

## Diversity of freshwater ascomycetes in freshwater bodies at Amphoe Mae Chan, Chiang Rai

Elvi KURNIAWATI<sup>a, b</sup>, Huang ZHANG<sup>c</sup>, Ekachai CHUKEATIROTE<sup>a</sup>,  
Liliek SULISTYOWATI<sup>b</sup>, Mohamed A. MOSLEM<sup>d</sup> & Kevin D. HYDE<sup>a, d\*</sup>

<sup>a</sup>*School of Science, Mae Fah Luang University, 333 M. 1. T. Tasud Muang District,  
Chiang Rai 57100, Thailand*

<sup>b</sup>*Faculty of Agriculture, University of Brawijaya, Jl. Veteran, Malang, Indonesia*

<sup>c</sup>*College of Environmental Science & Engineering,  
Kunming University of Science & Technology, 650093, Kunming, China*

<sup>d</sup>*Botany and Microbiology Department, College of Science,  
King Saud University, Riyadh, Saudi Arabia*

**Abstract** – The diversity of freshwater fungi on submerged wood has been documented in three water resources of Mae Chan, Chiang Rai, Thailand. Sixty samples were collected from each site and examined for fungi. In total, 60 fungal taxa were identified including 29 ascomycetes, 27 anamorphic taxa and 4 unidentified species. The data obtained from the three sites were then used to calculate frequency of occurrence, species richness, Margalef index, Simpson's index (D), Shannon-Weiner index (H') and species evenness (E). The fungal diversity from Site 2 (waterfall with abundant trees) was higher than that from Site 1 (river near agricultural zone) and Site 3 (waterfall with scrub). Interestingly, *Acrogenospora sphaerocephala*, *Canalisporium caribense*, *Corynesporium* sp., *Didymella aptrootii*, *Fluminicola bipolaris*, *Halosarphaeia aquadulcis*, *Helicomycetes roseus* and *Savoryella lignicola* were frequently recorded in all sites. This paper is the first report to provide new data concerning the distribution of freshwater fungi in northern Thailand. The relationship between fungal diversity and freshwater habitats is also discussed.

**Ecology / environment / hyphomycetes / riparian vegetation / submerged wood**

## INTRODUCTION

Freshwater fungi play an important role in the degradation and conversion of woody organic matter in streams and rivers; some species produce enzymes capable of breaking down cellulose and lignin (Wong *et al.*, 1998; Abdel-Raheem & Shearer, 2002; Simonis *et al.*, 2008; Seena *et al.*, 2008). Fungal diversity has attracted considerable attention, and presently over 600 species of freshwater fungi are known (Shearer, 2001; Cai *et al.*, 2003; Goh & Tsui, 2003; Shearer *et al.*, 2007; Zhang *et al.*, 2008; Raja *et al.*, 2009b). Numerous studies have been carried out on the diversity of freshwater fungi on submerged wood, including streams (Hyde & Goh, 1997; Ho *et al.* 2001, 2002; Duarte *et al.* 2008; Seena *et al.* 2008), rivers (Hyde *et al.* 1998; Hyde & Goh, 1999; Cai *et al.* 2003; Fryar *et al.* 2004), lakes (Hyde & Goh, 1998a; Cai *et al.*, 2002; Luo *et al.*, 2004), reservoirs (Goh & Hyde,

1999; Bisht *et al.*, 2000). Meanwhile, data is available from both tropical (Hyde & Goh, 1997, 1998a,b; Yuen *et al.*, 1998; Goh & Hyde, 1999; Hyde *et al.*, 1999; Tsui *et al.*, 2000; Cai *et al.*, 2003; Luo *et al.*, 2004; Tsui & Hyde, 2004; Fryar *et al.*, 2004, 2005) and temperate locations (Shearer, 1993; Hyde & Goh, 1999; Cai *et al.*, 2002; Van Ryckegem & Verbeken, 2005a,b).

There have been some studies of freshwater fungi in Thailand. Sivichai *et al.* (2000, 2002) showed that submerged wood in Thailand supports a high diversity of anamorphic fungi, generally dematiaceous taxa. Sivichai & Boonyene (2004) listed the common fungi on wood of various hosts in Thailand, while Kodsueb *et al.* (2008) studied fungal succession of freshwater fungi. These studies established that Thai freshwater habitats support a diverse fungal community and several were new taxon (Pinruan *et al.*, 2008).

The relationship between biodiversity and ecosystem functioning has been of interest in recent years. It has been assumed that the maintenance of high diversity might provide a buffer for ecosystem processes (Duarte *et al.*, 2008). The environmental factors that effect microbial diversity have been a major theme for the study of freshwater fungi as well as its effects on the species richness on substrates. Tsui & Hyde (2004) noted the significant correlation between environmental factors and the diversity of fungi.

Here, we report an investigation into the richness and biodiversity of freshwater fungi in three water bodies of Chiang Rai, Thailand. In addition, this present study also aims at understanding the relationship between the distribution of freshwater fungi and their natural habitats.

## MATERIALS AND METHODS

### Study sites

Three water bodies at Mae Chan were selected for study based on easy access, different riparian vegetation and agricultural run off (Figs 1A-C). Site 1 is a river near a village and agricultural zone. Site 2 (Huai Kang Pla Waterfall) is a stream and waterfall with water out-flows from a waterfall and has abundant trees. It is far from any agricultural area. Site 3 (Tat Thong Waterfall) is a stream and waterfall with scrub. The water is slow-moving. The parameters recorded from stream habitats include altitude, longitude, pH, temperature, dissolved oxygen and description of riparian vegetation (Table 1).

### Collection of samples and incubation

The sampling was conducted between February and October 2008. Sixty submerged wood samples that had been submerged for a long period (observed by the degree of degradation or presence of algae on the wood surface) were randomly collected from each site. The collected samples included branches, segments of trunks and twigs from a variety of unidentified angiosperms. The size of the wood samples ranged from 15-30 cm long and 1-5 cm in diameter.

All of samples were placed separately in Zip lock plastic bags with sterile moist tissue paper and incubated at room temperature. The woody substrates were examined under a dissecting microscope for fruiting bodies on day 7 after incubation until 2 months.



Fig. 1. Collection sites showing the water condition and riparian vegetation. **A.** Site 1 - river near a village and agricultural zone before Huai Kang Pla Waterfall. **B.** Site 2 - Huai Kang Pla Waterfall. **C.** Site 3 - Tat Thong Waterfall.

Table 1. Geographical, physical and chemical data of the experimental fields

<i>Stream</i>	<i>Location</i>	<i>pH</i>	<i>Temperature °C</i>	<i>Dissolved oxygen</i>	<i>Riparian vegetation</i>
Site 1	Alt: 488 N : 20°06'47.4" E : 009°46'52.0"	7.30	27-28.1	118% (9.22 mg/l)	Rice field, bamboo
Site 2	Alt: 488 N : 20°05'28.4" E : 009°46'52.6"	7.42	24-25	119.7% (9.54 mg/l)	Wood, bamboo
Site 3	Alt: 497 N : 20°07'08"9" E : 009°49'21".5"	7.60	21-22.6	93.4% (8.08 mg/l)	Scrub, bamboo

## Identification

Identification was achieved by direct comparison of specimens and by use of keys. Microscopic examination followed preparing slides of fungi mounted in water or lactic acid. Measurements were made from fresh material mounted in water and any fungi found were recorded, identified to genus or species level and where possible photographed. The conidia and ascospores were measured at their widest and longest points.

A slide was prepared for one representative for each distinct fungal species. Slides were rendered semi-permanent by the addition of lacto-phenol and deposited the herbarium of MFLU.

## Data analysis

The number of fungal species was recorded and used to calculate for frequency of occurrence of each species for each sampling site. Biodiversity of fungi was assessed based on the following diversity indices: Margalef species richness (Da), Simpson's index (D), Shannon-Winner Index (H') and evenness indices (E) (Hyde & Sarma, 2006; Tsui *et al.*, 2003).

$$\text{Frequency (F)} = \frac{Ni \times 100\%}{N}$$

$$\text{Margalef index: Da} = (S-1) / \ln N$$

$$\text{Simpson's index: } D = \sum \frac{n(n-1)}{N(N-1)}$$

$$\text{Shannon index: } H' = - \sum_{i=1}^s P_i \ln P_i, \text{ where } P_i = N_i/N$$

$$\text{Evenness: } E = \frac{H'}{\ln N}$$

$n$  = number of occurrences of  $i$ th species;

$N$  = the total number of fungal occurrence;

$S$  = the number of species;

$P_i$  = the frequency of fungal species  $i$ th occurring on wood;

Based on the percentage occurrence of different species they were grouped as very frequent (> 10%), frequent (5-10%), infrequent (1-5%) and rare (< 1%).

## RESULTS

The fungal taxa identified from all submerged woody substrates at the three sites with their frequency of occurrence are listed in Table 2. The species richness, Margalef index ( $D_a$ ), Simpson's index ( $D$ ), Shannon-Weiner index ( $H'$ ) and Species Evenness ( $E$ ) were calculated and are also listed in Table 2. A total of 60 freshwater taxa were identified on the woody substrata including 29 ascomycetes, 27 anamorphic taxa and also 4 species whose taxonomic placement was uncertain. 39 taxa were identified from Site 1 (river near agricultural zone), 51 from Site 2 (waterfall with abundant trees) and 25 from Site 3 (waterfall with scrub). Most ascomycetes belonged to the families *Annulatascaceae*, *Halosarpheiaceae* and *Lophiostomaceae*. The most frequent genera of hyphomycetes were *Acrogenospora*, *Camposporium*, *Corynesporium* and *Helicomyces*. The most frequent taxa at each site differed, but some dominant species were common in all sites. In Site 1, *Corynesporium* sp. (9.2%), *Savoryella lignicola* (8.04%) and *Helicomyces roseus* (7.47%) were dominant species. *Fluminicola bipolaris* (10.23%), *Halosarpheia aquadulcis* (6.98%) and *Didymella aptrootii* (6.51%) were frequent in Huai Kang Pla Waterfall (Site 2). In Tat Thong Waterfall (Site 3), *Acrogenospora sphaerocephala* (12.39%), *Corynesporium* sp. (12.39%) and *Diaporthe* sp. (7.08%) were dominant (Table 2). Three frequent taxa (> 10%) common to all sites were *Acrogenospora sphaerocephala* (12.39%), *Corynesporium* sp. (12.39%) and *Fluminicola bipolaris* (10.23%). *Halosarpheia aquadulcis* was also present in the three sites, but it was not very frequent (< 10%). Other common species found at the three sites were *Acrogenospora sphaerocephala*, *Canalisporium caribense*, *Corynesporium* sp., *Didymella aptrootii*, *Fluminicola bipolaris*, *Halosarpheia aquadulcis*, *Helicomyces roseus*, *Massarina thalassioidea*, *Pseudohalonectria lignicola* and *Savoryella lignicola*.

Among the 60 taxa recorded, the highest number of taxa (51) was found at Huai Kang Pla Waterfall (Site 2). The highest diversity coincided with the observed species richness. The Evenness was least (0.671) at Site 1, while it was 0.735 at Site 2 indicating that there were less dominant species from Site 2. Site 3 showed the lowest Simpson's Index (0.053) and Shannon index (2.976) as well as the least species richness (Table 2).

Table 2. Frequency of occurrence of freshwater fungi at Amphoe Mae Chan,  
Chiang Rai, northern Thailand

Name of the species	Frequency of occurrence (%)		
	Site 1	Site 2	Site 3
<i>Aniptodera</i> sp.	4.32	1.86	–
<i>Acremonium</i> sp.	0.57	0.47	–
<i>Acrodictys erecta</i> (Ellis & Everh.) M.B. Ellis	0.57	0.47	–
<i>Acrogenospora sphaerocephala</i> (Berk. & Broome) M.B. Ellis	6.32	7.91	12.39
<i>Annulatascus velatisporus</i> K.D. Hyde	2.3	2.33	–
<i>Aquaphila albicans</i> Goh, K.D. Hyde & W.H. Ho	–	0.93	1.77
<i>Aquaticola</i> sp1.	4.02	5.12	–
<i>Aquaticola</i> sp2.	–	0.47	–
<i>Astrosphaeriella striatispora</i> K.D. Hyde & J. Fröhl.	1.15	1.86	2.65
<i>Astrosphaeriella</i> sp.2	1.15	1.86	–
<i>Astrosphaeriella stellata</i> (Pat.) Sacc.	2.3	2.79	4.42
<i>Berkleasmium</i> sp.	–	–	2.65
<i>Brachiosphaera tropicalis</i> Nawawi	1.15	–	–
<i>Brachysporiella guyana</i> M.B. Ellis	–	1.4	–
<i>Camposporium cambrense</i> S. Hughes	–	0.93	–
<i>Camposporium</i> sp.1	2.93	1.4	6.19
<i>Camposporium</i> sp.2	1.72	1.4	–
<i>Canalisporium caribense</i> Nawawi & Kuthub.	6.32	4.19	6.19
<i>Caryospora</i> sp.	–	0.93	–
<i>Cataractispora aquatica</i> K.D. Hyde, S.W. Wong & E.B.G. Jones	0.57	–	–
<i>Chalara</i> sp.	4.02	–	–
<i>Corynesporium</i> sp.	9.2	7.44	12.39
<i>Diaporthe</i> sp.	6.9	3.72	7.08
<i>Didymella aptrootii</i> K.D. Hyde & S.W. Wong	3.45	6.51	3.54
<i>Dictyosporium subtramianii</i> B. Sutton	2.3	2.33	–
<i>Ellisembia</i> sp.	1.15	–	1.77
<i>Endophragmiella</i> sp.	1.72	2.33	2.65
<i>Exserticlava</i> sp.	1.15	0.93	–
<i>Fluminicola bipolaris</i> S.W. Wong, K.D. Hyde & E.B.G. Jones	6.32	10.23	2.65
<i>Fusarium</i> sp.	0.57	–	–
<i>Gliocladium</i> sp.	–	0.93	–
<i>Gonytrichum macrocladum</i> M.B. Ellis	–	0.93	–
<i>Halosarpheia aquadulcis</i> S.Y. Hsieh, H.S. Chang et E. B. G. Jones	5.75	6.98	6.19
<i>Helicomyces roseus</i> Link	7.47	4.19	7.08
<i>Helicoon</i> sp.	–	0.93	–
<i>Leptosphaeria</i> sp.	–	0.93	–
<i>Lophiostoma frondisubmersum</i> Ces. & De Not	2.87	1.39	2.65
<i>Lophiotrema</i> sp.	0.57	–	–
<i>Mariannaea aquaticola</i> L. Cai & K.D. Hyde	1.15	1.4	–
<i>Massarina thalassioidea</i> K.D. Hyde & Aptroot	0.57	6.97	2.65
<i>Massarina</i> sp.1	–	0.47	0.88
<i>Melanochaeta</i> sp.	0.57	–	0.88
<i>Mycosphaerella</i> sp.	–	0.93	0.88
<i>Nawawia filiformis</i> M.B. Ellis	–	1.4	–

Table 2. Frequency of occurrence of freshwater fungi at Amphoe Mae Chan, Chiang Rai, northern Thailand

Name of the species	Frequency of occurrence (%)		
	Site 1	Site 2	Site 3
<i>Nectria</i> sp.	–	1.4	–
<i>Ophiobolus</i> sp.	0.57	0.47	–
<i>Ophioceras commune</i> Shearer, J.L. Crane & W. Chen	3.45	2.79	2.65
<i>Pleospora</i> sp.	–	2.32	0.88
<i>Pseudohalonectria lignicola</i> Minoura & Muroi	–	6.04	–
<i>Savoryella lignicola</i> E.B.G. Jones & R.A. Eaton	8.04	5.58	4.42
<i>Stilbella</i> sp.	2.87	3.72	7.96
<i>Teichospora</i> sp.	0.57	0.47	–
<i>Tetraploa aristata</i> Berk. & Broome	1.15	–	–
<i>Trichoderma</i> sp.	–	0.93	–
<i>Tubeufia</i> sp.	–	0.93	–
<i>Xylomyces chlamydosporus</i> Goos, R.D. Brooks & Lamore	1.15	2.33	–
Unidentified sp.1	–	0.47	–
Unidentified sp.2	0.57	–	–
Unidentified sp.3	–	0.47	0.88
Unidentified sp.4	–	0.47	–
Total number of species	191	272	121
Margalef index (Da)	7.2349	9.0977	5.212
Simpson's index (D)	0.04	0.0338	0.0535
Shannon-Weiner index (H')	3.5252	4.1196	2.9768
Species Evenness (E)	0.6711	0.7348	0.632

## DISCUSSION

### Fungal communities at the three sites

Thailand has many widely distributed freshwater fungi, such as *Massarina thalassioidea*, *Halosarphaea aquadulcis* and *Helicomyces roseus* (Sivichai *et al.* 2000; 2002). These species were also recorded in this study. It should be noted however that some species such as *Didymella aptrootii*, *Fluminicola bipolaris* and *Pseudohalonectria lignicola* (ascomycetes) are new records in Thailand. Species composition was very diverse in all sites and all communities consisted of a large number of infrequent species and a few dominant species.

The species richness in the streams studied was higher than that found in temperate streams. Similar conclusions have previously been reported. Goh & Hyde (1996) discussed the differences between tropical and temperate stream fungi and showed that a higher diversity occurred in streams in the tropics. Hyde & Goh (1999) studied submerged wood in an English stream and found that this temperate stream had a lower fungal diversity (25 species) than tropical streams. Hyde & Goh (1998a) identified 39 taxa from Lake Barrine in Australia, while 34 taxa were recorded from Seychelles (Hyde & Goh, 1998b) and 58 taxa from a stream in South Africa (Hyde *et al.*, 1998).

### Species diversity in relation to riparian vegetation

The diversity of freshwater fungi can be affected by many factors, such as pH (Dangles & Chauvet, 2003; Kladwang *et al.*, 2003), seasonality (Ho *et al.*, 2002), salinity (Cai *et al.*, 2002) and incubation methods (Sridhar *et al.*, 2010). Raja *et al.* (2009a) have investigated the latitudinal distribution patterns of these fungi in northern and southern sites of Florida Peninsula and showed that both latitude and pH are important factors to the distribution patterns of freshwater ascomycetes. The effects of seasonality on freshwater lignicolous fungi has been investigated in Hong Kong and it was found that higher species richness, fewer dominant fungi and more infrequent fungi occurred on submerged wood during the hot wet season than the cool dry season (Ho *et al.*, 2002). However, few studies have examined the effect of riparian vegetation (Vijaykrishna & Hyde, 2006). The three sites studied were near by locations and pH, temperature and dissolved oxygen were similar (Table1). Samples collected from the three sites were incubated in the same way. The riparian vegetation at each site however, differed (agricultural rice fields vs. wood vs. scrub). The highest species diversity was found at the site with abundant trees (Site 2), followed by the agricultural zone (Site 1), while the scrub zone (Site 3) had least diversity. Vijaykrishna & Hyde (2006) studied freshwater in tropical Australia and found that the highest species diversity was in the pristine forest zone, followed by agricultural zone, while the least diverse was in the re-growth zone. In conclusion we identified different freshwater fungal communities from three sites and the differences are probably due to the differences in riparian vegetation.

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