Freshwater hyphomycetes in a preserved insular system from Brazil: first records at Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo State

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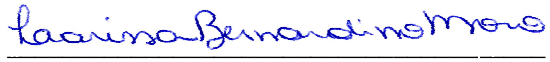

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Freshwater hyphomycetes in a preserved insular system from Brazil: first records at the Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo State, Brazil

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ABSTRACT - **(**Freshwater hyphomycetes in a preserved insular system from Brazil: first records at Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo State). To survey freshwater hyphomycetes from insular water bodies at the Parque Estadual da Ilha do Cardoso State Park, in Cananéia municipality, São Paulo State, Brazil, submerged mixed leaf litter samples were collected from six sites from June 2012 to February 2013. The samples were taken to the laboratory and incubated in Petri dishes containing sterile distilled water at room temperature (20ºC). From the 5th day on, until at least one month, fragments of the leaf litter samples were examined under the microscope, resulting in the morphological identification of 33 taxa. Among those, two are new records for the state of São Paulo and four for Brazil. Descriptions and pictures of each new record are presented.
Keywords: asexual ascomycetes, Atlantic rainforest, biodiversity

RESUMO - (Hifomicetos de água doce em sistema insular preservado do Brasil: primeiros registros no Parque Estadual da Ilha do Cardoso, município de Cananéia, Estado de São Paulo). Para o levantamento de hifomicetos de água doce em corpos de água insulares no Parque Estadual da Ilha do Cardoso, no município de Cananéia, Estado de São Paulo, Brasil, foram coletadas amostras de folhedo misto submerso de seis locais de junho de 2012 a fevereiro de 2013, levados ao laboratório e incubados em placas de Petri contendo água destilada estéril a temperatura ambiente (20ºC). A partir do 5º dia, até pelo menos um mês, fragmentos das amostras de folhas foram analisados microscopicamente, resultando na obtenção de 33 táxons, identificados por características morfológicas. Entre eles, dois são novos registros para o Estado de São Paulo e quatro são novos registros para o Brasil. Descrições e figuras de cada novo registro são apresentadas.
Palavras-chave: ascomicetes assexuais, biodiversidade, Mata Atlântica

Introduction

The term 'aquatic hyphomycetes' has not taxonomical value, but it characterizes, ecologically, a group of anamorphic fungi or asexual ascomycetes that must have, at least, a part or all of their life cycle occurring in freshwater, including also fungi exclusively originated in the aquatic environment (Chan *et al.* 2000).

As a diverse phylogenetically diverse group (Belliveau & Barlocher 2005, Duarte *et al.* 2015), aquatic anamorphic fungi are classified into four groups according to their tolerance to submergence or requirement of water for the conidiogenesis: aeroaquatic hyphomycetes, terrestrial aquatic hyphomycetes, submerged aquatic hyphomycetes and ingoldian hyphomycetes (Goh & Hyde 1996).

Aquatic anamorphs have been considered one of the most important ecological group of fungi active during the decomposition process of submerged leaf litter, as consumers or improving the palatability of the substrates by nutritional enrichment for other organisms of the trophic chain, such as shredding invertebrates (Bärlocher 1992, Cheng *et al.* 1997, Suberkropp 1992, 2003). Therefore, these fungi may play a very important role in nutrient cycling (Belliveau & Barlocher 2005).

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The importance of the aquatic anamorphs has been especially emphasized in lotic systems such as streams with well-aerated, clean, moderately turbulent waters, with its conidia dispersed in foams or associated with decaying organic substrates (Ingold 1975). Eventually, some aquatic anamorphs may be found in terrestrial environments, such as in roots of plants, including grasses and riparian ferns (Sati & Belwal 2005), also in lentic waters (Suzuki & Nimura 1961, Casper 1965), and more recently in urban, eutrophicated waters (Schoenlein-Crusius *et al.* 2009, Schoenlein-Crusius *et al.* 2014).

Several studies have expanded the knowledge of the geographical distribution of anamorphic fungi in the Brazilian Atlantic rainforest (Schoenlein-Crusius & Milanez 1989, Schoenlein-Crusius & Milanez 1990, Schoenlein-Crusius & Milanez 1998, Grandi 1998, 1999, Gusmão *et al.* 2001, Grandi & Gusmão 2002, Silva *et al.* 2019). However, among them, only Silva *et al*. (2019) included sampling points in a lentic system on an island.

The first study of the diversity of freshwater hyphomycetes in Brazilian insular areas was conducted by Moro *et al.* (2018) for the Ilhabela State Park (PEIb). In this same island, two new species of freshwater Hyphomycetes, *Polylobastipora setulosa* (Moro *et al.* 2015a) and *Campylospora brasiliensis* (Moro *et al.* 2015b) were described, as well as the first report from Brazil *Peyronelina glomerulata* PJ Fisher, J. Webster & DF Kane (Moro *et al.* 2015c).

At the Ilha do Cardoso island, there are already studies about the diversity of fungi from the following phyla: Basidiomycota (Bononi 1979a,b,c, 1984, Bononi *et al.* 1984, Bononi & Capelari 1984, Capelari 1989, Gugliotta & Capelari 1995, Gugliotta & Bononi 1999), Glomeromycota (Trufem *et al.* 1989, Trufem *et al.* 1994) and more recently, Chytridiomycota and Blastocladiomycota (Jerônimo 2014, Jerônimo *et al.* 2015). However, until now, there are no records about aquatic anamorphic fungi, being the improvement of the knowledge concerning its diversity, the main aim of the present study. The data of the measurements of some abiotic parameters are also presented for historical documentation about the island.

Materials and methods

Sampling - The State Park of Ilha do Cardoso (PEIC) is located in a homonymous island, at the southeastern coast of São Paulo State (25º03'05"-25º18'18"S and 47º53'48"-48º05'42"W). Within the total area of 22.500 hectares, several types of environments can be found: primary and secondary Atlantic rainforest, dunes, mangroves, sandbank formations, and one larger stream, the Perequê river which runs through most of the island, many small ponds, tributaries, rivers rapids, waterfalls and brooks (Secretaria de Estado do Meio Ambiente 1998).

In the Ilha do Cardoso island, the following sampling sites were visited: 1 - Perequê waterfall, 2 - Cor de Chá river, 3 - Córrego, 4 - Poço das Antas, 5 - Perequê river.

The site 1 - Perequê waterfall and the site 5 - Perequê river were visited for collecting during all field trips: in June, August and November of 2012 and February of 2013. Site 2 - Cor de Chá river presented water flow enough to be sampled only once, in February 2013, while site 3 - Córrego, was visited during the first three field trips, and site 4 - Poço das Antas, only during the last two (November 2012 and February 2013).

During the field trips on the shores and about 30 cm deep at each site, 10 leaves were selected from the submerged mixed leaf litter using tweezers. The leaves and water taken from the site were placed in previously sterile 200 mL polyethylene bottles. During the entire study, 72 samples were collected and transported to the laboratory.

At each sampling site, the temperature (ºC), pH, conductivity ($\mu S.cm^{-1}$) and dissolved oxygen (mgO_2/L) of the water was measured with and Horiba U_{10} and U_{50} equipment.

Incubation - In the laboratory, the leaf samples were coarsely fragmented individually using sterile scissors to fit loosely in sterile Petri dishes. Sterile water was added to the Petri dishes to cover sufficiently the leaf fragments (Ingold 1975). Thus, 172 Petri dishes were obtained, which were incubated at room temperature (around 20 ºC) for 4 to 8 weeks. Whenever the proliferation of bacteria was perceptible or the leaf fragments emerged, aliquots of sterile water were added to the Petri dishes or the entire water was replaced in order to keep the substrates as clean as possible.

Taxonomical identification - From the 5th day on, fragments of the leaf litter samples were analyzed under a microscope for least one month and then until no new fungi were observed. Fragments of the incubated leaf samples were mounted onto slides with a drop of sterile water and cover glasses to be analyzed under an optical microscope model Olympus BX50.

The steps of the conidial production were followed and morphological relevant features were searched to identify the taxa by specific identification keys (Ingold 1975, Marvanová 1997, Santos-Flores & Betancourt-López 1997). For documentation and *ex-situ* preservation purposes, slides were also prepared with PVLG (polyvinyl alcohol lactoglycerol) resin, deposited at the Herbarium Maria Eneida Kaufmann Fidalgo in the Instituto de Botânica, in municipality of São Paulo, São Paulo State, Brazil. Digital images were obtained with the aid of an optical microscope Leica DM LB2, equipped with a Leica DFRC 280 digital camera.

Frequencies of taxa occurrence were calculated based on Dajoz (1973) and Zak & Willig (2004), dividing the number of samples in which the species was registered by the total number of analyzed samples, multiplied by 100. The fungi were grouped in the following constancy categories - C: Constant ≥ 50%; A: Accessory ≥ 25% e < 50%; O: Occasional < 25%.

Results

Abiotic factors - The water temperature was about 18 ºC in August/2012 and 23 ºC in February/2013 in all sites (table 1). Regarding the pH of the water, the values varied from 5.58 to 8.75 at the Cachoeira do Perequê, and from 5.26 to 6.64 at the Rio Perequê. In the water of the Córrego the pH values varied from 5.48 to 7.12 and at the Poço das Antas the values varied from 6.14 to 6.28. The only measurement of the pH of Rio Cor de Chá, revealed a very low value, corresponding to 3.84, and a conductivity value of 0.394 $S.cm^{-1}$. Dissolved oxygen values remained constant among the collection sites: from 8.75 to 10.01 mgO_2/L at Perequê waterfall; 6.73 to 8.90 mgO_2/L at Perequê river; 6.50 to 7.82 mgO_2/L at Córrego; 7.50 a 10.45 mgO_2/L at Poço das Antas. Only one measurement was performed at Cor de Chá river, resulting in 10.17 mgO_2/L.

Fungal diversity - A total of 33 taxa of aquatic Hyphomycetes were observed in water bodies of the Parque Estadual da Ilha do Cardoso, with 97 occurrences (table 2). In the first sampling, 18 taxa (26 occurrences) were recorded. The 2nd sampling presented 15 taxa and 3rd sampling 14 taxa, and the numbers of occurrences were close (22 and 19, respectively). On the 4th sampling, 17 taxa (30 occurrences) were recorded.

The Perequê waterfall was the site with the highest number of unique taxa (07) and presented 40 occurrences. The 1st and 2nd samplings were those with the highest number of occurrences (11 and 14, respectively), although with lower numbers compared to the numbers of occurrence of the 3rd and 4th sampling are close (07 and 08, respectively).

The Perequê river presented 24 occurrences and two unique taxa, *Blodgettia indica* Subram. (figure 1a-b) and *Pyramidospora casuarinae* Sv. Nilsson. The 1st sampling was with the largest number of occurrences (11). Among the 2nd, 3rd and 4th samplings there was no significant variation (04, 03 and 05, respectively).

The Córrego registered 13 occurrences of aquatic hyphomycetes and the Poço das Antas 14. Córrego presented *Pyramidospora constricta* N. Singh (figure 3d) and Poço das Antas presented *Ingoldiella hamata* D.E. Shaw (figure 2d).

In the Cor de Chá river, only one sampling was carried out, totaling the record of six occurrences. Nevertheless, this location presented 04 exclusive taxa: *Dactylellina appendiculata* (Anastasiou) M. Scholler, Hagedorn & A. Rubner (figure 3b), *Isthmolongispora minima* Matsush. (figure 2f), *Pleiochaeta amazonensis* Matsush. (figure 3c), and *Subulispora longirostrata* Nawawi & Kuthub (figure 4a).

All examined taxa are new records for the Parque Estadual da Ilha do Cardoso. The following five species are described because they are new records for Brazil and one for the State of São Paulo.

Taxonomy

Dactylellina appendiculata (Anastasiou) M. Scholler, Hagedorn & A. Rubner, Sydowia 51 (1): 110 (1999).

Figure 3b

Conidiophore not observed. Conidia hyaline, spindle-shaped, 42.5-62.5 µm × 10-12.5 µm, 1-3-septed, producing 1-2 lateral appendages, hyaline, more than 87.5 µm long., 2-2.5 µm wide.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Cor de Chá river, in submerged mixed leaf litter, 26-II-2013, L.B. Moro (SP 466899).

Notes: although among the analyzed specimens the presence of curved conidia was not observed, only straight conidia, all other morphological features are in accordance with the original description.

Table 1. Some abiotic water factors measured at the sites during the sampling of submerged leaf litter in the Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo State, Brazil.

Sites/samplings	Abiotic factors			
	Temperature (°C)	pH	Conductivity (S.cm^{-1})	Dissolved Oxygen (mgO_2/L)
Perequê waterfall				
June/2012	19.3	8.75	0.044	8.75
August/2012	17.9	5.58	0.047	8.38
November/2012	20.9	8.14	0.046	8.13
February/2013	23.2	5.68	0.047	10.01
Perequê river				
June/2012	19.4	6.64	0.046	6.73
August/2012	18.6	6.44	0.049	*
November/2012	21.6	5.26	0.054	*
February/2013	23.1	6.40	0.049	8.90
Córrego				
June/2012	19.6	7.12	0.043	7.82
November/2012	21.7	5.48	0.057	*
February/2013	23.9	5.78	0.052	6.50
Poço das Antas				
August/2012	18.4	6.26	0.049	8.77
November/2012	21.4	6.14	0.052	7.50
February/2013	23.3	6.28	0.047	10.45
Cor de Chá river				
February/2013	24.0	3.84	0.394	10.17

*Measurement not available

The species was originally described by Anastasiou (1964) with the name *Dactyllela appendiculata*, with the presence of appendages. It may have curved conidia when originated from curved spore primordium or straight conidia when originated from a straight spore primordium. Later, Nawawi (1976) described a new genus *Laridospora* recombining *Dactyllela appendiculata* as *Laridospora appendiculata*, however observing only curved conidia and appendages. Rubner & Gams considered *L. appendiculata* as *Monacrosporium tentaculatum*, considering only its predatory behavior. The ecology and conidial appendages related to aquatic habitat were not taken into account, considering that *M. tentaculatum* is not an aquatic species (Rubner 1996). At last, through molecular biology, Scholler *et al.* (1999) proposed *M. tentaculatum* as synonymous with *Dactyllelina appendiculata*. This is the first record of *D. appendiculata* in Brazil.

Isthmolongispora intermedia Matsush., Microfungi of the Solomon Islands and Papua-New Guinea: 32 (1971).

Figure 2e

Conidiophore not observed. Conidial uniseriate chains connected by narrow isthmi, fusiform, 3-celled, hyaline, central cell rectangular, ends of the navicular cells, 25-33 × 2.5-3.0 µm.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Perequê river, in submerged mixed leaf litter, 19-VI-2012, L.B. Moro; Perequê waterfall, in submerged mixed leaf litter, 30-VIII-2012, GH Jerônimos; Poço das Antas, in submerged mixed leaf litter, 6-XI-2012, L.B. Moro (SP 466902).

Notes: the genus *Isthmolongispora* was described by Matsushima (1971) for the Solomon Islands and Papua New Guinea, together with *I. intermedia* and

Table 2. Hyphomycetes taxa observed in the Parque Estadual da Ilha do Cardoso, municipality of Cananéia, São Paulo State, Brazil. Frequency (%), occurrence and constancy (C: Constant, A: Accessory; O: Occasional) of species.

Taxa	colletion 1 (06/2012)			colletion 2 (08/2012)			colletion 3 (11/2012)				colletion 4 (02/2013)					Frequency (%)	Occurrences	Constancy
	Perequê waterfall	Perequê river	Córrego	Perequê waterfall	Perequê river	Poço das Antas	Perequê waterfall	Perequê river	Córrego	Poço das Antas	Perequê waterfall	Perequê river	Córrego	Poço das Antas	Cor de Chá river			
Alatospora acuminata Ingold	X			X												13.3	2	O
Anguillospora longissima (Sacc. & P. Syd.) Ingold	X	X		X											X	26.6	4	A
Anguillospora pseudolongissima Ranzoni	X			X					X							20	3	O
Blodgettia indica Subram.Fig. 1a-b												X				6.6	1	O
Camposporium anntenatum Harkn. Fig. 1c								X			X					13.3	2	O
Campylospora chaetocladia Ranzoni	X										X					13.3	2	O
Clavariopsis aquatica De Wild. Fig. 2a			X				X									13.3	2	O
Dactylellina appendiculata (Anastasiou) M. Scholler, Hagedorn & A. Rubner*. Fig. 3b															X	6.6	1	O
Dendrosporium lobatum Plakidas & Edgertonex J.L. Crane Fig. 2b				X							X	X				20	3	O
Flagellospora curvula Ingold	X	X		X				X								26.6	4	A
Flagellospora penicillioides Ingold	X															6.6	1	O
Helicomyces sp.		X														6.6		O
Helicosporium sp.						X										6.6		O
Heliscus submersus H.J. Huds. Fig. 2c				X												6.6	1	O
Ingoldiella hamata D.E. Shaw Fig. 2d										X						6.6	1	O
Isthmolongispora intermedia Matsush.*. Fig. 2e		X		X						X						20	3	O
Isthmolongispora minima Matsush.*. Fig. 2f															X	6.6	1	O
Isthmolongispora quadricellularia Matsush*. Fig. 2g		X													X	13.3	2	O
Jaculispora submersa Hudson, H.J. & Ingold, C.T. Fig. 2h				X												6.6	1	O
Lunulospora curvula Ingold Fig. 3a	X	X	X	X	X	X	X		X		X		X	X		73.3	11	C
Lunulospora cymbiformis K. Miura	X	X		X			X									26.6	4	A
Pleiochaeta amazonensis Matsush.*. Fig. 3c															X	6.6	1	O
Pyramidospora casuarinae Sv. Nilsson		X														6.6	1	O
Pyramidospora constricta N. Singh*. Fig. 3d									X							6.6	1	O
Pyramidospora robusta C.G. Moreira & I.H. Schoenlein-Crusius Fig. 3e												X	X	X		20	3	O
Scutisporus brunneus K. Ando & Tubaki Fig. 3f		X		X				X	X				X			33.3	5	A

continue

Table 2 (continuation)

Taxa	colletion 1 (06/2012)			colletion 2 (08/2012)			colletion 3 (11/2012)				colletion 4 (02/2013)					Frequency (%)	Occurrences	Constancy
	Perequê waterfall	Perequê river	Córrego	Perequê waterfall	Perequê river	Poço das Antas	Perequê waterfall	Perequê river	Córrego	Poço das Antas	Perequê waterfall	Perequê river	Córrego	Poço das Antas	Cor de Chá river			
Tetraploa aristata Berk. & Broome Fig. 4b											X					6.6	1	O
Tripospermum camelopardus Ingold, Dann & P.J. McDougall Fig. 4c		X		X	X	X	X			X	X	X	X	X		66.6	10	C
Tripospermum myrti (Lind) S. Hughes	X	X					X			X						26.6	4	A
Triscelophorus acuminatus Nawawi				X	X		X				X		X	X		40	6	A
Triscelophorus monosporus Ingold Fig. 4d	X	X	X	X	X	X	X			X	X	X	X	X		80	12	C
Number of fungal taxa at each site	11	12	3	14	4	4	7	3	4	5	8	5	6	5	6			
Number of fungal taxa in each sampling		18			15				14				17					
Number of occurrences		26			22				19				30					

*new records

I. minima. The genus is characterized by the presence of free conidiophores and conidial serials, formed by cells connected by a small isthmus. Currently, the genus is composed of nine other species. This is the first record of the species for Brazil.

Isthmolongispora minima Matsush., Microfungi of the Solomon Islands and Papua-New Guinea: 32 (1971).

Figure 2f

Conidiophore not observed. Conidial uniseriate chains connected by narrow isthmi, fusiform, hyaline, 2-celled, 10-15.7 × 2.1-4.3µm per cell, entire conidia 28.6-22.8 µm long.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Cor de Chá river, in submerged mixed leaf litter, 26-II-2013, L.B. Moro (SP 466900).

It is the first record of this species from São Paulo State.

Isthmolongispora quadricellularia Matsush. Icones Microfungorum a Matsushima lectorum: 90 (1975).

Figure 2g

Conidiophore not observed. Conidial uniseriate chains connected by narrow isthmi, solitary, hyaline, 4-celled, 54.5-60 × 3-4 µm.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Perequê river, in submerged mixed leaf litter, 19-VI-2012, L.B. Moro; Cor de Chá river, in submerged mixed leaf litter, 26-II-2013, L.B. Moro (SP 466901).

Notes: the conidia of *I. quadricellularia* are morphologically similar to those of the genus *Wiesneriomyces* Koord. The difference between these fungi is that in *I. quadricellularia* the conidiophores are free, and *Wiesneriomyces* presents sporodochium. However, *W. laurinus* (Tassi) P.M. Kirk may have 5-9 cells composing the conidia in culture medium or 7-8 cells in the natural substrate as noted by Matsushima (1975), more than 15 cells as described by Ellis (1971). Matsushima (1975) described 4 cells or rarely 5 cells for *I. quadricellularia* conidia. In the present research, although the conidiophore was not observed, the 4 cells conidia agree with the original description of Matsushima (1975) for *I. quadricellularia*, allowing the identification of the collected fungus.

I. quadricellularia is concomitantly cited for two completely different biomes in Brazil: the Atlantic Rain Forest of Ilha do Cardoso in the southeast State of São Paulo (this paper) and in the Caatinga region of the northeast state of Bahia (Fiuza & Gusmão 2014). The geographical distribution of these taxa seems to be broader than first supposed. It is the first record of the species for São Paulo State.

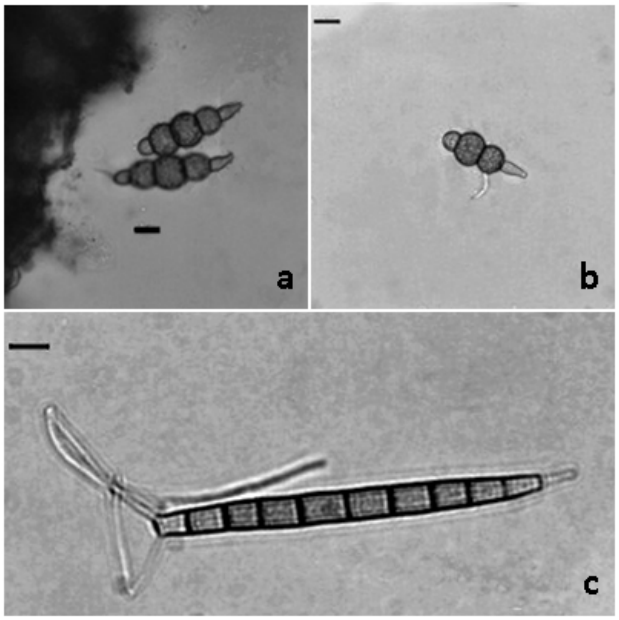

Figure 1. Mature conidia of a-b. *Blodgettia indica*. c. *Camposporium antennatum*. Scale bars = 10 µm.

Pleiochaeta amazonensis Matsush., Matsushima Mycological Memoirs 7: 61 (1993).
Figure 3c

Conidiophore not observed. Conidia solitary, brown, cylindrical-fusiform, multi-sepated, filiform apex curved, composed of a main axis of 160-185 µm 9-10 µm long and wide at the thinnest part, 17-19 septa; two side branches, thin, cylindrical, light brown, 65-110 × 2.0-2.5 µm, 7-9 septa.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Cor de Chá river, in submerged mixed leaf litter, 26-II-2013, L.B. Moro (SP 466903).

Notes: the genus *Pleiochaeta* was described by (Sacc.) S. Hughes (1951), having the species *P. setosa* as the type species. In addition to this, other five species were described. This is a dematiaceous fungi, with cylindrical-fusiform conidia with appendages (Castañeda Ruíz *et al.* 1996). This is the first report of the species for Brazil.

Pyramidospora constricta N. Singh, Transactions of the British Mycological Society 59 (2): 336 (1972).
Figure 3d

Conidiophore hyaline, single, 45-70 µm in length, bearing one terminal conidia. Conidia formed by a main axis of 30-40 µm in length with lateral branches 8-12 µm in length.

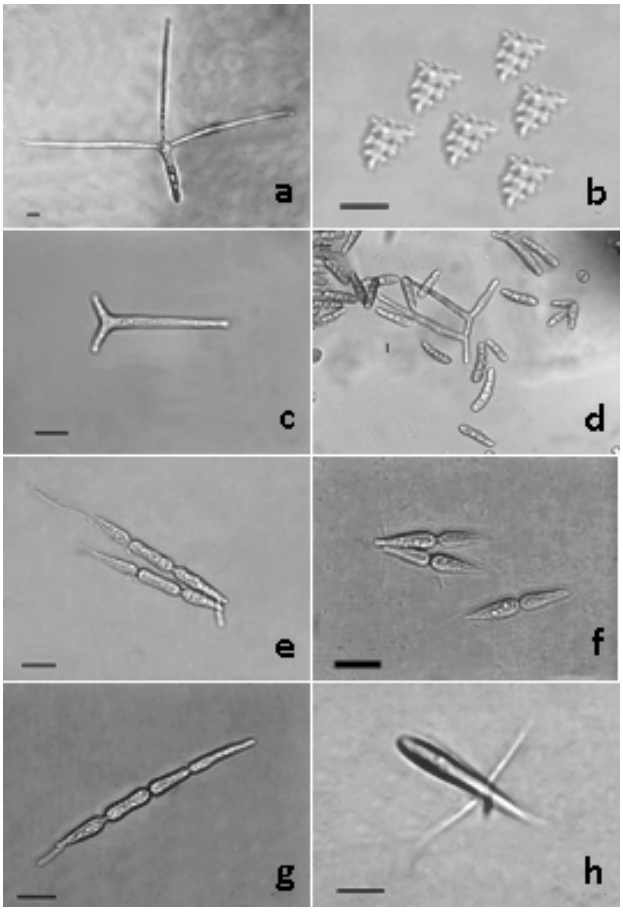

Figure 2. Mature conidium(ia) of a. *Clavariopsis aquatica*. b. *Dendrosporium lobatum*. c. *Heliscus submersus*. e. *Isthmolongispora intermedia*. f. *Isthmolongispora minima*. g. *Isthmolongispora quadricellularia.* h. *Jaculispora submersa.* Initial fase of development of d. *Ingoldiella hamata.* Scale bars = 10 µm.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, Córrego, in submerged mixed leaf litter, 6-XI-2012, L.B. Moro (SP 466898).

Notes: the genus *Pyramidospora* was described originally by Nilsson (1962), with the type species *P. casuarinae*, isolated from Venezuela, the main feature of this genus is the presence of a sizeable narrowing in the conidiophore next to where it joins to the conidium, forming a twist in this region (Singh 1972). Currently the genus consists of nine species. This is the first record of the species for Brazil.

Discussion

Abiotic factors - The water temperature followed the climatic conditions (table 1). The values of water

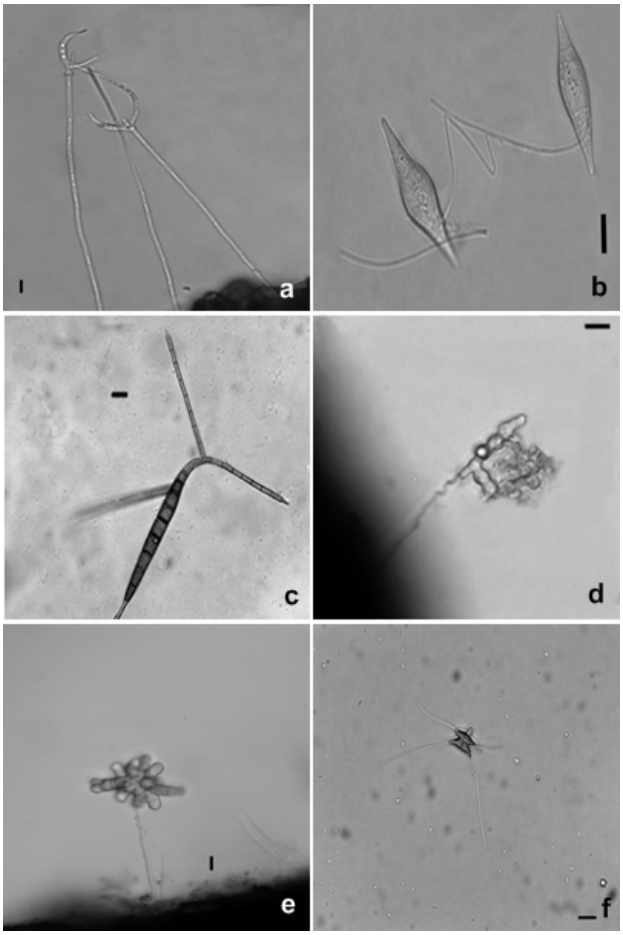

Figure 3. Conidia and conidiophore of a. *Lunulospora curvula.* e. *Pyramidospora robusta.* Mature conidia of b. *Dactyllelina appendiculata.* c. *Pleiochaeta amazonensis.* f. *Scutisporus brunneus.* Initial stage of conidia of d. *Pyramidospora constricta.* Scale bars =10 µm.

temperature during the samplings are comparable to other studies in the Atlantic rainforest biome. Schoenlein-Crusius et al. (2009) cited temperatures between 20 °C to 26 °C in the Parque Estadual das Fontes do Ipiranga (PEFI), State of São Paulo. Nascimento (2007) pointed out temperatures around 14.3 °C to 21.6 °C in the Parque Estadual da Serra da Cantareira, also in the State of São Paulo. These values are considered as ideal for the occurrence of aquatic Hyphomycetes, which are described as having an optimum temperature for growth between 15 ºC to 25 ºC (Ingold 1975).

Although there has been a high variation in pH values between sampling sites (3.84 to 8.75), the pH values were similar to those described by Nascimento (2007), around 5.8 to 6.6 and Schoenlein-Crusius *et al.* (2009), 3.36 to 9.08.

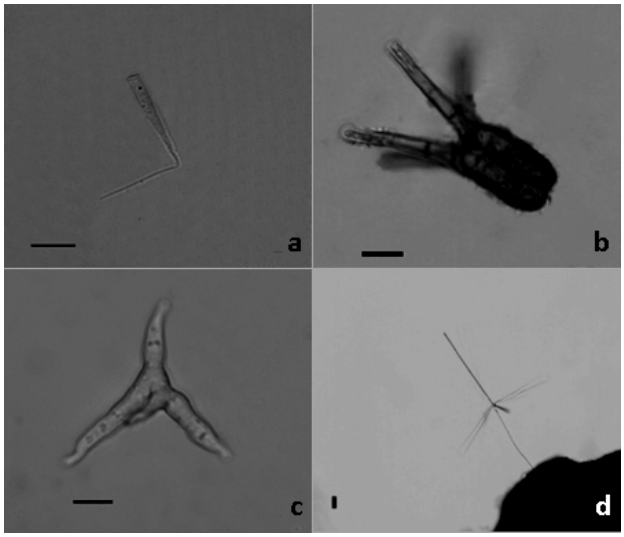

Figure 4. Mature conidia of a. *Subulispora longirostrata.* b. *Tetraploa aristata.* c. *Tripospermum camelopardus.* Conidia and conidiophores of d. *Triscelophorus monosporus.* Scale bars = 10 µm.

The conductivity, in general, can be characterized as low, ranging from a maximum of 0.057 to 0.043 $S.cm^{-1}$. The values of dissolved oxygen were expected to be high in view of the fact that most sites are running waters, with the exception of Poço das Antas, which is a pond. The values varied from 6.73 to 10.45 mgO_2/L (table 1). The values of conductivity and dissolved oxygen were larger in relation to previous studies in the Atlantic rainforest. Nascimento (2007) obtained values between 0.02 to 0.03 $\mu S.cm^{-1}$ of conductivity and 5.1 to 7.8 mgO_2/L for dissolved oxygen in the Parque Estadual da Cantareira.

The values of the abiotic parameters of freshwater the island are compatible with those usually reported for the Brazilian Atlantic Rainforest.

Fungal diversity - According to Dajoz (1973), species with a frequency of occurrence ≥ 50% are classified as constant, those with a frequency between 25 and 50% are accessories and those with a frequency < 25% as occasional, showing an interesting connection between the number of samplings and fungal taxa.

Among the total mycota, the most frequent species were *Triscelophorus monosporus* (80%), *Lunulospora curvula* (73.3%) and *Tripospermum camelopardus* (66.6%), classified as constant (table 2).

Several studies corroborate the high frequency of *T. monosporus* (Schoenlein-Crusius & Martin 1990, Schoenlein-Crusius *et al.* 1990, Schoenlein-Crusius *et al.* 1992a, Pires-Zottarelli *et al.* 1993, Schoenlein-Crusius & Martin 1998, Malosso 1999, Schoenlein-

Crusius 2002, Schoenlein-Crusius *et al.* 2014) and *L. curvula* (Schoenlein-Crusius *et al.* 1990, Schoenlein-Crusius & Milanez 1990, Schoenlein-Crusius *et al.* 1992, Malosso 1995, Schoenlein-Crusius & Milanez 1998, Malosso 1999, Schoenlein-Crusius 2002, Schoenlein-Crusius *et al.* 2014). *T. camelopardus* is less reported, being registered to the Guarapiranga dam by Malosso (1999).

A total of six taxa showed a frequency index between 26.6-40%: *Anguillospora longissima*, *Flagellospora curvula*, *Lunulospora cymbiformis*, *Scutisporus brunneus*, *Tripospermum myrti* and *Triscelophorus acuminatus*, being classified as accessory. These species were frequently reported in several studies from Brazil (Schoenlein-Crusius *et al.* 2009, Fiuza & Gusmão 2011, Fiuza & Gusmão 2013, Fiuza *et al.* 2015, Schoenlein-Crusius *et al.* 2015).

Twenty four taxa were less frequent (6.6-20%), and classified as occasional. *Blodgettia indica* has recently been isolated in leaves of *Caesalpinia echinata* and *Campomanesia phaea* submerged in a lake in Parque Municipal Alfredo Volpi (Moreira & Schoenlein-Crusius 2012). *Flagellospora penicillioides* was isolated from submerged mixed leaf litter from the Cerrado biome by Schoenlein-Crusius (2002). *Heliscus submersus* was also isolated in submerged mixed leaf litter on Monjolinho river and Jacaré river in São Carlos, SP (Malosso 1999), *Ingoldiella hamata* was cited by Schoenlein-Crusius (2002) in submerged mixed leaf litter in Itirapina, SP. *Jaculispora submerged*, was recently described by Fiuza & Gusmão (2013) in the Serra da Jibóia, a Brazilian semi-arid region. *Pyramidospora casuarinae*, was registered as the first citation to South America by Schoenlein-Crusius *et al.* (2009), *Subulispora longirostrata* was registered colonizing leaves *M. cabussu* Hoehne and *T. pulchra* Cogn. in Paranapiacaba, Santo André, SP (Gusmão *et al.* 2001, Grandi & Gusmão 2002), *Tetracladium marchalianum* was recently cited for the first time to Brazil by Schoenlein-Crusius *et al.* (2014) in submerged mixed leaf litter in Parque Municipal do Ibirapuera, SP and *Tetraploa aristata* was observed by Gruppi (2008) on mixed leaf litter samples collected in the Parque Central in Santo André, SP and by Barbosa *et al.* (2013) in submerged leaf litter in the city of Santa Terezinha, Bahia in a Brazilian semi-arid region.

The remaining taxa occurred sporadically between samplings, highlighting *Pyramidospora robusta* Moreira & Schoenlein-Crusius (2012) which was observed in submerged leaf litter collected from an urban park named Parque Alfredo Volpi in the city of São Paulo.

The results of this study confirm that the diversity of aquatic Hyphomycetes in insular environments is fairly higher than has been registered by other studies in the Brazilian Atlantic rainforest for instances. In the Parque Estadual das Fontes do Ipiranga-PEFI, an Atlantic Rainforest reminiscent located in São Paulo State, Schoenlein-Crusius *et al.* (2009), observed 24 taxa of aquatic Hyphomycetes, from submerged mixed leaf litter in 10 different sampling sites. In the northeast Atlantic Rainforest, from Monte da Pioneira, Serra da Jibóia north zone, Fiuza & Gusmão (2011) identified 10 aquatic Hyphomycetes in stream foam.

For the Ilhabela State Park, 39 species of freshwater Hyphomycetes have been observed. Among them, *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragmia triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamort and *Scutisporus brunneus* K. Ando & Tubaki were reported as new records from São Paulo State. *Isthmolongispora biramifera* Matsush., *Lateriramulosa ainflata* Matsush., *Phalangispora nawawii* Kuthub. and *Triscelophorus ponapensis* Matsush. corresponded to new records for Brazil. Fiuza *et al*. (2017) in a checklist of Ingoldian fungi in Brazil reported 85 taxa. Nineteen taxa are reported from the Amazon, 53 from the Atlantic Forest, 39 from the Caatinga and 21 from the Cerrado. Silva *et al.* (2019) reported 23 taxa of aquatic hyphomycetes associated to submerged leaf litter of tropical lotic and lentic environments in the Atlantic Forest of Northeast Brazil.

The identified taxa are all first occurrences to the Parque Estadual da Ilha do Cardoso. Besides, these results contribute to extending the knowledge concerning the occurrence and distribution of aquatic hyphomycetes in areas of the Atlantic Rainforest of São Paulo State and in Brazil, introducing studies of this group in insular areas.

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Literature cited

Anastasiou, C.J. 1964. Some aquatic fungi imperfect from Hawaii. Pacific **Science** 18, 202-206.

Barbosa, F.R., Raja, H.A., Shearer, C.A. & Gusmão, L.F.P. 2013. Some Freshwater Fungi from the Brazilian Semi-Arid Region, Including Two New Species of Hyphomycetes. Cryptogamie, Mycologie 34: 243-258.

Bärlocher, F. 1992. Research on aquatic hyphomycetes: historical background and overview. *In*: F. Bärlocher (ed.). The ecology of aquatic Hyphomycetes, Springer Verlag, Berlin, pp. 1-15.

Belliveau, M. & Bärlocher, F. 2005. Molecular evidence confirms multiple origin of aquatic Hyphomycetes. Mycological Research 109: 1407-1417.

Bononi, V.L., Guzmán, G. & Capelari, M. 1984. Basidiomicetos do Parque Estadual da Ilha do Cardoso V: Gasteromicetos. Richia 11: 91-97.

Bononi, V.L. & Capelari, M. 1984. Basidiomicetos do Parque Estadual da Ilha do Cardoso: Tremellales. Richia 11: 109-114.

Bononi, V.L. 1979a. Basidiomicetos do Parque Estadual da Ilha do Cardoso: I. Espécies hidnóides. Rickia 8: 63-74.

Bononi, V.L. 1979b. Basidiomicetos do Parque Estadual da Ilha do Cardoso: II. Hymenochaetaceae. Rickia 8: 85-99.

Bononi, V.L. 1979c. Basidiomicetos do Parque Estadual da Ilha do Cardoso: III. Espécies clavarióides, teleforóides e estereóides. Rickia 8: 105-121.

Bononi, V.L. 1984. Basidiomicetos do Parque Estadual da Ilha do Cardoso. IV. Adições às famílias Hymenochaetaceae, Stereaceae e Thelephoraceae. Rickia 11: 43-52.

Capelari, M. 1989. Agaricales do Parque Estadual da Ilha do Cardoso (exceto Tricholomataceae). Dissertação de Mestrado, Universidade de São Paulo, São Paulo.

Casper, S.J. 1965. Hyphomyzeten-Studien I. Die Süsswasser Hyphomyzeten des Stechlinsee-Gebietes. Limnologica 3: 257-270.

Castañeda-Ruíz, R.F. 1986. Fungi Cubense. Instituto de Investigaciones Fundamentales em Agricultura Tropical, Havana.

Castañeda-Ruíz, R.F., Guarro, J. & Cano, J. 1996. Notes on conidial fungi. IV. *Hyalopleiochaeta* anam. gen. nov. and some notes on *Pleiochaeta*. Mycotaxon 57: 457-462.

Cheng, Z.L., Andre, P. & Chiang, C. 1997. Hyphomycetes and macroinvertebrates colonizing leaf litter in two Belgian streams with contrasting water quality. Limnetica 13: 57-63.

Silva, G.V.R, Castañeda-Ruiz, R.F. & Malosso, E. 2019. Comparison of aquatic hyphomycetes communities between lotic and lentic environments in the Atlantic rain forest of Pernambuco, Northeast Brazil. Fungal Biology 123: 660-668. Available in https://doi.org/10.1016/j.funbio.2019.05.013 (access in 15-10-2019).

Dajoz, R. 1973. Ecologia geral. Vozes, São Paulo.

Duarte, S., Batista, D., Barlocher, F., Cássio, F. & Pascoal, C. 2015. Some new DNA barcodes of aquatic hyphomycetes species. Mycoscience 56: 102-108.

Ellis, M.B. 1971. Dematiaceous Huphomycetes. Kew, England: Commonwealth Mycological Institute.

Fiuza, P.O. & Gusmão, L.F.P. 2013. Ingoldian fungi from the semi-arid Caatinga biome of Brazil. Mycosphere 4: 1133-1150.

Fiuza, P.O. & Gusmão, L.F.P. 2011. Hyphomycetes aquáticos na Serra da Jibóia, Bahia, Brasil. *In*: Anais do XV SEMIC, Ciência, Saúde e Esporte-Seminário da Iniciação Científica da Universidade Estadual de Feira de Santana, pp.158-161.

Fiuza, P.O. & Gusmão, L.F.P. 2014. Hifomicetos aquáticos associados a folhas submersas de *Calophyllum brasiliense* Cambess na Chapada Diamantina, Bahia, Brasil. *In*: A.E.F. Molano (org.). Anais do VIII Congreso Latinoamericano de Micología. Revista Actualidades Biológicas 36: 285.

Fiuza, P.O., Ottoni-Boldrini, B.M.P., Monteiro, J.S., Catena, N.R., Hamada, N. & Gusmão, L.F.P. 2015. First records of Ingoldian fungi from the Brazilian Amazon. Brazilian Journal of Botany 38: 615-621.

Goh, T.K. & Hyde, K.D. 1996. Biodiversity of freshwater fungi. Journal of Industrial Microbiology 17: 328-345.

Grandi, R.A.P. 1998. Hyphomycetes decompositores do folhedo de *Alchornea triplinervia* (Spreng.) Müll. Arg. Hoehnea 25: 133-148.

Grandi, R.A.P. & Gusmão, L.F.P. 2002. Hyphomycetes decompositores do folhedo de *Tibouchina pulchra* Cogn. Revista Brasileira de Botânica 25: 79-87.

Grandi, R.A.P. 1999. Hifomicetos decompositores do folhedo de *Euterpe edulis* Mart. Hoehnea 26: 87-101.

Gruppi, V. 2008. Diversidade de fungos microscópicos em folhedo submerso de lagos do Parque Central no município de Santo André, SP, Brasil. Monografia, Universidade Metodista, São Bernardo.

Gugliotta, A.M. & Bononi, V.L.R. 1999. Polyporaceae do Parque Estadual da Ilha do Cardoso, São Paulo, Brasil. Boletim do Instituto de Botânica, n. 12.

Gugliotta, A. & Capelari, M. 1995. Polyporaceae from Ilha do Cardoso, SP, Brazil. Mycotaxon 56: 107-113.

Gusmão, L.F.P., Grandi, R.A.P. & Milanez, A.I. 2001. Hyphomycetes from leaf litter of *Miconia cabussu* in the Brazilian Atlantic Rain forest. Mycotaxon 79: 201-213.

Ingold, C.T. 1975. An Illustrated Guide to Aquatic and Water-borne Hyphomycetes (Fungi Imperfecti) with notes on their Biology. Freshwater Biological Association, Amblesidep, pp. 1-96.

Jerônimo, G.H. 2014. Avaliação da diversidade de Blastocladiomycota e Chytridiomycota do Parque Estadual da Ilha do Cardoso (PEIC), Cananéia, Estado de São Paulo, Brasil. Dissertação de Mestrado, Instituto de Botânica, São Paulo.

Jerônimo, G.H., Jesus, A.L., Marano, A.V., James, T.Y., Souza, J.I., Rocha, S.C.O. & Pires-Zottarelli, C.L.A. 2015. Diversidade de Blastocladiomycota e Chytridiomycota do Parque Estadual da Ilha do Cardoso, Cananéia, SP, Brasil. Hoehnea 42: 135-163.

Kuthubutheen, A.J & Nawawi, A. 1988. A new species of Wiesneriomyces (Hyphomycetes) from submerged decaying leaves. Transactions of the British Mycological Society 90: 619-625.

Malosso, E. 1999. Hyphomycetes em ambientes aquáticos lótico e lêntico - ocorrência e biomassa. Dissertação de Mestrado, Universidade Federal de São Carlos, São Carlos.

Malosso, E. 1995. Ocorrência de Hyphomycetes (FungiImperfecti) e Fungos Zoospóricos em Ambientes Aquáticos (Rio do Monjolinho, São Carlos, SP). Tese de Doutorado, Universidade Federal de São Carlos, São Carlos.

Marvanová, L. 1997. Freshwater hyphomycetes: a survey with remarks on tropical taxa. *In*: K.K. Janardhanan, C. Rajendran, K. Natarajan & D.L. Hawksworth (eds.). Tropical mycology. Science Publishers, Enfield, pp. 169-226.

Matsushima, T. 1975. *Icones Microfungorum a Matsushima Lectorum*. Matsushima, Kobe.

Moreira, C.G. & Schoenlein-Crusius, I.H. 2012. Nova espécie e novos registros para o Brasil de hifomicetos em folhedo submerso coletados no Parque Municipal Alfredo Volpi, São Paulo, SP, Brasil. Hoehnea 39: 521-527.

Moro, L.B., Delgado, G. & Schoenlein-Crusius, I.H. 2018. Freshwater hyphomycetes from Ilhabela State Park, Brazil. Current Research in Environmental & Applied Mycology 8: 204-216.

Moro, L.B., Delgado, G. & Schoenlein-Crusius, I.H. 2015a. *Polylobatispora setulosa*, a new freshwater Hyphomycete from Ilhabela, Sao Paulo state, Brazil. Mycosphere 6: 13-18.

Moro, L.B., Schoenlein-Crusius, I.H., Jesus, A.L. & Pires-Zottarelli, C.L.A. 2015b. *Campylospora brasiliensis*, a new species of freshwater fungi from Brazil. Phytotaxa 208: 287-295.

Moro, L.B., Delgado, G. & Schoenlein-Crusius, I.H. 2015c. *Clathrosporium retortum*, a novel aeroaquatic fungus in the Sordariomycetidae (Ascomycota) from Brazil. Phytotaxa 239: 17-29.

Nascimento, C.A. 2007. Diversidade de Chytridiomycota do Parque Estadual da serra da Cantareira, Estado de São Paulo. Dissertação de Mestrado, Instituto de Botânica, São Paulo.

Nawawi, A. 1976. A new genus of Hyphomycetes. Transactions of the British Mycological Society 66: 344-347.

Nilsson, S. 1964. Freshwater Hyphomycetes. Symbolae Botanicae Upsalienses 18: 1-130.

Pires-Zottarelli, C.L., Schoenlein-Crusius, I.H. & Milanez, A.I. 1993. Quantitative estimation of zoosporic fungi and aquatic Hyphomycetes on leaves submerged in a stream in the Atlantic rainforest, in the state of São Paulo, Brazil. Brazilian Journal of Microbiology 24: 192-197.

Rubner, A. 1996. Revision of predacious Hyphomycetes in the *Dactylella-Monacrosporium* complex. Studies in Mycology 39: 1-134.

Santos-Flores, C.J. & Betancourt-Lopes, C. 1997. Aquatic and water-borne hyphomycetes (Deuteromycotina) in streams of Puerto Rico (including records from other Neotropical locations). College of Arts and Sciences, University of Puerto Rico, Mayaguez.

Sati, S.C. & Belwal, M. 2005. Aquatic hyphomycetes as endophytes of riparian plant roots. Mycologia 97: 45-49.

Schoenlein-Crusius, I.H., Moreira, C.G. & Bicudo, D.C. 2009. Aquatic Hyphomycetes in the Parque Estadual das Fontes do Ipiranga - PEFI, São Paulo, Brazil. Revista Brasileira de Botânica 32: 411-426.

Schoenlein-Crusius, I.H. & Milanez, A.I. 1990. Hyphomycetes aquáticos no Estado de São Paulo, Brasil. Revista Brasileira de Botânica 13: 61-68.

Schoenlein-Crusius, I.H. & Milanez, A.I. 1998. Fungal succession on leaves of *Alchornea triplinervia* (Spreng.) M. Arg. Submerged in a stream of an Atlantic Rainforest in the State of São Paulo, Brazil. Revista Brasileira de Botânica 21: 253-259.

Schoenlein-Crusius, I.H. 2002. Aquatic Hyphomycetes from cerrado regions in the state of São Paulo, Brazil. Mycotaxon 81: 457-462.

Schoenlein-Crusius, I.H., Moreira, C.G., Takahashi, J.P. & Gomes, E.P.C. 2014. Riqueza dos fungos ingoldianos e aquáticos facultativos no Parque Municipal do Ibirapuera, São Paulo, SP, Brasil. Hoehnea 41: 61-76.

Schoenlein-Crusius, I.H., Pires-Zottarelli, C.L.A. & Milanez, A.I. 1992. Aquatic fungi in leaves submerged in a stream in the Atlantic Rainforest. Revista de Microbiologia 23: 167-171.

Schoenlein-Crusius, I.H., Moreira, C.G. & Gomes, E.P.C. 2015. Riqueza dos fungos ingoldianos e dos fungos aquáticos facultativos do Parque Municipal da Aclimação, São Paulo, SP, Brasil. Hoehnea 42: 239-251.

Schoenlein-Crusius, I.H., Pires-Zottarelli, C.L.A. & Milanez, A.I. 1990. Sucessão fúngica em folhas de *Quercus robur* L. (Carvalho) submersas em um lago situado no município de Itapecerica da Serra, SP. Revista de Microbiologia 21: 61-67.

Scholler, M., Hagedorn, G. & Rubner, A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. *S*ydowia 51: 89-113.

Secretaria do Meio Ambiente. 1998. Instituto Florestal. Plano de gestão ambiental – Fase 1 do Parque Estadual da Ilha do Cardoso. São Paulo, pp. 1-47.

Singh, N. 1972. *Pyramidospor aconstricta* sp. nov., a new aquatic Hyphomycete. Transactions of the British Mycological Society 59: 336-339.

Suberkropp, K. 2003. Methods for examining interactions between freshwater fungi and macroinvertebrates. Fungal Diversity Research Series 10: 159-171.

Suberkropp, K. 1992. Interactions with invertebrates. *In*: F. Barlocher (ed.). The Ecology of Aquatic Hyphomycetes, Springer-Verlag, Berlin, pp. 118-134.

Suetrong, S., Rungjindamai, N., Sommai, S., Rung-Areerate, P., Sommrithipol, S. & Jones, E.B.G. 2014. *Wiesneriomyces* a new lineage of Dothideomycetes (Ascomycota) basal to Tubeufiales. Phytotaxa 176: 283-297.

Sugiyama, M. & Mantovani, W. 1994. Fitossociologia de um trecho de mata de restinga na Ilha do Cardoso, SP. *In*: Anais do III Simpósio de ecossistemas da Costa Brasileira. ACIESP, Serra Negra, SP, pp. 49-57.

Suzuki, S. & Nimura, H. 1961. Relation between the distribution of aquatic Hyphomycetes in Japanese lakes and lake types. Botanical Magazine 74: 51-55.

Trufem, S.F.B., Malatinszky, S.M.M. & Otomo, H.S. 1994. Fungos micorrízicos arbusculares em rizosferas de plantas do litoral arenoso do Parque Estadual da Ilha do Cardoso, SP, Brasil: 2. Acta Botânica Brasílica 8: 219-229.

Trufem, S.F.B., Otomo, H.S. & Malatinszky, S.M.M. 1989. Fungos micorrízicosvesículo-arbusculares em rizoseras de plantas em dunas do Parque Estadual da Ilha do Cardoso, São Paulo, brasil. (1) Taxonomia. Acta Botânica Brasílica 3 supl. 141, pp. 141-152.

Zak, J.C. & Willig, M.R. 2004. Fungal biodiversity patterns. *In*: G. Mueller & G. Bills (eds.). Measuring and Monitoring Biological Diversity: Standard Methods for Fungi. Smithsonian Institution Press, Washington, D.C., pp. 59-75.

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