

Occurrence and distribution of the heterotrophic straminipiles from Brazilian Atlantic Rainforest areas

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How to cite: Pires-Zottarelli, C.L.A., Jesus, A.L., Colombo, D.R.S., Ventura, P.O., Boro, M.C. Occurrence and distribution of the heterotrophic straminipiles from Brazilian Atlantic Rainforest areas. *Hoehnea* 50: e392022. <https://doi.org/10.1590/2236-8906e392022>

ABSTRACT – (Occurrence and distribution of the heterotrophic straminipiles from Brazilian Atlantic Rainforest areas). We present herein the occurrence and distribution of the heterotrophic straminipiles collected from soil and water, submerged leaves and surface sediment from freshwater bodies at “Mosaico de Unidades de Conservação Juréia-Itatins”, a preserved conservation unit of the Brazilian Atlantic Rainforest. From 180 collected samples, we isolated 41 taxa belonging to Hyphochytriomycota and Oomycota using the multiple baiting technique, with several new taxa to the science, new occurrences to São Paulo State or Brazil, and all new citation to the studied area. These taxa were identified based on morphological and/or molecular data, with several genic regions made available in GenBank. The calculated community structure of these zoosporic organisms showed a diverse community, with few dominant taxa, without significant differences ($P \leq 0.05$) among the different types of samples.

Keywords: diversity, Ecology, Hyphochytriomycota, Oomycota, Taxonomy

RESUMO – (Ocorrência e distribuição dos estramenópilos heterotróficos de áreas de Mata Atlântica Brasileira). Apresentamos aqui a ocorrência e a distribuição dos estramenópilos heterotróficos coletados de solo, e de água, folhas submersas e sedimento superficial de corpos de água doce no “Mosaico de Unidades de Conservação Juréia-Itatins”, uma unidade de conservação preservada de Mata Atlântica brasileira. Das 180 amostras coletadas, isolamos 41 táxons pertencentes aos filos Hyphochytriomycota e Oomycota, usando a técnica de iscagem múltipla, com vários deles novos táxons para a ciência, novas ocorrências para o estado de São Paulo ou Brasil, e todos primeira ocorrência para o local estudado. Os táxons foram identificados com base em dados morfológicos e/ou moleculares, com várias regiões gênicas disponibilizadas no GenBank. O cálculo da estrutura da comunidade mostrou uma comunidade diversa, com poucos táxons dominantes, sem diferença significativa ($P \leq 0,05$) entre os diferentes tipos de amostras.

Palavras-chave: diversidade, ecologia, Hyphochytriomycota, Oomycota, taxonomia

Introduction

The heterotrophic straminipiles are fungal-like organisms inserted within the Kingdom Straminipila, in the supergroup SAR, together with the Alveolata and Rhizaria (Adl *et al.* 2019). It is represented by the phyla Hyphochytriomycota, Labyrinthulomycota and Oomycota that are characterized by their heterokont, predominantly biflagellate zoospores.

Members of these phyla are widespread and can be found specially as saprotrophs of different kinds of substrates, in freshwater, marine and terrestrial ecosystems. Some of them are important parasites of a variety of host organisms, such as algae, animals, fungi, oomycetes and plants (Beakes *et al.* 2014, Beakes & Thines 2017, Marano *et al.* 2017).

These phyla have similarities with the true zoosporic fungi, like as osmotrophic nutrition, flagellate spores

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(zoospores) and ecological niches, however, molecular studies have shown that the heterotrophic straminipiles are phylogenetically distant from these fungi (Beakes *et al.* 2012, Beakes & Thines 2017, Marano *et al.* 2017).

Hyphochytriomycota and Oomycota were studied in the “Mosaico de Unidades de Conservação Juréia-Itatins” from 2016 to 2017, an important Atlantic Rainforest area located in São Paulo State, Brazil, with the aim to assess the species composition and community structure of these phyla from soil and freshwater samples. Although several studies have already been carried out in Brazilian Atlantic Rainforest (Beneke & Rogers 1962, 1970, Rogers *et al.* 1970, Lyra & Milanez 1974, Pires-Zottarelli *et al.* 1995, 1996, Schoenlein-Crusius & Milanez 1998, Schoenlein-Crusius *et al.* 2006, Pires-Zottarelli & Rocha 2007, Miranda & Pires-Zottarelli 2008, 2012, Jesus *et al.* 2013, 2015, 2016a, b, Rocha *et al.* 2016a, b, 2018, and others), there was no study with these phyla in the studied area.

Material and Methods

Studied area, sampling and laboratory analyses – From August/2016 to October/2017, samples of soil, as well as water, submerged leaves and surface sediments from freshwater bodies were collected at “Mosaico de Unidades de Conservação Juréia-Itatins”, São Paulo State, Brazil (Figure 1). The aliquots of these samples were plated and baited using cellulosic, keratinous and chitinous substrates according to the technique described in Milanez (1989). These samples were incubated at room temperature (~22°C)

and the baits observed after five days under a Leica DMLB2 compound microscope (Hessen, Wetzlar, Germany). The specimens observed were isolated and purified onto MP₅ culture medium (4 g maltose, 1 g peptone, 15 g agar and 1000 mL deionized water) and CMA culture medium (17 g cornmeal agar and 1000 mL deionized water) with addition of 0,05 g of streptomycin sulfate and 0,1 g of penicillin. The asexual and/or sexual structures of the obtained specimens were observed under the microscope, measured in water cultures (n=50) and identified morphologically according to specific literature, such as Scott (1961), Seymour (1970), Karling (1977), Plaats-Niterink (1981), Johnson *et al.* (2002), and original descriptions. Due to difficulties in culturing some taxa on agar media, these were purified by multiple transfers to new substrates. The identified specimens were photographed with a Leica MC170 HD camera using Leica Qwin 3.1 software (Hessen, Wetzlar, Germany), and at least one isolate was incorporated at CCIBt (“Coleção de Culturas de Algas, Cianobactérias e Fungos do Instituto de Botânica” – nowadays “Instituto de Pesquisas Ambientais”) and/or Herbarium SP. Taxonomical placement of the genera followed Beakes & Thines (2017). *Aphanodictyon* and *Phragmosporangium*, genera not mentioned by these authors, followed Dick (2001) and Rocha *et al.* (2018), respectively, at family level.

DNA extraction, PCR amplification and sequencing – For DNA extraction, small fragments of culture medium with the mycelium of the specimens were transferred to erlenmeyer flasks (250 mL) containing 30 mL of MP₅ liquid medium. Cultures were incubated at ~22 C for 5-10 days,

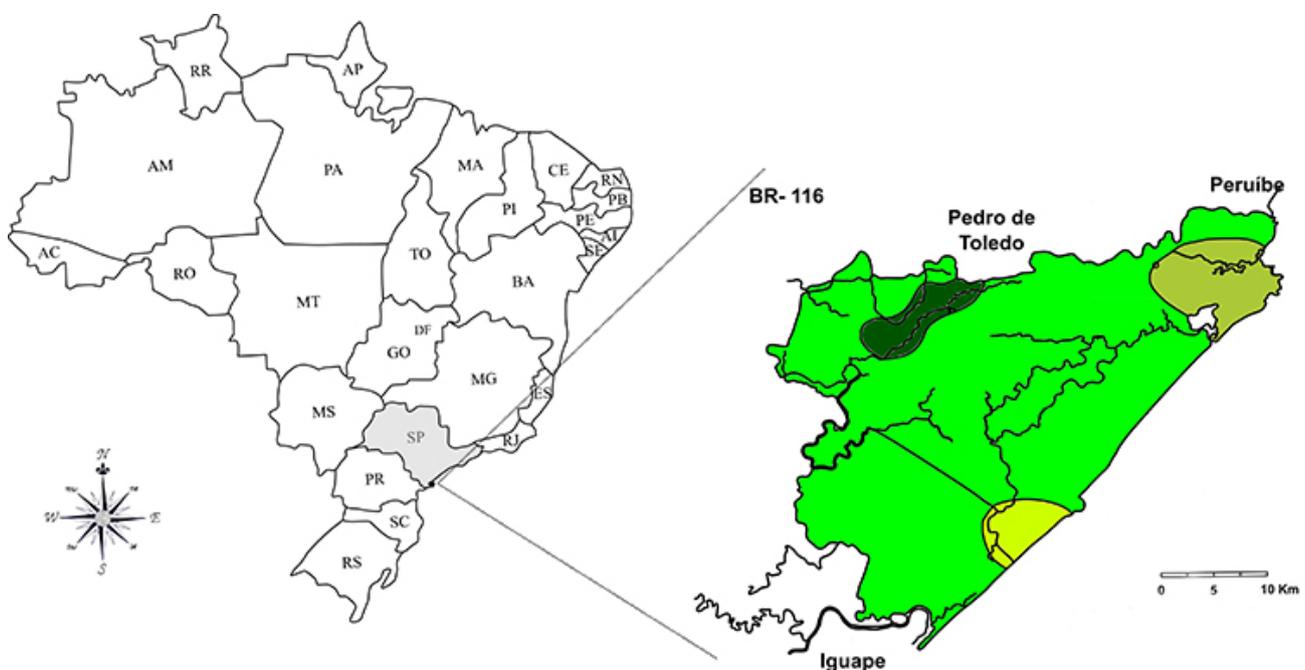


Figure 1. Map of the “Mosaico de Unidades de Conservação Juréia-Itatins”, São Paulo State, Brazil, showing the municipalities sampled (adapted from Souza and Souza 2004).

after which the biomass was transferred to 2 mL microfuge tubes. The tubes were centrifuged for 15 min at 13000 rpm, the supernatant removed, and the resulting pellet was used for DNA extraction. The pellets were treated according to the protocol described in the PureLink Genomic DNA kit (Invitrogen). Partial LSU, SSU, and ITS1-5.8S-ITS2 regions of the nuclear rDNA locus were amplified using the LR0R/LR6-O (Riethmüller *et al.* 2002), NS4/SR1R (White *et al.* 1990) and UN-up18S42/UN-lo28S22 (Robideau *et al.* 2011) primers, respectively, and/or COI mtDNA using OomCoxI-Levup/OomCoxI-Levlo (Robideau *et al.* 2011). Amplicons were purified using the protocol in Schmitz and Riesner (2006), and sequencing was performed using the same PCR primer sets in an ABI 3730 DNA Analyser (Life Technologies, Carlsbad, California). The sequences were edited manually using Sequencher 4.1.4. The obtained sequences were compared with similar sequences of GenBank using the BLASTn, with our sequences have been published in this database (<https://www.ncbi.nlm.nih.gov/genbank/>).

Ecological data analyses – The total frequency (TF) was calculated based on the occurrence of each taxon considering the total sampling/types of samples (16 in total – four sampling during the study period and four types of samples) according to Marano *et al.* (2008). The total abundance (TA) of the identified species was based on the number of occurrences considering the presence or absence of them in the samples. The community structure was estimated by (i) the species richness (S), (ii) Shannon diversity index $H' = -\sum_{i=1}^S p_i \log_2(p_i)$, where p_i is the abundance of the

species i in the community, (iii) evenness $E = H'/H'_{\max}$, where H'_{\max} is the maximum value of the diversity for the number of present taxa, (iv) Simpson's dominance index $D = 1/\sum_{i=1}^S p_i^2$ based on the species abundance (Hammer *et al.* 2001, Zak & Willig 2004). The Wilcoxon test was used to verify if the abundance and diversity measured were equal between the samples (Hammer *et al.* 2001).

Results

Species composition and community structure – One hundred and eighty samples (45 water, 45 soil, 45 submerged leaves, 45 surface sediment) were analyzed. From these, 209 specimens were obtained, and 41 taxa identified belonging to the phyla Hyphochytriomycota (1) and Oomycota (40), with 38 at species level and 03 to genus level, as shown in the Table 1. Total frequency and abundance of them are also mentioned. Thirty-eight species were sequenced (ITS, SSU and LSU rDNA and/or COI mDNA) and most of the sequences available in GenBank. Twelve taxa have already been published as new species and/or new citation for Brazil or the studied area (Pires-Zottarelli *et al.* 2019, Dima *et al.* 2021, Pires-Zottarelli *et al.* 2022).

From 41 identified taxa, 21 have been occurred in water (53 occurrences), 17 in submerged leaves (40 occurrences), 18 in surface sediment (39 occurrences) and 25 in soil samples (77 occurrences). Although the species richness (S) and occurrence were higher in soil, there was no significant differences ($P \leq 0.05$) among the samples considering the indices (Table 2). *Achlya* sp1, *Aphanomyces stellatus*,

Table 1. Straminipiles from soil and water, submerged leaves and surface sediment from freshwater bodies at “Mosaico de Unidades de Conservação Juréia-Itatins” collected from August/2016 to October/2017. TF%: Total Frequency. TA: Total Abundance.

Taxa/ Samples	Water	Submerged leaves	Sediment	Soil	TF%	TA
STRAMINIPILA KINGDOM						
HYPHOCHYTRIOMYCOTA						
HYPHOCHYTRIALES						
Hyphochytriaceae						
<i>Hyphochytrium catenoides</i>				1	6.25	1
OOMYCOTA						
Leptolegniellaceae <i>incertae sedis</i>						
<i>Aphanodictyon papillatum</i>				1	6.25	1
<i>Leptolegniella keratinophila</i>		1		5	37.50	6
<i>Leptolegniella exogena</i>				1	6.25	1
<i>Leptolegniella</i> sp 1				3	12.50	3
PERONOSPORALES <i>s. l.</i>						
Pythiaceae <i>sensu lato</i>						
<i>Myzocytiopsis humana</i>		3	2	4	75	9

Table 1 (continued)

Taxa/ Samples	Water	Submerged leaves	Sediment	Soil	TF%	TA
<i>Pythiogeton abundans</i>	3		1		12.50	4
<i>Pythium acanthicum</i>			1	4	50	5
<i>Pythium graminicola</i>			2		12.50	2
<i>Pythium periplocum</i>	1	2			37.50	
<i>Pythium splendens</i>			1		6.25	1
Peronosporaceae.s. l.						
<i>Phytophthora undulata</i>		1			6.25	1
<i>Phytopythium chamaehyphon</i>	2	1	4		37.50	7
<i>Phytopythium palingenes</i>	4	3		2	18.75	9
<i>Phytopythium vexans</i>				4	12.50	4
SAPROLEGNIALES						
Saprolegniaceae s. l.						
<i>Achlya catenulata</i>	2	3			25	5
<i>Achlya flagellate</i>	2		2	4	56.25	7
<i>Achlya klebsiana</i>	3	3	2		37.50	8
<i>Achlya diffusa</i>	2	4	1		18.75	7
<i>Achlya orion</i>	4			4	37.50	8
<i>Achlya proliferoides</i>	2				12.50	2
<i>Achlya truncatiformis</i>				1	6.25	1
<i>Achlya</i> sp 1	6	6	7	7	75	26
<i>Aplanopsis terrestris</i>				1	6.25	1
<i>Brevilegnia longicaulis</i>			1	2	12.50	3
<i>Brevilegnia</i> sp 1				2	12.50	2
<i>Dictyuchus pseudodictyon</i>		3	1		37.50	4
<i>Leptolegnia eccentrica</i>				3	12.50	1
<i>Newbya androgyna</i>	1			2	25	3
<i>Pythiopsis irregularis</i>				3	12.50	3
<i>Saprolegnia aenigmatica</i>	3	1	1		37.50	7
<i>Saprolegnia blelhamensis</i>				2	6.25	2
<i>Saprolegnia glomerata</i>	2	1	3		37.50	6
<i>Saprolegnia milanezii</i>	1				6.25	1
<i>Saprolegnia atlantica</i>	3			1	25	4
Verrucalvaceae						
<i>Aphanomyces brasiliensis</i>			1	1	25	2
<i>Aphanomyces helicoides</i>	3	1	1		37.50	5
<i>Aphanomyces raphanin</i>	1				6.25	1
<i>Aphanomyces stellatus</i>	1	3	5	1	75	10
<i>Phragmosporangium uniseriatum</i>	1	1		12	75	14
<i>Plectospira myriandra</i>	6	3	3	4	100	16
Total of species	21	17	18	25		
Total of occurrences	53	40	39	77		209

Table 2. Species richness (*S*), evenness (*E*), Shannon diversity (*H'*) and Simpson dominance (*D*) indices in each of the samples collected. The same letters indicate that the values do not differ significantly ($P \leq 0.05$).

Index	Water	Submerged leaves	Sediment	Soil
<i>S</i>	21a	17a	18a	25a
<i>E</i>	0.85a	0.85a	0.79a	0.77a
<i>H'</i>	2.89a	2.67a	2.65a	2.96a
<i>D</i>	0.06a	0.08a	0.09a	0.06a

Phragmosporangium uniseriatum e *Plectospora myriandra* were the species more frequent and abundant, considering all samples.

For each taxa presented below, geographic location, basionym, origin and GenBank accession numbers (not yet mentioned or published elsewhere) are provided. Images of the species not previously illustrated by our team are presented. New citations of the species for the São Paulo State and/or the studied area are mentioned herein.

KINGDOM STRAMINIPILA

PHYLUM HYPHOCHYTRIOMYCOTA

HYPHOCHYTRIALES

HYPHOCHYTRIACEAE

1. *Hyphochytrium catenoides* Karling. Am. J. Bot. 26: 512. 1939.

Figures 2 a, 2 b

Specimen examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W, from soil samples baited with snake skin, 27 June 2017. Culture not obtained.

Identification: This species of *Hyphochytrium* is characterized by a polycentric thallus, usually hypha-like, with a linear series of intercalary and terminal swellings and zoosporangia. The zoosporangia are normally spherical or ovoid and delimited by cross septa. The zoospores anteriorly uniflagellate with numerous lipidic granules emerge by exit tubes from the zoosporangia as a naked mass, maturing outside the zoosporangia (Karling 1939). The characteristics of our specimen are in agreement with the original description. This species was previously mentioned in Brazil by Moreira (2006) from submerged leaves of *Tibouchina pulchra* Cogn. in Atlantic Rainforest area in São Paulo city. This is a new citation for the studied area.

PHYLUM OOMYCOTA

LEPTOLEGNIELLACEAE – *Incertae sedis*

2. *Aphanodictyon papillatum* Huneycutt ex M.W. Dick, Trans. Br. Mycol. Soc. 57: 422. 1971.

Figures 2 c, 2 d

Specimen examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1"S, 47°14'53.0"W, from soil samples baited with snake skin, 25 Oct 2017, SP512792. Culture not obtained.

Identification: *Aphanodictyon papillatum* grow only as saprotrophic in keratinous substrate and their pure culture has never been obtained. We tried many times in several media cultures, however no culture was obtained. It is characterized by delicate, much-branched hyphae, normally globose to subglobose zoosporangia. Their biflagellate zoospores are discharged like as *Dictyuchus* genus, leaving a net of cysts wall into the zoosporangia. The oogonia are normally globose to subglobose, some irregular, with papillate ornamentations; androgynous or diclinous antheridia; thick oospores normally 1-3 by oogonia (Huneycutt 1948). The characteristics of our specimen are in agreement with the original description. This species was previously mentioned in Brazil from soil samples in Piauí State (Rocha 2002), with their distribution in the country published by Milanez *et al.* (2007) and Rocha *et al.* (2010). This is a new citation for São Paulo State.

3. *Leptolegniella exogena* Karling. Nova Hedwigia 45: 433. 1987.

Specimen examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W, from soil samples baited with snake skin, 27 June 2017, SP512793, CCIBt4682. GenBank – SSU: ON860582, LSU: ON860619.

Identification: This holocarpic species presented a coarse branched occasionally constricted and septate mycelium, which is intramatrical or extramatrical in the substrate. Zoosporangia undifferentiated of vegetative mycelium. Zoospores biflagellate normally emerging in succession and forming a globular mass at the exit orifice or encysting into the sporangia. Development of the resting spores is unique for this species, where the protoplasm aggregated into the hyphae contract to form exogenous resting spores that remain attached to the hyphae wall (Karling 1987). Our specimen presented all the important characteristics of this beautiful species. Sequences of this genus/species from were obtained for the first time from pure culture. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

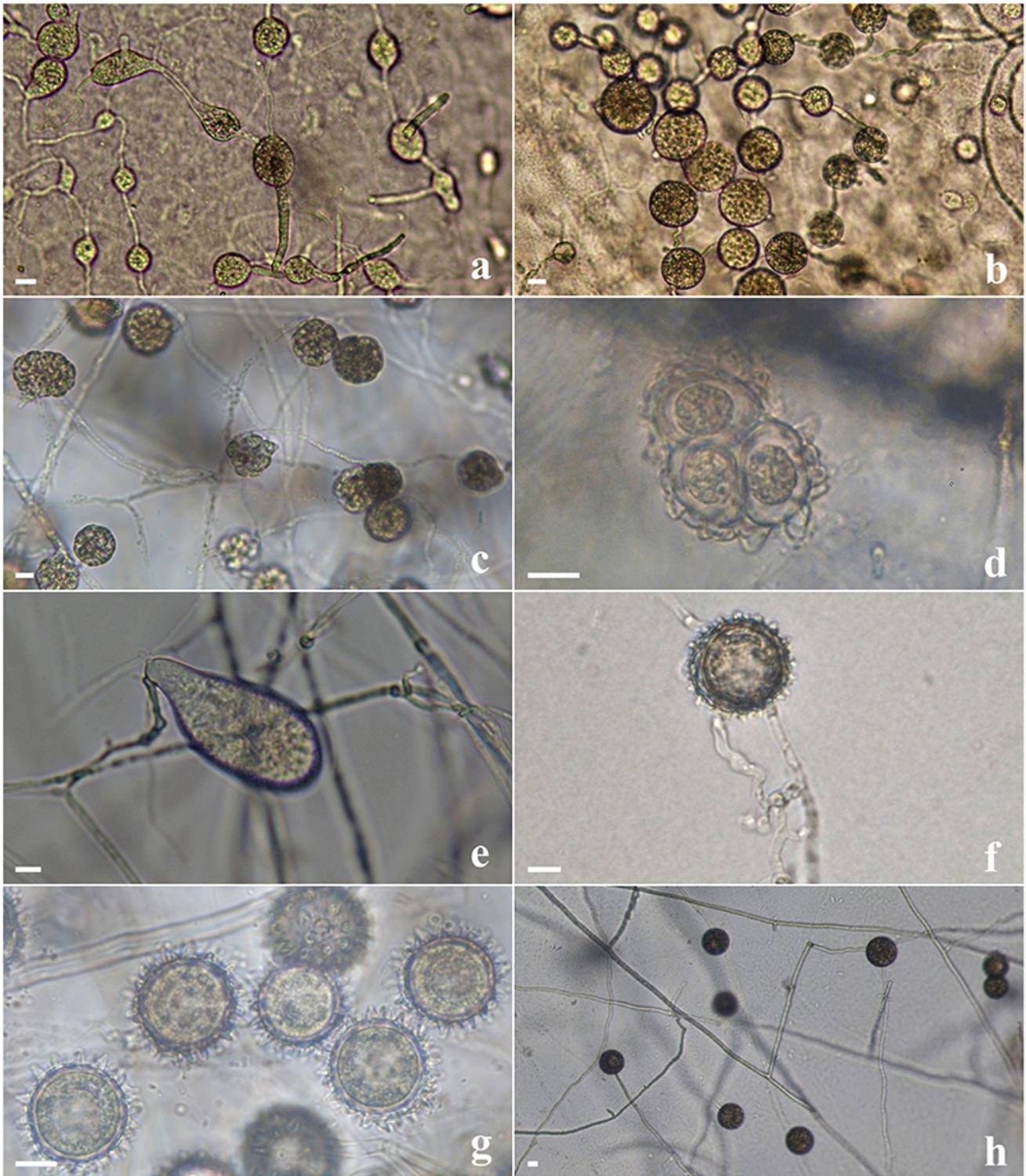


Figure 2. a-b. *Hyphochytrium catenoides* Karling. a. Young thallus on snake skin. b. Polycentric thallus on snake skin with intercalary and terminal swellings and zoosporangia connected by hyphae. c-d. *Aphanodictyon papillatum* Huneycutt ex M.W. Dick. c. Zoosporangia in snake skin. d. Papillate oogonium with thick oospores on snake skin. e. *Pythiogeton abundans* J.H. Huang, Chi Y. Chen & Yi S. Lin. Zoosporangium. f-g. *Pythium acanthicum* Drechsler. f. Intercalary ornamented oogonium with oospore and monoclinal antheridium. g. Details of the oogonia with conical projections. h. *Pythium splendens* Hans Braun. Globose hyphal swellings with dark granular content in the mycelium. Scale bars = 10µm

4. *Leptolegniella keratinophila* Huneycutt, J. Elisha Mitchell. Sci. Soc. 68: 110. 1952.

Specimens examined: BRAZIL. SÃO PAULO: Peruíbe, Pedro de Toledo, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W; 24°21'59.1"S, 47°18'32.7"W; 24°34'21.1"S, 47°14'53.0"W, respectively, from submerged leaves of freshwater bodies and soil samples baited with snake skin, 24 Aug 2016, 27 June 2017 and 25 Oct 2017, SP512794. Culture not obtained.

Identification: This holocarpic species described by Huneycutt (1952) is characterized by a poorly developed extramatrical and an extensive intramatrical mycelium, in keratinous substrate. Zoosporangia undifferentiated of vegetative mycelium. Resting spores is typical for this species and produced within undifferentiated hyphae. Pure culture of this species has not been obtained. Our specimens are in agreement with the original description. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

5. *Leptolegniella* sp1

Specimens examined: BRAZIL. SÃO PAULO: Peruíbe and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from soil samples baited with snake skin, 24 Aug 2016 and 25 Oct 2017, CCIBt4424. Sequences are not available herein, because this isolate is being studied considering it does not fit into any previously taxa described.

PERONOSPORALES SENSU LATO

PYTHIACEAE SENSU LATO

6. *Myzocytiopsis humana* (Karling) M.W. Dick, Mycol. Res. 101: 879. 1997.

Basionym: *Lagenidium humanum* Karling

Specimens examined: BRAZIL. SÃO PAULO: Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1"S, 47°18'32.7"W; 24°33'11.1"S, 47°13'34.5"W; 24°34'21.1"S, 47°14'53.0"W, respectively, from submerged leaves and surface sediment of freshwater bodies and soil samples baited with snake skin, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4304. GenBank – ITS: ON860609, SSU: ON860583, LSU: ON860620.

Identification: The specimens presented septate thallus with exit tubes and numerous refractive bodies, forming disarticulated zoosporangium as mentioned in the original description of Karling (1947). Sexual reproduction was not observed. They grew in keratinous substrate (snake skin) and very well in medium culture (MP₃). It is a new citation for the studied area. It was previously mentioned for Brazil by Jesus *et al.* (2013) from water samples of the Atlantic

Rainforest of São Paulo city. Illustration of the species in Jesus *et al.* (2013).

7. *Pythiogeton abundans* J.H. Huang, Chi Y Chen & Yi S. Lin, Mycoscience 54: 132. 2013.

Figure 2 e

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W, from water and surface sediment samples of freshwater bodies baited with *Sorghum* sp seeds, 27 Jun 2017, CCIBt4340. GenBank – ITS: ON860610.

Identification: The production of the spherical, unsymmetrical and bursiform zoosporangia, their insertion in the hyphae at right angles, the internal proliferation and the zoospores discharge of them confirmed the morphological identification of the isolate at genus level, as mentioned by Sparrow (1960). Our specimens were identified at species level taking into account the morphological and molecular data, considering that *P. abundans* not produce sexual structures as mentioned by Huang *et al.* (2013). It was previously mentioned for Brazil from freshwater samples, as well as dead trout fish in Campos do Jordão, São Paulo State (da Paixão 2021). This is a new citation for the studied area.

8. *Pythium acanthicum* Drechsler, J. Wash. Acad. Sci. 20: 408. 1930.

Figures 2 f, 2 g

Specimens examined: BRAZIL. SÃO PAULO, Peruíbe, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W; 24°21'59.1"S, 47°18'32.7"W; 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from surface sediment of freshwater bodies and soil samples baited with *Sorghum* sp seeds, 24 Aug 2016, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt 4309. Genbank – ITS: ON860611, LSU: ON860621.

Identification: The production of the zoosporangia, the discharge and the maturation of the zoospores into the vesicle, the oogonia with conical projections and blunt tip and monoclinal antheridia, as mentioned by Plaats-Niterink (1981), are characteristics of this species. The morphology of our specimens, as well as the rDNA sequences, allowed us to identified the isolate as *P. acanthicum*. It is a new citation for the studied area.

9. *Pythium graminicola* Subraman, Bull. Agric. Res. Inst. Pus. 177: 1. 1928.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1"S, 47°18'32.7"W and 24°33'11.1"S, 47°13'34.5"W, respectively, from surface

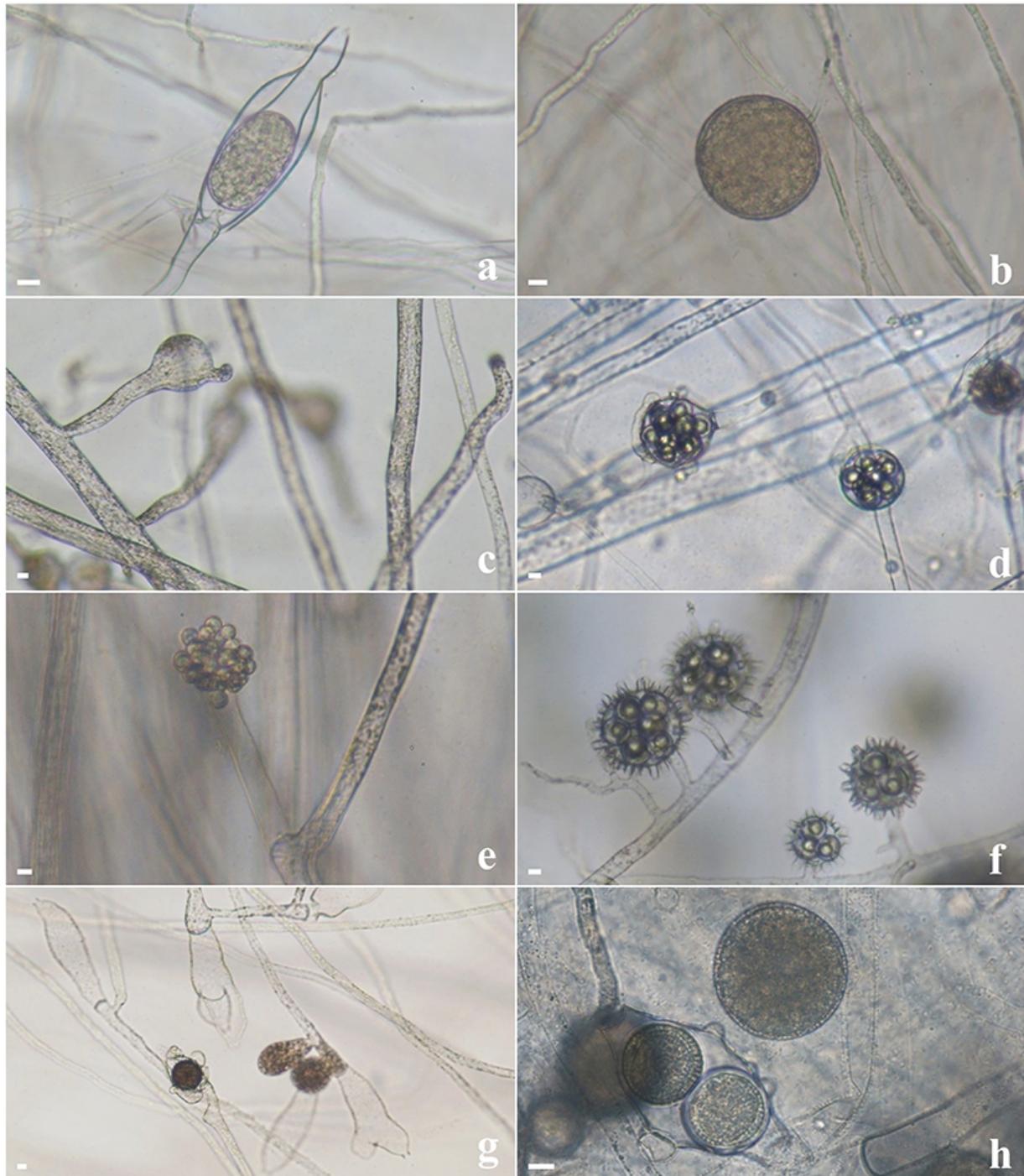


Figure 3. a-b. *Phytophthora undulata* (H.E. Petersen) M.W. Dick. a. Zoosporangium with internal proliferation. b. Chlamydo-spore. c-d. *Achlya diffusa* J.V. Harv. Ex T.W. Johnson. C. “Balloon-like” hyphal-swelling. d. Oogonia with eccentric oospores and declinous antheridia. e-f. *Achlya truncatiformis* M.W. Dick & Mark A. Spencer. E. Achlyoid discharge of the zoosporangia. f. Oogonia with truncate oogonial papillae and eccentric oospores. g-h. *Pythiopsis irregularis* R.L. Seym. g. Empty zoosporangia and initial development of oogonium and antheridia. h. Ornamented oogonium with subcentric oospores. Scale bars = 10µm

sediment samples of freshwater bodies baited with *Sorghum* sp seeds, 14 Feb 2017 and 27 Jun 2017, CCIBt4330. GenBank – ITS: ON860612, LSU: ON860622.

Identification: This species is characterized by inflated filamentous zoosporangia, and by oogonia with predominantly monoclinous antheridia (Plaats-Niterink

1981). Our specimens were identified taking into account the morphological and molecular data. It is a new citation for the studied area. Illustration of the species in Jesus *et al.* (2013).

10. *Pythium periplocum* Drechsler, J. Wash. Acad. Sci. 20: 405. 1930.

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water and submerged leaves samples of freshwater bodies baited with snake skin and *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4344, CCIBt 4420. GenBank – ITS: ON860613, LSU: ON860623.

Identification: *Pythium periplocum* is characterized by inflated filamentous zoosporangia and spines oogonia (Plaats-Niterink 1981). Our specimens produced many zoosporangia, however few oogonia. The molecular data helped us to identify the specimens. It is a new citation for the studied area. Illustration of the species in Gonçalves *et al.* (2016a).

11. *Pythium splendens* Hans Braun, J. Agric. Res. 30: 1061. 1925.

Figure 2 h

Specimen examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1''S, 47°14'53.0''W, from surface sediment samples of freshwater bodies baited with *Sorghum* sp seeds, 25 Oct 2017, CCIBt4399. GenBank – ITS: ON860614, LSU: ON860624.

Identification: Our specimen produced globose and abundant hyphal swellings, with dark granular content, like as mentioned by Plaats-Niterink (1981). The molecular data helped us to identify the specimen. This is a new citation for São Paulo State.

PERONOSPORACEAE SENSU LATO

12. *Phytophthora undulata* (H.E. Petersen) M.W. Dick, Mycotaxon 35: 449. 1989.

Basionym: *Pythium undulatum* H.E. Petersen

Figures 3 a, 3 b

Specimen examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1''S, 47°13'34.5''W, from submerged leaves samples of freshwater bodies baited with snake skin and

corn leaves, 27 Jun 2017, CCIBt4303. GenBank – ITS: ON860615, LSU: ON860625.

Identification: This species is easy recognize by elongate and proliferous zoosporangia, and dark yellow chlamydospores usually intercalary in the hyphae (Plaats-Niterink 1981). Our molecular data confirm the morphological identification. It is a new citation for the studied area.

13. *Phytophthora chamaehyphon* (Sideris) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque, Persoonia 34: 36. 2014.

Basionym: *Pythium chamaehyphon* Sideris

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W and 24°33'11.1''S, 47°13'34.5''W, respectively, from water, surface sediment and submerged leaves samples of freshwater bodies baited with corn leaves, onion skin and *Sorghum* sp seeds, 14 Feb 2017 and 27 Jun 2017, CCIBt4338, CCIBt4407.

Identification: Description, comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Dima *et al.* (2021).

14. *Phytophthora palingenes* (Drechsler) Abad, de Cock, Bala, Robideau & Levésque, Persoonia 34: 37. 2014.

Basionym: *Pythium palingenes* Drechsler

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1''S, 47°14'53.0''W, from water and submerged leaves of freshwater bodies and soil samples baited with onion skin, snake skin and *Sorghum* sp seeds, 25 Oct 2017, CCIBt4397, CCIBt4428.

Identification: Comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Dima *et al.* (2021).

15. *Phytophthora vexans* (de Bary) Abad, de Cock, Bala, Robideau & Levésque, Persoonia 34: 37. 2014.

Basionym: *Pythium vexans* de Bary

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from soil samples baited with onion skin, snake skin and *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4383 CCIBt4398, CCIBt4422.

Identification: Comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Dima *et al.* (2021).

SAPROLEGNIALES

SAPROLEGNIACEAE *SENSU LATO*

16. *Achlya catenulata* Pires-Zottar., A.L. Jesus, Marano & J.I. Souza, Phytotaxa 212: 223. 2015.

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water and submerged leaves of freshwater bodies samples baited with shrimp exoskeleton and *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4302. GenBank – ITS: OK271001, SSU: OK235689; LSU: OK270960.

Identification: This Brazilian species was described by Jesus et al. (2015) from freshwater and saline water samples of the Atlantic Rainforest of São Paulo State. It is characterized by catenulate smooth-walled oogonia, usually in chains, with declinuous antheridia and eccentric oospores, which normally not mature. Our specimens are in agreement with the original description. It is a new citation for the studied area. Illustration of the species in Jesus *et al.* (2015).

17. *Achlya diffusa* J.J.V. Harv. ex T.W. Johnson, The genus *Achlya*: morphology and taxonomy 64: 1956. Figures 3 c, 3 d

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W, from water, surface sediments and submerged leaves of freshwater bodies samples baited with *Sorghum* sp seeds, 14 Feb 2017, CCIBt4396, CCIBt4405, CCIBt4429. GenBank – ITS: OK270977, OK270979, OK270978, respectively; SSU: NA, NA, OK235673, respectively, LSU: OK270937, OK270939, OK270938, respectively; COI: OK120427, OK120429, OK120428, respectively.

Identification: This species is characterized by inflated “balloon-like” hyphal swellings, and eccentric oospores, which usually not mature (Johnson 1956). The characteristics of our specimens are in agreement with the original description. This is a new citation for São Paulo State.

18. *Achlya flagellata* Coker, Saprolegniaceae with notes on other water molds: 116. 1923.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W; 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water and surface sediments of freshwater bodies and soil samples baited with shrimp exoskeleton and *Sorghum* sp seeds, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4400, CCIBt4404, CCIBt4425. GenBank – ITS:

OK270982, OK270981, OK270980, respectively; SSU: NA, NA, NA, respectively; LSU: OK270942, OK270941, OK270940, respectively; COI: NA, NA, OK120430, respectively.

Identification: This species is very common in aquatic and terrestrial Brazilian ecosystems (Milanez et al. 2007). It is characterized by production of oogonia with oospheres usually not maturing, when mature are eccentric (Johnson *et al.* 2002). The molecular data confirm our morphological identification. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

19. *Achlya klebsiana* Pieters, Bot. Gaz. 60: 486. 1915.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water, surface sediments and submerged leaves of freshwater bodies samples baited with snake skin and *Sorghum* sp seeds, 14 Feb 2017 and 25 Oct 2017, CCIBt4333, CCIBt4339, CCIBt4357, CCIBt4421. GenBank – ITS: OK270984, OK270983, OK270985, OK270986, respectively; SSU: NA, OK235675, NA, NA, respectively; LSU: OK270944, OK270943, OK270945, OK270946, respectively; COI: OK120432, OK120431, OK120433, NA, respectively.

Identification: This species is characterized by oogonia with eccentric oospores and usually declinuous and monoclinal antheridia, when monoclinal arising from a point remote from the oogonium to which attached as mentioned by Johnson (1956). In Johnson *et al.* (2002) this species, together with *Achlya flagellata*, are considered synonym of *Achlya debaryana*, however this is not accepted by many specialists, including us. The molecular data obtained confirm our impression. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

20. *Achlya orion* Coker & Couch, J. Elisha Mitchell Sci. Soc. 36: 100. 1920.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W; 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water of freshwater bodies and soil samples baited with *Sorghum* sp seeds, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4310, CCIBt4384. GenBank – ITS: OK270987, OK270988, respectively; SSU: NA; LSU: OK230947, OK270948, respectively; COI: OK120434, OK120435, respectively.

Identification: This species very common in Brazilian ecosystems (Milanez *et al.* 2007) is easy recognize by usually

spherical oogonia with long, slender, bent or recurved stalks, commonly androgynous or monoclinal antheridia, and eccentric oospores (Johnson *et al.* 2002). The characteristics of our specimens are in agreement with the description. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

21. *Achlya proliferoides* Coker, Saprolegniaceae with notes on other water molds 115, 1923.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W and 24°33'11.1''S, 47°13'34.5''W, respectively, from water of freshwater bodies samples baited with *Sorghum* sp seeds, 14 Feb 2017 and 27 Jun 2017, CCIBt4418, CCIBt4419. GenBank – ITS: OK270989, OK270990, respectively; SSU: NA, OK235677, respectively; LSU: OK270949, OK270950, respectively; COI: OK120436, OK120437, respectively.

Identification: This species is characterized by predominantly declinuous antheridia coiling in the hyphae which may or may not bear oogonia, and eccentric oospores (Johnson *et al.* 2002). Our specimens produced all the described characteristics. It is a new citation for the studied area. Illustration of the species in Nascimento & Pires-Zottarelli (2012).

22. *Achlya truncatiformis* M.W. Dick & Mark A. Spencer, Mycol Res. 106: 557. 2002. Figures 3 e, 3 f

Specimen examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1''S, 47°14'53.0''W, from soil samples baited with *Sorghum* sp seeds, 25 Oct 2017, CCIBt4341. GenBank – ITS: OK270991; SSU: OK235678; LSU: OK270951; COI: OK120438.

Identification: The description of *Achlya truncatiformis* is based on *Achlya recurva sensu* Latham, which is characterized by typical truncate oogonial papillae and eccentric oospores (Spencer *et al.* 2002). Our specimen is in agreement with the description of these authors. It is a new citation for the studied area, considering that specimens of *Achlya recurva* with this type of ornamentation and eccentric oospores were mentioned for Brazilian ecosystems of Piauí and São Paulo State (Milanez *et al.* 2007).

23. *Achlya* sp 1

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W; 24°33'11.1''S, 47°13'34.5''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from water, surface sediment, submerged leaves of freshwater bodies and soil samples baited with snake skin

and *Sorghum* sp seeds, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4337, CCIBt4381, CCIBt4408. Sequences are not available herein.

Identification: The specimens are being studied considering its does not fit into any previously described species.

24. *Aplanopsis terrestris* Höhnk, Veröff. Inst. Meeresf. Bremerhaven, Sonderband 1: 127. 1952.

Specimens examined: BRAZIL. SÃO PAULO: Pedro de Toledo, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W, from soil samples baited with snake skin, 14 Feb 2017, CCIBt4307. GenBank – ITS: OK270992, LSU: OK270953.

Identification: This species is characterized by no zoosporangia produced; oogonia wall usually smooth, however few papillae were observed, with centric and subcentric oospores and commonly androgynous antheridia (Johnson *et al.* 2002, Rocha *et al.* 2016a). The morphological and molecular data confirm the identification of this species. It was previously mentioned for Brazil by Rocha *et al.* (2016a) from soil samples of the Atlantic Rainforest of São Paulo State. It is a new citation for the studied area. Illustration of the species in Rocha *et al.* (2016a).

25. *Brevilegnia longicaulis* T.W. Johnson, Mycologia 42: 244. 1950.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W, from soil samples baited with *Sorghum* sp seeds, 14 Feb 2017, CCIBt4336, CCIBt4356. GenBank – ITS: OK270995, OK270994, respectively; SSU: NA, OK235684, respectively. LSU: NA, OK270955, respectively.

Identification: This species is recognized by discharge and behavior brevilegnoid, rarely dictyucoid, of the zoospores, antheridia always declinuous attaching in oogonia that produce eccentric oospores. When dictyucoid behavior occurs the zoosporangium partly disintegrated and disarticulating (Johnson *et al.* 2002). It was previously mentioned for Brazil by Rocha *et al.* (2016a) from soil samples of the Atlantic Rainforest of São Paulo State. It is a new citation for the studied area. Illustration of the species in Rocha *et al.* (2016a).

26. *Brevilegnia* sp 1

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1''S, 47°18'32.7''W and 24°34'21.1''S, 47°14'53.0''W, respectively, from soil samples baited with *Sorghum* sp seeds, 14 Feb 2017 and 25 Oct 2017, CCIBt4332, CCIBt4350. Sequences not available herein.

Identification: The specimens are being studied considering it does not fit into any previously described species of the genus.

27. *Dictyuchus pseudodictyon* Coker & Braxton, J. Elisha Mitchell Sci. Soc. 46: 228. 1931.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1"S, 47°18'32.7"W; 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from submerged leaves of freshwater bodies samples baited with *Sorghum* sp seeds, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4377, CCIBt4378. GenBank – ITS: OK271002, OK271003, respectively; SSU: OK235690, NA; LSU: OK270961, NA, respectively.

Identification: The zoospores cysts left inside the zoosporangia, forming true and false network, declinuous antheridia involving the oogonium and eccentric oospores are important features of this species (Johnson *et al.* 2002). Our morphological and molecular data of the specimens confirm the identification. It is a new citation for the studied area. Illustration of the species in Miranda & Pires-Zottarelli (2012).

28. *Leptolegnia eccentrica* Coker, J. Elisha Mitchell Sci. Soc. 42: 215. 1927.

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1"S, 47°14'53.0"W, from soil samples baited with snake skin, 25 Oct 2017, CCIBt4403. GenBank – ITS: ON860617, LSU: ON860627.

Identification: Papillate oogonia, with papillae in variable number, monoclinous and androgynous antheridia, as well as eccentric oospores are characteristic of this species (Johnson *et al.* 2002). The characteristics of our specimens are in agreement of the species' description. It is a new citation for the studied area. Illustration of the species in Rocha *et al.* (2018).

29. *Newbya androgyna* (W. Archer) Pires-Zottar. & S.C.O. Rocha, Mycol. Progr. 17: 696. 2018.

Basionym: *Saprolegnia androgyna* W. Archer

Specimens examined: BRAZIL. SÃO PAULO, Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water of freshwater bodies and soil samples baited with *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4306. GenBank – ITS: OK271005; SSU: OK235692; LSU: OK270963.

Identification: Rare terminal or intercalary zoosporangia, filiform and/or naviculate oogonia with papillate ornamentation, subcentric oospores, and androgynous antheridia are important features of this species (Johnson *et al.* 2002, Rocha *et al.* 2018). The characteristics of our specimens are in agreement with the descriptions of these authors. It is a new citation for the studied area. Illustration of the species in Rocha *et al.* (2018).

30. *Pythiopsis irregularis* R.L. Seym., Mycotaxon 92: 2. 2005.

Figures 3 g, 3 h

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°21'59.1"S, 47°18'32.7"W and 24°33'11.1"S, 47°13'34.5"W, respectively, from soil samples baited with snake skin, 14 Feb 2017 and 27 Jun 2017, CCIBt4308, CCIBt4311. GenBank – ITS: OK271009; SSU: OK235700; LSU: OK270967.

Identification: This species produces oogonia with short, broad, conspicuous papillae or bullations, centric and subcentric oospores and androgynous antheridia (Johnson *et al.* 2002). The characteristics of our specimens are in agreement of the original description, with molecular data corroborating the morphological identification. It is a new citation for the studied area.

31. *Saprolegnia aenigmatica* Sand.-Sierra & Diég.-Urib., PLoS ONE 10:9. 2015. .

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water, surface sediment and submerged leaves of freshwater bodies samples baited with *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4380, CCIBt4406, CCIBt4417. Comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2022).

32. *Saprolegnia atlantica* Pires-Zottar., Rocha S.C.O. & A.L. Jesus, Mycol. Progr. 21 (3, n° 41): 6. 2022.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water of freshwater bodies and soil samples baited with *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4335, CCIBt4351. Description, comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2022).

33. *Saprolegnia blelhamensis* (M.W. Dick) Milko, Mikol. Fitopatol. 13: 290. 1979.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1"S, 47°14'53.0"W, from soil samples baited with *Sorghum* sp seeds, 25 Oct 2017, CCIBt4354, CCIBt4423. Description, comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2022).

34. *Saprolegnia glomerata* (Tiesenh.) A. Lund, Mém. Acad. Roy. Sci. Lett. Sec. Sci 5: 14. 1934.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water and surface sediment of freshwater bodies samples baited with *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4352, CCIBt4353, CCIBt4402, CCIBt4549. Description, comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2022).

35. *Saprolegnia milanezii* Pires-Zottar. & S.C.O. Rocha, Phytotaxa 270: 289. 2016.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W, from water of freshwater bodies samples baited with *Sorghum* sp seeds, 27 Jun 2017, CCIBt4379. Comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2022).

VERRUCALVACEAE

36. *Aphanomyces brasiliensis* Pires-Zottar. & A.L. Jesus, Phytotaxa 415: 214. 2019.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°34'21.1"S, 47°14'53.0"W, from soil samples baited with snake skin 25 Oct 2017, CCIBt4359, CCIBt4545. GenBank – SSU: OK235679. Description, comments, illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2019).

37. *Aphanomyces helicoides* Minden, Krypt. Fl. Branderburg (Lepizig) 5: 559. 1912 (1915).

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water and surface

sediment of freshwater bodies samples baited with onion skin, shrimp exoskeleton, snake skin and *Sorghum* sp seeds, 27 Jun 2017 and 25 Oct 2017, CCIBt4331. GenBank – SSU: OK235681. Illustration and sequence code of ITS rDNA region are available in Pires-Zottarelli *et al.* (2019).

38. *Aphanomyces raphani* J.B. Kendr., Phytopathology 17: 43. 1927.

Specimens examined: BRAZIL. SÃO PAULO: Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°33'11.1"S, 47°13'34.5"W, from water of freshwater bodies samples baited with corn leaves, 27 Jun 2017, CCIBt4409. GenBank – SSU: OK235682. Illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2019).

39. *Aphanomyces stellatus* de Bary, J. Wiss. Bot. 2: 178. 1860.

Specimens examined: BRAZIL. SÃO PAULO, Peruíbe and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W; 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water, surface sediment and submerged leaves of freshwater bodies and soil samples baited with snake skin, 24 Aug 2016, 27 Jun 2017 and 25 Oct 2017, CCIBt4305. GenBank – SSU: OK235682. Illustration and sequences codes of ITS and LSU rDNA regions are available in Pires-Zottarelli *et al.* (2019).

40. *Phragmosporangium uniseriatum* R.L. Seym., Mycotaxon 92: 8. 2005.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo, Peruíbe and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W; 24°21'59.1"S, 47°18'32.7"W; 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water and submerged leaves of freshwater bodies and soil samples baited with snake skin, shrimp exoskeleton and *Sorghum* sp seeds, 24 Aug 2016, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4334. GenBank – ITS: ON860616, LSU: ON860626.

Identification: This is the unique species of the genus. *Phragmosporangium uniseriatum* presents abundant production of the zoosporangia and oogonia, oogonial wall with inner surface smooth and outer surface smooth, irregular or papillated; antheridia androgynous and monoclinal and centric and subcentric oospores (Johnson *et al.* 2002, Pires-Zottarelli *et al.* 2007). The morphological characteristics of our specimens are in agreement with the descriptions of these authors, with the ITS rDNA sequences corroborating this identification. It is a new citation for the studied area. Illustration of the species in Pires-Zottarelli *et al.* (2007).

41. *Plectospira myriandra* Drechsler, J. Agric. Res. 34: 295. 1927.

Specimens examined: BRAZIL. SÃO PAULO, Pedro de Toledo, Peruíbe and Iguape, Mosaico de Unidades de Conservação Juréia-Itatins, 24°23'51.5"S, 47°07'14.4"W; 24°21'59.1"S, 47°18'32.7"W; 24°33'11.1"S, 47°13'34.5"W and 24°34'21.1"S, 47°14'53.0"W, respectively, from water, surface sediment and submerged leaves of freshwater bodies and soil samples baited with onion skin and corn leaves, 24 Aug 2016, 14 Feb 2017, 27 Jun 2017 and 25 Oct 2017, CCIBt4342. GenBank – ITS: ON860618.

Identification: Abundant production of the oogonia, yellowish subcentric oospores, as well as declinuous and monoclinal antheridia are important characteristics of this species (Johnson *et al.* 2002, Miranda & Pires-Zottarelli 2012, Jerônimo *et al.* 2017). The morphological characteristics of our specimens are in agreement with the descriptions of these authors. Molecular data corroborated the morphological identification. It is a new citation for the studied area. Illustration of the species in Jerônimo *et al.* (2017).

Discussion

This study allowed us to analyze the occurrence and distribution of the heterotrophic straminipiles of a preserved Brazilian Atlantic Rainforest area. All the identified taxa were isolated from freshwater and soil samples using baits as substrate. The majority of the species was found as saprotrophs, however some of them with pathogenic potential in plants and animals (Plaats-Niterink 1981, Sati 1991, Sandoval-Sierra & Diégues-Uribeondo 2015, Carraschi *et al.* 2018, Ferreira *et al.* 2021, Dima *et al.* 2021, and others), with several of them with genic regions made available for the first time in GenBank. Many of them have already mentioned for Brazil (Gomes & Pires-Zottarelli 2006, Milanez *et al.* 2007, Mendes *et al.* 1998, Miranda & Pires-Zottarelli 2008, 2012, Nascimento & Pires-Zottarelli 2012, Jesus *et al.* 2013, Rocha *et al.* 2016a, b, 2018, Jesus *et al.* 2016a, Gonçalves *et al.* 2016a, b), however we contributed with new taxa for the science and new occurrences for the studied area, São Paulo State or Brazil.

Aphanomyces stellatus, *Phragmosporangium uniseriatum* and *Plectospira myriandra* were the most frequent taxa in agreement with other studies in Atlantic Rainforest areas (Pires-Zottarelli *et al.* 2007, Miranda & Pires-Zottarelli 2012), together with *Achlya* sp1 (possibly a new taxon for science).

The calculated species richness and community structure evidenced a diverse microbiota, with few dominant taxa. Although a higher number of species and occurrences were obtained in the soil, there was no statistical difference among the samples. These results are in agreement with Nascimento *et al.* 2011, 2012; Jerônimo *et al.* 2015.

The novelties in relation to this study corroborate the importance of diversity studies, especially in Atlantic

Rainforest, a rich Brazilian biome understudied in terms of heterotrophic zoospore organisms. The obtained sequences enrich the genome database of these organisms.

Acknowledgements

We are indebted to Instituto Florestal, for the permission given to collect samples at “Mosaico de Unidades de Conservação Juréia-Itatins” and to Otto Hartung, for his valuable help during sampling. We also wish to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), for the financial support (Process N° 2016/11146-4), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the grant (Process N° 304091/2018-9) given to C.L.A. Pires-Zottarelli.

Conflict of interest

The authors declare no competing interests.

Authors' Contributions

Carmen Lidia Amorim Pires-Zottarelli and Ana Lucia de Jesus: collected freshwater and soil samples, baited them and identified the species using morphological and/or molecular data.

Carmen Lidia Amorim Pires-Zottarelli: wrote the first draft of the manuscript

Ana Lucia de Jesus, Débora Rodrigues da Silva Colombo, Marcela Castilho Boro and Poliana de Oliveira Ventura: help in the final version of the manuscript.

Débora Rodrigues da Silva Colombo and Poliana de Oliveira Ventura: helped us with morphological and/or molecular analysis.

Marcela Castilho Boro: preserved and included the cultures in CCIBt, as well as prepared the plates.

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Editora Associada: Elaine Malosso

Submissão: 05/08/2022

Aceito: 09/02/2023

Preprint: 01/03/2023

