Fungal succession on senescent leaves of *Castanopsis diversifolia* in Doi Suthep-Pui National Park, Thailand

Duong, L.M.[1,2,4]*, McKenzie, E.H.C.[3], Lumyong, S[4] and Hyde, K.D.[5,6]

[1]Department of Microbiology and Biotechnology, Faculty of Biology, Hanoi National University of Education, 136 Xuanthuy, Caugiay, Hanoi, Vietnam
[2]Mushroom Research Centre, 128 Bahn Phadeng, Papae, Maetaeng, Chiang Mai 50150, Thailand
[3]Landcare Research, Private Bag 92170, Auckland, New Zealand
[4]Department of Biology, Faculty of Science, Chiang Mai University, 50200 Chiang Mai, Thailand
[5]School of Science, Mae Fah Luang University, Tasud, Chiang Rai 57100, Thailand
[6]International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Balongsi, Kunming 650224, PR China

Duong, L.M., McKenzie, E.H.C., Lumyong, S. and Hyde, K.D. (2008). Fungal succession on senescent leaves of *Castanopsis diversifolia* in Doi Suthep-Pui National Park, Thailand. Fungal Diversity 30: 23-36.

Senescent leaves of *Castanopsis diversifolia* were collected and subjected to different treatments to follow fungal colonization and succession. Sixty sterilized leaves were hung 4 metres above the ground under the *C. diversifolia* canopy. Sixty sterilized leaves and 60 unsterilised leaves were laid on the forest floor. Ten leaves were sterilized and incubated in plastic containers as a control for sterility. The study yielded 112 taxa (19 ascomycetes, 4 basidiomycetes, 1 myxomycete and 88 anamorphic taxa) during the 4-month incubation period. The sterile hanging leaves harboured the highest diversity with 65 taxa, while unsterile leaves on the forest floor yielded 55 taxa and sterile leaves on the forest floor yielded 53 taxa. Highest fungal diversity was reached at different times for each treatment. Fungal distribution patterns, origins and functional roles in leaf decomposition are discussed.

Keywords: fungal ecology, leaf litter fungi, microfungi

Article information
Received 7 September 2007
Accepted 3 January 2008
Published online 31 May 2008
*Corresponding authors: Lam Minh Duong; e-mail: duong.minhlam@gmail.com
K.D. Hyde; e-mail: kdhyde1@gmail.com

Introduction

Leaf litter is an important component of all ecosystems, being a major source of soil organic matter (Reed *et al*., 2007). Leaf litter provides a protective layer against microhabitat fluctuations, erosion and soil compaction and creates a microclimate that is favorable for fungal fruiting-body production. Removal of litter affects fungal growth and diversity (Eaton *et al*., 2004; Sayer, 2005).

Fungal succession is the sequential occupation of the same site by thalli either of different fungi or of different associations of fungi (Rayner and Todd, 1979). This term, however, has recently been questioned by Fryar (2002) who concluded that a sequence of fruiting-bodies would be a more accurate description in succession studies. Sequence of fruiting-bodies on litter as a measure of fungal succession has been used by some authors (e.g. Yanna *et al*., 2002; Tang *et al*., 2005). Fungal succession has been divided into different types, e.g. substratum succession (Prach *et al*., 2007) and resource (mixed substrates) succession (Frankland, 1998; Twieg *et al*., 2007). Suzuki *et al*. (2002) distinguished between micro-scale (substratum) and macro-scale (the whole community level or ecological level) successions. Fungal successsion studies have been carried out at both levels and were reviewed by Frankland (1998).

Fungal successions have been studied mainly on substrata and environments in

temperate forests. Successions of fungal communities on decaying leaves have been studied by e.g. Bonet *et al*. (2004), Osono, (2005); Twieg *et al*., 2007. Only a few such successional studies have been carried out in tropical and subtropical areas (Promputtha *et al*., 2002; Tang *et al*., 2005; Paulus *et al*., 2006).

Castanopsis diversifolia forms part of the evergreen tropical forests of northern Thailand. The fungi occurring on *Castanopsis* have been documented with about 360 records worldwide of 220 species on 35 different *Castanopsis* species (Farr *et al*., 2008). Tang *et al*. (2005) reported 38 taxa during a successional study of fungi on decaying leaves of *Castanopsis fissa* in Hong Kong. To date, only 13 taxa (*Acremonium* sp., *Beltrania rhombica*, *Beltraniella portoricensis*, *Chaetopsina fulva*, *Cladosporium* sp., *Dictyochaeta* sp., *Emarcea castanopsidicola*, Hypocreales sp., *Idriella lunata*, *Lophodermium* sp., *Ophioceras* sp., *Selenosporella curvispora* and *Zygosporium echinosporum*) have been recorded on *C. diversifolia* (Duong *et al*., 2004a, b). In the present study fungal succession was followed on senescent leaves of *C. diversifolia* during the decay process. The aims of this study were to establish: 1) the composition of fungal communities during *C. diversifolia* leaf decay; 2) the species richness at different stages of leaf decay; 3) the fungal distribution on different parts of the leaf (e.g. lamina, petiole, midrib; substrate specificity); and 4) the origin of fungi involved in leaf decomposition.

Materials and Methods

Collecting site and experiment design

The leaf samples were collected at Doi Suthep-Pui National Park, 1146 metres above sea level, 18º48.402′ North, 98º54.617′ East, Chiang Mai, Thailand.

Castanopsis diversifolia (Kurz) King ex Hook. f. is a tree to 10-20 m high. Leaves are partly deciduous, 8-24 × 4-12 cm, ovate, or oblong with tapering tips and a rounded or slightly pointed base, smooth or with scattered teeth near the apex. Young leaves are brown and hairy, mature leaves are leathery, smooth above, and usually sparsely hairy, at least on the midrib below. The petioles are 1.2-2 cm long and stout.

Four individual *Castanopsis diversifolia* trees were selected on the basis of adequate leaf fall. One-hundred and ninety recently fallen senescent, partly green leaves were collected from the forest floor on 23 June 2004. Sixty leaves were air-dried (unsterilised), the other 130 leaves were sterilised in an oven at 70ºC for 7 days. On 30 June 2004, 15 sterilised and 15 unsterilised leaves were placed on the ground under each of the four selected trees. Fifteen sterilised leaves were hung individually in each *C. diversifolia* tree, about 4 m above the ground. Leaves were tied to a thin, red, plastic thread and fastened with nails to the tree branches. The remaining ten sterile leaves were incubated separately for 10 days in sterile Zip-Lock plastic bags containing sterile tissue paper wetted with sterile water to serve as controls. All leaves on the ground were marked with thin strips of blue plastic so they could later be recognised in the field. Three leaves from each treatment and from each tree, were collected on days 7, 21, 51, 86, and 120 after the start of the experiment. They were placed in separate sterile plastic bags moistened with sterile tissue paper. Fungi were observed and examined during 12 days of incubation.

Fungal examination

The aim of fungal observation was to examine fungi occurring on leaf litter at the time when they were collected. Two-days of incubation induced sporulation of fungi on the samples. All the leaves were examined within 12 days. Different parts of the leaf (blade vs. petiole vs. midrib) were checked for ascomata and colonies under a stereomicroscope (Olympus SZH10). Morphological characters were described from slides mounted in sterile water and observed by differential interference microscopy. Semi-permanent slides were prepared for representative fruiting structures of each distinct fungal taxon, mounted in 90% lactophenol and sealed with nail varnish.

Isolation and herbarium specimens

Single-spore isolation was undertaken for each fungal taxon detected following the method of Goh (1999). Herbarium specimens

were prepared by removing a section of the leaf on which fungal colonies occurred, and drying at 37°C for 3 days. Specimens of new taxa were deposited at Mushroom Research Centre Herbarium (MRC).

Statistical analysis

Each fungal taxon was recorded on a leaf as being present or absent. The number of occurrences of each taxon was counted, and the frequency of occurrence of a fungus was calculated for leaves at each decay stage using the following formula.

$$\text{Occurrence frequency of taxon A} = \frac{\text{occurrence of taxon A}}{\text{number of leaves examined}} \times 100$$

Taxa with a percentage of occurrence higher than, or equal to 10% were considered to be common species. The overlap and complementarity of microfungi from different leaf treatment types and different decay stage in the study were calculated using the Sørensen quotient:

$$\text{Overlap (\%)} = \frac{\text{number of taxa shared between A and B}}{\text{total number of taxa observed in A and B}} \times 100$$

$$\text{Complementarity (\%)} = 100 - \text{overlap}$$

in which A denotes the number of microfungal species in one kind of leaf treatment or decay stage and B denotes the number of microfungal species in another leaf treatment type or decay stage.

Species diversity was measured using species diversity indices (species richness and species evenness). Species richness refers to the number of species in a community and species evenness refers to the contribution (relative abundance or equability) of individuals. The Shannon diversity is often used to estimate species diversity in a given community; it comprises two different indices. The Shannon diversity index accounts for abundance of species present (H). This depends much upon the species number in the community. The Shannon evenness accounts for equability of the species present (E). Shannon evenness ranges from 0 to 1. A community with a Shannon evenness equal to 1 means that every species in the community has the same frequency of occurrence. The Shannon diversity index is calculated according to the formula:

$H = -\sum p_i . \ln p_i$ (p_i: proportion of i^{th} species).

The Shannon evenness is calculated according to the equation:

$E = H/\ln S$ (S: total species number).

Similarities among the fungal communities from different leaf treatment types and different stages of decay were estimated using cluster analysis, using the PC-ORP program (McCune and Mefford, 1999) and paired t-test. Shannon indices were used to estimate species diversity of microfungi on *Castanopsis diversifolia*.

Results

Total fungal numbers on the leaf treatments

No fungal taxa were observed on the 10 sterile leaves in the control experiment, which indicates that sterilization was effective.

A total of 180 *Castanopsis diversifolia* leaves from the three treatments were examined and 799 identifications of 112 fungal taxa were made. An average of 4.44 fungi were identified from each leaf. A list of species encountered and their frequencies are given in Table 1. Common species with frequencies of occurrence ≥ 10% were *Idriella* sp. (28%), *Subulispora procurvata* (20%), *Zygosporium gibbum* (19.5%), *Beltrania rhombica* (18%), *Parasympodiella laxa* (17%), *Cladosporium oxysporum* (15.5%), *B. portoricensis* (15%), *C. sphaerospermum* (14.5%), *Subramaniomyces fusisaprophyticus* (13.5%), Coelomycete sp. 6 (11%), *Beltraniella odinae* (10.5%), *Chalara pteridina* (10.5%), and Coelomycete sp. 2 (10.5%).

The Sørensen comparisons between the three treatments are presented in Table 2. Fungal communities from each leaf treatment and from all leaves were analysed. Diversity indices (Shannon diversity and evenness indices) are shown in Table 3. The highest diversity of fungi during leaf decomposition was observed on day 7 for sterile hung leaves, and on day 51 for sterile and unsterile leaves on the forest floor (Table 4).

Table 1. List of species found from three treatments of *Castanopsis diversifolia* leaves (Numbers refer to the number of times that each fungus was encountered; maximum 60 per treatment).

Species	Sterile hanging leaves		Sterile leaves on the ground		Unsterile leaves on the ground	
	Petioles and midribs	Leaf blades	Petioles and midribs	Leaf blades	Petioles and midribs	Leaf blades
Acremonium sp.		1			5	7
Albonectria albosuccinea						2
Alternaria sp.		1				
Annulatascaceae sp.						2
Ardhachandra cristaspora						2
Arthrinium sp.						9
Aspergillus sp.		1				
Asterina sp.		2				
Bacillispora aquatica	6					
*Beltrania mangifera*e						1
Beltrania rhombica		1		14		17
Beltraniella odinae				2		11
Beltraniella portoricensis		4		14		9
Beltraniella sp.						1
Bipolaris cynodontis		1		1		
Cercosporula sp.		1				
Chaetendophragmia triangularia				1		
Chaetosphaeria sp.				2		1
Chalara pteridina		6		9		4
Cladosporium cladosporioides		1				
Cladosporium oxysporum		26		3		
Cladosporium sp. 1		1				
Cladosporium sp. 2						3
Cladosporium sphaerospermum		24		1		1
Cladosporium tenuissimum		1				
Clonostachys candelabrum				1		
Clonostachys compactiuscula		6		5		3
Coelomycete sp. 1		3		3		13
Coelomycete sp. 2						8
Coelomycete sp. 3	8				4	
Coelomycete sp. 4		5		3		12
Coelomycete sp. 5				2		
Coelomycete sp. 6				2		4
Coelomycete sp. 7				2		3
Coelomycete sp. 8						1
Cryptophiale udagawae				6		8
Cylindrocladium gracile	2	1		10		
Cylindrocladium pseudogracile						1
Cylindrum griseum		5		1		
Dendrodochium cylindricum		2		1		4
Dictyochaeta cylindrospora		2				
Dictyochaeta heteroderae				1		
Dictyochaeta simplex		2		9		3
Dictyochaeta sp. 1				1		
Dictyochaeta sp. nov.						7
Dictyochaeta stipiticolla		1		4		
Dictyochaeta-like	1					
Emarcea castanopsidicola		7				3
Endophragmiella sp.		3				
Fusarium sp.		3				
Geotrichum candidum		1		2		
Gnomonia gnomon					5	1
Hansfordia sp.		1				3
Haplographium sp.		1		1		
Helicosporium talbotii				3		1
Hyponectria buxi				3		2
Idriella fertilis		1		4		

Table 1(continued). List of species found from three treatments of *Castanopsis diversifolia* leaves (Numbers refer to the number of times that each fungus was encountered; maximum 60 per treatment).

Species	Sterile hanging leaves		Sterile leaves on the ground		Unsterile leaves on the ground	
	Petioles and midribs	Leaf blades	Petioles and midribs	Leaf blades	Petioles and midribs	Leaf blades
Idriella sp. 1	1	11		17	2	19
Idriella sp. 2		1				
Kionochaeta spissa				8		2
Kramasamuha sibika		2				
Lachnum sp.						1
Lauriomyces bellulus	4		1			
Lecanicillium lecanii		1		1		15
Lichenopeltella salicis		1				
Lophodermium australiense						2
Lophodermium sp.						3
Marasmius sp. 1				2	1	1
Marasmius sp. 2				1		
Menisporopsis novaezelandiae				11		2
Microdochium phragmitis		2		10		
Microthyrium sp.	1					
Monochaetia sp.		3				
Mycena sp. 1				1		1
Mycena sp. 2				1		
Mycosphaerella sp.		6				4
Oedocephalum sp.		4				
Ophioceras commune				4		11
Parasympodiella laxa	3	20			1	6
Penicillium sp.		2		2		9
Periconia cookei		3				
Periconia paludosa						4
Periconia sp. 1		1				
Periconia sp. 2						6
Pestalosphaeria hansenii		3				
Pestalotiopsis tecomicola		11				
Phaeoisaria sp.				2		
Phomopsis sp.		1		2		
Pithomyces karoo		3				
Pseudobotrytis terrestris				1		
Pseudohalonectria phialidica				7		
Ramularia gei		6				
Stemonitis sp.				1		3
Stictis sp. nov.						3
Stomiopeltis sp.						3
Subramaniomyces fusisaprophyticus		11		10		3
Subulispora procurvata		11	1	16		8
Thozetella sp.		1		4		
Thysanophora sp.		1				
Tritirachium bulbophorum	1					
Unidentified hyphomycete sp. 1		4				
Unidentified ascomycete sp. 1						1
Unidentified ascomycete sp. 2		1				
Unidentified hyphomycete sp. 2						1
Verticillium sp.				1		
Wiesneriomyces javanieus		1		3		
Xenocylindrocladium sp.	1		3			
Zygosporium gibbum		35				
Zygosporium minus		1				

Table 2. Pair-wise overlapping fungi from three *Castanopsis diversifolia* leaf treatments. Singlets were not excluded from the analysis.

	Sterile leaves on the ground	Unsterile leaves on the ground
Sterile hanging leaves	21.2	15
Sterile leaves on the ground		21.3

Table 3. Diversity indices of fungal communities on decaying leaves of *Castanopsis diversifolia*. Petiole and midrib (PM) and leaf blade (L).

Treatment	All treatments	Sterile hanging leaves		Sterile leaves on the ground		Unsterile leaves on the ground	
Leaf part	All components	PM	L	PM	L	PM	L
Shannon diversity (H)	4.135	1.986	3.393	1.122	3.521	1.611	3.625
Shannon evenness (E)	0.876	0.862	0.836	0.865	0.895	0.899	0.909

Table 4. Diversity indices of fungi from three treatments during decomposition of *Castanopsis diversifolia* leaves.

Day	Sterile hanging leaves		Sterile leaves on the ground		Unsterile leaves on the ground	
	Shannon diversity (H)	Shannon evenness (E)	Shannon diversity (H)	Shannon evenness (E)	Shannon diversity (H)	Shannon evenness (E)
7	3.189	0.928	2.918	0.958	2.2	0.955
21	2.945	0.915	2.919	0.944	3.16	0.929
51	3.089	0.917	3.267	0.97	3.235	0.942
86	2.905	0.914	3.188	0.956	3.127	0.96
120	2.731	0.963	2.99	0.953	2.848	0.95

Fungal communities on different leaf treatments

The fungal communities observed in the three leaf treatments were different in species composition and species abundance. The common species of different leaf treatments are listed in Table 5.

In total, 65 taxa (6 ascomycetes and 59 anamorphic taxa) were encountered on the 60 sterilized leaves hanging in the canopy (291 identifications). Twenty-nine taxa (44.5%) were encountered once (singlets). Ten out of 65 taxa were collected on the petioles and midribs (27 identifications) (Table 1). Sixteen taxa were common throughout the study period with a percentage occurrence higher than 10%, the most common of which were *Zygosporium gibbum* (58.5%), *Cladosporium oxysporum* (43.5%), *C. sphaerospermum* (40%), and *Parasympodiella laxa* (38.5%) (see Table 1).

Fifty-three fungal species were identified on the sterile leaves of *Castanopsis diversifolia* on the ground (227 identifications) comprising 6 ascomycetes, 4 basidiomycetes, 1 myxomycete, and 42 anamorphic taxa. Three of these species were found on petioles and midribs, *Lauriomyces bellulus* (exclusively), *Subulispora procurvata* and *Xenocylindrocladium* sp. Seventeen singlets (32%) and 10 doublets (19%) were found from these samples. Thirteen of these species were common with a percentage occurrence equal to or higher than 10% (Table 1).

On the 60 unsterile leaves on the ground, 55 fungal species were identified (281 identifications), comprising: 14 ascomycetes, 2 basidiomycetes, 1 myxomycete, and 38 anamorphic fungi. Six species, *Acremonium* sp., *Coelomycetes* sp. 5, *Gnomonia gnomon*, *Idriella* sp., *Marasmius* sp. and *Parasympodiella laxa* were also found on petioles and midribs. Twelve singlets (22%) and 6 doublets (11%) were found. Seventeen common species with more than 10% frequency were: *Idriella* sp. 1 (31.5%), *Beltrania rhombica* (28.5%), *Lecanicillium lecanii* (25%), Coelomycete sp. 2 (21.5%), *Acremonium* sp. (20%), Coelomycete sp. 6 (20%), *Beltraniella odinae* (18.5%), *Ophioceras commune* (18.5%), *Arthrinium* sp. (15%), *Beltraniella portoricensis* (15%),

Table 5. Common species on different treatments of *Castanopsis diversifolia* leaves (percentage occurrence).

Species	Sterile hanging leaves (%)	Sterile leaves on the ground (%)	Unsterile leaves on the ground (%)
Acremonium sp.	1.5	-	20
Arthrinium sp.	-	-	15
Bacillispora aquatica	10	-	-
Beltrania rhombica	1.5	23.5	28.5
Beltraniella odinae	-	3.5	18.5
Beltraniella portoricensis	6.5	23.5	15
Chalara pteridina	10	15	6.5
Cladosporium oxysporum	43.5	5	-
Cladosporium sphaerospermum	40	1.5	1.5
Clonostachys compactiuscula	10	8.5	5
Coelomycete sp. 2	5	5	21.5
Coelomycete sp. 3	-	-	13.5
Coelomycete sp. 5	13.5	-	6.5
Coelomycete sp. 6	8.5	5	20
Cryptophiale udagawae	-	10	13.5
Cylindrocladium gracile	5	16.5	-
Dictyochaeta simplex	3.5	15	5
Dictyochaeta sp. 2	-	-	11.5
Emarcea castanopsidicola	11.5	-	5
Idriella sp. 1	20	28.5	31.5
Kionochaeta spissa	-	13.5	3.5
Lecanicillium lecanii	1.5	1.5	25
Menisporopsis novaezelandiae	-	18.5	3.5
Microdochium phragmitis	3.5	16.5	-
Mycosphaerella sp.	10	-	6.5
Ophioceras commune	-	6.5	18.5
Parasympodiella laxa	38.5	-	10
Penicillium sp.	3.5	3.5	15
Periconia sp. 2	-	-	10
Pestalotiopsis tecomicola	18.3	-	-
Pseudohalonectria phialidica	-	11.5	-
Ramularia gei	10	-	-
Subramaniomyces fusisaprophyticus	18.5	16.5	5
Subulispora procurvata	18.5	26.5	13.5
Zygosporium gibbum	58.5	-	-

Penicillium sp. (15%), Coelomycete sp. 3 (13.5%), *Cryptophiale udagawae* (13.5%), *Subulispora procurvata* (13.5%), *Dictyochaeta* sp. 2 (11.5%), *Parasympodiella laxa* (10%), and *Periconia* sp. 2 (10%).

Similarity analysis

The leaves were divided into three subsets with different treatments, with the objective of establishing the origins of the fungal communities. The fungal communities differed in treatments. Overlap of fungal communities in the three leaf treatments, from the first to the last collecting time of sterile hung leaves, sterile and unsterile leaves on the forest floor are shown in Figures A-D. Species compositions from three leaf treatments were not significantly different [*p* value from paired t-test of sterile hung leaves (SH), sterile leaves on the forest floor (SF) and unsterile leaves (UF): SH-SF, SH-UF and SF-UF were 0.204, 0.483 and 0.131, respectively)]. However, species compositions of different collecting times during leaf decomposition differed significantly from one to another (Table 6).

Table 6. Paired t-test of fungal communities occurring on *Castanopsis diversifolia* leaves (Singletons were excluded from the analysis).

		Day 7	Day 21	Day 51	Day 86	Day 120
Sterile hanging leaves	Day 7	1	0.259	0.155	0.5	0.0037
	Day 21		1	0.295	0.304	0.01
	Day 51			1	0.153	0.0118
	Day 86				1	0.0005
	Day 120					1
Sterile leaves on the ground	Day 7	1	0.19	0.13	0.039	0.323
	Day 21		1	0.35	0.14	0.172
	Day 51			1	0.162	0.106
	Day 86				1	0.0185
	Day 120					1
Unsterile leaves	Day 7	1	0	0	0.0014	0.029
	Day 21		1	0.287	0.0337	0.015
	Day 51			1	0.0059	0.0043
	Day 86				1	0.2055
	Day 120					1

Discussion

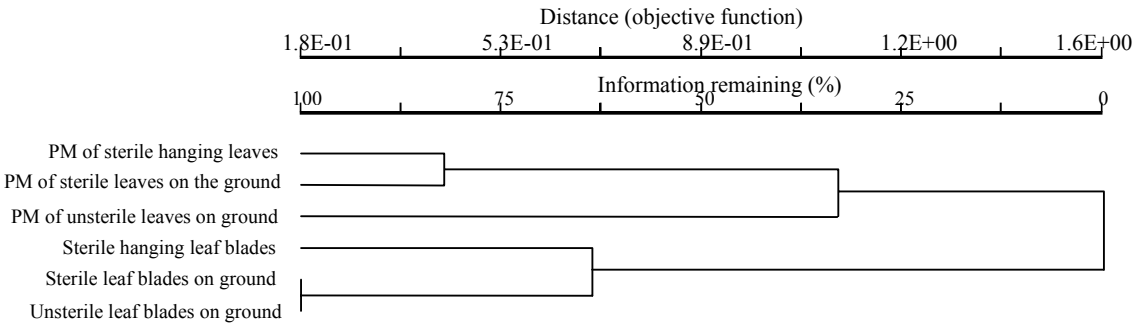
Fungal diversity on Castanopsis diversifolia leaf litter

In studies of fungal diversity on leaf litter, different methodologies have repeatedly led to different communities. Indirect isolation approaches often result in more taxa (including non-sporulating morphospecies) than direct observation (Bills and Polishook, 1994a; Bills, 1996; Polishook *et al*., 1996; Paulus and Hyde, 2003). Indirect isolation methods have been criticized by some authors (Paulus and Hyde, 2003) because they recover both active fungi and dormant spores that do not participate in leaf decay processes. For this reason we chose incubation of exposed leaves and assessment of only those fungi that show active growth on the natural substrate.

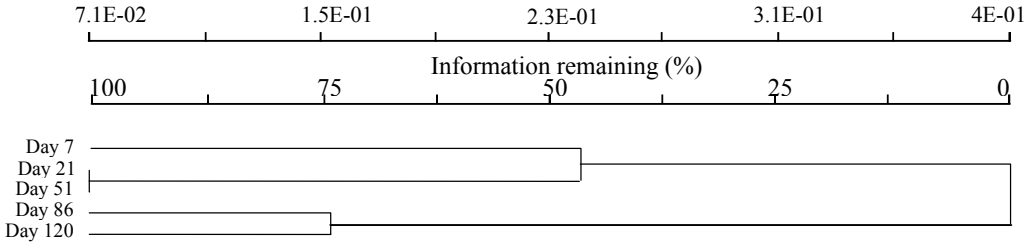
In this study, 180 senescent leaves of *Castanopsis diversifolia* were examined and 112 fungal taxa were encountered. This is a relatively high diversity in comparison with previous studies on other hosts (Bills and Polishook, 1994b; Promputtha *et al*., 2002; Tang *et al*., 2005; Paulus *et al*., 2006). Promputtha *et al*., (2002) studied the fungal succession on decaying leaves of *Manglietia garrettii* (*Magnoliaceae*) at Doi Suthep-Pui National Park and identified only 22 fungal taxa. On senescent leaves of *Castanopsis fissa*, allowed to decompose for 4 months in Hong Kong Island, Tang *et al*. (2005) encountered 38 fungal taxa. A different array of species occur on *Castanopsis fissa* (in Hong Kong) and *C. diversifolia* (in Thailand) with only three overlapping taxa (*Beltrania rhombica*, *Cryptophiale udagawae*, and *Subulispora procurvata*). These three species are common leaf litter fungi (Polishook *et al*., 1996; Parungao *et al*., 2002; Duong *et al*., 2004a; Rambelli *et al*., 2004; Paulus *et al*., 2006). On *Ficus pleurocarpa* (*Moraceae*) in northern Queensland, Paulus *et al*. (2006) found 104 fungal taxa using a direct observation method, but this included only four taxa that were also identified in the present study (*Beltrania rhombica*, *Beltraniella portociensis*, *Microdochium phragmitis* and *Wiesneriomyces laurinus*).

Fungal diversity and origin on different leaf treatments

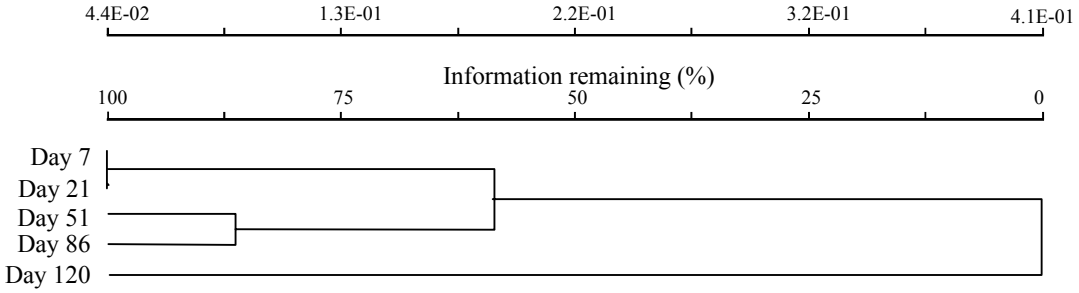
The highest diversity was found on the sterile hanging leaves (65 species), followed by the unsterile leaves placed on the forest floor (55 species), with lowest diversity on the sterile leaves on the forest floor (53 species). After leaf fall many competitors from different sources would be expected to colonize leaves that lie on the ground. Hanging leaves would, however, be colonized either by airborne or by rain-splash translocated inocula from the canopy. So far no studies of phyllosphere fungi have been carried out in Doi Suthep-Pui National Park.



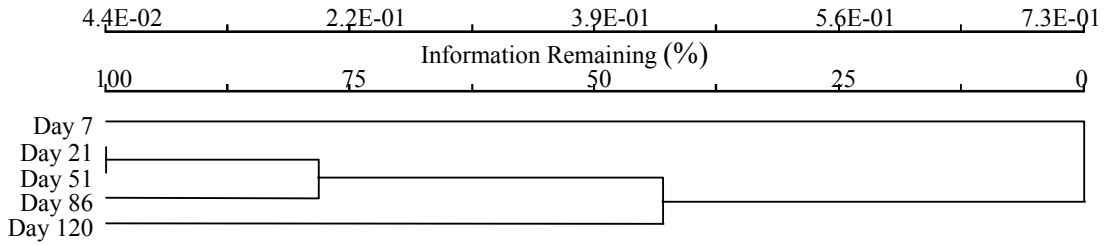
A. Sørensen coefficient and group average method of fungal communities on different leaf tissue types of *Castanopsis diversifolia*. Petiole and midrib (PM).



B. Sørensen coefficient and group average method of fungal communities on sterile hung leaves during leaf decomposition.



C. Sørensen coefficient and group average method of fungal communities on sterile leaves on the forest floor during leaf decomposition.



D. Cluster analysis using Sorensen coefficient and group average method of fungal communities on unsterile leaves on the forest floor during leaf decomposition. Singletons are excluded.

Figs A-D. Cluster analysis of fungal communities of different treatment during leaf decomposition.

The high diversity in fungal communities on sterile hanging leaves requires an explanation. The hanging leaves receive less water than those on the ground, even in conditions of high humidity and rain. Thus, they decay more slowly than leaves on the ground. The most common species in all studied periods were *Cladosporium oxysporum*, *C. sphaerospermum*, *Zygosporium gibbum*, and *Parasympodiella laxa*. Many rare fungi were also found on hanging leaves. The high diversity of fungi on hanging leaves may have been due to limited moisture that prevented extensive growth of fungal colonies and hence less interaction and competition between taxa. The high number of fungi recorded on hanging leaves may reflect a high diversity of canopy fungi in Doi Suthep-Pui National Park, a hypothesis that requires further study.

Fungal communities at different decaying stages

Succession of fungal communities is often divided into early, middle and late decomposers (Frankland 1998; Tang *et al*., 2005; Paulus *et al*., 2006). Our findings support the distinction of different stages of the leaf decay process (Figs B, C, D). Only a few taxa overlapped at the different collecting times. Significant differences in fungal communities during leaf decomposition were, however, only found at certain stages within a treatment. Fungal communities in sterile hanging leaves differed significantly when day 7 was compared to day 120 (Table 6), while those of sterile leaves on the ground changed significantly between day 86 and day 120 ($p = 0.018$, Table 6). Fungal communities on unsterile leaves on the ground changed significantly at each sampling (Table 6, Fig. D).

During decomposition of *C. diversifolia* leaves on the ground four different communities could be distinguished, corresponding with different phases of decay. Fungal communities on sterile leaves on the forest floor changed over time, representing four stages of decay (Fig. C). The changes, however, were not statistically supported ($p > 0.05$, Table 6). Fungal communities at day 7 and 21 grouped together, including fungi representing the early periods of leaf decay. Fungi on unsterile leaves grouped into four groups, in which fungi that were encountered on day 21 and 51 grouped together. Fungi on day 7 included early decomposers *Beltrania odinae*, *Mycosphaerella* sp., *Ophioceras commune* and *Stomiopeltis* sp., which might have been endophytic fungi that changed their life styles to decomposers. A change of endophytic fungi to saprobes has been observed in previous studies (Frankland, 1998; Tang *et al*., 2005). Fungal communities on day 21 and 51 grouped together as a second group of decomposers during leaf decay process.

The dominant species on leaves differed with treatments (Table 5). The dominant species on the sterilized hanging leaves included *Bacillispora aquatica*, *Cladosporium oxysporum*, *C. sphaerospermum*, *Pestalotiopsis tecomicola* and *Zygosporium gibbum*. These species were not present or present at very low frequencies on leaves placed on the forest floor. Among the fungi colonising hanging leaves, *Cladosporium* spp. and *Zygosporium* spp. are considered to be typically airborne, while *Bacillispora aquatica* and *Pestalotiopsis temicola* are more likely to be splash-inoculated. Shortly after rain the hanging leaves dry out and expose the fungi to drought stress. Thus, colony expansion is limited and a dense mosaic of colonies of different species can develop. *Cladosporium* species, in particular, are well-known airborne fungi and they were abundant in the site studied (Table 1). *Pestalotiopsis* species are common pathogenic, phyllosphere or endophytic taxa (Osono, 2002, 2007). *Zygosporium* species are also airborne, and have been well studied (Paulus *et al*., 2006; Rao, *et al*., 2007; Tsai *et al*., 2007).

Arthrinium sp., *Beltrania rhombica*, Coelomycete sp. 3, *Cryptophiale udagawae*, *Dictyochaeta* sp. 2, *Ophioceras commune* and *Periconia* sp. 2 were dominant species on leaves on the forest floor and not encountered on hanging leaves. *Arthrinium* sp. was abundant on unsterilized leaves in this study, but not on sterile hanging leaves. *Beltrania rhombica*, *Cryptophiale*, *Dictyochaeta* and *Periconia* spp. have been encountered on leaf litter in previous studies (Polishook *et al*., 1996; Parungao *et al*., 2002; Tempesta *et al*., 2003; Rambelli *et al*., 2004; Tang *et al*., 2005; Paulus *et al*., 2006).

Basidiomycetes were found only on leaves on the forest floor (both sterile and unsterile) at late stages of decay. Basidiomycetes have been shown to be late litter decomposers in previous studies in temperate forests (Watkinson *et al*., 2006; Osono, 2007) and are well-known to be present as mycelium or rhizomorphs in the soil (Anderson *et al*., 2001; Lamour *et al*., 2007). *Marasmius* spp. and *Mycena* spp. were found on leaves in the present study and are common in litter in northern Thailand forests (Wannathes *et al*., 2004). Basidiomycetes decompose polysaccharides, lignin and litter phenolics (Ghosh *et al*., 2003) and have strong competetive capacities (Frankland, 1998). In previous studies of fungi occurring on leaf litter in tropical forests no basidiomycetes were recorded (Promputtha *et al*., 2002, 2004; Tang *et al*., 2005), although they may have been present as mycelium or rhizomorphs. Competition by basidiomycetes may account for the reduced number of micro-fungi on leaves on the ground (Frankland, 1998; Kwaśna, 2003).

The lowest fungal diversity was found on sterile leaves on the forest floor. Sterilization of recently dead leaves eliminates the endophytes and, therefore, taxa found on these leaves must arrive from outside. Various factors influence fungal attachment and germination on leaves in the phyllosphere. Living leaves of *C. diversifolia* are protected from fungal colonization by various mechanisms such as cutin, hairs, and active metabolites (phenolic compounds). Fungal communities on unsterile leaves can originate from endophytes, pathogens, air, and soil. In this situation, a higher number of fungi might be expected. The fungi that were present only on unsterile leaves on the forest floor may have been endophytes or leaf pathogens. They include the ascomycetes *Albonectria albosuccinea*, Annulatascaceae sp., *Lachnum* sp., *Lophodermium australiense*, *Lophodermium* sp., *Stictis* sp., and the anamorphic ascomycetes *Ardhachandra cristapora*, *Arthrinium* sp., *Cylindrocladium pseudogracile*, *Periconia paludosa*, *Periconia* sp. 2, and *Subramaniomyces fusisaprophyticus*. *Davidiella* spp. and *Cladosporium* spp. anamorphs (Braun *et al*., 2003) have been reported as endophytic fungi from various plants. *Mycosphaerella* species have been recorded on fallen leaves of *Eucalyptus* and *Quercus* at early stages of decay and were followed by other saprobes and basidiomycetes (Crous, 1998; Frankland, 1998; Hunter *et al*., 2006; Tang *et al*., 2005).

Some of the taxa on the hanging leaves were also found on sterile leaves on the forest floor (*Cladosporium oxysporum*, *C. sphaerospermum*) but with low frequencies. Fungi colonising both sterile leaves lying on the forest floor and hanging leaves indicate airborne or rain splash transmission. Some other typically airborne taxa, abundant on hanging leaves but rarely found on unsterile leaves on the ground included *Alternaria* sp., *Cladosporium* spp., *Parasympodiella laxa*, *Ramularia* sp. and *Zygosporium gibbum*.

Fungi ubiquitous in all three treatments, such as *Bacillispora aquatica*, *Beltrania rhombica*, *Beltraniella portoriciensis*, *Chalara pteridina*, *Clonostachys compactiuscula*, *Dactylaria hemibeltranioidea*, *Dictyochaeta simplex*, *Microdochium caespitosum*, *Subulispora procurvata* and *Volutella* sp. 1 were also found at all stages of decay. These fungi participate in leaf decay throughout the period studied and sporulate rapidly.

Host and substrate specificity and factors influencing fungal distribution

Evidence for host-specificity or host-recurrence of fungi have been noted from various plants (Zhou and Hyde, 2001; Parungao *et al*., 2002; Photita *et al*., 2003). However, the basis for such preference is poorly understood. Research has provided evidence that saprobic and endophytic taxa are often the same species (Osono, 2002; Promputtha *et al*., 2005). Cannon (2002) found that endophytic fungi in tropical forests are less host-specific than those of temperate forests.

Substrate preferences of fungi has been studied by many mycologists. Among the saprobic fungi on *Magnolia liliifera* leaves only 5 out of the 37 encountered taxa grew on midribs and petioles (Promputtha *et al*., 2004); on banana in Doi Suthep-Pui, Chiang Mai, Thailand, Photita *et al*., (2003) found 80 fungal species of which 51 species were on petioles; thirty of these overlapped on petioles and leaf

blades. Among the 112 taxa found in the present study, only eleven taxa ocurred on petioles and midribs, and five taxa were encountered on both midribs and leaf blades. We assume that these five taxa first grew on the leaf blade near the midrib and then covered the midrib, mostly at the leaf tip. The similarities of fungal communities from different leaf substrates (leaf lamina, petioles and midribs) are shown in Figures A-D. Fungal species composition on leaf blades, petioles and midribs differed significantly ($p = 1.9e^{-11}$). Fungal communities on petioles and midribs of different leaf treatments were more similar to each other than to those of leaf blades, pointing to a distinct ecological niche. Fungi on petioles and midribs may have higher capacity for colonizing and decomposing woody substances.

The factors that influence the presence of a fungus on a given host are difficult to determine. Some factors have been suggested that might influence the fungal communities on leaves. The leaf surfaces (waxy, different kinds of hairs) are one of the most important substrata for fungal attachment (Eaton *et al*., 2004). The phylloplane mycoflora is important during early stages of decay (Osono, 2002). Other factors affecting fungal communities include leaf thickness, toughness, metabolites (from the plant itself or from endophytes and other fungi) such as glycol, chlorohydrins and bromohydrin (Varma *et al*., 2004; Paulus *et al*., 2006) or phenolic compounds in plant cells (Polishook *et al*., 1996; Sallé *et al*., 2005; Paulus *et al*., 2006). *Castanopsis diversifolia* has mechanisms that protect the leaves against fungal colonization such as hairs on both sides when young, smooth surface, leather leaf tissue when mature and phenolic compounds.

Acknowledgment

Duong is pleased to acknowledge Mushroom Research Foundation, Chiang Mai, Thailand for his PhD research grant at Chiang Mai University. Professor Walter Gams is thanked for reading the manuscript and giving valuable comments.

References

Anderson, I.C., Chambers, S.M., John, W.G. and Cairney, J.W.G. (2001). Distribution and persistence of Australian *Pisolithus* species genets at native sclerophyll forest field sites. Mycological Research 105: 971-976.

Bills, G.F. (1996). Isolation and analysis of endophytic fungal communities from woody plants. In: *Endophytic fungi in grasses and woody plants*: *systematics*, *ecology and evolution* (ed. S.C. Redlin). St. Paul, MN: APS Press: 31-65.

Bills, G.F. and Polishook, J.D. (1994a). Abundance and diversity of microfungi in leaf litter of lowland forest in Costa Rica. Mycologia 86: 187-198.

Bills, G.F. and Polishook, J.D. (1994b). Microfungi from decaying leaves of *Heliconia mariae* (*Heliconiaceae*). Brenesia 41-42: 27-43.

Bonet, J.A. Fischer, C.R. and Colinas, C. (2004). The relationship between forest age and aspect on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the central Pyrenees. Forest Ecology and Management 203: 157-175.

Braun, U., Crous, P.W., Dugan, F., Groenewald, J.Z. and de Hoog, G.S. (2003). Phylogeny and taxonomy of *Cladosporium*-like hyphomycetes, including *Davidiella* gen. nov., the teleomorph of *Cladosporium* s. str. Mycological Progress 2: 3-18.

Cannon, P.F. (2002). Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. Mycologia 94: 210-220.

Crous, P.W. (1998). *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. Mycologia Memoir 21: 1-170.

Duong, L.M., Lumyong, S. and Hyde, K.D. (2004a). Fungi on leaf litter. In: *Thai Fungal Diversity* (eds. E.B.G. Jones, M. Tanticharoen and K.D. Hyde). BIOTEC, Thailand: 163-171.

Duong, L.M., Lumyong, S., Hyde, K.D. and Jeewon, R. (2004b). *Emarcea castanopsidicola* gen. et sp. nov. from Thailand, a new xylariaceous taxon based on morphology and DNA sequences. Studies in Mycology 50: 253-260.

Eaton, R.J., Barbercheck, M., Buford, M. and Smitha, W. (2004). Effects of organic matter removal, soil compaction, and vegetation control on collembolan populations. Pedobiologia 48: 121-128.

Farr, D.F., Rossman, A.Y., Palm, M.E. and McCray, E.B. (2008). Fungal Databases, Systematic Botany and Mycology Laboratory, ARS, USDA. Retrieved April 14, 2008, from http://nt.ars-grin.gov/fungaldatabases.

Frankland, J.C. (1998). Fungal succession—unravelling the unpredictable. Mycological Research 102: 1-15.

Fryar, S.C. (2002). Fungal succession or sequence of fruit bodies? In: *Fungal succession* (eds. K.D. Hyde and E.B.G. Jones). Fungal Diversity 10: 5-10.

Ghosh, A., Frankland, J.C., Thurston, C.F. and Robinson, C.H. (2003). Enzyme production by *Mycena galopus* mycelium in artificial media and in *Picea sitchensis* F1 horizon needle litter. Mycological Reseach 107: 996-1008.

Goh, T.K. (1999). Single-spore isolation using a hand-made glass needle. Fungal Diversity 2: 47-63.

Hunter, G.C., Crous, P.W., Wingfield, B.D., Pongpanich, K. and Wingfield, M.J. (2006). *Pseudocercospora flavormarginata* sp. nov. from *Eucalyptus* leaves in Thailand. Fungal Diversity 22: 733-738.

Kwaśna, H. (2003). The effect of felling on the occurrence of microfungi stimulatory to *Armillaria* rhizomorph formation in thin roots of *Quercus robur*. Journal of Phytopathology 151: 185-189.

Lamour, A., Termorshuizen, A.J.,Volker, D. and Jeger, M.J. (2007). Network formation by rhizomorphs of *Armillaria lutea* in natural soil: their description and ecological significance. FEMS Microbiology Ecology 62: 222-232.

McCune, B and Mefford, M.J. (1999). Multivariate analysis of ecological data, version 4. MjM software design, Gleneden Beach, Oregon, USA.

Osono, T. (2002). Phyllosphere fungi on leaf litter of *Fagus crenata*: occurrence, colonization, and succession. Canadian Journal of Botany 80: 460-469.

Osono, T. (2005). Colonization and succession of fungi during decomposition of *Swida controversa* leaf litter. Mycologia 97: 589-597.

Osono, T. (2007). Endophytic and epiphytic phyllosphere fungi of red-osier dogwood (*Cornus stolonifera*) in British Columbia. Mycoscience 48: 47-52.

Parungao, M.M., Fryar, S.C. and Hyde, K.D. (2002). Diversity of fungi on rainforest litter in north Queensland, Australia. Biodiversity and Conservation 11: 1185-1194.

Paulus, B. and Hyde, K.D. (2003). Estimation of microfungal diversity in tropical rainforest leaf litter using particle filtration: the effects of leaf storage and surface treatment. Mycological Research 107: 748-756.

Paulus, B., Gadek, P. and Hyde, K.D. (2006). Successional patterns of microfungi in fallen leaves of *Ficus pleurocarpa* (*Moraceae*) in an Australian tropical rain forest. Biotropica 38: 42-51.

Photita, W., Lumyong, P., McKenzie, E.H.C., Hyde, K.D. and Lumyong, S. (2003). Saprobic fungi on dead wild banana. Mycotaxon 80: 345-356.

Polishook, J.D., Bills, G.F. and Lodge, D.J. (1996). Microfungi from decaying leaves of two rain forest trees in Puerto Rico. Journal of Industrial Microbiology 17: 284-289.

Prach, K., Pyšek, P. and Jarošík, V. (2007). Climate and pH as determinants of vegetation succession in Central European man-made habitats. Journal of Vegetation Science 18: 701-710.

Promputtha, I., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and Hyde, K.D. (2002). Fungal succession on senescent leaves of *Manglietia garrettii* in Doi Suthep Pui National Park, northern Thailand. In: *Fungal succession* (eds. K.D. Hyde and E.B.G. Jones). Fungal Diversity 10: 89-100.

Promputtha, I., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and Hyde, K.D. (2004). Fungal saprobes on dead leaves of *Magnolia liliifera* (*Magnoliaceae*) in Thailand. Cryptogamie Mycologie 25: 315-321.

Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E.H.C., and Hyde, K.D. (2005). Ribosomal DNA fingerprinting in the identification of non-sporulating endophytes from *Magnolia liliifera* (*Magnoliaceae*). Fungal Diversity 20: 167-186.

Rambelli, A., Mulas, B., Pasqualetti, M. (2004). Comparative studies on microfungi in tropical ecosystems in Ivory Coast forest litter: behaviour on different substrata. Mycological Research 108: 325-336.

Rao, C.Y., Riggs, M.A., Chew, G.L., Muilenberg, M.L., Thorne, P.S., Van Sickle, D., Dunn, K.H. and Brown, C. (2007). Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after hurricanes Katrina and Rita. Applied and Environmental Microbiology 73: 1630-1634.

Rayner, A.D.M. and Todd, N.K. (1979). Population and community structure and dynamics of fungi in decaying wood. Advances in Botanical Research 7: 334-420.

Reed, S.C., Cleveland, C.C., and Townsend, A.R. (2007). Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. Biotropica 39: 585-592.

Sallé, A., Monclus, R, Yart, A. and Lieutier, F. (2005). Effect of phenolic compounds on the *in vitro* growth of two fungi associated with *Ips typographus*. Forest Pathology 35: 298-304.

Sayer, E.J. (2005). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biological Reviews 80: 1-31.

Suzuki, A., Uchida, M., and Kita, Y. (2002). Experimental analyses of successive occurrence of ammonia fungi in the field. In: *Fungal Succession* (eds. K.D. Hyde and E.B.G. Jones). Fungal Diversity 10: 141-165.

Tang, A.M.C., Jeewon, R. and Hyde, K.D. (2005). Succession of microfungal communities on decaying leaves of *Castanopsis fissa*. Canadian Journal of Microbiology 51: 967-974.

Tempesta, S., Pasqualetti, M., Fonck, M. and Mulas, B. (2003). Succession of microfungi in *Phillyrea angustifolia* litter in a Mediterranean maquis in Sardinia. Plant Biosystems 137: 149-154.

Tsai, F.C., Macher, J.M. and Hung, Y.Y. (2007). Biodiversity and concentrations of airborne fungi in large US office buildings from the BASE study. Atmospheric Environment 41: 5181-5191.

Twieg, B.D., Durall, D.M. and Simard, S.W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. New Phytologist 176: 437-447.

Varma, A., Abbott, L., Werner, D. and Hampp, R. (2004). Plant Surface Microbiology. Springer. pp616.

Wannathes, N., Desjardin, D.E., Retnowati, A., Tan, Y.S. and Lumyong, S. (2004). A redescription of *Marasmius pellucidus*, a species widespread in South Asia. Fungal Diversity 17: 203-218.

Watkinson, S.C., Bebber, D., Darrah, P.R., Fricker, M.D.,Tlalka, M. and Boddy, L. (2006). The role of wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: *Fungi in Biogeochemical Cycles* (eds. G.M. Gadd). Cambridge University Press, Cambridge: 151-181.

Yanna, Ho, W.H. and Hyde, K.D. (2002). Fungal succession on fronds of *Phoenix hanceana* in Hong Kong. Fungal Diversity 10: 185-211.

Zhou, D.Q. and Hyde, K.D. (2001). Host-specificity, host-exclusivity and host-recurrence in saprobic fungi. Mycological Research 105: 1449-1457.