



Fungal diversity on fallen leaves of *Ficus* in northern Thailand*

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Abstract: Fallen leaves of *Ficus altissima*, *F. virens*, *F. benjamina*, *F. fistulosa* and *F. semicordata*, were collected in Chiang Mai Province in northern Thailand and examined for fungi. Eighty taxa were identified, comprising 56 anamorphic taxa, 23 ascomycetes and 1 basidiomycete. Common fungal species occurring on five host species with high frequency of occurrence were *Beltraniella nilgirica*, *Lasiodiplodia theobromae*, *Ophioceras leptosporum*, *Periconia byssoides* and *Septonema harknessi*. *Colletotrichum* and *Stachybotrys* were also common genera. The leaves of different *Ficus* species supported diverse fungal taxa, and the fungal assemblages on the different hosts showed varying overlap. The fungal diversity of saprobes at the host species level is discussed.

Key words: *Ficus*, Fallen leaves, Saprobes, Fungal diversity

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INTRODUCTION

Fungi are chemoheterotrophic organisms that play an important role as decomposers in natural ecosystems (Duarte *et al.*, 2006; Gadd, 2007). Fungi have additional economic importance as biocontrol agents, chemical producers of bioactive compounds used in the pharmaceutical and many other industries (Yuen *et al.*, 1999; Bucher *et al.*, 2004; Duarte *et al.*, 2006). The economic impact of fungi in the environment is likely related to the total fungal species pool (Hyde, 2001; Piepenbring, 2007). Research on fungal diversity therefore provides a basis for estimating the functional role of fungi in ecosystems, exploiting these economic fungi and estimating the total fungal species richness in a region or globally (Arnold *et al.*, 2001; Mueller and Schmit, 2007).

Although the 1.5 million species estimated by Hawksworth (2001) are commonly accepted (Hyde, 2001; Hyde *et al.*, 2007), the actual quantity of fungal species is unclear (Arnold *et al.*, 2001; Hyde, 2001; Hyde *et al.*, 2007; Schmit and Mueller, 2007). Schmit and Mueller (2007) estimated that there is a minimum of 712000 fungal species worldwide. This estimate was primarily based on the observed ratio between vascular plant species diversity and fungal diversity in a certain area. Meta-analysis of fungal diversity in small plots (Schmit *et al.*, 2005) demonstrated that the numbers of tree species and macrofungal species diversities are correlated (Mueller *et al.*, 2007). The actual number of fungi is still unknown; however, only 5%~13% of the total estimated global fungal species have been described. The undescribed fungi may occur in poorly studied countries, hosts, habitats, niches, or tissues, and are mostly microfungi (Hyde, 2001; Schmit and Mueller, 2007). To determine the accuracy of fungal estimates, more information is needed concerning species diversity in poorly studied areas and hosts, especially in poorly studied areas

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(Dulymamode *et al.*, 2001; Hyde, 2001).

There have been several studies on fungi in poorly studied habitats in Thailand (Jones and Hyde, 2004). This has included studies on larger fungi in the forests of northern Thailand (Le *et al.*, 2007a; 2007b), fungi on peat swamp palms (Pinnoi *et al.*, 2006; Pinruan *et al.*, 2007), fungi on other monocotyledons (Photita *et al.*, 2001; Thongkantha *et al.*, 2008), fungi on leaf litter of angiosperms (Promputtha *et al.*, 2004; 2005; Duong *et al.*, 2006; 2008), and fungi on decaying wood (Kodsueb *et al.*, 2008a; 2008b). There has been, however, no study on the litter of *Ficus* species.

Whether fungi are host-specific or generalists are significant in estimating species numbers (Zhou and Hyde, 2001; Hyde *et al.*, 2007), is particularly important in studying leaf litter. In tropical forests, plant litter comprises a mixture of diverse host leaves, unlike that in many temperate forests where stands contain low tree diversity. Several studies on the saprobic microfungal diversity associated with plants have been carried out (Hyde *et al.*, 2001; Yanna *et al.*, 2001; Dulymamode *et al.*, 2001; Paulus *et al.*, 2006a; 2006b), and to a large extent, fungi tend to be host-specific at the host genus level (Taylor *et al.*, 2001). Paulus *et al.* (2006a; 2006b) studied 6 tree hosts from 4 families and found very little overlap. The low overlap may be because the hosts studied were from different families. The next question to ask is whether litter of different species of the same host genus has host-specific fungi or generalists? We, therefore, chose to study the saprobes on 5 species of *Ficus* to establish data on the fungal communities involved in the decay of litter from different species of the same host genus.

The genus *Ficus* (Moraceae) includes some 750 species of woody plants occurring in most tropical and subtropical forests throughout the world (Weiblen, 2000). *Ficus* is an evergreen or deciduous tree, frequently growing on other trees (strangling)

with smooth pale gray bark and abundant white latex (Gardner *et al.*, 2000). There is little known concerning the fungal diversity on this host genus (Suryanarayanan and Vijaykrishna, 2001; Paulus *et al.*, 2006a). Paulus *et al.* (2006a; 2006b) reported on the fungal diversity in leaf litter of 2 species of *Ficus* in Australia, while Suryanarayanan and Vijaykrishna (2001) isolated 28 endophyte fungi from *Ficus benghalensis*. *Ficus* is the second largest tree genus in northern Thailand after *Syzygium* with 30 species of *Ficus* growing in the region (Gardner *et al.*, 2000).

The purpose of the present study is to assess the diversity of saprobic fungi on 5 species of *Ficus* in northern Thailand in order to (1) investigate fungal distribution on saprobic fallen leaves of *Ficus* species; and (2) evaluate the different fungal communities involved in the decay of litter from different species of the same host genus.

MATERIALS AND METHODS

Survey design

To examine the effect of host species on micro-fungal assemblages, 5 species of *Ficus* were chosen for the study. Individual trees of each species were located at each of the sites selected for sampling (Table 1). Thirty fallen leaves at various stages of decay were haphazardly collected from each *Ficus* species from each site within 2 d. All samples were placed in Ziplock plastic bags containing tissue paper moistened with sterile distilled water and sealed and incubated at room temperature. Material was examined in the laboratory on Days 5, 15 and 25 after sampling.

Assessment of microfungal diversity

For the assessment of fungal presence, all leaves were cut into 5 cm×5 cm quadrates for observation.

Table 1 Location of *Ficus* species surveyed in Chiang Mai Province in northern Thailand

Plant species	Location
<i>Ficus altissima</i>	MRC, T. Pa Pae, A. Mae Taeng, Chiang Mai Province, Thailand, 19°07'12" N, 98°44'2.64" E.
<i>F. virens</i>	Out door of MRC, T. Pa Pae, A. Mae Taeng, Chiang Mai Province, Thailand, 19°07'12" N, 98°44'2.64" E.
<i>F. benjamina</i>	About 5 km east of MRC, M. Mae Taeng, Chiang Mai Province, Thailand, 19°05'12" N, 98°44'2.64" E.
<i>F. fistulosa</i>	Near Mae Malai, M. Mae Taeng, Chiang Mai Province, Thailand, 19°05'0" N, 98°5'42" E.
<i>F. semicordata</i>	Tung Cho National Park, forest trail, Tung Joaw Village, Mae Teng Dist., Chiang Mai Province, Thailand, 19°08'4.2" N, 98°38' 54" E.

Fruiting bodies of microfungi were observed under a stereoscope. A slide was then prepared for one representative fruiting structure of each taxon and placed in water and observed under a compound microscope. Micrographs were taken and stains were added as appropriate. Slides were made semipermanent by the addition of 90% (v/v) lactic acid. Species identifications were made using morphological characters. Herbarium specimens and slides of all taxa are maintained at the Mushroom Research Foundation Herbarium with some duplicates in Zhejiang University Herbarium.

Definitions and statistical analyses

Taxa were recorded as either present or absent from each leaf. The number of leaves, on which a particular fungal species was observed, designated the 'occurrence of a fungus' and was used to calculate the 'percentage occurrence' of a taxon on leaves of one tree species using the following formula (Tsui *et al.*, 2001; Yanna *et al.*, 2002):

Percent occurrence of taxon A (%) = occurrence of taxon A / occurrence of all taxa in one tree species × 100.

Fungal species diversity on each *Ficus* species was calculated using Shannon-Wiener's index H' :

$$H' = -\sum P_i \ln P_i,$$

where P_i is the frequency of fungal species i occurring on specific host leaves (Begon *et al.*, 1993; Wong and Hyde, 2001).

Sørensen's index of similarity (S) was applied (Magurran, 1988; Tsui *et al.*, 2001) to compare the similarity of the species on leaves of different *Ficus* spp.: $S = 2c/(a+b)$, where a is the total number of species on host A leaves, b is the total number of species on host B leaves, and c is the number of species on both host leaves. Similarity is expressed with values between 0 (no similarity) and 1 (absolute similarity).

RESULTS

Species richness and dominant fungi on *Ficus* spp.

Examination of decaying leaves of 5 species of *Ficus* from northern Thailand yielded 80 fungal taxa, comprising 56 anamorphic taxa, 23 ascomycetes and 1 basidiomycete. We have identified many taxa to

species level, although in several cases the morphology of the taxa in our study was not identified to species (unidentified species, Table 2). Five species, *Beltraniella nilgirica*, *Lasiodiplodia theobromae*, *Ophioceras leptosporum*, *Periconia byssoides* and *Septonema harknessi* were common species occurring on all five host species (>3% occurrence, Table 2). In addition, *Colletotrichum* and *Stachybotrys* were common genera, with several species occurring on the *Ficus* leaves (Table 2). Figs. 1~5 also show some rare fungal taxa on the different host species.

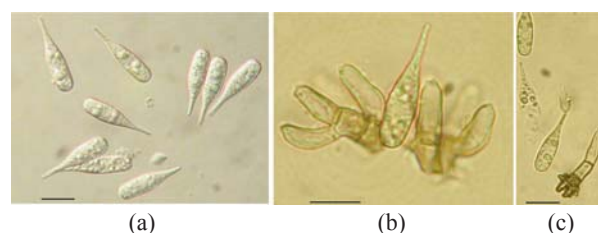


Fig.1 *Ellisiopsis galesiae* on fallen leaves of *Ficus altissima*. (a) Morphology of conidia; (b) and (c) Morphology of conidiophores. Bar=10 μ m

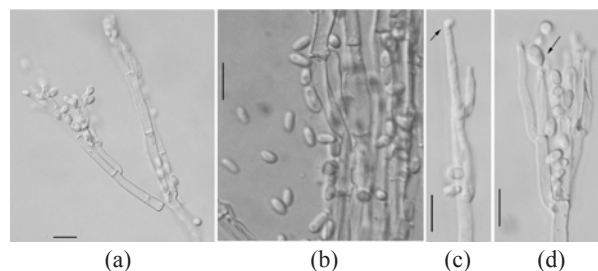


Fig.2 Morphology of *Sagenomella striatispora* on fallen leaves of *Ficus fistulosa*. (a) Characters of conidiophore; (b) Characters of conidia and conidiophore; (c) and (d) developing conidia from conidiophores. Arrow indicates the young conidium. Bar=10 μ m

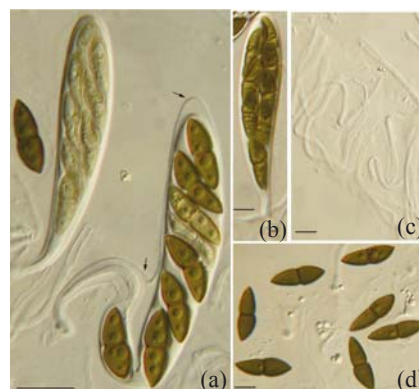


Fig.3 Morphology of *Kirschsteiniothelia* spp. on fallen leaves of *Ficus semicordata*. (a) Characters of asci. Arrows indicate the outer layer of the ascus wall; (b) Characters of ascus; (c) Characters of pseudoparaphyses; (d) Characters of ascospores. (a) Bar=20 μ m; (b)~(d) Bar=10 μ m

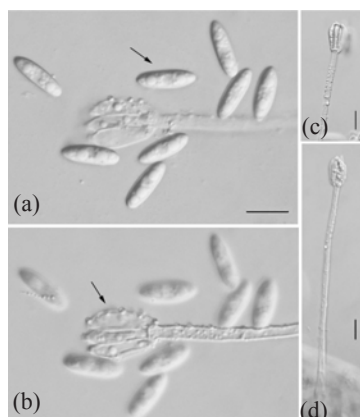


Fig.4 Morphology of *Stachybotrys bisbyi* on fallen leaves of *Ficus benjamina*. (a) Characters conidia. Arrow indicates the smooth wall of the conidium; (b) Characters of conidiophore. Arrow indicates the rough wall of conidiophore cell; (c) and (d) Characters of conidiophores. Bar=10 μ m



Fig.5 Morphology of *Stachybotrys renisporea* on fallen leaves of *Ficus virens*. (a) Characters of conidiophore; (b) and (c) Characters of conidia and conidiophores. Arrow indicates the succession of conidium; (d) and (e) Characters of conidia. Bar=10 μ m

Table 2 Percent occurrence (%) of fungal taxa on leaves of 5 species of *Ficus* in northern Thailand

Fungus name	FV	FF	FA	FS	FB	Fungus name	FV	FF	FA	FS	FB
Ascomycetes						Mitosporic fungi					
<i>Amphisphaeria fallax</i>			2.3			<i>Ellisiopsis galleisiae</i>			2.3		
<i>Anthostomella limitata</i>	2.2	2.4				<i>Fusariella hughesii</i>	4.4		4.7		
<i>Anthostomella nannorrhopis</i>			2.3	3.4	5.8	<i>Fusariella obstipa</i>			2.3		
<i>Chaetomium sulphureum</i>			4.7			<i>Fusarium chlamydosporum</i>		4.8			
<i>Diaporthe adunca</i>			4.7			<i>Fusarium oxysporum</i>	5.5				
<i>Diaporthe per juncta</i>		2.4		3.4		<i>Fusarium solani</i>	4.4				
<i>Eutypella scoparia</i>		2.4		3.4		<i>Giulia tenuis</i>				1.7	
<i>Glomerella acutata</i>						<i>Kellermania yuccifolia</i>		2.4			
<i>Glomerella cingulata</i>	1.1		1.2			<i>Kontospora halophila</i>				1.7	
<i>Guignardia spp.</i>	1.1	2.4	1.2		2.9	<i>Koorchaloma jamaicensis</i>		2.4			
<i>Hyponectria populi</i>				3.4		<i>Lasiodiplodia theobromae</i>	3.3	4.8	3.6	6.8	5.8
<i>Kirschsteiniotelia spp.</i>				1.7		<i>Menispora ciliata</i>				3.4	
<i>Lasiosphaeria breviseta</i>	4.4					<i>Menispora glauca</i>				3.4	
<i>Lophiostoma minusporum</i>				6.8		<i>Microdochium phragmitis</i>		4.8	4.7		
<i>Massarina hepaticarum</i>			4.7			<i>Mycoenterolobium platysporum</i>	2.2		2.3		
<i>Melanospora brevirostris</i>				3.4		<i>Periconia byssoides</i>	4.4	4.8	4.7	6.8	4.3
<i>Munkovalsaria appendenta</i>	1.1			1.7	1.4	<i>Pestalotiopsis gigas</i>	2.2				2.9
<i>Mycoenterolobium platysporium</i>	2.2		2.3			<i>Pestalotiopsis owenii</i>	4.4				2.9
<i>Mycosphaerella dianthi</i>		4.8			2.9	<i>Phaeoisaria clematidis</i>			2.3		
<i>Mycosphaerella freycineitae</i>	4.4			3.4		<i>Phoma eupyrena</i>					5.8
<i>Nectria sinopica</i>			1.2			<i>Phoma valerianae</i>	2.2	2.4			
<i>Ophioceras leptosporum</i>	4.4	4.8	4.7	6.8	5.8	<i>Phomopsis asparagi</i>	2.2		1.2		1.4
<i>Rhytisma acerinum</i>				3.4		<i>Plectrophomella nypae</i>	2.2				
Mitosporic fungi						<i>Sagenomella striatispora</i>		2.4			
<i>Acremonium charticola</i>			2.3			<i>Schwarzmannia goebeliae</i>	1.1				
<i>Ajrekarella polychaetriae</i>	2.2					<i>Septonema harknessi</i>	4.4	4.8	4.7	5.1	5.8
<i>Alternaria alternata</i>		4.8			4.3	<i>Sphaeridium candidum</i>		2.4			2.9
<i>Beltrania querna</i>			1.2			<i>Stachybotrys bisbyi</i>					2.9
<i>Beltraniella nilgirica</i>	4.4	4.8	3.6	6.8	5.8	<i>Stachybotrys nephrospora</i>					2.9
<i>Cercospora apii</i>	2.2				2.9	<i>Stachybotrys oenantes</i>	4.4		2.3		2.9
<i>Chaetospermum artocarp</i>		4.8				<i>Stachybotrys renisporea</i>	3.3				
<i>Chaetospermum chaetosporum</i>					5.8	<i>Stachybotrys sansevieriae</i>			2.3	3.4	
<i>Cladosporium cladosporioides</i>	3.3	3.6		6.8		<i>Torula graminis</i>					5.8
<i>Colletotrichum dematium</i>	2.2	4.8	3.6		2.9	<i>Torula herbarum</i>	4.4				
<i>Colletotrichum gloeosporioides</i>			3.6			<i>Trichothecium roseum</i>		2.4	3.6	3.4	
<i>Colletotrichum graminicola</i>			4.7			<i>Volutella ciliata</i>	4.4		3.6	6.8	
<i>Cylindrocolla urticae</i>					5.8	<i>Wiesneriomyces javanicus</i>	2.2	4.8		3.4	
<i>Dicyma state of Ascotricha chartarum</i>	2.2		2.3			<i>Xylohypha nigrescens</i>			4.7		
<i>Dinemasporium strigosum</i>	2.2	4.8			2.9	<i>Zygosporium chartarum</i>					2.9
<i>Discosia artocreas</i>		2.4				Basidiomycete					
<i>Discosia brasiliensis</i>					1.4	<i>Halocyphina spp.</i>			1.2		

FV: *Ficus virens*; FF: *F. fistulosa*; FA: *F. altissima*; FS: *F. semicordata*; FB: *F. benjamina*

Fungal diversity

Communities of fungal taxa on different *Ficus* species leaf litter were relatively distinct. The number of taxa varied from 24 (*F. semicordata*) to 33 (*F. virens* and *F. altissima*) (Table 3). The fungal communities on *F. virens* and *F. altissima* were the most similar but were different from those on *F. semicor-*

data (Table 4). The percentages of ascomycetes on *F. virens* and *F. altissima* were similar (24% and 30%, respectively). *F. semicordata* had the highest percentage of ascomycetes (46%) among the host species. The percentages of anamorphic taxa on *F. virens* and *F. altissima* were also similar.

Table 3 Diversity of fungi on different *Ficus* species

<i>Ficus</i> species	Number of species			Total	Index <i>H</i>
	Ascomycetes	Mitosporic fungi	Basidiomycetes		
<i>Ficus virens</i>	8	25		33	3.3525
<i>F. fistulosa</i>	6	19		25	3.0887
<i>F. altissima</i>	10	22	1	33	3.3196
<i>F. semicordata</i>	11	13		24	3.0881
<i>F. benjamina</i>	5	21		26	3.1496

Table 4 Index of similarity among different *Ficus* species

	<i>Ficus virens</i>	<i>F. fistulosa</i>	<i>F. altissima</i>	<i>F. semicordata</i>	<i>F. benjamina</i>
<i>Ficus virens</i>		0.41	0.45	0.35	0.44
<i>F. fistulosa</i>			0.31	0.41	0.43
<i>F. altissima</i>				0.35	0.31
<i>F. semicordata</i>					0.28
<i>F. benjamina</i>					

DISCUSSION

Studies of fungal diversity on *Ficus* have not been carried out until recently, although this genus is very common in forests throughout the world (Weiblen, 2000; Paulus et al., 2006a; 2006b). Suryanarayanan and Vijaykrishna (2001) studied fungal endophyte diversity on different host tissues of *F. benghalensis* in India. They found little overlap between fungal endophyte composition on the leaves and aerial roots. Paulus et al. (2006a; 2006b) studied saprobic microfungi in leaf litter of Australia tropical rainforests, including two *Ficus* species. They found that fungal assemblages of *F. destruens* and *F. pleurocarpa* grouped closely together. The overlapping species were *Beltraniella portoricensis*, *Beltrania* cf. *concurvispora*, *Cylindrocladium coulhouii*, *Diclypchaeta* cf. *novae-guineensis*, *Cylindrosympodium cryptocaryae*, *Idriella acerosea*, *Idriella lunata*, *Menisporopsis theobromae*, *Ophiognomonina elasticae*, *Parasypodiella elongate* and *Trichoderma viride*. In the present study, we investigated fungal diversity on leaves of five *Ficus* species. Our results show that the

overlap of fungal species was much higher than that in previous studies on fallen leaves from different hosts in different families. Similarity ranged from 28% to 45%. Some species, such as *Ophioceras leptosporum*, *Beltraniella nilgirica*, *Lasiodiplodia theobromae*, *Periconia byssoides* and *Septonema harknessi*, were shared by all hosts with high occurrence, indicating that a 'core fungal group' might be responsible for the decay of *Ficus* leaves (Wong and Hyde, 2001). In this study some fungi are new records on *Ficus*. Among these, many species are widespread, such as *Diaporthe adunca*, *Lasiochaeria breviseta*, *Colletotrichum gloeosporioides* and *Torula herbarum*. At least one species, *Munkovalsaria appendenta*, is a new record for Thailand. Further studies are needed to find more fungi on the leaves of *Ficus*, for our investigation was limited, with one tree of each host being sampled once.

Some previous research results showed that the hosts or tissues are important factors that influence the fungal communities (Ho et al., 2001; Paulus et al., 2003; Rambelli et al., 2004) and that some saprobes might be host-specific (Hyde et al., 1997). Wong and

Hyde (2001) investigated fungal diversity on *Gramineae*, and their results showed that fungal composition on host species level varied. Studies on microfungi of tree leaves in tropical regions suggested that the fungal assemblages on different host species were variable, and some other terms, such as 'host exclusivity', 'host fidelity', 'host affinity', 'host affinity', 'host preference' and 'host recurrence', were used to describe plant-fungal associations with definitive host (Zhou and Hyde, 2001). Taylor *et al.* (2001) noted that saprobic fungi were specific at a host genus level. In the present study, our results indicated that saprobic fungal diversity was high at the host species level, with some rare fungal taxa occurring on each of the different host species.

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