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ORIGINAL ARTICLE

Cultivable cellulolytic fungi isolated from the gut of Amazonian aquatic insects

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ABSTRACT

Filamentous fungi have been targeted by bioprospecting studies because they are effective producers of extracellular enzymes that can potentially be used by the bioindustry. In this study, we isolated filamentous fungi from the guts of Amazonian aquatic insect larvae to evaluate their cellulolytic activity. We collected 69 larvae of shredder insects of three genera: *Phylloicus* (Trichoptera: Calamoceratidae), *Triplectides* (Trichoptera: Leptoceridae) and *Stenochironomus* (Diptera: Chironomidae) in ten streams from a protected area in the central Brazilian Amazon. Production of mycelia was elaborated in PDA (Potato Dextrose Agar) medium. The isolates were transferred to a synthetic medium with carboxymethyl cellulose, and Congo red was used to determine the enzymatic index. The hydrolysis halo, indicating the production of cellulases, was observed in 175 fungal isolates (70% of the total), of which 25 had an enzymatic index ≥ 2.0 and belonged to seven fungal genera. The fungal taxa *Cladosporium*, *Gliocephalotrichum, Penicillium*, *Pestalotiopsis*, *Talaromyces*, *Trichoderma* and *Umbelopsis* were isolated from guts of *Phylloicus*, *Triplectides* and *Stenochironomus*, which are traditionally used in biotechnological applications. Our results indicate the cellulolytic potential of fungi associated with the guts of aquatic Amazonian insects.

KEYWORDS: **c**ellulase, enzymatic hydrolysis, *Phylloicus*, shredders, Amazonia

Fungos celulolíticos cultiváveis isolados do intestino de insetos aquáticos da Amazônia

RESUMO

Fungos filamentosos tem sido alvo de estudos de bioprospecção devido à sua grande eficiencia em produzir enzimas extracelulares, as quais tem grande potencial para uso em bioindústrias. Neste estudo, fungos filamentosos foram isolados do intestino de larvas de insetos aquáticos da Amazônia, para avaliar sua atividade celulolítica. Foram coletadas 69 larvas de insetos aquáticos fragmentadores de três gêneros: *Phylloicus* (Trichoptera: Calamoceratidae), *Triplectides* (Trichoptera: Leptoceridae) e *Stenochironomus* (Diptera: Chironomidae) em dez igarapés de uma área protegida na Amazônia central brasileira. O crescimento dos fungos isolados foi feito em meio de cultura Ágar Batata Dextrose (BDA). Os isolados fúngicos foram transferidos para o meio sintético com Carboximetil celulose e utilizou-se vermelho Congo para determinar o índice enzimático. O halo de hidrólise, indicando a produção de celulases, foi observado em 175 isolados fúngicos (70% do total), dos quais 25 tiveram um índice enzimático ≥ 2,0 e pertencem a sete gêneros fúngicos. Os táxons fúngicos *Cladosporium*, *Gliocephalotrichum, Penicillium*, *Pestalotiopsis*, *Talaromyces*, *Trichoderma* e *Umbelopsis* foram isolados dos intestinos das larvas de *Phylloicus*, *Triplectides* e *Stenochironomus* e são tradicionalmente utilizados em aplicações biotecnológicas. Os resultados indicam um potencial celulolítco destes fungos associados ao intestino de insetos aquáticos amazônicos.

PALAVRAS-CHAVE: celulase, hidrólise enzimática, *Phylloicus*, fragmentadores, Amazônia

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INTRODUCTION

Cellulases produced by fungi are widely used in industry, and the demand for efficient microorganisms has increased over time. Cellulases are currently employed in the production of food, animal feed, second generation ethanol, fruit and vegetable juices, paper, wine and textiles as well as in pulp extraction, starch processing, breweries, laundry and agriculture (Bhat 2000; Choi *et al.* 2015; Singh *et al.* 2016). Fungal enzymes are particularly advantageous when compared to animal or plant equivalents, as their production is less costly since the enzymes are secreted as complexes that work in synergy (Dashtban *et al.* 2009). Moreover, fungal enzymes have different physicochemical characteristics (e.g., thermostable enzymes of thermophilic organisms), and they are easier to produce on a large scale (Dalmaso *et al.* 2015).

Many insects that digest wood, foliage and debris (e.g., Isoptera, Coleoptera, Orthoptera, Hymenoptera) (Martin 1983) use lignocellulose as their main food source and are highly efficient in obtaining glucose from cellulose degradation (Sun and Zhou 2011). Naturally, wood-boring insects have been common subjects of studies aiming at the prospection of lignocellulolytic enzymes (Geib *et al.* 2010). In addition, aquatic leaf-mining Diptera larvae (Koroiva 2013) and other larvae of shredder insects (Rogers and Dora-Peterson 2010) have been considered as potential sources of lignocellulolytic enzymes for the second-generation biofuel industry (Cook and Dora-Peterson 2010; Huang *et al.* 2010). Although insects can produce endogenous cellulases (Watanabe and Tokuda 2010; Shelomi *et al.* 2014; Pothula *et al.* 2019), the polysaccharide hydrolysis is mainly performed by extracellular enzymes produced by microorganisms that colonize the digestive tracts of the insects, especially by fungi like *Aspergillus* Micheli ex Haller, *Fusarium* Link and *Penicillium* Link (Rojas-Jiménez and Hernández 2015). Several filamentous fungi, such as *Cladosporium* Link, *Penicillium*, and *Trichoderma* Persoon, have been found in the guts of Coleoptera and Diptera such as Silvanidae (Coleoptera) (David *et al.* 1974), Tenebrionidae (Coleoptera) (Prabha *et al.* 2011), Platypodidae (Coleoptera) (Henriques *et al.* 2009) and Culicidae (Diptera) (Fonseca *et al.* 2008; Pereira *et al.* 2009; Maketon *et al.* 2014). However, the evolution and dynamics of microorganism communities and their insect hosts are not yet fully understood (Bobay and Raymann 2019).

In general, microorganisms participate in the digestive processes of insects that have diets with nutritional deficiency, such as insects that feed on phloem or on complex molecules like cellulose (Watanabe and Tokuda 2010). Their capacity to degrade cellulose, in particular, is high and widely distributed among different taxa. For example, microorganisms associated with the digestive tracts of various termite species degrade cellulose and lignin, providing the insects with glucose and fatty acids that will be used as an energy source (Breznak 2002). In contrast to our understanding of insect gut-associated bacteria, which have been widely documented (e.g., Schaaf and Dettner 1997; Lundgren and Lehman 2010; Vojvodic *et al.* 2013), less is known about the relationships between insects and associated fungi (McCreadie *et al.* 2011; Shao *et al.* 2015). In freshwater environments, an elaborate symbiosis occurs in aquatic shredder insects. These insects feed on allochthonous organic matter (decaying wood and leaves) only after it has been conditioned, i.e., colonized by microorganisms, which convert this tissue into a more palatable food through extracellular enzymes (Gessner *et al.* 1999; Abelho 2001; Gulis and Bärlocher 2017).

In the Amazon region, where insect diversity is one of the highest in the world, knowledge on fungus-insect systems and cellulase-producing fungi associated with aquatic insects is still incipient (Rios-Velasquez *et al.* 2002; Alencar *et al.* 2003; Fonseca *et al.* 2008; Pereira *et al.* 2009; Santos *et al.* 2018a; Santos *et al.* 2018b). The study of the gut mycobiota of aquatic insects can lead to the discovery of metabolic processes and interactions with potential biotechnological applications. Thus, the aim of this study was to investigate the cellulase activity of filamentous fungi isolated from the gut of aquatic shredder insects, expecting to identify new cellulolytic enzymes in poorly studied species of Amazonian insects.

MATERIAL AND METHODS

Collecting insects

Larvae of aquatic shredder insects belonging to the genus *Phylloicus* Müller (Trichoptera: Calamoceratidae), *Triplectides* Kolenati (Trichoptera; Leptoceridae) and *Stenochironomus* Kieffer (Diptera: Chironomidae) were collected in ten forest streams located in the Adolpho Ducke Forest Reserve, municipality of Manaus, Amazonas state (Brazil) (02°56'21"N, 59°57'43"W) (Figure 1). The stream beds are composed of sand and leaves, and the streams are shaded by riparian vegetation (e.g., Mendonça *et al.* 2005). The values of pH ranged from 4.4 to 5.4. The electrical conductivity of the water ranged from 7 to 23 μS cm^{-1}, water velocity from 0.04 to 0.17 cm s^{-1}, dissolved oxygen concentration from 5.8 to 6.8 mg L^{-1}, and the water temperature ranged from 24.8 to 26.8 °C.

Insect larvae were collected randomly in August 2016 with the aid of an aquatic entomological net and identified to the genus level in the field according to Hamada *et al.* (2014). In total, we captured 69 larvae: 24 *Phylloicus* spp., 20 *Triplectides* spp., and 25 *Stenochironomus* spp. Each larva was removed from its shelter (leaf or stick), sterilized for 30 seconds in 70% ethanol, and stored in a microtube with sterile distilled water. Larvae were processed shortly after collection to avoid the decomposition or excretion of the contents of their digestive tracts.

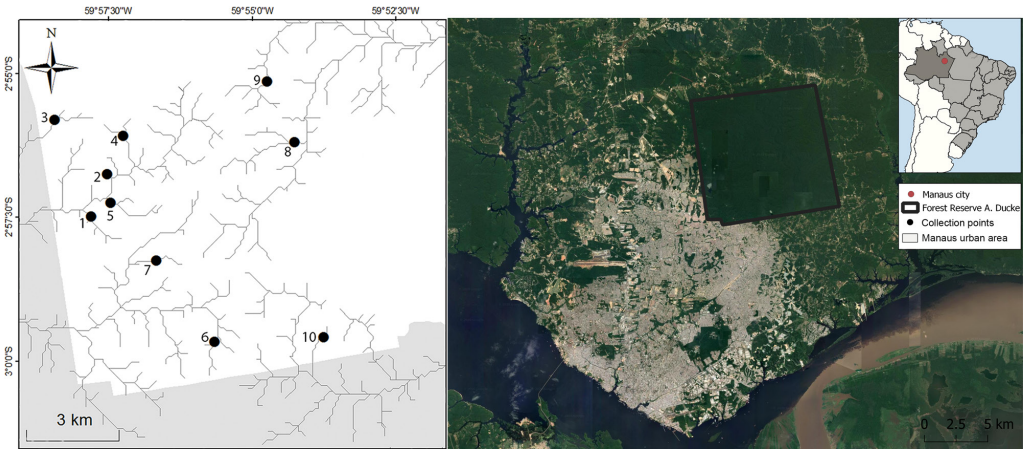

Figure 1. Location of the Adolpho Ducke Forest Reserve adjacent to the city of Manaus, Amazonas state, Brazil (satellite image and inset map). The outline map shows the southwestern portion of the Reserve with its stream outlines and the sampling sites of aquatic shredder insect larvae (1 to 10). This figure is in color in the electronic version.

Isolation, purification and morphological characterization of filamentous fungi

Larvae were dissected under a stereomicroscope using sterilized pins and scissors in the Laboratory of Citotaxonomy and Aquatic Insects of Instituto Nacional de Pesquisas da Amazônia - INPA. Each larva had its guts removed and transferred to a microtube containing 1 mL of sterile distilled water. After homogenizing, a 100 µL aliquot of each sample was inoculated in a Petri dish containing Potato Dextrose Agar (PDA) medium supplemented with 0.1 mg L^{-1} chloramphenicol and incubated at 27 °C for seven days. For purification, the filamentous fungi that showed no contamination were transferred separately to new Petri dishes containing PDA. After obtaining pure fungal cultures, preservation was carried out by the Castellani method (Castellani 1939). Contaminated strains that were not recovered after the purification step were not considered in data analysis and they are not represented in the results. The fungal isolates were identified based on macromorphological characteristics such as color, shape, size, and border, among other relevant characteristics (Lacap *et al.* 2003; Ibrahim *et al.* 2017). To observe the micromorphological characteristics, the fungal isolates were microcultured, stained with lactophenol cotton blue and examined under a Leica Microsystems optical microscope with an attached Leica DFC295 camera and the Leica Application Suite (LAS, 4.2.0) software.

Cellulolytic activity screening

The fungal isolates were evaluated for cellulase production in carboxymethyl cellulose (CMC) medium ($NaNO_3$: 3.0 g L^{-1}; K_2HPO_4: 1.0 g L^{-1}; $MgSO_4$: 0.5 g L^{-1}; KCl: 0.5 g L^{-1}; $FeSO_4.7H_2O$: 10.0 mg L^{-1}; CMC: 10.0 g L^{-1}; agar: 20.0 g L^{-1}) (Ruegger and Tauk-Tornisielo 2004). The plates were incubated at 27 °C for four days and then subjected to thermal shock for 16 h at 50 °C. The degradation halo was observed using 10 mL of Congo red solution (2.0 g L^{-1}) for 30 min and 5 mL of 0.5 M NaCl solution in 0.1 M Tris-HCl buffer, pH 8.0 (Nogueira and Cavalcante 1996). These assays were performed in triplicate. We calculated the enzymatic index (E.I.) of all isolates that produced a degradation halo. The E.I. was calculated as the ratio between the mean halo diameter and the mean colony diameter among the three replicates (Hankin and Anagnostakis 1975). The fungal isolates that had high enzymatic activity, here defined as E.I. ≥ 2.0 (Lealem and Gashe 1994), were further identified by molecular analysis.

Molecular analyses

The fungal isolates were cultivated in natural potato broth for five days in a rotary shaker (150 rpm) at 27 °C. DNA extraction was performed using a Fungi/Yeast Genomic DNA Isolation kit (Norgen Biotek, ON, CAN) following the manufacturer's instructions. The fungal ITS1-5.8S-ITS2 regions were amplified by PCR using the forward primer ITS1 5′-TCCGTAGGTGAACCTGCGG-3′ and the reverse primer ITS4 5′-TCCTCCGCTTATTGATATGC-3′ (White *et al.* 1990). Amplification was accomplished in a 50 µL reaction mixture containing 2 µL of genomic DNA, 0.3 µL of Taq DNA polymerase - Platinum® 5U/µL, 1.25 µL of reaction buffer (10×), 2.5 µL of dNTPs (2.5 mM each), 2.5 µL of MgCl (50 mM), 0.5 µL of each primer (20 µM), and 15.95 µL of sterile Milli-Q water with the following reaction program: 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. Amplicons were confirmed by electrophoresis on a 1.0% (w/v) agarose gel, purified with GFX™ PCR and DNA Gel Band Purification Kit (GE Healthcare, USA) and sequenced following the BigDye® V3.1 protocol in an ABI 3500 sequencer (Thermo Fisher Scientific, MA, USA). Analyses were carried out in

the Molecular Biology Laboratory of Universidade Federal do Amazonas - UFAM.

Taxonomy

The consensus sequence of both DNA strands was obtained using Geneious® 9.0.5 (Kearse *et al.* 2012). The alignment of the consensus sequences was performed against the database of fungal genomes deposited in GenBank using the Basic Local Alignment Searching Tool (BLAST) (Altschul *et al.* 1990) of the National Center for Biotechnology Information (NCBI). In addition, the UNITE database was also used (Nilsson *et al.* 2018; https://unite.ut.ee/).

RESULTS

The intestinal content of 46 of the 69 aquatic insect larvae collected resulted in fungal growth (Table 1). A total of 248 fungi were isolated from the guts of *Phylloicus*, *Triplectides* and *Stenochironomus* larvae. Among these fungal isolates, 175 produced cellulases: 84 from guts of *Phylloicus* larvae, 55 from *Triplectides* larvae, and 36 from *Stenochironomus* larvae (Table 1). Out of the 175 isolates that produced cellulases, 25 had an E.I. ≥ 2.0 (22.4% of all fungi isolated from guts of *Stenochironomus* larvae, 8% of *Triplectides* and 6.2% of *Phylloicus*) (Table 1).

Most of the identified fungal isolates were Eurotiomycetes, of which 15 were identified as *Penicillium* (Eurotiales: Aspergillaceae), with E.I. from 2.0 to 4.0. (A1TB1), which had been previously identified as *Penicillium* sp. based on morphology, but was assigned to *Talaromyces purpurogenus* (Stoll) Samson, Yilmaz, Frisvad and Seifert (Eurotiales: Trichocomaceae) based on molecular analysis (Table 2). Another representative group was Dothideomycetes, with six isolates identified as *Cladosporium* (Capnodiales: Davidiellaceae). Ascomycete was represented by one isolate of the genus *Pestalotiopsis* Steyaert (Xylariales: Sporocadaceae), and Sordariomycetes was represented by one isolate of the genus *Trichoderma* (Hypocreales: Hypocreaceae) (Table 2; Figure 2).

The molecular taxonomic identification confirmed most of our morphology-based identifications (Table 2). Five isolates could be identified only to the genus level: *Gliocephalotrichum* Ellis and Hesseltine (Hypocrales: Nectriaceae) and

Umbelopsis Amos and Bernett (Mucorales: Umbelopsidaceae). Five isolates could be further identified to the species level: *Trichoderma harzianum* Rifai, *Talaromyces purpurogenus*, *Penicillium adametzii* Zalessky, and *P. citrinum* Thom.

Table 1. Total number of fungal isolates and number of isolates positive for cellulolytic activity in aquatic shredder insects belonging to three genera sampled in Ducke Reserve, in the central Brazilian Amazon. E.I. = enzymatic index.

Aquatic insect	Number of insects		Number of fungal isolates		
	Total	N with fungal growth	Total	Positive	E.I. ≥ 2.0
Phylloicus spp.	24	19	112	84	7
Triplectides spp.	20	19	87	55	7
Stenochironomus spp.	25	8	49	36	11
Total	69	46	248	175	25

DISCUSSION

Overall, we found that the guts of *Phylloicus*, *Triplectides* and *Stenochironomus* larvae analyzed in this study are colonized by *Cladosporium*, *Gliocephalotrichum*, *Penicillium*, *Pestalotiopsis*, *Talaromyces* Benjamin, *Trichoderma* and *Umbelopsis* fungi that have species that are traditionally used in biotechnological applications (Jalmi *et al.* 2012; Copete-Pertuz *et al.* 2019; Geetha *et al.* 2019; Liuzzi *et al.* 2019; Salazar-Cerezo *et al.* 2019; Ge *et al.* 2020; Slaný *et al.* 2020).

Interestingly, among the isolates with E.I. ≥ 2.0, fungi of the genus *Penicillium* were more common and more efficient producers of cellulase in solid medium than *Trichoderma*, which is the most well-studied fungal group for cellulase, comprising very powerful decomposers of crystalline cellulose (Galliano *et al.* 1988; Ahamed and Vermette 2008; Gusakov 2011). *Penicillium* strains can be as good as *Trichoderma* strains in the production of cellulases, based on indicators such as production level and cellulase hydrolytic performance per unit of activity or per milligram of protein (Gusakov 2011; Syed *et al.* 2013; Carvalho *et. al.* 2014). More recently, Santos *et al.* (2018b) investigated the filamentous fungi isolated from the guts of *Phylloicus* larvae using both classical and molecular methods and found 21 isolates belonging to the genus *Penicillium*. Our study further corroborates the association between filamentous fungi and aquatic insect larvae.

Other fungal genera with known cellulolytic activity were isolated in this study. *Gliocephalotrichum* is commonly regarded as a saprophytic fungus (Lombard *et al.* 2014) and this is the first record of its association with insect guts. *Umbelopsis*, which here produced cellulases in a solid medium with carboxymethyl cellulose, colonizes substrates rich in simple carbohydrates and has been found associated with Curculionidae insects (Silva *et al.* 2015). *Talaromyces* is also known to be associated with insects such as Cynipidae (Hymenoptera) (Seifert *et al.* 2004) and has been recorded in the midguts of *Aedes aegypti* (Diptera) (Angleró-Rodríguez *et al.* 2017). Some species of *Talaromyces* (*T. emersonii* CBS 814.70 and *T. cellulolyticus* Fujii) are able to produce cellulolytic enzymes (Inoue *et al.* 2014). *Talaromyces purpurogenus*, which is important in bioindustry due to its ability to produce cellulolytic enzymes (Belancic *et al.* 1995), was recently placed in the genus *Penicillium* (Samson *et al.* 2011), and *Talaromyces* received all species of the *Penicillium*

Table 2. Cellulolytic filamentous fungi isolated from the guts of *Phylloicus* (Trichoptera), *Triplectides* (Trichoptera) and *Stenochironomus* (Diptera) shredder aquatic insects, in the central Amazon region of Brazil. The table shows the morphological and molecular identification of the isolates that had an enzymatic index ≥ 2.0 after growing on a synthetic carboxymethylcellulose medium. M = morphological identification; G = molecular genotyping; E.I. = enzymatic index (mean index ± SD, n = 3); (*) = percentage of similarity between our sequences and those available in the NCBI and UNITE databases.

Sample #	Host insect	Fungal isolate	Species	Type of identification	E.I.	Identity*	Accession number
1	Phylloicus	A1PA3	*Cladosporium* sp.	M	2.3 ± 0.3		
2	Phylloicus	A1PB5	*Pestalotiopsis* sp.	G	3.1 ± 0.8	97%	KF887030.1
4	Phylloicus	A2PA4	*Penicillium* sp.	G	3.9 ± 0.2	99%	JQ889696.1
5	Phylloicus	A2PC2	*Cladosporium* sp.	G	2.8 ± 0.5	87%	MH655007.1
11	Phylloicus	A5PA3	*Gliocephalotrichum* sp.	G	2.9 ± 0.4	94%	MH397480.1
17	Phylloicus	A8PB5	*Penicillium* sp.	M	2.1 ± 0.2		
20	Phylloicus	A9PB4	*Trichoderma harzianum*	G	2.3 ± 0.1	100%	MN262498.1
2	Triplectides	A1TB1	*Talaromyces purpurogenus*	G	3.0 ± 0.0	99%	MK108916.1
2	Triplectides	A1TB3	*Penicillium* sp.	G	2.0 ± 0.4	97%	MK775828.1
8	Triplectides	A4TA1	*Penicillium* sp.	M	2.8 ± 0.3		
8	Triplectides	A4TA3	*Umbelopsis* sp.	G	3.7 ± 0.5	98%	MF101390.1
17	Triplectides	A8TA5	*Penicillium adametzii*	G	4.0 ± 0.6	99%	JN714932.1
17	Triplectides	A8TA7	*Penicillium* sp.	G	3.9 ± 0.4	94%	KF848945.1
17	Triplectides	A8TA9	*Penicillium* sp.	G	2.5 ± 0.0	93%	MH268036.1
3	Stenochironomus	A1SC3	*Penicillium* sp.	G	2.5 ± 0.1	85%	KC181929.1
4	Stenochironomus	A2SA2	*Penicillium* sp.	M	2.1 ± 0.8		
4	Stenochironomus	A2SA4	*Penicillium* sp.	M	2.8 ± 0.8		
4	Stenochironomus	A2SA5	*Penicillium* sp.	M	2.2 ± 0.1		
5	Stenochironomus	A2SB2	*Cladosporium* sp.	M	2.0 ± 0.5		
5	Stenochironomus	A2SB3	*Penicillium adametizii*	G	2.0 ± 0.3	99%	JN714932.1
7	Stenochironomus	A3SA1	*Cladosporium* sp.	M	3.1 ± 0.5		
8	Stenochironomus	A4SA1	*Penicillium* sp.	G	2.3 ± 0.1	97%	MN238763.1
10	Stenochironomus	A4SC2	*Penicillium citrinum*	G	2.1 ± 0.4	99%	KM278038.1
12	Stenochironomus	A5SB3	*Cladosporium* sp.	M	2.5 ± 0.1		
13	Stenochironomus	A5SC1	*Cladosporium* sp.	M	2.9 ± 0.4		

subgenus *Biverticillium* (Samson *et al.* 2011), since these two taxa form a monophyletic group (Yilmaz *et al.* 2014).

Our results expand the knowledge on the diversity and functions of fungi associated with the guts of aquatic insects, since few similar studies have been carried out with Amazonian species. Santos *et al.* (2018b) identified fungi isolated from the gut of *Phylloicus* larvae in from the Brazilian states of Pará and Tocantins, in the Amazonian and Cerrado savanna biomes, respectively, thus suggesting the occurrence of distinct associated fungi communities across ecosystems. While there are studies on fungi associated with insects, studies on the gut microbiome are lacking (e.g., Fonseca *et al.* 2008; Pereira *et al.* 2009). Moreover, studies on the gut mycobiota of aquatic insects in Amazonia have been concentrated on the class Trichomycetes (e.g., Rios-Velásquez *et al.* 2002; Alencar *et al.* 2003).

Our results suggest that the mycobiota of aquatic shredder insect guts isolated in this study can effectively degrade cellulose. The presence of cellulolytic fungi is probably related to the conditioning of ingested leaf material through the decomposition of structural compounds by fungal enzymes, which softens the material and increases the palatability of the leaves (Graça *et al.* 2001; Aßmann *et al.* 2011; Casotti *et al.* 2015; Biasi *et al.* 2017; Reis *et al.* 2018). Graça *et al.* (2001) demonstrated experimentally that insects preferred conditioned leaves over unconditioned leaves. Likewise, Reis *et al.* (2018) showed that high concentrations of tannins in unconditioned leaves are inhibitory and not consumed by

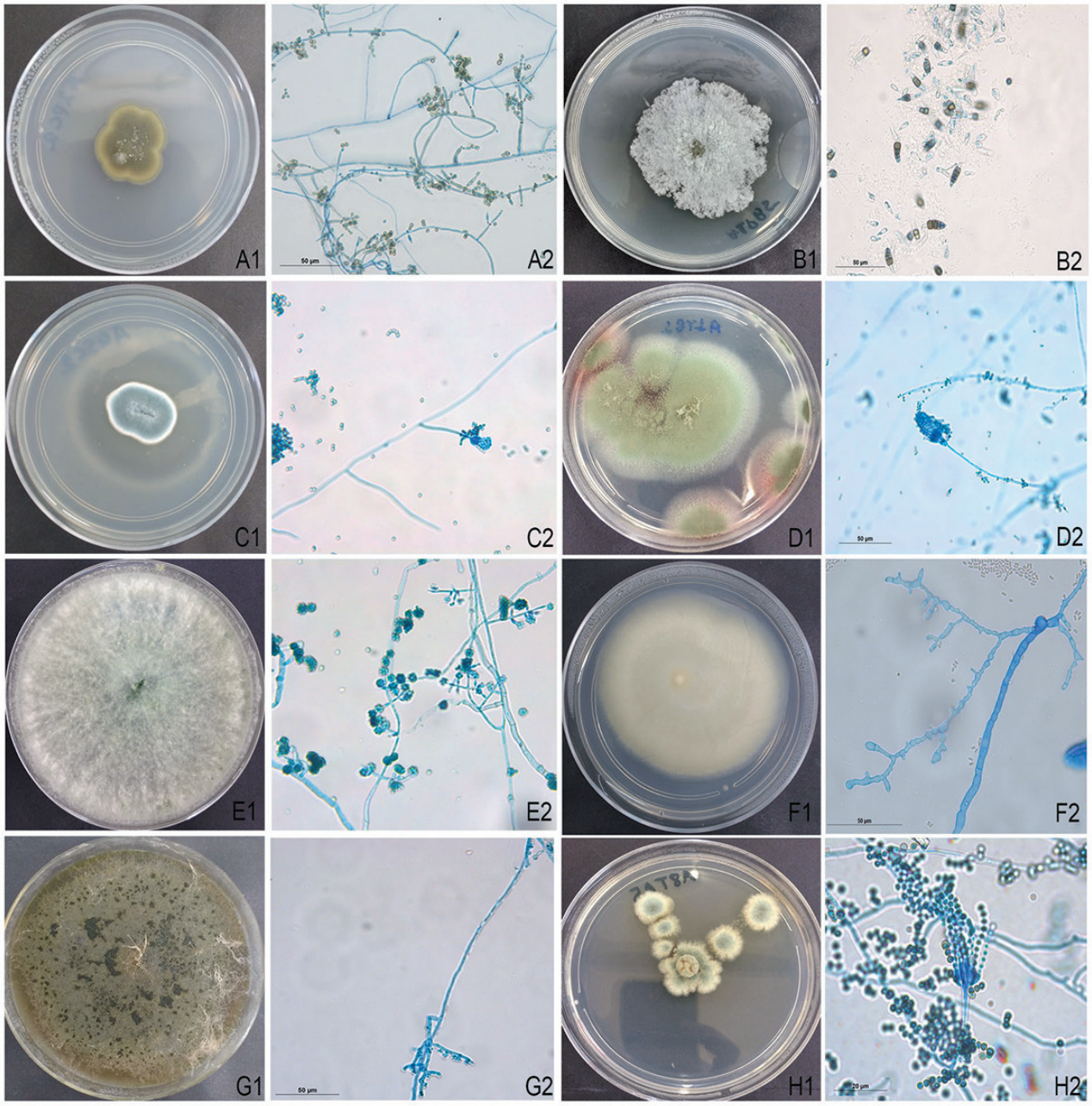

Figure 2. Macro and microscopic views of filamentous fungi isolated from the guts of *Phylloicus* (Trichoptera), *Triplectides* (Trichoptera) and *Stenochironomus* (Diptera) plated in PDA medium for seven days at 27 ºC. A1/A2 – *Cladosporium* sp.; B1/B2 – *Pestalotiopsis* sp.; C1/C2 – *Penicillium citrinum*; D1/D2 – *Talaromyces purpurogenus*; E1/E2 – *Trichoderma harzianum*; F1/F2 – *Umbelopsis* sp.; G1/G2 - *Gliocephalotrichum* sp.; H1/H2 – *Penicillium adametzii*. This figure is in color in the electronic version.

insect larvae. Gonçalves-Júnior *et al.* (2017) compared the characteristics of five common leaves found in streams in the Adolpho Ducke Forest Reserve and noted that shredder insects were more frequent on the leaves of *Mabea speciosa* Müll.Arg (Euphorbiaceae), which had a higher polyphenol content, lower amount of cellulose and higher concentration of fungal biomass than the leaves of the other plants. These results indicate that aquatic shredder insect larvae feed on leaves conditioned by fungi, which initiate the decomposition process through extracellular enzymes, facilitating digestion by the larvae (Graça *et al.* 2001; Aßmann *et al.* 2011; Casotti *et al.* 2015; Biasi *et al.* 2017; Reis *et al.* 2018).

CONCLUSIONS

This study contributes to the knowledge of the mycobiota associated with the guts of aquatic insect larvae, however it remains elusive to what extent the fungal microbiota

influences its host life's. Overall, we expect that future research on shredder aquatic insects microbiota will contribute to our understanding of interactions fungi–host. The presence of cellulolytic fungi in the guts of aquatic insects reinforces the biotechnological potential for cellulolytic-enzyme prospection. Our study also brings new information on the identities and functional traits of the symbiotic fungi of aquatic-insects in the Amazon that have not been previously studied. As available data about symbionts increase, so does the understanding of the ecological relationships between these fungi and their insect hosts. More research is essential to better understand the processes that occur in the fungus-insect system, in addition to the biotechnological potential of these processes.

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