fungal groups such as Ascomycetes and Hyphomycetes (Graça et al., 2016). Insect species of the genus *Triplectides* (Trichoptera: Leptoceridae) are shredders during their aquatic larval stage, consuming substrates such as leaves and dead wood (Oliveira and Pes, 2014; Cortez and Gonçalves, 2015). Microbial colonization of those substrates improve palatability and increase nitrogen content of food (Graça et al., 2001). The genus is highly diverse in tropical streams where a variety of substrates are available for fungal colonization that may be ingested by these insects along with food. The hypothesis arises that there is a diversity of fungi associated with the DT of larvae of insects of the genus *Triplectides* in low-order streams in the Brazilian Cerrado and Amazon Forest.

The fungal composition and diversity in the DT of insects may clarify the role of fungi in the physiology of the host (Gao et al., 2018) and shed light onto the ecological role of symbiosis between the two groups and biotechnological potential of those fungal communities. Thus, this study aimed to verify whether there is a possible specificity of occurrence of fungi in the DT of those insects and to test the potential for the production of xylanases and cellulases, which also shows the potential nutritional role, in food digestion in the DT of the insect.

Material and Methods

Characterization of the study areas

The study was carried out in five streams located in a conservation area and its surroundings in the north part of the Brazilian Cerrado (Tocantins, Brazil) and in four streams in an Amazon Forest (Pará, Brazil) biomes (Figure 1). A 200 m stretch of the body of the stream was sampled using a D-frame net (0.500 mm mesh and 0.465 m^2 area) in each stream selected. Larvae of *Triplectides* (Trichoptera: Leptoceridae) were collected in the substrate available (especially leaf packages) and identified by specialists based on the taxonomic descriptions by Hamada et al. (2014). Each individual larva was transferred to a sterile tube containing 1 mL sterile saline solution and stored for 2 to 4 h in ice until processing in the laboratory.

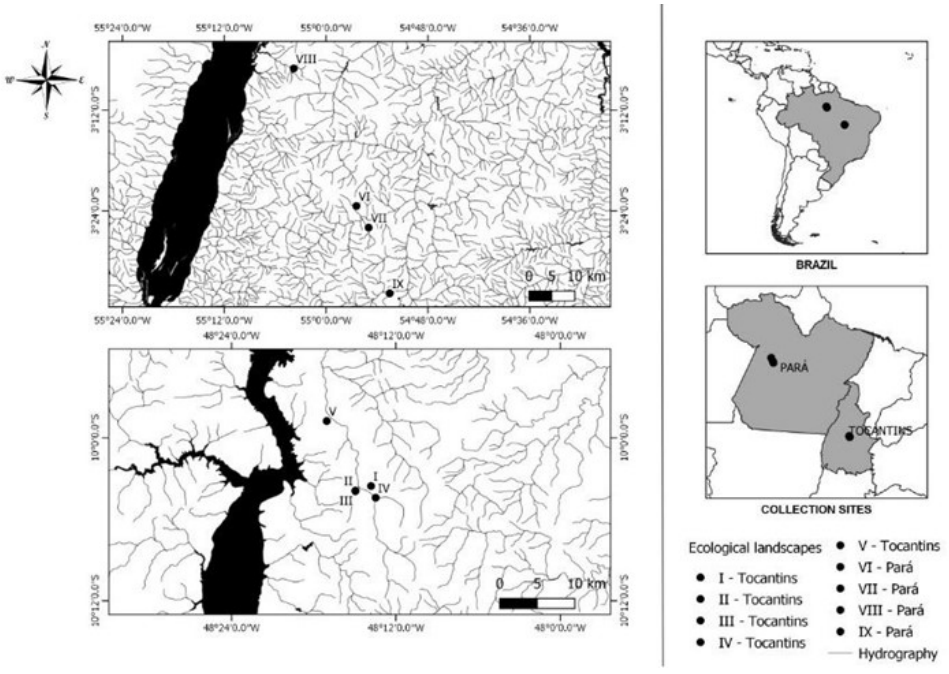

Figure 1. Maps with sampling sites of *Triplectides* (Trichoptera: Leptoceridae) in low-order streams in the Cerrado (Lajeado State Park and surroundings, state of Tocantins, Brazil) and low-order streams in the Amazon Forest (Tapajós National Forest, state of Pará, Brazil) biomes.

Fungal isolation and purification

In the laboratory, the individual larvae were aseptically dissected using a stereoscopic microscope. The intestinal tract was added to 1.0 mL sterile saline solution in an Eppendorf tube. Next, a 0.1 mL aliquot was seeded on a Petri dish containing potato-dextrose agar (PDA) supplemented with 100 µg mL^{-1} chloramphenicol in triplicates. The dishes were incubated at 28 °C for up to 60 days. The fungal isolates obtained were individually transferred to Petri dishes containing PDA and incubated at 25 °C for seven days for purification. Fungal strains were kept in storage according to adaptations of Castellani (1939).

DNA extraction, amplification and sequencing

The fungal isolates were transferred from storage to dishes containing PDA for 24-28 h and then transferred to 3% ME (Malt Extract) broth for cellular increase for seven days of growth in a rotating shaker (100 rpm) at room temperature. Next, approximately 40 mg of mycelium were collected for DNA extraction with the Wizard™ Genomic DNA Purification Kit (Promega, USA), following the modified protocol by Burghoorn et al. (2002). After extraction, the DNA was analyzed in a NanoDrop 2000 (Thermo Scientific, Brazil) spectrophotometer. Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990) were employed for amplification of the ITS (Internal Transcribed Spacer) region of rDNA (~600 bp) following the amplification conditions proposed by Santos et al. (2016). The amplified ITS fragments were submitted to electrophoresis in 1.0% agarose gel containing GelRed™ (Biotium Inc., USA) and visualized under ultraviolet light in a photo documentation system (Loccus Biotechnology, Brazil). The 1 kb DNA Ladder (Promega, USA) was used as a molecular weight marker.

The amplified products were sequenced in both directions using the same PCR starters in an ABI 3500 XL (Life Technologies, USA) automated sequencer according to the Sanger or chain termination method (Sanger et al., 1977) using a BigDye Terminator v3.1 sequencing kit (Life Technologies, USA). Sequencing was performed by the company Myleus Biotechnology, located in Belo Horizonte – MG, Brazil. Additionally, the amplification of the genes β-tubulin (Bt2a and Bt2b) was used for fungus taxa with low intraspecies variation according to the protocols established by Godinho et al. (2013). All sequences were compared with sequences deposited at the GenBank database using a local alignment algorithm for nucleotide sequences BLAST (Basic Local Alignment Search) (Altschul et al., 1990) and at the CBS (*Centraalbureau voor Schimmelcultures* Fungal Biodiversity Centre) database (http://www.cbs.knaw.nl).

Xylanolytic and cellulolytic screening of the fungal community

The fungal community in the DT of *Triplectides* was tested for the production of xylanase and cellulase through screening in solid medium containing xylan or carboxymethylcellulose (CMC) as the only carbon source. The production of the enzyme was assessed via the growth of the strain on a dish and the revelation of hydrolysis halo using Congo red stain (Zhang et al., 2006). The strains were reactivated in PDA and then repeated in triplicate to a medium with xylan (Xylan, Beechwood purified) or carboxymethylcellulose (CMC) and trace elements composed of ($C_6H_8O_7 \cdot H_2O$ 50; $ZnSO_4 \cdot 7H_2O$50; Fe (NH_4) 2 $(SO_4)_2 \cdot 6H_2O$ 10; $CuSO_4 \cdot 5H_2O$ 2.5; $MnSO_4 \cdot H_2O$ 0.05; H_3BO_3 0.05; $Na_2MoO_4 2H_2O$ 0.05; Salt solution: $Na_3C_6H_5O_7 \cdot 5H_2O$ 150; KH_2PO_4 250; NH_4NO_3 100; $MgSO_4 \cdot 7H_2O$ 10; $CaC_{l2} \cdot 2H_2O$ 5) and biotin (0.1 mg ml^{-1}) 5 mL; 0.2 mL chloroform (Vogel, 1956).

Next, staining was conducted with Congo red (0.25%) for 30 min and washing was performed with NaCl (1 M) for 15 min. The fungi that exhibited lighter color halos around the colony in the selective medium were considered producers of xylanase or cellulase. A digital caliper was used to measure the diameter of the colonies and the halos. The enzymatic index (EI) was determined by dividing the diameter of the halo by the diameter of the colony (Nogueira and Cavalcanti, 1996).

Statistical analysis

Diversity was measured via the indices of Simpson (1-D), Shannon (H '), Margalef, and Chao-1, which were calculated for the number of sampled larvae from streams in the Cerrado and Amazon Forest. The larvae were considered the sampling unit, being the biomes, and not the streams, the variable of interest. The indices were calculated with 95% confidence using the software PAST version 4.01 (Hammer et al., 2001).

Results

A total of 49 fungal isolates were obtained from 21 larvae of *Triplectides* (Trichoptera: Leptoceride) and identified among 32 species of 12 genera, besides two taxa with inconclusive taxonomy (Table 1). The average fungal isolates per DT was 2.14 CFU/DT in the Cerrado biome streams and 1.72 CFU/DT in the Amazon Forest streams. In the Cerrado, 32 strains were obtained belonging to 23 species of 11 genera, whereas in the Amazon Forest, 17 strains of 11 species of five genera were obtained. The genus *Roussoella* was only found in the DT of insects in Amazon Forest streams, while seven genera only occurred in the DT of insects in Cerrado streams. The genus *Penicillium* was the most frequent and occurred both in the Cerrado and in the Amazon Forest, with 20 strains (40%) isolated in different DTs. The genera *Cladosporium* (8%), *Talaromyces* (8%), and *Trichoderma* (8%) exhibited similar frequency of occurrence. The genera *Aspergillus* and *Clonostachys*, with one occurrence each, were isolated only in the Cerrado biome.

Among the species, *Penicillium caseifulvum*, *Penicillium paxilli*, and *Neopestaloptiopsis formicarum* occurred in the DT of larvae from streams in the Cerrado and Amazon Forest. All other species occurred in only one of the biomes, i.e., 20 species occurred exclusively in the Cerrado and eight, in the Amazon Forest. The most frequent species were *P. paxilli* in the Cerrado, with four strains, and *T. palmae* in

Table 1. Identification of fungi from the DT of *Triplectides* (Trichoptera: Leptoceridae) larvae in Cerrado and Amazon Forest Biomes Brazil and their respective enzymatic indices (EI).

Biomes		Code*	Taxa	N° GenBank accession	No. of bp analyzed	Query cover (%)	Identity (%)	My GenBank number	EI** (Xylanase)	EI** (Cellulase)
CERRADO	I	T5TA7	*Penicillium caseifulvum*	NR_163685.1	414	100	100	MT521710.1	1.14±0.04	1.04±0.01
		T5TA9	*Cladosporium kenpengii*	KY646222.1	399	100	99	MT521711.1	2.97±0.07	1.32±0.04
		T5TB4a	*Penicillium paxilli*	MH856391.1	380	100	100	MN737735.1	0	1.29±0.11
		T5TB5	*Penicillium vancouverense****	JN606663.1	369	97	97		1.14±0.06	1.08±0.55
		T5TB7	*Penicillium paxillii*	MH856391.1	499	100	99	MN737736.1	1.15±0.04	1.99±0.43
		T5TB14	*Talaromyces stollii****	JX315633.1	380	99	100		1.59±0.15	1.13±0.03
		T5TC1	*Byssochlamys spectabilis*	NR_130679.1	402	100	100	MN737737.1	0	0
		T5TC2	*Penicillium mallochii*	NR_111674.1	395	99	99	MN737738.1	1.14±0.01	1.85±0.05
		T5TC3	*Penicillium mallochii*	NR_111674.1	459	100	100	MN737739.1	0	2.01±0.74
		T5TC4	*Trichoderma dorotheae*	MH863050.1	449	100	100	MN737740.1	0	0
		T5TC5	*Diaporthe passifloricola****	NR_147595.1	478	97	99		1.17±0.03	1.07±0.01
		T5TC6	Fungal species A						1.27±0.01	1.22±0.03
	II	T6TA1	*Trichoderma virens ****	MH857855.1	330	100	100		0	1.06±0.02
		T6TA2	*Penicillium exudans*	NR_153273.1	340	100	100	MN737741.1	0	1.12±0.05
		T6TA3	*Cladosporium subuliforme*	MH864124.1	442	100	100	MT521712.1	2.89±0.38	2.12±0.36
		T6TA13	*Neopestalotiopsis formicarum*	NR_145242.1	456	100	97	MT521713.1	0	1,07±0.02
		T6TB2	*Penicillium mallochii*	NR_111674.1	462	99	100	MN737742.1	1.07±0.01	1.38±0.02
		T6TC2	*Penicillium citrinum*	NR_121224.1	420	100	100	MN737743.1	1.16±0.05	1.38±0.03
		T6TC5	*Talaromyces stollii*	NR_111781.1	475	100	100	MN737744.1	1.25±0.10	0
	III	T7TA1	*Penicillium citrinum*	MH856132.1	398	100	100	MN737745.1	0	1.63±0.04
		T7TA4	*Byssochlamys spectabilis*	NR_130679.1	488	98	97	MT521714.1	1.33±0.18	0
		T7TB3.2	*Aspergillus stellatus*	NR_131287.1	399	100	100	MT521715.1	1.79±0.04	0
		T7TB5	*Penicillium atrofulvum****	JN606677.1	318	100	100		0	1.58±0.33
		T7TC2	*Penicillium rubens*	NR_111815.1	461	100	100	MN737746.1	1.2±0.07	1.27±0.11
	IV	T9TA4	*Penicillium paxilli*	MH856391.1	468	100	100	MT521716.1	0	
		T9TC3	*C. verrucocladosporioides****	MH863939.1	434	100	100		1.88±0.05	1.34±0.04
		T9TC4	*Myxospora musae*	NR_155387.1	467	98	100	MN737747.1	0	1.49±0.07

*Fungal codification of the Collection of Microbial Cultures Carlos Rosa CCR of the Federal University of Tocantins, Brazil. **Arithmetic mean ± standard deviation. ***Identified with β-tubulin and not deposited.

Table 1. Continued...

Biomes		Code*	Taxa	N° GenBank accession	No. of bp analyzed	Query cover (%)	Identity (%)	My GenBank number	EI** (Xylanase)	EI** (Cellulase)
CERRADO	V	T10TA5B	*Penicillium paxilli*	MH856391.1	423	99	100	MN737748.1	1.35±0.17	1.34±0.09
		T10TB2	*Cladosporium sp.****	LN834420.1	475	100	100		1.94±0.10	1.57±0.15
		T10TB3	*Paraphaeosphaeria arecacearum*	NR_145166.1	483	98	100	MT521717.1	1.25±0.03	1.19±0.02
		T10TB4	*Trichoderma neotropicale*	MH865818.1	462	100	100	MT521718.1	0	0
		T10TC2	*Clonostachys rosea ****	AF358160.1	274	100	96		1.23±0.04	1.09±0.01
AMAZON FOREST	VI	P2TA3	*Diaporthe stewartii*	MH855768.1	422	100	98	MT521719.1	0	0
		P2TA6	*Penicillium paxilli*	MH856391.1	315	100	100	MN737731.1	1.17±0.07	1.6±0.17
		P2TA11	*Roussoella solani*	NR_145198.1	465	97	99	MT521720.1	0	0
		P2TA13	*Roussoella mexicana*	KT950848.1	253	100	100	MN401284	1.17±0.08	0
		P2TA15	*Penicillium cairnsense****	NR_121508.1	385	100	100		1.37±0.02	1.38±0.14
		P2TA18	*Roussoella solani*	NR_145198.1	474	100	99	MT521721.1	1.31±0.10	0
		P2TB6	*Penicillium maximae*	NR_121343.1	463	100	100	MN737732.1	1.55±0.35	1.33±0.01
		P2TC9	*Penicillium paxilli*	MH856391.1	427	97	100	MT521722.1	0	1.24±0.04
		P2TC12	*Diaporthe pterocarpicola*	NR_111713.1	379	92	100	MN737733.1	1.15±0.04	0
	VII	P4TA2	*Neopestalotiopsis formicarum*	NR_145242.1	403	96	100	MT521723.1	1.11±0.03	1.04±0.02
	VIII	P6TC2	*Penicillium rolfsii*	MH856397.1	461	100	100	MN737734.1	0	1.13±0.09
		P6TC6	*Neopestalotiopsis formicarum****	NR_145242.1	326	96	99		0	0
	IX	P7TB6	*Talaromyces palmae*	NR_103617.1	471	100	98	MT521724.1	0	0
		P7TB11	*Talaromyces palmae*	NR_103617.1	417	100	98	MT521725.1	0	1.19±0.08
		P7TB15	*Penicillium caseifulvum*	NR_163685.1	413	100	100	MT521726.1	1.07±0.03	1.14±0.05
		P7TC6	*Talaromyces palmae*	NR_103617.1	515	100	98	MT521727.1	1.27±0.10	0
		P7TC7	Fungal species B						1.11±0.02	0

*Fungal codification of the Collection of Microbial Cultures Carlos Rosa CCR of the Federal University of Tocantins, Brazil. **Arithmetic mean ± standard deviation. ***Identified with β-tubulin and not deposited.

the Amazon Forest, with three strains. Twenty-two species, including four species of the genus *Cladosporium*, seven species of *Penicillium*, three species of *Trichoderma* and *Diaporthe*, in addition to *Aspergillus stellatus*, *Myxospora musae*, *Paraphaeosphaeria arecacearum*, *Clonostachys rosea*, and *Roussoella mexicana* occurred as singletons.

The Chao-1 index showed that, in both biomes, the ideal sampling number was not reached. Due to the overvaluation of species considered singletons in the calculation of richness, the Cerrado biome (54 fungal species) showed a greater estimate of richness than the Amazon Forest (15 fungal species). The Margalef richness index was also higher in the Cerrado, as were the Simpson and Shannon diversity indices (Table 2).

Differences in the physicochemical parameters of the water in the nine streams sampled were detected between the Cerrado and the Amazon Forest (Table 3). The mean altitude of Cerrado streams was above 400 m, while those in the Amazon Forest had a mean altitude of 99 m as they were in the Amazon plain. The mean temperature of the streams in the Cerrado was lower than in the Amazon Forest by about 2 °C. The waters in the Amazon Forest streams were acidic and had higher electric conductivity and turbidity.

A total of 62% (Figure 2A) of the fungal community in the Cerrado exhibited enzyme activity for xylanase and strains *Cladosporium kenpeggii* MT521711.1 and *Cladosporium subuliforme* MT521712.1 showed the highest values of enzymatic indices (EI) (Table 1). Seventy-eight percent strains exhibited cellulolytic activity and the highest EI values were shown by *Cladosporium subuliforme* MT521712.1 and *Penicillium mallochii* MN737739.1 (Table 1). In the Amazon Forest (Figure 2B), 71% of strains exhibited xylanolytic activity and 35% exhibited positive activity for cellulase. The highest EI values were shown by strains *Penicillium maximae* MN737732.1 to xylanase and *Penicillium paxilli* MN737731.1 to cellulase (Table 1).

Discussion

The DTs of *Triplectides* larvae found in streams in the Amazon Forest and Cerrado host a diversified community

Table 2. Number of insects sampled (n) and richness and diversity of filamentous fungi in the DT of *Triplectides* (Trichoptera: Leptoceridae) in Cerrado and Amazon Forest Biomes, Brazil.

Biomes		Geographical coordinates	Sampled larvae (n)	Richness (S)	Chao -1	Margalef	Simpson (1 – D)	Shannon (H)
Cerrado	I	(10°03'33.40"S; 48°13'49.30"W)	n=3	10	54	6.5	0.95	3.06
	II	(10°03'53.60"S; 48°14'58.00"W)	n=3	7				
	III	(10°03'55.90"S; 48°14'57.70"W)	n=3	5				
	IV	(10°04'25.00"S; 48°13'29.10"W)	n=2**	3				
	V	(09°58'46.30"S; 48°17'03.20"W)	n=3	5				
		Richness	n= 14	23*				
Amazon Forest	VI	(03°23'25.2"S; 54°56'26.3"W)	n=3	7	15	3.7	0.90	2.34
	VII	(03°25'59.1"S; 54°54'59.6"W)	n=1**	1				
	VIII	(03°07'04.3"S; 55°03'49.5"W)	n=1**	1				
	IX	(03°33'48.2"S; 54°52'30.90"W)	n=2**	3				
		Richness	n= 7	11*				

Note: *Total value excluding taxon repetition; **Number of samples collected was lower due to lack of larvae during the field trip.

Table 3. Physicochemical parameters of sampled streams of Cerrado and Amazon Forest, Brazil.

Parameters*	Cerrado	Amazon Forest
Altitude (m)	452.2 ± 94.16	99 ± 18.67
Temperature (°C)	23.03 ± 23.03	25.84 ± 0.90
Turbidity	1.91 ± 1.04	2.83 ± 1.68
Dissolved oxygen (mg L^{-1})	8.72 ± 0.21	8.71 ± 1.46
pH	6.46 ± 0.57	4.02 ± 0.13
Electrical conductivity (mS cm^{-1})	6.97 ± 4.23	18.74 ± 0.64
Width (m)	2.19 ± 1.25	3.01 ± 1.60
Depth (m)	0.14 ± 0.07	0.20 ± 0.11
Current velocity (m s^{-1})	0.30 ± 0.10	0.21 ± 0.15

*Arithmetic mean ± standard deviation.

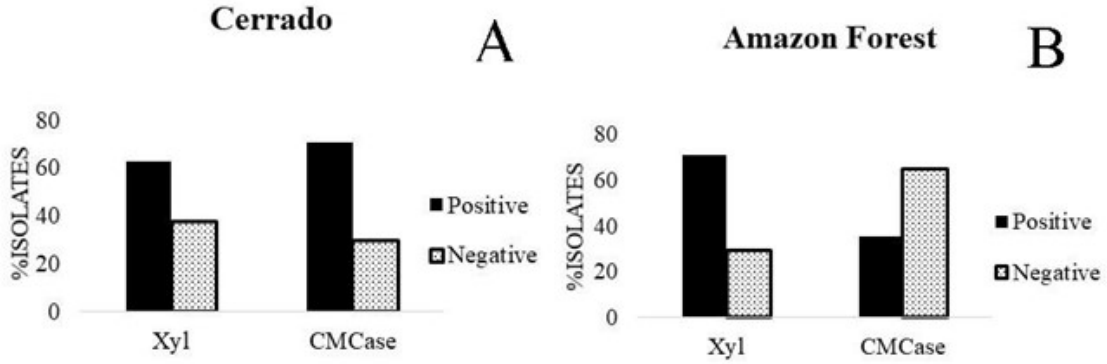

Figure 2. Percentage of fungal isolates from the DT of *Triplectides* (Trichoptera: Leptoceridae) in Cerrado (A) and Amazon Forest (B) biomes producers and non-producers of xylanase (Xyl) and cellulase (CMCase).

of fungi, as the diversity indices show (Table 2). *Triplectides* sp. larvae are usually found in patches of leaves located on the bed of streams and pools of water and are classified as a trophic group of leaf shredders (Kiffer et al., 2016). Therefore, it is expected that, when feeding on leaves, those larvae ingest a variety of microorganisms, including fungi colonizing such substrates, which may make up their mycobiome (Mohammed et al., 2018). That phenomenon is known as conditioning of the plant material and may occur by fungal colonization of the leaf litter in the environment. Those fungi may originate from the plant itself and from soil and water after the abscission of leaves. Seasonality affects the fall of leaf litter in tropical biomes (Atlantic Forest, Amazon Forest, and Cerrado), an effect that possibly impacts the quality and amount of substrates (Tonin et al., 2017). This might reflect on the distribution of shredder species in their microhabitat and also in the choice of the food (Abos et al., 2006). Since the present study did not select the type of leaf where the insect was collected (age, senescence, stage of decomposition, etc.), it is possible that the larvae had fed on leaves of different types that probably contain different microbial communities, thus forming a diverse microbiome.

Another possible origin of the high diversity of fungal groups in DT of larvae is the differences in diets of species and life cycles of the insects. There is strong evidence that substrates determine the intestinal microbiota of larvae and thus the diversity of the intestinal microbiota is fully related to the diet and life cycles of each insect species (Arias-Cordero et al., 2012; Mohammed et al., 2018; Alves Júnior et al., 2019). Recent studies show that the microbiomes differ in the stages of larval development (Chen et al., 2016; Gao et al., 2018; Yao et al., 2019), with higher diversity in the initial phase and greater richness in the adult phase (Gao et al., 2018). Since the larvae collected in this work were possibly at distinct larval stages, a diversified microbiome was expected. Also the physiology and physicochemical characteristics of the DTs of insects may vary between species and, thus, different larvae may host distinct microbial communities (Ceja-Navarro et al., 2014; Mason et al., 2017). In the field, it is not possible to distinguish larvae of different *Triplectides* species, specially in the two biomes where there are few thorough taxonomic studies of aquatic insects, and the distinction of species is accomplished by examination of adult stages (Pes et al., 2005).

The fungal community was composed mostly of singletons, i.e., with a low frequency of occurrence of a large number of species. One of the possible explanations for this fact is related to the diversity of substrates on which the larvae were collected. That is supported by the literature, the high frequency of singletons may be related to the high diversity of fungi associated with plant substrates (Martins et al., 2017; Malta et al., 2019; Ferreira et al., 2019). It also reinforces evidence that the diets of larvae of the same taxon of Trichoptera may vary due to the differences in riparian vegetation in their aquatic habitats.

A high number of filamentous fungi morphospecies per DT (6.2 ± 6.4) of larvae of *Phylloicus* was counted in streams of the Amazon Forest (Santos et al., 2018). In the present study, the mean number of taxa per DT was 2.33, much lower than the value reported by Santos et al. (2018). That is likely due to the high diversity of the insect genus *Phylloicus* that presents 25 spp described for Brazil (Santos et al., 2019). This study, in turn, investigated the mycobiome of *Triplectides*, which has about 14 species described in Brazil (Pes et al., 2014). It is highly probable that the set of *Triplectides* larvae collected belonged to a narrower group of species. Studies (Clair, 1994; Pimentel et al., 2020) report both *Phylloicus* and *Triplectides* as little selective and the availability of food items is the greatest influence on their diet. *Phylloicus* are detritivores, being shredders only for breaking down leaves for shelter construction (Pimentel et al., 2020), another explanation for the higher diversity of fungi in DT of this genus as compared to *Triplectides*.

The present results indicated common taxa in both biomes, as is the case of the genus *Penicillium*, which was the most frequent in this research (40%) and occurred both in the Cerrado and in the Amazon Forest. The genus *Penicillium* was also found in the DT of larvae of *Phylloicus* in the same sites of Cerrado and Amazon Forest biomes (Santos et al., 2018) as also in larvae of *Triplectides*, *Phylloicus* and *Stenochironomus* aquatic insects in streams sampled in another site of the Amazon Forest in the State of Amazonas,

Brazil, together with *Aspergillus* and *Trichoderma* (Belmont-Montefusco et al., 2020a; Belmont-Montefusco et al., 2020b). In addition, the species *Paraphaeosphaeria arecacearum*, found in the DT of *Triplectides* in Cerrado, was also isolated in the DT of *Phylloicus* in the same Cerrado sites by Santos et al. (2018). Thus, despite the differences among the environmental factors of each biome and even among the larval species studied, such species may have a mycobiome in common, formed by fungi ubiquitous in the environment, which reinforces the possibility that those fungi are important to the insects.

Many of the fungal taxa found in the DT of *Triplectides* are of broad occurrence in aquatic and terrestrial environments (Gutiérrez et al., 2015; Song et al., 2018). The genus *Penicillium* is found widely in nature in soil and plants, in the air, and in decaying vegetation (Godinho et al., 2015; Mohammadian et al., 2017). The genus *Cladosporium* is very diverse, common, and widespread, including endophytic, pathogenic, phytopathogenic, and saprophytic species (Bensaci et al., 2015).

The most frequent species were *Penicillium paxilli* in the Cerrado and *Talaromyces palmae* in the Amazon Forest. The species *T. palmae* belongs to an endophytic group (Sette et al., 2006), which includes *P. paxilli* that was first isolated from leaves (Rukachaisirikul et al., 2007). Based on those findings and considering the diet of insect larvae is based on leaves, it can be suggested that some of the fungi in the insects diet may be of endophytic or epiphytic origin. Endophytic fungi are a very diversified group present in most plants (Marques et al., 2018; Ramírez-Camejo, 2024) and, according to Peay et al. (2016), the diversity of endophytic fungi associated with leaves is higher in tropical forests when compared with larger spatial scales. And some endophytic fungi may be good producers of enzymes, such as cellulases and xylanases (Amirita et al., 2012; Corrêa et al., 2014).

The community of fungi associated with the DT of *Triplectides* in Cerrado has higher richness and diversity than those in the Amazon forest, which may be influenced by several factors. Particularities of streams such as current velocity, width, and depth (Landeiro et al., 2010) and leaf characteristics are important and must be taken into account in studies on tropical streams (Li et al., 2009; Landeiro et al., 2010). Current velocity may also influence the feeding of insects inhabiting streams (Boyero et al., 2006). Moreover, the taxonomy and functionality of the composition of communities of Trichoptera in Cerrado streams may be determined by factors such as physical structure of the streams and water quality (Ferreira et al., 2017). That may explain the differences in composition of the fungal community of *Triplectides* DT between those two biomes. Streams in the Amazon Forest and Cerrado, besides having distinct abiotic characteristics and plant physiognomies, can host different species of *Triplectides*, thus resulting in different mycobiomes. In the Cerrado, a higher diversity of species of *Triplectides* may have been sampled, since fourteen insects were sampled in the Cerrado whereas only seven were collected in the Amazon Forest. In addition to those, factors such as altitude, which was different in the streams studied, may have an influence. According to Camacho et al. (2009), the abundance and richness of shredder species vary with the altitude due to the variation in temperature. According to Casotti et al. (2015), the characteristics of the leaves may induce the behavior of shredders, such as *Triplectides*, as these organisms are able to choose the most palatable resources. The Cerrado vegetation has leaves with waxes, hair, and other characteristics (Fank-de-Carvalho et al., 2010) that make them less palatable than forest vegetation (Landeiro et al., 2010; Reis et al., 2019). *Triplectides* larvae may feed preferably in leaves heavily conditioned by fungi in Cerrado streams, explaining the higher fungal diversity in Cerrado than Amazon Forest.

The occurrence of filamentous fungi producers of cellulases and xylanases in the DT of *Triplectides* larvae indicates that those organisms may play a role in the breakdown of lignocellulosic matter ingested by the larva. The percentage of CMCase-producing strains in the Cerrado (78%) was more than two-fold that in the Amazon Forest (35%). The differences in vegetation composition may have influenced the enzymatic profile of the fungal community in the biomes studied since different plant species host different fungal species (Ferreira et al., 2015). Also, the lower palatability of leaves from Cerrado vegetation may account for differences in fungal enzymatic capabilities in leaves of these two biomes. The percentages of strains that broke down xylan in the Cerrado (62%) and in the Amazon Forest (71%) indicate that the fungal community is more xylanolytic than cellulolytic. That is certainly due to the characteristic of the *Triplectides* insect that feeds both on leaves and on wood (Oliveira and Pes, 2014; Cortez and Gonçalves, 2015). And, as plant cell walls are composed of cellulose, hemicellulose (mainly xylan), and lignin (Walia et al., 2017), xylan requires several xylanolytic enzymes for full hydrolysis (Okeke, 2014).

In this work, it was observed that some taxa are potentially higher enzyme-producers than others. The genera *Cladosporium* and *Penicillium* exhibited the highest enzymatic indexes for xylanase and cellulase, having, in this case, greater extracellular enzyme activity (Oliveira et al., 2006). The species that had the highest indices were *Cladosporium kenpeggii*, which is considered a new, little-studied species (Marin-Felix et al., 2017), as well as *Cladosporium subuliforme* (Ramos-García et al., 2016) and *Penicillium malochii* (Rivera et al., 2012). According to the literature, *Penicillium* species may produce enzymes able to break down lignocellulosic material (Andersen et al., 2016; Mohammed et al., 2018), as well as species of *Cladosporium* (Andersen et al., 2016; Marques et al., 2018).

It is likely that the larvae acquire the fungi from their diet, which, along with the environment where they live, impact the formation of the mycobiome of the larvae (Yao et al., 2019). In that case, fungi may represent an additional food item so that many fungi can be considered a reasonable source of amino acids and nitrogen in insect diets (Mason et al., 2017). In addition to being part of larva diet, the symbiotic role of that fungal community may be related to the action of enzymes able to delignify the material made up of, mainly, cellulose, hemicellulose (Gao et al., 2018; Alves Júnior et al., 2019), and xylans, enriching the diet of the insect, thus exerting a nutritional role (Mohammed et al., 2018). Our results point to the

hypothesis that the fungal community found in DT of *Triplectides* larvae helps in processing the food both during conditioning in the ecosystem and in the DT. The presence of xylanolytic and cellulolytic fungi in the DT of aquatic shredder insects supports the hypothesis of a nutritional role of fungi as a symbiont and reinforces the importance of ecological studies for the discovery of biotechnological potential for enzyme production of fungal strains new to science.

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