

Diversity of aero-aquatic hyphomycetes from six streams in Doi Inthanon and Khao Yai tropical forests, Thailand

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Abstract – Randomly collected naturally decaying submerged/semi-submerged wood from six freshwater streams in two forests Doi Inthanon (DI) and Khao Yai National Parks (KY), Thailand was examined for aero-aquatic hyphomycetes following five field collections in July 2009 to November 2010. A total of 1,171 fungal occurrences belonging to 32 species in 13 genera were identified from DI (31%), and KY (28%), with 41 % common to both sites. Of these, eighteen were new records (56.25% of all species) for Thailand. Percentage occurrence of fungi at DI streams 1-3 were 30.77%, 35.99%, and 33.33%, respectively; while at streams 4-6 at KY, percentage occurrence was 31.11, 35.56, and 33.33%, respectively. The most common species at both forests (with >10% frequency of occurrence, FO) were *Candelabrum brocciatum* (34.5%), followed by *Pseudaegerita corticalis* (15.4%), and *Cancellidium applanatum* (12.5%). These fungi were found at five streams in the two forests while *Helicomyces roseus* (6.3%), *Candelabrum microsporium* (4.3%) and *Helicosporium panachaenum* (4.3%) are regarded as common species. Seven species were regarded as rare, occurring only once per site with FO = 0.1% (*Candelabrum* sp., *Helicoma atroseptatum*, *H. perelegans*, *H. resiniae*, *Helicoma* sp., *Helicosporium aureum* and *H. gracile*). A comparison of the fungal community at the two forests and six streams shows little difference in species composition.

Aquatic fungi / species abundance / species diversity / submerged wood / frequency of occurrence

INTRODUCTION

Freshwater hyphomycetes are characterized as those that dwell in freshwater ecosystems for all or a part of their life cycle. Nonetheless, this definition is vague, as it includes all fungi that may be present in a freshwater environment regardless of their origins. They can be categorized into four groups based on their occurrence in various aquatic habitats: terrestrial-aquatic hyphomycetes, amphibious hyphomycetes, aquatic hyphomycetes, and aero-aquatic hyphomycetes (Goh & Hyde, 1996a; Chan *et al.*, 2000a; Descals & Moralejo, 2001).

Among these categories, the aero-aquatic hyphomycetes, which is the target group of this study, produce distinctive asexual spores when exposed to air and inhabit submerged leaves and wood in static to slow-flowing freshwater environments (Beverwijk, 1951; Abdullah & Webster, 1980). The multicellular dispersal units of aero-aquatic hyphomycetes are morphologically diverse, but all have one feature in common; they trap air between their cells and thus float on the surface of water (Goh & Hyde, 1996a). These fungi can be isolated by collecting old submerged leaves from a stream or pond bottom or a swampy area under aerobic conditions (Bärlocher, 1992).

Since 1983-2012, aero-aquatic hyphomycetes have been increasingly studied, as evidenced by the increased number of publications on these fungi and several new species and genera from all over the world have been identified (Hyde *et al.*, 2011). Most studies have taken place in temperate regions resulting in the description of many novel species (Abdullah *et al.*, 1996, 1997, 1998a, 1998b, 2000, 2005; Abdullah & Webster, 1983; Hennebert, 1998; Marvanová & Bärlocher, 1998; Voglmayr, 1997a, 1997b, 1997c, 1998, 2004; Voglmayr & Delgado-Rodríguez, 2001; Voglmayr & Fisher, 1997; Voglmayr & Krisai-Greilhuber, 1996, 1997; Voglmayr *et al.*, 1999; Yamaguchi *et al.*, 2009, 2012).

A number of studies on aero-aquatic hyphomycetes on leaf litter from tropical and semi-tropical regions have been undertaken (Castañeda & Kendrick, 1991; Chang, 2001; Goh, 1997; Goh & Hyde, 1996b; Nawawi & Kuthubutheen, 1987, 1988, 1990; Voglmayr & Delgado-Rodríguez, 2001, 2003; Voglmayr & Yule, 2006); while they have also been reported on submerged wood as part of general mycological surveys (Cai *et al.*, 2003; Ho *et al.*, 2001, 2002; Hyde & Goh, 1998; Jones & Pang, 2012; Kurniawati *et al.*, 2010; Tsui & Hyde, 2004; Tsui *et al.*, 2000; Vijaykrishna & Hyde, 2006; Yamaguchi *et al.*, 2012). In Thailand, such studies have identified a number of aero-aquatic hyphomycetes on wood (Pinnoi *et al.*, 2006; Pinruan *et al.*, 2007; Sivichai & Hywel-Jone, 1999; Sivichai *et al.*, 2000, 2002; Zhang *et al.*, 2011), while Pinnoi *et al.* (2006) and Pinruan *et al.* (2007) recorded *Cancellidium applanatum*, *Helicoma* sp., *Helicosporium* sp., and *Helicosporium gigasporum* on palm materials, while Sivichai & Hywel-Jone (1999), and Sivichai *et al.* (2000, 2002) listed *Biflagellospora japonica*, *B. siamensis*, *B. papillata*, *B. gracilis*, *Cancellidium applanatum*, *Helicomycetes roseus*, *Helicosporium* *grisum*-like, *H. gigasporum*, *Helicosporium* sp., *H. vegetum*-like, *Candelabrum brocchiatum*, *Helicoma* sp., *Helicoön* sp., and *Spirosphaera* sp. on submerged test blocks. A dedicated study of aero-aquatic hyphomycetes on wood has rarely been undertaken.

The objectives of this study were: (i) to survey and increase the fungal records for aero-aquatic hyphomycetes isolated from natural wood at two selected rainforests in Thailand; (ii) to compare the species (common, rare, overlapping and exclusive species) collected at the two forests (Doi Inthanon and Khao Yai National Parks) and six selected sub-streams, and (iii) to assess their frequency of fungal occurrence and fungal diversity.

MATERIALS AND METHODS

Collection sites. — Three streams in Doi Inthanon National Park (henceforth referred as to DI) and Khao Yai National Park (henceforward described as KY) were chosen as the six representative sub-sites/streams of a tropical rainforest (Fig. 1).

DI is located in the northern part of Thailand and covers an area of approximately 482 square kilometers at Chaing Mai Province (Fig. 1A). DI contains a variety of forests (the deciduous and evergreen nature of the vegetation), including virgin forest, pine and mixed forest, and is rich in flora and fauna (Chayamarit & Puff, 2007; Santisuk, 1988). Selected sites within DI were (1) the Ang Ka Nature Trail (N 18°35', E 098°29', 2,542 m in elevation), (2) Pha Doksiao Waterfall (N 18°32', E 098°31', 1,212 m in elevation), and (3) Wang Muang-Wang Khwai Waterfall (N 18°30', E 098°40', 405 m in elevation).

KY is the main conserved forest in Thailand (Fig. 1B). It covers four provinces in central and northeastern Thailand (Saraburi, Nakhon Ratchasima and Prachin Buri Provinces in the east, Nakhon Ratchasima in the north, and Nakhon Nayok Province in the south) and represents one of the largest humid tropical rainforest in Thailand. The vegetation of this forest was classified into five types, viz. dry evergreen forest, dry mixed deciduous forest, tropical rain forest, hill evergreen forest, and grassland and secondary forest (Smitinand, 1968). Its topography is mountainous, with peaks reaching approximately 1,351 meters above sea level. The three streams chosen in KY are 4, 5 and 6 respectively (4) the Lum Ta Khong Stream (N 14°20', E 101°21', 518 m in elevation), (5) Tat Ta Phu Waterfall (N 14°24', E 101°22', 611 m in elevation), and (6) Wang Champi Waterfall (N 14°26', E 101°21', 668 m in elevation).

Sample collection. — Five collections were made between July 2009 and November 2010. Forty naturally submerged to semi-submerged woody substrates (twigs or branches) were randomly selected, placed in polythene bags and transported to the laboratory. Sample size ranged from ca 1-5 cm in diameter × 15-30 cm long. Water temperature (ranging between 8.5-28°C), pH level (about 6.0-7.0) and collection dates (from 1st to 5th) are listed in Table 1.

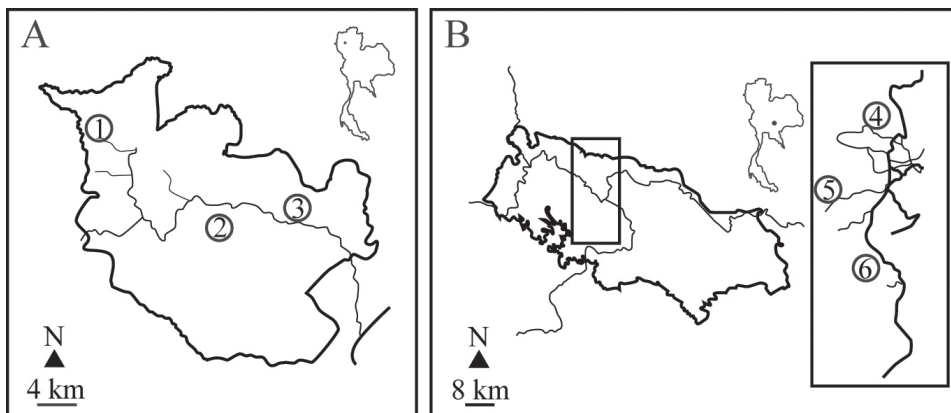


Fig. 1. Map of the collection sites in Doi Inthanon National Park (1A) and Khao Yai National Park (1B) including the six sub-streams: The stream 1) Ang Ka Nature Trail; The stream 2) Pha Doksiao Waterfall; The stream 3) Wang Muang-Wang Khwai Waterfall; The stream 4) Lum Ta Khong Stream; The stream 5) Tat Ta Phu Waterfall, and The stream 6) Wang Champi Waterfall.

Table 1. Collection dates with the pH and temperature of the water in the six streams

Sub-stream*		Date sampled				
		1 st	2 nd	3 rd	4 th	5 th
The stream 1	AK	Jul 28, 2009, 13°C, pH 6	Oct 30, 2009, 11.5°C, pH 6	Jan 12, 2010, 8.5°C, pH 6-7	Apr 20, 2010, 12°C, pH 5	Aug 28, 2010, 15°C, pH 6
The stream 2	PD	Jul 29, 2009, 19°C, pH 7	Oct 29, 2009, 18°C, pH 7	Jan 12, 2010, 17.5°C, pH 7	Apr 19, 2010, 21°C, pH 7	Aug 27, 2010, 20°C, pH 7
The stream 3	WMK	Jul 29, 2009, 20°C, pH 7	Oct 29, 2009, 19°C, pH 7	Jan 12, 2010, 19°C, pH 7	Apr 19, 2010, 25°C, pH 7	Aug 27, 2010, 24°C, pH 7
The stream 4	LTK	Aug 4, 2009, 23°C, pH 7	Nov 4, 2009, 22°C, pH 7	Jan 15, 2010, 21°C, pH 6-7	Apr 5, 2010, 28°C, pH 7	Nov 13, 2010, 23°C, pH 6
The stream 5	TTP	Aug 4, 2009, 22°C, pH 7	Nov 4, 2009, 20°C, pH 7	Jan 16, 2010, 16°C, pH 6	Apr 6, 2010, 23°C, pH 6	Nov 13, 2010, 23°C, pH 7
The stream 6	WC	Aug 4, 2009, 23°C, pH 7	Nov 4, 2009, 22°C, pH 7	Jan 15, 2010, 19°C, pH 6	Apr 5, 2010, 28°C, pH 7	Nov 13, 2010, 28°C, pH 7

*LTK: Lum Ta Khong Stream; TTP: Tat Ta Phu Waterfall; WC: Wang Champi Waterfall; AK: Ang Ka Nature Trail; PD: Pha Doksiao Waterfall; WMK: Wang Muang-Wang Khwai Waterfall

Fungal isolation and morphological identification. — The collected samples were placed in the 25 × 10 × 10 cm plastic boxes with a layer of water over the surface and incubated with moist tissue paper at 20°C in a cabinet.

Samples were examined under a stereomicroscope on incubation and re-examined at 14-day intervals for 3 months, to determine the presence of sporulating aero-aquatic hyphomycetes. Single spores were isolated into axenic culture, grown on corn meal agar (CMA, from Criterion™ Dehydrated Culture Media, Santa Maria, California) and potato carrot agar (PCA, extract from 20 g/L potato, extract from 20 g/L carrot, 2% agar) supplemented with antibiotics (streptomycin 0.5 g/l and penicillin G 0.5 g/l). Germinated conidia were subsequently transferred to potato dextrose agar plates (PDA, from Criterion™ Dehydrated Culture Media, Santa Maria, California).

For morphological identification, sporulating aero-aquatic hyphomycetes on substrata were mounted in water with lactophenol. Measurements of aero-aquatic hyphomycetes were taken from fresh material mounted in water and photographed using a Nomarski differential interference contrast microscope. Permanent slides were deposited at the BIOTEC Bangkok Herbarium (BBH, Thailand) and NITE Biological Resource Center collection (NBRC, Japan). Fungal isolates were kept in the BIOTEC Culture Collection (BCC, Thailand) and NBRC, Japan.

Statistical analyses. — Fungi identified from each woody sample were recorded only once when found in the same sample. The occurrence frequency of an aero-aquatic fungus was calculated for a wood sample using the following formula:

$$\text{Percentage of occurrence frequency} = \frac{\text{No. of wood samples colonized by a specific fungus} \times 100}{\text{No. of wood samples examined}}$$

The average number of aero-aquatic hyphomycetes per wood sample and percent colonization for each species were calculated using the following formulas:

$$\text{Average no. of fungi per wood sample} = \frac{\text{Total no. of fungal isolations}}{\text{No. of wood samples with sporulating fungi}}$$

Table 2. Percentage of fungal occurrence, number of species, number of collections and their distributions in the six streams

Aero-aquatic Fungi	% Frequency of occurrence, (% FO)								
	The stream no.			Overall percentage occurrence at DI	The stream no.			Overall percentage occurrence at KY	Overall percentage occurrence at DI and KY (OP ^{FO})
	1	2	3		4	5	6		
<i>Aegerita</i> -like	–	–	–	–	–	0.5	1	0.5	0.3
<i>Cancellidium applanatum</i>	–	0.5	0.5	0.3	4	44.5	25.5	24.7	12.5
<i>Candelabrum brocciatum</i>	8.5	31.5	35	25	30.5	49.5	52	44	34.5
<i>Candelabrum clathrosphaeroides</i> *	3	0.5	–	1.2	–	–	–	–	0.6
<i>Candelabrum microsporum</i> *	–	2	14.5	5.5	2.5	4.5	2	3	4.3
<i>Candelabrum</i> sp.#	–	–	–	–	–	0.5	–	0.2	0.1
<i>Clathrosporium compactum</i> *	–	10.5	1.5	4	–	–	–	–	2
<i>Clathrosporium intricatum</i> *	–	–	–	–	–	0.5	2	0.8	0.4
<i>Helicoma atroseptatum</i> *#	–	–	–	–	0.5	–	–	0.2	0.1
<i>Helicoma perelegans</i> *#	0.5	–	–	0.2	–	–	–	–	0.1
<i>Helicoma resinae</i> *#	–	–	–	–	0.5	–	–	0.2	0.1
<i>Helicoma</i> sp.#	–	–	0.5	0.2	–	–	–	–	0.1
<i>Helicoma</i> -like	1	–	–	0.3	–	–	–	–	0.2
<i>Helicomycetes</i> cf. <i>macrofilamentosus</i>	–	–	–	–	0.5	6.5	2	3	1.5
<i>Helicomycetes roseus</i>	0.5	6	13	6.5	5	5.5	7.5	6	6.3
<i>Helicoön gigantisporum</i>	–	–	–	–	1.5	–	–	0.5	0.3
<i>Helicosporium aureum</i> *#	–	0.5	–	0.2	–	–	–	–	0.1
<i>Helicosporium</i> cf. <i>pannosum</i>	–	–	1.5	0.5	–	–	0.5	0.2	0.3
<i>Helicosporium gigasporum</i>	–	–	3	1	0.5	1	3	1.5	1.3
<i>Helicosporium gracile</i> *#	–	–	–	–	–	–	0.5	0.2	0.1
<i>Helicosporium griseum</i> *	–	0.5	3	1.2	1	–	–	0.3	0.8
<i>Helicosporium guianense</i> *	1	0.5	2	1.2	–	–	–	–	0.6
<i>Helicosporium lumbricopsis</i> *	–	6.5	5.5	4	3.5	3.5	4	3.7	3.8
<i>Helicosporium panachaeum</i> *	–	10	6.5	5.5	6	1.5	1.5	3	4.3
<i>Helicosporium virescens</i> *	–	–	–	–	0.5	–	3.5	1.3	0.7
<i>Peyronelina glomerulata</i> *	0.5	0.5	1	0.7	1	1.5	–	0.8	0.8
<i>Pseudaegerita corticalis</i> *	61	1.5	–	20.8	–	24.5	5.5	10	15.4
<i>Pseudoclathrosphaerina</i> cf. <i>evamariae</i>	12	–	–	4	–	–	–	–	2
<i>Pseudoclathrosphaerina evamariae</i> *	12	–	–	4	–	0.5	–	0.2	2.1
<i>Pseudoclathrosphaerina</i> sp.	7.5	0.5	–	2.7	–	0.5	–	0.2	1.4
<i>Pseudoclathrosphaerina spiralis</i> *	5	–	–	1.7	–	–	–	–	0.8
<i>Spirosphaera</i> sp.	–	–	–	–	–	0.5	0.5	0.3	0.2
Percentage of wood colonized	72	54	59	62	48	83	70	67	64
Average no. of fungi per wood samples	1.6	1.3	1.5	1.5	1.2	1.8	1.6	1.6	1.5
Total no. of species at each site	12	14	13	22	14	16	15	23	32
Total no. of species at each forest	–	22	–	–	–	23	–	–	–
Shannon-Weaver (<i>H</i>)	1.58	1.77	1.88	2.31	1.72	1.73	1.74	1.87	–
Simpson (<i>D</i>)	0.67	0.75	0.78	0.85	0.69	0.76	0.72	0.75	–
Evenness (<i>J</i>)	0.63	0.67	0.73	0.75	0.56	0.63	0.64	0.60	–
Total genera in the study	–	–	–	–	–	13	–	–	–
Total species in the study	–	–	–	–	–	32	–	–	–
Total no. of wood samples	–	–	–	–	–	1,200	–	–	–
Total no. of fungal strains	–	–	–	–	–	1,171	–	–	–

FO: Frequency of occurrence; Asterisk (*): The new record species in Thailand; Number sign (#): The rare species in Thailand; Bold: The common species found for this study; DI: Doi Inthanon National Park comprises of three sub-streams: the stream 1-3; KY: Khao Yai National Park comprises three sub-streams: the stream 4-6; OP^{FO}: Overall percentage occurrence consists of two forests: Doi Inthanon and Khao Yai National Parks

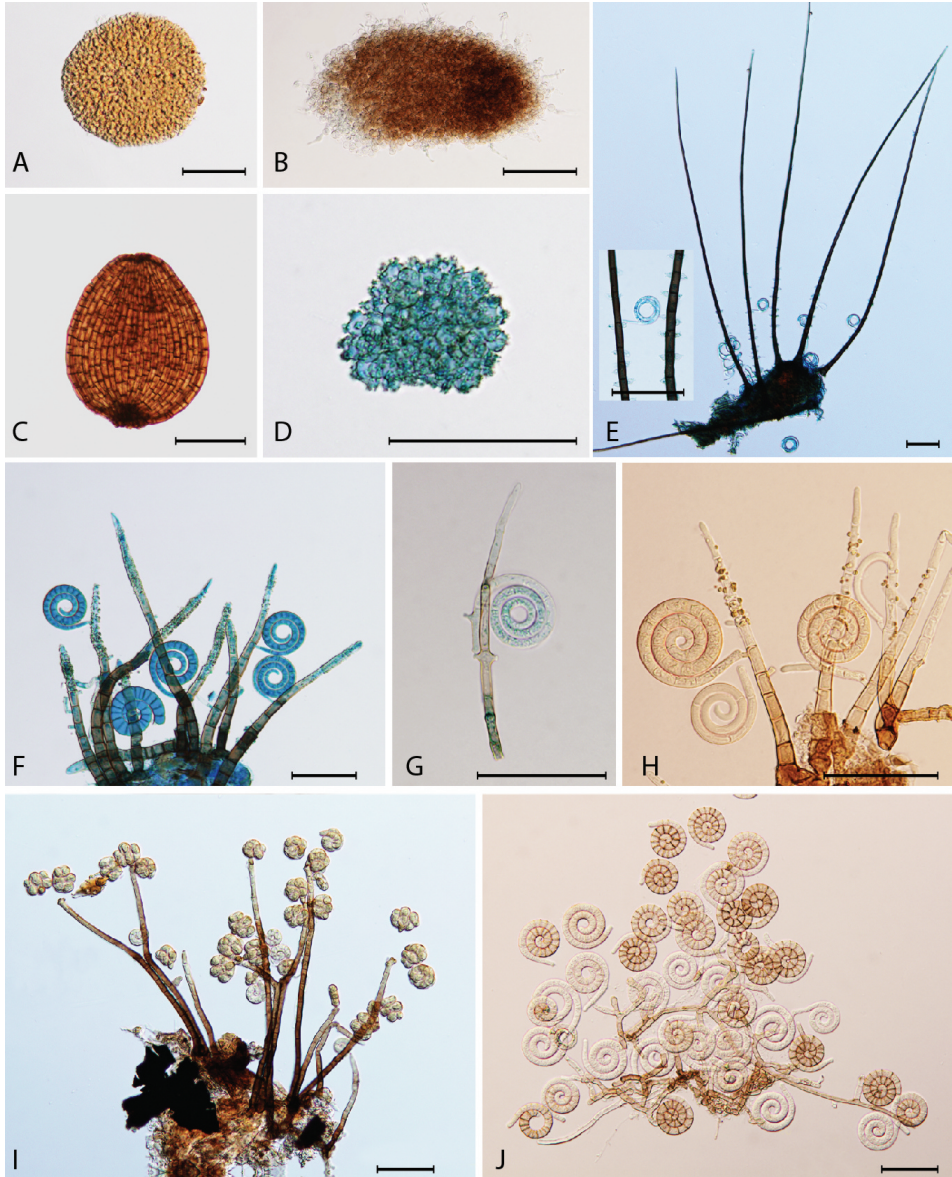


Fig. 2. The three most common fungi at the two forests: **A.** *Candelabrum brocchiatum*, **B.** *Pseudaegerita corticalis* and **C.** *Cancellidium applanatum*. **D-J.** Rare species at the two forest sites: **D.** *Candelabrum* sp., **E.** *Helicosporium aureum*, **F.** *Helicoma atroseptatum*, **G.** *Helicoma gracile*, **H.** *Helicoma* sp., **I.** *Helicoma resiniae* and **J.** *Helicoma perelegans*. Bars: A-J = 50 μ m.

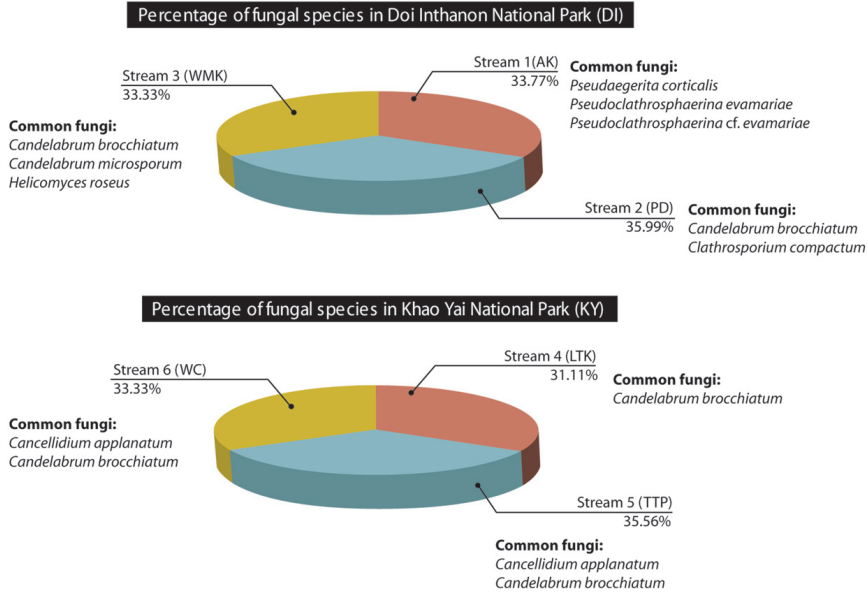


Fig. 3. Percentage of fungal records at each stream in DI and KY National Parks.

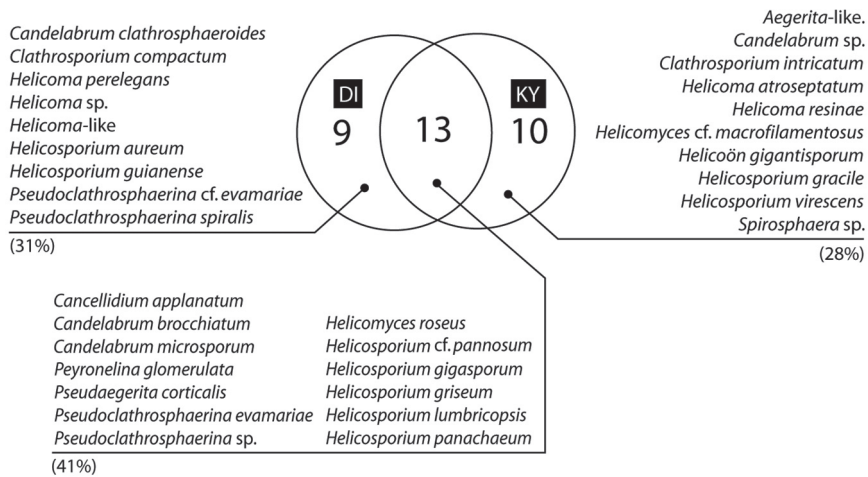


Fig. 4. Distribution of aero-aquatic fungi by national park. Numbers within each circle indicate the number of fungal species found at each forest. Numbers in the overlapping region indicate those common to both forests (DI and KY).

Percentage colonization = $\frac{\text{Total no. of wood samples with sporulating fungi} \times 100}{\text{Total no. of examined wood samples}}$

Shannon-Weaver (*H*), Simpson (*D*), Evenness (*J*) and Sørensen's (*S*) indices were calculated through the Vegan Community Ecology Package version 1.17-6 (Oksanen *et al.*, 2011).

RESULTS

Diversity of aero-aquatic fungi at Doi Inthanon and Khao Yai National Parks

Table 2 lists the fungi from 1,200 woody samples at both DI and KY, which yielded 1,171 fungal occurrences, belonging to 32 species in 13 genera. Eighteen species listed with an asterisk represent new records for Thailand (56.3% of all species). Stream 5 supported the highest number of fungi followed by streams 6, 2, 4, 3, and 1 with 16, 15, 14, 14, 13 and 12 species, respectively.

H, *D* and *J* indices were the highest at DI for stream 3 (*H* = 1.88, *D* = 0.78, and *J* = 0.73), and lowest at KY for the stream 1 (*H* = 1.58, *D* = 0.67, and *J* = 0.63). Seven species (21.9% of total species) were represented by only one record and can be regarded as infrequent or rare with an overall percentage occurrence of $OP^{FO} = 0.1\%$, and included three genera: *Candelabrum*, *Helicosporium* and *Helicoma* (Table 2, Fig. 2). In contrast (Table 2, Fig. 2), the three most common species found were *Candelabrum brocchiatum*, *Pseudaegerita corticalis* and *Cancellidium applanatum* with an OP^{FO} of 34.5, 15.1, and 12.5%, respectively.

In DI, twenty-two species, belonging to ten genera, were collected with *C. brocchiatum*, *Helicomycetes roseus*, *Helicosporium guianense* and *Peyronelina glomerulata*, occurring at all three streams; while the common species at the individual streams in DI were: stream 1 *Pseudaegerita corticalis* (61%), *Pseudoclathrosphaerina evamariae* (12%) and *Pseudoclathrosphaerina cf. evamariae* (12%); stream 2 *C. brocchiatum* (31.5%) and *Clathrosporium compactum* (10.5%); and stream 3 *C. brocchiatum* (35%), *C. microsporium* (14.5%), and *H. roseus* (13%). As shown in Fig. 3, stream 2 supported the greatest fungal species with 14 (35.99% all of species), stream 1: 12 (33.77%), and stream 3: 13 (33.33%).

In KY, twenty-three species, belonging to twelve genera, were collected with *C. applanatum*, *C. brocchiatum*, *C. microsporium*, *Helicomycetes cf. macrofilamentosus*, *H. roseus*, *Helicosporium gigasporum*, *H. lumbricopsis*, and *H. panachaeum* found at all three streams. The most common at each stream were: stream 4 *C. brocchiatum* (30.5%); stream 5 *C. applanatum* (44.5%) and *C. brocchiatum* (49.5%); and stream 6 *C. applanatum* (25.5%), and *C. brocchiatum* (52%). Stream 5 supported the greatest number of species (Fig. 3).

The overlapping/exclusive fungi found in two forests and among their sub-streams

Nine and 10 fungi were only found exclusively at DI and KY forests, respectively, while 13 were common to both forests (Fig. 4).

In DI streams, 5, 1 and 3 fungi were found only in streams 1, 2 and 3, respectively, with four species common to all three streams. For the KY streams, 4, 3 and 2, fungi were found only at streams 4, 5 and 6 respectively, with 8 common to all streams (Fig. 5).

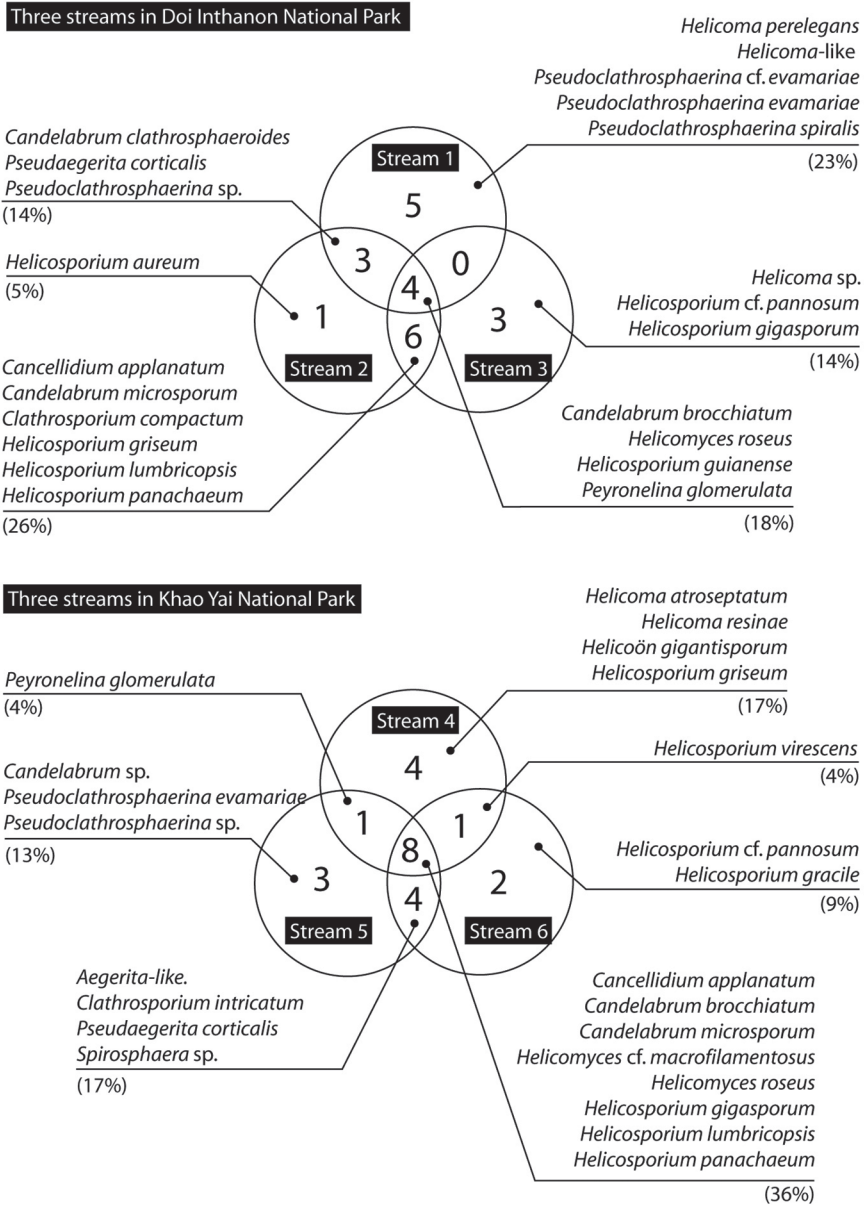


Fig. 5. Distribution of aero-aquatic fungi in the six streams in the two national parks. Numbers within each circle indicate the number found in that stream. Numbers in the overlapping regions indicate species found common between the different streams.

Table 3. Sørensen's index (%) showing similarity indices among the six different streams at the two forests

Collection sites (The stream no.)	Similarity (%)					
	1	2	3	4	5	6
1	–	53.9	32	23.1	42.9	22.2
2	–	–	74.1	57.1	60	48.3
3	–	–	–	66.7	55.2	57.1
4	–	–	–	–	60	62.1
5	–	–	–	–	–	77.4
6	–	–	–	–	–	–

Fungal similarities between streams in DI and KY, Thailand

In Table 3, the similarity in fungal communities associated with wood samples between the different sub-streams ranged from 22.2 to 77.4%. The closest fungal similarity was between streams 6 and 5 (77.4%) and lowest for streams 1 and 6 (22.2%), respectively.

DISCUSSION

Biodiversity of aero-aquatic fungi in six different selected streams at DI and KY

Thirty-two aero-aquatic fungi (in 13 genera) were recorded on woody substrata collected at two forests in Thailand, with only 13 species (41%) common to both locations. *Cancellidium applanatum* (at 5 streams), *Candelabrum brocchiatum* (6), *Helicomycetes roseus* (6), *Helicosporium panachaeum* (5) and *Pseudaegerita corticalis* (4) were the most common species found during this study. Of the nine and ten species found only at DI and KY respectively, most had a low frequency of occurrence (0.3-0.5%) or occurred only at one stream.

The topography of the adjacent vegetation through which the streams passed varied greatly: stream 1 had slow moving water, low temperature (8.5-15°C), surrounded by many shrubs, and was largely shaded by trees and at an elevation of 2,542 m. The second stream was in the lower montane rainforest, at elevation of 1,212 m., and water temperatures ranged between 17.5-20°C), while stream 3 was in mixed deciduous forest, at elevation of 405 m., and water temperatures ranged between 19-25°C. The water at both sites was fast-moving, especially in rainy season and showed static to slow-moving water in dry season. Despite different aspects of the streams there was little variation in species diversity.

Sivichai *et al.* (2000, 2002) also studied the diversity of freshwater fungi colonizing submerged test blocks of *Dipterocarpus alatus* and *Xylia dolabriformis* at KY forest, with *Helicomycetes roseus* and *Candelabrum brocchiatum* occurring at a frequency of occurrence of 73.3% and 1.5% at stream 2 (a site common to both studies). However, *Scutisporus brunneus* and *Helicosporium vegetum* were not

found. A variety of factors may account for the differences in biodiversity observed in freshwater habitats, tree canopy over the stream, sampling strategies, riparian vegetation, decaying wood abundance, host-specificity, geographical location, temperature, rainfall, water velocity, water chemistry, nutrient status and the chemical composition (Fröhlich & Hyde, 2000; Jones, 2000; Kane *et al.*, 2002; Kurniawati *et al.*, 2010; Taylor & Hyde, 2003; Tsui *et al.*, 2000; Wood-Eggenschwiler & Bärlocher, 1985; Zhang *et al.*, 2011).

Thai most common aero-aquatic fungi on wood compared to those from tropical and temperate locations

In our study, we examined the naturally collected wood on return to the laboratory, at 48 hours and at one week while there was still a film of water on the wood surface. During this period, a number of some ascomycete and aquatic hyphomycetes sporulated on the wood surface, but not recorded in this paper (data not shown).

A large number of aquatic and aero-aquatic fungi associated with decomposing submerged leaves and spores trapped in air bubbles in stream ecosystems (Bärlocher, 1992), and many have been recorded from tropical and subtropical regions (Au *et al.*, 1992; Bhat & Chien, 1990; Chan *et al.*, 2000a, b). However, few have documented aero-aquatic hyphomycetes from submerged woody substrata (Cai *et al.*, 2003; Ho *et al.*, 2001; Hyde & Goh, 1998; Tsui *et al.*, 2000; Tsui & Hyde, 2004; Vijaykrishna & Hyde, 2006).

Table 4, lists the most common aero-aquatic fungi from tropical and temperate locations, with the most frequent species *C. brocchiatum* (10 from 12 studies), *Helicomycetes torquatus* (6 out of 12), *Helicosporium griseum* (4 out of 12), *Helicomycetes roseus* (9 out of 12) and *Cancellidium applanatum* (2 out of 12); however, these were general surveys and did not focus on not aero-aquatic fungi.

Candelabrum brocchiatum, has dichotomously branched conidia with contiguous lobes, and was the most frequent species recovered from both forest (DI, KY) at all six freshwater streams in the current study. This species is considered an ubiquitous species in Thai forests (Sivichai *et al.*, 2000, 2002), and other tropical areas: Australia, Brunei, Hong Kong and Malaysia (Ho *et al.*, 2001; Hyde & Goh, 1998; Tsui *et al.*, 2000; Vijaykrishna & Hyde, 2006) and in temperate waters in Japan (Tsui & Hyde, 2003).

Cancellidium applanatum was also cosmopolitan tropical aero-aquatic and originally isolated from balsawood test blocks from a lake in Japan (Tubaki, 1975). However, it has since been recovered from submerged decaying leaves from wood in Malaysia (Webster & Davey, 1980), Queensland, Australia (Shaw, 1994) and Brunei (Fryar *et al.*, 2004), but not common in temperate waters (Table 4).

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Table 4. The most common aero-aquatic fungi recorded from published studies in tropical and temperate zones

<i>Place and Reference</i>	<i>Samples</i>	<i>Habitat origin</i>	<i>The most common species (% occurrence frequency)</i>
Tropical regions			
Thailand (This study)	Unidentified woods	Six Streams in Doi Inthanon National and Khao Yai Parks	<i>Candelabrum brocchiatum</i> (34.5) <i>Pseudaegerita corticalis</i> (15.4) <i>Cancellidium applanatum</i> (12.5) <i>Helicomycetes roseus</i> (6.3) <i>Candelabrum microsporium</i> (4.3) <i>Helicosporium panachaeum</i> (4.3)
Thailand (Sivichai <i>et al.</i> , 2000)	Submerged Test Blocks: <i>Dipterocarpus alatus</i> <i>Xylia dolabriformis</i>	Stream at Km 29.2 in Khao Yai National Park	<i>Helicomycetes roseus</i> (70) <i>Scutisporus brunneus</i> (20.84) <i>Helicosporium griseum</i> -like (7.5) <i>Candelabrum brocchiatum</i> (5.83) <i>Helicosporium vegetum</i> -like (5)
Thailand (Sivichai <i>et al.</i> , 2002)	Submerged Test Blocks: <i>Dipterocarpus alatus</i> <i>Xylia dolabriformis</i>	Stream at Tad Ta Phu in Khao Yai National Park	<i>Helicomycetes roseus</i> (73.3) <i>Cancellidium applanatum</i> (26.7) <i>Scutisporus brunneus</i> (26.7) <i>Helicosporium vegetum</i> -like (17.5) <i>Candelabrum brocchiatum</i> (15)
Australia (Vijaykrishna and Hyde, 2006)	Unidentified woods	Stream at the Barron River	<i>Helicomycetes roseus</i> (1.9) <i>Helicosporium hiospiroides</i> (0.7) <i>Helicosporium gigasporum</i> (0.3) <i>Helicosporium griseum</i> (0.3) <i>Helicosporium decumbens</i> (0.3)
Australia (Hyde and Goh, 1998)	Unidentified woods	Lake	<i>Candelabrum brocchiatum</i> (41) <i>Helicosporium griseum</i> (7) <i>Helicosporium guianensis</i> (4) <i>Clathrosphaerina</i> sp. (1) <i>Helicoma depressispora</i> (1) <i>Helicomycetes roseus</i> (1)
Brunei (Ho <i>et al.</i> , 2001)	Unidentified woods	Stream	<i>Helicomycetes roseus</i> (5.0) <i>Candelabrum brocchiatum</i> (2.1) <i>Helicomycetes torquatus</i> (0.7)
Hong Kong (Tsui <i>et al.</i> , 2000)	Unidentified woods	Stream	<i>Candelabrum brocchiatum</i> (11.3) <i>Helicosporium griseum</i> (10.3) <i>Helicosporium pallidum</i> (6) <i>Helicomycetes torquatus</i> (4) <i>Helicomycetes roseus</i> (3.3)
Hong Kong (Tsui and Hyde, 2004)	Unidentified woods	Stream in Tai Ho Bay	<i>Candelabrum brocchiatum</i> (8.5) <i>Helicomycetes torquatus</i> (8.5) <i>Helicoma</i> sp. (2.1) <i>Helicosporium</i> sp. (2.1)
Malaysia (Ho <i>et al.</i> , 2001)	Unidentified woods	Stream	<i>Helicomycetes roseus</i> (1.9) <i>Candelabrum brocchiatum</i> (1.9) <i>Helicosporium lumbricoides</i> (1.9) <i>Helicomycetes torquatus</i> (1.0) <i>Spirosphaera floriformis</i> (1.0)
Philippines (Cai <i>et al.</i> , 2003)	Bamboo and unidentified woods	River	<i>Helicosporium gigasporum</i> (3.5) <i>Candelabrum brocchiatum</i> (2)
Temperate regions			
China (Cai <i>et al.</i> , 2002)	Unidentified woods and tree root	Pool	<i>Helicomycetes torquatus</i> (3) <i>Helicomycetes roseus</i> (1)
Japan (Tsui and Hyde, 2003)	Unidentified woods	River	<i>Candelabrum brocchiatum</i> (0.025) <i>Helicomycetes torquatus</i> (0.025) <i>Helicosporium abuense</i> (0.025) <i>Helicosporium</i> sp. (0.025)
UK (Kane <i>et al.</i> , 2002)	Beech (<i>Fagus sylvatica</i>)	River	<i>Helicomycetes scandens</i> (2.78)

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REFERENCES

- AU D.W.T., HODGKISS I.J. & VRIJMOED L.L.P., 1992 — Decomposition of *Bauhinia purpurea* leaf litter in a polluted and unpolluted Hong Kong waterway. *Canadian Journal of Botany* 70(5): 1061-1070.
- ABDULLAH S.K., CANO J., DESCALS E. & GUARRO J., 1998a — A new species of *Helicoon* from Mallorca, Spain. *Mycologia* 90(5): 916-920.
- ABDULLAH S.K., CANO J., DESCALS E. & GUARRO J., 2000 — The aero-aquatic *Helicodendron microsporium* n. sp. from Mallorca, Spain. *Mycological Research* 104(3): 375-377.
- ABDULLAH S.K., GENE J. & GUARRO J., 1997 — A new species of *Pseudaegerita* from Italy. *Mycotaxon* 65: 493-497.
- ABDULLAH S.K., GENE J. & GUARRO J., 1998b — New and interesting aero-aquatic mitosporic fungi from Italy. *Mycotaxon* 66: 267-272.
- ABDULLAH S.K., GENE J. & GUARRO J., 2005 — A synopsis of the aero-aquatic genus *Pseudaegerita* and description of two new species. *Mycological Research* 109(5): 590-594.
- ABDULLAH S.K., GUARRO J. & FIGUERAS M.J., 1996 — New and interesting *Helicoon* species from Spain. *Mycotaxon* 60: 449-454.
- ABDULLAH S.K. & WEBSTER J., 1980 — Aquatic and aero-aquatic hyphomycetes from Ireland. *The Irish Naturalists' Journal* 20(2): 49-55.
- ABDULLAH S.K. & WEBSTER J., 1983 — The aero-aquatic genus *Pseudaegerita*. *Transactions of the British Mycological Society* 80(2): 247-254.
- BÄRLOCHER F., 1992 — The Ecology of Aquatic Hyphomycetes. Berlin, Springer-Verlag, p. 225.
- BEVERWIJK van A.L., 1951 — Zalewski's '*Clathrophaera spirifera*'. *Transactions of the British Mycological Society* 34(3): 280-290.
- BHAT D.J. & CHIEN C.Y., 1990 — Water-Borne hyphomycetes found in Ethiopia. *Transactions of the Mycological Society of Japan* 31: 147-158.
- CAI L., TSUI C.K.M., ZHANG K. & HYDE K.D., 2002 — Aquatic fungi from Lake Fuxian, Yunnan, China. *Fungal Diversity* 9: 57-70.
- CAI L., ZHANG K., MCKENZIE E.H.C. & HYDE K.D., 2003 — Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. *Fungal Diversity* 13: 1-12.
- CASTAÑEDA Ruiz R.F. & KENDRICK B., 1991 — Ninety-nine Conidial Fungi from Cuba and three from Canada. Waterloo, Department of Biology-University of Waterloo, pp. 1-132.
- CHAN S.Y., GOH T.K. & HYDE K.D., 2000a — Ingoldian fungi in Hong Kong. In: Hyde KD, Ho WH & Pointing SB. (eds.), *Aquatic Mycology across the Millennium*. 5. Hong Kong, Fungal Diversity Press, pp. 89-107.
- CHAN S.Y., GOH T.K. & HYDE K.D., 2000b — Ingoldian fungi in Lam Tsuen River and Tai Po Kau Forest Stream, Hong Kong. In: Hyde KD, Ho WH & Pointing SB. (eds.), *Aquatic Mycology across the Millennium*. 5. Hong Kong, Fungal Diversity Press, pp. 109-118.
- CHANG H.S., 2001 — *Helicoon doliiformis* sp. nov. and two similar helicosporous hyphomycetes from Taiwan. *Botanical Bulletin of Academia Sinica* 42: 149-152.
- CHAYAMARIT K. & PUFF C., 2007 — Plants of Doi Inthanon National Park. National Park. Bangkok, Prachachon Company, Limited, pp. 8-29.
- DESCALS E. & MORALES E., 2001 — Water and asexual reproduction in the ingoldian fungi. *Botanica Complutensis* 25: 13-71.
- FRÖHLICH J. & HYDE K.D., 2000 — Palm Microfungi. Fungal. *Fungal Diversity Research Series* 3. Hong Kong, Fungal Diversity Press, p. 393.
- FRYAR S.C., BOOTH W., DAVIES J., HODGKISS I.J. & HYDE K.D., 2004 — Distribution of fungi on wood in the Tutong River, Brunei. *Fungal Diversity* 17: 17-38.
- GOH T.K., 1997 — Tropical Freshwater Hyphomycetes. In: Hyde KD. (ed.), *Biodiversity of Tropical Microfungi*. Hong Kong, Hong Kong University Press, pp. 189-227.
- GOH T.K. & HYDE K.D., 1996a — Biodiversity of freshwater fungi. *Journal of Industrial Microbiology & Biotechnology* 17(5-6): 328-345.

- GOH T.K. & HYDE K.D., 1996b — *Helicoon gigantisporum* sp. nov., and an amended key to the genus. *Mycological Research* 100(12): 1485-1488.
- HENNEBERT G.L., 1998 — New species of the aeroaquatic hyphomycete genus *Clathrosporium* and their relationship with *Strumella* Sacc. *Canadian Journal of Botany* 76(9): 1596-1607.
- HO W.H., HYDE K.D., HODGKISS I.J. & Yanna, 2001 — Fungal communities on submerged wood from streams in Brunei, Hong Kong, and Malaysia. *Mycological Research* 105(12): 1492-1501.
- HO W.H., YANNA, HYDE K.D. & HODGKISS I.J., 2002 — Seasonality and sequential occurrence of fungi on wood submerged in Tai Po Kau Forest Stream, Hong Kong. *In: Hyde KD & Jones EBG. (eds.), Fungal Succession*. 10. Hong Kong, Fungal Diversity Press, pp. 21-43.
- HYDE K.D. & GOH T.K., 1998 — Fungi on submerged wood in Lake Barrine, north Queensland, Australia. *Mycological Research* 102(6): 739-749.
- HYDE K.D., MCKENZIE E.H.C. & KOKO T.W., 2011 — Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. *Mycosphere* 2(1): 1-88.
- JONES E.B.G., 2000 — Marine fungi: some factors influencing biodiversity. *Fungal Diversity* 4: 53-73.
- JONES E.B.G. & PANG K.-L., 2012 — Tropical aquatic fungi. *Biodiversity and Conservation* 21: 2403-2423.
- KANE D.F., TAM W.Y. & JONES E.B.G., 2002 — Fungi colonising and sporulating on submerged wood in the River Severn, UK. *In: Hyde KD & Jones EBG. (eds.), Fungal Succession*. 10. Hong Kong, Fungal Diversity Press, pp. 45-55.
- KURNIAWATI E., ZHANG H., CHUKEATIROTE E., SULISTYOWATI L., MOSLEM M.A. & HYDE K.D., 2010 — Diversity of freshwater ascomycetes in freshwater bodies at Amphoe Mae Chan, Chiang Rai. *Cryptogamie Mycologie* 31(3): 323-331.
- MARVANOVÁ L. & BÄRLOCHER F., 1998 — Hyphomycetes from Canadian streams. IV. *Spirosphaera dimorpha* sp. nov. *Mycotaxon* 68: 33-40.
- NAWAWI A. & KUTHUBUTHEEN A.J., 1987 — *Clathrosporium intricatum* gen. et sp. nov. an aeroaquatic hyphomycete. *Transactions of the British Mycological Society* 89(3): 407-411.
- NAWAWI A. & KUTHUBUTHEEN A.J., 1988 — *Beverwykella cerebriformis* sp. nov., an aeroaquatic hyphomycete from Malaysia. *Transactions of the British Mycological Society* 90(3): 487-491.
- NAWAWI A. & KUTHUBUTHEEN A.J., 1990 — *Nidulispora* gen. nov., a hyphomycete genus with crateriform conidia. *Mycotaxon* 36(2): 329-336.
- OKSANEN J., BLANCHET F.G., KINDT R., LEGENDRE P., O'HARA R.B., SIMPSON G.L., SOLYMOS P., STEVENS M.H.H. & WAGNER H., 2011 — Vegan: Community Ecology Package. R package version 1.17-7.
- PINNOI A., LUMYONG S., HYDE K.D. & JONES E.B.G., 2006 — Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn peat swamp forest, Narathiwat, Thailand. *Fungal Diversity* 22: 205-218.
- PINRUAN U., HYDE K.D., LUMYONG S., MCKENZIE E.H.C. & JONES E.B.G., 2007 — Occurrence of fungi on tissues of the peat swamp palm *Licuala longicalycata*. *Fungal Diversity* 25: 157-173.
- SANTISUK T., 1988 — An account of the vegetation of northern Thailand. *Geocological research*. 5. Stuttgart, Franz Steiner Verlag Wiesbaden.
- SHAW D.E., 1994 — The aero-aquatic fungus *Cancellidium applanatum* K. Tubaki in Queensland. *Mycologist* 8: 162-163.
- SIVICHAI S. & HYWEL-JONES N.L., 1999 — *Biflagellospora* (aero-aquatic hyphomycetes) from submerged wood in Thailand. *Mycological Research* 103(7): 908-914.
- SIVICHAI S., JONES E.B.G. & HYWEL-JONES N.L., 2000 — Fungal colonisation of wood in a freshwater stream at Khao Yai National Park, Thailand. *In: Hyde KD, Ho WH & Pointing SB. (eds.), Aquatic Mycology across the Millennium*. 5. Hong Kong, Fungal Diversity Press, pp. 71-88.
- SIVICHAI S., JONES E.B.G. & HYWEL-JONES N.L., 2002 — Fungal colonisation of wood in a freshwater stream at Tad Ta Phu, Khao Yai National Park, Thailand. *In: Hyde KD & Jones EBG. (eds.), Fungal Succession*. 10. Hong Kong, Fungal Diversity Press, pp. 113-129.
- SMITINAND T., 1968 — Vegetation of Khao Yai National Park. *Natural History Bulletin of the Siam Society* 22: 289-305.
- TAYLOR J.E. & HYDE K.D., 2003 — Microfungi of Tropical and Temperate Palms. *Fungal Diversity Research Series 12*. Hong Kong, Fungal Diversity Press, p. 459.
- TSUI C.K.M. & HYDE K.D., 2003 — Fungi on submerged wood in the Koito River, Japan. *Mycoscience* 44: 55-59.
- TSUI C.K.M. & HYDE K.D., 2004 — Biodiversity of fungi on submerged wood in a stream and its estuary in the Tai Ho Bay, Hong Kong. *Fungal Diversity* 15: 205-220.
- TSUI C.K.M., HYDE K.D. & HODGKISS I.J., 2000 — Biodiversity of fungi on submerged wood in Hong Kong streams. *Aquatic Microbial Ecology* 21(3): 289-298.

- TUBAKI K., 1975 — Notes on the Japanese Hyphomycetes VII. *Cancellidium*, a new hyphomycete genus. *Transactions of the Mycological Society of Japan* 16: 357-360.
- VIJAYKRISHNA D. & HYDE K.D., 2006 — Inter- and intra stream variation of lignicolous freshwater fungi in tropical Australia. *Fungal Diversity* 21: 203-224.
- VOGLMAYR H., 1997a — Two new aero-aquatic species of the hyphomycete genus *Helicodendron* from Austria. *Plant Systematics and Evolution* 205(3-4): 185-193.
- VOGLMAYR H., 1997b — *Helicoön myosuroides* sp. nov. and *Helicoön dendroides* sp. nov., two new aero-aquatic hyphomycetes. *Mycological Research* 101(3): 337-340.
- VOGLMAYR H., 1997c — *Helicodendron praetermissum* sp. nov. and *Spirosphaera carici-graminis* sp. nov., aero-aquatic fungi on monocotyledonous debris. *Canadian Journal of Botany* 75(10): 1772-1777.
- VOGLMAYR H., 1998 — *Candelabrum desmidiaceum* and *Candelabrum clathrosphaeroides* spp. nov., additions and key to *Candelabrum*. *Mycological Research* 102(4): 410-414.
- VOGLMAYR H., 2004 — *Spirosphaera cupreorufescens* sp. nov., a rare aeroaquatic fungus. *Studies in Mycology* 50(1): 221-228.
- VOGLMAYR H., BONNER L.J. & DICK M.W., 1999 — Taxonomy and oogonial ultrastructure of a new aero-aquatic peronosporomycete, *Medusoides* gen. nov. (Pythiogetonaceae fam. nov.). *Mycological Research* 103(5): 591-606.
- VOGLMAYR H. & DELGADO-RODRIGUEZ G., 2001 — *Dendroclathra caeruleofusca* gen. nov. et sp. nov., an aeroaquatic hyphomycete from Cuba. *Canadian Journal of Botany* 79(9): 995-1000.
- VOGLMAYR H. & DELGADO-RODRIGUEZ G., 2003 — New species, notes and key to the aeroaquatic genera *Beverwykella* and *Ramicephala* gen. nov. *Mycological Research* 107(2): 236-244.
- VOGLMAYR H. & FISHER P.J., 1997 — *Helicodendron fuscum* and its allies. *Mycological Research* 101(9): 1122-1126.
- VOGLMAYR H. & KRISAI-GREILHUBER I., 1996 — *Dicranophora fulva*, a rare mucoraceous fungus growing on boletes. *Mycological Research* 100(5): 583-590.
- VOGLMAYR H. & KRISAI-GREILHUBER I., 1997 — *Pseudoclathrosphaerina evamariae* gen. et sp. nov. and *Sympodioclathra globosa* gen. et sp. nov., two aeroaquatic fungi similar to *Clathrosphaerina*. *Mycologia* 89(6): 942-951.
- VOGLMAYR H. & YULE C.M., 2006 — *Polyancora globosa* gen. sp. nov., an aeroaquatic fungus from Malaysian peat swamp forests. *Mycological Research* 110(10): 1242-1252.
- WEBSTER J. & DAVEY R.A., 1980 — Two aero-aquatic hyphomycetes from Malaysia. *Transactions of the British Mycological Society* 75(2): 341-345.
- WOOD-EGGENSCHWILER S., BÄRLOCHER F., 1985 — Geographical distribution of Ingoldian fungi. *Verhandlungen des Internationalen Verein Limnologie* 22: 2780-2785.
- YAMAGUCHI K., DEGAWA Y. & NAKAGIRI A., 2009 — An aero-aquatic fungus, *Peyronelina glomerulata*, is shown to have teleomorphic affinities with cyphelloid basidiomycetes. *Mycoscience* 50(3): 156-164.
- YAMAGUCHI K., TSURUMI Y., SUZUKI R., CHUASEEHARONNACHAI C., SRI-INDRASUTDHI V., BOONYUEN N., OKANE I., SUZUKI K. & NAKAGIRI A., 2012 — *Trichoderma matsushimae* and *T. aeroaquaticum*: two aero-aquatic species from Thailand and Japan with *Pseudaegerita*-like propagules. *Mycologia* 104(5): 1109-1120.
- ZHANG H., JONES E.B.G., ZHOU D., BAHKALI A.H. & HYDE K.D. 2011 — Checklist of Freshwater Fungi in Thailand. *Cryptogamie Mycologie* 32(2): 199-217.

