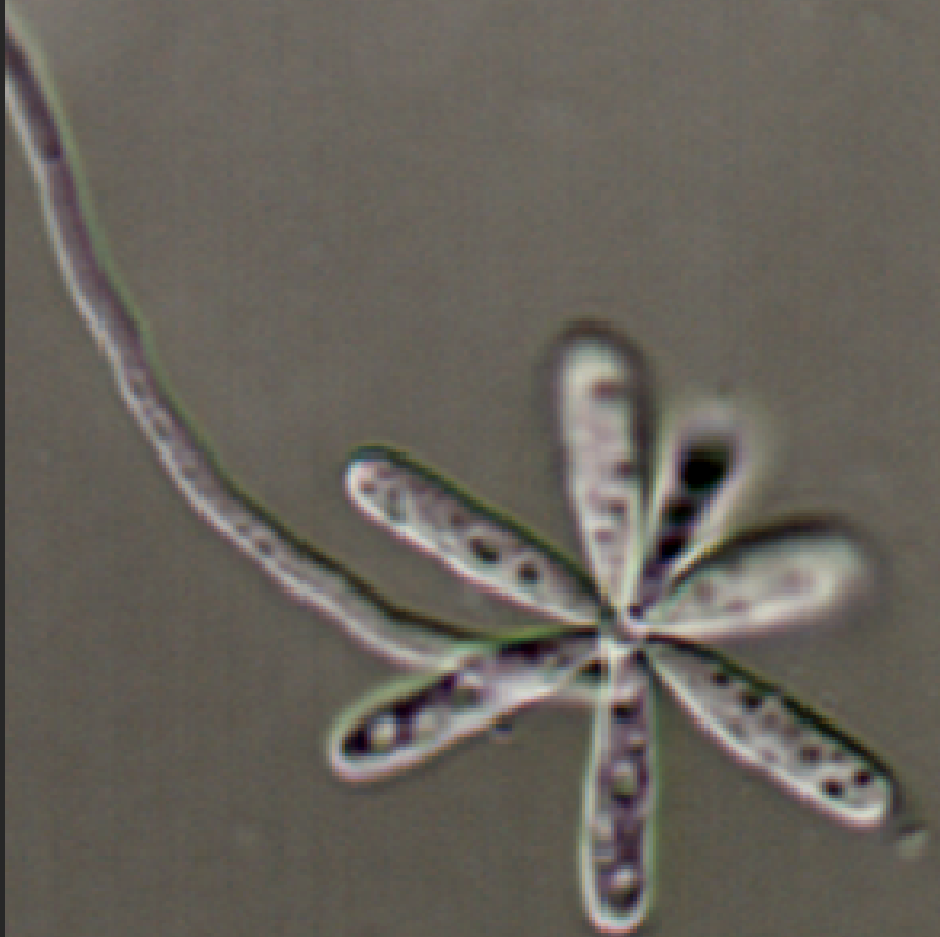


Larissa Bernardino Moro

2015

Diversidade de Hyphomycetes aquáticos
nos corpos d'água da Ilha de São Sebastião
(Ilhabela) e da Ilha do Cardoso (Cananéia),
SP, Brasil



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Orientadora

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Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

SÃO PAULO

2015

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Que nada nos defina. Que nada nos sujeite.

Que a liberdade seja a nossa própria substância.

Simone de Beauvoir

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Resumo

O grupo ecológico dos Hyphomycetes (ou hifomicetes) aquáticos é constituído pelos fungos anamórficos (antigos deuteromicetos ou imperfeitos), que se caracterizam pela formação exclusiva assexuada de conídios livres, em sinemas ou em esporodóquios. Com base na forma dos conídios e no ciclo de vida, os Hyphomycetes aquáticos foram divididos nos seguintes grupos ecológicos: os fungos ingoldianos, que apresentam conídios com formas tetrarradiadas ou várias ramificações típicas, somente produzidas na água; os aeroaquáticos que são fungos que suportam condições submersas, porém produzem conídios na interface água/ar; os fungos aquáticos terrestres, que incluem várias espécies encontradas na água da chuva ou orvalho acumulada na superfície de folhas e os fungos aquáticos submersos que são capazes de atuar como sapróbios em substratos vegetais submersos ou terrestres. O presente estudo visa analisar a diversidade das espécies de Hyphomycetes aquáticos no Parque Estadual de Ilhabela (PEIb) e no Parque Estadual de Ilha do Cardoso (PEIC), SP, através do levantamento taxonômico em ambientes aquáticos de água doce lóticos, com ênfase especial para as quedas d'água, iniciando o estudo da taxonomia desses fungos em ecossistemas insulares e avaliar a técnica de Descals para obter culturas destes fungos. Para isso, foram realizadas quatro expedições para a coleta de folheto misto submerso nos diversos ambientes lóticos no PEIb e no PEIC. Concomitantemente, alguns fatores abióticos da água foram medidos utilizando-se uma sonda U₁₀ da Horiba. No laboratório, as amostras de folheto misto submerso foram fragmentadas e distribuídas em placas de Petri contendo água destilada esterilizada, e incubadas a 20°C. A partir do quinto dia de incubação, fragmentos de folhas foram observados ao microscópio ótico à procura de características taxonomicamente relevantes que permitissem a identificação dos fungos observados, com base em literatura específica. A análise molecular foi necessária para a confirmação da identificação de alguns táxons. As observações foram realizadas repetidamente até que nenhum novo táxon fosse verificado nos fragmentos. Sob microscópio óptico, conídios foram coletados com uma pipeta e transferidos para placas de Petri com meio extrato de malte ágar (MEA) 0,1%, marcando-se as suas posições com caneta. Após 24h de incubação a 21°C, os conídios foram transferidos para novo meio de cultura (MEA) para prosseguir o seu desenvolvimento. Foram obtidos 59 táxons de Hyphomycetes aquáticos para as ilhas amostradas, sendo 35 táxons de fungos ingoldiano, 8 táxons de fungos aeroaquáticos e 16 táxons de fungos aquáticos submersos e aquáticos terrestres. Para o PEIb, 40 táxons foram identificados, incluindo oito novas ocorrências, sendo quatro novos registros para o bioma Mata Atlântica do Estado de São Paulo e quatro para o Brasil, além de duas espécies novas: *Campylospora brasiliensis* e *Polylobatispora setulosa*. Para o PEIC, 38 táxons foram registrados, sendo seis novas ocorrências, respectivamente um para o bioma Mata Atlântica do Estado de São Paulo, cinco para o Brasil, além de uma espécie nova: *Clathrosporium retortum*. Entre os aeroaquáticos, cinco espécies são novos registros, três para o Brasil e duas para o Estado de São Paulo. Foram obtidos 15 isolados em culturas puras graças ao aperfeiçoamento da técnica de Descals. A diversidade dos Hyphomycetes aquáticos mostrou-se significativa nos ambientes insulares, em especial nas cachoeiras. A contribuição do presente estudo ampliou o conhecimento sobre a distribuição geográfica dos fungos ingoldianos no Brasil em 14% e em relação aos fungos aeroaquáticos, ao redor de 80%, salientando a importância dos sistemas insulares como ambientes propícios para estudos de diversidade dos Hyphomycetes aquáticos.

Palavras-chave: áreas insulares - diversidade – fungos anamórficos - Mata Atlântica.

Abstract

The ecological group of the aquatic Hyphomycetes (or “hifomicetos”) is constituted by anamorphic fungi (formerly known as Deuteromycetes ou imperfect fungi), characterized by the exclusive asexual formation of conidia which may be free or contained in synema or sporodochia. Based on the shape and life cycle, the aquatic Hyphomycetes are divided in the following ecological groups: ingoldian fungi, that present conidia with tetrarradiate shapes or several typical ramifications, only produced in the water; the aeroaquatic fungi, which tolerate submersion, however the conidia are produced only at the water/air interface; the terrestrial aquatic fungi, which include several species usually found in the rainwater or dew accumulated on leaf surfaces; and the submerged aquatic fungi, that are able to act as saprobes on submerged or terrestrial substrates. The present study aims at analyzing the diversity of species of aquatic Hyphomycetes in Parque Estadual de Ilhabela (PEIb) and the Parque Estadual de Ilha do Cardoso (PEIC), SP, through the taxonomic survey on freshwater lotic aquatic environments, with special emphasis to the waterfalls, starting the taxonomy study of these fungi in island ecosystems and evaluate the technique of Descals for cultures of these fungi. For this, four expeditions were made to collect submerged mixed leaf litter samples in several lotic environments at the PEIb and at the PEIC. Concomitantly, some abiotic factors of the water were measured with an U₁₀ Horiba probe. In the laboratory, fragmented leaf samples were distributed in Petri dishes containing sterile distilled water and incubated at 20°C. From the 5th day on, the leaf fragments were microscopically observed to search for taxonomical relevant features, in order to identify the observed fungi by specific literature. These observations were made repeatedly, until no new fungi were verified in the leaf fragments. Molecular procedures were also necessary for the confirmation of the identification of some taxa. Under a microscope, conidia were taken with a pipette and transferred to Petri dishes containing malt extract agar media (MEA) at 0,1%, marking its position with a pen. After 24 hours of incubation at 21°C, the conidia were again transferred to new culture MEA media to proceed their development. A total of 59 taxa of aquatic Hyphomycetes were obtained for the island being 35 taxa of ingoldian fungi, eight aeroaquatic fungi and 16 submerged aquatic fungi and terrestrial aquatic fungi. For PEIb, 40 taxa were identified, including eight new records, respectively four new records the São Paulo state and four to Brazil, besides two new species: *Campylospora brasiliensis* and *Polylobatispora setulosa*. For PEIC, 38 taxa were registered, including six new records, respectively one new for São Paulo state and five to Brazil besides one new species: *Clathrosporium retortum*. Among the aeroaquatic fungi, three species are new records to Brazil and two to São Paulo state. Fifteen pure cultures were obtained due to the improvement of Descal’s technique. The diversity of the aquatic Hyphomycetes showed to be high in insular environments, especially in waterfalls. The contribution of the present study increased the knowledge about the geographical distribution of the ingoldian fungi in Brazil by 14%, and regarding the aeroaquatic fungi by 80%, highlighting the importance of insular systems as propitious environments for the diversity of aquatic Hyphomycetes.

Keywords: anamorphic fungi-diversity-insular area- Atlantic Rainforest.

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Esta tese refere-se ao estudo da diversidade de Hyphomycetes aquáticos em ambientes lóticos (cachoeiras, rios, córregos) de áreas de Mata Atlântica bem preservadas dos parques insulares de Ilhabela (extremo norte do Estado de São Paulo) e da Ilha do Cardoso (extremo sul do referido Estado).

A introdução da tese aborda os Hyphomycetes aquáticos, além de definir cada grupo, apontando o papel ecológico destes fungos. Revisão bibliográfica e compilação dos principais trabalhos no Brasil e no mundo são apresentadas, seguida da descrição das áreas de coleta, dados históricos de cada ilha e a seleção das pesquisas micológicas realizadas em cada Parque. Após a introdução são apresentados os objetivos do trabalho e a descrição detalhada da metodologia utilizada no presente estudo.

Os resultados da tese estão detalhados em cinco capítulos, todos formatados em artigo nas normas das revistas às quais foram publicados, submetidos ou serão submetidos.

O primeiro capítulo aborda a diversidade de Hyphomycetes aquáticos no Parque Estadual de Ilhabela, incluindo novas citações. Nesta ilha foram visitados 17 diferentes locais de coleta, na maioria cachoeiras.

*O segundo capítulo inclui uma espécie nova para a Ciência, *Polylobatispora setulosa*, coletada no Parque Estadual de Ilhabela. Neste trabalho também foi incluída uma segunda espécie, *Acumispora verruculosa*, identificada no mesmo local e com medidas diferentes das descritas originalmente. Chaves de identificação para os gêneros são apresentadas, assim como pranchas com fotos.*

O terceiro capítulo descreve uma espécie nova do gênero Campylospora, C. brasiliensis, com base em análises moleculares, comparando-a com as sequências de todas as espécies do gênero.

O quarto capítulo contempla a diversidade geral de Hyphomycetes aquáticos no Parque Estadual da Ilha do Cardoso, incluindo novas citações. Para esta ilha, as coletas seguiram o leito do Rio Perequê, no núcleo que recebe o mesmo nome, região central de pesquisas científicas e de fácil acesso para as coletas.

No quinto capítulo é apresentada uma espécie nova para a Ciência, Clathrosporium retortum e a diversidade de fungos aeroaquáticos tanto no Parque Estadual de Ilhabela quanto no Parque Estadual da Ilha do Cardoso.

Em anexo estão as normas dos periódicos aos quais os artigos foram submetidos e a tabela dos Hyphomycetes aquáticos registrados no Parque Estadual de Ilhabela.

1. INTRODUÇÃO GERAL

1.1. Fungos anamórficos

Os fungos anamórficos produzem os conídios por mitose, sendo considerados Ascomycota ou Basidiomycota (Alexopoulos *et al.* 1996, Kirk *et al.* 2008).

Estes fungos já foram conhecidos como fungos imperfeitos ou fungos mitospóricos e pertenceram a grupos taxonômicos definidos como Deuteromycotina ou Deuteromycota (Kirk *et al.* 2008).

Contudo a taxonomia deste grupo de fungos sempre foi controversa, pois para os seres vivos, os taxonomistas utilizam as estruturas sexuais para ordená-los e relacioná-los aos seus ancestrais. Essas estruturas não são produzidas pelos fungos anamórficos, uma vez que muitos só exibem estruturas assexuais, não sendo possível assim, relacioná-los com seus ancestrais. Por conseguinte, todas as propostas de classificação são artificiais. Os nomes de ordens ou famílias não são utilizados, mantendo-se apenas gênero e espécie. Uma exceção é permitida para o uso dos nomes das classes, com o intuito de facilitar as atividades didáticas (Grandi 1998a).

Os fungos anamórficos, apesar de suas diferenças evolutivas, são mais bem agrupados em função de sua ecologia e morfologia (Webster & Weber 2007). Por conseguinte, de acordo com Kirk *et al.* (2008), os fungos anamórficos são organizados em três grupos morfológicos que, no passado, eram classes da divisão Deuteromycotina:

- a) **Coelomycetes** – produzem conídios em estruturas como picnídios e acérvulos; saprófitos ou parasitas de plantas, fungos, líquens, vertebrados; organizados em 1000 gêneros e 7000 espécies.

- b) **Agonomycetes**– forma micelial estéril, contudo podem produzir estruturas de resistência como clamidósporos e esclerócios;
- c) **Hyphomycetes**– forma micelial produzindo conídios livres, em uma hifa separada (conidióforo livre) ou em conidióforos agrupados (sinema ou esporodóquio); crescem em tecido vegetal, insetos ou outros artrópodes, outros fungos incluindo líquens e diversos outros ambientes (Seifert *et al.* 2011) sendo terrestres ou aquáticos.

Esta tese contemplará o grupo ecológico dos Hyphomycetes que ocorrem em água doce.

1.2. Hyphomycetes aquáticos

Independentemente da validade taxonômica, o termo Hyphomycetes é encontrado na literatura como sendo constituído pelos fungos anamórficos que produzem conidióforos e conídios livres ou conidióforos agrupados em **sinema** (feixes eretos de conidióforos) ou **esporodóquio** (aglomerado de conidióforos curtos) (Alexopoulos *et al.* 1996, Kirk *et al.* 2008).

Embora não tenham sido descritos de ambientes aquáticos, Saccardo (1880) descreveu o primeiro membro do grupo, *Heliscus lugdunensis* Sacc. et Therry, isolado de cascas de pinheiro perto de Lyon na França e no norte da Itália.

De Wildeman (1893,1894,1895) descreveu três novas espécies de Hyphomycetes aquáticos tetrarradiados, *Clavariopsis aquatica* De Wild., *Lemonniera aquatica* De Wild. e *Tetracladium marchalianum* De Wild., em lagoas e áreas pantanosas, nas folhas de salgueiro e macrófitas aquáticas. O autor foi o primeiro a descrever as estruturas conidiogênicas desses fungos em água.

No início da década de 40, Ingold (1942) identificou 16 espécies de Hyphomycetes aquáticos em folhas submersas de um pequeno córrego que drena o reservatório de Cropston perto de Leicester, Inglaterra.

Ranzoni (1956) identificou a primeira fase sexual de um Hyphomycetes aquático como um Ascomycota. *Flagellospora penicillioides* Ingold (Hyphomycetes aquático) é a fase anamórfica de *Nectria penicillioides* Ranzoni fase teleomorfa conhecida apenas em meio de cultura e não é aquática.

Ingold (1959) documentou o primeiro Hyphomycetes aquático com grampos de conexão presentes nos conídios os quais são características exclusivas dos membros do filo Basidiomycota. Contudo, não foi possível a identificação da espécie naquele momento. Apenas em 1972, Shaw descreveu o novo gênero e espécie como *Ingoldiella hamata* de folhas submersas não identificadas, em um pequeno riacho ao norte de Queensland, Austrália, com grampos de conexão presentes nos conídios. Em 1992, Webster documentou que mais Hyphomycetes aquáticos estão relacionados com Ascomycota, do que com Basidiomycota.

Análises moleculares confirmaram que os Hyphomycetes aquáticos são polifiléticos e suas fases sexuadas são relacionadas principalmente a Ascomycota (Belliveau & Bärlocher 2005, Baschien *et al.* 2006, Campbell *et al.* 2009, Duarte *et al.* 2015).

Portanto, sob o ponto de vista taxonômico, os Hyphomycetes aquáticos constituem um grupo artificial e filogeneticamente heterogêneo, sendo considerados Ascomycota ou Basidiomycota (Webster 1992). Uma classificação em quatro grupos ecológicos baseados nos métodos de esporulação e crescimento micelial contribui para uma visão mais clara dos diferentes grupos. Os quatro grupos ecológicos são:

Hyphomycetes aeroaquáticos, Hyphomycetes aquáticos terrestres, Hyphomycetes aquáticos submersos (facultativos) e os fungos ingoldianos (Goh & Hyde 1996), cuja conceituação será apresentada nos itens a seguir.

1.2.1. Fungos ingoldianos

São Hyphomycetes aquáticos com paredes finas e hialinas, apresentando conídios com formas estreladas, tetrarradiadas, sigmóides ou esféricas. Esses conídios são extremamente leves o que facilita o acúmulo nas espumas que se formam naturalmente na água. Estes fungos dependem exclusivamente do ambiente aquático para reprodução, sendo considerados aquáticos típicos (Ingold 1975, Dix & Webster 1995, Webster & Descals 1981). Receberam o nome de fungos ingoldianos em homenagem ao seu principal estudioso, Dr. Cecil Terence Ingold (Descals *et al.* 1978).

Duas características são cruciais para os fungos ingoldianos, a capacidade de esporular na água e de crescerem em folhas em decomposição em córregos e rios. Além disso, a maioria dos fungos ingoldianos produz uma quantidade relativamente grande de conídios multirradiados (frequentemente tetrarradiados) ou esporos sigmóides (Ingold 1975, Dix & Webster 1995, Webster & Descals 1981). Estas características morfológicas facilitam a fixação do esporo em folhas e outras superfícies lisas (Webster 1959, Kearns & Bärlocher 2008, Krauss *et al.* 2011).

Conídios sigmóides frequentemente aderem-se ao substrato pelas suas extremidades com o auxílio de mucilagem e se posicionam na direção da corrente de água de modo que são menos susceptíveis a serem retirados da superfície do substrato (Figura 1A). Conídios tetrarradiados e ramificados atuam como uma âncora e permitem a adesão ao substrato ou a espuma da superfície da água (Ingold 1942, 1953). Conídios

tetrarradiados também podem se fixar ao substrato em três pontos das suas projeções “formando um tripé fortemente adesivo” (Webster & Descals 1981), como mostra a Figura 1B. Uma massa mucilaginosa adesiva também é produzida em cada ramo do conídio em contato com uma superfície (Read 1990, Read *et al.* 1992) o que permite ao conídio aderir firmemente ao substrato. Read (1990) indica que a colonização do substrato pelos fungos ingoldianos pode envolver quatro fatores:

- i. a forma dos conídios e fixação,
- ii. a rápida germinação,
- iii. a produção de mucilagem na superfície dos conídios e tubos germinativos, e
- iv. a formação de apressórios.

Essas características podem conferir vantagens para os fungos ingoldianos frente a outros grupos de fungos presentes no ambiente aquático (Wong *et al.* 1998).

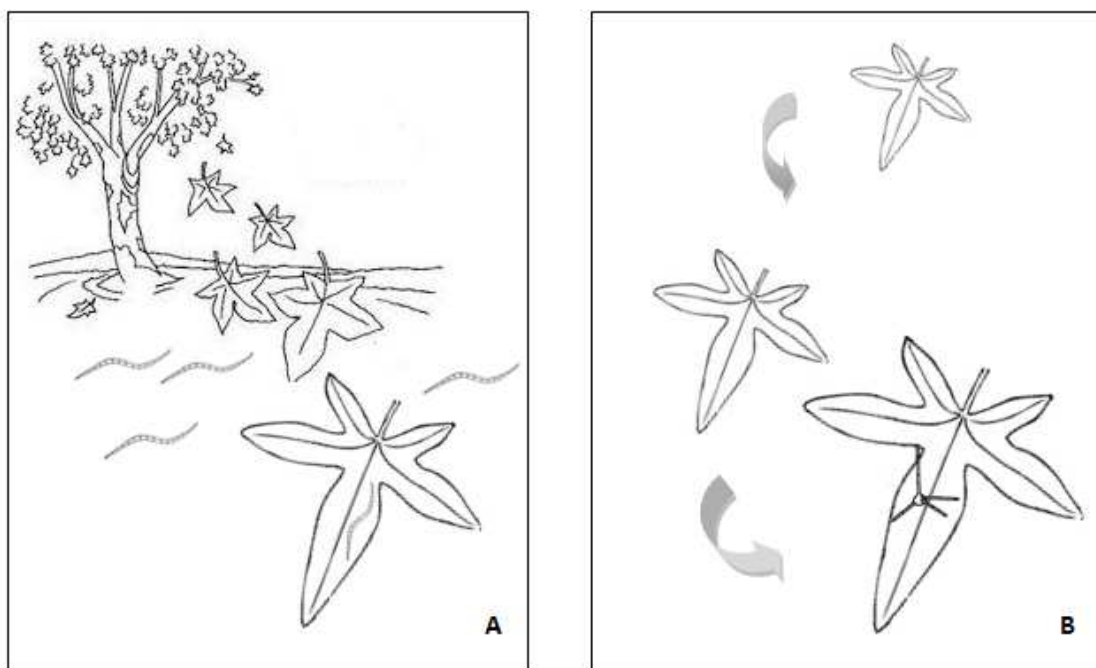


Figura 1. Adesão dos conídios de fungos ingoldianos ao substrato. A: Adesão de conídio do tipo sigmóide (adesão em cada extremidade do conídio). B: Adesão de conídio do tipo tetrarradiado (adesão em três pontos do conídio).

As adaptações destes fungos ao ambiente aquático favorecem a sua ocorrência nos ambientes lóticos (Sridhar *et al.* 2000), como rios, riachos e córregos, onde o fluxo de água é contínuo (Thomas 1996), com água limpa (Pascoal *et al.* 2005). Porém, estes fungos também foram relatados em corpos de água lênticos, tais como lagos (Suzuki & Nimura 1961, Casper 1965), reservatórios (Marvanová & Marvan 1963) e até em águas poluídas (Sridhar *et al.* 2000). Também foram encontrados como endofíticos de raízes de plantas próximas a córregos (Sridhar & Bärlocher 1992) e em plantas ribeirinhas (Sati & Arya 2010).

Esses fungos são conhecidos por lançar uma grande quantidade de conídios em baixo d'água com formas características que, a princípio, facilitam a identificação das espécies. No entanto, sempre que possível, a conidiogênese deve ser observada para acompanhar o desenvolvimento completo dos fungos ingoldianos (Pascoal *et al.* 2005), subsidiando a identificação correta dos táxons.

1.2.2. Fungos aeroaquáticos

O termo aeroaquático foi proposto por Agathe van Beverwijk para fungos anfíbios que produzem conídios apenas acima do nível da água (Beverwijk 1951), ou seja, não esporulam quando o substrato vegetal está submerso, porém esporulam quando este entra em contato com o ar atmosférico (Abdullah & Webster 1980, Kalgutkar & Mcintyre, 1991).

Seus conídios possuem formas tridimensionais, como espirais, e alguns desses conídios se tornam sigmóides depois de liberados dos conidióforos (Goh & Tsui 2003). Tais conídios funcionam como uma armadilha para as bolhas de ar, o que permite que

flutuem e se dispersem na lâmina d'água (Michaelides & Kendrick 1982, Premdas & Kendrick 1991).

Alguns fungos aeroaquáticos possuem fase teleomorfa conhecida, que são de origem terrestre e pertencem a vários grupos dos filos Ascomycota e Basidiomycota (Abdullah 1980, Goos *et al.* 1985, Voglmayr 1994).

Atualmente, os estudos com os fungos aeroaquáticos estão sendo realizados agrupando as espécies em seus respectivos filios, alguns com base molecular, como é o caso de *Polyancora globosa* Voglmayr & Yule pertencente à ordem Xylariales (Voglmayr & Yule 2006) do filo Ascomycota, a partir da análise parcial da região LSU e ITS do DNA ribossomal, ou com base morfológica, como observado em *Peyronelina glomerulata* Fisher, Webster & Kaneque foi descoberta a sua conexão com a fase teleomórfica pertencente ao gênero *Flagelloscypha*, do filo Basidiomycota, por Yamagushi *et al.* (2009) a partir de observações da formação do basidioma em placas de cultura antigas.

Esses fungos normalmente ocorrerem em ambientes lênticos, onde o fluxo de água é mais lento, como nos lagos, lagoas, pântanos e riachos com pouca correnteza (Thomas 1996, Premdas & Kendrick 1991), colonizando substratos orgânicos submersos, mas também podem ser encontrados em todos os tipos de habitats aquáticos e/ou terrestres (Shearer *et al.* 2004). São capazes de sobreviver a baixas concentrações de oxigênio (Shearer *et al.* 2007) e até em condições anaeróbicas, onde não são capazes de esporular (Fisher 1978).

Field & Webster (1983) testaram a capacidade de sobrevivência de cinco espécies do gênero *Helicodendron* Peyronel, sob tais condições, verificando que 100%

das espécies sobreviveram por seis meses. Esta sobrevivência pode estar relacionada com o espessamento da parede das hifas ao invés da produção de estruturas de resistência como os clamidósporos, que são raros para este grupo de fungos (Webster & Weber 2007).

1.2.3. Fungos aquáticos terrestres

O termo Hyphomycetes aquáticos terrestres foi criado por Ando em 1992 para fungos conidiais isolados de gotas de chuva associados às partes de plantas terrestres intactas (Goh & Hyde 1996, Goh & Tsui 2003). Diversos estudos anteriores foram realizados para este grupo de fungos. Bandoni (1981) descreveu conídios de Hyphomycetes aquáticos terrestres em água da chuva drenadas de árvores vivas, incluindo *Tripospermum* e algumas espécies não identificadas. Mackinnon (1982) descobriu que a esporulação da maioria dos fungos encontrada em seu estudo tinha correlação positiva com a precipitação pluviométrica. Ando & Tubaki (1984), descreveram vários novos gêneros e espécies de Hyphomycetes aquáticos terrestres de amostras de água de chuva coletadas no Japão.

Ando & Tubaki (1984) relataram que estes Hyphomycetes pudessem viver na superfície intacta das folhas, sendo capazes de usar diversas formas de água (chuva, neblina, orvalho) para a esporulação. Contudo, estes estudos e as suas hipóteses intrigantes receberam pouca atenção na última década (Gönczöl & Révay 2006), uma vez que as pesquisas são mais voltadas para a taxonomia do que para a biologia desse grupo de fungos.

Esse grupo produz conídios radiados morfológicamente similares aos conídios produzidos pelos fungos ingoldianos, mas diferenciam-se principalmente pela ausência

de conidióforos macronemáticos (Ando 1992, Goh & Hyde 1996). Os conídios em sua maioria são hialinos e de paredes finas; contudo, espécies dematiáceas foram descritas (Goh & Hyde 1996).

1.2.4. Fungos aquáticos submersos (facultativos)

Os Hyphomycetes aquáticos submersos foram primeiro abordados por Ingold (1975). Esses fungos também são conhecidos como lignícolas ou aquáticos facultativos (Goh & Hyde 1996), apresentando muitos fungos dematiáceos (Webster & Descals 1981, Goh & Hyde 1996).

Incluem os Hyphomycetes, Coelomycetes e Ascomycetes saprofitos e/ou parasitas encontrados em ambientes aquáticos e terrestres (principalmente substratos lignificados), atuando como organismos facultativos. Embora algumas espécies possam esporular em condições submersas, um vasto número esporula apenas quando os substratos não estão mais sob a água (Goh & Hyde 1996, Goh & Tsui 2003).

Do mesmo modo que é usual para a identificação dos Hyphomycetes aquáticos também para reconhecer os fungos aquáticos submersos são utilizadas as características dos conidióforos, células conidiogênicas e dos conídios. A morfologia dos conidióforos de Hyphomycetes aquáticos submersos varia muito, podendo ser mononemáticos, esporoquial e alguns formam sinema. Outra característica relevante para a identificação deste grupo de fungos é a pigmentação dos conidióforos que pode ser marrom ou hialino (Hu *et al.* 2014). Os conídios dos Hyphomycetes aquáticos submersos são de várias formas, ou seja, ovóides, cilíndricos, obclavados, piriformes ou fusiformes. No entanto, os conídios ramificados não são incomuns e podem também ser encontrados em amostras de espuma (Goh & Hyde 1996).

1.3. Importância ecológica dos Hyphomycetes aquáticos

Por serem sapróbios, os Hyphomycetes aquáticos são responsáveis pela decomposição da matéria orgânica, atuando nas transformações bioquímicas nos ecossistemas, uma vez que a atividade de degradação libera compostos mais simples, os quais são reabsorvidos pelas plantas (Remacle 1981). Esses fungos também aumentam a palatabilidade dos substratos para consumo dos detritívoros, dinamizando a ciclagem de nutrientes ou servindo de alimento por meio da produção de biomassa (Bärlocher & Kendrick 1981, Bärlocher 1985, Christensen 1989, Bärlocher 1992, Kendrick 1992, Alexopoulos *et al.* 1996, Moore-Landecker 1996, Cheng *et al.* 1997).

Os fungos aquáticos têm se destacado pela grande capacidade de produzirem rico maquinário enzimático com a produção de uma variedade de enzimas capazes de degradar os principais polissacarídeos da folha (Suberkropp & Krug 1980). Essas enzimas são capazes de degradar os açúcares simples, celulose, e outros polímeros das plantas, resultando na esqueletização das folhas (Tubaki 1957, Thornton 1963, 1965, Nilsson 1964, Singh 1982, Chandrashekar & Kaveriappa 1988). Dados experimentais demonstram que os Hyphomycetes aquáticos têm atividade dependente do pH para a degradação da celulose, da xilana e da pectina (Chamier & Dixon 1982, Suberkropp *et al.* 1983, Chandrashekar & Kaveriappa 1988). Existem pelo menos, indícios de que algumas espécies possam degradar lignina e causar podridão mole ou macia (Jones 1981, Fisher *et al.* 1983, Zemek *et al.* 1985).

Alguns invertebrados preferem alimentar-se de folhas colonizadas, possivelmente em decorrência do enriquecimento nutricional que os fungos proporcionam ao substrato (Bärlocher 1992, Cheng *et al.* 1997).

Os Hyphomycetes aquáticos constituem um importante elo do fluxo de energia em ecossistemas lóticos entre serrapilheira e invertebrados aquáticos (Bärlocher & Kendrick 1981, Bärlocher 1985), relacionada com a palatabilidade dos substratos foliares para demais componentes da cadeia trófica.

1.4. Importância econômica dos Hyphomycetes aquáticos

Os fungos são bem conhecidos por produzirem antibióticos (Demain 2000), e continuam a serem investigados como fonte de produtos naturais e de metabólitos secundários (Hoffmeister & Keller 2007) para o seu potencial uso em medicina, indústria e na agricultura (Bill *et al.* 1994, Liu *et al.* 2008); porém os estudos ainda são raros para o grupo dos Hyphomycetes aquáticos, talvez devido ao seu nicho ecológico peculiar.

Existem alguns trabalhos descrevendo relações intra e interespecíficas entre Hyphomycetes aquáticos envolvendo a produção de substâncias inibidoras difusas (Khan 1987, Shearer & Zare-Maivan 1988, Bärlocher 1991). Chamier *et al.* (1984) relataram inibição de bactérias por fungos ingoldianos em experimentos de campo. O primeiro estudo sobre o isolamento e determinação estrutural de compostos antimicrobianos de *Anguillospora longissima* Ingold, resultou na descoberta do metabólito “Anguillosporal” (Harrigan *et al.* 1995). “Quinaphthin” é um composto antimicrobiano descrito a partir da espécie aeroaquática *Helicoon richonis* (Boud.) Linder (Fisher *et al.* 1988, Adriaenssens *et al.* 1994) e o “Heliconols” A-C é um antimicrobiano descrito a partir de *Helicodendron giganteum* Glen Bott (Mudur *et al.* 2006). “Clavariopsinas” A e B foram isoladas de *Clavariopsis aquatica* De Wild., apresentando atividade antifúngica contra *Aspergillus fumigatus* Fresen. e, em menor

extensão, contra *A. niger* Tiegh. E *Candida albicans* (C.P. Robin) Berkhout (Kaida *et al.* 2001).

Gulis & Stephanovich (1999), em um estudo *in vitro*, verificou os efeitos antibióticos de culturas filtradas de 29 espécies de Hyphomycetes aquáticos contra bactérias gram-negativas e gram-positivas, leveduras e Hyphomycetes. Trinta e oito por cento dos isolados foram ativos em pelo menos um dos testes efetuados para atividades biológicas. Quinze espécies inibiram o crescimento de bactérias e quatro demonstraram atividade antifúngica. Do total de isolados, 35% possuíam atividade antibacteriana.

Com o intuito de verificar a atividade antagonista de cinco espécies de Hyphomycetes aquáticos coletados de raízes de plantas ribeirinhas contra sete fungos fitopatógenos, Sati & Arya (2010) verificaram que *Tetrachaetum elegans* Ingold apresentou atividade antagonista contra *Colletotrichum falcatum* Went, *Fusarium oxysporum* Schlecht., *Pyricularia oryzae* Cavara, *Sclerotinia sclerotiorum* (Lib.) De Barye *Tilletia indica* Mitra. Além disso, *Heliscus lugdunensis* Saccardo & Therry apresentou a mesma atividade para dois fungos fitopatogênicos, *Rhizoctonia solani* Huhn e *Colletotrichum falcatum* Went. Este estudo evidencia a potencialidade da utilização dos Hyphomycetes aquáticos em programas de controle biológico.

Uma potencialidade recente dos fungos aquáticos é a utilização de *Heliscus lugdunensis* Saccardo & Therry na degradação do contaminante ambiental utilizado na produção de policarbonato, o bisfenol A (BPA), que está sob investigação por seu efeito negativo sobre o ecossistema aquático. Em estudo de Omoike *et al.* (2013), foi verificada a capacidade das células fúngicas de *H. lugdunensis* de utilizar BPA, tendo o fungo removido mais de 70% do BPA depois de 12 dias. Esses resultados sugerem que

H. lugdunensis seja capaz de biodegradar BPA e utilizá-lo como fonte de energia, sendo um promissor biomaterial na degradação do BPA no ambiente aquático.

1.5. Obtenção de culturas de Hyphomycetes aquáticos

A obtenção de culturas puras em estudos de taxonomia é uma etapa importante para aumentar quantitativamente o material disponível para identificação e caracterização morfológica do fungo isolado. No atual estado da arte, principalmente para os Hyphomycetes aquáticos, a obtenção de cultura é um passo necessário para executarestudos moleculares e auxiliar na conexão genética do fungo anamórfico isolado com o seu teleomorfo (Descals 1997).

Diversas técnicas para isolamento de Hyphomycetes aquáticos são descritas. Ingold (1975) inoculou amostras de espuma dos corpos d'água em ágar com antibiótico para reduzir o crescimento de bactérias. Cavalcanti & Milanez (2007) inocularam um mililitro de água de amostra em placas de Petri com meio de Martin (1950) com cloranfenicol.

Anderson & Shearer (2011) utilizaram um microscópio de dissecação para a retirada individual de conídios presentes em folhas incubadas e os inocularam em meio Agar-água com antibiótico.

Fiuza (2013) incubou fragmentos de folhas em placas de Petri com água destilada e inseriuporções de meio de cultura de extrato de malte a 0,2% nas amostras incubadas. Após a observação da colonização das porções de meio de cultura, esses foram transferidos para uma nova placa de Petri com meio de cultura para purificação.

Uma técnica interessante foi proposta por Descals em 2005 segundo a qual, inicialmente, colocam-se algumas folhas coletadas em campo em placas de Petri esterilizadas contendo água destilada para incubação por 1 a 3 dias. Os conídios em suspensão na água ou recentemente aderidos nas folhas podem ser coletados com o auxílio de um tubo capilar ou uma pipeta de Pasteur no microscópio de dissecação e transferidos para placas de Petri contendo meio de isolamento. Sobre o reverso da placa de Petri deve ser desenhada uma pequena escada ao lado do local de inoculação do conídio. Após 24 a 48 h, deve ser verificada a germinação dos conídios isolados através da observação da presença de tubos germinativos. Os conídios germinados devem ser transferidos, com o auxílio de uma agulha, para o próximo degrau da escada e assim sucessivamente. Esse processo deve continuar até a obtenção da colônia livre de contaminantes.

Esta técnica foi escolhida para a obtenção de culturas puras desta tese, por permitir uma transferência controlada do inoculo, obtendo-se rapidamente as colônias sem contaminantes.

1.6. Cenário dos estudos com os Hyphomycetes aquáticos

Mais de 60 anos depois dos estudos pioneiros realizados por Saccardo (1880) e De Wildeman (1893, 1894, 1895) que descreveram *Heliscus lugdunensis* Sacc. et Therry, *Clavariopsis aquatica* De Wild., *Lemonniera aquatica* De Wild. E *Tetracladium marchalianum* De Wild., respectivamente, os estudos com Hyphomycetes aquáticos foram retomados por Ingold (1942), descreveu 16 espécies de Hyphomycetes aquáticos de folhas submersas de um pequeno córrego que drena o reservatório de Cropston na Inglaterra. Webster & Descals listaram mais de 150 espécies conhecidas até 1981.

Contudo, estima-se que existam em torno de 300 espécies descritas de Hyphomycetes aquáticos, reportadas para águas continentais, entre as quais 280 espécies (143 gêneros) são conhecidas nos trópicos (Goh 1997). Na América do Sul, incluindo Argentina, Brasil, Chile, Equador, Peru e Venezuela, estimou-se que em torno de 90 táxons tenham sido descritas (Schoenlein-Crusius & Grandi 2003).

Embora a maioria dos estudos tenham sido realizados em regiões temperadas (Sivichai *et al.* 2002, Mavanová *et al.* 2003, Abdullah *et al.* 2005, Shearer *et al.* 2007), o conhecimento real sobre a distribuição dos Hyphomycetes aquáticos é espacialmente dispar devido o nível desigual de amostragem entre regiões geográficas. Áreas temperadas da América do Norte e Europa, o Reino Unido, Chile, Japão e Malásia são relativamente bem amostrados (Shearer *et al.* 2007). Por outro lado, os Hyphomycetes aquáticos são relativamente pouco estudados na região tropical.

Wood-Eggenschwiler & Bärlocher (1985), indicaram em uma revisão de que muitas espécies de Hyphomycetes aquáticos são cosmopolitas, embora sejam descritas espécies de ocorrência em regiões pantropicais ou pantemperadas, ou mesmo espécies endêmicas, e em alguns casos, algumas espécies possuem uma distribuição restrita.

Para a região tropical, diversos autores apresentam levantamentos taxonômicos. Na Índia, os Hyphomycetes aquáticos foram estudados por Ingold & Webster (1973), Thakur (1977), Patil & Kapadnis (1980), Subramanian & Bhat (1981), Talde (1981, 1983), Galiyah & Manoharachary (1987), Agrawal *et al.* (1990), Sati & Tiwari (1997), Sati *et al.* (2002), Borse & Patil (2006, 2007), Patil (2009), Pawara *et al.* (2009), Wagh *et al.* (2009), Nemade *et al.* (2009) e Patil *et al.* (2010, 2011).

Sakayaroj *et al.* (2005), realizaram uma pesquisa na reserva selvagem de Ton Nga Chang, Songkhla, Tailândia, e identificaram 48 espécies de Hyphomycetes aquáticos. Estudos no país insular asiático Sri Lanka foram feitos por Gunasekera & Rukmani (1984), identificando 11 táxons em nível de espécie e 4 em nível de gênero. Matsushima (1971) identificou diversas espécies de Hyphomycetes aquáticos nas Ilhas Solomão e em Papua Nova Guiné. Ranzoni (1979), em seu estudo nas ilhas Oahu, Kauai, Maui e Hawaii, identificou 17 Hyphomycetes aquáticos, e dentre esses, 10 espécies novas citações para o Hawaii.

Para a América Central, Santos-Flores & Betancourt-López (1997), estudando os Hyphomycetes aquáticos (ingoldianos e aquáticos facultativos) em córregos em Porto Rico, identificaram 143 espécies de fungos, elaborando um compêndio com as descrições dos táxons. Visando ampliar o conhecimento sobre a diversidade dos Hyphomycetes aquáticos no México, mais precisamente no Estado de Tabasco, Hernández *et al.* (2007) identificaram 44 espécies e destas, 17 novos registros para o país. Em Cuba, Voglmayr & Delgado-Rodríguez (2001) descreveram um novo gênero e nova espécie de fungo aeroaquático, *Dendroclathra caeruleofusca* Voglmayr & Delgado-Rodríguez em folhas submersas de *Bambusa vulgaris* L. no rio San Juan, na Reserva da Biosfera Sierra del Rosario.

Na América do Sul, nos parques Nacionais de Quebrada Martinera, Quebrada Guatopo, Quebrada Ingenio, Río Tocome, Río Cabriales e Río Cupira, da Venezuela, Smits *et al.* (2007) identificaram 50 espécies de Hyphomycetes aquáticos, sendo 30 espécies novas citações para o país. Na Colômbia, Luna-Fontalvo (2009), com o objetivo de verificar os fungos anamórficos associados a folhas submersas em decomposição de *Anacardium excelsum* (Bertero & Balb. ex Kunth) Skeels e

Pithecollobium longifolium (Humb. & Bonpl. ex Willd.) Standley, identificou cinco táxons de Hyphomycetes aquáticos, *Clavatospora tentacula* Sv. Nilsson, *Clavariopsis aquatica* De Wildeman, *Campylospora chaetocladia* Ranzoni, *Helicomycetes* sp. e *Flagellospora curvula* Ingold.

No Brasil, o estudo dos Hyphomycetes aquáticos foi iniciado há mais de 25 anos, com a observação de algumas espécies durante a sucessão fúngica em folhas de *Ficus microcarpa* L. f. submersas em um lago artificial no Parque Estadual das Fontes do Ipiranga, SP (Schoenlein-Crusius & Milanez 1989). Posteriormente, os fungos ingoldianos foram observados durante a sucessão fúngica nas folhas de *Quercus robur* L. submersas em um lago no município de Itapeçerica da Serra, SP (Schoenlein-Crusius *et al.* 1990).

Os fungos observados em folheto misto, coletados esporadicamente em cachoeiras (“Cachoeira do Altarújo” no município de Rio Claro, SP) e córregos (“Rancho dos Nefelibatas” no município de Águas de Lindóia, SP) constam nas primeiras descrições taxonômicas (11 táxons) de espécies que ocorrem no Brasil (Schoenlein-Crusius & Milanez 1990a,b).

Na Reserva Biológica do Alto da Serra de Paranapiacaba, no município de Santo André, no Estado de São Paulo, algumas espécies de fungos ingoldianos foram observadas durante a sucessão de fungos na decomposição de folhas submersas de *Alchornea triplinervia* Spreng. (M.) Arg. (Schoenlein-Crusius & Milanez 1998).

Ainda na mesma região, foi conduzido o estudo comparativo da micota aquática associada à *Ficus microcarpa* L., *Quercus robur* L. e *Alchornea triplinervia* (Spreng.) Mull. Arg., submersas em um riacho, avaliando-se a elevada diversidade de espécies

(Schoenlein-Crusius *et al.* 1992), a viabilidade da quantificação dos conídios para dimensionar a capacidade de colonização dos substratos (Pires-Zottarelli *et al.* 1993) e estabelecer as possíveis correlações entre a diversidade da micota aquática (Hyphomycetes aquáticos e fungos zoospóricos), com os teores de alguns macro e microelementos presentes nos substratos durante a decomposição (Schoenlein-Crusius *et al.* 1998, 1999). Na mesma reserva, *Dendrosporomyces splendens* (Nawawi) Nawawi, uma espécie aquática típica, foi isolada de folheto de *Alchornea triplinervia* (Spreng.) M. Arg. e *Euterpe edulis* Mart. (Grandi 1998b, 1999). Sete táxons também foram isolados de folhas em decomposição de *Miconia cabussu* Hoehne. (Gusmão *et al.* 2001) e cinco de *Tibouchina pulchra* Cogn. (Grandi & Gusmão 2002).

Ainda na Mata Atlântica de Paranapiacaba, Moreira (2002) acompanhou a sucessão de fungos (geofungos e Hyphomycetes aquáticos) durante a decomposição de folhas de *Tibouchina pulchra* Cogn. submersas em um riacho. Observou-se a tendência de haver relação positiva entre o teor de matéria orgânica remanescente nas folhas e um maior número de táxons de fungos, devido à maior disponibilidade de matéria orgânica.

Na mesma Reserva foi realizado um estudo (I.H. Schoenlein-Crusius *et al.* comunicação pessoal), no qual foram realizadas coletas trimestrais de folheto misto submerso em locais ao longo de um riacho de porte médio, medindo-se concomitantemente alguns parâmetros abióticos da água. Através da técnica das lavagens sucessivas foram obtidos 21 táxons de fungos ingoldianos, com destaques para *Lunulospora curvula* Ingold, *Triscelophorus monosporus* Ingold e *Tripospermum myrti* (Lind.) Hughes, considerados típicos de ambientes tropicais e de maior ocorrência em épocas mais chuvosas. Nesse estudo foram obtidas pela primeira vez no Brasil algumas culturas de fungos.

Uma revisão detalhada dos estudos sobre a ecologia dos fungos incluindo os Hyphomycetes aquáticos na Reserva Biológica do Alto da Serra de Paranapiacaba consta em Schoenlein-Crusius *et al.* (2009a) e Capelari *et al.* (2009).

Com relação aos estudos realizados em regiões urbanas, no município de São Paulo alguns foram realizados na Represa do Guarapiranga e no Parque Estadual das Fontes do Ipiranga. Wellbaum *et al.* (1999), conduziram estudo preliminar sobre a diversidade de fungos filamentosos em ambiente aquático e terrestre na Ilha dos Eucaliptos localizada na Represa do Guarapiranga, verificando a influência do tipo de vegetação (eucalipto, mata de transição e mata nativa) sobre a diversidade dos fungos filamentosos isolados em meio de cultura. Posteriormente, Wellbaum *et al.* (2007), ampliaram o número de locais de coleta para comparar a micota presente nas folhas submersas com a das folhas presentes às margens secas da represa. Os autores obtiveram 20 táxons exclusivos para o ambiente aquático e 34 táxons de organismos que habitam o ambiente aquático e terrestre. Como os fungos foram isolados através da distribuição de discos de folhas sobre meios de cultura, foram obtidos predominantemente táxons de origem terrestre como *Pestalotia* sp., *Pestalotiopsis* spp., *Trichoderma* spp., *Cylindrocladium scoparium* Morgan e *Epicoccum nigrum* Link.

No Parque Estadual das Fontes do Ipiranga, que é uma das últimas reservas de Mata Atlântica de planalto no Estado de São Paulo, folhas senescentes de *Tibouchina pulchra* Cogn. foram acondicionadas em sacos de tela de náilon e submersas no Lago das Garças (hipereutrófico) e no Lago das Ninféias (meso-oligotrófico), Moreira (2006) e Moreira & Schoenlein-Crusius (2007) obtiveram 63 táxons de fungos das folhas coletadas no lago hipereutrófico e 79 táxons no lago meso-oligotrófico. Foi concluído que o estado trófico dos lagos influenciou não apenas quantitativamente, mas também

qualitativamente sobre os padrões de distribuição da sucessão fúngica, concentração de ergosterol e taxa de decomposição das folhas submersas.

Na mesma unidade de conservação, foi avaliada a diversidade de Hyphomycetes aquáticos em ambientes com diferentes níveis tróficos, através da coleta de amostras de folheto misto submerso de dez locais. Com base na observação de características microscópicas, obtiveram-se 24 táxons, com predomínio de *Anguillospora crassa* Ingold, *Lunulospora curvula* Ingold, *Tetrachaetum elegans* Ingold e *Camposporium pellucidum* (Grove) Hughes. O número de táxons foi maior em um lago meso a oligotrófico e menor em local submetido à contaminação orgânica. O predomínio e a intensa proliferação das espécies cosmopolitas, contrapondo-se aos raros registros de outras, indica possíveis efeitos do estado trófico sobre a micota, mesmo em locais considerados preservados. De acordo com a análise multivariada pode-se considerar que a maior variabilidade limnológica decorreu da trofia do sistema e, em seguida, do período climático (Schoenlein-Crusius *et al.* 2009b).

No Parque Municipal Alfredo Volpi, que é um dos resquícios da Mata Atlântica no planalto paulista, foram desenvolvidos dois estudos referentes à velocidade de decomposição e geofungos associados a folhas submersas. Fagundes (2008) obteve 39 táxons em *Caesalpinia echinata* Lam., o pau-brasil. Dias (2008) obteve 21 táxons de fungos associados às folhas de *Campomanesia phae* (O. Berg.) Landrum, o cambuci, durante a decomposição, com maior ocorrência de *Rhinocladiella* sp. (6), *Pyramidospora* sp.2 (6), *Tricelophorus* sp.2 (8) e *Endophragmiella* sp. (6).

Ainda em áreas urbanas, no Parque Municipal do Ibirapuera, São Paulo, SP, Schoenlein-Crusius *et al.* (2014) visando revelar a riqueza de espécies de Hyphomycetes aquáticos nas águas do Parque, obtiveram 27 táxons, sendo 15 de fungos

ingoldianos e 12 aquáticos facultativos, com predomínio de *Lunulospora curvula* Ingold, *Triscelophorus monosporus* Ingold, *Tripaspermum myrtii* (Lind.) S. J. Hughes, *Anguillospora longissima* (Sacc. et Syd.) Ingold e *Triscelophorus acuminatus* Nawawi.

Em alguns lagos do Parque Central, em Santo André, SP, Gruppi (2008) estudou a diversidade dos fungos microscópicos em folhas submersas para verificar diferenças entre locais de coleta, aplicação de diferentes métodos de isolamento e interações entre os fungos e variações de alguns parâmetros abióticos da água. Foram obtidos 38 táxons de fungos, divididos em 26 Hyphomycetes, 8 ingoldianos, um Coelomycetes, dois representantes de Zygomycota e um fungo zoospórico. Dentre estes, 28 táxons foram isolados em meio de cultura, sete através de incubação das folhas na água e cinco em folhas incubadas em aquários. Os fatores abióticos medidos na água provavelmente não limitaram a ocorrência de fungos, mas a presença de vegetação abundante no entorno dos locais de coleta influenciou a incidência de fungos, reforçando a importância da manutenção dos programas de plantio de novas árvores e planejamento paisagístico no parque, o que pode ser recomendado para ambientes aquáticos recém-criados.

Malosso (1999) comparou a diversidade dos Hyphomycetes aquáticos e o teor de ergosterol nas folhas submersas na Represa do Guarapiranga em São Paulo e no Sistema dos Rios Jacaré-Monjolinho em São Carlos, SP, verificando maior ocorrência e diversidade dos fungos no sistema lótico. Os fungos mais frequentes foram *Lunulospora curvula* Ingold, *Lemonniera aquática* H. J. Hudson, *Anguillospora longissima* Ingold e *Triscelophorus monosporus* Ingold.

Para a Mata Atlântica nordestina, Cavalcanti & Milanez (2007), visando ampliar o conhecimento sobre a diversidade de fungos em ambientes aquáticos, destacaram as espécies *Curvularia tuberculata* Jain, *Dendrosporium lobatum* Plakidas & Edgerton ex

Crane, *Dichotomophthoropsis nymphaeorum* (Rand) M. B. Ellis, *Phaeoisaria glauca* (Ellis & Everh.) Hoog & Papendorf e *Trichurus spiralis* Hasselbring por terem sido pouco referidas e não descritas anteriormente no Brasil.

Para o bioma caatinga na região nordeste brasileira, Almeida *et al.* (2012), durante o estudo de fungos conidiais aquáticos facultativos associados a substratos vegetais submersos na Serra da Fumaça e na Serra da Jibóia, Estado da Bahia, identificaram 17 espécies, sendo cinco novos registros: *Actinocladium verruculosum* W.P. Wupara para o continente americano, *Xylomyces aquaticus* (Dudka) K. D. Hyde & Goh para o Neotrópico, *Actinocladium longiramosum* (R.F. Castañeda) R.F. Castañeda e *Dischloridium inaequiseptatum* (Matsush.) Hol.-Jech. para a América do Sul e *Cacumisporium pleuroconidiophorum* (Davydkina & Melnik) R.F. Castañeda, Heredia & Iturr. para o Brasil.

Ainda na Serra da Jibóia, Barbosa *et al.* (2013), descreveram duas novas espécies de Hyphomycetes coletados de material submerso: *Dactylaria saccardoana* F.R. Barbosa & Gusmão e *Quadracaea stauroconidia* F.R. Barbosa & Gusmão.

No mesmo bioma, nas áreas Brejo Paraibano-PB, Serra da Jibóia-BA, Chapada do Araripe e Serra de Ibiapaba-CE, Fiuza & Gusmão (2013) identificaram 27 espécies de fungos ingoldianos, sendo 22 novos registros, sendo para o Continente Americano (3); para o Neotrópico (1); para a América do Sul (3), para o Brasil (5), para a Região semi-árida brasileira (7), para o Ceará (2) e para a Paraíba (1).

Ainda na região semiárida, como objetivo determinar a biodiversidade fúngica em três reservatórios eutróficos no estado da Paraíba, região nordeste do Brasil, Lima *et*

al. (2014) obtiveram 33 táxons de fungos, com predomínio de Hyphomycetes demátiáceos e hialinos.

Recentemente, Fiuza *et al.* (2015) descreveram os primeiros registros de fungos ingoldianos para a região amazônica brasileira. Neste estudo, os autores obtiveram 17 taxons, destes um é novo registro para as Américas, outras três para o Brasil e 13 para a região amazônica brasileira.

1.7. Caracterização da Mata Atlântica

A Mata Atlântica é formada por um conjunto de formações florestais (Florestas Ombrófila Densa, Ombrófila Mista, Estacional Semidecidual, Estacional Decidual e Ombrófila Aberta) e ecossistemas associados como as restingas, manguezais e campos de altitude, que se estendem originalmente por aproximadamente 1.300.000 km² em 17 estados do território brasileiro. Hoje os remanescentes de vegetação nativa estão reduzidos a cerca de 22% de sua cobertura original (Figura 2) e encontram-se em diferentes estágios de regeneração. Apenas cerca de 7% estão bem conservados em fragmentos acima de 100 hectares. Mesmo reduzida e muito fragmentada, estima-se que na Mata Atlântica existam cerca de 20.000 espécies vegetais (cerca de 35% das espécies existentes no Brasil), incluindo diversas espécies endêmicas e ameaçadas de extinção. Essa alta diversidade biológica é maior que a de alguns continentes (17.000 espécies na América do Norte e 12.500 na Europa) (Ministério do Meio Ambiente-Mata Atlântica 2014). Este bioma está entre os mais ameaçados hotspots de biodiversidade no mundo (Myers *et al.* 2000) e por isso a região da Mata Atlântica é altamente prioritária para a conservação da biodiversidade mundial. Em relação à fauna, os levantamentos já realizados indicam que a Mata Atlântica abriga 849 espécies de aves, 370 espécies de

anfíbios, 200 espécies de répteis, 270 de mamíferos e cerca de 350 espécies de peixes (Ministério do Meio Ambiente-Mata Atlântica 2014).

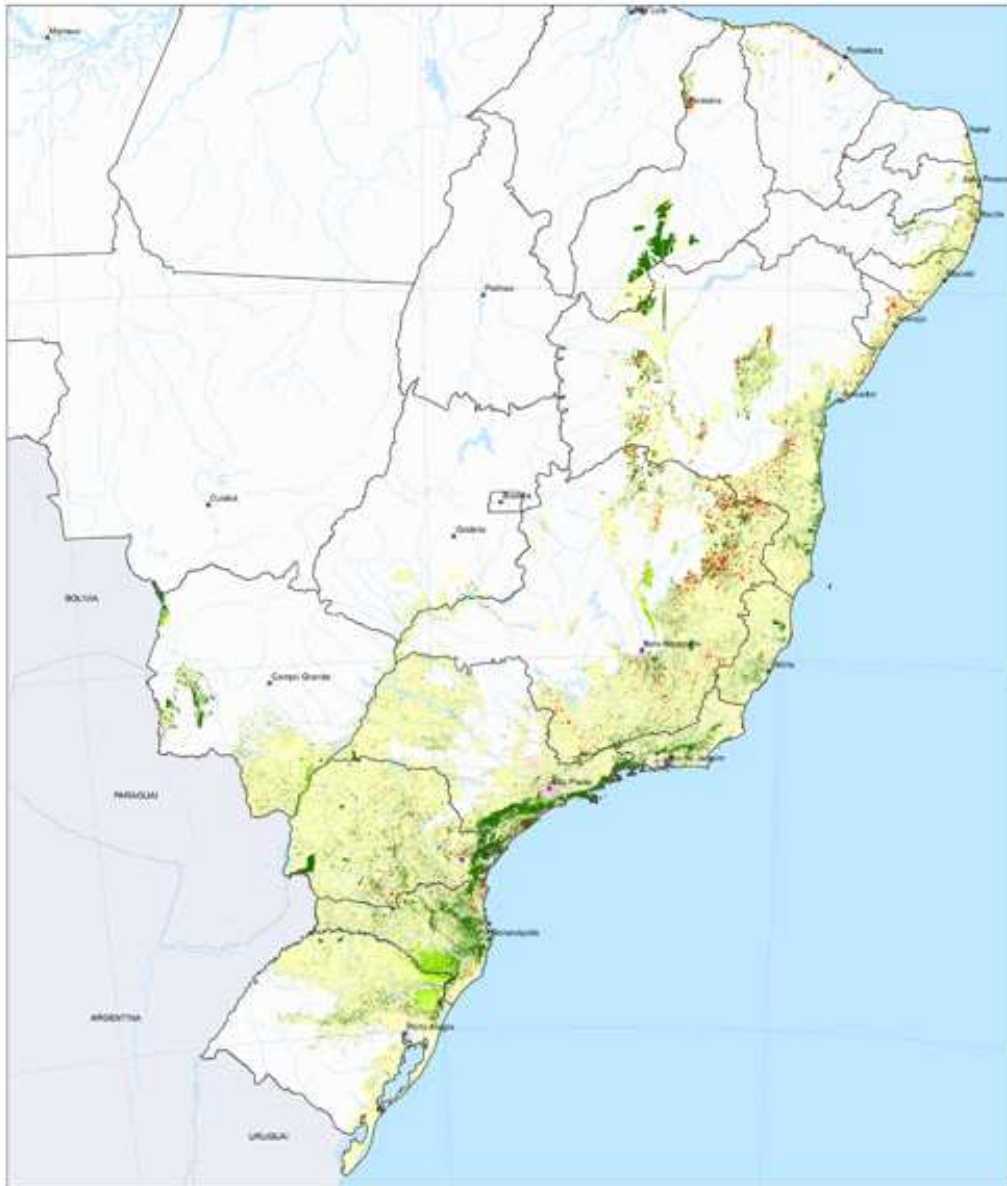


Figura 2. Mapa dos remanescentes florestais da Mata Atlântica (2011-2012). Em verde, as áreas de remanescente florestais da Mata Atlântica. Em amarelo, as áreas inicialmente cobertas pela Mata Atlântica.

Fonte: SOS Mata Atlântica/INPE.

1.8. Caracterização das áreas de estudo

1.8.1. Ilha de São Sebastião - Ilhabela

a. Dados históricos

A ocupação humana mais antiga no arquipélago de Ilhabela foi a dos sambaqueiros, grupos que tinham como atividade de subsistência principalmente a pesca, a caça de animais de pequeno porte e coleta de moluscos, crustáceos e de vegetais. Esses grupos deixaram como vestígios de sua ocupação os sambaquis, sítios arqueológicos formados pelo acúmulo de conchas de moluscos (Prefeitura Municipal de Ilhabela 2014a).

O arquipélago de Ilhabela pode ser caracterizado como uma área onde houve um intenso processo civilizatório, decorrente do início da sua ocupação pelos portugueses, pelos piratas e corsários europeus e argentinos interessados em saquear e dominar aquele espaço no início da época colonial (Simões 2005).

A Ilha de São Sebastião, quando descoberta por Américo Vespúcio em 1502, recebeu o nome do santo do dia, São Sebastião (Instituto Brasileiro de Geografia e Estatística-IBGE 2013). Anteriormente a ilha era denominada Maembipe pelos tupinambás, que eram o povo indígena natural da Ilhabela (Prefeitura Municipal de Ilhabela 2014b). Contudo, o litoral norte só começou a ser povoado pelos colonizadores a partir de 1608, quando os primeiros sesmeiros se estabeleceram no canal de São Sebastião para desenvolver a atividade canavieira. Após o período canavieiro, o cultivo de café teve seu início na ilha. Este cultivo, assim como a canavieira, contava com a

mão de obra escrava, que cuidava de todo o processo, desde a plantação até o ensacamento (Simões 2005).

As atividades da lavoura do café duraram, em Ilhabela, oitenta anos, sendo um período caracterizado por enorme crescimento econômico, intensificando as atividades comerciais e culturais e enriquecendo os fazendeiros locais. A população na ilha chegou a alcançar a casa dos dez mil habitantes. No entanto, a produção cafeeira na ilha chegou até a atingir a cota altimétrica de 400 metros (altitude acima do nível médio do mar), ocasionando a devastação da Mata Atlântica nativa (Secretaria do Meio Ambiente 2005, Peres & Barbosa 2008). Atualmente a área compreendida pelo parque possui seus limites definidos por cotas altimétricas que variam de 200 metros (na região ao longo do canal de São Sebastião), 100 metros (a partir da Ponta da Sela ao sul e Ponta das Canas ao norte) e a 0 metro (à medida que se aproxima da Ponta do Boi) (Parque Estadual de Ilhabela-SMA 2014).

Após a abolição da escravatura em 1888, houve um declínio econômico tendo a população subsistindo da produção agrícola, da pesca e da caça, configurando o modo de vida que hoje é chamado de caiçara (Simões 2005, Peres & Barbosa 2008).

Em 2012, o Instituto Histórico, Geográfico e Arqueológico de Ilhabela (IHGAI), ligado à Secretaria da Cultura, realizou estudos para a identificação e cadastramento de sítios arqueológicos em todo o arquipélago, através do projeto de Gestão e Diagnóstico do Patrimônio Arqueológico de Ilhabela (Gedai) (Prefeitura Municipal de Ilhabela 2012). Os levantamentos documentais resultaram em descobertas inéditas para a ilha, que ajudaram a desvendar a história das praias da região, como a do Bonete, além de preservar a história do arquipélago (Prefeitura Municipal de Ilhabela 2013).

b. Parque Estadual de Ilhabela (PEIb)

O Parque Estadual de Ilhabela (Figura 3) está localizado no município-arquipélago de Ilhabela. Este município compõem, em sua totalidade, as ilhas de São Sebastião, Búzios, Vitória, as ilhotas dos Pescadores, Sumítica, Serraria, Cabras, Figueira, Castelhanos, Lagoa e Enchovas, com uma área de 348 km², que compreendem a sua dimensão territorial, distribuída entre as coordenadas geográficas 23°46'28" Sul e 45°21'20" Oeste (Simões 2005, Parque Estadual de Ilhabela-SMA 2014). Constitui um importante remanescente de Mata Atlântica, com aproximadamente 27.025 hectares (Parque Estadual de Ilhabela-SMA 2014).

A Ilha de São Sebastião, separada pelo continente pelo Canal de São Sebastião, caracteriza-se por ser a maior ilha do litoral sudeste brasileiro (Pollete 1993), possuindo uma área de aproximadamente 337km² (Lima *et al.* 2008).

O Parque foi fundado com o intuito de preservar a vegetação da Mata Atlântica, de restinga, os campos de samambaias, os animais em extinção (como a jacutinga e o macuco), as espécies endêmicas (como o cururuá), sendo criado em 1977, pelo Decreto Estadual 9.414 (Maldonado 1997, Parque Estadual de Ilhabela-SMA 2014), medida que visava à recuperação de áreas degradadas pelas atividades econômicas que ocorreram no local por décadas e a preservação dos ecossistemas sobreviventes à exploração colonial. Esta Unidade de Conservação está sob a administração do Instituto Florestal da Secretaria Estadual do Meio Ambiente, e compreende cerca de 80% da área do município (Simões 2005, Parque Estadual de Ilhabela-SMA 2014).

Segundo a classificação climática de Koeppen, o Parque Estadual de Ilhabela apresenta o tipo climático **Am** que caracteriza o clima tropical chuvoso, com inverno

seco onde o mês menos chuvoso tem precipitação inferior a 60mm. O mês mais frio tem temperatura média superior a 18°C (Miranda *et al.* 2014).

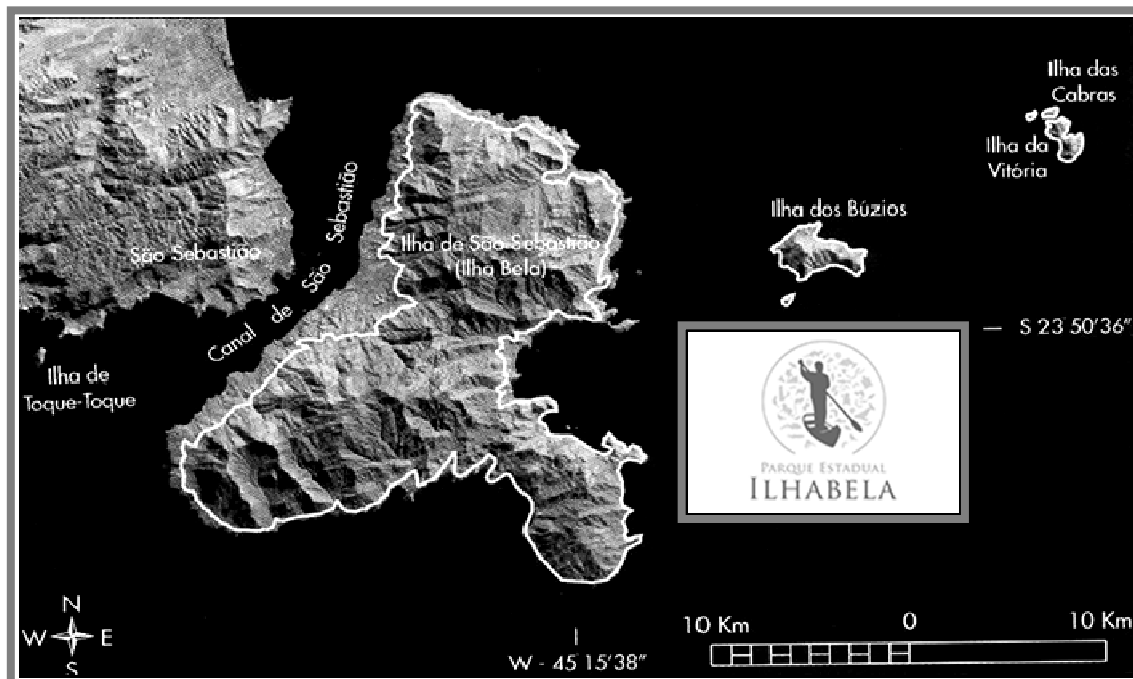


Figura 3. Mapa de localização do Parque Estadual de Ilhabela. Contorno em branco demarcando a área do Parque.

Fonte: <http://www.ecoturismoaventura.com.br/brasil-mapa/ilhabela.htm>

c. Estudos sobre fungos no Parque Estadual de Ilhabela

Paralelamente ao presente estudo foi conduzido o primeiro levantamento de líquens no Parque Estadual de Ilhabela pelo Pesquisador científico Dr. Michel Navarro Benatti do Instituto de Botânica, que identificou 12 gêneros de 5 famílias de fungos liquenizados folhosos (Benatti, 2013) e 34 táxons de *Parmotrema* (Benatti 2014) no parque.

Até o presente momento, não há registro de outros trabalhos sobre fungos no Parque Estadual de Ilhabela.

1.8.2. Ilha do Cardoso

a. Dados históricos

A ocupação da Ilha do Cardoso é datada no período de 5.260 ± 100 anos AP (antes do presente) a 3.170 ± 95 anos AP, constituídos de uma população de canoieiros, conchófagos e ictiófagos (Ab' Saber & Besnard 1953, Mendonça 2000). Quando os portugueses chegaram, a nação Carijó, do grupo tupi-guarani já ocupava o território a aproximadamente 300 anos (Soares & Lana 1994, *apud* Sugiyama 2003), sendo a principal fonte de alimentação a pesca (Behr 1992, *apud* Sugiyama 2003). No ano de 1501 a Vila de Cananéia foi fundada por Américo Vespúcio (Mendonça 2000).

No período provincial, a Ilha do Cardoso foi um dos lugares mais habitados na Vila de Cananéia, com diversas fazendas, fábricas de aguardente, olarias e estaleiros destinados à construção naval (Almeida 1946).

Nos séculos XVII e XVIII, a região de Cananéia era próspera com a mineração, agricultura de arroz e mandioca, construção naval e exportações (Mendonça 2000).

No século XIX, houve um grande êxodo para fora da ilha devido à decadência econômica da região decorrente do declínio dos portos de Iguape e Cananéia, além de mudanças nas formas de subsistência da população remanescente que aumentaram a exploração de palmito que já ocorria na região desde 1840 (Pio *et al.* 1985 *apud* Mendonça 2000). A agricultura passou a ser substituída pela pesca de subsistência e para o comércio de pescado levando ao êxodo da área rural para a urbana (Sugiyama 2003)

No período de criação do Parque, em 1962, a Ilha do Cardoso apresentava uma considerável ocupação por comunidades caiçaras com base agrícola. Contudo, não há registro de populações indígenas autóctones da ilha nos últimos 350 anos (Olmos *et al.* 2004).

Nos tempos modernos, os primeiros indígenas foram introduzidos na ilha no final de 1992, sendo um grupo de quatro Mbyá provenientes das ilhas do Superagüi e das Peças, no Paraná, migrados de barco para o PEIC na localidade denominada Sítio Grande. Esta migração foi promovida por membros de ONGs socioambientais da região de Cananéia (Oesp 18/11/2001, *apud* Olmos *et al.* 2004).

Outros Guaranis começaram a migrar para o local, sempre contando com o transporte oferecido por terceiros, uma vez que não contavam com embarcação própria nem sabiam navegar (Olmos *et al.* 2004).

Em 1995 havia 46 índios vivendo na Ilha do Cardoso (incluindo quatro provenientes de Itanhaém), 76 em 1999 e 35 em 2002. Em 2004, existiam cerca de 45 índios Guarani Mbyá distribuídos em pelo menos duas clareiras diferentes, situadas na região centro-norte do PEIC, classificada no Plano de Manejo como zona de recuperação (Secretaria do Meio Ambiente 2002, Olmos *et al.* 2004). Praticavam a agricultura tradicional de roças de coivara, plantando principalmente batata, mandioca (aipim) e milho (Felipim 2001).

b. Parque Estadual da Ilha do Cardoso (PEIC)

O Parque Estadual da Ilha do Cardoso (Figura 4) está situado no Município de Cananéia, extremo sul do Estado de São Paulo. Possui uma área de 13.500 hectares e encontra-se nas coordenadas geográficas 48°05'42" Oeste e 25°18'18" Sul. O Parque foi

criado pelo Decreto Estadual nº 40.319 de 03/07/1962, estando sob responsabilidade do Instituto Florestal da Secretaria de Estado de Meio Ambiente de São Paulo (Parque Estadual da Ilha do Cardoso-SMA 2014).

O Parque possui grande diversidade florestal de Mata Atlântica costeira como restinga, florestas costeiras e manguezais. A leste o parque é banhado pelo Oceano Atlântico, a oeste pelo Canal de Ararapira, ao norte pela Baía de Trapandé e ao sul pela Barra de Ararapira (Parque Estadual da Ilha do Cardoso-SMA 2014).

A criação do Parque propiciou a preservação de suas diversas formações vegetais do domínio Mata Atlântica, como restinga, florestas costeiras e manguezais, além de ampliar o desenvolvimento de pesquisas em diversas linhas realizadas pelo Instituto de Botânica, Instituto Florestal e Universidades (Sugiyama 2003, Parque Estadual da Ilha do Cardoso-SMA 2014).

Segundo a classificação de Koeppen, o clima da Ilha do Cardoso é do tipo **Cfa**, mesotérmico úmido, sem estação seca, com alta pluviosidade e umidade relativa elevada (Sugiyama & Mantovani 1993, Miranda *et al.* 2014).

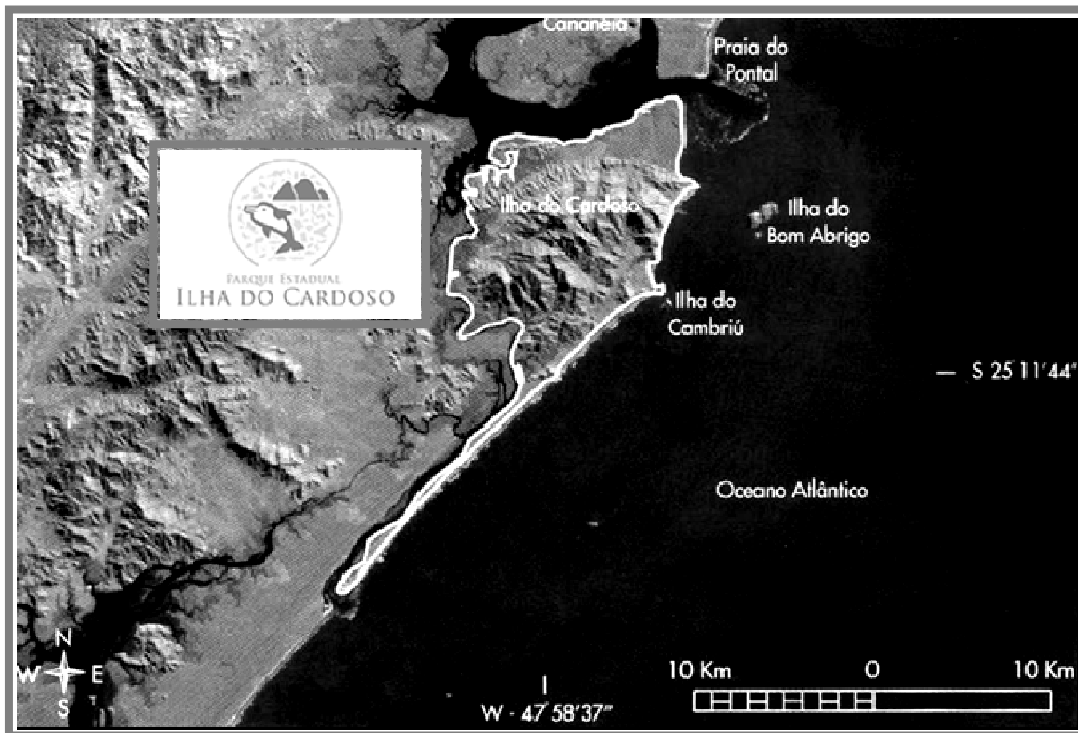


Figura 4. Mapa da localização do Parque Estadual da Ilha do Cardoso. Contorno em branco demarcando a área do Parque.

Fonte: <http://www.ecoturismoaventura.com.br/brasil-mapa/ilha-do-cardoso.htm>.

c. Estudos sobre fungos no Parque Estadual da Ilha do Cardoso

Os primeiros estudos da diversidade fúngica no Parque Estadual da Ilha do Cardoso foram realizados por Bononi (1979a,b,c, 1984), com representantes do filo Basidiomycota das famílias Hymenochaetaceae, Stereaceae e Thelephoraceae, além das famílias da ordem Aphyllophorales. Ainda para o mesmo filo, Bononi *et al.* (1984), em coletas bimestrais de 1975 a 1983 no PEIC, identificaram 14 espécies pertencentes a 8 famílias de gasteromicetos. No mesmo ano, Bononi & Capelari, identificaram 11 espécies de Tremellales no PEIC. Capelari (1989) efetuou o levantamento de 12 famílias, 28 gêneros e 73 espécies de Agaricales e Gugliotta & Capelari (1995) identificaram 33 táxons de Polyporaceae classificados em 32 espécies e 19 gêneros.

Gugliotta & Bononi (1999), em um levantamento das espécies de Polyporaceae que ocorrem na Ilha do Cardoso, identificaram 32 espécies e 19 gêneros.

Para o filo Glomeromycota, Trufem *et al.* (1989) identificaram 14 espécies de fungos micorrízicos vesículo-arbusculares em dunas do PEIC em coletas realizadas no período de 1988-1989. Trufem *et al.* (1994) coletaram 410 amostras de solo de rizosfera de plantas do litoral arenoso da Ilha do Cardoso, com finalidade de se conhecer a micota de fungos micorrízicos arbusculares-fMA, obtendo 24 espécies.

Souza (2010) avaliando a diversidade de fungos anamórficos do solo em áreas de preservação, incluindo o PEIC, verificou 59 espécies de 40 gêneros, com destaque para *Trichoderma* sp., *Paecilomyces carneus* (Duche et Heim) Brown et Smith, *Chloridium virescens* var. *caudigerum* (Höhn.) W. Gams & Hol. e *Penicillium funiculosum* Thom, como os táxons mais frequentes.

Com relação aos organismos aquáticos da Ilha do Cardoso, Jerônimo *et al.* (2015) analisou a riqueza e a diversidade dos fungos zoospórios pertencentes aos fillos Blastocladiomycota e Chytridiomycota, coletados do solo e em corpos d'água. Obteve 256 isolados, sendo 112 da água e 144 do solo, abrangendo 34 táxons (31 pertencentes ao filo Chytridiomycota e 3 ao filo Blastocladiomycota).

Águas moderadamente turbulentas, bem aeradas e limpas favorecem a produção de maior quantidade de conídios dos Hyphomycetes aquáticos, justificando a escolha de águas correntes para este estudo, onde se prevê a captura dos mesmos para fins de cultivo. Além disso, trata-se de um trabalho inédito em áreas insulares brasileiras e para estas reservas de Mata Atlântica do litoral do Estado de São Paulo, pois o grupo dos Hyphomycetes aquáticos, alvo deste trabalho, jamais foi explorado em estudos

anteriores. Dessa forma, estudos sobre biodiversidade são cruciais para aumentar o conhecimento sobre a micodiversidade, especialmente no Bioma da Mata Atlântica, que é um importante *hotspot* de biodiversidade mundial e que sofre grande pressão antrópica.

2. OBJETIVOS

- ✦ Analisar a diversidade das espécies de Hyphomycetes aquáticos no Parque Estadual de Ilhabela e no Parque Estadual de Ilha do Cardoso, SP, através do levantamento taxonômico em ambientes aquáticos de água doce lóticos, com ênfase especial para as quedas d'água, iniciando o estudo da taxonomia desses fungos em ecossistemas insulares.

- ✦ Avaliar a aplicabilidade da técnica de cultivo proposta por Descals (2005) para obtenção de culturas axênicas de Hyphomycetes aquáticos, para fins de documentação taxonômica e futuros estudos moleculares.

3. MATERIAL E MÉTODOS

3.1. Áreas de estudo

Para o levantamento dos Hyphomycetes aquáticos em sistemas insulares foram selecionadas duas unidades de conservação estaduais: Parque Estadual de Ilhabela (PEIb), na Ilha de São Sebastião, município de Ilhabela (SP), litoral norte e o Parque Estadual da Ilha do Cardoso (PEIC), município de Cananéia (SP), litoral sul (Figura 5) (Parque Estadual de Ilhabela-SMA 2014, Parque Estadual da Ilha do Cardoso-SMA 2014).

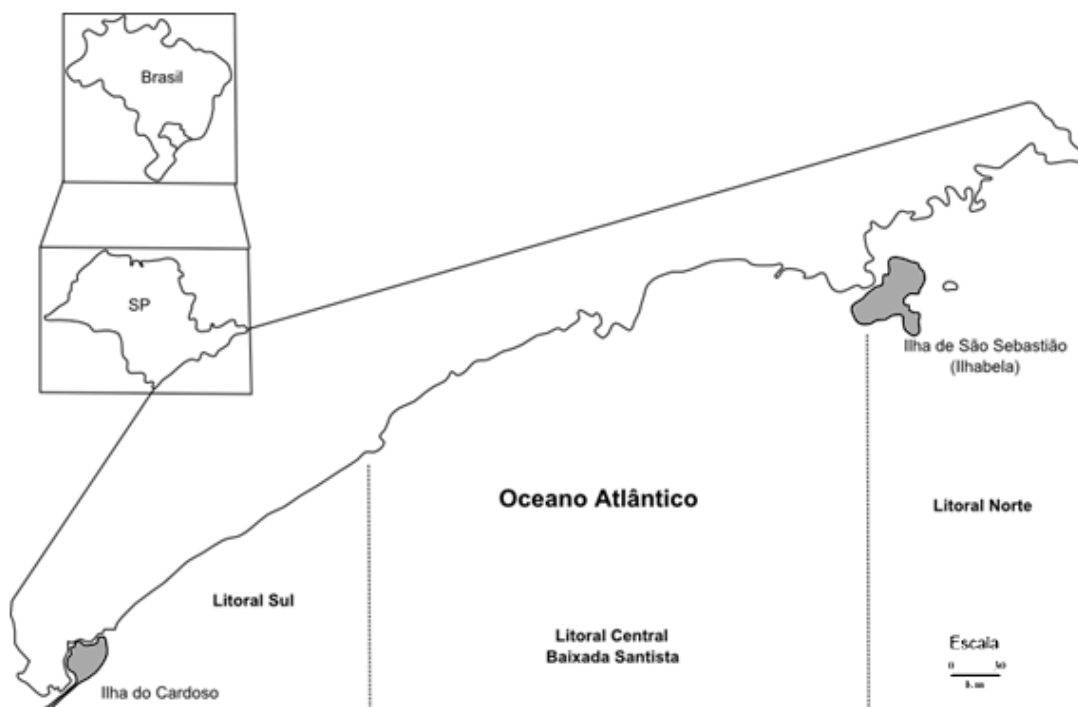


Figura 5. Localização da Ilha do Cardoso e de Ilhabela no litoral de São Paulo.

Fonte: Modificado de Coelho *et al.* (2010).

3.2. Expedições de coleta

Na Ilhabela as coletas foram realizadas em junho (1ª coleta), setembro (2ª coleta) e dezembro de 2012 (3ª coleta) e em maio de 2013 (4ª coleta), visitando-se 17 diferentes locais de coleta (Figura 6), durante todo o período de estudo. Os locais de coleta localizaram-se de forma bem distribuída pela ilha, abrangendo tanto o lado voltado para a Baía de São Sebastião (exemplos: locais de 1 a 12) quanto o lado voltado para o mar aberto do Oceano Atlântico (por exemplo, os locais de 13 a 17). Na 1ª coleta foram visitadas a Cachoeira da Lage, a Cachoeira do Veloso e a Cachoeira dos Três Tombos; na 2ª coleta, Poço da Pedra, Poço da Escada, Poço da Ducha, Poço do Jabuti, Poço do Jequitibá, Córrego lado norte da ilha e Cachoeira do Centro da Ilha; na 3ª coleta os locais Córrego do Engenho d'água, Cachoeira da Toca, Cachoeira da água Branca e Ribeirão do Pombo e na 4ª coleta os locais Cachoeira do Gato, Rio da Cachoeira do Gato, Córrego do Cemitério 1 e 2, Rio dos Castelhanos, Rio Caminho da Praia Vermelha, Cachoeira do Cocáia e Cachoeira do Tesouro da Colina (Figura 8).

Na Ilha do Cardoso foram realizadas coletas em junho (1ª coleta), agosto (2ª coleta) e novembro de 2012 (3ª coleta) e fevereiro de 2013 (4ª coleta), visitando-se 5 locais de coleta (Figura 7), localizados em torno do Rio Perequê, que representa o principal rio de água doce da ilha. Na 1ª coleta foram visitados os locais Cachoeira do Perequê, Rio Perequê e Córrego; na 2ª coleta, os locais Cachoeira do Perequê, Rio Perequê e Poço das Antas; na 3ª coleta os locais Cachoeira do Perequê, Rio Perequê, Córrego e Poço das Antas e na 4ª coleta os locais Cachoeira do Perequê, Rio Perequê, Córrego, Poço das Antas e Rio Cor de Chá.

Portanto, em Ilhabela, o delineamento de coleta foi o de distribuição geográfica, ou seja, abrangendo o maior número de locais, não repetindo os mesmos. Já na Ilha do

Cardoso (Figura 9), seguiu-se o esquema de coleta repetida nos mesmos locais (exceto para o Rio Cor de Chá que foi incluída na quarta coleta).

Os procedimentos das atividades em campo foram os mesmos nas duas ilhas estudadas. As amostras de folheto misto submerso foram coletadas em triplicata para cada local de coleta em todas as expedições ao campo. Em cada local de coleta, com auxílio de uma pinça, foram selecionadas cerca de 10 folhas submersas em decomposição, que foram colocadas em potes de polipropileno de boca larga com capacidade para 200 mL, aos quais foi acrescentada água do local suficiente para manter as folhas submersas (Figura 10 A-C). Caso, no local de coleta, fosse observada presença de espumas naturais, estas também eram coletadas junto com a água e as folhas submersas. Em seguida, as amostras foram acondicionadas em caixas térmicas e encaminhadas para o laboratório do Núcleo de Pesquisa em Micologia do Instituto de Botânica de São Paulo.

A fim de caracterizar os locais de coleta, alguns fatores abióticos (oxigênio, condutividade elétrica, pH e temperatura) foram mensurados com o auxílio de sondas multiparâmetro da marca Horiba U10 e U51 (Figura 11).



Figura 6. Locais de Coleta na Ilha de São Sebastião - Ilhabela. 1. Cachoeira da Lage. 2. Cachoeira do Veloso. 3. Cachoeira dos Três Tombos. 4. Ribeirão do Pombo. 5. Cachoeira da Água Branca. 6. Cachoeira da Toca. 7. Cachoeira do Centro da Ilha. 8. Córrego do Engenho d'água. 9. Cachoeira da Cocáia. 10. Poço da Pedra, Poço da Escada, Poço da Ducha, Poço do Jequitibá e Poço do Jabuti. 11. Cachoeira do Tesouro da Colina. 12. Córrego Lado Norte da Ilha. 13. Rio caminho da praia Vermelha. 14. Rio dos Castelhanos. 15. Córrego do cemitério (braço 1 e 2). 16. Rio da Cachoeira do Gato. 17. Cachoeira do Gato.

Fonte: Modificado de <http://www.ecoturismoaventura.com.br/brasil-mapa/ilhabela.htm>.



Figura 7. Locais de Coleta na Ilha do Cardoso. 1. Cachoeira do Perequê. 2. Rio Cor de Chá. 3. Córrego. 4. Poço das Antas. 5. Rio Perequê.

Fonte: Modificado de Yokoya *et al.* (1999).



Figura 8. Alguns locais de coleta na Ilha de São Sebastião-Ilhabela. A. Rio Caminho da Praia Vermelha. B. Cachoeira da Lage. C. Cachoeira dos Três Tombos. D. Rio dos Castelhanos. E. Cachoeira do Veloso. F. Cachoeira do Cocáia. G. Cachoeira do Gato. H. Cachoeira da Toca.



Figura 9. Locais de coleta na Ilha do Cardoso (PEIC-Núcleo Perequê). A. Núcleo Perequê. B. Cachoeira do Perequê. C. Rio Perequê. D. Córrego. E. Poço das Antas. F. Rio Cor de Chá.



Figura 10. A-C. Coleta de amostras em campo. D-F. Incubação das amostras no laboratório.



Figura 11. Mensuração dos fatores abióticos: A. Utilização da sonda multiparâmetro Horiba U10. B. Sonda multiparâmetro Horiba U51.

3.3. Incubação das amostras de folhas

No laboratório, as folhas de cada amostra, assim que chegavam do campo, foram acondicionadas em placas de Petri esterilizadas, sobre as quais foi anotado o local, o número e a data da coleta. Em seguida as placas foram completadas com água destilada esterilizada (Figura 10D-F) segundo Ingold (1975). Em cada placa foram colocadas, em média, cinco folhas ou fragmentos de folhas, dependendo do tamanho das mesmas. Estas placas foram mantidas em sala climatizada a aproximadamente 20°C.

3.4. Análise microscópica e identificação taxonômica

Do 5º ou 7º dia em diante de incubação, foi iniciada a análise microscópica do micélio, dos conidióforos e conídios dos Hyphomycetes aquáticos. O preparo das lâminas para observação microscópica consistiu na montagem de fragmentos de folhas (especialmente as áreas das bordas para aumentar a chance de se obter estruturas como conidióforos) ou de gotas da água de incubação entre lâminas e lamínulas de vidro, acrescidas somente de água destilada esterilizada quando necessário (Figura 12).

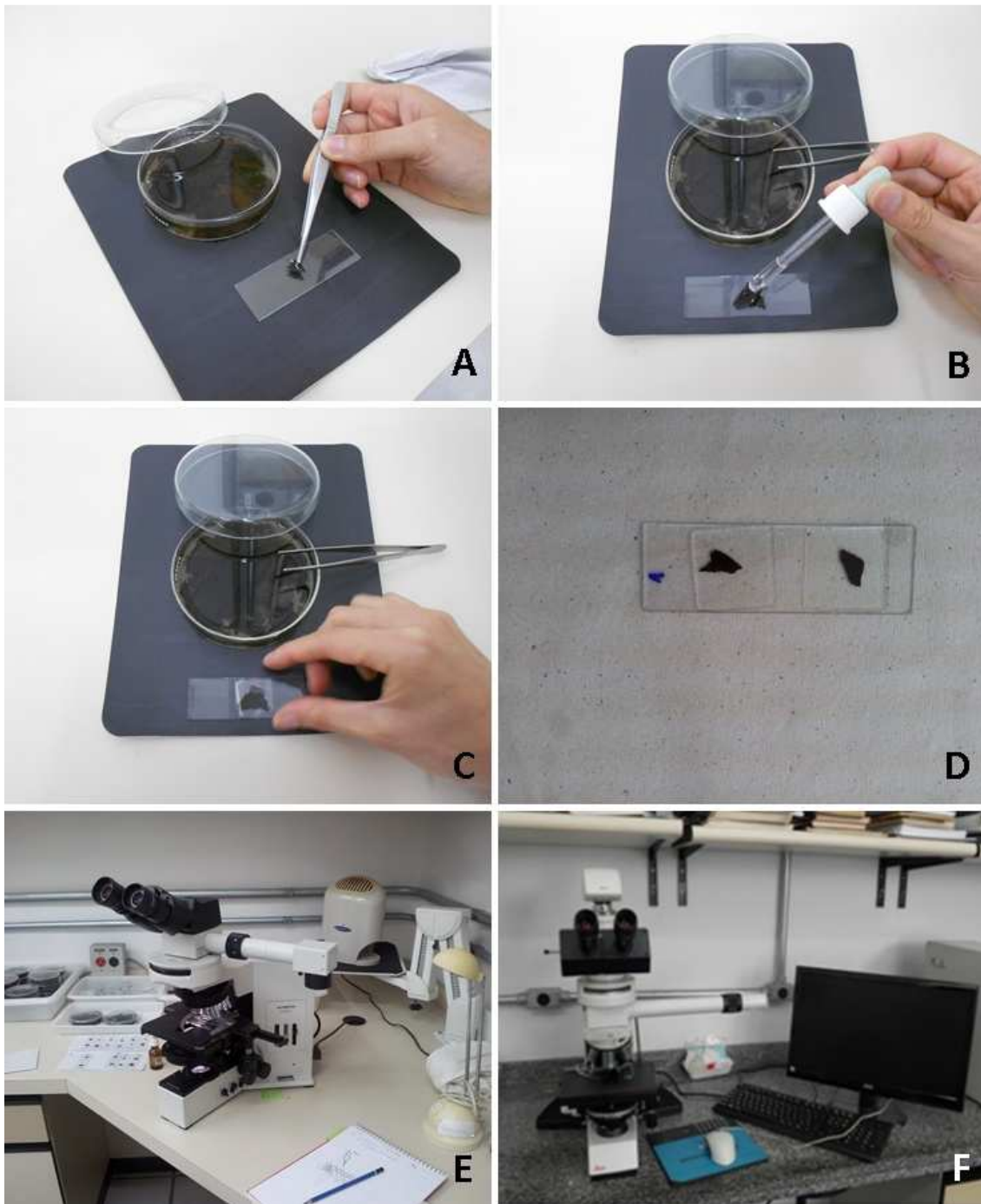


Figura 12. Preparo de lâminas com fragmentos de folha: A-C. Confeção de lâminas. D. Lâmina pronta para observação. E. Observação das estruturas em microscópio óptico para observação e medidas. F. Microscópio óptico para documentação fotográfica.

Para obter conídios que poderiam estar flutuando na lâmina d'água, a superfície da água das placas de Petri com folhas incubadas foi "varrida" com o auxílio de uma lamínula de vidro fixado em uma pinça. Em seguida, essa lamínula foi colocada sobre

uma lâmina de vidro contendo uma gota de resina PVLG-álcool polivinil lacto glicerol (Morton *et al.* 1993) ou água destilada e observada ao microscópio óptico (Figura 13). Ao passar com a lamínula sobre a água, os conídios que estavam flutuando na lâmina d'água ficaram aderidos à mesma. Esse procedimento permitiu a observação de um maior número de conídios, principalmente dos aeroaquáticos.

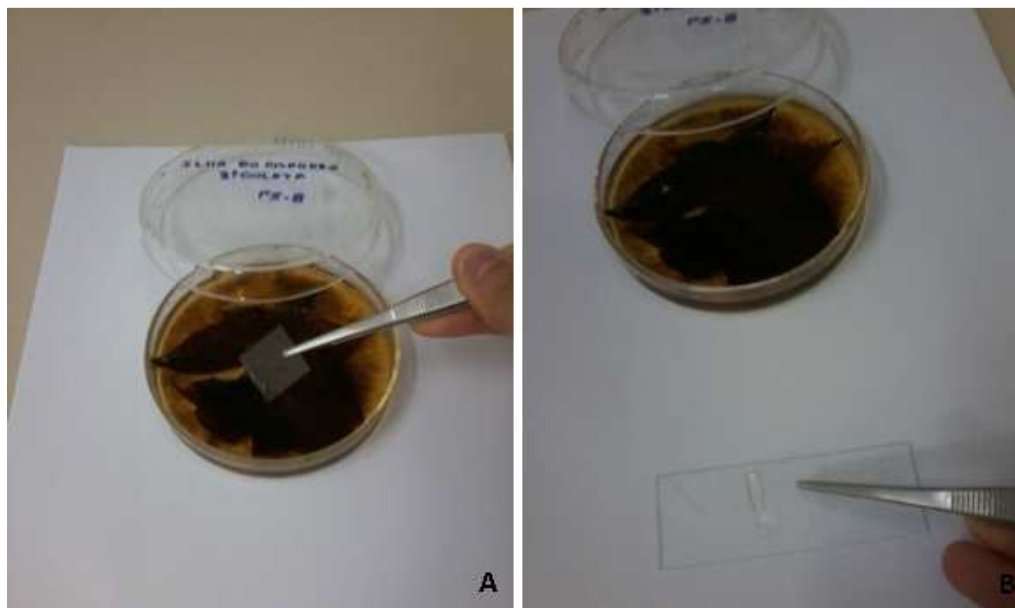


Figura 13. Preparo de lâminas com a técnica de “varrer” a superfície d’água: A. Superfície d’água sendo “varrida” com o auxílio de uma lamínula. B. Colocação da lamínula com os conídios aderidos sobre uma lâmina de vidro.

Para a observação de características de valor taxonômico, foi utilizado o microscópio óptico modelo Olympus BX50 (Figura 12E) e consultadas as chaves de identificação específicas (Ingold 1975, Marvanová 1997, Santos-Flores & Betancourt-Lópes 1997), sendo a documentação realizada através de fotografias digitais das estruturas dos fungos no microscópio Leica DM LB2 com auxílio de um analisador de imagens Leica DFC 280 (Figura 12F) e através de ilustrações confeccionadas em câmara clara.

As observações ao microscópio foram feitas por no mínimo um mês ou até que se repetissem as espécies, ou seja, até que não se observasse algum novo fungo nas amostras.

Para a preservação das estruturas, lâminas permanentes foram confeccionadas. Para isso, foram retirados fragmentos de folhas incubadas nas placas de Petri com o auxílio de uma pinça ou gotas de água contendo conídios retirados com uma pipeta de Pasteur. Estes então foram colocados em lâminas com resina PVLG e cobertas com uma lamínula (Morton *et al.* 1993). Caso a lâmina estivesse adequada a ser incorporada ao Laminário do Herbário Maria Eneida P. Kaufmann Fidalgo (SP) do Instituto de Botânica, estas tinham as bordas vedadas com esmalte de unha incolor.

Adicionalmente para os resultados do Capítulo IV, foi calculada a frequência da ocorrência dos táxons baseada em Dajoz (1973), calculando o número de áreas com a espécie i pelo número de áreas estudadas $\times 100$. Espécies com frequência de ocorrência $\geq 50\%$ foram classificadas como constante; com frequência entre 25 e 50% como acessórias e aquelas com frequência $< 25\%$ como ocasionais (Dajoz 1973).

3.5. Obtenção de culturas

O método para a obtenção de culturas puras dos Hyphomycetes aquáticos baseou-se no descrito por Descals (2005), segundo o qual, quando é observada a presença de quantidade satisfatória de conídios nas placas de Petri a partir da análise microscópica, estes devem ser retirados das placas com o auxílio de pipeta de Pasteur, transferidos para a superfície de meio de extrato de malte Agar-MEA 0,1% (Descals 1997) acrescido de cloranfenicol. No reverso das placas, deve ser desenhada a figura de uma escada de parede e a posição dos conídios deve ser marcada no primeiro degrau da mesma.

Assim que o conídio germina, é deslocado para o próximo degrau da “escada”, até estar distante das contaminações causadas por bactérias e por outros fungos, obtendo-se assim, o inóculo para uma cultura axênica.

No intuito de aperfeiçoar a obtenção de culturas axênicas, foram realizadas algumas modificações do método descrito acima: gotas de água ou fragmentos das folhas contendo os conídios dos Hyphomycetes aquáticos foram colocadas em placas de Petri com meio de cultura MEA 0,1% com cloranfenicol (Descals 1997). Em câmara de fluxo laminar vertical, assim que as gotas foram depositadas sobre o meio, foi feita uma leve agitação para espalhá-las sobre toda a superfície. Em seguida, o reverso destas placas foi visualizado ao microscópio ótico para marcar a localização dos conídios de interesse com auxílio de uma caneta de retroprojeter de determinada cor. Com outra cor de caneta de retroprojeter foram marcados os conídios de fungos contaminantes. Assim foi possível avaliar a distância de um conídio de interesse com um contaminante, facilitando a repicagem. As placas foram mantidas em câmara climática para BOD, sob temperatura de 21°C. Assim que os conídios começavam a germinar (24 horas aprox.), alíquotas do novo micélio foram transferidas para novas placas de Petri contendo meio MEA 1,6% [8g de extrato de malte Acumedia®; 6,5g de Agar bacteriológico Acumedia®; 0,0125g de cloranfenicol cristalizado Sigma Aldrich®; 500ml de água destilada] (modificado de Thornton 1963) (Figura 14).

Além do MEA 0,1% (Descal 1997) e MEA 1,6% (modificado de Thornton1963), o meio Ágar Lignocellulose (LCA) [Glicose 1g; KH₂PO₄ 1g; MgSO₄.7H₂O 0,2g; KCl 0,2g; NaNO₃ 2g; estrato de levedura 0,2g; ágar 13g; água destilada 1L] proposto por Miura & Kudo (1970) para Hyphomycetes aquáticos, também foi testado, apresentando um crescimento considerado bom quando comparado com o MEA 0,1%. Contudo, foi

observado que com MEA 0,1% com cloranfenicolos *Hyphomycetes* aquáticos obtiveram um crescimento mais rápido, sendo então este último escolhido para a sequência do estudo.



Figura 14. Isolamento: A e B. Inoculação dos conídios em meio de cultura. C e D. Marcação do posicionamento dos conídios de interesse. E. Retirada dos conídios para novo meio de cultura. F. Culturas axênicas obtidas pelo processo.

Em câmara de fluxo laminar vertical, os conídios dos fungos aeroaquáticos, que podiam ser observados a olho nu na lâmina d'água das amostras incubadas em placas de

Petri (Figura 15 A-B), foram capturados com auxílio de uma agulha esterilizada e depositados sobre a superfície do meio de extrato de malte (0,1%) contido em placas de Petri e incubados em câmara climática a 21°C por 7 a 10 dias.

A confirmação de que as culturas obtidas eram de Hyphomycetes aquáticos, foi realizada através do retorno de alíquotas (aproximadamente 1cm²) das colônias para placas de Petri contendo 10mL de água destilada esterilizada, para produção de conídios que variava de 7 a 15 dias de incubação em sala climatizada a aproximadamente 20°C (Figura 15 C). Lâminas foram preparadas com fragmentos do micélio e da água destilada para verificar a presença de conídios ao microscópio óptico.

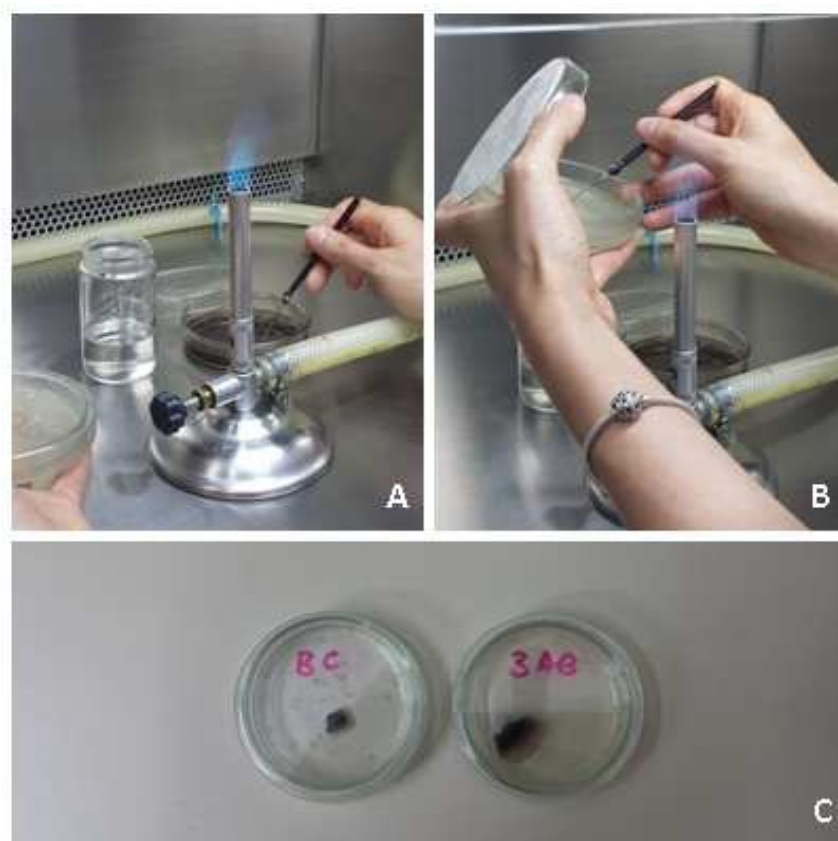


Figura 15. A. Captura dos conídios flutuantes com o auxílio de uma agulha esterilizada. B. Inoculação dos conídios em meio de cultura. C. Retorno de alíquotas das colônias para confirmação do isolamento.

3.6. Preservação das culturas

A preservação das culturas dos Hyphomycetes aquáticos foi realizada através da aplicação dos métodos já em uso na Coleção de Culturas de Fungos do Instituto de Botânica (Schoenlein-Crusius *et al.* 1994, Figueiredo 2001) (Figura 16), a seguir relatados.

✧ *Repicagens periódicas*

De acordo com este método, os fungos são repicados de 3 a 4 vezes por ano, mantendo-se os mesmos em tubos de ensaio ou em frascos do tipo penicilina, contendo meio MEA 1,6% com cloranfenicol (Descal 1997), inclinado, em ambiente refrigerado (em torno de 15°C em B.O.D.).

✧ *Óleo Mineral*

Esse método consiste em recobrir culturas jovens, desenvolvidas pelo método clássico em tubos de ensaio com meio MEA 1,6% com cloranfenicol (Descal 1997), com uma camada de óleo mineral esterilizado. Esses tubos são mantidos em temperatura de $\pm 15^{\circ}\text{C}$.

As culturas preservadas por esses métodos são reativadas periodicamente, repicando-se as mesmas sobre novos meios de cultura e também submergindo alíquotas em água destilada esterilizada para acompanhar a produção de conídios e de micélio. Deste modo são avaliadas a longevidade e o grau de pureza que determinado método proporciona na preservação das culturas.

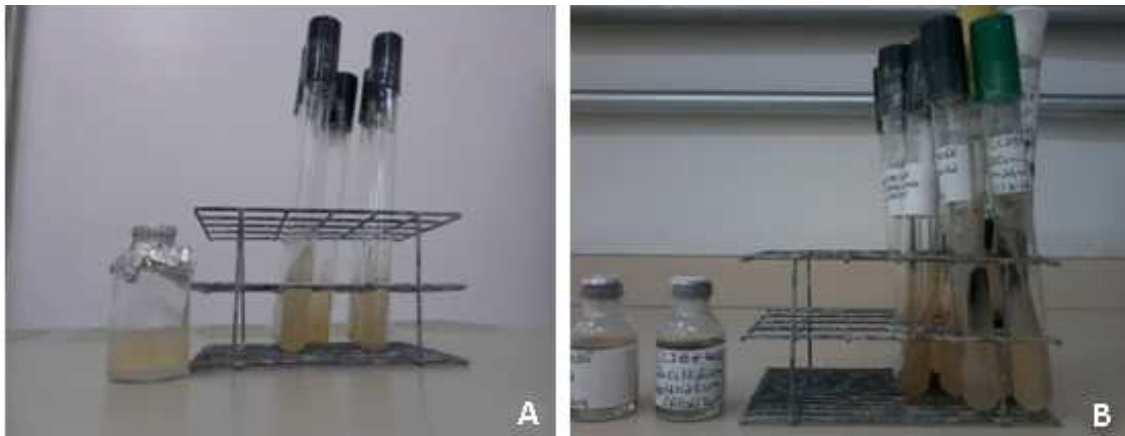


Figura 16. Preservação de culturas: A. Frascos para a técnica do óleo mineral e tubos inclinados com meio de cultura (MEA 1,6%) sem o fungo. B. Isolados preservados em óleo mineral e tubos inclinados.

3.7. Estudos moleculares

✧ *Produção de biomassa e extração de DNA*

A biomassa fúngica foi obtida pelo cultivo de cada isolado em frascos Erlenmeyer contendo 20 ml de meio líquido MEA 1,6%. Após incubação no período de 7 a 10 dias a 21°C, a biomassa foi transferida para tubos Eppendorf® (1,5 mL) e foram centrifugados a 13000 rpm por 15 minutos, a fim de obter *pellets* do micélio. Os sobrenadantes foram descartados e 1 ml de água deionizada estéril foi adicionado aos tubos e em seguida foram levados ao Vortex a 2500 rpm. Os tubos foram novamente centrifugados e os sobrenadantes descartados. Os *pallets* de micélio foram tratadas de acordo com o protocolo descrito no kit de DNA genômico de PureLink® (Invitrogen™). A eletroforese foi realizada utilizando gel de agarose 1% (p/v), aplicação de 3-5 µl de amostra + 2 µl de tampão de carregamento de Gel tipo I (Sigma®) e um padrão de 2 µl de 123 bp DNA Ladder (Invitrogen™) + 2 µl de tampão de carregamento do Gel. As condições da eletroforese foram de 100 mV por 40 min.

✧ *Amplificação, purificação e sequenciamento*

As regiões do rDNA foram amplificadas por PCR com *primers* apresentados na Tabela 1. A amplificação do DNA foi realizado com o kit PCR SuperMix® (Invitrogen™) em um C1000 Touch™ Thermal Cycler Bio-Rad. As condições foram: volume de 25 µl; desnaturalização inicial a 94°C por 3 min., seguido por 35 ciclos de 94°C por 1 min., 54°C por 30s, 72°C por 1 min. e uma extensão final a 72°C por 7 min.. Os produtos da PCR foram analisados por eletroforese em gel de agarose a 1% usando 1-2 µL Low DNA Mass Ladder (Invitrogen™) + 2-4 µL de Gel carregando Buffer como padrão e 2-3 µL de DNA. A amplificação foi purificada com kit AxyPrep™ PCR Clean-up (Axygen®). O seqüenciamento foi realizado utilizando os mesmos *primers* mencionados na Tabela 1 em um analisador de DNA de 3730 ABI (Life Technologies™) para o Centro de Estudos do Genoma Humano, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil.

As análises filogenéticas estão detalhadas nos Capítulos III e V, pois esta etapa foi específica para cada isolado estudado.

Tabela 1: Táxons analisados e suas regiões do rDNA amplificados por PCR com seus respectivos primers e referências.

Táxon	Região	Primer	Referência
<i>Campylospora brasiliensis</i>	ITS	UN-up18S42 CGTAACAAGGTTTCCGTAGGTGAAC (ITS 5')	Robideau <i>et al.</i> (2011)
		UN-lo28S22 GTTTCTTTTCCTCCGCTTATTGATATG (ITS 3')	
<i>Clathrosporium retortum</i>	ITS	UN-up18S42 CGTAACAAGGTTTCCGTAGGTGAAC (ITS 5')	Robideau <i>et al.</i> (2011)
		UN-lo28S22 GTTTCTTTTCCTCCGCTTATTGATATG (ITS 3')	
<i>Clathrosporium retortum</i>	LSU	LR0R - ACCCGCTGAACTTAAGC (LSU 5')	Vilgalys & Hester (1990)
		LR5 -TCCTGAGGGAACTTCG (LSU 3')	

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5. RESULTADOS

Diversidade

Foram coletadas 72 amostras no Parque Estadual da Ilha do Cardoso e 87 amostras no Parque Estadual de Ilhabela. Um total de 59 táxons de Hyphomycetes aquáticos foi obtido.

Para o PEIb foram identificados 40 táxons (Tabela 2), sendo 21 exclusivos da Ilha em comparação ao PEIC. *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragmia triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamorte, *Scutisporus brunneus* K. Ando & Tubaki, são novos registros para o Estado de São Paulo e *Isthmolongispora biramifera* Matsush., *Lateriramulosa a-inflata* Matsush., *Phalangispora nawawii* Kuthub. e *Triscelophorus ponapensis* Matsush. são novos registros para o Brasil. Além disso, foi reportado um novo registro para o Brasil do fungo aeroaquático *Peyronelina glomerulata* P.J. Fisher, J. Webster & D.F. Kane. e duas novas espécies para a Ciência: *Campylospora brasiliensis* L.B. Moro & I.H. Schoenlein-Crusius e *Polylobatispora setulosa* L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius.

As descrições dos novos registros para o Brasil e/ou Estado de São Paulo e fatores abióticos dos locais de coleta, bem como as descrições das espécies novas encontram-se nos Capítulos I, II, III e V.

Para o PEIC foram identificados 38 táxons (Tabela 3), sendo 19 táxons exclusivos da Ilha em comparação ao PEIb. *Isthmolongispora quadricellularia* Matsush. é um novo registro para o Estado de São Paulo e *Isthmolongispora intermedia* Matsush., *Isthmolongispora minima* Matsush., *Dactylellina appendiculata* (Anastasiou) M. Scholler, Hagedorn & A. Rubner, *Pleiochaeta amazonensis* Matsush. e *Pyramidospora constricta* N. Singh primeiras citações para o Brasil. Entre os fungos aeroaquáticos, 7 táxons foram obtidos, sendo *Beverwykella clathrata* Voglmayr, *Cancellidium applanatum* Tubaki e *Candelabrum brocchiatum* Tubaki novos registros para o Estado de São Paulo e *Helicoon septatissimum* Godeas & Aramb. novo registro para o Brasil. Uma espécie nova para a Ciência: *Clathrosporium retortum* L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius.

As descrições dos novos registros para o Brasil e/ou Estado de São Paulo e fatores abióticos dos locais de coleta, bem como a descrição da espécie nova encontram-se nos Capítulos IV e V.

Tabela 2: Listagem dos táxons de Hyphomycetes aquáticos encontrados no Parque Estadual de Ilhabela.

<i>Acumispora verruculosa</i> Heredia, R.F. Castañeda & R.M. Arias [•]
<i>Alatospora acuminata</i> Ingold ⁺
<i>Anguillospora crassa</i> Ingold ⁺
<i>Anguillospora filiformis</i> Greath. ⁺
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold ⁺
<i>Anguillospora pseudolongissima</i> Ranzoni ⁺
<i>Articulospora tetracladia</i> Ingold ⁺
<i>Camposporidium cristatum</i> Nawawi & Kuthub. [•]
<i>Camposporium antennatum</i> Harkn. [•]
<i>Camposporium pellucidum</i> (Grove) S. Hughes [•]
<i>Campylospora chaetocladia</i> Ranzoni ⁺
<i>Campylospora brasiliensis</i> L.B. Moro & I.H. Schoenlein-Crusius ^{*+}
<i>Campylospora filicladia</i> Nawawi ⁺
<i>Campylospora parvula</i> Kuzuha ⁺
<i>Chaetendophragma triangularis</i> Matsush. [•]
<i>Clavariopsis aquatica</i> De Wild. ⁺
<i>Clavatospora tentacula</i> Sv. Nilsson ⁺
<i>Dendrosporium lobatum</i> Plakidas & Edgerton ex J.L. Crane ⁺
<i>Flabellospora acuminata</i> Descals ⁺
<i>Flagellospora curvula</i> Ingold ⁺
<i>Flagellospora penicillioides</i> Ingold ⁺
<i>Heliscus submersus</i> H.J. Huds. ⁺
<i>Isthmolongispora biramifera</i> Matsush. ⁺
<i>Isthmolongispora quadricellularia</i> Matsush. ⁺
<i>Lateriramulosa a-inflata</i> Matsush. ⁺
<i>Lunulospora curvula</i> Ingold ⁺
<i>Lunulospora cymbiformis</i> K. Miura ⁺
<i>Peyronelina glomerulata</i> P.J. Fisher, J. Webster & D.F. Kane [◇]
<i>Phalangispora nawawii</i> Kuthub. [•]
<i>Physalidiella elegans</i> (Mosca) Rulamort [•]
<i>Polylobatispora setulosa</i> L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius [*]
<i>Scutisporus brunneus</i> K. Ando & Tubaki [•]
<i>Tetracladium marchalianum</i> De Wild., ⁺
<i>Tetracladium setigerum</i> (Grove) Ingold ⁺
<i>Tricladium gracile</i> Ingold ⁺
<i>Tripospermum camelopardus</i> Ingold [•]
<i>Tripospermum myrti</i> (Lind) S. Hughes [•]
<i>Triscelophorus acuminatus</i> Nawawi ⁺
<i>Triscelophorus monosporus</i> Ingold ⁺
<i>Triscelophorus ponapenses</i> Matsush. ⁺

*Espécie nova; ◇fungos aeroaquáticos; +fungos Ingoldianos; •Fungos considerados facultativos (aquáticos terrestres e aquáticos submersos).

Tabela 3: Listagem dos táxons de Hyphomycetes aquáticos encontrados no Parque Estadual da Ilha do Cardoso.

<i>Alatospora acuminata</i> Ingold ⁺
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold ⁺
<i>Anguillospora pseudolongissima</i> Ranzoni ⁺
<i>Beverwykella clathrata</i> Voglmayr [◇]
<i>Blodgettia indica</i> Subram. [•]
<i>Cancellidium applanatum</i> Tubaki [◇]
<i>Candelabrum brocciatum</i> Tubaki [◇]
<i>Camposporium antennatum</i> Harkn. [•]
<i>Campylospora chaetocladia</i> Ranzoni ⁺
<i>Clathrosporium retortum</i> L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius ^{*◇}
<i>Clavariopsis aquatica</i> De Wild. ⁺
<i>Dactylellina appendiculata</i> (Anastasious) M. Scholler, Hagedorn & A. Rubner [•]
<i>Dendrosporium lobatum</i> Plakidas & Edgerton ex J.L. Crane ⁺
<i>Flagellospora curvula</i> Ingold ⁺
<i>Flagellospora penicillioides</i> Ingold ⁺
<i>Helicoön septatissimum</i> Godeas & Aramb. [◇]
<i>Helicomycetes</i> sp. [◇]
<i>Helicosporium</i> sp. [◇]
<i>Heliscus submersus</i> H.J. Huds. ⁺
<i>Ingoldiella hamata</i> D.E. Shaw ⁺
<i>Isthmolongispora intermedia</i> Matsush. ⁺
<i>Isthmolongispora minima</i> Matsush. ⁺
<i>Isthmolongispora quadricellularia</i> Matsush. ⁺
<i>Jaculispora submersa</i> Hudson, H.J.; Ingold, C.T. ⁺
<i>Lunulospora curvula</i> Ingold ⁺
<i>Lunulospora cymbiformis</i> K. Miura ⁺
<i>Pleiochaeta amazonensis</i> Matsush. [•]
<i>Pyramidospora casuarinae</i> Sv. Nilsson ⁺
<i>Pyramidospora constricta</i> N. Singh ⁺
<i>Pyramidospora robusta</i> C. G. Moreira & I.H. Schoenlein ⁺
<i>Scutisporus brunneus</i> K. Ando & Tubaki [•]
<i>Subulispora longirostrata</i> Nawawi & Kuthub.
<i>Tetracladium marchalianum</i> De Wild. ⁺
<i>Tetraploa aristata</i> Berk. & Broome [•]
<i>Tripospermum camelopardus</i> Ingold, Dann & P.J. McDougall [•]
<i>Tripospermum myrti</i> (Lind) S. Hughes [•]
<i>Triscelophorus acuminatus</i> Nawawi ⁺
<i>Triscelophorus monosporus</i> Ingold ⁺

*Espécie nova; ◇fungos aeroaquáticos; +fungos Ingoldianos; •Fungos considerados facultativos (aquáticos terrestres e aquáticos submersos).

Isolamento em Meio de Cultura

Foram realizadas mais de 20 tentativas de isolamento em meio de cultura, seguindo criteriosamente a técnica proposta por Descals (2005), até que a modificação descrita no item 3.5 fosse utilizada para a obtenção das culturas.

Foi possível obter neste estudo, 15 isolados em meio de cultura de Hyphomycetes aquáticos (Tabela 4). Destes, 6 táxons identificados em nível específico, com destaque paraduasnovasespécies (marcadas com asterisco na Tabela 2), que foram incluídas na Coleção de Fungos, Algas e Cianobactérias do Instituto de Botânica de São Paulo. Também foram identificadosdois táxons em nível de gênero e dois táxons não identificados que aindaestão em estudo.

Tabela 2: Hyphomycetes aquáticos isolados em meio de cultura e seus respectivos números CCIBt e acesso no Genbank.

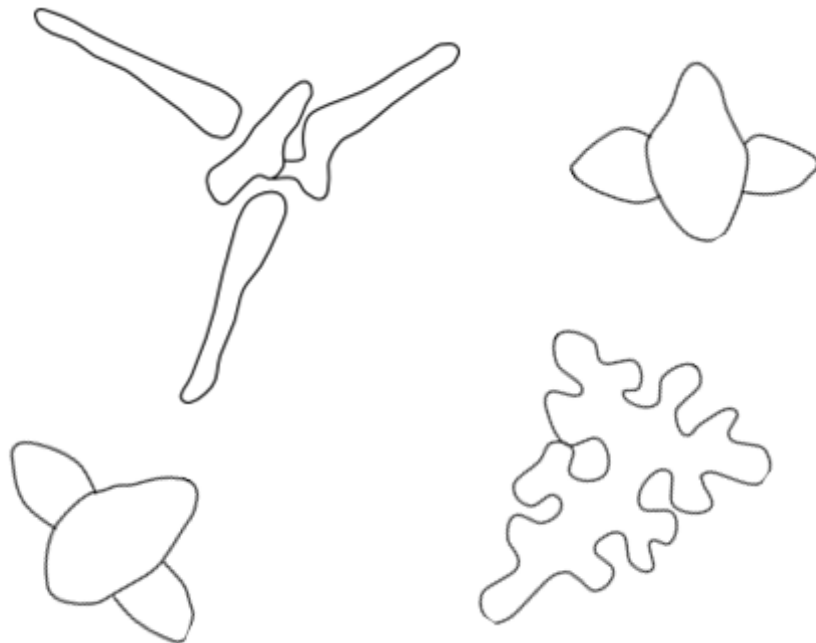
Espécie	Número do isolado	Número de acesso Genbank
<i>Acumispora verruculosa</i>	CCIBt 4066	✖
<i>Campylospora brasiliensis</i> *	CCIBt 4075	KP262470
<i>Campylospora brasiliensis</i> *	CCIBt 4076	KP262471
<i>Campylospora brasiliensis</i> *	CCIBt 4077	KP262472
<i>Campylospora brasiliensis</i> *	CCIBt 4078	KP262473
<i>Cancellidium applanatum</i>	CCIBt 4036	✖
<i>Ingoldiella hamata</i>	CCIBt 3995	✖
<i>Ingoldiella hamata</i>	CCIBt 4035	✖
<i>Beverwykella clathrata</i>	CCIBt 4105	—
<i>Helicosporium</i> sp.	CCIBt 4118	✖
<i>Helicomycetes</i> sp.	CCIBt 4117	✖
<i>Clathrosporium retortum</i> *	CCIBt 4122	KT223312\KT223314
<i>Clathrosporium retortum</i> *	CCIBt 4123	KT223313\KT223315
Não identificado 1	—	✖
Não identificado 2	—	✖

✖ Sequências obtidas, porém não incluídas no Genbank; * espécies novas obtidas.

***Freshwater Hyphomycetes in preserved insular areas
from Brazil: First records in Ilhabela State Park,
municipality of Ilhabela, São Paulo State***

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Freshwater Hyphomycetes in preserved insular areas from Brazil: First records in Ilhabela State Park, municipality of Ilhabela, São Paulo State

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Abstract – (Freshwater Hyphomycetes in preserved insular areas from Brazil: First records in Ilhabela State Park, municipality of Ilhabela, São Paulo State). During the period from June/2012 to May/2013, water and submerged mixed leaf litter samples were collected from 22 waterfalls and rivers of the Ilhabela State Park, to survey the diversity of freshwater Hyphomycetes. From submerged mixed leaf litter samples, incubated in Petri dishes containing pond and sterile distilled water, 39 species of freshwater Hyphomycetes were isolated, all new records for the park. *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragmia triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamort and *Scutisporus brunneus* K. Ando & Tubaki are reported 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 to Brazil. Descriptions, comments and illustrations are presented for these species.

Diversity\island environments\submerged substrate\taxonomy\waterfalls

INTRODUCTION

Aquatic Hyphomycetes are anamorphic fungi that are characterized by the exclusive formation of conidiophores and conidia in a free manner or grouped into synema or sporodochia, as asexual stages of Ascomycota and some of Basidiomycota (Alexopoulos *et al.* 1996; Kirk *et al.* 2008).

These typical aquatic fungi may be divided into four main ecological groups: ingoldian fungi (Descals *et al.* 1978), aeroaquatic fungi (Beverwijk 1951), terrestrial aquatic fungi (Ando 1992) and submerged aquatic fungi (Ingold 1975).

Ingoldian fungi are exclusively aquatic, depending on the water to complete their life cycles (Ingold 1975). Colonizing strategies include the effective mechanism of attaching the conidia related to tetra- and sigmoidal shape, rapid germination, rapid growth and, sporulation reinforced by turbulence and rapid water flow (Webster & Towfik 1972; Sanders & Webster 1980).

Aeroaquatic fungi generally produce conidia with helicoidal forms (helicospores), sporulating only when the substrate comes into contact with the air (Beverwijk 1951; Webster & Descals 1981; Ando & Tubaki 1984; Alexopoulos 1996). Terrestrial aquatic fungi include species of fungi isolated from rain drops associated with terrestrial plant or rainwater accumulated on the surface of leaves (Ando 1992).

Submerged aquatic fungi are also called lignicolous or facultative aquatic Hyphomycetes (Ingold 1975; Goh & Hyde 1996). Dematiaceous Hyphomycetes, are able to grow on submerged plant substrates as saprobes or optional form terrestrial, sporulated under terrestrial conditions or, in some cases, also submerged (Webster & Descals 1981; Goh & Hyde 1996; Descals & Moralejo 2001; Révay & Gönczöl 2007).

The aquatic Hyphomycetes are saprobic organisms, being responsible for high amounts of the decomposition of organic matter, acting in biochemical transformations in the ecosystems (Remacle 1981). These fungi are also 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 the best known for their diversity (Ranzoni 1979). However, for the tropics, the number of studies is lower, but it is expected that the diversity of these fungi may reach significant rates upon the approach of a broader geographical distribution (Rossman 1994; Hawksworth 1991).

To Brazil, the studies were initiated in the 1980 decade, but with emphasis on the South-East, more specifically in the São Paulo State in the Atlantic rainforest and coastal plateau (Schoenlein-Crusius & Milanez 1989; Schoenlein-Crusius & Milanez 1990), in the “Cerrado” (Schoenlein-Crusius 2002) and in urban waters (Moreira & Schoenlein-Crusius-2012; Schoenlein-Crusius *et al.* 2014). More recently, surveys has

been conducted in the northeast in the “Caatinga” biome (Fiuza & Gusmão 2013; Barbosa *et al.* 2013) and in the Amazon region too (Gusmão & Monteiro 2013; Fiuza *et al.* 2015).

Insular systems located at the east Brazilian coast, occupied by preserved Atlantic forest and lotic environments, are promising areas for studies regarding aquatic Hyphomycetes. The aim of this research is to begin the studies of aquatic Hyphomycetes in Brazilian insular environments, and, in addition, to expand and contribute to the knowledge of the diversity of these fungi in Brazilian Atlantic rainforest.

MATERIALS AND 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 and 45° 21' 20" W) which is an important remanescant area of Atlantic rainforest, approaching approximately 27.025 hectares. The Park is under the administration of the Instituto Florestal of São Paulo (Parque Estadual de Ilhabela 2013).

Collection

Samples of submerged mixed leaf litter were taken from 22 water bodies from June/2012 to May/201. Each sample was composed by about 10 leaves of submerged mixed leaf litter with water taken from each site collection, placed in 200 ml polypropylene flask totalizing 87 samples\4 field trip. For the characterization the collection area, some abiotic factors (temperature, pH, conductivity and dissolved oxygen) were measured at each site with a multi-parameter probes Horiba®U10 and U51.

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

From the 5th day of incubation on, slides for microscopical observation were prepared with aliquots of the leaves. The slides were observed with an optical microscope Olympus BX50 to search for conidiophores and conidia in order to identify the taxa with support of specific literature (Ingold 1975; Marvanová 1997; Santos-Flowers & Betancourt-López 1997). The identification procedures lasted until no different taxa were obtained anymore. The photographic documentation was carried out with the aid of an optical microscope Leica DM LB2 with coupled camera Leica DFC 280. Permanent slides were prepared with PVLG-resin polyvinyl alcohol in lactoglycerol (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 AND DISCUSSION

Abiotic factors

The water temperature varied according to the seasons, being higher in the summer, 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 0.077 $\mu\text{S}\cdot\text{cm}^{-1}$) are compatible with the ones in well preserved areas (Table 1). The level of dissolved oxygen in the water proved to be high (8.85 to 12.87 mgO_2/L), ones in probably due to the turbulence. The latter abiotic factor is considered the most important for the aquatic Hyphomycetes, once well oxygenated waters may favors the growth and sporulation of this group of fungi (Nilsson 1964; Webster & Towfik 1972).

Fungal diversity

Thirty nine taxa of aquatic Hyphomycetes were identified. 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 for São Paulo State: *Isthmolongispora biramifera* Matsush., *Lateriramulosa a-inflata* Matsush., *Phalangispora nawawii* Kuthub. and *Triscelophorus ponapensis* Matsush. are new records to Brazil.

Morphological description

Camposporidium cristatum Nawawi & Kuthub., Mycotaxon 32: 161 (1988)

Figures 1-2

Conidiophore erect, macronematous, solitary, simple, cylindrical, septate, straight or slightly flexed, brown to dark brown, 30-35 μm in length. Conidiogenics cells blastic, terminal, integrated, cylindrical. Conidia solitary, cylindrical, 9-12 septate, smooth walls or rarely with small verruculous, apex rounded, truncated at base, subhyaline to light brown, 75-90 x 9-10 μm ; basal cell truncated, fouscus, 3-4 x 6-7 μm ; apical cells rounded and clearer, 4.5-7 x 5-6 μm , with 4-5 appendages, 0- septate, hiyaline to subhyaline, 37-80 μm .

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Cachoeira do Gato” river, in submerged mixed leaf litter, 15\2013, LB Moro. (SP446218).

Remarks: Nawawi & Kuthubutheen (1988) described the conidiophores of *C. cristatum* may reach more than 245 μm in length. However, in the present study, measures were lower.

Camposporidium was described by Nawawi & Kuthubutheen (1988) in Malaysia with the type species *C. cristatum*. In the decade of 1990 they described two other species of the genus, *C. ghindense* (Bhat) R.F. Castañeda, Guarro & Cano and *C. hughesii* R.F. Castañeda & Guarro. After the original description, *C. cristatum* was reported to Brazil, for the semi-arid region of the “Caatinga” Biome (Barbosa &

Gusmão 2011), and in this survey for the first time to the Atlantic Rainforest biome and for the São Paulo State.

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

Figure 3

Conidiophore not observed. Conidia triangular, light brown, smooth, total length 50-62.5 μm , wider base 7.5 μm , 5-septate, small constricted at the septa, with visible scars at the base, 2-3 lateral appendages, hyaline, smooth, 25-37.5 μm .

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Cachoeira do Gato” river, “Cemitério” stream, in submerged mixed leaf litter, 15\2013, LB Moro. (SP446222).

Remarks: *C. triangularis* appears to be most closely related to *C. britannica*, differing from this species by producing larger conidia and by being constricted at the septa. In *C. britannica* the second and third cell from base are golden brown, while in *C. triangularis* these cells are light brown.

The genus *Chaetendophragmia* was described by Matsushima (1971) with the type species *C. triangularis*, isolated from decaying leaves of *Castanopsis* sp. in Bulolo, Papua New Guinea. Other eight species and two varieties were described for the genus. In Brazil, *C. triangularis* was identified in an Atlantic rainforest fragment of Serra da Jibóia, Bahia, in leaves of *Clusia melchiorii* Gleason (Barbosa *et al.* 2009). This is the first record for the State of São Paulo.

Isthmolongispora biramifera Matsush., *Matsushima Mycological Memoirs* 7: 55 (1993)

Figure 4

Conidiophore not observed. Conidia compost to one central axis fusiformis and two lateral branches, hyaline, 7-cells connected by narrow isthmus; central axis 28.2-31.85 μm , central cell 2.7-3.6 μm wide, 3-cells; lateral branches 14.5-19.1 μm in length, 2-cells in each branch.

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\2013, LB Moro.

Remarks: Although the measures of length of central axis and lateral branches of *I. biramifera* described by Matsushima (1993) are smaller than those observed in this study, all the other characteristics indicate this species, since the morphology of conidia are singular.

The genus *Isthmolongispora* was described by Matsushima (1971) in unidentified leaf litter in Wau, Papua New Guinea. In addition to the species type, *I. intermedia*, ten other species are described. The genus is characterized, in relation to conidia, for being bi or multicellular, separated by small isthmus. This is the first record of this species for Brazil.

Lateriramulosa a-inflata Matsush., Icones Microfungorum a Matsushima lectorum: 92 (1975)

Figure 5

Conidiophore not observed. Conidia hyaline, short branches; (1) a cylindrical shaft in the middle of two branches, (2) a branch in the middle of the cylindrical shaft, (3) branch obclavate e (4) angularly bent branch; (1) 6-9 μm long by 1.2-1.5 μm wide; (2) e (3) 9-11 long, wider base 2-2.5 μm ; (4) branch with 7-11 μm long, wider base 2-2.5 μm .

Material examinad: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Gato” waterfall, in submerged mixed leaf litter, 14\2013, LB Moro.

The genus *Lateriramulosa* was described by Matsushima (1971) in Rabaul, Papua New Guinea, being characterized by hyaline conidia, formed by 3 main branches and a central cylindrical axis. The genus consists of four more species, namely *L. bi-inflata* Matsush., *L. minitriangularia* Matsush., *L. quadriradiata* Miura and Okano and *L. uni-inflata* Matsush., as the type species. This is the first record of the taxa for Brazil.

Phalangispora nawawii Kuthub., Transaction of the British Mycological Society 89(3): 419 (1987)

Figure 6

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

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\2013, LB Moro. (SP 445864).

The genus *Phalangispora* was described by Nawawi & J. Webster (1982), with *P. constricta* as type species. In addition to the type species and *P. nawawii*, more recently, in 2002, *P. bharathensis* T.S.K. Prasad & Bhat was described. For Latin America, *P. nawawii* was described by Smits *et al.* (2007) on submerged leaf litter of rivers in Venezuela. This is the first record of the species for Brazil.

Physalidiella elegans (Mosca) Rulamort, Bulletin de la Société Botanique du Centre-Ouest 21: 512 (1990)

Figure 7

Conidiophore not observed. Conidia solitare, smooth, 3-cells, central cell dark brown, obovoid or ellipsoid, 7-11 x 6-7 μm . Two lateral hemispherical cells, sub-hyaline, 3-4 x 3-4 μm , disposed on the main cell as two flaps.

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\IV\2013, LB Moro. (SP446219).

Remarks: the conidial central cell of the *P. elegans* described by Castro *et al.* (2012) measured 6-7 × 5-6 μm, therefore smaller than the here studied specimen. However, the measures are in accordance to Ellis (1971) who described varying from 7-11 × 6-7 μm.

Physalidiella was described by Rulamort (1990), and *P. elegans* as the type species, with *P. matsushimae* (R.F. Castañeda & W.B. Kendri.) M. Morelet composing the genus. *P. elegans* was described for Latin America, in leaves of *Inga* sp. from Peru, by Matsushima (1993). To Brazil, in “Ilha do Combu”, Pará State, the Amazon region (Castro *et al.* 2012) and decaying leafy substrates in the Serra da Jibóia, State of Bahia (Fiuza *et al.* 2010). This is the first record for the State of São Paulo,

Scutisporus brunneus K. Ando & Tubaki, Trans. Mycol. Soc. Japan 26(2): 153 (1985)

Figures 8-9

Conidiophore not observed. Conidia consisting by four cells, septa cross-shaped, hyaline to subhyaline, 7-10(-11) x (6-)-8-9 μm, appendages filiforms, projected from each cell of the body, smooth, hyaline, 0-septate, 20-37 x 0,5-0,7 μm; basal cuneiform cell, 4,0-7,0 x 2,0-3,0 μm.

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço do Jabuti”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Toca” waterfall and “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\IV\2013, LB Moro.

The genus *Scutisporus* was described by Ando and Tubaki (1985) being only *S. brunneus* described until now. Although *S. brunneus* was described for terrestrial environments, associated with decaying leaves of *Pinus densiflora* (Ando & Tubaki 1985), this species has been common in aquatic environments, in submerged leaf litter or in foams (Tubaki 1965, Silva & Briedis 2009). For Latin America the species was described in Peru by Matsushima (1987) in lignicolous substrates; in Venezuela by Smits *et al.* (2007) and Silva & Briedis (2009) in foam; in Ecuador by Matsushima (1993) on decomposing leaves in the Yuturi river. To Brazil it was described by Barbosa & Gusmão (2011) in submerged leaf litter in the State of Bahia and by Fiuza & Gusmão (2013) in foam in the semi-arid region of the State of Ceará and more recent from region Amazon by Fiuza *et al.* (2015). This is the first record of the taxa in the State of São Paulo.

Triscelophorus ponapensis Matsushima, T. 1981. Matsushima Mycological Memoirs 2. Matsushima Mycological Memoirs. 2:1-68

Figure 10

Conidiophore not observed. The mature conidia consists of a main axis and 2-3 verticillate lateral branches forming a whorl next to the base of this axis, hyaline; main axis 12-26 x 4-6 μm , 2-4 septate; lateral branches 8-15 x 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” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro.

Triscelophorus ponapensis was isolated by Matsushima (1981) of *Pandani* sp. leaf. *Triscelophorus* Ingold is characterized by the typical tetraradiate conidia. The genus was described by Ingold (1943) with the type species *T. monosporus* and currently the genus has eight species. Although several species of the genus are commonly described for Brazil (Schoenlein-Crusius & Milanez 1990, Schoenlein-Crusius *et al.* 1990, 1992, 1998, Malosso 1999, Schoenlein-Crusius 2002, Fiuza & Gusmão 2013, Silva *et al.* 2014, Fiuza *et al.* 2015). This is the first record of *T. ponapensis* for Brazil.

Other species of freshwater Hyphomycetes found in Ilhabela State Park, Ilhabela-São Paulo State:

Acumispora verruculosa Heredia, R.F. Castañeda & R.M. Arias, Mycotaxon 101: 90 (2007)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\V\2013, LB Moro (SP445-979, CCIBt 4066).

Alatospora acuminata Ingold, Transactions of the British Mycological Society 25 (4): 384 (1942)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Gato” waterfall, in submerged mixed leaf litter, 14\V\2013, LB Moro.

Anguillospora crassa Ingold, Transactions of the British Mycological Society 41 (3): 367 (1958)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Poço do Jabuti”, in submerged mixed leaf litter, 04\IX\2012, LB Moro.

Anguillospora filiformis Greath., South African Journal of Botany 27: 202 (1961)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro.

Anguillospora longissima (Sacc. & P. Syd.) Ingold, Transactions of the British Mycological Society 25 (4): 402 (1942)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall and “Veloso” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\V\2013, LB Moro.

Anguillospora pseudolongissima Ranzoni, Farlowia 4: 362 (1953)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, “Poço da Escada”, “Poço da Ducha”, “Poço do Jequitibá”, “Poço do Jabuti” and “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Caminho da Praia Vermelha” river, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Articulospora tetracladia Ingold, Transactions of the British Mycological Society 25 (4): 376 (1942)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Veloso” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro.

Camposporium antennatum Harkn., Bulletin of the California Academy of Sciences 1: 37 (1884)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Ducha” e “Lado Norte da Ilha” stream, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Toca” waterfall and “Ribeirão do Pombo” stream, in submerged mixed leaf litter, 06\XII\2012, LB Moro.

Camposporium pellucidum (Grove) S. Hughes, Mycological Papers 36: 9 (1951)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Toca” waterfall and “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro.

Campylospora chaetocladia Ranzoni, Farlowia 4: 373 (1953)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Poço da Pedra”, “Poço da Escada”, “Poço do Jabuti”, “Lado Norte da Ilha” stream and “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Toca” waterfall and “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Cemitério” stream, in submerged mixed leaf litter, 14\V\2013, LB Moro.

Campylospora brasiliensis L.B. Moro & I.H. Schoenlein-Crusius, *Phytotaxa* 208 (4): 293 (2015)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Toca” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro, (SP446282).

Campylospora filicladia Nawawi, *Transactions of the British Mycological Society* 63 (3): 604 (1974)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Poço da Pedra”, “Poço do Jequitibá” and “Poço do Jabuti”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Gato” waterfall, “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Caminho da Praia Vermelha” river and “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Campylospora parvula Kuzuha, *Journal of Japanese Botany* 48 (7): 220 (1973)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço das Pedras”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Toca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro.

Clavariopsis aquatica De Wild., *Annales de la Société Belge de Microscopie* 19: 201 (1895)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço das Escadas”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Clavatospora tentacula Sv. Nilsson, *Symbolae Botanicae Upsalienses* 18 (2): 89 (1964)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Água Branca” waterfall, in submerged mixed leaf litter,

06\XII\2012, LB Moro; “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Dendrosporium lobatum Plakidas & Edgerton ex J.L. Crane, Transactions of the British Mycological Society 58 (3): 423 (1972)

Figures 11-12

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” Waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Gato” waterfall and “Castelhanos” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Caminho da Praia Vermelha” river, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Flabellospora acuminata Descals, Transactions of the British Mycological Society 78 (3): 411 (1982)

Figure 13

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro.

Flagellospora curvula Ingold, Transactions of the British Mycological Society 25 (4): 402 (1942)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro.

Flagellospora penicillioides Ingold, Transactions of the British Mycological Society 27: 41 (1944)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Gato” waterfall, “Cachoeira do Gato” river, “Cemitério” stream and “Castelhanos” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Caminho da Praia Vermelha” river, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Heliscus submersus H.J. Huds., Transactions of the British Mycological Society 44: 91 (1961)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Poço da Escada” and

“Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Engenho d’água” stream and “Toca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro.

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

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra” and “Poço da Escada”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Gato” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Lunulospora curvula Ingold, Transactions of the British Mycological Society 25 (4): 404 (1942)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Poço da Pedra”, “Poço da Escada”, “Poço da Ducha”, “Poço do Jabuti”, “Lado Norte da Ilha” river and “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Água Branca” waterfall and “Ribeirão do Pombo” stream, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Gato” waterfall, “Cemitério” stream and “Castelhanos” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Caminho da Praia Vermelha” river and “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Lunulospora cymbiformis K. Miura, J. Jap. Bot.: 68 (1972)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Poço do Jequitibá” and “Lado Norte da Ilha” river, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Gato” waterfall, in submerged mixed leaf litter, 14\V\2013, LB Moro.

Polylobatispora setulosa L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius, Mycosphere 6: 1 (2015)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, “Cachoeira do Gato” river, in submerged mixed leaf litter, 14\V\2013, LB Moro (SP445-978).

Tetracladium marchalianum De Wild., Annales de la Societé Belge de Microscopie 17: 39 (1893)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Velosos” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\2012, LB Moro; “Gato” waterfall, in submerged mixed leaf litter, 14\V\2013, LB Moro.

Tetracladium setigerum (Grove) Ingold, Transactions of the British Mycological Society 25 (4): 369 (1942)

Material examined: BRASIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, in submerged mixed leaf litter, 27\VI\ 2012, LB Moro; “Gato” waterfall, in submerged mixed leaf litter, 14\V\ 2013, LB Moro.

Tricladium gracile Ingold, Transactions of the British Mycological Society 27: 39 (1944)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Três Tombos” waterfall, in submerged mixed leaf litter, 27\VI\ 2012, LB Moro.

Tripospermum camelopardus Ingold, Dann & P.J. McDougall, Transactions of the British Mycological Society 51 (1): 53 (1968)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, “Poço da Escada”, “Poço da Ducha”, “Poço do Jequitibá”, “Poço do Jabuti”, “Lado Norte da Ilha” river and “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Gato” waterfall, “Cachoeira do Gato” river, “Cemitério” stream and “Castelhanos” river, in submerged mixed leaf litter, 14\V\2013, LB Moro; “Cocáia” waterfall, in submerged mixed leaf litter, 15\V\2013, LB Moro.

Tripospermum myrti (Lind) S. Hughes, Mycological Papers 46: 18 (1951)

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Engenho d’água” stream, “Toca” waterfall and “Água Branca” waterfall, in submerged mixed leaf litter, 06\XII\2012, LB Moro.

Triscelophorus acuminatus Nawawi, Transactions of the British Mycological Society 64 (2): 346 (1975)

Figure 14

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Poço da Pedra”, “Poço da Escada” and “Poço da Ducha”, in submerged mixed leaf litter, 04\IX\2012, LB Moro; “Engenho d’água” stream, in submerged mixed leaf litter, 06\XII\2012, LB Moro; “Gato” waterfall, “Cachoeira do

Gato” river and “Castelhanos” river, in submerged mixed leaf litter, 14\2013, LB Moro; “Caminho da Praia Vermelha” river, in submerged mixed leaf litter, 15\2013, LB Moro.

Triscelophorus monosporus Ingold, Transactions of the British Mycological Society 26 (3-4): 148 (1943)

Figure 15

Material examined: BRAZIL. São Paulo: Ilhabela, São Sebastião Island, Parque Estadual de Ilhabela, “Lage” waterfall, “Veloso” waterfall and “Três Tombos” waterfall, in submerged mixed leaf litter, 27\2012, LB Moro; “Poço da Pedra”, “Poço da Escada”, “Poço da Ducha”, “Poço do Jequitibá”, “Poço do Jabuti” and “Centro da Ilha” waterfall, in submerged mixed leaf litter, 04\2012, LB Moro; “Engenho d’água” stream, “Toca” waterfall, “Água Branca” waterfall and “Ribeirão do Pombo” stream, in submerged mixed leaf litter, 06\2012, LB Moro; “Gato” waterfall, “Cachoeira do Gato” river and “Cemitério” stream, in submerged mixed leaf litter, 14\2013, LB Moro; “Caminho da Praia Vermelha” river, “Cocáia” waterfall e “Tesouro da Colina” waterfall, in submerged mixed leaf litter, 15\2013, LB Moro.

Thirth nine were obtained from the 14 visited waterfalls and 8 were obtained from the eight collected streams, reinforcing the preference of the ingoldian fungi to fast running, well aerated waters. Among the waterfalls, the “Gato” waterfall presented the highest number of fungal taxa – 18, followed by “Poço da Pedra” (12 taxa), “Lage” waterfall and “Três Tombos” waterfall, both with 11 taxa and “Veloso” waterfall and “Água Branca” waterfall, each with 10 taxa. Among the streams, the “Cachoeira do Gato” river was the site with the highest number of fungal taxa (11). There was not such a very expressive 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.

The diversity of aquatic Hyphomycetes in the waterfalls and stream of Ilhabela may be considered higher in comparison to other ones reported in other studies conducted in the Brazilian Atlantic Rainforest. For instances, 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, in the state of São Paulo. In the “Parque Estadual das Fontes do Ipiranga – PEFI”, an important remaining 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.* 2015, per.Comm.).

The survey conducted in the Ilhabela State Park contributes to the expansion of knowledge of the diversity of aquatic Hyphomycetes in areas of Atlantic Rainforest in

Brazil and in insular areas, with 22.5% of the identified species described or first reported for Brazil and/or State of São Paulo, and 100% for the Ilhabela State Park. Thus, the results encourage the preservation of the water bodies in the Ilhabela island, as an important remaining of the Brazilian Atlantic Rainforest, in order to assure the conservation of the biodiversity.

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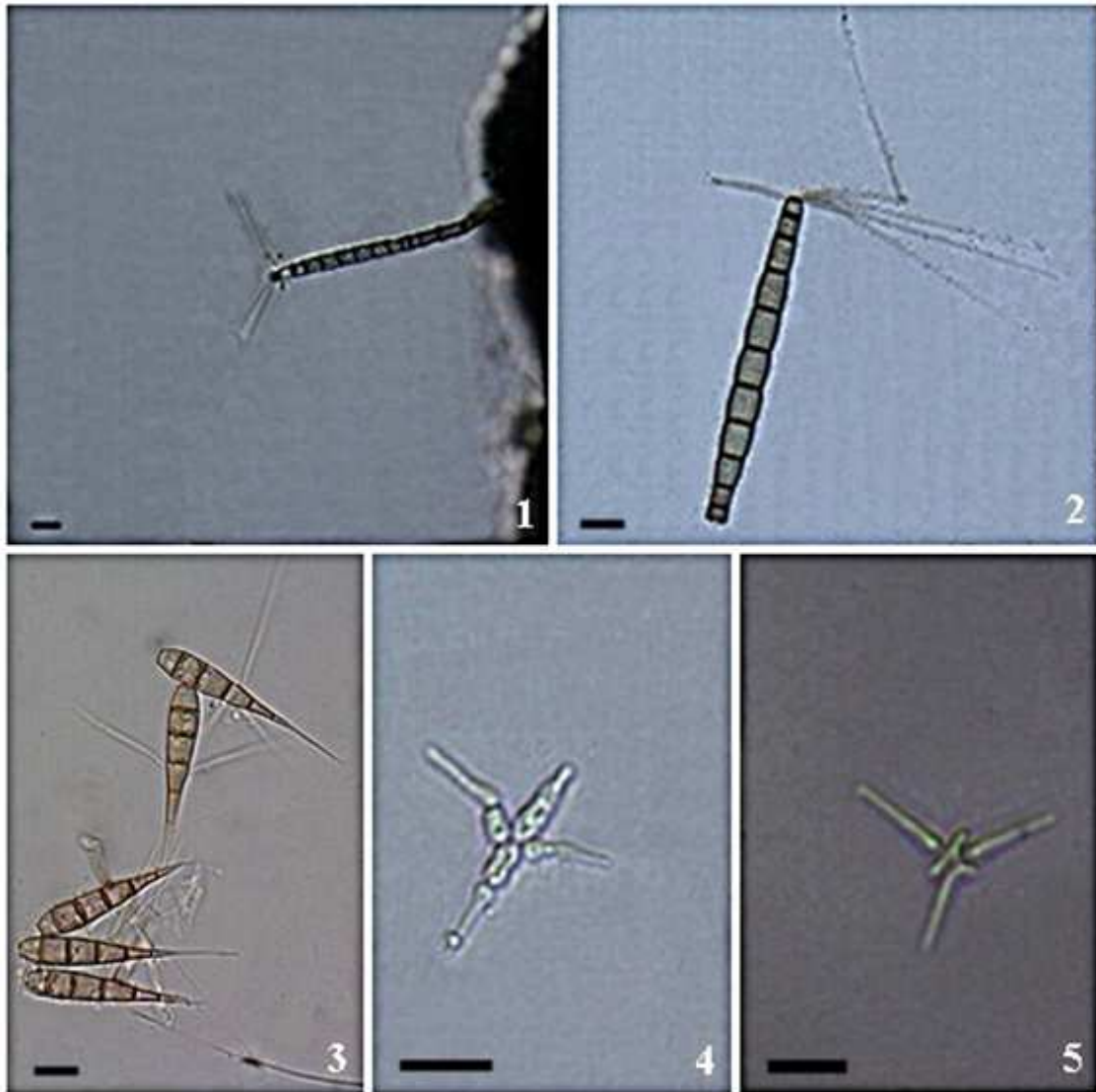
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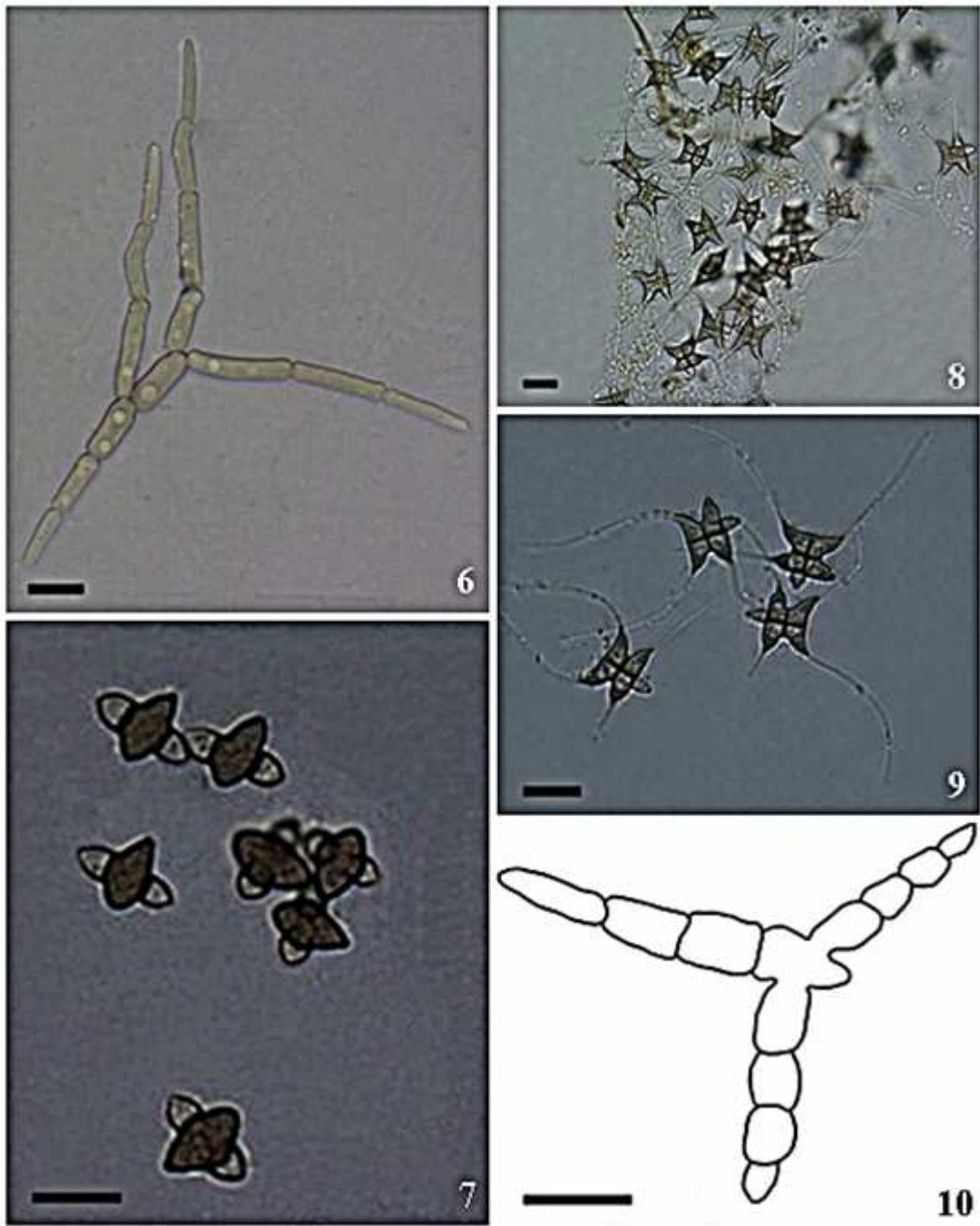
Table 1: Variation (maximum-minimum) of abiotic factors on the collections in the Ilhabela State Park (PEIb).

	Collection 1	Collection 2	Collection 3	Collection 4
	06/2012	09/2012	12/2012	05/2013
Temperature (°C)	17.9-20	16.8-18.8	23.1-24.2	18.8-20.6
pH	6.85-8.03	4.98-5.95	4.41-5.67	6.06-5.67
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	0.027-0.040	0.042-0.077	0.027-0.054	0.024-0.036
Dissolved oxygen (mg/L)	11.93-12.87	9.01-10.27	*	8.85-9.92

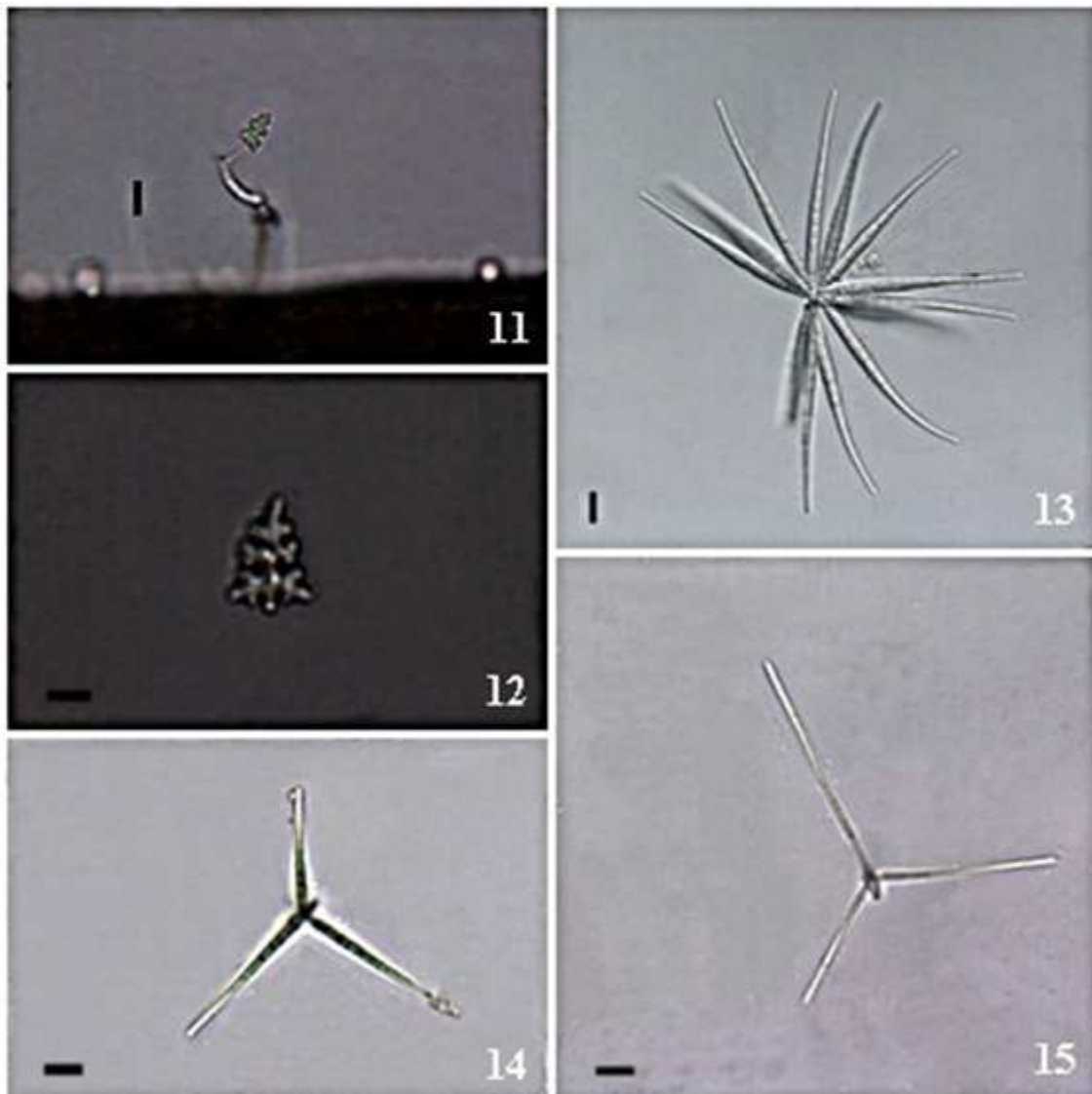
*It has not been possible to measure the dissolved oxygen values in this collection.



Figs 1–5. 1–2. *Camposporidium cristatum*. 1. Conidia and conidiophore. 2. Mature conidia. 3. Mature conidia of *Chaetendophragmia triangularis*. 4. Mature conidia of *Isthmolongispora biramifera*. 5. Mature conidia of *Lateriramulosa a-inflata*. Scale bars: 10 μ m.



Figs 6–10. 6. Mature conidia of *Phalangispora nawawii*. 7. Mature conidia of *Physalidiella elegans*. 8–9. Mature conidium of *Scutisporus brunneus*. 10. Mature conidia of *Triscelophorus ponapensis*. Scale bars: 10 μ m



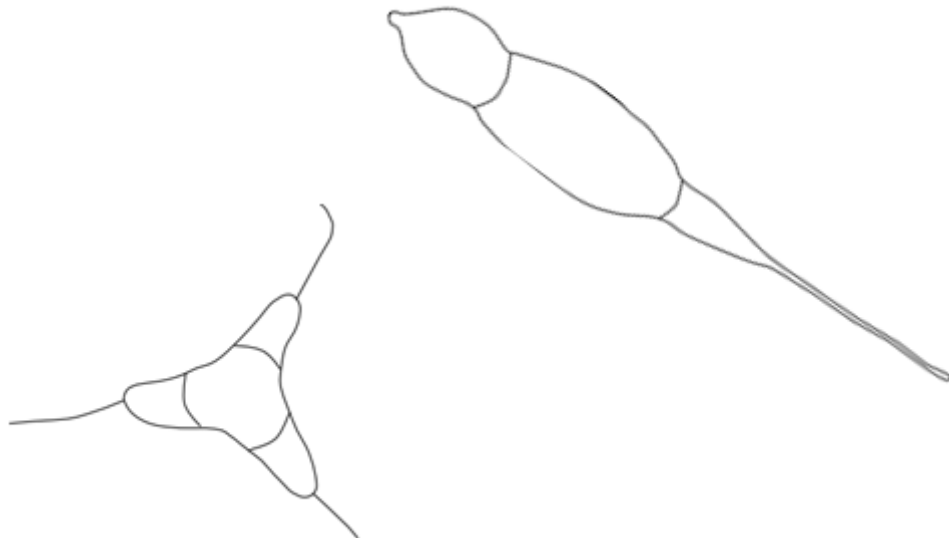
Figs 11–15. 11–12. *Dendrosporium lobatum*. 11. Conidiophore and conidia development. 12. Mature conidia. 13. Mature conidia of *Flabellospora acuminata*. 14. Mature conidia of *Triscelophorus acuminatus*. 15. Mature conidia of *Triscelophorus monosporus*. Scale bars: 10 μ m.

*Polylobatispora setulosa, a new freshwater hyphomycete
from Ilhabela, Sao Paulo state, Brazil*

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Abstract

Polylobatispora setulosa sp. nov. is described and illustrated from submerged mixed leaf litter samples collected at streams in Ilhabela, São Paulo state, Brazil. The fungus is distinct in having setulae at the tip of the conidial lobes. *Acumispora verruculosa*, isolated from identical substrate and characterized by the presence of fusiform, acuminate, rostrate, verrucose, 2-septate, hyaline to greenish conidia, is recorded for the third time from the Americas. They are compared with morphologically similar species and identification keys are provided.

Keywords – anamorphic fungi – freshwater fungi – systematics – tropical fungi

Introduction

Among freshwater hyphomycetes, the so called typical aquatic or Ingoldian fungi have been studied extensively in Brazil, mainly in well-preserved areas of the “cerrado” region (Schoenlein-Crusius 2002), the Atlantic rainforest of the state of São Paulo (Schoenlein-Crusius & Grandi, 2003) and also in a few urban waters (Schoenlein-Crusius et al. 2009, 2014). Recently, however, increasing attention has been paid to other groups of freshwater fungi, particularly those hyphomycete genera frequently reported from terrestrial as well as freshwater habitats and known as facultative-aquatic (Goh & Hyde 1996) or inmigrants (Shearer et al. 2007). While the semi-arid “caatinga” biome in the northeast Brazil has proven to be rich in freshwater fungi (Barbosa & Gusmão 2011, Barbosa et al. 2013, Carneiro et al. 2012), other poorly studied or unexplored areas are also believed to harbour a high diversity of this group of fungi.

Between the years 2012 and 2013, an extensive survey of freshwater hyphomycetes inhabiting submerged materials was carried out in the islands off the coast of the state of São Paulo, including the island of São Sebastião in the municipality of Ilhabela. Two interesting anamorphic taxa were collected on mixed submerged leaf litter samples. They are morphologically similar to the genera *Acumispora* and *Polylobatispora*, but the latter was found distinct enough from previously described species to be considered as a new taxon. Taxonomic descriptions and illustrations are presented here along with identification keys for both genera.

Materials and Methods

Sampling and morphology

Samples of submerged mixed leaf litter (approx. 10g) and local water were collected in sterile polyethylene bottles (about 200 ml) and brought to the laboratory. The leaves were cut into pieces in approximately 1 cm diam. and incubated in Petri dishes containing sterile distilled water. They were kept in climatic chambers at around 20°C following Ingold (1975). From the seventh day of incubation and for at least one month, slides of leaf fragments were prepared using polyvinyl alcohol lacto glycerol resin as mounting medium (Morton et al.1993). Fungal structures were observed and measured using an Olympus BX50 light microscope and images were taken with a Leica DM LB2 microscope fitted with a Leica DFC 280 camera.

Isolates

In order to obtain pure cultures of fungi, water droplets containing conidia of the target fungi were captured with a Pasteur pipette and spread over the surface of Malt Extract Agar (MEA) Petri dishes containing Chloramphenicol (final conc. 25mg/L). After 24 h of incubation in climatic chambers at 21°C, the reverse side of the dishes was observed using a stereoscope to verify hyphal growth the position of the conidia was marked with a permanent marker pen. Under aseptic conditions and with the aid of a sterile stainless steel needle, the conidia were removed from the surface of the medium, inoculated onto a new MEA Petri dish and incubated under similar conditions. Permanent slides are deposited in the Herbarium "Maria P. Eneyda Kauffmann Fidalgo" (SP) and fungal cultures are deposited in the Fungal Collection of the Instituto de Botânica, São Paulo (CCIBt).

Scanning electron microscopy

For scanning electron microscopy (SEM), mycelia with conidiophores and conidia grown on MEA were prepared according to the modified method described by Pan et al. (1994) They were fixed overnight in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4), washed thoroughly with distilled water, dehydrated through an ethanol series (10%, 40%, 60%, 80% and 100%), each series lasting 30 minutes and dried in silica gel. After preparation, the stubs were covered with double-sided carbon tape, and the specimens were mounted on stubs, sputter-coated with gold, and examined using a PHILIPS XL series XL 20, S/W, 5.21. scanning electron microscope at 10KV.

Taxonomy

Polylobatispora setulosa L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius sp. nov. Fig. 1–6
Mycobank MB811045

Etymology – Latin, *setulosa*, provided with *setulae*.

Colonies on natural substrate inconspicuous. *Conidiophores* macronematous, septate, hyaline, forked, bottle-shaped, 10.0–25.74 × 1.43–2.5 µm. *Conidiogenous cells* lageniform, enteroblastic-phialidic, hyaline to greenish. *Conidia* hyaline, triangular, 3-lobed, 8.6–14.3 µm diam., with thick dark walls and a hyaline setula at each tip, 5.7–12.87µm long; with a central, fuscous halo, 5.7–7.0 µm diam.

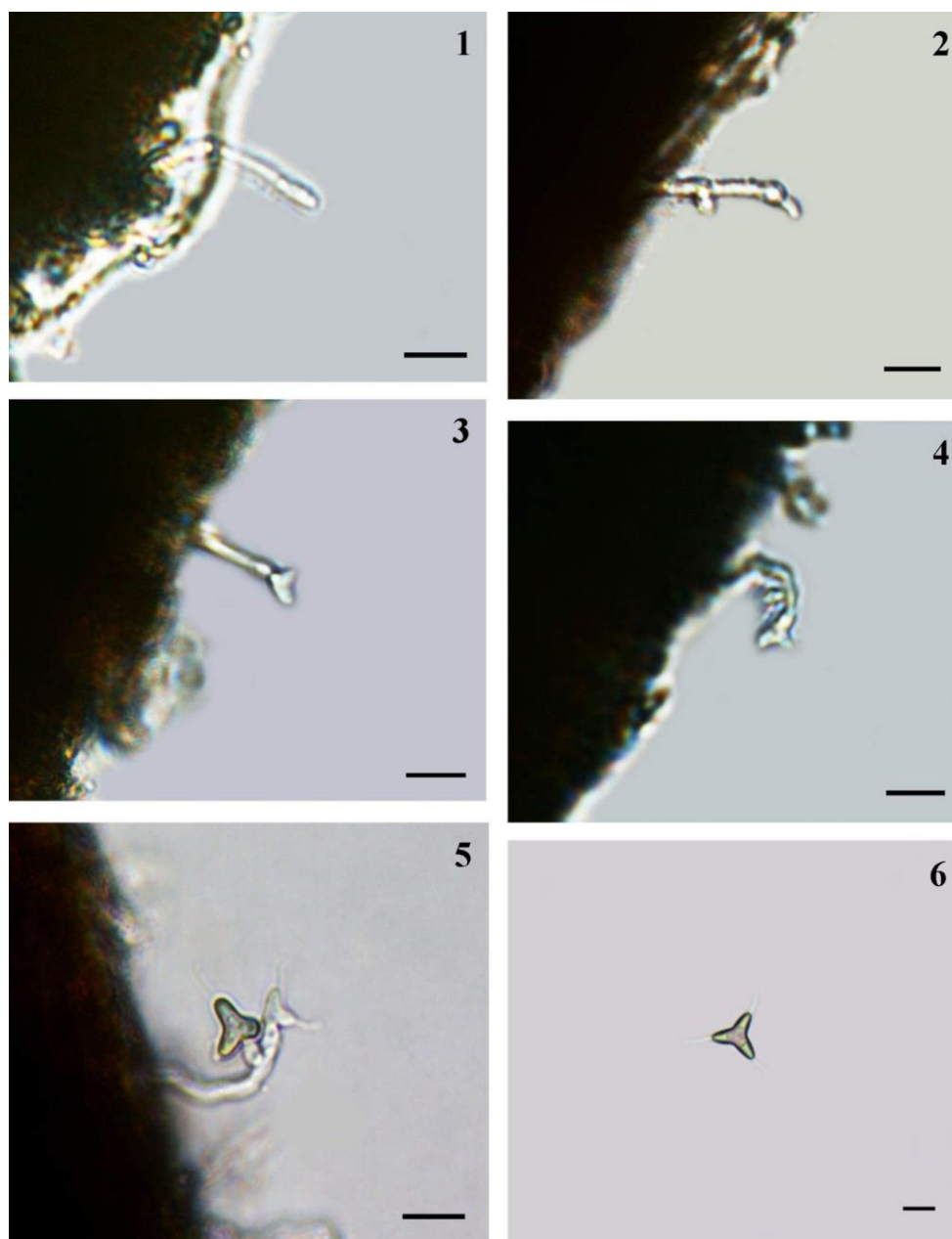
Teleomorph – unknown.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião island, “Cachoeira do Gato” stream, on submerged mixed leaf litter, 14 May 2013, L.B. Moro (Holotype: SP445-978).

Note – Pure culture of *Polylobatispora setulosa* was not possible to obtain.

Acumispora verruculosa Heredia, R.F. Castañeda & R.M. Arias. Mycotaxon 101: 90, 2007.

Fig. 7–12



Figs 1–6 – *Polylobatispora setulosa*. SP 445-978 (holotype). 1-5 Developmental stages of conidiophores and conidia. 6 Mature conidium with setulae at the tip of each lobe and central halo. Scale bar = 10 μ m.

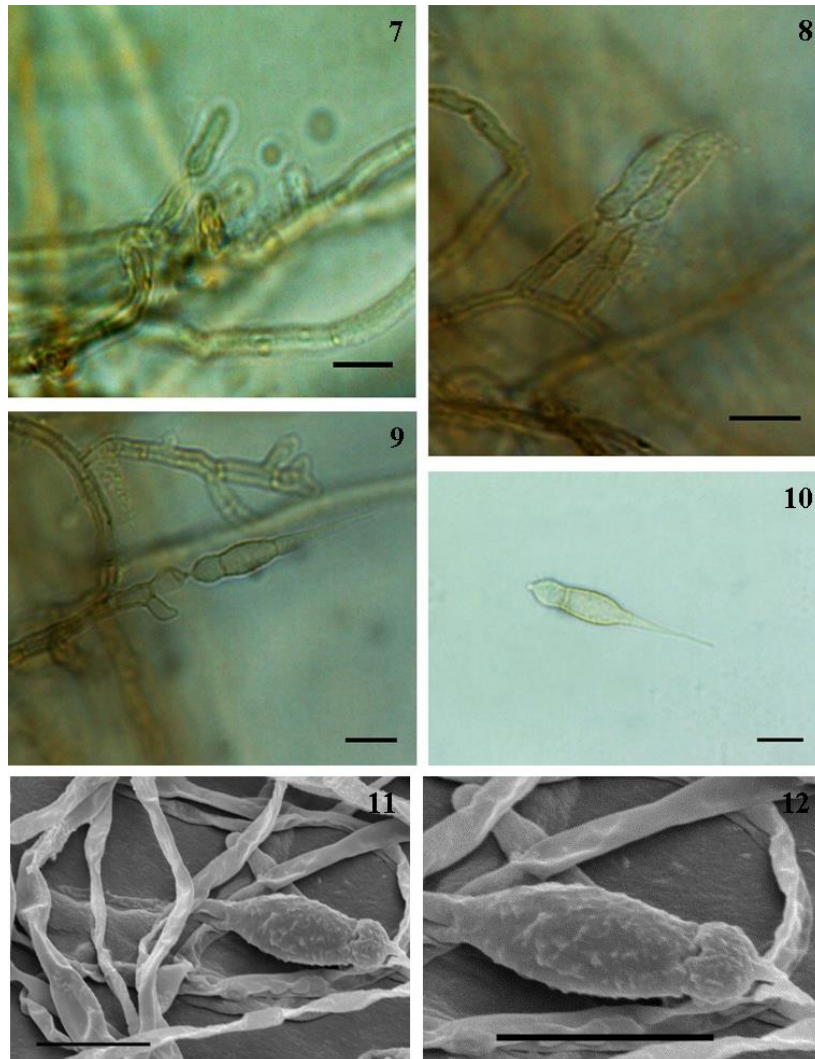
Colonies on MEA dark gray to greenish, velvety, slow growing, reaching less than 2 cm diam. after 2 weeks of incubation at 21 °C. *Mycelium* composed of branched, septate, hyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, erect, simple or branched, 0–4-septate, light brown to brown, 9.0–51.5 \times 2.86–4.30 μ m. *Conidiogenous cells* mono- or polyblastic, integrated, terminal and intercalary, proliferating sympodially, denticulate; conidiogenous loci apical or lateral, often protuberant and denticle-like; conidial secession rhexolytic. *Conidia* fusiform, 2-septate, constricted at the septa, with rounded basal cell and slender apical cell, acuminate, rostrate, hyaline to greenish, 30–45 (–50) \times 5.0–8.75 μ m, verruculose, with the basal cell often bearing a denticle-like detachment scar.

Teleomorph – unknown.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião island, “Cachoeira do Gato” stream, on submerged mixed leaf litter, 14 May 2013, L.B. Moro (SP445-979, CCIBt 4066).

Discussion

The genus *Polylobatispora* was originally described from leaves collected in Malaysia (Matsushima 1996) with two species: *P. deltoidea* Matsush., the type species, and *P. quinquecornuta* Matsush., based on the presence of cylindrical or bottle-shaped (doliiform), conidiophores and enteroblastic-phialidic, hyaline, conidiogenous cells that produce single, light brown, stauroconidia with a small scar formed after detachment from the conidiophore. *Polylobatispora deltoidea* presents characteristic triangular conidia with three lobes and *P. quinquecornuta* 5-lobed conidia. New records of these species are unknown. The main difference between *Polylobatispora deltoidea*, *P. setulosa* and *P. quinquecornuta* is the number of lobes: the first two species possess 3 lobes and the latter 5 lobes. The 3-lobed *P. setulata* differs from *P. deltoidea* by slightly larger conidia and the presence of flexible setulae, one at the tip of each lobe.



Figs 7–12 – *Acumispora verruculosa*. 7-9 Conidiophores, conidiogenous cells and conidia. 10 Mature conidium. 11 SEM image of mature conidium. 12 Details of the conidia wall. Scale bar = 10µm.

Identification key to species of the genus *Polylobatispora*

- 1a Conidia 5-lobed.....*P. quinquecornuta*
- 1b Conidia 3-lobed.....2
- 2a Conidia 6–8 µm diam., setulae absent.....*P. deltoidea*
- 2b Conidia 8.6–14.3 µm diam., setulae present.....*P. setulosa*

Table 2 Conidial morphology in species of *Polylobatispora*

Species	Conidia			References
	Shape	Diameter (μm)	Presence\absence of setulae	
<i>P. deltoidea</i>	3-lobed	6-8	absent	Matsushima (1996)
<i>P. quinquecornuta</i>	5-lobed	11-15	absent	Matsushima (1996)
<i>P. setulosa</i>	3-lobed	8.6-14.3	present	this paper

Matsushima (1980) described the genus *Acumispora* to accommodate three species collected on dead leaves in Taiwan: *A. uniseptata* Matsush., the type species, *A. biseptata* Matsush., and *A. phragmospora* Matsush., on the basis of a peculiar acuminate conidial shape and conidia, with different number of septa that secede rhexolytically from monoblastic or polyblastic, sympodially proliferating, denticulate conidiogenous cells on simple or irregularly branched conidiophores. Heredia et al. (2007) described *A. verruculosa* Heredia, R.F. Castañeda & R.M. Arias from submerged leaves collected at the waterfall “Los Tuxtlas” in Veracruz, Mexico, with distinctly rough-walled conidia. This fungus was recently reported for the first time from South America on submerged leaves and branches collected in aquatic environments at the urban area of Belém do Pará (“Ilha do Combu”, “Parque Ecológico Gunma” and “Parque Estadual do Utinga”) located in the Amazon biome (Silva et al. 2013). Our collection is the third from the continent with the conidia slightly larger than in earlier collections indicating that there are variations in conidial size: earlier collections are $25.4\text{--}33.5 \times 4.4\text{--}5.5 \mu\text{m}$ whereas the present collection has conidia measuring $30\text{--}45 \times 5.0\text{--}8.75 \mu\text{m}$. Taxonomic key and diagnostic features of described species in *Acumispora* are given below.

Identification key to species of *Acumispora*

- 1a. Conidia with verruculose walls.....*A. verruculosa*
 1b. Conidia smooth-walled.....2
 2a. Conidia 1-septate.....*A. uniseptata*
 2b. Conidia more than 1-septate.....3
 3a. Conidia 3-6 septate.....*A. phragmospora*
 3b. Conidia 2-septate.....*A. biseptata*

Table 1 Diagnostic characteristics of the genus *Acumispora*

Species	Conidia					References
	Nº of septa	Length (μm)	Width (μm)	Color	Wall texture	
<i>A. uniseptata</i>	1	23-28	5.5-6.5	Hyaline to very light brown	smooth	Matsushima (1980)
<i>A. biseptata</i>	2	16-25	3.8-5	Hyaline to very light brown	smooth	Matsushima (1980)
<i>A. phragmospora</i>	3-6	20-35	4-5	Brown to subhyaline	smooth	Matsushima (1980)
<i>A. verruculosa</i>	2	25.4–33.5	4.4–5.5	Pale brown	verruculose	Heredia et al. (2007)
<i>A. verruculosa</i> (present collection)	2	30-45	5.0-8.75	Hyaline to greenish	verruculose	This paper

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*Campylospora brasiliensis, a new species of freshwater
fungi from Brazil*

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Campylospora brasiliensis, a new species of freshwater fungi from Brazil

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Abstract

Campylospora brasiliensis is a new freshwater fungus that has been collected from submerged mixed leaf litter in the Brazilian Atlantic Rainforest. A molecular phylogenetic analysis based on ITS sequences of the rDNA reveals it as a new species. Comparison with other species of *Campylospora* is provided.

Key words: anamorphic fungi, ingoldian fungi, ITS sequences, phylogeny, taxonomy

Introduction

Among the freshwater fungi, the ecological group of ingoldian fungi presents conidia with typical tetra- or polyradiate and varyingly branching. The conidia are produced almost exclusively on conidiophores ranging from the leaf tissue into water. These fungi contribute to decomposition of leaves or woody debris by the release of extracellular enzymes and so increase their palatability for shredders, through nutritional enrichment (Bärlocher 1992, Cheng *et al.* 1997, Goh 1997).

The genus *Campylospora* (Ranzoni 1953: 373) has been cited worldwide, e.g. in Africa: South African Republic (Webster *et al.* 1994); Americas: Argentina (Arambarri & Spinedi 1984), Brazil (Schoenlein-Crusius & Milanez 1990, Malosso 1995, Schoenlein-Crusius 2002, Fiuza & Gusmão 2013), Ecuador (Matsushima 1993), Hawaii (Ranzoni 1979), Peru (Matsushima 1993), Venezuela (Nilsson 1962, Cressa & Smits 2007, Smits *et al.* 2007, Silva & Briedis 2009), U.S.A. (California, Ranzoni 1953); Asia: India (Sridhar & Kaveriappa 1992), Thailand (Tubaki *et al.* 1983, Sakayaroj 2005); Europe: England (Ingold 1974), Spain (Casas & Descals 1997), Austria (Regelsberger *et al.* 1987); Oceania: Australia (Cowling 1963), New Zealand (Aimer & Segedin 1985).

The distribution of the species of *Campylospora* is known from biodiversity studies based on microscopic examination of natural leaf litter samples or of conidia obtained in the laboratory by enhancing the fungal sporulation in previously colonized leaves in water samples. However, several misinterpretations in identifying the species occur frequently, leading to biased data on distribution of the genus (Fiuza & Gusmão 2013, Marvanová & Laichmanová 2014). Thus, complete characterization and description of new species is only appropriate if based on the taxa isolated in pure culture (Fiuza & Gusmão 2013).

During the survey of aquatic hyphomycetes inhabiting submerged mixed leaf litter in waterfalls situated in the “Parque Estadual de Ilhabela”, an important fragment of Brazilian Atlantic Rainforest, conidial fungus, morphologically matching the genus *Campylospora*, was detected. Supported by phylogenetic analysis from ITS region the rDNA, *C. brasiliensis* is described as a new species.

Material & Methods

Collection of samples

At Toca waterfall, Cemitério stream, Caminho da Praia Vermelha river, Tesouro da Colina waterfall, all situated in

the “Parque Estadual de Ilhabela”, samples of submerged mixed leaf litter (around 10 leaves) and local water were collected in sterile polyethylene bottles (200 ml). Once at the laboratory, the leaves were fragmented in approximately 4cm diam. pieces, incubated in Petri dishes containing sterile distilled water, kept in climatic room at around 20°C. From the 7th incubation day onwards, slides of leaf fragments were prepared with distilled water and the permanent slides were prepared with polyvinyl alcohol lacto glycerol resin—PVLG (Morton *et al.* 1993). The slides were observed under an Olympus BX50 light microscope to search for taxonomically relevant features that allow the identification of anamorphic species according to current literature (Ingold 1975, Marvanová 1997, Santos-Flores & Betancourt-Lopez 1999) or specific for the genus *Campylospora* (Marvanová & Laichmanová 2014). Photographs of the fungus were taken with a Leica DMLB2 microscope and a Leica DFC 280 camera.

Isolation

In order to obtain cultures, water droplets containing conidia of the target fungi were transferred with a Pasteur pipette, and spread over the surface of Malt Extract Agar 0.1% (MEA 0,1%) medium with chloramphenicol (Descal 1997), previously disposed in Petri dishes. After 24 hours of incubation in climatic chambers at 21°C, the reverse side of the dishes was observed under low power of optical microscope to verify hyphal growths and to mark the position of the conidia with a permanent marker pen. Under aseptic conditions and with the aid of a sterile stainless steel needle, the conidia were removed from the media surface and inoculated onto a new dish, also with MEA, and incubated under similar conditions.

Permanent slides are deposited in the Herbarium SP and the fungal cultures in the Fungal Collection of the Instituto de Botânica (“Coleção de Culturas de algas, cianobactérias e fungos do Instituto de Botânica de São Paulo” —CCIBt).

Biomass production, DNA extraction, amplification, sequencing and phylogenetic analysis

Biomass was obtained by cultivating each isolate in Erlenmeyer flask containing 20 ml of MEA liquid medium. After incubation for 7–10 days at 21°C, the biomass was transferred to Eppendorf® tubes and was harvested by centrifugation at 13000 rpm for 15 min in order to obtain mycelia pellets. The supernatants were discarded and 1 ml of sterile deionized water was added to the tubes followed by vortexing at 2500 rpm. Tubes were again centrifuged, and the supernatant discarded. Pellets were treated according to the protocol described in the PureLink Genomic DNA kit (Invitrogen®). Electrophoresis was performed using 1% (p/v) agarose gel, applying 3–5 µL of the sample + 2 µL of Gel Loading Buffer Type I (Sigma®), and a standard of 2 µL of 123 bp DNA Ladder (Invitrogen®) + 2 µL of Gel Loading Buffer. Electrophoresis conditions were 100 mV for 40 min. The complete ITS regions of rDNA were amplified by PCR with the primers UN-up18S42 (ITS forward) and UN-lo28S22 (ITS reverse) (Robideau *et al.* 2011). DNA was amplified with the PCR SuperMix kit (Invitrogen®) in a C1000 Touch™ Thermal Cycler Bio-Rad. The conditions were as follows: volume 25 µl; initial denaturalization at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 30 s, 72 °C for 1 min, 35 and a final extension at 72 °C for 7 min. PCR products were analysed by electrophoresis on 1% agarose gel using 1–2 µL Low DNA Mass Ladder (Invitrogen®) + 2–4 µL of Gel Loading Buffer as standard and 2–3 µL of DNA. Amplification was purified with AxyPrep PCR Clean-up kit (Axygen®). Sequencing was performed using the above mentioned primers in an ABI 3730 DNA Analyser (Life Technologies™) at the Centro de Estudos do Genoma Humano, Instituto de Biociências, University of São Paulo, São Paulo, Brazil.

Editing and contiguity of the assembly of the DNA sequences were performed using Sequencher4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA). For phylogenetic reconstruction the sequences of *Campylospora* spp. provided by Marvanová & Laichmanová (2014), obtained from GenBank, were used. The sequences of *C. brasiliensis* were submitted to GenBank and their accession numbers are listed in Table 1.

Sequences were aligned using Clustal X 2.1 (Larkin *et al.* 2007) and manually checked and adjusted. The best fitting model of evolution were Kimura 2-parameter + Gama (K2+G) and the phylogenetic tree for ITS rDNA was constructed using neighbour-joining (NJ) (Saitou & Nei 1987) and Maximum Likelihood (ML) (Swofford *et al.* 1996; Saitou 1996) with the MEGA 5 program (Tamura *et al.* 2011). Sequence divergence was analysed using the Kimura 2-parameter model (Kimura 1980). Branch support was tested by 1000 replications on bootstrapped data sets (Felsenstein 1985). *Calonectria hurae* (Lombard *et al.* 2010) was used as outgroup.

TABLE 1. CCIBt culture collection number and GenBank accession number of *Campylospora brasiliensis* isolates, sequenced in this study.

Species	Strain No.	GenBank No.
<i>Campylospora brasiliensis</i> ^{1*}	CCIBt 4075	KP262470
<i>Campylospora brasiliensis</i> ²	CCIBt 4076	KP262471
<i>Campylospora brasiliensis</i> ³	CCIBt 4077	KP262472
<i>Campylospora brasiliensis</i> ⁴	CCIBt 4078	KP262473

*ex-holotype

Results

Phylogenetic analyses

A total of 21 ITS sequences of *Campylospora* spp. were aligned with an outgroup taxon. The ML and NJ tree are shown in the Fig. 1. The topology of ML tree was almost identical to that of NJ tree. The newly identified fungus is supported with high bootstrap values (99% in ML, 99% in NJ; Fig. 1). Phylogenetically, *C. brasiliensis* is more closely related to *C. parvula* (Kuzuha 1973), and this relationship is well supported (86% in ML, 95% in NJ; Fig. 1). These two species are close to *C. leptosoma* (Marvanová & Laichmanová 2014) (91% in ML, 98% in NJ; Fig. 1), which in turn are close to *C. filicladia* (Nawawi 1974) (96% in ML, 99% in NJ; Fig. 1).

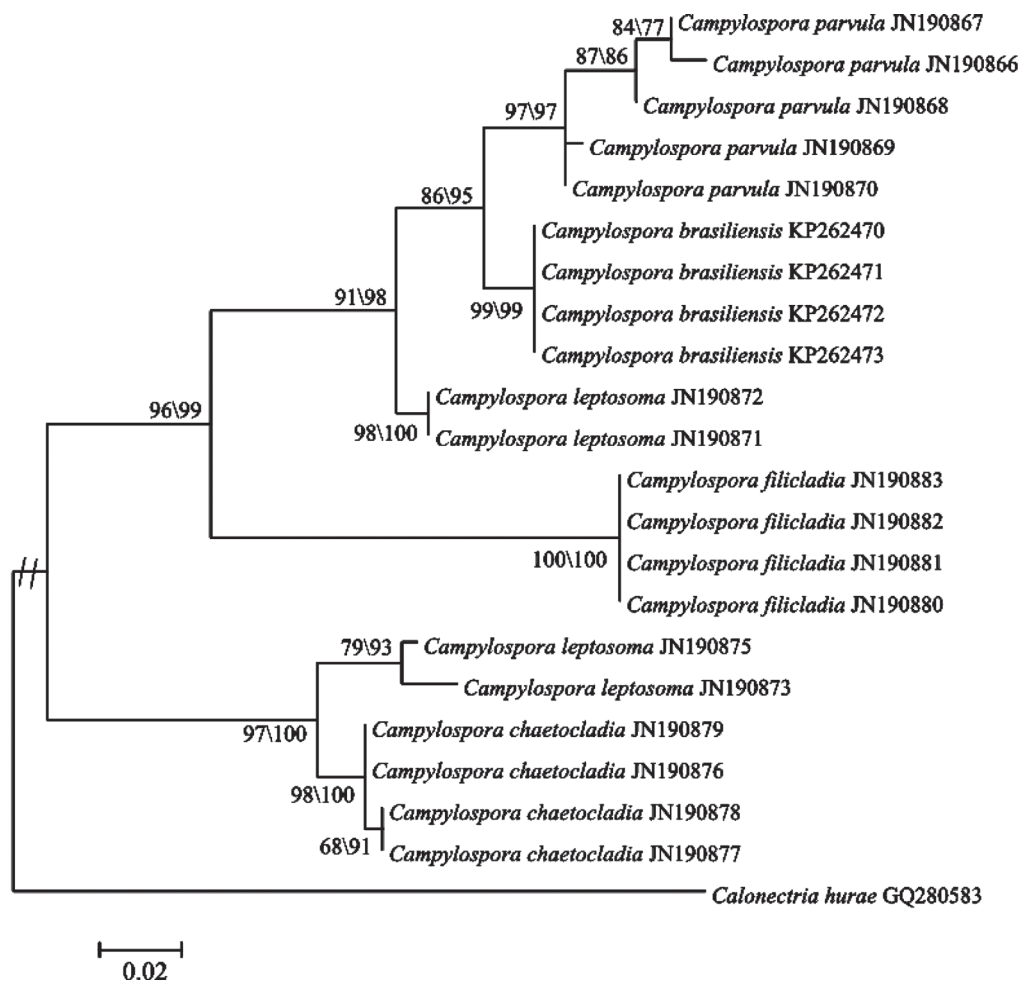


FIGURE 1. Phylogenetic tree reconstructed by Maximum Likelihood (ML) method and Neighbor Joining (NJ) method based on ITS sequences using 1000 corrections. *Calonectria hurae* was used as outgroup. Numbers refer to bootstrap support (%) (ML/NJ) and the bar show the number of substitutions per site. GenBank sequence accession numbers follow the taxon name.

TABLE 2. Estimates of evolutionary divergence (%) between *Campylospora* species based on ITS sequences. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 253 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

Species-GenBank number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1 <i>Campylospora brasiliensis</i> KP262470	0%																				
2 <i>Campylospora brasiliensis</i> KP262471	0%																				
3 <i>Campylospora brasiliensis</i> KP262472	0%	0%																			
4 <i>Campylospora brasiliensis</i> KP262473	0%	0%	0%																		
5 <i>Campylosporachaetocladia</i> JN190876	18%	18%	18%	18%	18%																
6 <i>Campylosporachaetocladia</i> JN190877	18%	18%	18%	18%	18%	0%															
7 <i>Campylosporachaetocladia</i> JN190878	18%	18%	18%	18%	18%	0%	0%														
8 <i>Campylosporachaetocladia</i> JN190879	18%	18%	18%	18%	18%	0%	0%	0%													
9 <i>Campylosporaparvula</i> JN190866	6%	6%	6%	6%	6%	20%	21%	21%	20%												
10 <i>Campylosporaparvula</i> JN190867	5%	5%	5%	5%	5%	19%	20%	20%	19%	1%											
11 <i>Campylosporaparvula</i> JN190868	5%	5%	5%	5%	5%	20%	21%	21%	20%	2%	1%										
12 <i>Campylosporaparvula</i> JN190869	3%	3%	3%	3%	3%	18%	19%	19%	18%	3%	3%	2%									
13 <i>Campylosporaparvula</i> JN190870	3%	3%	3%	3%	3%	18%	18%	18%	18%	3%	2%	2%	0%								
14 <i>Campylosporaleptosoma</i> JN190871	4%	4%	4%	4%	4%	14%	15%	15%	14%	7%	6%	7%	5%	5%							
15 <i>Campylosporaleptosoma</i> JN190872	4%	4%	4%	4%	4%	14%	15%	15%	14%	7%	6%	7%	5%	5%	0%						
16 <i>Campylosporaleptosoma</i> JN190873	19%	19%	19%	19%	19%	4%	5%	5%	4%	22%	20%	22%	20%	19%	15%	15%					
17 <i>Campylosporaleptosoma</i> JN190875	18%	18%	18%	18%	18%	3%	4%	4%	3%	20%	19%	20%	18%	18%	15%	15%	2%				
18 <i>Campylosporafilicladia</i> JN190880	14%	14%	14%	14%	14%	20%	20%	20%	20%	16%	17%	18%	15%	16%	14%	14%	20%	19%			
19 <i>Campylosporafilicladia</i> JN190881	14%	14%	14%	14%	14%	20%	20%	20%	20%	16%	17%	18%	15%	16%	14%	14%	20%	19%	0%		
20 <i>Campylosporafilicladia</i> JN190882	14%	14%	14%	14%	14%	20%	20%	20%	20%	16%	17%	18%	15%	16%	14%	14%	20%	19%	0%	0%	
21 <i>Campylosporafilicladia</i> JN190883	14%	14%	14%	14%	14%	20%	20%	20%	20%	16%	17%	18%	15%	16%	14%	14%	20%	19%	0%	0%	0%

Campylospora leptosoma formed two well supported clades, one with *C. brasiliensis* and *C. parvula* (91% in ML\98% in NJ) and another with *C. chaetocladia* (Ranzoni 1953) (97% in ML, 100% in NJ). The formation of two distinct clades of *C. leptosoma* was also observed by Marvanová & Laichmanová (2014), however, only the clade with *C. parvula* was well supported.

Analyses of evolutionary divergence were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution. The evolutionary divergence among *Campylospora* spp. based on ITS sequences are shown in Table 2. For the isolates of *C. brasiliensis* they were zero, and all isolates of *C. chaetocladia* presented an evolutionary divergence of 18% in relation to *C. brasiliensis*. However, also in relation to *C. brasiliensis*, the isolates of *C. parvula* varied from 3 to 6%; the isolates of *C. leptosoma* varied from 4 to 19%, and those of *C. filicladia* presented an evolutionary divergence of 14%.

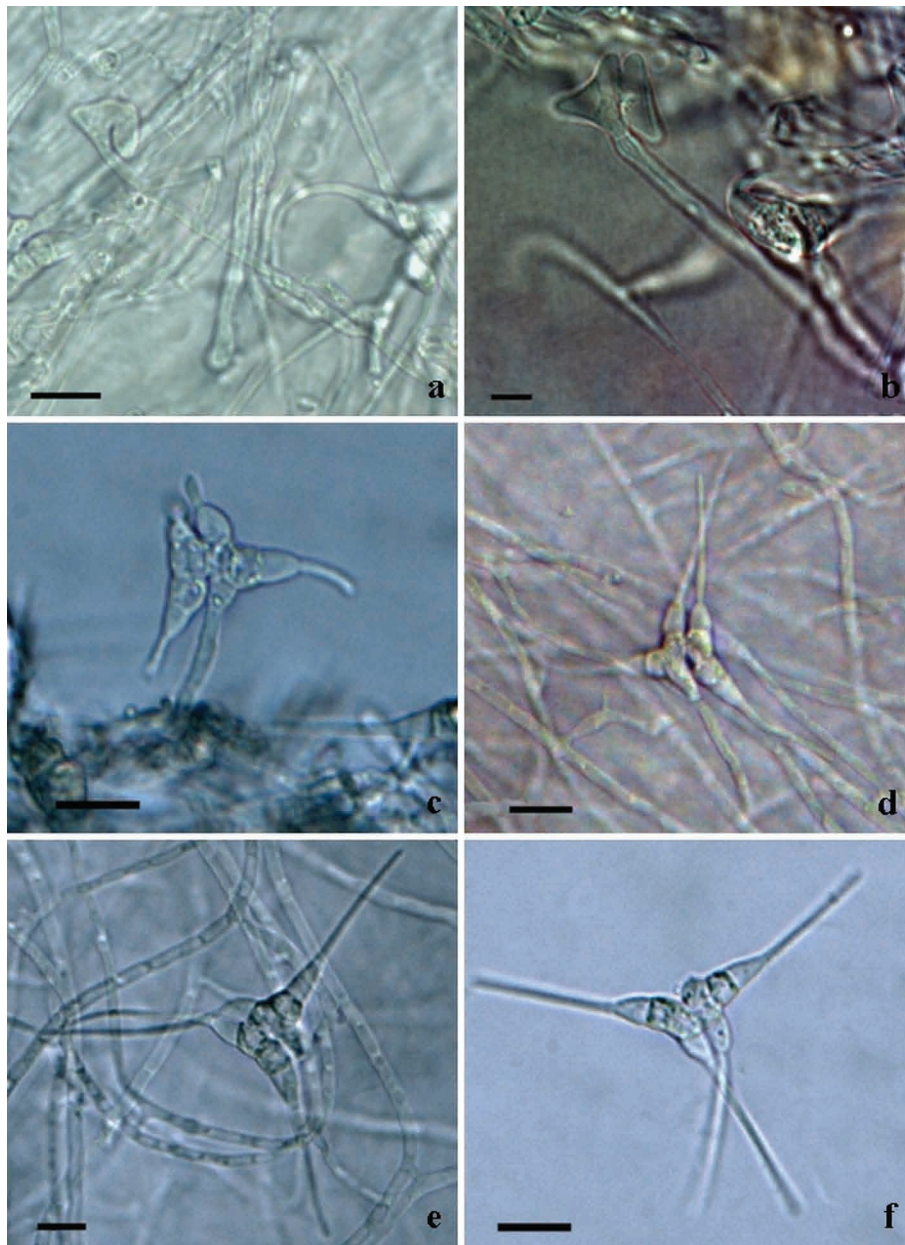


FIGURE 2. *Campylospora brasiliensis* (SP446282). **a–b.** Conidial primordium. **c.** Developing conidium on conidiophore. **d–f.** Mature conidia. Scale bar = 10 μ m.

Taxonomy

Campylospora brasiliensis L.B. Moro & I.H. Schoenlein-Crusius, *sp. nov.* Fig.2
 MycoBankMB811052

TABLE 3. Morphological characteristics of the genus *Campylospora*.

Species	Conidia			References
	Triangular part	Opposite part	Appendages	
			Distinguishing features of appendages	
<i>C. chaetocladia</i>	9-14x 15-25 µm	fusoid, 17-25 x 4.5-6.5 µm	29-56 x 1.5-2 µm	integrated with the conidial end cells Marvanová and Laichmanová (2014)
<i>C. parvula</i>	10-14 x 12-15µm	fusoid, 16-20.5 x 4-6µm	7-12µm	short, thin, one finger-like Marvanová and Laichmanová (2014)
<i>C. filicladia</i>	4.5-7.5 x 9-12 µm	allantoid, 9-13 x 3-4.5 µm	7-17 x 0.3-0.7µm	hair-like Marvanová and Laichmanová (2014)
<i>C. leptosoma</i>	6-10 x 11-23 µm	allantoid, 13-18 x 3-4 µm	9-35 x 1-1.5µm	base constricted Marvanová and Laichmanová (2014)
<i>C. brasiliensis</i>	10-11.4 x 17.2-21.5 µm	fusoid, (15.7) 20-21.5x (5) 5.7- 7.7 µm	21.5-30 x 1.4-1.7 µm	base discrete, unstricted This paper

Holotype:—BRAZIL. São Paulo: Ilhabela, São Sebastião island, Toca waterfall, in submerged mixed leaf litter, 27 June 2012, coll. *Moro L.B.*, (SP446282).

Ex-holotype:—CCIBt4075.

Asexual fungi, hyphomycetes. Colonies on MEA radiating, in sectors, one with creeping beige mycelium, and other with velvety off-white mycelium; central region of the colony elevated. Hyphae hyaline, thin-walled, septate, branched, 2.1–2.8 µm wide. Inflated cells hyaline, globose to ellipsoidal, 4.3–11.4 × 4.3–6.4 µm with thick walls. Conidia form abundantly after submerging pieces of culture into standing distilled water. Conidiophores not well differentiated from the vegetative hyphae, 2.1–2.9 µm wide. Conidiogenous cells integrated, conidial initial strongly recurved, with one septum (Fig. 1a). The proximal part develops two blunt conical outgrowths (Fig. 1b) which later become arms of the triangular part; the recurved part is finally transformed into the fusoid part. Later on, each of the end cells of the triangular and the fusoid part produces a thin, straight or slightly curved appendage (Fig. 1c). In mature conidia (Fig. 1d–f) the triangular part is 2–3 septate, 17.2–21.5 × 10–11.4 µm, fusoid part 3-septate, (15.7) 20–21.5 × (5) 5.7–7.7 µm, conidia central cells subhyaline to pale beige, with hyaline cells attached to the appendices. Appendages hyaline, discrete, not constricted at the base, straight or gently curved, 21.5–30 × 1.4–1.7 µm, mostly crossing over one another, resembling half opened scissors. Conidial detachment schizolytic, proliferation of conidiogenous cell has not been seen.

Sexual state:—Unknown.

Etymology:—referring to the country that the species was found.

Other cultures examined:—BRAZIL. São Paulo: Ilhabela, São Sebastião island, stream Cemitério, in submerged mixed leaf litter, 14 May 2013, *Moro L.B.*, CCIBt4076; BRAZIL. São Paulo: Ilhabela, São Sebastião island, Caminho da Praia Vermelha river, in submerged mixed leaf litter, 14 May 2013, *Moro L.B.*, CCIBt4077; BRAZIL. São Paulo: Ilhabela, São Sebastião island, Tesouro da Colina waterfall, in submerged mixed leaf litter, 15 May 2013, *Moro L.B.*, CCIBt4078.

Table 3 presents comparatively the main morphological features among the *Campylospora* species.

Discussion

The genus *Campylospora* was described by Ranzoni (1953) having *C. chaetocladia* as the type-species, collected from California, USA. Kuzuha (1973) described *C. parvula* from Japan, which differs from the type species by presenting thinner and shorter appendages, one of which is perpendicular towards its parent cell. In Malaysia, Nawawi (1974) described the third species, *C. filicladia*, which differs from *C. chaetocladia* and *C. parvula* by comparatively smaller conidia very constant in size, shape, and number of cells and by long and thin appendages. More recently, Marvanová & Laichmanová (2014) described *C. leptosoma* also from Malaysia. These authors discussed morphology of this species and published the first phylogenetic analysis of *Campylospora* spp.. In their NJ tree they placed the *C. leptosoma* strains into two clades: one as sister clade of *C. parvula* and the other one of *C. chaetocladia*. In the present study, these two clades show similar relationships: the first is close to *C. parvula* and *C. brasiliensis*, the second to *C. chaetocladia* (Fig. 1). According to our opinion, this can be a reason of considering two species being involved within *C. leptosoma*. However, because of lack of significant morphological differences among the isolates as pointed out by Marvanová & Laichmanová (2014) and the relative paucity of isolates (all from Malaysian part of Borneo, East Asia), we agree that further phylogenetic analyses of more isolates with broader geographic distribution of this species are necessary for confirmation or rejection of the “two species” hypothesis.

Although they are in distinct clades, at the first sight *C. brasiliensis* resembles morphologically *C. chaetocladia*. However, the important character appearing in *C. chaetocladia*, i.e. the strongly backwardly bent conidial appendages during conidial development, has not been observed in *C. brasiliensis*. The appendages according the Fig. 1d–f do not merge with the supporting cell, as they do in *C. chaetocladia*. Here their base is discrete, i.e. clearly distinct, delimited by a septum from the conidial end cells (in Fig. 1e probably out of focus). It seems that *C. brasiliensis* is morphologically also somewhat similar to *C. leptosoma*. From the latter it differs by the conoid end cells, the unconstricted bases of the appendages, and an overall more robust appearance, resembling *C. chaetocladia*.

On genus level, the *Campylospora* species are well recognisable owing to their characteristic shape. On the species level there is a great variability in the conidia. Therefore it is important to pay attention to details of morphological characters. Molecular methods may become useful tool for the identification of *Campylospora* species, when more isolates with various geographical origins were sequenced. For these techniques, it is necessary to obtain pure cultures,

what is often not possible in biodiversity studies due the number of the isolates and the difficulties in producing pure cultures. To assist in the morphological identification, particularly between the very similar *C. brasiliensis* and *C. chaetoclada*, apart from conidia measurements, the first may be identified by the presence of subhyaline to pale beige conidia central cells, with hyaline cells attached to the appendices.

Campylospora brasiliensis is the first new species of ingoldian fungi in insular habitats in Brazil, and its description expands the knowledge about freshwater fungi in South America.

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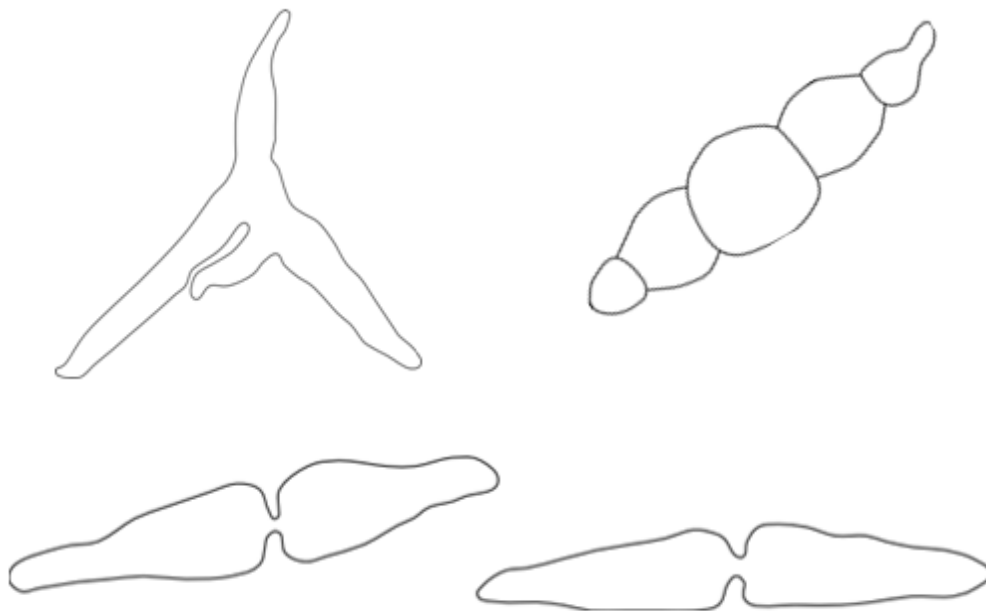
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***Freshwater Hyphomycetes in preserved insular system
from Brazil: First records at the Ilha do Cardoso State
Park, municipality of Cananéia, São Paulo State***

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Freshwater Hyphomycetes in preserved insular system from Brazil: First records at the Ilha do Cardoso State Park, municipality of Cananéia, São Paulo State

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Abstract – (Freshwater Hyphomycetes in preserved insular system from Brazil: First records at the Ilha do Cardoso State Park. In order to survey aquatic anamorphic fungi in insular water bodies in the Ilha do Cardoso State Park, in the municipality of Cananéia in the state of São Paulo, Brazil, submerged mixed leaf litter samples were collected from six sites from June of 2012 to February of 2013, brought to the laboratory, and incubated at room temperature (20°C) in Petri dishes containing sterile distilled water. From the 5th day on, until at least one month, fragments of the leaf litter samples were microscopically analyzed, resulting in 33 taxa, identified by morphological features. Among those, one is a new record to the State of São Paulo and six are new records to Brazil. Descriptions and figures of each new record are presented.

Asexual ascomycetes\Atlantic rainforest\biodiversity\insular waters

INTRODUCTION

The term aquatic Hyphomycetes does not have any 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 group (Belliveau & Bärlocher 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 fungal group 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 shredder invertebrates (Bärlocher 1992; Cheng *et al.* 1997; Suberkropp 1992, 2003). Therefore, these fungi may play a very important role in the nutrient cycling (Belliveau & Bärlocher 2005).

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 to 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 these 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). However these studies did not include insular areas, where the vegetation is in general pristine or highly preserved.

The State Park of Ilha do Cardoso (PEIC) is located in a homonymous island, at the southeastern coast of São Paulo State (25°03'05"-25°18'18"S and 47°53'48"-48°05'42"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).

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 *et al.* 2015). However, until now, there are no records about aquatic anamorphic fungi in Ilha do Cardoso State

Park, being the improvement of the knowledge concerning its diversity, the main aim of the present study.

MATERIALS AND METHODS

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”, and 5-“Perequê” river.

The site 1-“Perequê” waterfall and site 5-“Perequê” river, were visited for collections 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 of 2013, while site 3-“Córrego”, was visited during the first three field trips, and site 4-“Poço das Antas”, only during the last two ones (November of 2012 and February of 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 collection site, the temperature ($^{\circ}\text{C}$), pH, conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) and dissolved oxygen (mgO_2/L) of the water was measured with and Horiba U₁₀ and U₅₀ equipment.

Incubation

In the laboratory, the leaf samples were individually coarsely fragmented using sterile scissors to fit loosely in sterile Petri dishes. To the Petri dishes sterile water was added enough to cover the leaf fragments following Ingold (1975). In this way, 172 Petri dishes were obtained, which were incubated at room temperature (around 20°C) for several weeks. Whenever proliferation of bacteria was perceptible or the leaf fragments began to emerge, 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 microscopically analyzed, until no new fungi were observed, for at least one month. Fragments of the incubated leaf samples were mounted in 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 in order to identify the taxa by specific identification keys (Ingold 1975; Marvanová 1997; Santos-Flores & Betancourt-López 1997) and keys for genera. For documentation and *ex-situ* preservation purposes, slides were also prepared with PVLG (polyvinyl alcohol lactoglycerol) resin, and deposited at the Herbarium “Maria Eneida

P. Kaufmann Fidalgo” in the Instituto de Botânica, in São Paulo. Digital images were obtained with the aid of an optical microscope Leica DM LB2, equipped with a Leica DFRC 280 digital camera.

Frequencies of the taxa were calculated based on Dajoz (1973) by the ratio between the number of sites where the fungal taxa occurred and the number of studied sites, multiplied by 100.

RESULTS AND DISCUSSION

Abiotic factors

The water temperature probably followed the climatic conditions, being around 18°C in colder months such as August/2012 and higher, 23°C in February/2013 in all sites (Table 1). The values of water temperature during the collections 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 a temperature 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°C to 25°C (Ingold 1975).

Regarding the pH of the water, the values varied greatly, from 5.58 to 8.75 at the “Perequê” waterfall, and lower, from 5.26 to 6.64 at the “Perequê” river. 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 little, around 6.14-6.28. Surprisingly, the only measurement of the pH of “Cor de Chá” river, revealed a very low value, corresponding to 3.84, as well as a very high value of the conductivity ($0.394\mu\text{S}\cdot\text{cm}^{-1}$). Although there has been a high variation in pH values between collection 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.

At the other sites, the conductivity, in general, can be characterized as low, ranging from a maximum of 0.057 to $0.043\mu\text{S}\cdot\text{cm}^{-1}$. The values of dissolved oxygen were expected to be high in view of the fact that most sites are streams, with exception of “Poço das Antas”, which is a pond. The values varied from 6.73 to 10.45 mgO₂/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 $0.03\mu\text{S}\cdot\text{cm}^{-1}$ of conductivity and 5.1 to 7.8mgO₂/L for dissolved oxygen in the “Parque Estadual da Cantareira”.

The values of the abiotic parameters of the freshwater at the island are compatible to other ones usually reported for the Brazilian Atlantic Rainforest, not being limited to fungi anyway.

Fungal diversity

A total of 33 taxa of aquatic Hyphomycetes were observed in water bodies of the Ilha do Cardoso State Park, with a total of 95 occurrences (Table 2). In the first collection 17 taxa (25 occurrences) were recorded. The 2nd and 3th collection presented equal numbers of taxa (14), and the numbers of occurrences were close (21 and 19, respectively). On the 4th collection 17 taxa (30 occurrences) were recorded.

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

The “Perequê” river presented 24 occurrences and two unique taxa, *Blodgettia indicata* Subram. and *Pyramidospora casuarinae* Sv. Nilsson. The 1st collection was with the largest number of occurrences (11). Among the 2nd, 3rd and 4th collections there was no significant variation (04, 03 and 05, respectively).

The “Córrego” and the “Poço das Antas” registered 13 occurrences of aquatic Hyphomycetes to each location. Córrego presented *Pyramidospora constricta* N. Singh as an unique taxon, and, at the same manner, Poço das Antas presented *Ingoldiella hamata* D.E. Shaw. The low number of occurrence can be related to the smallest number of collections, which for both places were done three times.

In the “Cor de Chá” River just a collection was made totaling the record of six occurrences. Nevertheless, this location presented 04 exclusive taxa: *Dactylellina appendiculata* (Anastasiou) M. Scholler, Hagedorn & A. Rubner, *Isthmolongispora minimum* Matsush., *Pleiochaeta amazonensis* Matsush. and *Subulispora longirostrata* Nawawi & Kuthub.

According to Dajoz (1973), species with a frequency of occurrence of $\geq 50\%$ are classified as constant, those with a frequency between 25 and 50% are accessories and those with a frequency of $< 25\%$ as occasional, showing an interesting connection between the number of collections and fungal taxa. The constancy index of species are shown in the Table 2.

Among the total mycota, the most frequent species were *Triscelophorus monosporus* Ingold (85.7%), *Lunulospora curvula* Ingold (78.6%) and *Tripospermum camelopardus* Ingold, Dann & P.J.McDougall (71.4%), classified as constant.

Several studies corroborate with the high frequency of *T. monosporus* (Schoenlein-Crusius & Milanez 1990; Schoenlein-Crusius *et al.* 1990; Schoenlein-Crusius *et al.* 1992; Pires-Zottarelli *et al.* 1993; Schoenlein-Crusius & Milanez 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 frequency index between 28.6-42.7%: *Anguillospora longissima* (Sacc. & P. Syd.) Ingold, *Flagellospora curvula* Ingold, *Lunulospora cymbiformis* K. Miura, *Scutisporus brunneus* K. Ando & Tubaki, *Tripospermum myrti* (Lind) S. Hughes and *Triscelophorus acuminatus* Nawawi, 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 (7.1-21.4%), and classified as occasional. These, *Blodgettia indica* Subram. has recently been isolated in leaves of *Caesalpinia echinata* Lam. and *Campomanesia phaea* (O. Berg.) Landrum submerged in a lake in “Parque Municipal Alfredo Volpi” (Moreira & Schoenlein-Crusius 2012). *Flagellospora penicillioides* Ingold was isolated from submerged mixed leaf litter from the “Cerrado” biome Luíz Antônio stream, SP by Schoenlein-Crusius (2002). *Heliscus submersus* H.J. Huds. was also isolated in submerged mixed leaf litter on Monjolino river and Jacaré river in São Carlos, SP (Malosso 1999), *Ingoldiella hamata* D.E. Shaw was described by Schoenlein-Crusius (2002) in submerged mixed leaf litter in Itirapina, SP. *Jaculispora submerged* Hudson, H.J. & Ingold, C.T., was recently described by Fiuza & Gusmão (2013) in the Serra da Jibóia, Brazilian semi-arid region. *Pyramidospora casuarinae* Sv. Nilsson, was registered as the first citation to South America by Schoenlein-Crusius *et al.* (2009), *Subulispora longirostrata* Nawawi & Kuthub. was registered colonizing leaves *M. cabussu* Hoehnee and *T. pulchra* Cogn. In Paranapiacaba, Santo André, SP (Gusmão *et al.* 2001; Grandi & Gusmão 2002), *Tetracladium marchalianum* De Wild. 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* Berk. & Broome was recorded by Gruppi (2008) on mixed leaf litter samples collected by “Parque Central” in Santo André, SP and Barbosa *et al.* (2013) in submerged leaf litter in the city of Santa Terezinha, BA in Brazilian semi-arid region. The remaining taxa occurred so sporadic between collections, highlighting *Pyramidospora robusta* Moreira & Schoenlein-Crusius (2012) which has recently been identified in submerged leaf litter of “Parque Alfredo Volpi”, SP, an urban environment.

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

Morphological description

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 x 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, LB Moro.

Remarks: Although among the analyzed specimens, the presence of curved conidia was not observed, but only straight conidia, all other morphological features are in accordance to the original description.

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 habitats, were not taken in 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 to *Dactyllelina appendiculata*. The present research 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-cellularia, hyaline, central cell rectangular, ends of the cells navicular, 25-33 x 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, LB Moro; “Perequê” waterfall, in submerged mixed leaf litter, 30-VIII-2012, GH Jerônimos; “Poço das Antas”, in submerged mixed leaf litter, 06-XI-2012, LB Moro.

The genus *Isthmolongispora* was described by Matsushima (1971) for the Solomon Islands and Papua New Guinea, together with *I. intermedia* and *I. minima*. The genus is characterized by the presence of free conidiophores and conidial serials, formed by cells connected by 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-cellularia, 10-15.7x 2.1-4.3 μm 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, LB Moro. It is the first record of this specie from Brazil.

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-cellularia, 54.5-60 x 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, LB Moro; “Cor de Chá” river, in submerged mixed leaf litter, 26-II-2013, LB Moro.

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. The genus *Wiesneriomyces* includes three species, *Wiesneriomyces laurinus* (Tassi) P.M. Kirk, the type species *Wiesneriomyces javanicus* Koord., and *W. conjunctospous* Kuthub. & Nawawi (Suetrong *et al.* 2014). However, *W. laurinus* (Tassi) P.M. Kirk may have 5-9 cells composing the conidia in culture medium or 7-8 cells in natural substrate as noted by Matsushima (1975), more than 15 cells as described by Ellis (1971). *W. javanicus* Koord. may have 6-8 cells which are connected by thin hyphal bridges (Matsushima 1971, 1975). *W. conjunctospous* Kuthub. & Nawawi was described conidia with 15-21 cells (Kuthubutheen & Nawawi 1988). 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 cell conidia agrees 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.

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

Figure 3c

Conidiophore not observed. Conidia solitary, brown, cylindrical-fusiform, multi-septated, 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 x 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, LB Moro.

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 genus of dematiaceous fungi, with cylindrical-fusiform conidia with appendages that vary in size and borne or not borne at the apical cell, for example, vary according to the species, besides of the presence of pigmented hyphae, conidiophores

and conidia (Castañeda-Ruíz *et al.* 1996). The specific epithet is *P. amazonensis*, due its reported to the Peruvian Amazon, observed for the first time 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.

Material examined: BRAZIL. São Paulo: Cananéia, Parque Estadual da Ilha do Cardoso, “Córrego”, in submerged mixed leaf litter, 06-XI-2012, LB Moro.

The genus *Pyramidospora* was described originally by Nilsson (1964), with the type species *P. casuarinae*, isolated from Venezuela, South America. 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 the region (Singh 1972). Currently the genus consists of eight species. This is the first record of the species for Brazil.

The results of this study confirm the cosmopolitan nature of this group of fungi, indicating 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”, a 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 collection 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.

The identified taxa are all first occurrences to the Ilha do Cardoso State Park. Besides, these results contribute to extend the knowledge about the occurrence and distribution of aquatic Hyphomycetes in areas of Atlantic Rainforest of São Paulo State and in the country, starting their studies in insular areas.

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Table 1. Some abiotic factors measured in the water with an U₁₀ Horiba equipment, at the sites during the collection of submerged leaf litter samples in the Ilha do Cardoso State Park.

Sites/collections	Abiotic factors			
	Temperature (°C)	pH	Conductivity(μS. cm ⁻¹)	DissolvedOxygen (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.

Table 2. Taxa observed in Ilha do Cardoso State Park. Frequency (%), occurrences and constancy of species: C – Constant; A – Accessory; O – Occasional.

Taxa	collection 1 (06/2012)			collection 2 (08/2012)			collection 3 (11/2012)				collection 4 (02/2013)					Frequency (%)	Occurrences	Constancy
	"Perequê" waterfall	"Perequê" river	"Corrego"	"Perequê" waterfall	"Perequê" river	"Poço das Antas"	"Perequê" waterfall	"Perequê" river	"Corrego"	"Poço das Antas"	"Perequê" waterfall	"Perequê" river	"Corrego"	"Poço das Antas"	"Cor de Chá" river			
<i>Alatospora acuminata</i> Ingold	x			x												14,3	2	O
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	x	x		x											x	28,6	4	A
<i>Anguillospora pseudolongissima</i> Ranzoni	x			x					x							21,4	3	O
<i>Blodgettia indica</i> Subram. Fig. 1a-b												x				7,1	1	O
<i>Camposporium antenatum</i> Harkn. Fig. 1c							x				x					14,3	2	O
<i>Campylospora chaetoclada</i> Ranzoni	x										x					14,3	2	O
<i>Clavariopsis aquatica</i> De Wild. Fig. 2a			x				x									14,3	2	O
<i>Dactylectella appendiculata</i> (Anastasiou) M. Scholler, Hagedorn & A. Rubner*														x		7,1	1	O
<i>Dendrosporium lobatum</i> Plakidas & Edgertonex J.L. Crane Fig. 2b				x						x		x				21,4	3	O
<i>Flagellospora curvula</i> Ingold	x	x		x				x								28,6	4	A
<i>Flagellospora penicillioides</i> Ingold	x															7,1	1	O
<i>Helicomyces</i> sp.		x														7,1	1	O
<i>Helicosporium</i> sp.						x										7,1	1	O
<i>Heliscus submersus</i> H.J. Huds. Fig. 2c				x												7,1	1	O
<i>Ingoldiella hamata</i> D.E. Shaw Fig. 2d									x							7,1	1	O
<i>Isthmolongispora intermedia</i> Matsush.*		x		x					x							21,4	3	O
<i>Isthmolongispora minima</i> Matsush.*														x		7,1	1	O
<i>Isthmolongispora quadricellularia</i> Matsush.*		x												x		14,3	2	O
<i>Jaculispora submersa</i> Hudson, H.J. & Ingold, C.T. Fig. 2h				x												7,1	1	O
<i>Lumulospora curvula</i> Ingold Fig. 3a	x	x	x	x	x	x	x		x		x		x			78,6	11	C
<i>Lumulospora cymbiformis</i> K. Miura	x	x		x			x									28,6	4	A
<i>Pleiochaeta amazonensis</i> Matsush.*														x		7,1	1	O
<i>Pyramidospora casuarinae</i> Sv. Nilsson		x														7,1	1	O
<i>Pyramidospora constricta</i> N. Singh*									x							7,1	1	O
<i>Pyramidospora robusta</i> C.G. Moreira & I.H. Schoenlein-Crusius Fig. 3e												x	x	x		21,4	3	O
<i>Scutisporus brunneus</i> K. Ando & Tubaki Fig. 3f		x		x					x				x			35,7	5	A
<i>Subulispora longirostrata</i> Nawawi & Kuthub. Fig. 4a														x		7,1	1	O
<i>Tetracladium marchalianum</i> De Wild.	x															7,1	1	O
<i>Tetraploa aristata</i> Berk. & Broome Fig. 4b																7,1	1	O
<i>Tripodsporium camelopardus</i> Ingold, Dann & P.J. McDougall Fig. 4c		x		x	x	x	x		x		x	x	x			71,4	10	C
<i>Tripodsporium myrti</i> (Lind) S. Hughes	x	x					x		x							28,6	4	A
<i>Triscelophorus acuminatus</i> Nawawi				x	x		x				x		x			42,9	6	A
<i>Triscelophorus monosporus</i> Ingold Fig. 4d	x	x	x	x	x	x	x		x		x	x	x			85,7	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 collection		18			15				14				17					
Number of occurrences		26			22				19				30					

*new records

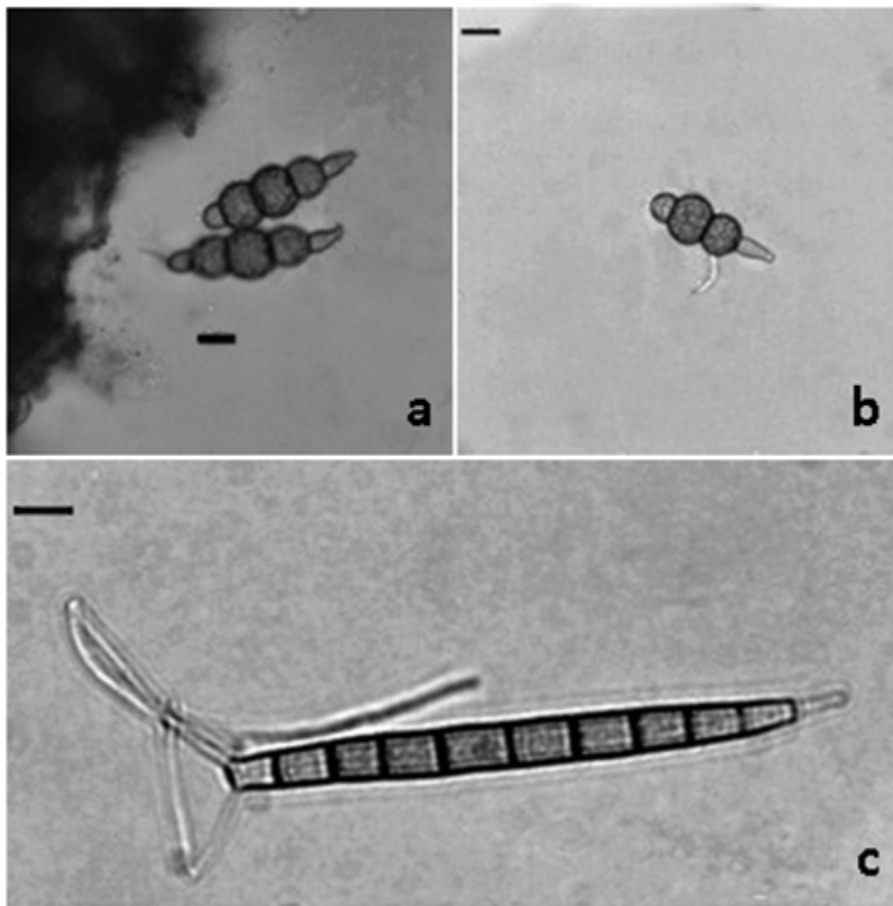


Figure 1: a-b. Mature conidia of *Blodgettia indica*. c. Mature conidia of *Camposporium antennatum*. Scale bar = 10 μ m.

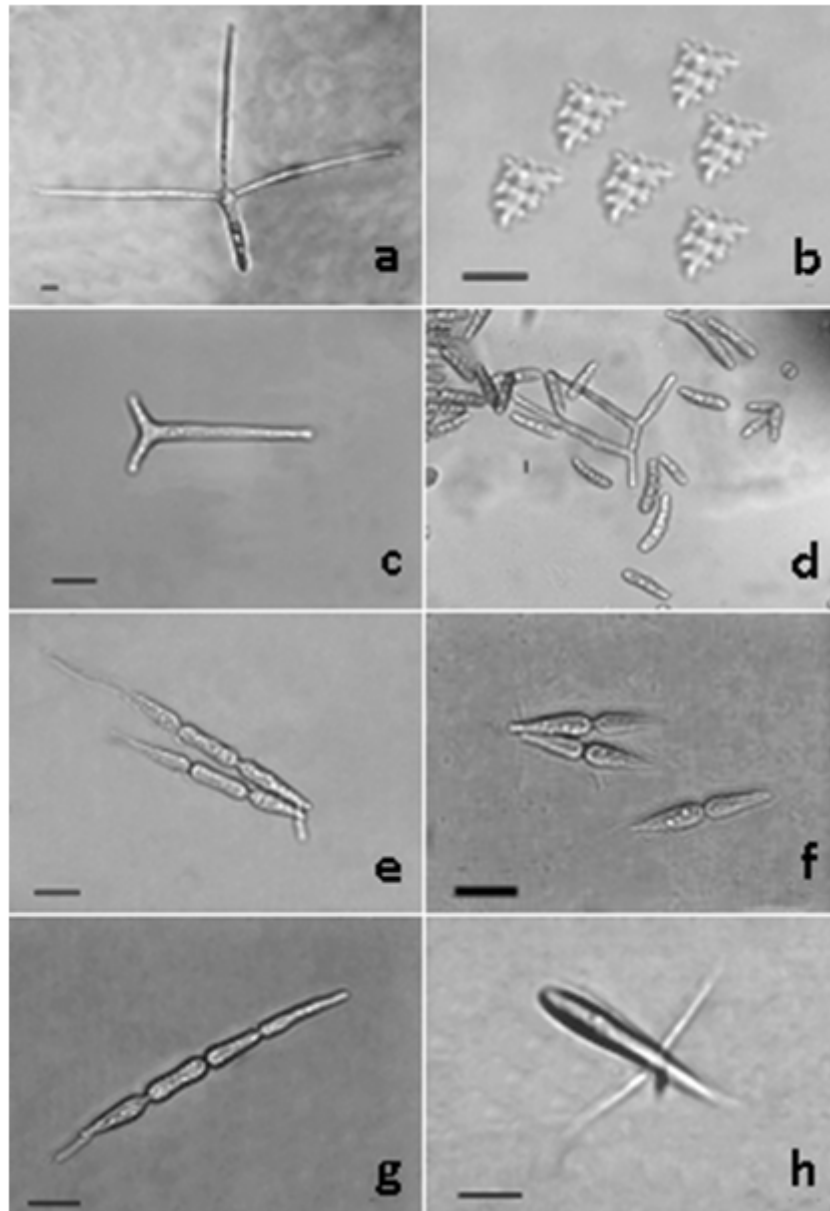


Figure 2: **a.** Mature conidia of *Clavariopsis aquatica*. **b.** Mature conidia of *Dendrosporium lobatum*. **c.** Mature conidia of *Heliscus submersus*. **d.** Initial stage of development of *Ingoldiella hamata*. **e.** Mature conidia of *Isthmolongispora intermedia*. **f.** Mature conidia of *Isthmolongispora minima*. **g.** Mature conidia of *Isthmolongispora quadricellularia*. **h.** Mature conidia of *Jaculispora submersa*. Scale bar = 10 μ m.

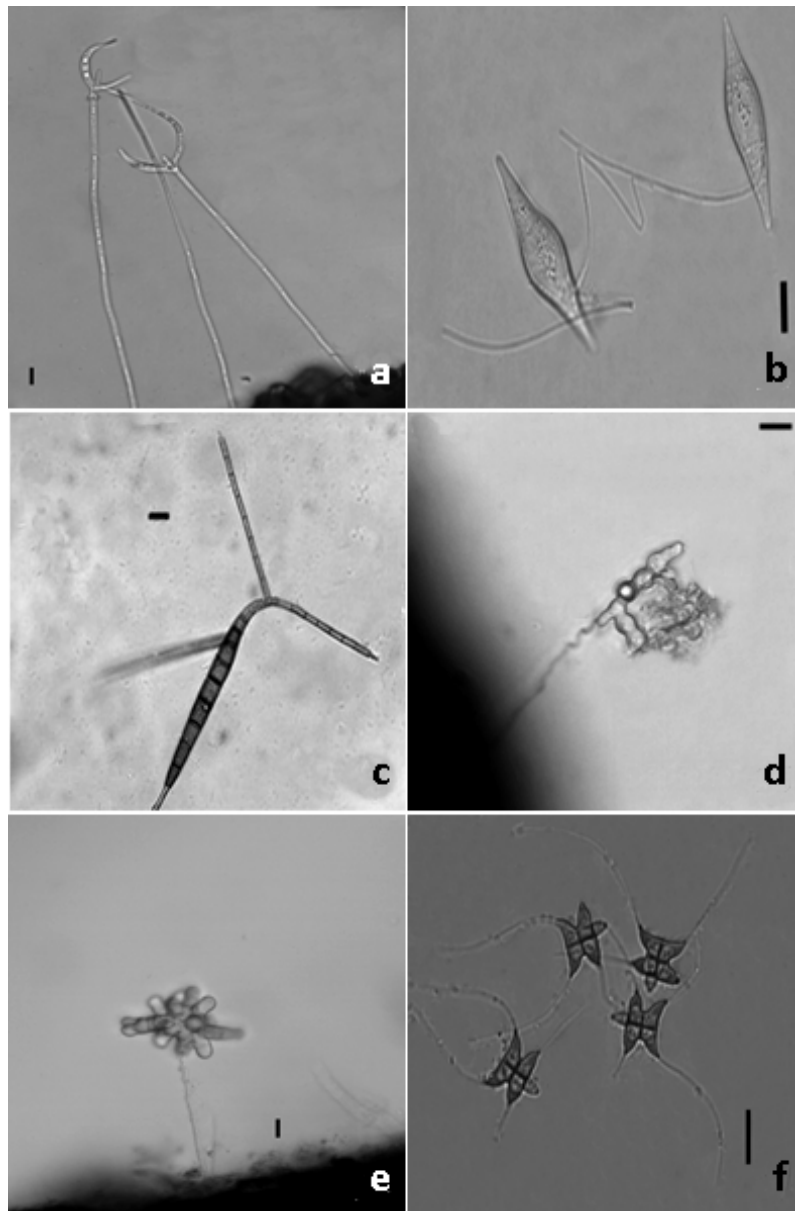


Figure 3: **a.** Conidia and conidiophore of *Lunulospora curvula*. **b.** Mature conidia of *Dactyllelina appendiculata*. **c.** Mature conidia of *Pleiochaeta amazonensis*. **d.** Initial stage of conidia of *Pyramidospora constricta*. **e.** Conidiophore and conidia of *Pyramidospora robusta*. **f.**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

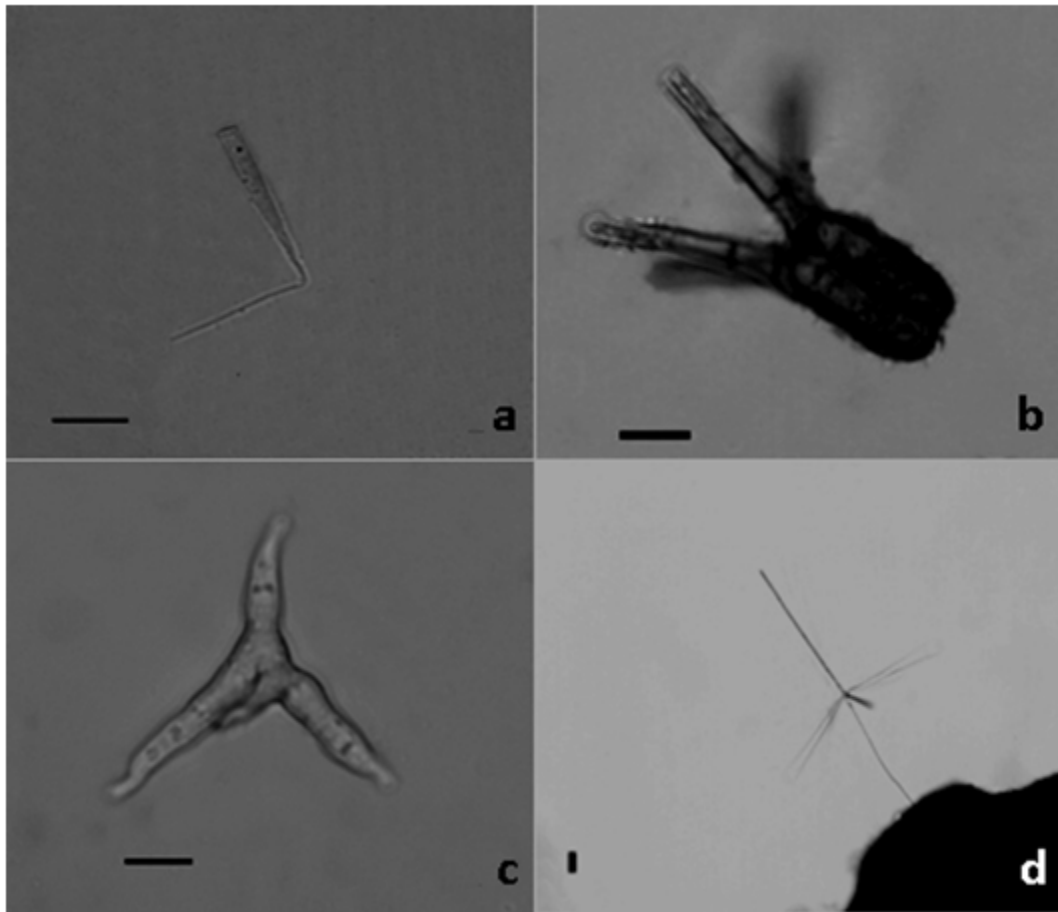


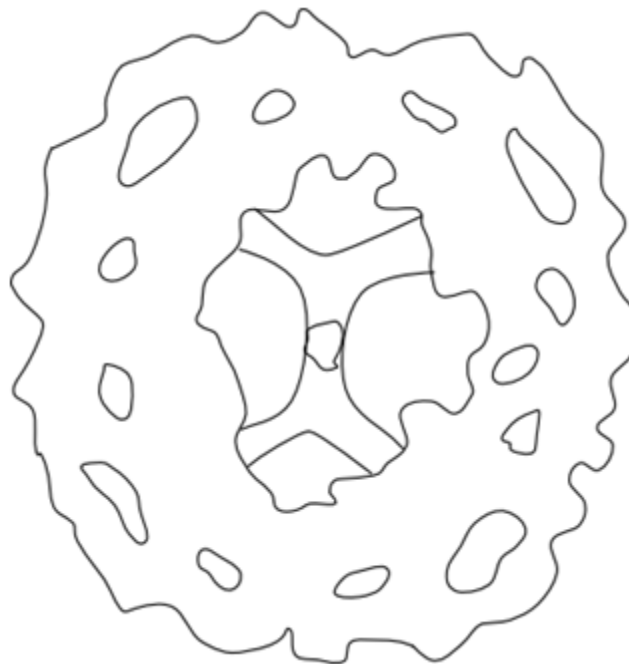
Figure 4: **a.** Mature conidia of *Subulispora longirostrata*. **b.** Mature conidia of *Tetraploa aristata*. **c.** Mature conidia of *Tripospermum camelopardus*. **d.** Conidia and conidiophores of *Triscelophorus monosporus*. Scale bar = 10 μ m.

Clathrosporium retortum, a novel aeroaquatic fungus in the
Sordariomycetidae (Ascomycota) from Brazil

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Clathrosporium retortum, a novel aeroaquatic fungus in the Sordariomycetidae (Ascomycota) from Brazil

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Abstract

Clathrosporium retortum sp. nov., collected on submerged mixed leaf litter samples at Ilha do Cardoso State Park, São Paulo state, Brazil, is described based on morphological and molecular data. The fungus is characterized by forming whitish, dense, subglobose to irregular propagules, hyaline to subhyaline when young, subhyaline to dark brown at maturity, that are formed by densely interwoven conidial filaments with each conidial cell repeatedly branching bilaterally or occasionally unilaterally. Phylogenetic analyses using partial LSU nrDNA sequence data suggest that *C. retortum* belongs in the Sordariomycetes (Ascomycota) where it forms a well-supported clade with *Clohesia corticola* in the Sordariomycetidae, but its ordinal or familial placement remains unresolved. Its phylogenetic placement confirms the polyphyletic nature of aeroaquatic fungi like *Clathrosporium*, as it was distantly related to one available sequence in GenBank named as *C. intricatum*, the type species, which is phylogenetically related to the Helotiales (Leotiomycetes). However, due to lack of authenticity of the identity of this sequence with the type specimen of *C. intricatum*, a broad concept of *Clathrosporium* is tentatively adopted here to accommodate the present fungus instead of introducing a new genus. *Beverwykella clathrata*, *Helicoön septatissimum* and *Peyronelina glomerulata* are recorded for the first time from Brazil. *Cancellidium applanatum* and *Candelabrum brocchiatum* are new records for the state of São Paulo.

Key words: biodiversity, hyphomycetes, insular areas, molecular, taxonomy

Introduction

Beverwijk (1951) first proposed the ecological group of aeroaquatic hyphomycetes to conceptualize fungi that are able to reproduce asexually only at the interface of air and water. The aeroaquatic fungi are known to occur in almost all types of aquatic environments (Bodiagyn & Prokhorov 2010). Moreover, they are adapted to sites with low concentrations of oxygen being prevalent in small water bodies, lentic-forest lakes and swamps (Bärlocher *et al.* 1978, Fisher & Webster 1979, 1981, Field & Webster 1983). Their propagules are morphologically diverse, but all species have one characteristic in common, which is the storage of air inside their propagules between the cells, enabling them to float on the air-water interface (Goh & Hyde 1996, Michaelides & Kendrick 1982, Webster & Descals 1981). Together with other microfungi, aeroaquatic hyphomycetes play an important role in nutrient cycling as leaf litter decomposers (Bodiagyn & Prokhorov 2010).

This ecological group of fungi has been poorly studied in the Neotropics with only a few scattered records or novelties from submerged wood or herbaceous debris in freshwater or terrestrial habitats (Allegrucci *et al.* 2009, Becerra *et al.* 2007, Castañeda & Kendrick 1991, Delgado & Mena 2004, Heredia *et al.* 2000, Matsushima 1983, 1993, Voglmayr & Delgado 2001, 2003, Zelski *et al.* 2014). In Brazil, such studies are still scarce, nevertheless there are results concerning the “Caatinga” biome in the country’s northeast (Barbosa & Gusmão 2011, Silva *et al.* 2014). In order to increase our knowledge about the diversity of freshwater hyphomycetes in Brazil, submerged mixed leaf litter samples were collected from lotic environments in biodiversity-rich insular areas of the southeastern Brazilian coast. Several interesting aeroaquatic taxa were recovered after incubation of material from the surveyed areas. Among these, one fungus producing whitish, dense propagules morphologically similar to *Clathrosporium* Nawawi & Kuthub. in shape and branching pattern (Hennebert 1998, Nawawi & Kuthubutheen 1987) was isolated twice at the same location.

Morphological and DNA sequence data belonging to the 28S nuclear ribosomal large subunit (LSU) and the internal transcribed spacer region (ITS) support the recognition of a novel aeroaquatic fungus that is described here as a new species of *Clathrosporium*. Five other aeroaquatic species were also found, three of them for the first time from Brazil and two others are reported as new for the state of São Paulo.

Material and Methods

Collection of samples

Samples of submerged leaf litter were collected at several waterfalls and streams in islands offshore the state of São Paulo, Brazil. Samples were collected in triplicates in each collection site from June, 2012 to May, 2013 at Ilhabela State Park, municipality of Ilhabela, and from June, 2012 to February, 2013 at Ilha do Cardoso State Park, municipality of Cananéia. The submerged mixed leaf litter samples were placed in polypropylene vials (200mL) and half filled with water gathered from the collection point. Once in the laboratory, the leaf litter samples were fragmented and incubated in Petri dishes containing sterile distilled water at room temperature around 20°C (Ingold 1975).

After seven days, segments of submerged leaves were taken from the Petri dishes and mounted on slides to be observed under an optical microscope, searching for taxonomically relevant features such as conidiophores and conidia. Once propagules were observed on the water they were collected by “sweeping” the water surface with the side of a sterile cover slip held by a clamp. The cover slip with the attached conidia was placed over a glass slide with a drop of polyvinyl alcohol lactic acid glycerol resin (PVLG) allowing permanent fixation and microscopic observation. Slides were then observed with an Olympus BX50 light microscope and images were taken with a Leica DFC 280 camera attached to a Leica DM LB2 microscope. Macro images were taken with a MP-E 65mm Canon macro lens with an EOS 70D Canon camera. Slides are deposited in the Herbarium “Maria P. Eneyda Kauffmann Fidalgo” (SP) of the Núcleo de Pesquisa em Micologia belonging to the Instituto de Botânica, São Paulo.

Isolation

Floating propagules were removed from the water with the help of a sterile dissecting needle, inoculated in Petri dishes containing malt extract agar (MEA 1.6%) with chloramphenicol and incubated at 21°C in climatic chambers. Another isolation technique was performed using water droplets containing conidia of the target fungi transferred with a Pasteur pipette and spread over the surface of MEA 0.1% medium with chloramphenicol previously poured on Petri dishes. After 24 hours of incubation in climatic chambers at 21°C, the reverse side of the dishes was observed under the stereoscope to verify hyphal growths and to mark the position of the conidia with a permanent marker pen. Under aseptic conditions and with the aid of a sterile stainless steel needle, the conidia were removed from the agar surface and inoculated onto a new MEA 1.6% Petri dish and incubated under similar conditions. Pieces of approximately 1cm² of medium with mycelium were cut out and placed on Petri dishes with autoclaved distilled water and incubated at room temperature around 21°C for 7–10 days to induce propagules development. Cultures have been preserved by periodical subculturing and mineral oil immersion technique (Smith & Onions 1994) and are deposited in the Culture Collection (CCIBt) of the Instituto de Botânica.

DNA extraction, PCR amplification and sequencing

Mycelium for DNA extraction was obtained by cultivating each isolate in Erlenmeyer flask containing 20 ml of MEA 1.6% liquid medium. After incubation for 7–10 days at 21°C, the mycelium was transferred to 1.5 ml centrifuge tubes (Eppendorf) and harvested by centrifugation at 13000 rpm for 15 min in order to obtain mycelia pellets. The supernatant was discarded and 1 ml of sterile deionized water was added to the tubes followed by vortexing at 2500 rpm. Tubes were again centrifuged and the supernatant discarded. DNA was extracted from the mycelial pellets with the PureLink Genomic DNA kit (Invitrogen) following the manufacturer’s protocol. Electrophoresis was performed using 1% (p/v) agarose gel applying 3–5 µL of the sample + 2 µL of Gel Loading Buffer Type I (Sigma), and a standard of 2 µL of 123 bp DNA Ladder (Invitrogen®) + 2 µL of Gel Loading Buffer. Electrophoresis conditions were 100 mV for 40 min. The ITS and LSU regions of nrDNA were amplified by PCR with the primers UN-up18S42 -CGTAACAAGGTTTCCGTAGGTGAAC- (ITS forward) and UN-lo28S22 -GTTTCTTTTCCCTCCGCTTATTGATATG- (ITS reverse) (Robideau *et al.* 2011) and primers LR0R -ACCCGCTGAACCTAAGC- (LSU forward) and LR5 -TCCTGAGGGAAACTTCG- (LSU reverse) (Vilgalys & Hester 1990). DNA was amplified with the PCR SuperMix kit (Invitrogen) in a C1000 Touch Thermal Cycler (Bio-

Rad). The conditions were as follows: volume 25 µl; initial denaturalization at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were quantified by electrophoresis on 1% agarose gel using 1–2 µL Low DNA Mass Ladder (Invitrogen) + 2–4 µL of Gel Loading Buffer as standard and 2–3 µL of DNA and purified with AxyPrep PCR Clean-up kit (Axygen). Sequencing of ITS and LSU regions were performed using the same primers in a ABI 3730 DNA Analyser (Life Technologies) at the Centro de Estudos do Genoma Humano, Instituto de Biociências, University of São Paulo, São Paulo, Brazil. Sequences were edited and assembled using Sequencher4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA).

Phylogenetic analyses

A representative taxon sampling was obtained after BLAST searches of the newly generated ITS and LSU nrDNA sequences in GenBank and additional sequences of taxa belonging to Sordariomycetidae and Hypocreomycetidae from recent phylogenetic studies (Untereiner *et al.* 2013, Voglmayr 2011, Zhang *et al.* 2006). Available sequences of some aeroaquatic fungi belonging to *Cancellidium* Tubaki, *Dendroclathra* Voglmayr & G. Delgado, *Spirosphaera* Beverw., *Clathrosporium* Nawawi & Kuthub., and *Pseudaegerita* J.L. Crane & Schokn. were also included. Sequences were aligned using MAFFT version 7 (Katoh 2013) and manually adjusted with BioEdit version 7.2.0 (Hall 1999). The best fitting model of evolution as determined by MEGA 5.2 (Tamura *et al.* 2011) using the corrected Akaike Information Criterion was Tamura-Nei (Tamura & Nei 1993) + Gamma (Yang 1994) (TN93+G) and the phylogenetic tree for LSU nrDNA was constructed also in MEGA using Maximum Likelihood (ML) (Saitou 1996, Swofford *et al.* 1996). Branch support was evaluated using non-parametric bootstrap support with 1000 pseudo replicates (Felsenstein 1985) and considered significant when bootstrap support BS ≥ 70% (Hillis & Bull 1993). LSU sequences of Helotiales (Leotiomycetes) species downloaded from GenBank were used as outgroup. Pairwise distances (p-distance) between ITS and LSU sequences were calculated using also MEGA. For Bayesian phylogenetic analysis, three independent runs were performed on the LSU dataset and posterior probabilities for internodes were calculated with the Metropolis coupled Markov chain Monte Carlo (MCMC) after 5 million generations. The Bayesian tree was constructed using MrBayes 3.2.2 (Ronquist *et al.* 2012).

Results

Phylogenetic analysis

The final alignment of the LSU sequence dataset consisted of 823 characters and 44 taxa and the single most-likely tree obtained from the ML analysis is shown in Fig. 1. Both LSU sequences were placed within the Sordariomycetes where they formed a strongly supported clade (99% BS and 1.00 BPP) sister to a sequence (AF132329) of the freshwater ascomycetes fungus *Clohiesia corticola* K.D. Hyde (Annulatasceae s.l.) in a well-supported clade (87% BS and 1.00 BPP). They were nested within a clade that includes members of Boliniales, Sordariales, Chaetosphaerales, Coniochaetales, Annulatasceae and other Sordariomycetidae *incertae sedis* similar to previously reported phylogenies (Cai *et al.* 2014, Réblová *et al.* 2010, Untereiner *et al.* 2013, Zhang *et al.* 2006).

LSU nrDNA and ITS sequences obtained for the two isolates were deposited in GenBank. The p-distance between both LSU sequences was 1% and between the ITS sequences was 2%. BLAST searches of the ITS regions of the two isolates did not show any closely similar sequences and therefore they were not used for analyses. Closest hits using the LSU sequences of both isolates revealed the highest similarity with members of Boliniales and Sordariales.

Taxonomy

Clathrosporium retortum sp. nov. L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius

Fig. 2.

MycoBank MB 812999

Etymology:—from the Latin *retortum* = twisted, convolute, referring to the irregular, densely interwoven propagule shape.

Type:—BRAZIL. São Paulo: Cananêia, Ilha do Cardoso State Park, “Poço das Antas”, on submerged mixed leaf litter, 26 Feb. 2013, Moro L.B., SP466253 (**Holotype**), ex- Holotype culture CCIBt 4122.

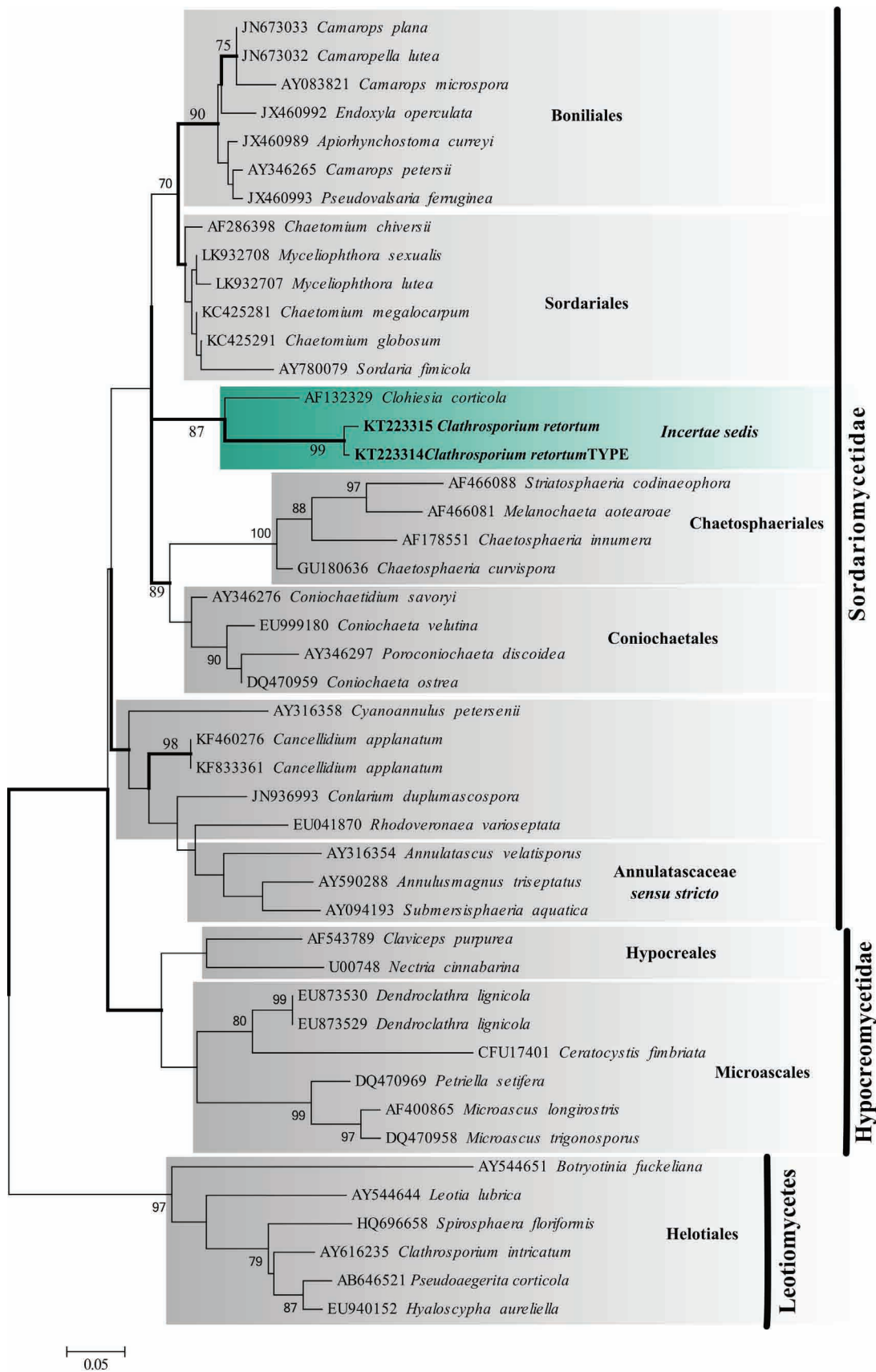


FIGURE 1. Phylogenetic tree reconstructed by Maximum Likelihood (ML) and Bayesian methods based on LSU nrDNA sequences showing the phylogenetic placement of *Clathrosporium retortum* within the Sordariomycetidae. Numbers refer to ML BS $\geq 70\%$ and BPP ≥ 0.95 support values are indicated by thickened branches. Members of Leotiomyces were used as outgroup taxa. Bar indicates nucleotide substitutions per site. GenBank accession numbers follow the taxon names.

Colonies on MEA slow growing, reaching 1.8–2.3 mm diam. in 15 days at 21°C, velvety, brown to dark brown or blackish brown, with cottony, brown aerial mycelium in the center, margin diffuse, slightly undulate, reverse blackish brown. Mycelium immerse and superficial, composed by septate, smooth, hyaline to fuscous hyphae, 2.5–5 µm wide. Conidiophores semimacronematous, mononematous, simple, erect, septate, smooth, hyaline to brown, up to 200 µm long, 1.25–5.7 (–7.5) µm wide. Conidiogenous cells holoblastic, monoblastic, integrated, terminal. Conidial secession schizolytic. Conidia acrogenous, subglobose to irregular, hyaline to subhyaline when young, subhyaline to dark brown at maturity, 55–150 µm diam, formed by branched, densely interwoven conidial filaments, with a flattened scar after detachment. Conidial filaments hyaline to subhyaline, smooth, septate, slightly or strongly constricted at the septa, each conidial cell branching bilaterally or unilaterally and coiling, each lateral branch repeatedly branching similarly, conidial cells sometimes breaking at maturity, 10–15.7 × 5.7–11.4 µm, 4.3–7.15 µm wide at the septa.

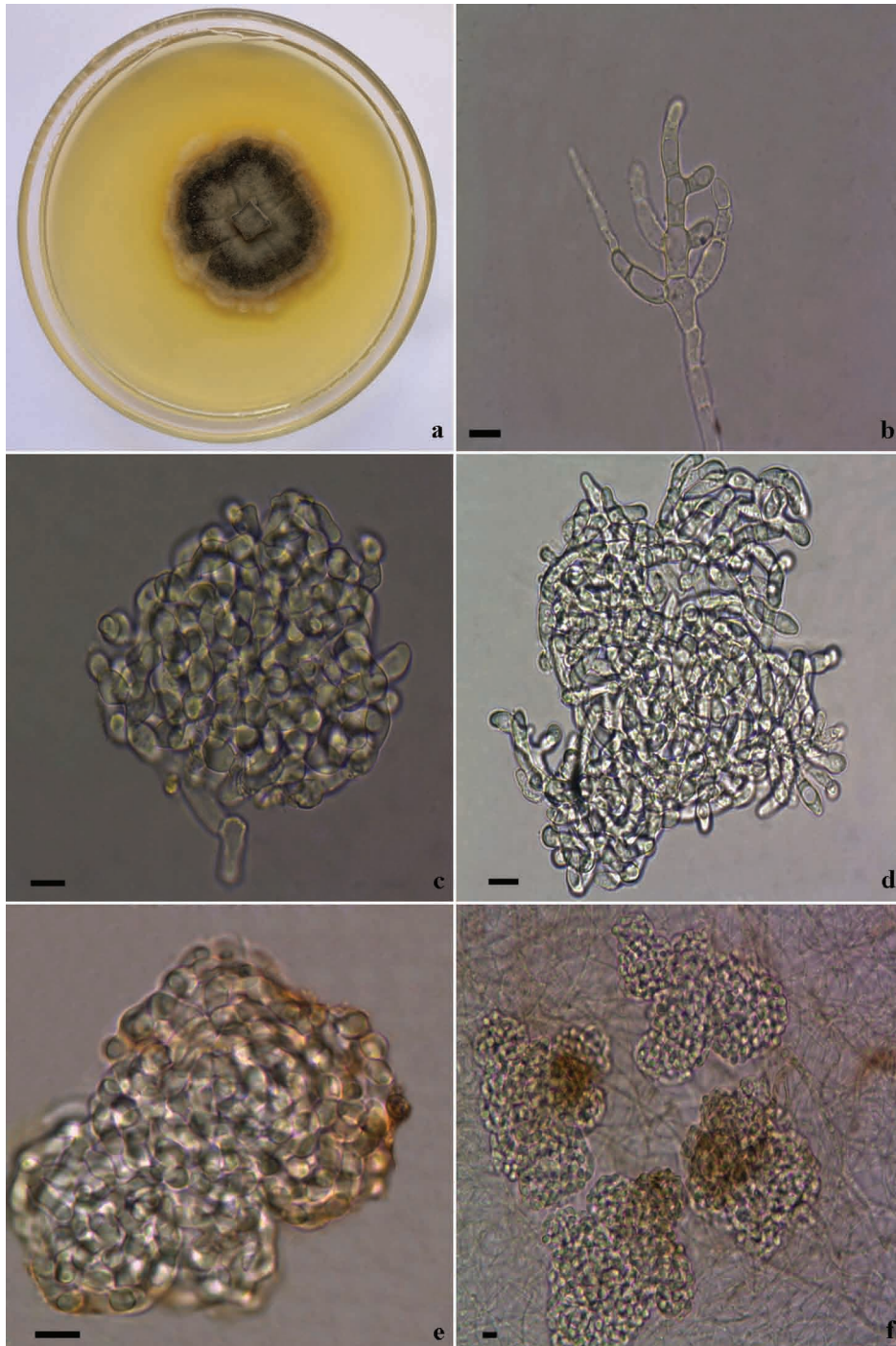


FIGURE 2. *Clathrosporium retortum* (ex-holotype CCIBt 4122). **a.** Colony on MEA after 15 days at 21°C. **b.** Conidial initial. **c.** Young conidia. **d–f.** Mature conidia. Scale bars = 10 µm.

Sexual morph:—Unknown.

Other specimens examined:—BRAZIL. São Paulo: Cananéia, Ilha do Cardoso State Park, “Poço das Antas”, on submerged mixed leaf litter, 06 Jun. 2012, Moro L.B., SP466254 (Paratype), ex- paratype culture CCIBt 4123.

Ex-type sequences:—ITS: KT223312, LSU: KT223314 (ex-holotype). ITS: KT223313, LSU: KT223315 (ex-paratype).

Five other interesting aeroaquatic species were discovered and identified by morphological characteristics, from the surveyed areas. They are described and illustrated as follows including comments on their morphology, phylogeny and Neotropical distribution.

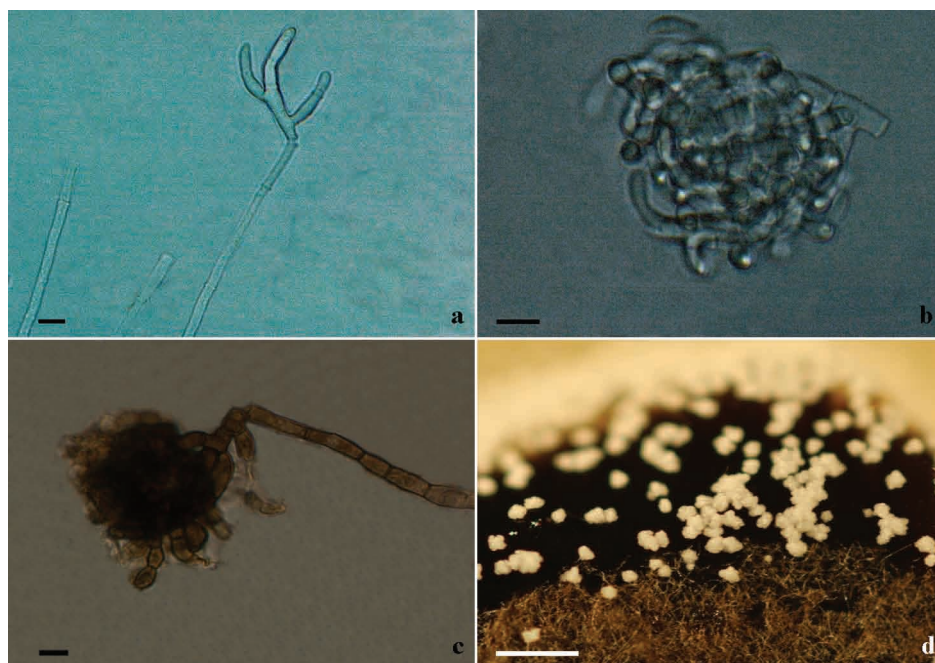


FIGURE 3. *Clathrosporium retortum* (ex-paratype CCIBt 4123). **a.** Conidial initial. **b.** Young conidia. **c.** Mature conidia. **d.** Colony over culture medium incubated in distilled water showing presence of propagules. Black scale bars = 10 μm , white scale bar = 500 μm .

Beverwykella clathrata Voglmayr, Mycol. Res. 107 (2): 239 (2003) (Fig. 4d, e)

Colonies on MEA slow growing reaching 24 mm diam. after 30 days, blackish, reverse black. Mycelium velvety, composed of septate hyphae, 2.5–5 μm wide. Conidiophores macronematous, septate, unbranched, erect, light fuscous at maturity, 35–50 \times 2.5–3.5 μm . Conidia hyaline when young, dark brown to black at maturity, clathrate, spherical to oval, 40–42.5 \times 52.5 μm diam.

Material examined:—Brazil. São Paulo: Cananéia, Ilha do Cardoso State Park, “Cor de chá” stream, on submerged mixed leaf litter, 26 Feb. 2013, Moro L.B. (SP446-321).

Remarks:—*Beverwykella clathrata* was first described from submerged leaves of *Pinus* sp. and evergreen broad leaf shrubs in the subtropical United States (Voglmayr & Delgado 2003). It is distinct among other *Beverwykella* species in having a clathrate outermost cell layer formed by moderately appressed cells and the conidium interior branching system produced by three regular dichotomous branchings, resulting in eight branches supporting the outermost cell layer. The morphological features of the specimen described here in agree well with the original description. This species is known so far only from the type locality in the United States and this is the second record of its presence worldwide. It is phylogenetically affiliated within Sordariomycetes (Voglmayr, pers. comm.), but a detailed study has not yet been published.

Cancellidium applanatum Tubaki, Trans. Mycol. Soc. Japan 16(4): 358 (1975) (Fig. 4a–b)

Colonies on MEA slow growing attaining 16 mm after 30 days at 23°C, dark brown. Conidiophore brown, septate, erect, branched, 50–55 \times 5–7 μm . Conidia obovate, flattened, dark brown, composed of parallel columns of cells, 95–165 \times 52.5–115 μm .

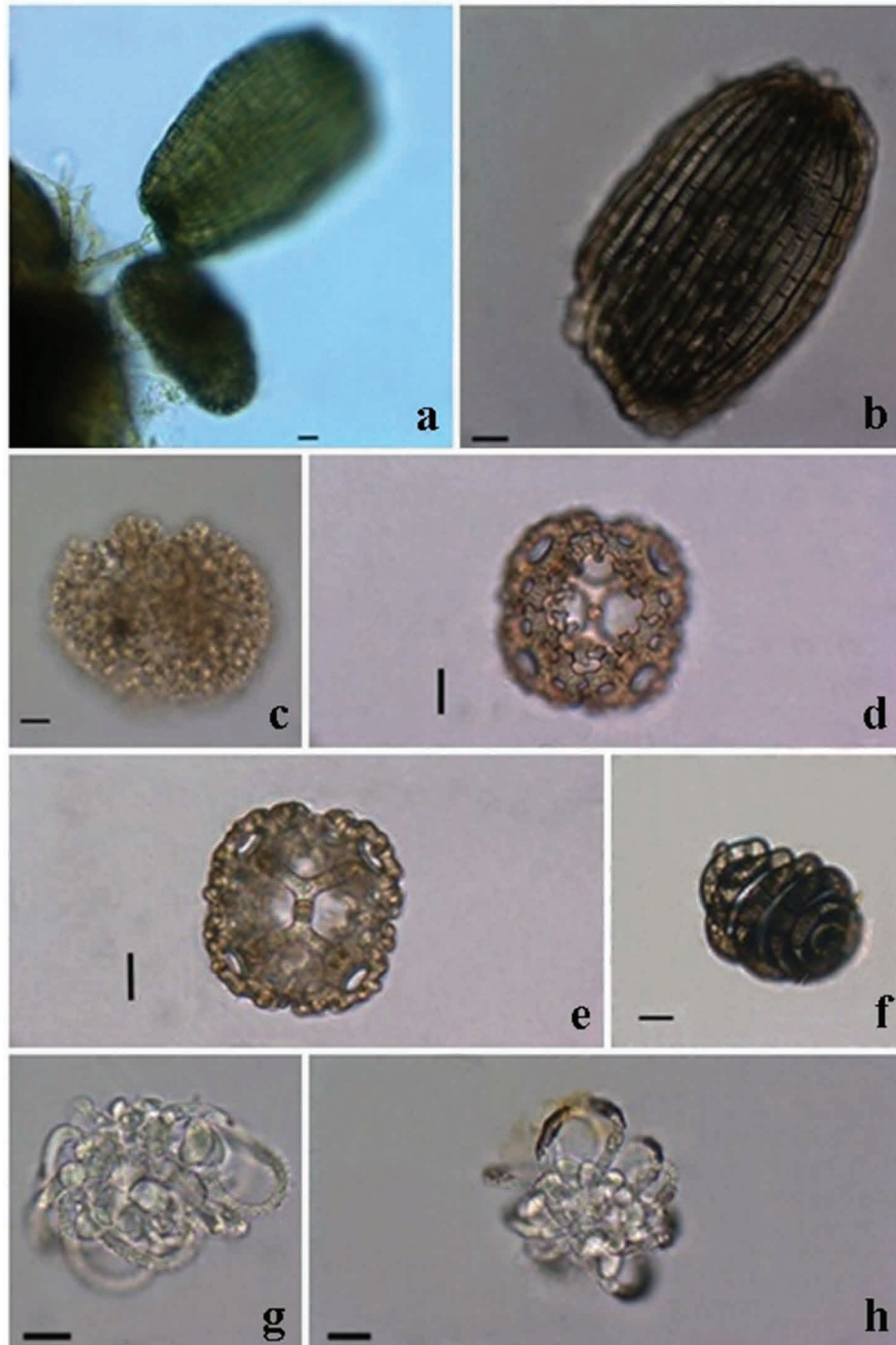


FIGURE 4. *Cancellidium applanatum*. **a–b.** Conidiophore and conidia. *Candelabrum brocciatum*. **c.** Conidium. *Beverwykella clathrata*. **d–e.** Conidia. *Helicoön septatissimum*. **f.** Conidium. *Peyronelina glomerulata*. **g–h.** Conidial propagules showing subglobose central cells and spiny arms. Scale bar = 10µm.

Material examined:—Brazil. São Paulo: Cananéia, Ilha do Cardoso State Park, “Perequê” stream and “Cor de chá” stream, on submerged mixed leaf litter, 26 Feb. 2013, Moro L.B. (SP445-865; CCIBt 4036).

Remarks:—*Cancellidium applanatum* is a pantropical aeroaquatic fungus with a peculiar morphology originally isolated from balsa wood collected from a lake in Japan (Tubaki 1975). Barbosa & Gusmão (2011) previously collected this fungus in Brazil on submerged leaf litter samples in the “Caatinga” biome of the state of Bahia. The morphological features of the present specimen are in agreement with the original description of Tubaki. This is the first record of this fungus from the Atlantic rainforest of the state of São Paulo. Other Neotropical records include Peru on submerged woody debris (Zelski *et al.* 2014).

Candelabrum brocchiatum Tubaki, Trans. Mycol. Soc. Japan 16 (2): 134 (1975) (Fig. 4c)

Conidiophores not observed. Conidia formed dichotomically or sometimes trichotomically in all directions resulting in globular conidia with warty cell ends; yellow to brownish orange at maturity; 62.5–122.5 µm diam.

Material examined:—Brazil. São Paulo: Cananéia, Ilha do Cardoso State Park, “Perequê” stream, on submerged leaf litter, 19 Jun. 2012, Moro L.B.; idem, 06 Nov. 2012, Moro L.B. (SP446-320A, SP446-320B).

Remarks:—*Candelabrum brocchiatum* is considered an ubiquitous species in Thai forests and other tropical areas (Sivichai *et al.* 2000, 2002). It resembles *C. microsporium* Castañeda and Kendrick (Castañeda & Kendrick 1991) in shape, color and conidial development; the only difference is the conidial diameter which ranges 35–60 µm in *C. microsporium* and 70–120 µm in *C. brocchiatum* (Voglmayr 1998). In Brazil, this species was previously described from the “Caatinga” biome from the state of Bahia (Barbosa & Gusmão 2011) and now for the first time from São Paulo state. Other Neotropical records include Cuba on submerged branches of *Calophyllum antillanum* Britt., and *Sizygium jambos* (L.) Alston (Delgado & Mena 2004). It has also been reported from Mexico on unidentified submerged branches (Heredia *et al.* 2000).

Helicoön septatissimum Godeas & Aramb., Mycotaxon 60: 481 (1996) (Fig. 4f)

Conidiophores not observed. Conidia solitary, brown to dark brown, helicoid. Conidial filament 7.5 µm wide, multiseptate, not constricted at septa, tightly coiled 9–10 times in a counter-clockwise direction to form a doliiform conidium, 60 µm long, 42.5–45 µm diameter of the coil.

Material examined:—Brazil. São Paulo: Cananéia, Ilha do Cardoso State Park, “Perequê” stream and “Cor de chá” stream, on submerged mixed leaf litter, 26 Feb. 2013, Moro L.B. (SP466252).

Remarks:—Among species of *Helicoön* Morgan, *H. septatissimum* is distinct in having doliiform, light brown to brown, multiseptate, 9–14 coiled conidia, 70–75 × 40–45 µm in size, with the conidial filament 8.5–10 µm wide (Abdullah *et al.* 1998, Zhao *et al.* 2007). The morphological features of this collection corroborate those cited by Godeas & Arrambarri (1996) for the type specimen except that the conidia are shorter and conidial filaments are slightly narrower. Apart from the type locality in neighboring Argentina this species has not been reported elsewhere and therefore it is first reported here from Brazil and second for South America.

Peyronelina glomerulata P.J. Fisher, J. Webster & D.F. Kane, Trans. Br. Mycol. Soc. 67 (2): 351 (1976) (Fig. 4g–h)

Conidiophores not observed. Conidia hyaline, globose, 19–21 × 24–28 µm diam., composed of hyaline central subglobose cells unrounded by 6–8 spiny, incurved appendages. Teleomorph: *Flagelloscypha*.

Material examined:—Brazil. São Paulo: Ilhabela, São Sebastião Island, Ilhabela State Park, “Cachoeira do Gato” waterfall, on submerged mixed leaf litter, 14 May 2013, Moro L.B. (SP466335).

Remarks:—Fisher *et al.* (1976) validly published *Peyronelina glomerulata* G. Arnaud based on British specimens after it was originally described without a Latin diagnosis. This peculiar aeroaquatic fungus has been connected to a cyphelloid *Flagelloscypha*-like teleomorph (Niaceae, Basidiomycota) based on cultural and molecular data (Yamaguchi *et al.* 2009). Almost all morphological features of the present collection agree well with the type specimen, except for conidial diameter which is slightly smaller. An online search shows that *P. glomerulata* has been wrongly reported from Brazil e.g. Minter & da Silva (2007) based on Batista *et al.* (1970) who actually reported a phomoid fungus, *Peyronella glomerata* (Corda) Goid. (≡ *Phoma glomerata* (Corda) Wollenw. & Hochapfel) from soil. Previous Neotropical records include Puerto Rico in foam and water (Santos & Betancourt 1997) and a *Peyronelina* sp. on rachis of dead leaf of *Roystonea regia* (Kunth) O.F. Cook from Cuba (Castañeda & Arnold 1985). This is the first report from Brazil.

Discussion

Nawawi & Kuthubutheen (1987) introduced the genus *Clathrosporium* to accommodate *C. intricatum*, an aeroaquatic

hyphomycete found growing on submerged decaying leaves in a freshwater stream in Malaysia. The fungus was originally characterized by forming clathrate, globose to subglobose, brown conidia that do not disarticulate on handling and are born on short, erect, simple, pale brown to brown, semimacronematous to micronematous conidiophores. The conidial initial consists of an elongating cell that soon after been delimited by a first septum curves in wardly and produces a pair of opposite bilateral branches. They elongate and branch repeatedly in the same manner until a conidium is produced by intertwining of the lateral branches whose tips rarely fuse. Hennebert (1998), after reexamining the type specimen deposited in IMI, concluded that the branch tips never fuse and therefore the propagules were not clathrate or hollow, and they can be dispersed as a unit or break apart into one or many-celled hyphal elements or arthroconidia at maturity or in dry conditions. He redescribed the genus and its type species based on these features, and introduced three additional species, *C. olivatum* (Sacc.) Hennebert, based on *Strumella olivatra* (Sacc.) Sacc. (\equiv *Illosporium olivatum* Sacc.), *C. delicatum* Hennebert and *C. compactum* Hennebert, differing in propagule size, color and conidial filament width.

Clathrosporium retortum agrees well with Hennebert's generic concept in having conidial filaments repeatedly branching bilaterally and forming dense, complex propagules resulting from the intertwining of branches that sometimes break apart in segments of one to several cells. The conidial initial formed similarly as previously described by Nawawi & Kuthubutheen for *C. intricatum* (Fig. 2b). The fungus, however, showed significant morphological and developmental variation among isolates. Isolate CCIBt 4123 (Fig. 3) was morphologically different from isolate CCIBt 4122 in forming greenish brown, smaller colonies on MEA, reaching 1.8–2.1 cm diam. after 15 days at 21°C, dark brown when older, shorter and narrower conidiophores up to 67.5 μ m long, 1.25–3.75 (–6.25) μ m wide, and smaller propagules 55–125 μ m diam. Hennebert (1968) noted remarkable variations in color, sporulation and conidium formation between strains of species belonging to the morphologically similar genus *Spirosphaera* Beverw. The conidial initial was also variable and quite atypical in producing bilateral but alternate branching; the first elongated cell producing a first branch proximal to the basal septum and another branch distally in the opposite side but not directly across from each other (Fig. 3b). Sometimes they also displayed alternate branching along different contiguous cells of the same filament or only unilateral branching as in *Spirosphaera* Beverw. (Beverwijk 1953, Hennebert 1968) (Fig. 3c).

Nevertheless, the LSU sequences of both isolates of *C. retortum* as well as their ITS regions (data not shown) grouped together with strong support in all analyses. The distances between the LSU and ITS sequences were minimal suggesting that despite their morphological differences both isolates are probably conspecific and belong to a single variable species. Additional collections outside the type locality, where both cultures originate, as well as additional markers of higher resolution are necessary to study the fungus intraspecific variability and to further clarify if more than one species is involved. Both LSU sequences of *C. retortum* were phylogenetically unrelated to the only currently available LSU sequence of *C. intricatum* (AY616235) that belongs in the Helotiales (Leotiomyces), thus confirming the polyphyletic nature of *Clathrosporium* as presently conceived. Voglmayr (2004), however, expressed doubts about the conspecificity of the specimen source of AY616235 with the type from Malaysia, a specimen that may represent a yet undescribed taxon. Unfortunately, other sequences belonging to the type or additional *Clathrosporium* species are not available for analysis and, therefore the phylogenetic placement of the genus requires further refinement. Due to these uncertainties about the identity of the generic type, Voglmayr's criterion to maintain *Clathrosporium* as well as *Spirosphaera* in a morphological sense despite their polyphyly (Voglmayr 2004) is applied here to tentatively accommodate the newly described aeroaquatic fungus instead of introducing a new genus.

Some aeroaquatic fungi currently placed in *Spirosphaera* Beverw. but morphologically attributable to *Clathrosporium* such as *S. carici-graminis* Voglmayr and *S. verruculosa* Abdullah, Y. Horie & Udagawa are also comparable with *C. retortum* in having conidia with bilateral branching (Abdullah *et al.* 1986, Voglmayr, 1997, 2004). The phylogenetic placement of *S. carici-graminis*, however, is within the Leotiomyces (Voglmayr, pers. comm.) and therefore is not related to *C. retortum* but consistent with the position of *S. floriformis* Beverw., the generic type, represented in our phylogeny by a LSU sequence of the ex-holotype (HQ696658). In the case of *S. verruculosa*, although its phylogenetic relationships is unknown, conidiophores and conidia are pale olive green to dark green and smaller in size (55–70 μ m) with roughened conidial filaments bearing minute warts (Abdullah *et al.* 1986).

In our phylogeny, the closest relative of *C. retortum* was *Clohiesia corticola*, a freshwater ascomycete of uncertain affinities within Sordariomycetidae (Cai & Hyde 2007) with whom it shares a similar habitat. Recently Cai *et al.* (2014), in a comprehensive phylogeny of freshwater Sordariomycetes based on LSU sequence data, recognized five different clades in the polyphyletic family Annulatascaceae and included *Cl. corticola* as the single member of Clade III, closer to Chaetophariales but distantly related to members of Annulatascaceae sensu stricto in Clade I. Similarly, members of that clade included in our analysis such as *Annulatascus velatispora* K.D. Hyde, *Annulusmagnus triseptatus* (S.W.

Wong, K.D. Hyde & E.B.G. Jones) *J. Campb.* & Shearer and *Submersisphaeria aquatica* K.D. Hyde were not related to the isolates of *C. retortum* or to *Cl. corticola*. Fungi belonging to the Annulatascaceae are the most commonly encountered species of freshwater Sordariomycetes, but their phylogenetic relationships are poorly resolved (Cai *et al.* 2014). The unexpected grouping of atypical aeroaquatic fungus as *C. retortum* producing complex propagules capable of enclosing air between their cells with an annulatascaceous fungus may reflect the lack of information about the affinities of aeroaquatic fungi and the still poor taxon sampling of freshwater taxa in the class as a whole. However, the well supported relationship between them does not exclude some level of affinity considering that aeroaquatic fungi are known to have evolved multiple times independently within various or distantly related lineages of ascomycetes as a result of adaptation to the aeroaquatic niche (Voglmayr 2008, Voglmayr *et al.* 2011). Further molecular data are needed to support this hypothesis as AF132329 is the only sequence available for *Cl. corticola* and the remaining two *Clohesia* species, *Cl. curvispora* L. Cai & K.D. Hyde and *Cl. lignicola* K.M. Tsui, K.D. Hyde & Hodgkiss (Cai & Hyde 2007, Tsui *et al.* 1998), still lack DNA sequence data.

Both *C. retortum* and *Cl. Corticola* grouped together within a clade supported only in Bayesian analyses that includes members of Boliniales, Chaetosphaeriales, Sordariales and Coniochaetales but separated from them into a distinct clade. Only the last two orders are known to include species from freshwater habitats primarily from woody substrata (Cai *et al.* 2014) but none of them form buoyant propagules typical of aeroaquatic fungi. The phylogenetic placement of *C. retortum* therefore should remain Sordariomycetidae incertae sedis until more taxa and additional sequence data become available. *Cancellidium applanatum* Tubaki, another aeroaquatic fungus of uncertain affinities within the subclass (Pratibha *et al.* 2014, Zelski *et al.* 2014) included in our phylogeny with two LSU sequences, was phylogenetically distant from *C. retortum*. Morphologically they are also dissimilar as *C. applanatum* is characterized by forming dictyoseptate, flattened, obovate to obcordate, dark brown to almost black, shiny conidia (Tubaki 1975).

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6. CONSIDERAÇÕES FINAIS

O levantamento taxonômico realizado no Parque Estadual de Ilhabela (PEIb) e no Parque Estadual da Ilha do Cardoso (PEIC) evidenciou elevada diversidade de Hyphomycetes aquáticos, representada por 59 táxons, sendo um pouco mais de 37% são novos registros para o Estado de São Paulo e/ou Brasil. Para o PEIb, 40 táxons foram obtidos, sendo 21 táxons exclusivos para a ilha. *Camposporidium cristatum* Nawawi & Kuthub., *Chaetendophragma triangularis* Matsush., *Physalidiella elegans* (Mosca) Rulamorte *Scutisporus brunneus* K. Ando & Tubaki, são novos registros para o Estado de São Paulo e *Isthmolongispora biramifera* Matsush., *Lateriramulosa a-inflata* Matsush., *Phalangispora nawawii* Kuthub. e *Triscelophorus ponapensis* Matsush. são novos registros para o Brasil. Com relação aos fungos aeroaquáticos, um novo registro de *Peyronelina glomerulata* P.J. Fisher, J. Webster & D.F. Kane. foi realizado para o Brasil.

Para o PEIC, 38 táxons foram observados, 19 táxons exclusivos para a ilha. *Isthmolongispora quadricellularia* Matsush. é um novo registro para o Estado de São Paulo e *Isthmolongispora intermedia* Matsush., *Isthmolongispora minima* Matsush., *Dactylellina appendiculata* (Anastasiou) M. Scholler, Hagedorn & A. Rubner, *Pleiochaeta amazonensis* Matsush. e *Pyramidospora constricta* N. Singh sendo primeiras citações para o Brasil. Entre os fungos aeroaquáticos, 8 táxons foram obtidos, sendo *Beverwykella clathrata* Voglmayr, *Cancellidium applanatum* Tubaki e *Candelabrum brocchiatum* Tubaki novos registros para o Estado de São Paulo e *Helicoon septatissimum* Godeas & Aramb. novo registro para o Brasil.

Três espécies novas para a ciência foram registradas: *Polylobatispora setulosa* L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius e *Campylospora brasiliensis* L.B. Moro & I.H. Schoenlein-Crusius para o PEIb e *Clathrosporium retortum* L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius para o PEIC.

Tanto o esquema de coletas sistemáticas nos mesmos locais adotado no levantamento no PEIC, quanto o esquema de coletas esporádicas em diferentes locais no PEIb, resultaram em dados representativos quanto a diversidade da micota aquática composta pelos Hyphomycetes aquáticos.

As cachoeiras e córregos, com águas turbulentas, são ambientes extremamente propícios para a ocorrência de Hyphomycetes aquáticos, mas não impedem a ocorrência dos fungos aeroaquáticos, que no presente estudo, foram bem representados por oito táxons, *Beverwykella clathrata* Voglmayr, *Cancellidium applanatum* Tubaki, *Candelabrum brocciatum* Tubaki, *Clathrosporium retortum* L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius, *Helicoön septatissimum* Godeas & Aramb., *Helicomycetes* sp., *Helicosporium* sp. e *Peyronelina glomerulata* P.J. Fisher, J. Webster & D.F. Kane.

Apesar de diversos trabalhos com este grupo de fungos no Brasil e no bioma Mata Atlântica, o presente é pioneiro nas áreas estudadas e em regiões insulares brasileiras.

O levantamento realizado nos parques contribuiu para a expansão do conhecimento da diversidade dos Hyphomycetes aquáticos em áreas de Mata Atlântica no Brasil e em áreas insulares. Para o Parque Estadual de Ilhabela, 100% das espécies descritas ou relatadas são novos registros para o parque, sendo que 27,5% das espécies identificadas são descritas pela primeira vez para o Brasil e/ou estado de São Paulo.

Para o Parque Estadual da Ilha do Cardoso, 100% das espécies descritas ou relatadas são novos registros para o parque, sendo que 29% das espécies identificadas são descritas pela primeira vez para o Brasil e/ou estado de São Paulo.

Compilando os dados de literatura, verifica-se que com relação aos fungos ingoldianos são conhecidos, até o momento, cerca de 70 espécies no Brasil. O presente estudo, com a citação de 10 novos táxons (incluindo duas novas espécies para a Ciência) está contribuindo com o acréscimo de 14% de novos táxons para o Brasil.

Entre os aeroaquáticos, eram conhecidas para o Brasil cerca de 5 táxons. O presente estudo contribui com o acréscimo de 80% do conhecimento da diversidade dos fungos aeroaquáticos no país.

Contudo, os resultados obtidos enfatizam a necessidade de preservar os corpos d'água do PEIb e do PEIC, importantes remanescentes da Mata Atlântica brasileira, afim de garantir a conservação da biodiversidade.

O método de isolamento baseado em Descals (2005) é aplicável com sucesso, desde que sejam introduzidas algumas modificações (marcação da posição dos conídios em placas de Petri) para a obtenção de culturas que de fato possam ser utilizadas para fins moleculares. Estas alterações foram satisfatórias para a obtenção das culturas axênicas, que totalizaram 15 isolados.

As ferramentas moleculares realizadas neste estudo para todos os isolados obtidos em meio de cultura, com exceção de *Beverlykella clathrata*, e seus sequenciamentos das regiões ITS e LSU, foi importante para auxiliar na identificação taxonômica, principalmente das novas espécies. Algumas das sequências obtidas neste estudo, estão disponíveis no GenBank, outras serão disponibilizadas futuramente, contribuindo para futuros estudos moleculares desses fungos.

ANEXOS

ANEXO I

INSTRUCTIONS TO AUTHORS

Cryptogamie, Algologie; Cryptogamie, Bryologie and Cryptogamie, Mycologie publish original papers, and reviews, on the systematics, biology and ecology of cryptogams (algae; bryophytes; fungi and lichens, respectively). Manuscripts written in French, English, German, Italian and Spanish are considered providing suitable referees fluent in the language of the manuscript are available. Authors not writing in their first language should have manuscripts checked for grammar and syntax by a suitable person before submission. There are no page limits for papers. Publication will be facilitated if authors check carefully that the manuscript and illustrations meet the requirements outlined below. Papers are reviewed by referees in whose field the paper lies. The choice of reviewers is at the discretion of the Editor. Final responsibility for the publication of papers rests with the Editor. Manuscripts that do not conform to will be returned for correction prior to review. On acceptance, papers become the copyright of the ADAC.

SUBMISSION — Articles should be submitted to the appropriate Editor-in-Chief, according to the subjects studied. Cryptogamie, Algologie – Editor in Chief: Line LeGall & Ian Probert – Tel. (33) 140793197 – algo@cryptogamie.com ; Cryptogamie, Bryologie – Editor in Chief: Denis Lamy – Tel. (33) 140793184 – denislamy@cryptogamie.com ; Cryptogamie, Mycologie – Editor in Chief: Bart Buyck – Tel. (33) 140793186 – myco@cryptogamie.com.

TEXT — The text of the manuscripts should be submitted to the Editor-in-Chief as attached document sent through e-mail. The use of MS Word compatible word processors would be appreciated. Each manuscript should include : – A title page containing the title of the paper and, if necessary, its English translation; the complete name (complete Christian name followed by the family name (in capitals)) and address of each author, and e-mail; author to whom correspondence should be sent; a running title of less than 50 letters, and a list of key words; – Two abstracts, the first in French (if necessary, some help can be provided by the Editorial Office), the second in English (a third one in the language of the text is accepted), each of no more than 180 words or 15 lines, summarizing the major results of the paper; – The main text, followed by references, legends for figures, and tables, in that order. The text should normally be divided into sections (e.g. Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References) appropriate to the nature of the paper. Indent the first line of all paragraphs. Symbols, units and nomenclature should conform to international usage. The SI system should be used throughout. The first mention of the Latin name of a species in the text should be accompanied by the nomenclatural authors (the authors should be abbreviated following Brummitt R.K. & Powell C.E. – Authors of plant names. Kew, Royal Botanic Gardens, 1992, 732 p.). Consult the current issue of the Journal for style of headings, subheadings and other conventions. Legends for figures and tables should be self-explanatory, and written in the language of the text and in English if it is not the language used for the text. Deposition of data to online, public databases Authors have to deposit all molecular sequence data newly acquired in GenBank or a related database (EMBL, DDBJ) prior to acceptance. Full alignment of datasets must also be made available online prior to acceptance of the paper (preferably submitted to the TreeBASE). For new species and taxonomic changes in Fungi, a Mycobank number must be added.

REFERENCES — References should be arranged alphabetically and then chronologically by author. Journals titles should be cited in full; and books, cited according to F.A. Stafleu & R.S. Cowan, 1976..., Taxonomic literature. Ed. 2. Utrecht/Antwerpen Bohn, Scheltema & Holkema. Conventions of style are provided in the following examples: AJISAKA T., NORO T., TRONO Jr G.C., YOUNG-MENG CHIANG & YOSHLDA T., 1994 —

Several *Sargassum* species (subgenus *Sargassum*) in East Asia with furcately branching leaves. In: Abbott IA. (ed.), *Taxonomy of Economic Seaweeds*. IV. La Jolla, California Sea Grant College, University of California, pp. 9-22. ALBRECHT A. & REISE K., 1994 — Effects of *Fucus vesiculosus* covering intertidal mussel beds in the Wadden Sea. *Helgoländer Meeresuntersuchungen* 48 (2-3): 243-256. MAGGS C.A. & HOMMERSAND M.H., 1993 — Seaweeds of the British Isles. 1 Rhodophyta. Part 3A Ceramiales. London, HMSO Books, The Natural History Museum, 464 p. MONTAGNE C., 1838 — Centurie de plantes cellulaires exotiques nouvelles. *Annales des sciences naturelles, Botanique*, sér. 2, 9: 38-57. In the text, references should be cited by the name of the author and the year of publication; use “et al.”, for more than two authors, and use “&” between two authors (e.g. Montagne, 1838; Maggs & Hommersand, 1993; Ajisaka et al., 1994).

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Preparation of manuscripts

General

Please read the guidelines below and additionally consult a recent article published in *Phytotaxa* and follow the style therein.

Language. The article has to be written in British or American English throughout the manuscript. Authors whose native language is not English are encouraged to ask colleagues familiar with the field of research and fluent in English (preferably a native speaker) to correct the language in the manuscript before submission. An article may be returned to the author without review if the language is not of an acceptable standard.

The author is also responsible for the correct usage of other languages, be it a Latin diagnosis or an abstract in a foreign language. The grammar of texts in foreign languages needs to be checked by the author before submission, and again after review if the English from which it is translated (e.g. an abstract) has changed. Latin scholars who are consulted for the correcting of diagnoses should be acknowledged.

Metric measures should be used. Please use the common font Times New Roman, 12 pt and as little formatting as possible (apply only **bold** and *italics* where necessary and indent paragraphs except the first). Special symbols can be used but need to be carefully checked by the author at proof stage, because they may be altered due to incompatibility of files.

Hyphens ‘-’ are used to link words such as personal names, topographical names, some prefixes and compound adjectives that could otherwise be confused (examples: well-established, 5-sided, Kingston-upon-Thames, Kingdon-Ward, co-operation, etc.).

En-dash or en-rule ‘—’ (a dash the length of the letter ‘n’) should be used for ranges or spans. In the context of *Phytotaxa* it is mainly used for ranges of numbers, most frequently size ranges, elevational ranges, dates and page numbers (e.g. 500–1000 m, 1–3 May, 1976–1977, figs 5–7). Remember also to apply them in the reference section for ranges of volumes, years and pages. The en-dash is also used in name associations (e.g. a Federal–State agreement) and in phenology (e.g. flowering May–September).

Em-dash or em-rule ‘—’ (the length of the letter ‘m’) is used infrequently; they are used for breaks in the text or subject. In contrast to parentheses an em-dash can be used alone; e.g. “What could these results mean—that John discovered the meaning of life?” Em-dashes are also used after a subheading, for instance:

“Type:—BRAZIL. Paraná: Ponta Grossa, Furnas Gêmeas, remnant *Araucaria* forest below large sandstone cliff, 25.145°S, 049.958°W, 950–1000 m, 16 February 2008, *Christenhusz et al. 4790* (holotype SP!, isotypes K!, MBM, NY!, P!, TI, TUR!, UCI, UPCB).”

Exclamation mark ‘!’ is used to indicate after the herbarium acronym to indicate that this voucher specimen has been seen by the author (see above).

Multiplication or times sign ‘×’. The multiplication sign × is not to be confused with the letter x. It should always be used in hybrid taxa (e.g. *Equisetum × schaffneri*) and in measurements of length and width (of leaves or petals, for example), for example: “leaves 1.0–4.2 × 0.4–0.8 cm”.

Dashes and hyphens should not be spaced. Please feel free to copy these symbols from this author guide and paste them into your manuscript. Using the correct symbols will speed up the editing process. Editors may return the manuscript to the author if dashes, hyphens and multiplication signs are not correctly used.

Italicisation. Generic names and all ranks below are italicised. Book and journal titles are also in italics, as well as diagnoses in Latin and Latin abbreviations (such as *sp. nov.*, *comb. nov.*, *nom. illeg.*, *et al.*). “*subsp.*”, “*ser.*”, “*var.*”, “*cv.*” and “*f.*” (for forma or filius) are not italicised, nor are names above the rank of genus. The abbreviation “*ssp.*” should be avoided and replaced by “*subsp.*” (for subspecies) to prevent confusion with the abbreviation *spp.* (= species in plural). As a general rule abbreviations are discouraged.

Abbreviations of certain words are standardised: ca. = circa, m = meter, cm = centimeter, dbh = diameter at breast height, elev. = elevation (altitude is not used for heights of land surfaces above sea level; altitude is used for heights above the earth surface, e.g. of an airplane), *sp. nov.* = new species, *comb. nov.* = new combination, *gen. nov.* = new genus, subsp. = subspecies, sect. = section, pers. comm. = personal communication, etc. Herbarium acronyms follow *Index Herbariorum* <http://sweetgum.nybg.org/ih/>

Citation of author names

Author abbreviations are seldom used (generally only for basionym authors and new taxa proposed in the manuscript); they are generally cited fully in the references. This means that the name is not abbreviated but the surname is written in full, followed in brackets by the year of publication, a colon, and the page number of the page where the name was published. This is treated as a normal citation, and thus the full article in which the species was published has to be cited in the references. (Include full title of the article, full journal title and full page range.) This is obligatory for all taxonomic articles and articles in which fewer than 30 taxa are mentioned. In articles that deal with a larger number of species (such as ecological or phylogenetic studies or checklists) this is not encouraged because it is impractical. If uncertain, please contact an editor about this matter.

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- *Hymenophyloopsis asplenioides* A.C.Sm. in Gleason (1931: 302). Smith is abbreviated here because it is published in Gleason, which is the proper article to cite.
- *Cyathea asplenioides* (A.C.Sm.) Christenhusz (2009: 39). Smith is abbreviated here because the basionym is already given above.
- *Cyathea gibbosa* (Klotzsch 1844: 542) Domin (1929: 262). Both the basionym and new combination are cited because the basionym is not given above.

In the references:

Christenhusz, M.J.M. (2009) New combinations and an overview

of *Cyathea* subg. *Hymenophyloopsis* (Cyatheaceae). *Phytotaxa* 1: 37–42.

Domin, C. (1929) *Pteridophyta*. České Akademie, Prague. 276 pp.

Gleason, H.A. (1931) Botanical results of the Tyler-Duida expedition. *Bulletin of the Torrey Botanical Club* 58: 227–344.

Klotzsch, J.F. (1844) Beiträge zu einer Flora der Äquinoctial-Gegenden der neuen Welt, Filices. *Linnaea* 18: 525–556.

Deposition of voucher specimens and GenBank numbers

Authors of new taxa are required to deposit type specimens in national or international public museums or collections, preferably ones listed in the *Index Herbariorum* that are provided with a corresponding acronym.

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Please apply the word 'elevation' when dealing with geological features. 'Altitude' is here defined as the distance above the surface of the Earth, whereas 'elevation' applies to the height of an earth surface above sea level.

For country names (always capitalised in specimen citations) and provinces (followed by a colon), the standard spellings in English followed by the UN apply (e.g. Kyrgyzstan not Kirghizia, Madagascar not Malagasy Republic etc.). For a standard list of countries and areas see: <http://unstats.un.org/unsd/methods/m49/m49alpha.htm>. Exceptions may be discussed with the editor.

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Names and affiliations of article author(s)

Names of all authors must be given below the title and should be typed in upper case (e.g. ADAM J. SMITH, BRIAN SMITH & CAROL SMITH). Inclusion of all major contributors to the work should be considered.

Below the names, the address(es) of professional affiliation for each author should be given in *italics* each starting on a separate line. E-mail address(es) should be provided if available. Affiliation addresses are linked to the author names by numbers in superscript and are provided in corresponding order.

Abstract

The abstract should cover concisely contents of the paper and should be phrased such that additional key words are not necessary. Any new names or new nomenclatural acts proposed in the article should be mentioned. No taxon

author names are to be included in the abstract. Introductory information should not be included in the abstract, neither should be the citation of references.

Abstracts in other languages using the Latin alphabet may also be included in addition to English and should be a direct translation of the English abstract. The spelling and grammar of these abstracts in other languages is the responsibility of the author. An abstract in another language should be corrected if there are any changes in the English abstract during the editorial process.

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Key words may be given when the abstract does not already cover these. The key words may not include words that are already in the title, and they should be given in alphabetical sequence.

Abstracts and key words are not included in short Communications.

Introduction

The introduction should place the study in context, and it should provide recent or historical background relevant to the study. This information should not be included in the abstract. Author names of a taxon should be cited only once, when the taxon/species first appears in the text.

Material & Methods

Materials and methodology used in empirical studies should be concisely provided. Herbaria consulted can be listed here, if not done so in the Acknowledgements. Field work should be highlighted. Floras and other taxonomic works consulted to identify the plant material involved in a study should be cited.

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Conclusion

The conclusion should state what the scientific contribution of your study is (ask yourself the question: 'What can we learn from this study and how do the results help us to understand the questions asked in the introduction and discussion?'). It is helpful for other researchers to point out further studies that may be needed in the future.

Taxonomy

A taxonomy section should start with each taxon in bold italics. Abbreviations of authors of new species should be given (following IPNI, not bold), and these should be followed by the correct designation (in italics, not bold, e.g. *comb. nov.*, *nom. nov.*, *spec. nov.*, *stat. nov.*, etc.). When species are not newly described, the author names should be followed by the year and page of publication (and the full article should be included in the references).

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Taxonomic descriptions should be organised describing the plants from below to above and from outside towards the inner parts. Of course, this is different for each taxon and can thus follow a variable. Subsections of descriptions can be highlighted using italics. Additional data (e.g. distribution, ecology, etymology, etc.) may follow. Often these are subheaded by '—' (m-dash).

Specimens are cited as follows:

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Lectotype (designated by Smith 2000/designated here):—FINLAND . Uusimaa: Helsinki, Kaisaniemi Park, 27 April 1976, *Virtanen 22* (H!).

Keys

Identification keys should be dichotomous, and the leads should (preferably) be opposite to each other in meaning so that the species can be easily distinguished. Please do not format the key; provide it in the following simple layout:

1. Bracts longer than pedicels; filaments with 1 acute lobe at apex on either side of anther ... *Ornithogalum nutans*
- Bracts shorter than pedicels; filaments without apical lobes on anther ... 2.
2. Inflorescence corymbose; tepals longer than 14 mm ... *Ornithogalum angustifolium*
- Inflorescence racemose; tepals shorter than 14 mm ... *Ornithogalum pyrenaicum*

Acknowledgements

The format for the Acknowledgements is variable, and anyone can be thanked for their contribution. Please consider co-authorship for people that contributed to the study in a major way, especially contributors of specimens or laboratory work.

References

All literature cited in the text (including full articles of taxon authors) should be included. Please check this carefully before submission because errors are common. References should be cited in the text as Smith (1999), Smith & Jones (2000) or Smith *et al.* (2001), the latter when there are three or more authors, or alternatively in a parenthesis (Adams 2000, Smith & Jones 2000, Jones 2001, Smith *et al.* 2001). The citation of floras, revisions and monographs used to identify the collections on which the study is based is strongly encouraged.

Please include DOI for papers that have these. This facilitates linking to papers that have online versions.

Journal article: Author, A. & Author, B.C. (YEAR) Title of the paper. *Title of the journal in full in italics* volume: x–y. For example:

Christenhusz, M.J.M., Zhang, X.-C. & Schneider, H. (2011) Linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa* 19: 7–54.

Book chapter: Author, A. & Author, B.C. (YEAR) Title of the chapter. *In*: Author, A., Author, B.C. & Author, D. (eds.) *Title of book in italics*. Publisher name, City, pp. x–y. For example:

Schneider, H., Kreier, H.-P., Janssen, T., Otto, E., Muth, H. & Heinrichs, J. (2010) Key innovations versus key opportunities: identifying causes of rapid radiations in derived ferns. *In*: Glaubrecht, M. (ed.) *Evolution in action*. Springer, Berlin, pp. 61–76.

Book: Author, A. & Author, B.C. (YEAR) *Title of book in italics*. Publisher name, location, xyz pp. For example:

Copeland, E.B. (1947) *Genera filicum*. Chronica Botanica, Waltham, Massachusetts, 247 pp.

Internet source: Author, A. (YEAR) *Title of website, database or other resources*, Publisher name and location (if indicated), number of pages (if known). Available from: <http://xxx.xxx.xxx/> (Date of access). For example:

IUCN (2010) *The IUCN red list of threatened species*, version 2010.4. IUCN Red List Unit, Cambridge U.K. Available from: <http://www.iucnredlist.org/> (accessed: 19 May 2011).

Dissertations resulting from graduate studies and non-serial proceedings of conferences/symposia are to be treated as books and cited as such. Articles not cited in the manuscript should not be included in the References section.

Figures and Tables

Legends of figures and tables should be listed after the list of references within the same file of the manuscript. Legends for tables and figures should start with **TABLE** or **FIGURE** followed by its number and a full stop. Illustrators and photographers should be mentioned in the figure legend, and if the illustrator is not one of the authors he/she should also be acknowledged. All figures and tables need to be referred to in the text.

Example:

FIGURE 1. Distribution map of *Psilotum nudum* in the Caribbean region.

When preparing illustrations, authors should bear in mind that the journal has a matter size of 25 cm by 17 cm and is printed on A4 paper. For species illustrations, line drawings are preferred, although good quality black and white or colour photographs are also acceptable. See a guide [here](#) for detailed information on preparing plates for publication; this guide was prepared by Dr Daniel Geiger for *Zootaxa*, but it applies equally to *Phytotaxa*.

Line drawings must be scanned at 600 to 1200 dpi as line art (bitmap, =1 bit); they must NOT be scanned as 8 bit or full colour images. Pictures and line drawings should be saved as TIFF files. In some cases PDF or DOC files are acceptable. JPG is not an accepted format. Do not scan line drawings as JPG files because this creates blurry or pixellated images. Sufficient resolution should be provided, but it is up to the author to do so. Low resolution figures can only be printed at a small size.

Tables, if any, should be provided at the end of the manuscript. Please use the table function in your word processor to build tables such that the cells, rows and columns remain aligned when font size and width of the table are changed. Please do not use the tab key or space bar to type tables.

References

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also <http://mapress.com/phytotaxa/content/2011/f/pt00019p134.pdf>)

Christenhusz, M.J.M., Zhang, X.-C. & Schneider, H. (2011a) Linear sequence of extant families and genera of lycophytes and ferns. *Phytotaxa* 19: 7–54. <http://mapress.com/phytotaxa/content/2011/f/pt00019p054.pdf>

Christenhusz, M.J.M., Reveal, J.L., Farjon, A., Gardner, M.F., Mill, R.R. & Chase, M.W. (2011b) A new classification and linear sequence of extant gymnosperms. *Phytotaxa* 19: 55–70.

<http://mapress.com/phytotaxa/content/2011/f/pt00019p070.pdf>.

ANEXO III

Tabela Complementar ao Capítulo I – Diversidade de Hyphomycetes aquáticos em cada local de coleta no Parque Estadual da Ilhabela (PEIb).

Táxons	Coleta 1 (27/06/2012)			Coleta2 (04/09/2012)						Coleta3 (06/12/2012)			Coleta4 (14e 15/05/2013)										
	Cachoeira da Lage	Cachoeira do Veloso	Cachoeira dos Três Tomboos	Pogo da Pedra	Pogo da Escada	Pogo da Duchá	Pogo do Jequitibá	Pogo do Jabuti	Riacho Lado norte	Cachoeira Centro da Ilha	Córrego do Eng. D'água	Cachoeira da Toca	Cachoeira da Água Branca	Córrego Ribeirão do Pombo	Cachoeira do Gato	Rio da Cachoeira do Gato	Córrego do Cemitério 1	Córrego do Cemitério 2	Rio dos Castelhanos	Rio Caminho da Praia Vermelha	Cachoeira do Cocóia	Cachoeirado Tesouro da Cozinha	
<i>Acumisporea verruculosa</i> Heredia, R.F. Catañeda & R.M. Arias															X	X							
<i>Alatospora acuminata</i> Ingold			X												X								
<i>Anguillospora crassa</i> Ingold				X					X														
<i>Anguillospora filiforme</i> Greath.	X																						
<i>Anguillospora longissima</i> (Sacc. & P. Sud.) Ingold	X	X														X							
<i>Anguillospora pseudolongissima</i> Ranzoni				X	X	X	X	X		X						X						X	
<i>Articulospora tetracladia</i> Ingold		X																					
<i>Chaetendrophragmia triangularis</i> Matsush.															X		X						
<i>Camposporidium cristatum</i> Nawawi & Kuthub.															X								
<i>Camposporium anntenatum</i> Harkn.						X			X			X	X										
<i>Camposporium pellucidum</i> (Grove) S. Hughes												X	X										
<i>Campylospora chaetocladia</i> Ranzoni	X	X	X	X	X			X	X	X		X	X				X						
<i>Campylospora brasiliensis</i> L.B. Moro & I.H. Shoenlein-Crusius																							
<i>Campylospora filicladia</i> Nawawi		X	X	X			X	X							X	X				X			X
<i>Campylospora parvula</i> Kuzuha				X								X											
<i>Centrospora aquática</i> De Wild.	X																						
<i>Clavatospora tentacula</i> Sv. Nilsson													X										X
<i>Dendrosporium lobatum</i> Plakidas & Edgerton	X														X				X	X			
<i>Flabellospora acuminata</i> Descals			X																				X
<i>Flagellospora curvula</i> Ingold	X	X	X																				
<i>Flagellospora penicillioides</i> Ingold									X			X		X	X	X			X	X			
<i>Heliscus submersus</i> H.J. Huds.	X	X	X		X					X	X	X											
<i>Istbmolongispora biramifera</i> Matsush.															X								
<i>Istbmolongispora quadricelularia</i> Matsush.				X	X										X								
<i>Lateriramulosa a-inflata</i> Matsush.															X								
<i>Lunulospora curvula</i> Ingold	X	X	X	X	X	X		X	X	X		X	X	X			X	X	X				X
<i>Lunulospora cymbiformis</i> K. Miura	X	X	X				X		X						X								

Táxons (continuação)	Coleta 1 (27/06/2012)			Coleta2 (04/09/2012)							Coleta3 (06/12/2012)				Coleta4 (14e 15/05/2013)									
	Cachoeira da Lage	Cachoeira do Veloso	Cachoeira dos Três Tomboos	Pogo da Pedra	Pogo da Escada	Pogo da Duchta	Pogo do Jequitibá	Pogo do Jabuti	Riacho Lado norte	Cachoeira Centro da Ilha	Córrego do Eng. D'água	Cachoeira da Toca	Cachoeira da Água Branca	Córrego Ribeirão do Pombo	Cachoeira do Gato	Rio da Cachoeira do Gato	Córrego do Cemitério 1	Córrego do Cemitério 2	Rio dos Castelhanos	Rio Caminho da Praia Vermelha	Cachoeira do Cocóia	Cachoeirado Tesouro da Colina		
<i>Phalangispora nawawii</i> Kuthub.				x									x										x	
<i>Physalidiella elegans</i> (Mosca) Rulamort															x									
<i>Polylobatispora setulosa</i> L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius															x	x								
<i>Scutisporus brunneus</i> K. Ando & Tubaki							x					x	x			x								
<i>Tetracladium marchalianum</i> De Wild.		x	x												x									
<i>Tetracladium setigerum</i> (Grove) Ingold	x														x									
<i>Tricladium gracile</i> Ingold			x																					
<i>Tripospermum camelopardus</i> Ingold, Dann & P.J. McDougall				x	x	x	x	x	x	x			x		x	x	x		x		x		x	
<i>Tripospermum myrtii</i> (Lind) S. Hughes											x	x	x											
<i>Triscelophorus acuminatus</i> Nawawi				x	x	x					x				x	x			x	x				
<i>Triscelophorus monosporus</i> Ingold	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x			x	x	x	x
<i>Triscelophorus ponapenses</i> Matsush.				x			x			x														