Freshwater hyphomycetes from Ilhabela State Park, Brazil

Moro LB¹, Delgado G² and Schoenlein-Crusius IH¹

¹ Instituto de Botânica, Núcleo de Pesquisa em Micologia, Av. Miguel Stéfano, 3687, 04301-902 São Paulo, SP, Brazil.
² EMLab P&K Houston, Houston, TX 77040 U.S.A.


Abstract

During the period of June, 2012 to May, 2013, water and submerged mixed leaf litter samples were collected from 22 waterfalls and rivers at Ilhabela State Park, municipality of Ilhabela, São Paulo State, Brazil, to survey the diversity of freshwater hyphomycetes. Thirty-nine species were collected for the first time from the park after incubating samples in Petri-dishes containing pond and sterile distilled water. *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragmia triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamort and *Scutisporus brunneus* K. Ando & Tubaki are recorded for the first time for São Paulo State and *Isthmolongispora biramifera* Matsush., *Lateriramulosa a-inflata* Matsush., *Phalangispora nawawii* Kuthub. and *Triscelophorus ponapensis* Matsush. are new records from Brazil. Descriptions, comments and illustrations are presented for each species.

Key words – Island environments – morphology – submerged substrate – taxonomy

Introduction

Aquatic hyphomycetes are asexual fungi characterized by producing distinctive sigmoid or multiradiate conidia which are capable of growing abundantly on deciduous leaves decaying in streams (Ingold 1975, Webster & Davey 1984, Webster 1987, Bärlocher 1992, Kirk et al. 2008, Sridhar 2009). Although they are adapted to running waters by their uncommon conidial shape, which facilitates dispersal as well as adherence to plant substrata, they have been reported to occur in diverse environments such as lentic freshwaters, brackish and marine environments, terrestrial niches, as endophytes or in urban, eutrophicated waters (Baldy et al. 2002, Murat et al. 2005, Schoenlein-Crusius et al. 2009, Elena et al. 2010, Sudheep & Sridhar 2010, Rama et al. 2014, Schoenlein-Crusius et al. 2014, Kalenitchenko et al. 2015, Chauvet et al. 2016, Schoenlein-Crusius et al. 2016). They are also responsible for high rates of decomposition of organic matter acting in biochemical transformations in the ecosystems (Remacle 1981). These fungi are related to the increase of palatability of substrates for detritivores promoting the cycling of nutrients or serving of food through the production of biomass (Christensen 1989, Kendrick 1992, Alexopoulos et al. 1996, Moore-Landecker 1996). Several studies are based on morphological differences of the conidia e.g. Ingold 1975, Webster & Descals 1981, Marvanová 1997, Santos-Flores & Betancourt-López 1997, Gulis et al. 2005, Fiuza et al. 2015. The aquatic hyphomycetes are cosmopolitan but most species have been found in cold and temperate climate regions which are still best known for their diversity (Ranzoni 1979). The low diversity in tropical and subtropical areas may be related to
ecological and methodological issues (Graça et al. 2016).

Studies on freshwater hyphomycetes in Brazil began in the eighties of the last century with emphasis made on the South-East region, specifically at the state of São Paulo including the Atlantic rainforest and coastal plateau (Schoenlein-Crusius & Milanez 1989, Schoenlein-Crusius & Milanez 1990), the “Cerrado” (Schoenlein-Crusius 2002) and in urban waters (Moreira & Schoenlein-Crusius 2012, Schoenlein-Crusius et al. 2014, Schoenlein-Crusius et al. 2016). More recently, surveys have been conducted in the North-East region of the “Caatinga” biome (Fiuza & Gusmão 2013, Barbosa et al. 2013) as well as the Amazon region (Gusmão & Monteiro 2013, Fiuza et al. 2015). A recent revision of the Brazilian Ingoldian fungi reported 85 fungal species, 19 from the Amazon, 53 from the Atlantic rainforest, 39 from the Caatinga and 21 from the Cerrado biome (Fiuza et al. 2017).

Insular systems located along the eastern coast of Brazil and occupied by preserved Atlantic forest and lotic environments are promising areas for studies of aquatic hyphomycetes. The aim of this research is to survey the aquatic hyphomycetes occurring in Brazilian insular environments for the first time and, additionally, to expand the present knowledge of the diversity of these fungi in the Brazilian Atlantic rainforest.

**Materials & Methods**

**Study area**

The São Sebastião Island is located in the municipality of Ilhabela with approximately 80% of its area belonging to the Ilhabela State Park (46° 23' 28"S 45° 21' 20"W) The park contains important remnants of Atlantic rainforest with approximately 27.025 ha and is under the administration of the Instituto Florestal of São Paulo (Parque Estadual de Ilhabela 2013).

**Sampling**

Submerged mixed leaf litter samples were collected in 22 water bodies from June, 2012 to May, 2013. Each sample was composed of about ten leaves of submerged mixed leaf litter with water taken from each site collection. They were placed in 200 ml polypropylene flasks totalizing 87 samples/four field trip. Some abiotic factors such as temperature, pH, conductivity and dissolved oxygen were measured at each site with a multi-parameter probes Horiba® U10 and U51. Once back in the laboratory, the leaves were transferred to sterile Petri dishes with sterile water (Ingold 1975) and incubated at room temperature of approximately 20°C.

**Identification and documentation**

Slides for microscopical observation were prepared after the fifth day of incubation with aliquots of the leaves. They were observed using an Olympus BX50 optical microscope for taxa identification with support of specific literature (Ingold 1975, Marvanová 1997, Santos-Flowers & Betancourt-López 1997). Photographs were taken using a Leica DM LB2 optical microscope coupled with a camera Leica DFC 280. Permanent slides were prepared with PVLG-resin polyvinyl alcohol in lactoglicerol (Morton et al. 1993) and kept in the collection of the Herbarium "Maria Eneyda P. Kauffmann Fidalgo" (Herbarium SP) of the Instituto de Botânica.

**Results**

**Abiotic factors**

The water temperature varied according to the season and was higher during the summer of December, 2012 (23.1–24.2°C). The pH ranged from 4.41 (December, 2012) to 8.03 (June, 2012). The low values of electrical conductivity (0.024 to 0.077µS.cm⁻¹) are compatible with those present in well preserved areas (Table 1). The level of dissolved oxygen in the water proved to be high (8.85 to 12.87mgO₂/L) and probably due to turbulence. The latter abiotic factor is considered the
most important for aquatic hyphomycetes once well aerated waters may favor the growth and sporulation of this group of fungi (Nilsson 1964, Webster & Towfik 1972).

Table 1: Variation (maximum-minimum) of abiotic factors during 4 field trips collections at Ilhabela State Park.

<table>
<thead>
<tr>
<th></th>
<th>Field trip 1 06/2012</th>
<th>Field trip 2 09/2012</th>
<th>Field trip 3 12/2012</th>
<th>Field trip 4 05/2013</th>
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</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>17.9–20</td>
<td>16.8–18.8</td>
<td>23.1–24.2</td>
<td>18.8–20.6</td>
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<tr>
<td>pH</td>
<td>6.85–8.03</td>
<td>4.98–5.95</td>
<td>4.41–5.67</td>
<td>6.06–5.67</td>
</tr>
<tr>
<td>Conductivity (μS.cm⁻¹)</td>
<td>0.027–0.040</td>
<td>0.042–0.077</td>
<td>0.027–0.054</td>
<td>0.024–0.036</td>
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<tr>
<td>Dissolved oxygen (mgO₂/L⁻¹)</td>
<td>11.93–12.87</td>
<td>9.01–10.27</td>
<td>*</td>
<td>8.85–9.92</td>
</tr>
</tbody>
</table>

*It was not possible to measure the dissolved oxygen values in this collection.

Fungal diversity

Thirty nine taxa of aquatic hyphomycetes were identified (Table 2). Among them, *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragmia triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamort and *Scutisporus brunneus* K. Ando & Tubaki are reported for the first time from São Paulo State. *Isthmolongispora biramifera* Matsush., *Lateriramulosa a-inflata* Matsush., *Phalangispora nawawii* Kuthub. and *Triscelophorus ponapensis* Matsush. are new records for Brazil.

Taxonomy


Conidiophores macronematous, solitary, erect, simple, cylindrical, septate, straight or slightly flexuous, brown to dark brown, 30–35 μm long. Conidiogenous cells monoblastic, integrated, terminal, cylindrical. Conidia solitary, cylindrical, 9–12-septate, smooth or rarely verruculose, apex rounded, truncated at base, subhyaline to light brown, 75–90 × 9–10 μm; basal cells truncated, fuscus, 3–4 × 6–7 μm; apical cells rounded and paler, 4.5–7 × 5–6 μm, with 4–5 appendages, 0-septate, hyaline to subhyaline, 37–80 μm long.


Notes – Nawawi & Kuthubutheen (1988) first described *Camposporidium* from Malaysia with *C. cristatum* as the type species. Conidiophores in the type specimen may reach up to 245 μm in length, but they were shorter, however, in the present specimen. *Camposporidium cristatum* was first reported in Brazil from the semi-arid region of the “Caatinga” Biome (Barbosa & Gusmão 2011), and now for the first time from the Atlantic Rainforest biome and São Paulo State.

*Chaetendophragmia triangularis* Matsush., Microfungi of the Solomon Islands and Papua-New Guinea (Osaka): 12 1971. Fig. 3

Conidiophores not observed. Conidia triangular, light brown, smooth, 50–62.5 μm long, 7.5 μm wide at base, 5-septate, slightly constricted at the septa, with visible scars at the base, 2–3 lateral appendages, hyaline, smooth, 25–37.5 μm long.


Notes – The genus *Chaetendophragmia* was first described by Matsushima (1971) from decaying leaves of *Castanopsis* sp. in Papua New Guinea, with *C. triangularis* as the type species. *Chaetendophragmia triangularis* is morphologically similar to *C. britannica* P.M. Kirk but differs in producing larger conidia constricted at the septa with the second and third proximal cells light brown while in *C. britannica* they are golden brown. In Brazil, *C. triangularis* was first identified
on leaves of *Clusia melchiorii* Gleason in a fragment of Atlantic rainforest at Serra da Jibóia, state of Bahia, (Barbosa et al. 2009). This is the first record for the state of São Paulo.

**Table 2** Species of freshwater hyphomycetes collected at Ilhabela State Park, Ilhabela, São Paulo State.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Field trip 1</th>
<th>Field trip 2</th>
<th>Field trip 3</th>
<th>Field trip 4</th>
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<td>(27/06/2012)</td>
<td>(04/09/2012)</td>
<td>(06/12/2012)</td>
<td>(14–15/05/2013)</td>
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<tr>
<td>Acumispora verruculosa Heredia, R.F. Cataheda &amp; R.M. Arias</td>
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<tr>
<td>Alatospora acuminata Ingold</td>
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<td>Anguillospora crassa Ingold</td>
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<td>Anguillospora filiforme Greath.</td>
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<td>Anguillospora longissima (Sacc. &amp; P. Sud.) Ingold</td>
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<tr>
<td>Anguillospora pseudolongissima Ranzoni</td>
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<td>Articulospora tetracladia Ingold</td>
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<tr>
<td>Chaetendrophragmia triangularis Matsush.</td>
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<tr>
<td>Camposporidium cristatum Nawawi &amp; Kuthub.</td>
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<td>Camposporium antennatum Harkn.</td>
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<td>Camposporium pellucidum (Grove) S. Hughes</td>
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<td>Campylospora chaetocladia Ranzoni</td>
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<tr>
<td>Campylospora brasiliensis L.B. Moro &amp; I.H. Shoelenlein-Crusius</td>
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<td>Campylospora filicladia Nawawi</td>
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<td>Campylospora parvula Kazuha</td>
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<tr>
<td>Centrospora aquatica De Wild.</td>
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<tr>
<td>Clavatospora tentaculata Sv. Nilsson</td>
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<td>Dendrosporum lobatum Plakidas &amp; Edgerton</td>
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<td>Flabellulospora acuminata Descals</td>
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<tr>
<td>Flagellulospora curvula Ingold</td>
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<tr>
<td>Flagellulospora penicilloides Ingold</td>
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<tr>
<td>Taxa (continuation)</td>
<td>Field trip 1 (27/06/2012)</td>
<td>Field trip 2 (04/09/2012)</td>
<td>Field trip 3 (06/12/2012)</td>
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<td>Heliscus submersus H.J. Huds.</td>
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<td>Isthmolongispora biramifera Matsush.</td>
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<td>Isthmolongispora quadricelularia Matsush.</td>
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<td>Lateriramulosa a-inflata Matsush.</td>
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<td>Lumulospora curvula Ingold</td>
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<tr>
<td>Lumulospora cymbiformis K. Miura</td>
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<tr>
<td>Phalangispora nawawii Kathub.</td>
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<tr>
<td>Physalidella elegans (Mosca) Rulamort</td>
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<tr>
<td>Polylobaapsora setulosa L.B. Moro, G. Delgado &amp; I.H. Schoenlein-Crusius</td>
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<tr>
<td>Scutisporus brunneus K. Ando &amp; Tubaki</td>
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<td>Tetractidiun marchalianum De Wild.</td>
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<td>Tetractidiun setigerum (Grove) Ingold</td>
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<td>Tricladiun gracile Ingold</td>
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<tr>
<td>Tricopsernum camelpodarum Ingold, Dann &amp; P.J. McDougall</td>
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<tr>
<td>Tricopsernum myrti (Lind) S. Hughes</td>
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<tr>
<td>Triscelophorus acuminatus Nawawi</td>
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<tr>
<td>Triscelophorus monosporus Ingold</td>
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<td>x x x x x x</td>
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<tr>
<td>Triscelophorus ponapenses Matsush.</td>
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</tbody>
</table>

Conidiophores not observed. Conidia composed of one central, fusiform, 3-celled axis and two hyaline, lateral branches totaling 7-cells connected by narrow isthmi; central axis 28.2–31.85 μm long, central cell 2.7–3.6 μm wide; lateral branches 14.5–19.1 μm long, 2-cells in each branch.


Notes – The length measurements of the central and lateral branches of the type specimen of *I. biramifera* described by Matsushima (1993) are smaller than those observed in this study. However, the remaining characteristics point out to this species considering the singular conidial
morphology. Matsushima (1971) described the genus *Isthmolongispora* on unidentified leaf litter collected in Papua New Guinea based on *I. intermedia* Matsush. The genus is characterized by possessing bi or multicellular conidia which each cell separated by small isthmi. This is the first record of this species for Brazil.

**Lateriramulosa a-inflata** Matsush., Icon. microfung. Matsush. lect. (Kobe): 92 1975.  
Fig. 5
Conidiophores not observed. Conidia hyaline composed of short branches: (1) a cylindrical shaft in the middle of two branches, 6–9 μm × 1.2–1.5 μm; (2) a branch in the middle of the cylindrical shaft, (3) an obclavate branch both of them 9–11 × 2–2.5 μm and (4) an angularly bent branch 7–11 × 2–2.5 μm.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião Island, Ilhabela State Park, “Gato” waterfall, on submerged mixed leaf litter, 14 May 2013, L.B. Moro (SP466895).

Notes – The genus *Lateriramulosa* was described by Matsushima (1971) from Papua New Guinea and it is characterized by having hyaline conidia formed by 3 main branches and a central cylindrical axis. The genus currently includes four more species, namely *L. bi-inflata* Matsush., *L. minitriangularia* Matsush., *L. quadriradiata* Miura & Okano and *L. uni-inflata* Matsush. as the type species, differing in conidial morphology. This is the first record of this taxon for Brazil.

Fig. 6
Conidiophores not observed. Conidia yellowish brown, becoming brownish-green in mass, branched in a chain of 13–16 cells connected by narrow isthmi, with a main axis and 2–3 laterals branches, 6–8 cells in the main axis, 2–4 cells in the lateral branches, 65–90 μm from base to apex, lateral branches 45–70 μm long; basal cells conical, 7–11 × 1.5–2 μm, cells along conidial chains cylindrical, 10–12 × 1.75–2 μm.


Notes – The genus *Phalangispora* was first described by Nawawi & Webster (1982), with *P. constricta* as the type species. In addition to the type species and *P. nawawii* Kuthub., *P. bharathensis* T.S.K. Prasad & Bhat was later described (Kuthubutheen 1987, Keshava Prasad & Bhat 2002). In the Neotropics Smits et al. (2007) reported *P. nawawi* on submerged leaf litter collected on rivers in Venezuela. This is the first record of this species for Brazil.

Fig. 7
Conidiophores not observed. Conidia single, smooth, consisting of 3-celled, central cell dark brown, obovoid or ellipsoid, 7–11 × 6–7 μm and two lateral hemispherical cells, sub-hyaline, 3–4 × 3–4 μm, disposed on the main cell as two flaps.


Notes – The conidial central cell of the specimen of *P. elegans* described by Castro et al. (2012) measured 6–7 × 5–6 μm and therefore it is smaller than the specimen studied here. However, measurements agree well with Ellis (1971) who described conidia ranging from 7–11 × 6–7 μm. *Physalidiella* was described by Rulamort (1990) with *P. elegans* as the type species and *P. matsushimae* (R.F. Castañeda & W.B. Kendr) M. Morelet transferred to the genus. Matsushima (1993) described *P. elegans* in the Neotropics on leaves of *Inga* sp. from Peru. The fungus has been reported from Brazil in Pará State, the Amazon region (Castro et al. 2012) and on decaying leafy substrates in Serra da Jibóia, state of Bahia (Fiuza et al. 2010). This is the first record for the State of São Paulo.

Conidiophores not observed. Conidia consisting of four cells, septa cross-shaped, hyaline to subhyaline, 7–10(–11) × (6–)8–9 µm, appendages filiform, projected from each cell of the body, smooth, hyaline, 0-septate, 20–37 × 0.5–0.7 µm; basal cell cuneiform, 4–7 × 2–3 µm.


Notes – The genus Scutisporus was first described by Ando & Tubaki (1985) with S. brunneus as the type species and has remained monotypic since then. Although S. brunneus was described from terrestrial environments associated with decaying leaves of Pinus densiflora (Ando & Tubaki 1985), this species has been commonly found in aquatic environments, on submerged leaf litter or foam (Tubaki 1965, Silva & Briedis 2009). In the Neotropics Matsushima (1987) reported this species from Peru on lignicolous substrates, Smits et al. (2007) and Silva & Briedis (2009) on foam from Venezuela and Matsushima (1993) on decomposing leaves from Ecuador. Barbosa & Gusmão (2011) described it from Brazil on submerged leaf litter in the State of Bahia and Fiuza & Gusmão (2013) on foam from the semi-arid region of the State of Ceará and more recently from the Amazon region (Fiuza et al. 2015). This is the first record of this taxon in the state of São Paulo.

Triscelophorus ponapensis Matsush., Matsush. Mycol. Mem. 2:19 1981. Fig. 10

Conidiophores not observed. Mature conidia consisting of a main axis and 2–3 verticillate lateral branches forming a whorl next to the base of the axis, hyaline; main axis 12–26 × 4–6 µm, 2–4-septate; lateral branches 8–15 × 3,5–5 µm, 1–4-septate.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, “Poço do Jequitibá” and “Centro da Ilha” waterfalls, on submerged mixed leaf litter, 04 September 2012, L.B. Moro (SP466896).

Notes – Triscelophorus ponapensis was isolated by Matsushima (1981) of leaf of Pandanus sp. Triscelophorus Ingold is characterized by its typical tetraradiate conidia and was first described by Ingold (1943) with T. monosporus as the type species and currently includes eight species. Several taxa within the genus have been previously described for Brazil (Schoenlein-Crusius & Milanez 1990, 1998, Schoenlein-Crusius et al. 1990, 1992, Malosso 1999, Schoenlein-Crusius 2002, Fiuza & Gusmão 2013, Silva et al. 2014, Fiuza et al. 2015). However, this is the first record of T. ponapensis in the country.

Discussion

Thirty-nine taxa were obtained from the 14 visited waterfalls and other eight were obtained from the eight surveyed streams reinforcing the preference of Ingoldian fungi for fast running, well-aerated waters. Among the waterfalls, the “Gato” waterfall presented the highest number of fungal taxa with 18, followed by “Poço da Pedra” with 12 taxa, “Lage” and “Três Tombos” waterfalls both with 11 taxa and “Veloso” and “Água Branca” waterfalls each with 10 taxa. Among the rivers, the “Cachoeira do Gato” river was the site with the highest number of fungal taxa including 11. There was not a significant variation between the number of fungal taxa in relation to the field trips, obtaining 17, 17, 15 and 19 taxa, respectively, between the first and fourth collections (Table 2).

The diversity of aquatic hyphomycetes in the waterfalls and streams of Ilhabela may be considered high in comparison with other studies conducted in the Brazilian Atlantic rainforest. For instance, Schoenlein-Crusius et al. (1992) reported 11 taxa of aquatic hyphomycetes isolated from leaves of Quercus robur L., Ficus microcarpa L.f. and Achornea triplonervi (Spreng) M.Arg. submerged in a fast running stream in the Atlantic rainforest of Paranapiacaba, state of São Paulo. In the “Parque Estadual das Fontes do Ipiranga – PEFI”, an important remaining of Atlantic Rainforest at the plateau of the city of São Paulo, 24 taxa of aquatic hyphomycetes were obtained.
from mixed leaf litter samples collected at 10 water bodies with different eutrophication levels (Schoenlein-Crusius et al. 2009). Later, at the same park, 33 taxa of aquatic hyphomycetes were reported from mixed leaf litter samples submerged in an artificial stream called “Córrego do Pirarungáua” after two years of monthly collections (Schoenlein-Crusius et al. 2016).

The surveys conducted at Ilhabela State Park contribute to the expansion of our knowledge about the diversity of aquatic hyphomycetes in areas of Atlantic rainforest in Brazil, particularly in insular areas, with 22.5% of the identified species described or first reported for Brazil and/or the State of São Paulo, and 100% for the Ilhabela State Park. These results encourage the preservation of the water bodies at Ilhabela Island as an important remnant of the Brazilian Atlantic rainforest in order to ensure the conservation of its biodiversity.

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