Rare hyphomycetes from freshwater environments from Chapada Diamantina, Bahia, Brazil

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Abstract: Aquatic litter-associated fungi are a relatively understudied group that includes mostly asexual stages of Ascomycota and Basidiomycota which are adapted to aquatic environment. They are important players in fundamental ecosystem-level processes, including decomposition of submerged organic matter. The aim of this work was to carry out a taxonomic survey of aero-aquatic hyphomycetes and other fungi associated with submerged decaying plant litter in Chapada Diamantina, Bahia, Brazil and provide descriptions, comments and illustrations, including worldwide geographical distribution for new records and rare species. Dactylaria fusifera, Helicoon myosuroides, Pseudaegerita websteri, Spadicoides subsphaerica and Verticicladus subiculifer are reported for the first time after the original descriptions; Pseudaegerita viridis is a new record for the Americas, Spirosphaera carici-graminis is a new record for South America and Ardhachandra aequilatera is a new record for the aquatic environments.

Key words: Aquatic hyphomycetes, Chapada Diamantina, taxonomy, tropical.

Introduction

Freshwater fungi are capable of completing the entire or part of their life cycle in aquatic ecosystems where they colonize a wide variety of substrates (Thomas 1996). They may be saprophytic, mutualistic, parasitic or endophytic and have a worldwide distribution (Jones & Pang 2012, Chauvet et al. 2015). Shearer et al. (2007) compiled literature data on fungi recorded from aquatic ecosystems (both freshwater and marine) and reported the total of 3047 taxa, with the Ascomycota (including asexual ascomycetes) being the
most diverse (1527 spp.), followed by Chytridiomycota (576 spp.), Ingoldian fungi (290 spp., that include species with affinity to asco- or basidiomycetes) and Basidiomycota (21 spp.). Recently, Jones & Pang (2012) compiled data on freshwater fungi from the tropics and recorded 660 species of asexual ascomycetes (including Ingoldian fungi and species lacking specific adaptations for water dispersal), 548–650 ascomycetes and 214 trichomycetes.

Freshwater hyphomycetes are asexual stages of ascomycetes and basidiomycetes that are adapted to growth, reproduction and dispersal in freshwater ecosystems. They can be separated into three ecological groups. (1) Ingoldian fungi (also known as aquatic hyphomycetes) regularly sporulate underwater and form tetradiate, multiradiate, variously branched or sigmoid conidia (Descals 2005, Gulis et al. 2005). (2) Aero-aquatic hyphomycetes mainly occur in stagnant waters, growing vegetatively on submerged substrates but sporulating only when the substrate gets in contact with air or conidiophores pierce through the water surface (Fisher 1977); they produce conidia with three-dimensional, helicoid or clathroid morphology that often trap air and float on the water surface (Michaelides & Kendrick 1982). (3) "Facultative aquatic" (Goh & Hyde 1996a, Descals & Moralejo 2001) hyphomycetes represent a heterogeneous group of fungi that often, though not always, form thick-walled dematiaceous conidia and are often associated with submerged woody substrates (i.e. lignicolous). Even though they can sporulate under submerged conditions, their conidia often lack specific morphological adaptations to water dispersal typical for Ingoldian fungi and aero-aquatic hyphomycetes.

The term "aero-aquatic" was originally proposed by van Beverwijk (1951) who referred to helicosporous genera studied by Linder (1929) and ecologically similar genera like Candelabrum and Papulaspora [nowadays Beverwykella pulmonaria (Beverw.) Tubaki] (van Beverwijk 1954). Later studies of aero-aquatic fungi led to discoveries of many new species from Asia, Australia, Europe, North, Central and South America (e.g. Webster & Davey 1980, Abdullah et al. 1986, Matsushima 1993, Abdullah et al. 1996, 1997, Castañeda-Ruiz & Gams 1997, Voglmayr 1997a, Voglmayr & Fisher 1997, Voglmayr & Krisai-Greilhuber 1997, Marvanová & Bärlocher 1998, Hyde & Goh 1998, Voglmayr 1998, Chang 2001, Voglmayr & Delgado-Rodríguez 2001, Voglmayr 2004, Abdullah et al. 2005, Voglmayr & Yule 2006). In Brazil, relatively few studies of aero-aquatic fungi have been carried out. However, recently, several new species have been described (Monteiro & Gusmão 2013, Monteiro et al. 2014a, Moro et al. 2015a) and new records of rare species have been published (Barbosa & Gusmão 2011, Monteiro 2014).

Facultative aquatic fungi were studied in Brazil more extensively than aero-aquatic fungi. In general, a higher number of species from this group has been recorded (Barbosa et al. 2013, Silva et al. 2014, Monteiro 2014) and multiple new species have been described from the Brazilian semiárid region and Brazilian Amazon (Silva & Gusmão 2013, Monteiro & Gusmão 2014, Monteiro et al. 2014b, Fiuza et al. 2015a).

This study aimed to carry out a taxonomic survey of aero-aquatic and facultative aquatic hyphomycetes in Chapada Diamantina, Bahia, Brazil and provide descriptions, comments and illustrations, including data on worldwide geographical distribution for new records and rare species.
Materials and methods

From November 2013 to January 2015 we made eight expeditions to “Serra da Tromba”, located in the Chapada Diamantina, a semi-arid region in northeastern Brazil. Submerged leaves of *Calophyllum brasiliense* Cambess and submerged wood, twigs and tree barks from unidentified hosts were collected from three streams in the Contas river basin. The samples were taken to the Laboratory of Mycology (LAMIC) in plastic bags, processed according to Castañeda-Ruiz (2005), incubated at room temperature (~24°C) with natural light conditions during one month and examined periodically under a dissecting microscope for the presence of reproductive structures. Some fungi were also isolated into potato dextrose agar, corn meal agar and malt extract agar and incubated at ~24°C. In those cases that isolates did not show sporulation on the agar, fragments of the pure culture isolates were submerged in a Petri dish with sterile distilled water until reproductive structures appeared. Measurements of reproductive structures and identification of specimens were performed using a compound microscope (Olympus BX51). The specimens were deposited in the Herbarium of “Universidade Estadual de Feira de Santana” (HUEFS).

**Results**

Fifty six taxa of freshwater hyphomycetes have been identified in this study, eight of them are rare. *Dactylaria fusifera*, *Helicoon myosuroides*, *Pseudaegerita websteri*, *Spadicoides subsphaerica* and *Verticicladus subiculifer* are reported for the first time after their original descriptions; *Pseudaegerita viridis* is a new record for the Americas, *Spirophaera carici-graminis* is a new record for South America and *Ardhachandra aequilatera* is a new record for the aquatic environments.

**Taxonomy**

**Aero-aquatic hyphomycetes**


**Conidiophores** macronematous, mononematous, unbranched, 1–2-septate, straight to flexuous, 39–45 × 3–3.5 µm, brown. **Conidiogenous cells** monoblastic, integrated, terminal. **Conidia** oval to rectangular, lenticular, with a dichotomous branching system, clathrate layer formed by the eight outermost branches produced by internal branches, edges rounded, conidia brown, 40–57 µm diam.

**Material examined:** Brazil. Bahia: Piatã, Chapada Diamantina, on submerged leaves of *Calophyllum brasiliense*, 30.X.2014, P.O. Fiuza (HUEFS 215942).

**Geographical distribution:** Brazil (Monteiro 2014) and USA (Voglmayr & Delgado-Rodríguez 2003).

*Beverwykella clathrata* is close to *B. cerebriformis* based on the ontogeny, size and shape of conidia (Voglmayr & Delgado-Rodríguez 2003). However, *B. cerebriformis* displays an outermost cell layer sealed by tightly appressed cells, while *B. clathrata* has a clathrate outermost cell layer (Voglmayr & Delgado-Rodríguez 2003). The material examined in this study is in agreement with the original description. This species was recorded several times on submerged leaves and twigs in Brazilian Amazon (Monteiro 2014, SpeciesLink 2016) and also in São Paulo (Moro et al. 2015a); it is the first record for the Brazilian semi-arid region.

**Conidiophores** macronematous, mononematous, unbranched, flexuous, 30–66 × 3–4 µm, brown. **Conidiogenous cells** monoblastic, integrated, terminal. **Conidia** helicoid, doliiform, cup-shaped, 27–30 µm diam., 12–18 µm long. Conidial filament septate, 4.5–6 µm wide in the middle of the conidium, gradually tapering towards the distal end (3 µm), coiled 4–6 times, brown.


**Geographical distribution**: Austria (Voglmayr 1997a) and Brazil (new record).

*Helicoon* was proposed by Morgan in 1892, and nowadays it is represented by 17 species (Seifert et al. 2011). The main diagnostic character is the spiral coil of the conidial filament that forms an elongated ellipsoidal body (Morgan 1892) in combination with conidium production directly on a conidiophore. *Helicoon myosuroides* is very close to *H. pluriseptatum* Beverw. but the latter has larger conidia (Voglmayr 1997a); furthermore, *H. pluriseptatum* has a conidial filament that is loosely coiled and not significantly tapering at the end (Voglmayr 1997a). The material examined in this study agrees well with the protologue. *Helicoon myosuroides* is reported for the first time after it was originally described from Austria.


**Conidia** crateriform, solitary, dry, with several dichotomously branched, curved, ascending arms, attenuated at the tips, with 6–7(9) eusepta each, ascending from several basal cells and encircling a hollow, air-filled space, light brown, becoming paler at the apex, 45–65(70) × 30–45 µm.

**Material examined**: Brazil. Bahia: Piatã, Chapada Diamantina, on submerged wood, 08.VII.2014, T.Cantillo (HUEFS 216614).

**Geographical distribution**: Brazil (Monteiro 2014) and Malaysia (Nawawi & Kuthubuthen 1990).

*Nidulispora* is a monotypic genus (Seifert et al. 2011). Until recently, this fungus was known only from the original description from Peninsular Malaysia (Nawawi & Kuthubuthen 1990, Kirk 2016). This is the second record from Brazil, following the recent finding on submerged leaves from Utinga State Park, Pará (Monteiro 2014). The peculiar arrangement of the arms forms a hollow space which traps air and allows conidia to float on the water surface, a common feature of aero-aquatic fungi.

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Conidiophores macronematous, mononematous, unbranched, 2–3-septate, light green in colour, 3–5 µm wide, up to 100 µm long. Conidia subglobose, 180–565 µm diam., olivaceous to dark green, composed of a highly branched system of torulose or subglobose cells, 5–7 µm diam., each successively budding out 1–3 (4) daughter cells, with empty spaces between them that trap air. The conidia disarticulate easily when mounted on slides. Colonies on potato dextrose agar up to 90 mm diam. in 7 days. Mycelium mostly immersed, rarely superficial, hyphae septate, olivaceous green, colony reverse blackish green. Conidia forming solitary or in groups, white at first, dark green with age. Phialidic state observed.

Material examined: Brazil. Bahia: Piatã, Chapada Diamantina, on submerged wood, 17.VII.2014, T.Cantillo (HUEFS 216602); Bahia: Piatã, Chapada Diamantina, on submerged bark tree, 09.I.2015, T.Cantillo (HUEFS 216603).

Geographical distribution: Brazil (new record), Hungary (Gönczöl & Révay 2003), New Zealand (Cooper 2005), Poland (Czeczuga & Orlowska 1996) and United Kingdom (Abdullah & Webster 1983).

**Pseudaegerita** is represented by eight species (Seifert et al. 2011). *Pseudaegerita viridis* is, by far, the most commonly found species of the genus (Cooper 2005) and one of the most common species of aero-aquatic hyphomycetes in Britain (Abdullah & Webster 1983) with 42 records in the Fungal Records Database of Britain and Ireland (FRDBI) (Kirk & Cooper 2009). It has been found on decaying twigs and wood of different plants deposited on the banks of rivers, streams and stagnant water bodies (Abdullah & Webster 1983). It has also been reported from water-filled tree holes in Hungary (Gönczöl & Révay 2003). The examined material is in agreement with the original description. Only in culture, the Brazilian material also develops a phialidic state, whereas it was reported from culture as well as from the natural substrate by Abdullah and Webster (1983). This is the first record from the Americas and from the tropical region.


Conidiophores macronematous, mononematous, unbranched, 1–2-septate, hyaline, 3.5–5 µm wide, up to 25 µm long. Conidia globose to subglobose, 52.5–105 µm diam., hyaline, composed of a branched system of globose cells, 3–4.5 µm diam., each successively budding out 1–4 daughter cells, with empty spaces between them that trap air. The conidia disarticulate easily when mounted on slides.

Material examined: Brazil. Bahia: Piatã, Chapada Diamantina, on submerged leaves of *Calophyllum brasiliense*, 08.I.2015, P.O.Fiuza (HUEFS 215946).

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**Geographical distribution:** Brazil (new record) and Spain (Abdullah et al. 2005).

*Pseudaegerita websteri* is easily distinguished from other species in the genus by forming white conidia on natural substrate and short conidiophores. This species is close to *P. corticalis* but the latter has larger and pigmented conidia (Abdullah et al. 2005). The material examined is in agreement with the original description. This is only the second record of *P. websteri* and the first from the tropics.

Fig. 3A–B

**Conidiophores** semimacronematous, mononematous, unbranched, septate, hyaline, 10–30 × 3–6 µm. **Conidiogenous cells** monoblastic, integrated, terminal. **Conidia** globose to subglobose, hyaline, 51–120 µm diam. Conidial filaments constricted at septa, 3–6 µm wide. Colonies in 0.1% malt extract agar up to 22 mm diam. in 30 days. Mycelium mostly immersed, hyphae septate, hyaline, colony white to cream. Conidia forming solitary or in groups, white.


**Geographical distribution:** Austria, Netherlands and USA (Voglmayr 1997b) and Brazil (new record).

*Spiroshera* was erected by van Beverwijk in 1953 based on *S. floriformis*. The genus includes nine species (Voglmayr 2004). *Spiroshera carici-graminis* differs from other species of the genus by its strongly constricted hyaline conidial filaments and cells that can give rise to up to two daughter filaments, while only one daughter filament is formed in other species (Voglmayr 1997b). *Spiroshera carici-graminis* is usually recorded in association with monocotyledonous plant remains (Voglmayr 1997b), but in this study, it is reported from decaying leaves of a dicotyledonous tree and bark material of an unidentified host. In the present study the species just showed sporulation when the media with fungi were put in contact with water. This is the first record for the Neotropics.

**Additional records of aero-aquatic hyphomycetes**


**Material examined:** Brazil. Bahia: Piatã, Chapada Diamantina, on submerged twigs, 15.I.2015, T.Cantillo. (HUEFS 216616).


**Material examined:** Brazil. Bahia: Piatã, Chapada Diamantina, on submerged leaves, 12.VII.2014, J.S.Monteiro (HUEFS 216617).


"Facultative aquatic" hyphomycetes associated with submerged decaying plant litter

_Ardhachandra aequilatera_ Matsush., Matsush. Mycol. Mem. 5: 3, 1987. Fig. 3D

Conidiophores macronematous, mononematous, unbranched, 1–3-septate, straight to flexuous, 28.5–65 × 3–6 µm, pale brown. Conidiogenous cells holoblastic, denticulate, sympodial, pale brown. Conidia fusiform in front view, lenticular in lateral view, guttulate, pale brown, 13.5–16.5 × 4.5–6 µm.

Material examined: Brazil. Bahia: Piatã, Chapada Diamantina, on submerged leaves of _Calophyllum brasiliense_, 08.VII.2014, P.O.Fiuza (HUEFS 215941).

Geographical distribution: Brazil (new record), Cuba (Matsushima 1987) and Taiwan (Matsushima 1987).

_Ardhachandra_ was erected by Subramanian and Sudha in 1978 with the type species _A. selenoides_. The genus is represented by six species (Mel’nik 2012) with conidia selenoid, fusiform or lenticular, produced singly and successively by conidiogenous cells with prominent denticles (Subramanian & Sudha 1978). _Ardhachandra aequilatera_ is easily distinguished from the type species that forms selenoid conidia (Subramanian & Sudha 1978). _Ardhachandra prolatafusiformis_ J.L.Chen & Tzean is close to _A. aequilatera_ but has longer conidia which are asymmetrical (unequal-sided) in front view (Chen & Tzean 1995). Two species of _Ardhachandra_ were recorded in Brazil: _A. critastospora_ (Matsush.) Subram. & Sudha was found associated with leaves of _Tibouchina pulchra_ (Cham.) Cogn. in the state of São Paulo (Grandi & Gusmão 2002) and leaves of _Clusia melchiorii_ Gleason in Bahia (Barbosa et al. 2009); _A. selenoides_ (de Hoog) Subram. & Sudha was recorded from leaves of _C. melchiorii_ and unidentified litter in Bahia (Barbosa et al. 2009, Marques et al. 2008). This is the first record of _A. aequilatera_ from the aquatic environment and for the Americas.

_Dactylaria fusifera_ (Berk. & M.A.Curtis) de Hoog, Stud. Mycol. 26: 17, 1985. Fig. 3E

Conidiophores macronematous, mononematous, unbranched, aseptate, rarely 1-septate, erect, subhyaline, with swollen foot, 22–40 × 5–7.5 µm. Conidiogenous cells polyblastic, denticulate, sympodial, 20–27.5 × 4–5 µm. Conidia cylindrical to fusiform, 1-septate, hyaline, 32.5–42 × 4–5 µm. Colonies on corn meal agar up to 40 mm diam. in 15 days. Mycelium mostly immersed, rarely superficial, hyphae septate, brown, colony dark brown.


Geographical distribution: Brazil (new record) and USA (Berkeley 1875).

_Dactylaria_ consist of 90 species (Seifert et al. 2011). The defining characteristics of the genus are conidiogenous cells with multiple cylindrical apical denticles (de Hoog 1985). _Dactylaria fusifera_ is closest to _D. candidula_ (Höhn.) G.C.Bhatt & W.B.Kendr., but the latter has fusiform conidia with constricted septa. The specimens found in this study match the description provided by de Hoog (1985), which is based on Berkeley’s material. This is a new record for the Neotropics and the first report after the original description.

Conidiophores macronematous, mononematous, unbranched, straight, septate, erect, smooth, brown, 62–115 × 3.5–4.5 µm. Conidiogenous cells polytretic, terminal and intercalary, integrated, minute pores clearly visible. Conidia subglobose, globose, or broadly ellipsoidal, aseptate, brown to dark brown, smooth, thick-walled, with an occasionally visible, slightly protuberant pore at the base, 4–4.5 × 3–3.5(4) µm.

**Material examined**: Brazil, Bahia: Piatã, Chapada Diamantina, on submerged wood, 3.V.2014, T.Cantillo (HUEFS 216612).

**Geographical distribution**: Brazil (new record) and USA (Li 2010).

*S. subsphaerica* was introduced with *S. bina* (Corda) S.Hughes as the type species and is very similar to the genus *Diplococcium* Grove, with the only difference being that in *Diplococcium* the conidia are produced in short chains (Goh & Hyde 1996b). Among other species of the genus with single-celled conidia, *Spadicoides subsphaerica* is characterized by small conidia (3.3–5.4 × 3.2–4.3 µm), subspherical or ellipsoidal, smooth, brown to dark brown. The Brazilian specimen had slightly larger conidiophores and conidia compared to the specimen from the U.S.


Conidiophores micronematous, mononematous, branched, straight, smooth, brown, 15–45 × 3–4 µm, 0–3-septate. Conidiogenous cells monoblastic, terminal, integrated, doliiform, brown, 6–10 × 3–4 µm. Conidia fusiform, 4–6 distoseptate, brown, 37.5–40.5 × 6–7.5 µm. Colonies on 2% malt extract agar up to 42 mm diam. in 15 days. Mycelium mostly immersed, rarely superficial, hyphae septate, brown, colony dark brown to black.


**Geographical distribution**: Brazil (new record) and South Africa (Matsushima 1996).

*Verticicladus* was erected by Matsushima in 1993. The genus includes three species: *V. amazonensis* Matsush., *V. hainanensis* M.T.Guo & Z.F.Yu and *V. subculifer* (Guo et al. 2015). *Verticicladus* is characterized by short conidiophores, verticillate conidiogenous cells and rhexolytic secession of cylindrical to fusiform phragmoconidia (Matsushima 1993). *Verticicladus subiculifer* is close to *V. amazonensis* but the conidiogenous cells of *V. subculifer* are doliiform with more pronounced apical constriction than in *V. amazonensis*. *Verticicladus subculifer* was originally described from terrestrial decaying leaves of *Podocarpus* sp., while in the present study it was recorded from submerged decaying leaves; *V. amazonensis* and *V. hainanensis* were also reported from aquatic habitats (Matsushima 1993, Guo et al. 2015). In the present study, some cultures of *V. subculifer* isolated on 2% malt extract agar showed faster sporulation under submerged conditions than without submergence. *Verticicladus subculifer* is a new record to the Americas.
Discussion

Freshwater hyphomycetes are successful colonizers of submerged decaying leaves of a variety of deciduous dicotyledonous trees (Goh & Hyde 1996a). They are important players in fundamental ecosystem-level processes, including decomposition of submerged organic matter. Of the three ecological groups that are involved in decomposition in freshwaters (Ingoldian fungi, aero-aquatic and facultative aquatic fungi) only two were addressed in this study (aero-aquatic and facultative aquatic fungi).

The first aero-aquatic hyphomycete recorded in Brazil was *Peyronelina glomerulata* P.J.Fisher, J.Webster & D.F.Kane in the state of Paraiba from a soil sample (Batista et al. 1970). Subsequent studies focused on freshwaters greatly expanded our knowledge on the biodiversity and distribution of aero-aquatic hyphomycetes in Brazilian biomes of Caatinga, Amazon and the Atlantic Forest (Barbosa et al. 2013, Monteiro & Gusmão 2013, Moro et al. 2015a). Nowadays, 19 species of aero-aquatic hyphomycetes are recorded from Brazil, 15 from Amazon (Monteiro 2014), six from Caatinga (Batista et al. 1970, Barbosa & Gusmão 2011) and seven from Atlantic Forest (Magalhães et al. 2011, Moro et al. 2015a). The present study yielded four new records for Brazil and seven species were reported for the first time from Caatinga. *Beverwykella clathrata*, *Cancellidium applanatum*, *Candelabrum brocchiatum* and *Inesiosporium longispirale* (observed in the present study) are now recorded in all three Brazilian biomes. *Cancellidium applanatum* and *Candelabrum brocchiatum* are broadly distributed worldwide, with the first one displaying mainly tropical distribution (Shaw 1994, Chuaseeharonnachai et al. 2013, Farr & Rossman 2016).

Facultative aquatic hyphomycetes have been studied in Brazil to greater extent than aero-aquatic ones, with a total of 338 species reported, 213 from Amazon (Monteiro 2014), 174 from Caatinga (Barbosa & Gusmão 2011, Silva & Gusmão 2013, Silva et al. 2014, Fiuza et al. 2015a, Fiuza et al. 2015b) and 39 from the Atlantic Forest (Schoenlein-Crusius & Grandi 2003, Schoenlein-Crusius et al. 2014, Schoenlein-Crusius et al. 2015, Moro et al. 2015b). In the present study, all new records are rare species (i.e. reported just once or twice worldwide), which suggests that more interesting or rare species could be recorded with the help of focused inventory programs in relatively understudied biomes.

Biodiversity inventories are important to increase our knowledge on the distribution of fungal species worldwide, especially for relatively understudied groups such as freshwater hyphomycetes and in less explored tropical regions. Furthermore, biodiversity studies and genetic barcoding can provide valuable information for ecological studies and environmental conservation programs.

Acknowledgements

The authors are grateful to the "Programa de Pesquisa em Biodiversidade" – (PPBio Semi-arid/ MCTI/CNPq) for financial support. POF thanks to CAPES-PDSE by scholarship of Sciences without Borders (Proc. 99999.000984/2015-09). TC thanks the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES, proc. 12636134/2014) and the 'Programa de Pós-graduação em Botânica PPGBot/UEFS'. LFPG is grateful to CNPq for grant (Proc. 303062/2014-2).
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Manuscript submitted April 20, 2016; accepted July 12, 2016.