Aquapteridospora Jiangxiensis sp. Nov., A New Aquatic Hyphomycetes From Freshwater Habitats in China and *A. Bambusinum* Comb. Nov.

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Research Article

Keywords: hyphomycetes, freshwater fungi, Ascomycota, taxonomy

Posted Date: October 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-967372/v1

Abstract

During an investigation of freshwater fungi in Jiangxi province, China, a new hyphomycetous species, *Aquapteridospora jiangxiensis*, was collected and isolated. *A. jiangxiensis* is characterized by its unbranched and guttulate conidiophores with multi-septa and swollen at the base, polyblastic conidiogenous cells with sympodial proliferations and denticles, guttulate conidia with a sheath. The new species was illustrated and a muti-loci (ITS, LSU, SSU, TEF1 and RPB2) phylogenetic tree was constructed. *Pleurophragmium bambusinum* was is transferred to *Aquapteridospora* based on molecular and morphological data. A key to the species of *Aquapteridospora* is presented in this paper.

Introduction

Yang et al. (2015) established the genus *Aquapteridospora* with *A. lignicola* Jiao Yang, K.D. Hyde & Maharachch as the type species, which was collected from a freshwater stream in northern Thailand. *Aquapteridospora* is characterized by its polyblastic conidiogenous cells with several sympodial proliferations, bearing tiny, protuberant, circular scars and fusiform conidia, with pale to dark brown central cells and subhyaline end cells, sometimes with a conspicuous sheath (Yang et al. 2015). Currently, there are three species belonging to the genus *Aquapteridospora*, i.e. *A. aquatica* X.D. Yu, W. Dong & H. Zhang, *A. fusiformis* Z.L. Luo, D.F. Bao, H.Y. Su & K.D. Hyde and *A. lignicola* (Luo et al. 2019; Dong et al. 2021).

Aquapteridospora is a typical freshwater genus, and all the known species of this genus were reported from freshwater habitats (Yang et al. 2015; Luo et al. 2019; Dong et al. 2021). We have been investigated the freshwater fungi in China for 20 years (Hu et al. 2007; Huang et al. 2016; Hu et al. 2017; Song et al. 2018a; Song et al. 2018b; Song et al. 2020a; Song et al. 2020b). When investigating the fungal diversity in a small stream in Jiangxi Province, China, a hyphomycetous fungus was collected. The fungus resembles species of *Aquapteridospora*. After a comprehensive study on its morphological characters and molecular phylogeny, we confirmed that the fungus was a new species of *Aquapteridospora*. Therefore, we describe the fungus as a new species in this paper.

Materials & Methods

Sample collection, fungal isolation and morphological studies

Unidentified wood samples submerged in a freshwater stream were collected in Jiangxi Province, China, and incubated in moist chambers at room temperature (ca. 25°C). Samples were examined for fungal fruiting bodies using a dissecting microscope once a week. The spores from the fruiting bodies were diluted and dropped on plates with potato dextrose agar (PDA) medium. After incubating in room temperature for 12 hours, the germinated spores were picked up and incubated in a new PDA plate to obtain pure cultures. Observations and photographs were prepared from materials mounted in water and examined with a Nikon Ni compound microscope (Hu et al. 2012a). The specimens examined were deposited in the Herbarium of Fungi, Jiangxi Agricultural University, Nanchang, China (HFJAU), and the living cultures were deposited in the Culture Collection of Jiangxi Agricultural University (JAUCC) and Dr. Lei Cai's personsal collection (LC).

Dna Extraction, Gene Amplification, Sequencing, And Phylogentic Analyses

Genomic DNA was extracted from fungal mycelia using CTAB method following the instruction described by Wu et al (2001). We amplified five nuclear DNA sequences. ITS (internal transcribed spacer) sequences were amplified with primers ITS4 and ITS5 (White et al. 1990). Partial sequences of LSU (large subunit ribosomal RNA) genes were amplified with primers LROR and LR6 (Vilgalys and Hester 1990; Rehner and Samuels 1995). Partial sequences of SSU (small subunit ribosomal RNA) genes were amplified with primers NS1 & NS4 (White et al. 1990). Partial sequences of TEF1 (translation elongation factor EF-1 alpha) genes were amplified with primers EF1-983F and EF1-2218R (Rehner 2001). Partial sequences of RPB2 (RNA polymerase II second largest subunt) genes were amplified with primers fRPB2-5F and fRPB20-7CR (Liu et al. 1999). PCR protocols followed the

conditions set by Hu et al (2012b). The PCR products were purified and sequenced by the same primers used for PCR at Tsingke Biotechnology Co., Ltd.

In this study, we generated five novel sequences (MZ871502, MZ871501, MZ855767, MZ855768, MZ855769) and retrieved 41 reference sequences from GenBank (Table 1) that were aligned using MAFFT v.7 (Katoh and Standley 2013).

Table 1
Reference strains used in this study and their GenBank numbers.

Species	Strain number	Genbank number				
		ITS	LSU	SSU	TEF1	RPB2
Pleurophragmium acutum	CBS 129113	MH865210	MH876650			
Pleurophragmium bambusinum	MFLUCC 12-0850	KU940161	KU863149		KU940213	
Aquapteridospora aquatica	MFLUCC 17-2371	MW286493	MW287767			
Aquapteridospora jiangxiensis *	JAUCC 3008	MZ871502	MZ871501		MZ855767	MZ855768
Aquapteridospora lignicola	MFLU 15-1172		KU221018			
Aquapteridospora fusiformis	MFLU 18-1601	MK828652	MK849798		MN194056	
Junewangia aquatica	HFJAU0700	MG213738	MG213737	MG213736		
Junewangia globulosa	CBS 126093	MH864078	MH875535			
Junewangia thailandica	MFLU 15-2682		MW287762			
Junewangia lamma	HMAS 44438	KU999961	KU751882	KX033523		
Junewangia lamma	HSAUP H4695	KU999971	KU751883	KX033533		
Junewangia sphaerospora	HSAUP myr4733	KU999981	KX033572	KX033543		
Junewangia queenslandic	HSAUP myr7722	KU999984	KX033575	KX033546		
Diluviicola aquatica	MFLUCC 15-0986	MF374356	MF374365		MF370961	MF370953
Dictyosporella hydei	IFRDCC 3075		MG813161			
Dictyosporella guizhouensis	MFLUCC 18-1232	MW286487	MW287760		MW396646	
Dictyosporella guizhouensis	MFLU 18-1505	MK593606	MK593605	MK593611		
Dictyosporella aquatica	S-777	MK828692	MK849843			
Dictyosporella aquatica	CBS H-22127		KT241022	KT241023		
Dictyosporella thailandensis	MFLUCC 15-0985	MF374355	MF374364	MF374373	MF370958	MF370952
Dictyosporella chiangmaiensis	MFLUCC 17-2345	MW286491	MW287765			
Sporidesmiella hyalosperma	KUMCC 15-0431	MK828690	MK849841	MK828306		

Remarks: Species name given in bold denote ex-type strains. "*" denotes the new species.

Species	Strain number	Genbank number				
		ITS	LSU	SSU	TEF1	RPB2
Sporidesmiella hyalosperma	MFLUCC 18-1013	MW286499	MW287773		MW396654	MW504070
Sporidesmiella hyalosperma	MFLUCC 18-1312	MK828688	MK849839			
Sporidesmiella obovoidia	MFLUCC 17-2372	MW286492	MW287766			
Sporidesmiella novae-zelandiae	S-951	MK828695	MK849847			
Sporidesmiella novae-zelandiae	S-1256	MK828693	MK849845			
Sporidesmium dulongense	MFLUCC 17-0116	MH795812	MH795817			
Sporidesmium lageniforme	MFLU 18-1594	MK828640	MK849782		MN194044	MN124533
Sporidesmium thailandense	MFLUCC 15-0964	MF374361	MF374370		MF370957	MF370955
Sporidesmium pyriformatum	MFLUCC 15-0627	KX710148	KX710143		MF135663	
Sporidesmium pyriformatum	MFLUCC 15-0620	KX710146	KX710141		MF135662	MF135649
Sporidesmium chiangmaiense	MFLUCC 18-0999	MW286497	MW287771			
Sporidesmium appendiculatum	MFLU 18-0981	MW286500	MW287774			
Sporidesmium melaleucae	CPC 32936	MH327818	MH327854			
Sporidesmium melaleucae	CPC 32707	MH327817	MH327853			
Longicollum biappendiculatum	PE0017-1a	KU975062	KU975071			
Pseudoproboscispora caudae-suis	CBS 146.51	MH856788	MH868307			
Pseudoproboscispora thailandensis	MFLUCC 15-0989	MF374360	MF374369	MF374377	MF370959	
Jennwenomyces navicularis	NCYU-JW1	MT224911	MT224910			
Cateractispora recepticuli	HKUCC 3710		AF132327			
Dothidea insculpta	CBS 189.58	AF027764	DQ247802	DQ247810		

Remarks: Species name given in bold denote ex-type strains. “*” denotes the new species.

The ML analyses were functioned with RAxML v7.2.6 (Stamatakis and Alachiotis 2010) using a GTRGAMMA substitution model with 1000 bootstrap replicates and evaluated by bootstrap support (MLBS). Trees were sampled 100 generations and the first 25% deleted as burn-in trees. Four simultaneous Markov chains were run 2,000,000 generations, resulting a total of 40002 trees (sampling 30002 of them). Posterior probabilities values of the BI analyses (BPP) over 0.95 were showed on the tree. The novel taxonomic descriptions and nomenclature were deposited in MycoBank (http://www.mycobank.org/).

Result

Phylogenetic analysis

The phylogenetic tree based on the five-locus analysis (Fig. 1) shows the relationship between the new species and other related taxa. The dataset including alignment gaps comprised 4415 characters: 646 for ITS, 843 for LSU, 999 for SSU, 873 for TEF1 and 1054 for RPB2. Branchs support of MLBS ≥70% and PP value≥0.90 are indicated above branches. The tree is rooted to *Dothidea insculpta* CBS 189.58. The new species *Aquapteridospora jiangxiensis* JAUCC3008 together with other *Aquapteridospora* species formed a well-supported clade (BPP = 1, MLBS = 100%).

Taxonomy

Aquapteridospora jiangxiensis J.E. Huang, H.Y. Song & D.M. Hu, sp. nov.

Mycobank No: 841242

Etymology: In reference to the host location, Jiangxi province, where the holotype was collected.

Holotype: HFJAU 3176

Saprobic on decaying submerged wood. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous. Colonies effuse consisting of conidiophores scattered over the substrate surface, brown to dark brown. Mycelium septate, hyaline, partly immersed and partly superficial on the natural substrate. Conidiophores 78–305 × 4–7 µm (mean = 144.3 × 4.7 µm, n = 20) macronematous, mononematous, cylindrical, erect, usually straight or slightly flexuous and sometimes flexuous, smooth, 3–15-septate, unbranched, dark brown in the middle and below, pale brown above, thick-walled, with abundant small guttulae, occasionally slight swollen at the base. Conidiogenous cells polyblastic, 20–68 × 4–6 µm (mean = 41.2 × 4.8 µm, n = 20), integrated, terminal, becoming intercalary, pale brown to brown, smooth, subclavate to subcylindrical, with several sympodial proliferations, bearing some conspicuous, rounded, brown to mid-brown denticles. Conidia 20–25 × 6–7.5 µm (mean = 22.5 × 6.5 µm, n = 25) µm, acrogenous or lateral, fusiform to subclavate, rounded at the apex, base truncate, 0.9–1.5 µm wide, straight to slightly curved, 3-septate, slightly constricted at septa and septa dark brown to black, with mid to dark brown central cells and pale to mid brown end cells, with abundant small guttulae, smooth-walled, sometimes with a sheath.

Culture characteristics: Conidia germinating on PDA within 24 h. Colonies growing on PDA, circular, reaching 40–60 mm diam. after 2–3 weeks at 28 °C; from above, flat, center grey-white with some dark brown spots and edge dark brown, mycelium superficial to immersed in media; from below, center grey-brown, near the edge black, with smooth margin.

Material examined: CHINA, Jiangxi Province, Shangrao, Dexing, Xiangtun Street, on submerged wood in a stream, 12 August 2018, J.E. Huang (Holotype, **HFJAU 3176**); ex-type living culture (JAUCC 3008).

Notes: The phylogenetic analysis based on combined data of ITS, LSU, SSU, TEF1 and RPB2 sequences showed that *Aquapteridospora jiangxiensis* clusters within *Aquapteridospora* clade with high bootstrap support (Fig. 1). Morphologically, *A. jiangxiensis* is characterized by its unbranched and guttulate conidiophores with multi-septa and swollen at the base, polyblastic conidiogenous cells with sympodial proliferations and denticles, guttulate conidia with a sheath, which fits well with the concepts of the genus *Aquapteridospora* (Yang et al. 2015). *Aquapteridospora jiangxiensis* shows obvious distrance to the other species of *Aquapteridospora* in the phylogenetic tree (Fig. 1). Morphologically, *A. jiangxiensis* differs from the other four species of *Aquapteridospora* by its fusiform to subclavate conidia with rounded apex and truncate base.

Aquapteridospora bambusinum **(D.Q. Dai & K.D. Hyde) J.E. Huang, H.Y. Song & D.M. Hu, comb. nov.**

Mycobank No: 841243

≡ *Pleurophragmium bambusinum* D.Q. Dai & K.D. Hyde, in Dai, Phookamsak, Wijayawardene, Li, Bhat, Xu, Taylor, Hyde & Chukeatirote, Fungal Diversity 82: 90 (2017).

Notes: *Pleurophragmium bambusicola* is characterized by polyblastic, sympodial, denticulate conidiogenous cells and 3-septate, brown, thick-walled conida (Dai et al. 2017), which fit the generic concepts of *Aquapteridospora* (Yang et al. 2015). On the phylogenetic tree (Fig 1), the type strain (MFLUCC 12-0805) of *Pleurophragmium bambusicola* clusters within *Aquapteridospora*

clade with high bootstrap support (BPP = 1, MLBS = 100%). Therefore, we transfer *Pleurophragmium bambusicola* to the genus *Aquapteridospora*.

Discussion

Aquapteridospora is described herein as a monotypic by Yang et al (2015). The type species *A. lignicola* resembles *Minimelanolocus manifestus* Hern.-Restr., R.F. Castañeda, Gené & Guarro, however *M. manifestus* has cymbiform to subfusiform conidia, which are fimbrillate at the base and lacks a sheath (Hernández-Restrepo et al. 2012; Yang et al. 2015). *Aquapteridospora lignicola* also resembles *Pleurophragmium indicum* M.A. D'Souza & Bhat., however *P. indicum* has ellipsoidal to obovoid conidia, with dark brown central cells and pale brown end cells and lacks a sheath (D'Souza and Bhat 2012; Yang et al. 2015). Molecular phylogenetic analysis is very important in inferring the taxonomic placement of hyphomycetous fungi. Yang et al (2015) conducted a molecular phylogeny of *Aquapteridospora* based on LSU sequences, and confirmed its phylogentic placement confirmed in Diaporthomycetidae genera incertae sedis. Recently, Dong et al (2021) conducted a molecular phylogeny study based on combined LSU, ITS, TEF and RPB2 sequences data, and established a new family Aquapteridosporaceae K.D. Hyde & Hongsanan (Distoseptisporales Z.L. Luo, K.D. Hyde & H.Y. Su) to accommodate a single genus *Aquapteridospora* (Hyde et al. 2021).

Five speces are accepted in *Aquapteridospora* in these paper, including the new species and new combination proposed in this tudy. A key to the five species of *Aquapteridospora* is presented.

Key to Aquapteridospora **species**

1. Conidia without sheath……2

1. Conidia with sheath……3
2. Conidia ellipsoidal, equally coloured, thick-walled, 13–21 × 5–7 µm…*A. bambusinum*
2. Conidia fusiform, obtuse at both ends, brown to dark brown in central cells and subhyaline at end cells, 14–18 × 5–7 µm……*A. fusiformis*
3. Conidia obtuse at both ends, 15–24 × 6–8 µm…….. *A. lignicola*
3. Conidia not obtuse at both ends……4
4. Conidia fusiform, slightly tapering towards the apex, 19–27.5 × 5–7.5 .. *A. aquatica*
4. Conidia fusiform to subclavate, rounded at the apex, base truncate, 20–25 × 6–7.5 µm……*A. jiangxiensis*

Declarations

The authors declare that there are not conflicts of interest.

Acknowledgments

Funds for research were provided by the National Natural Science Foundation of China (NSFC 32070023 and NSFC 32060014), the Key Projects of Youth Fund of Jiangxi Science and Technology Department of China (20192ACBL21017), Natural Science Foundation of Education Department of Jiangxi Province of China (GJJ190168).

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Figures

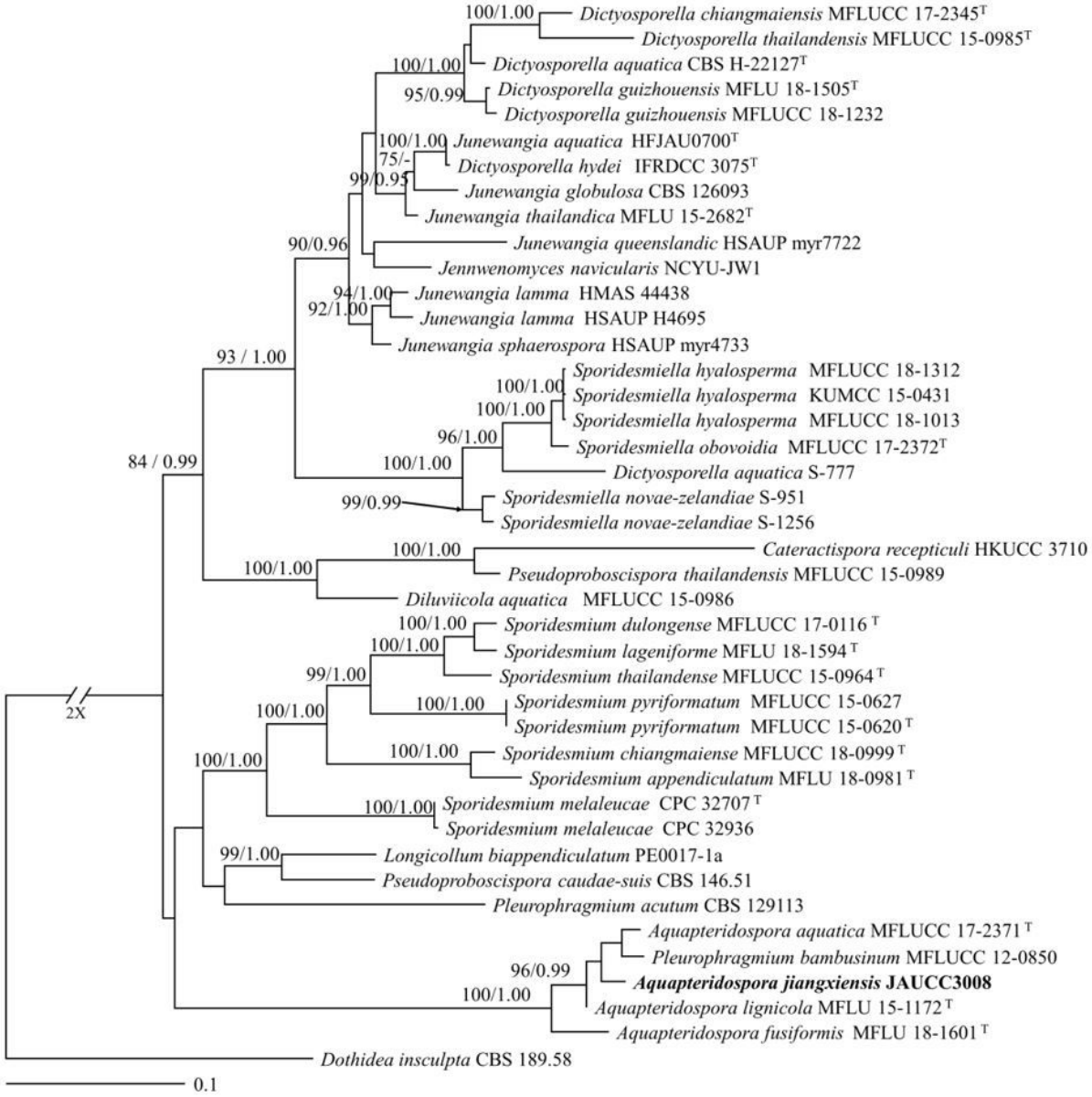

Figure 1

RAxML tree generated from combined LSU, ITS, SSU, TEF1 and RPB2 sequence data. Bootstrap support values for maximum likelihood equal to or greater than 50% are placed near the branches The tree is rooted to Dothidea insculpta CBS 189.58. “T” indicate ex-type strains and new species introduced in this study are indicated in bold.

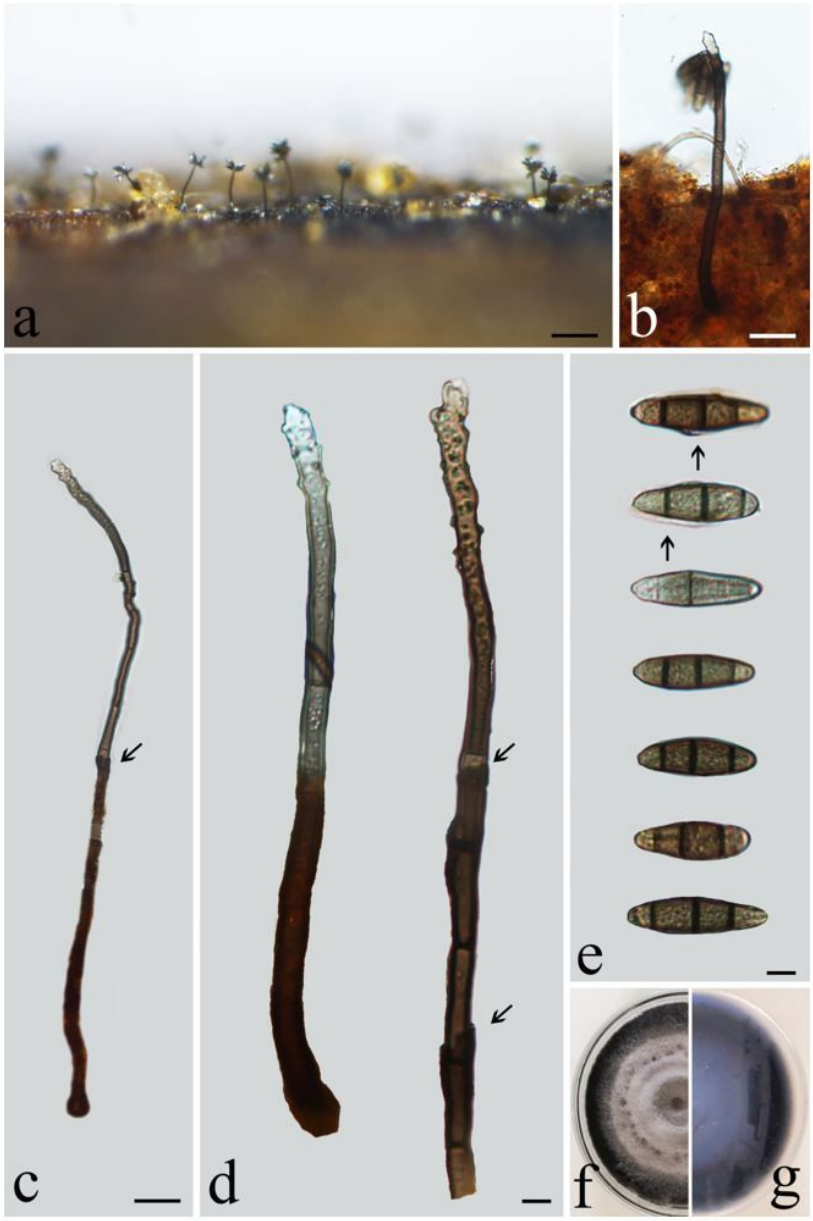

Figure 2

Aquapteridospora jiangxiensis (HFJAU 3176, holotype). a Colonies on submerged wood. b Conidiophore and conidia. c, d Conidiophores with conidiogenous cells. Note the proliferation (arrowed in c, d). e Conidia. f, g Colony on PDA from above and below. Scale bars: a = 100 µm, b, c = 20 µm, d, e = 5 µm.