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Aquapteridospora lignicola gen. et sp. nov., a new hyphomycetous taxon (Sordariomycetes) from wood submerged in a freshwater stream

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Abstract – *Aquapteridospora lignicola* gen. et sp. nov. was found on wood submerged in a freshwater stream in Chiang Rai Province, Thailand. Phylogenetic analysis of LSU sequence data placed the isolate in *Diaporthomycetidae*, genera *incertae sedis*. *Aquapteridospora* is a hyphomycete with 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. *Aquapteridospora* is compared to *Pleurophragmium* and *Minimelanolocus* which share some similar characters. A new genus and species is introduced to accommodate this new lineage with notes on its taxonomy and phylogeny.

Asexual fungi / *Diaporthomycetidae* / LSU / *Minimelanolocus* / Phylogeny / *Pleurophragmium*

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INTRODUCTION

Freshwater hyphomycetes are conidial asexual morphs which for the whole or part of their life cycle rely on freshwater (Goh & Hyde, 1996; Seifert *et al*., 2011). They are taxonomically diverse, well-documented and occur on almost any type of submerged plant litter (Goh & Hyde, 1996; Cai *et al*., 2008; Belliveau & Bärlocher, 2005; Jones *et al*., 2014). There are about 2,900 named hyphomycete genera (Seifert *et al*., 2011), however, many genera have not been assigned to modern classification schemes based on phylogenetic analyses; many asexual genera are polyphyletic or paraphyletic (Shenoy *et al*., 2007, 2010; Maharachchikumbura *et al*., 2015).

We are studying the taxonomy and diversity of freshwater fungi along a north – south latitudinal gradient in the Asian region (Hyde *et al*., 2016) and in this paper report on an interesting fungus collected on submerged wood in Chiang Rai, Thailand. The taxon is similar to species of *Pleurophragmium* (D'Souza & Bhat, 2012) and *Minimelanolocus* (Liu *et al*., 2015), but the phylogenetic analysis of LSU sequence data placed the isolate close to *Ellisembia* and *Sporidesmium* species, and thus it represents a new taxon. We therefore introduce *Aquapteridospora lignicola* gen. et sp. nov. with an illustrated account and supported by molecular data.

MATERIALS AND METHODS

Collection and examination of specimens. Specimens of submerged, decaying wood were collected from a stream flowing in Tham Luang Nang Non Cave, Chiang Rai Province, Thailand, in November 2014. Materials were brought to the laboratory in plastic bags and incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. The samples were processed and examined following the method described in Taylor & Hyde (2003). Morphological observations were made using a Motic SMZ 168 Series dissecting microscope for fungal fruiting bodies. The fungus was examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS6 software.

Single spore isolations were made onto potato dextrose agar (PDA) and later transferred onto malt extract agar (MEA) following a modified method of Chomnunti *et al*. (2014). Specimens (dry wood material with fungal material) are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Axenic cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and the Guizhou Culture Collection (GZCC). Facesoffungi numbers and Index Fungorum numbers are provided as outlined in Jayasiri *et al*. (2015) and Index Fungorum (2015).

DNA extraction, PCR amplification and sequencing. Total genomic DNA was extracted from fresh fungal mycelia (500 mg) scraped from the margin of a colony on a MEA plate incubated at 25 °C for 14 days (Guo *et al*., 2000). The primer pairs LROR and LR5 as defined by Vilgalys & Hester (1990) were used to amplify a segment of the large subunit rDNA (LSU). The amplifications were

performed in 25 μL of PCR mixtures containing 9.5 μL ddH_2O, 12.5 μL 2 × PCR Master Mix (TIANGEN Co., China), 1 μL of DNA template and 1 μL of each primer (10 μM). The amplification condition for LSU consisted of initial denaturation at 94 °C for 4 min; followed by 35 cycles of 45 s at 94 °C, 45 s at 56 °C and 1 min at 72 °C, and a final extension period of 10 min at 72 °C. The PCR product was observed on 1% agarose electrophoresis gel stained with ethidium bromide. Purification and sequencing of PCR product was carried out using the above mentioned PCR primer at Invitrogen Biotechnology Co., China.

Phylogenetic analysis. Sequences were optimized manually to allow maximum alignment and maximum sequence similarity (Table 1). Phylogenetic analysis of the sequence data consisted of maximum likelihood (ML) using raxmlGUI v. 1.3 (Silvestro & Michalak, 2011). For maximum likelihood analyses, the tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model. The resulting trees were printed with FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/), and the layout was created in Adobe Illustrator CS v. 6. Sequences generated in this study are deposited in GenBank.

Table 1. GenBank accession numbers of isolates included in this study. Newly sequenced isolate in bold.

Species	*Culture accession no.*	*GenBank accession*
		LSU
Annulatascus velatisporus	HKUCC 3701	AF132320
Annulusmagnus triseptatus	CBS 128831	GQ996540
Aquapteridospora lignicola	**MFLUCC 15-0377= GZCC 15-0051**	**KU221018**
Asteridiella obesa	VIC 31239	JX096809
Bambusicularia brunnea	INA-B-92-45	KM484948
Barbatosphaeria arboricola	CBS 127690	HQ878592
Barbatosphaeria varioseptata	CBS 137797	KM492869
Botryotinia fuckeliana	AFTOL-ID 59	AY544651
Buergenerula spartinae	ATCC 22848	DQ341492
Bullimyces aurisporus	AF316-1b	JF775590
Bullimyces costaricensis	AF317-1b	JF775592
Camaropella pugillus	SMH3846	EU481406
Chaetosphaeria innumera	SMH2748	AY017375
Colletotrichum asianum	LC0037	JN940408
Cryptadelphia groenendalensis	SMH3767	EU528001
Cryptadelphia groenendalensis	SH12	EU528007
Cumulospora marina	MF46	GU252135
Dactylaria parvispora	P024	EU107296
Dothidea sambuci	DAOM 231303	AY544681
Ellisembia adscendens	NN44654	DQ408561
Ellisembia leonensis	NN44360	DQ408566
Ellisembia minigelatinosa	NN47497	DQ408567
Eutypa lata	CBS 208.87 = AFTOL-ID 929	DQ836903
Fluminicola coronata	HKUCC 3717	AF132332
Fragosphaeria purpurea	CBS 133.34	AB189154

Table 1. GenBank accession numbers of isolates included in this study. Newly sequenced isolate in bold (*continued*)

Species	*Culture accession no.*	*GenBank accession*
		LSU
Graphium fimbriasporum	CMW 5605	KM495388
Hypocrea americana	AFTOL-ID 52	AY544649
Irenopsis vincensii	VIC 31751	JX133163
Jattaea mookgoponga	STE-U 6184	EU367458
Jobellisia fraterna	SMH 2863	AY346285
Jobellisia luteola	SMH 2753	AY346286
Kohlmeyeriella tubulata	PP1105	AF491265
Lecythothecium duriligni	CBS 101317	AF261071
Lepteutypa cupressi	IMI 052255	AF382379
Lopadostoma turgidum	LT2	KC774618
Magnaporthe salvinii	M21	JF414887
Matsusporium tropicale	NBRC32 × 499	GU252141
Meliola centellae	VIC 31244	JQ734545
Ophioceras leptosporum	CBS 894.70	JX134690
Ophiodiaporthe cyatheae	YMJ 1364	JX570891
Ophiostoma piliferum	AFTOL-ID 910	DQ470955
Papulosa amerospora	AFTOL-ID 748	DQ470950
Pleurophragmium subfusiforme	P011	EU107293
Pleurophragmium triseptatum	P018	EU107292
Pleurostoma ootheca	CMU 23858 = CBS 115329	AY761079
Pseudoplagiostoma variabile	CBS 113067	GU973611
Pyricularia borealis	CBS 461.65	DQ341511
Seiridium phylicae	CPC 19962	NG 042759
Sordaria fimicola	CBS 508.50	AY681160
Sporidesmium parvum	NN45992	DQ408558
Stilbospora macrosperma	CBS 121883	JX517299
Sydowiella stellatifolii	CBS 119342	EU552156
Thyridium vestitum	AFTOL-ID 172	AY544671
Tirisporella beccariana	BCC36737	JQ655450
Valsella salicis	AR 3514	EU255210
Woswasia atropurpurea	CBS 133167	JX233658
Xylomelasma sordida	CBS 131683	KM492871

RESULTS

Phylogenetic analyses

The alignment comprised 57 strains belonging to *Sordariomycetes*, with *Botryotinia fuckeliana* (AFTOL-ID 59) and *Dothidea sambuci* (DAOM 231303) as the outgroup taxa. The manually adjusted dataset comprised 889 characters including gaps. Most of the core orders in *Diaporthomycetidae* were included in our phylogenetic analysis and the best scoring RAxML tree is shown in Figure 1. The *Aquapteridospora* clusters in *Diaporthomycetidae*, as a sister taxon to *Ellisembia*

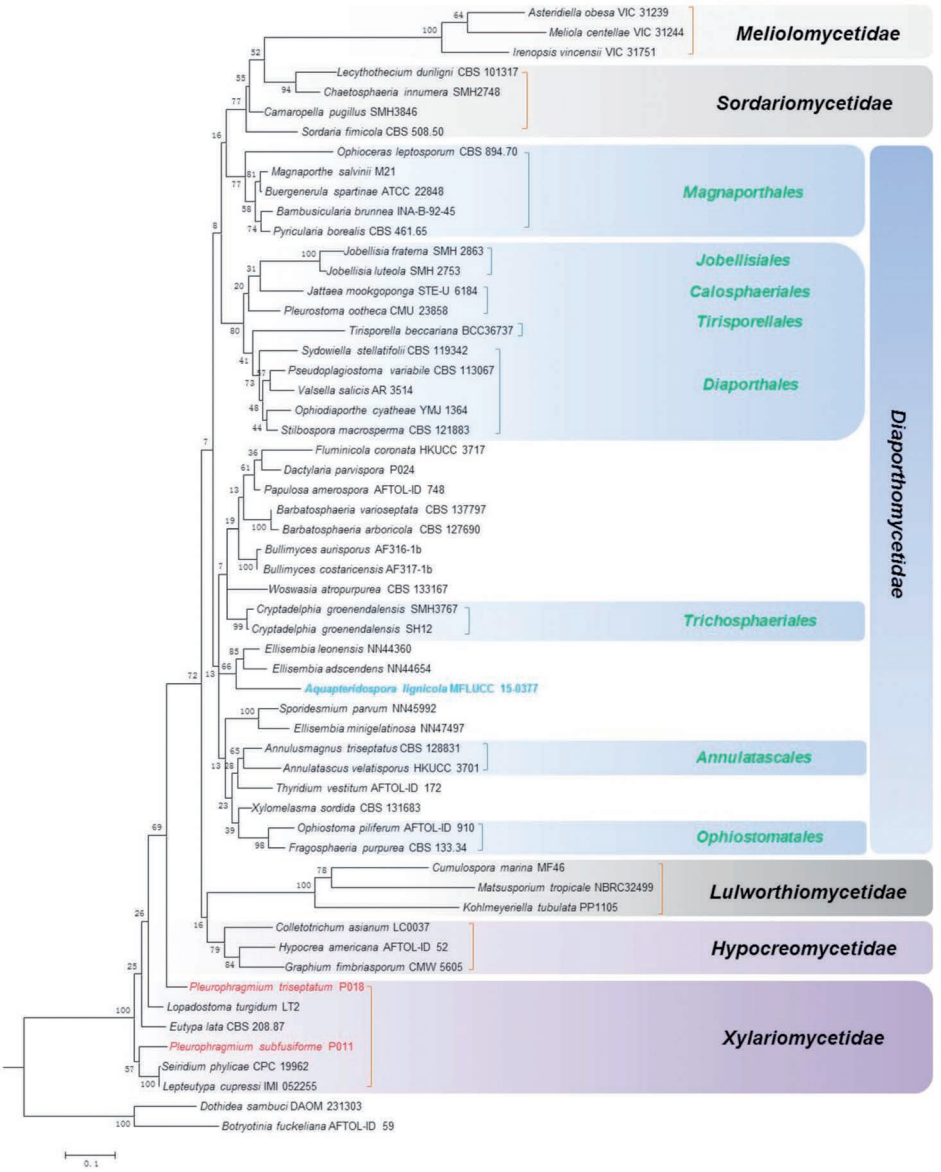

Fig. 1. Maximum likelihood (ML) majority rule consensus tree for the analyzed Sordariomycetes isolates based on a dataset of LSU sequence data. RAxML bootstrap support values (ML) are given at the nodes (ML). The scale bar represents the expected number of changes per site. The tree is rooted with *Botryotinia fuckeliana* and *Dothidea sambuci.* The original isolate numbers are noted after the species names. The new strain is in blue bold and morphologically similar strains are in red. Orders and subclasses are indicated as coloured blocks.

adscendens and *E. leonensis*. However, *Ellisembia*, *Sporidesmium* and phenotypically allied genera are polyphyletic. Furthermore, *Ellisembia* and *Sporidesmium* species have affinities to both *Dothideomycetes* and *Sordariomycetes*. The morphology of *Aquapteridospora* resembles *Pleurophragmium*, however the latter genus clusters in *Xylariomycetidae*. Therefore, considering the distinct morphology and phylogeny, the new genus *Aquapteridospora* is proposed and its phylogentic placement confirmed in *Diaporthomycetidae* genera *incertae sedis*.

Taxonomy

Aquapteridospora J. Yang, K.D. Hyde & Maharachch., **gen. nov.**

Index Fungorum Number: IF551731

Facesoffungi number: FoF 01640

Etymology: from *pteridospora* meaning winged spore and *aqua* referring to its presence in water.

Saprobic on decaying plant substrates submerged in freshwater. Asexual morph: *Colonies* on the natural substrate effuse, hairy, dark brown. *Mycelium* superficial or partly immersed, composed of branched, septate, pale brown to brown, smooth, thin-walled hyphae. *Conidiophores* macronematous, mononematous, solitary, erect, straight or slightly flexuous, unbranched, cylindrical, septate, smooth, thick-walled, dark brown at the base, paler towards apex. *Conidiogenous cells* polyblastic, terminal becoming intercalary, pale brown, integrated, with several sympodial proliferations, bearing tiny, protuberant, circular scars. *Conidia* solitary, fusiform, smooth, obtuse at both ends, 3-euseptate, slightly constricted at septa, with pale to dark brown central cells and subhyaline end cells, guttulate, acropleurogenous, sometimes with sheaths. Conidial secession schizolytic. Sexual morph: Undetermined.

Type species: *Aquapteridospora lignicola* J. Yang, K.D. Hyde & Maharachch.

Aquapteridospora lignicola J. Yang, K.D. Hyde & Maharachch.*,* **sp. nov. Fig. 2**

Index Fungorum Number: IF551732

Facesoffungi number: FoF 01641

Etymology: From *lignicola* meaning "dwelling on wood"

HOLOTYPUS: MFLU 15-1172

Saprobic on decaying plant substrates. Asexual morph: *Colonies* on the natural substrate effuse, hairy, dark brown. *Mycelium* superficial or partly immersed, composed of branched, septate, pale brown to brown, smooth, thin-walled hyphae. *Conidiophores* 70-200 × 4-7 µm ($\bar{x}$ = 146 × 6 µm, n = 20), macronematous, mononematous, solitary, erect, straight or slightly flexuous, unbranched, cylindrical, septate, smooth, thick-walled, dark brown at the base, paler towards apex. *Conidiogenous cells* polyblastic, terminal, becoming intercalary, pale brown, integrated, with several sympodial proliferations, bearing tiny, protuberant, circular scars. *Conidia* solitary, fusiform, smooth, obtuse at both ends, 3-euseptate, slightly constricted at septa, with pale to dark brown central cells and subhyaline end cells, guttulate, acropleurogenous, sometimes with a conspicuous sheath, 15-24 × 6-8 µm ($\bar{x}$ = 21 × 7 µm, n = 45). Conidial secession schizolytic. Sexual morph: Undetermined.

Culture characters: Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colony on MEA slow-growing, reaching 5-10 mm diam. at 14 days, with dense white mycelium on surface, in the center becoming sparse and dark brown at the edge; in reverse with a white middle and dark brown,

Fig. 2. *Aquapteridospora lignicola* (MFLU 15-1172, holotype). **a.** Substrate. **b-c.** Colony on wood. **d-e, g.** Conidia on conidiophores. **f, h.** Conidiophores. **i-k.** Apices of the conidiophores showing the conidiogenous cells and developing conidia. **l-s.** Conidia, some with mucilaginous sheath. **t.** Germinated conidium. **u-v.** Cultures on MEA, u from above, v from below. Scale bars: b = 200 µm, c = 50 µm, d, g-h, t = 30 µm, e-f = 40 µm, i = 15 µm, j-s = 10 µm.

smooth margin. After 2 months of incubation, the colony on MEA contained only superficial, branched, septate, smooth, mycelia and produced the asexual morph.

Habitat and distribution: On submerged wood in freshwater, Thailand.

Material examined: THAILAND, Chiang Rai Province, stream flowing in Tham Luang Nang Non Cave, on submerged wood, 25 November 2014, Jing Yang (MFLU 15-1172, **holotype**), extype living culture, MFLUCC 15-0377, GZCC 15-0051.

Notes: *Aquapteridospora* is described herein as a monotypic genus that was collected on a submerged decaying twig in a stream in Thailand. *Aquapteridospora lignicola* most closely resembles *Minimelanolocus manifestus* (Hernández-Restrepo *et al*., 2012) and *Pleurophragmium indicum* (D'Souza & Bhat, 2012). *Minimelanolocus manifestus* has cymbiform to subfusiform conidia, which are fimbrillate at the base and lacks a sheath (Hernández-Restrepo *et al*., 2012), while *Pleurophragmium indicum* has ellipsoidal to obovoid conidia, with dark brown central cells and pale brown end cells and lacks a sheath (D'Souza & Bhat, 2012). Conidiogenous cells of *Aquapteridospora lignicola* are similar to *Minimelanolocus manifestus*, but differ from the minutely, denticulate conidiogenous cells of *Pleurophragmium indicum*. Molecular analysis confirms that the new genus cannot be placed in *Minimelanolocus* or *Pleurophragmium*, which belong in Dothideomycetes and Pezizomycotina, respectively. *Aquapteridospora lignicola* clusters in the *Diaporthomycetidae*, and is close, but distinct from *Ellisembia* and *Sporidesmium*.

DISCUSSION

Aquapteridospora lignicola is morphologically similar to *Minimelanolocus, Pseudospiropes* and *Pleurophragmium*. The genus *Minimelanolocus* was established by Castañeda-Ruiz *et al*. (2001) with *M. navicularis* (R.F. Castañeda) R.F. Castañeda as the type species. Based on the morphology of the conidiogenous loci and conidial septation, *Minimelanolocus* was segregated from *Pseudospiropes* (Ellis, 1971), *Helminthosporium* and *Belemnospora* (Kirk, 1981). The characters *Minimelanolocus* shares with *Aquapteridospora* are polyblastic, terminal, integrated conidiogenous cells with holoblastic sympodial proliferations and the conidiogenous loci are inconspicuous or slightly prominent, narrow, opaque, with refractive to somewhat obscure dehiscence scars (Castañeda-Ruiz *et al*., 2001), but differs in having few enteroblastic percurrent proliferations. In *Pseudospiropes*, the conidia are distoseptate and conidiogenous loci are broad, protuberant, thickened and strongly melanized, apparently with several layers, forming a discoid black scar (Castañeda-Ruiz *et al*., 2001). Costantin (1888) established *Pleurophragmium* for a single species, *P. bicolor* Costantin. In this genus the conidiogenous cells are denticulate with pointed denticles (Hughes, 1958; Ellis, 1971, 1976). Moreover, the conidial characters of *Aquapteridospora lignicola* show variation in shape, size, septation, sheath and melanization of the central, basal and apical cells.

Phylogenetic analysis of LSU sequence data provides evidence that *Aquapteridospora lignicola* is distinct from *Minimelanolocus*, *Pseudospiropes* and *Pleurophragmium*. *Aquapteridospora lignicola* is close to some species of the genus *Ellisembia* and *Sporidesmium*, but they are quite different morphologically. The conidiogenus cells of the new fungus is polyblastic, while they are monoblastic in *Ellisembia leonensis*, *E. adscendens*, *E. minigelatinosa* and *Sporidesmium parvum*.

Moreover, *E. minigelatinosa* has up to five doliiform percurrent proliferations (Wu & Zhuang, 2005). *Ellisembia leonensis*, *E. adscendens* and *E. minigelatinosa* all produce distoseptate conidia, but *Aquapteridospora lignicola* has euseptate conidia (Wu & Zhuang, 2005). Furthermore, *Aquapteridospora lignicola* differs from these taxa in the shape and size of conidiophores and conidia. There is no sequence data for the type species of *Ellisembia*. The phylogenetic placement of *Ellisembia* and *Sporidesmium* are shown to be polyphyletic, which is similar to the results of Shenoy *et al*. (2006). Based on analysis of sequence data, Su *et al*. (pers. comm.) showed that *Ellisembia* and *Sporidesmium* are not monophyletic and species are distributed in three major subclasses in *Sordariomycetes*. However, most *Ellisembia* and *Sporidesmium* species cluster in *Sordariomycetidae* and *Xylariomycetidae*. The type sequence or morphology of most of these taxa are not available for comparison, except for *Sporidesmium knawiae* which clusters in *Xylariomycetidae*. The morphology of *S. knawiae* is distinct from the type species of *Sporidesmium.*

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