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# Dispersion, an Important Radiation Mechanism for Ectomycorrhizal Fungi in Neotropical Lowland Forests?

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## 1. Introduction

Mycorrhizas are symbiotic associations between plant roots and fungi. They are divided into different categories depending on morphological characteristics and the identity of the fungi and plants (Smith & Read, 2008). In tropical rainforests, most tree species are associated with arbuscular mycorrhizas (AM) (Alexander, 1989). Neotropical tree species belonging to confirmed ectomycorrhizal (EcM) host genera are diverse and generally scattered in a wide range of vegetation types (Table 1). *Pakaraimaea dipterocarpacea*, an endemic tree species from Guayana region (Maguire et al., 1977; Maguire & Ashton, 1980; Moyersoen, 2006), is one of the few known locally dominant EcM tree species in the Neotropical lowland forests. This tree is phylogenetically related to the most important EcM tropical tree family in SE Asia: the Dipterocarpaceae. The disjunct distribution of *P. dipterocarpacea* suggests that the capacity to associate with EcM evolved in the ancestors of Dipterocarpaceae before the splitting of South America from Gondwana, c. 135 million year ago (Ma) (Moyersoen, 2006). There are alternative proposals to explain *P. dipterocarpacea* EcM status such as independent acquisition of EcM association in South America or a more recent transoceanic long-distance dispersal of EcM Dipterocarpaceae to the Neotropics (Alexander, 2006). Biotrophic fungi such as EcM can be an important source of information for improved understanding of disjunct distributions (Pirozynski, 1983). The phylogeny of *P. dipterocarpacea* EcM fungi might reflect the disjunct distribution of this endemic tree species. A pioneering survey on *P. dipterocarpacea* indicated that broad host range fungal lineages distributed across the tropics are associated with this host plant (Moyersoen, 2006), but no phylogeographic studies on *P. dipterocarpacea* EcM fungi are available to date.

Apart from human transport, there are three possible hypotheses to predict the phylogeography of EcM fungal community associated with *P. dipterocarpacea*, i.e. vicariance, dispersion and/or migration. Both migration and vicariance assume that there is a close and specialized relationship between the fungus and the plant partners in EcM symbiosis (Pirozynski, 1983; Halling et al., 2008). Any discovery of fungi with an old Gondwanan origin associated with *P. dipterocarpacea* would support the vicariance hypothesis. Radiations of EcM fungi across continents are explained by co-migrations of both the fungus and the host partners (Halling et al., 2008; Pirozynski, 1983) and the capacity of the fungi to switch

hosts (Halling, 2001). The possible long term co-existence of Neotropical Dipterocarpaceae with other EcM host families in the same region (including the EcM Fabaceae *Dicymbe*) might have favored this radiation scenario (Moyersoen, 2006). Dispersion assumes that EcM fungi are able to cross environmental barriers, usually by spore dispersal. Long distance dispersion, presumably by *trans*-Tasman airflow, was proposed to explain the disjunct distribution of *Pisolithus* (Moyersoen et al., 2003) and *Inocybe* (Matheny et al., 2009) species between Australia and New Zealand. The biotrophic status of EcM fungi is an important constraint for the movement of these fungi and the relative importance of long distance dispersion versus migration and vicariance in global EcM fungi distribution is under debate (Halling et al., 2008).

	Species in VG* (total species)	For each entire genus			
		Life forms	Vegetation range	Altitudinal range	Geographical range
<b>Caesalpinaceae</b>					
<b>Amherstieae</b>					
<i>Dicymbe</i>	11 (19)	Small to large trees	Rainforests, shrublands, shrublands on rocky substrate, gallery forests	200-2700m	Southwestern Colombia, Southern Venezuela, Guyana, Surinam, Northwestern Brazil
<b>Fabaceae s.str.</b>					
<i>Aldina</i>	18 (22)	Small to large trees	Rainforests, shrublands, gallery forests, white sand shrub savannas, forest-savanna ecotone, savannas, riparian forests, swamp forests, shrublands on rocky substrate	100-1800m	Southwestern Colombia, Southern Venezuela, Guyana, Northwestern Brazil
<b>Dipterocarpaceae</b>					
<i>Pakaraimaea</i>	1 (1)	Trees, or shrubs	Forests on sandy soil, shrublands on rocky slopes	500-1100m	Southern Venezuela, Guyana
<i>Pseudomonotes</i>	0 (1)	Trees	Rainforests on clayey to sandy soil	200-300m	Southwestern Colombia
<b>Pisonieae</b>					
<i>Guapira</i>	13 (ca 50)	Trees or shrubs	Evergreen, semideciduous or deciduous forests, gallery forests, riparian forests, savanna-dry forest ecotone, white sand shrublands	50-1300m	Wide distribution from Mexico, West Indies to Brazil, Paraguay, and from Peru, Ecuador to French Guiana, Trinidad-Tobago
<i>Neea</i>	30 (ca 85)	Trees or shrubs	Rainforests, seasonally dry evergreen forests, semievergreen forests, riparian forests, savanna-dry forest ecotone, white sand savannas, white sand shrublands, shrublands	50-2000m	Neotropics

	Species in VG* (total species)	For each entire genus			
		Life forms	Vegetation range	Altitudinal range	Geographical range
			on rocky substrate, gallery forests, flooded forests		
<i>Pisonia</i>	1 (ca 40)	Small trees, shrubs or climbers	Varied	ca 300m	New World and Old World, subtropical and tropical
<b>Coccolobeae</b>					
<i>Coccoloba</i>	29 (400)	Shrubs, trees with scram- bling branches or lianas	Riparian forests, gallery forests, rainforests, savanna-forest ecotone, savannas, semideciduous forests, deciduous forests, shrublands on rocky substrate, white sand shrublands, seasonnally flooded or flooded forests	1-2000m	Neotropics
<b>Gnetaceae</b>					
<i>Gnetum</i>	6 (ca 40)	Lianas	Riparian forests, gallery forests, flooded forest margins, rainforests, vegetation on rocky slopes or white sand soils, swamps, savanna- forest ecotone	50-1800m	New World and Old World, subtropical and tropical

Table 1. Richness and distribution of confirmed EcM plant genera in the Neotropical lowland forests. \*Venezuelan Guayana. Source : Steyermark et al. (1995)

The objective of this study was to evaluate the different hypotheses about the possible origin of EcM fungi associated with *P. dipterocarpacea*. For comparisons between *P. dipterocarpacea* EcM fungi and other tropical tree species in the same region or elsewhere, EcM fungi diversity and community structure was evaluated in a plot, where clumps of *P. dipterocarpacea* are present together with individuals of the EcM tree *Aldina* sp., Fabaceae, Papilionoïdeae. *Inocybe* was selected for further phylogenetic analysis, because of its global importance as an EcM fungal lineage, the general knowledge about its biogeography and its hypothesized paleotropical origin (Kuyper, 1986; Matheny et al., 2009; Ryberg, 2009).

A great diversity of EcM fungi, comparable to other tropical or temperate EcM rich communities, was found in this study. The EcM community structure was similar to that found in another forest dominated by EcM Fabaceae *Dicymbe* sp. in the same region. This study was the first evidence that Neotropical host tree species belonging to the Dipterocarpaceae and the EcM Fabaceae share EcM fungi both within the same forest and across forest stands. The floristic similarities and phylogenetic relationships of the EcM fungi between *P. dipterocarpacea* and *Dicymbe* forests suggested that fungal dispersion is an

important radiation mechanism for EcM fungi in the region. Close phylogenetic relationships between *Inocybe* species associated with *P. dipterocarpacea* and African strains confirmed EcM phylogeographic links between the two continents. This study should be extended to examine a new *P. dipterocarpacea* dominated forest. The possible importance of dispersion in EcM fungal radiation and the link with paleotropical fungi should be tested in additional fungal lineages.

## 2. Methods

### 2.1 Study site

The sampling site was as described by Moyersoen (2006), located at 4°20'N, 61°48'W, altitude 500 m, near Icabarú Village, in Gran Sabana, Estado Bolívar, Venezuela, (Fig. 1). The precise location of the 20 X 20 m plot was selected based on a previous report of a stand of *P. dipterocarpacea* ssp. *nitida* described by Maguire & Steyermark (1981) in the same area (Fig. 2-a). A second EcM tree, *Aldina* sp., also occurs in the plot (Moyersoen, 2006). Four separate sampling expeditions were conducted, in November 2003 (one day), March 2006 (one day), July 2007 (5 days) and July 2008 (9 days), respectively.

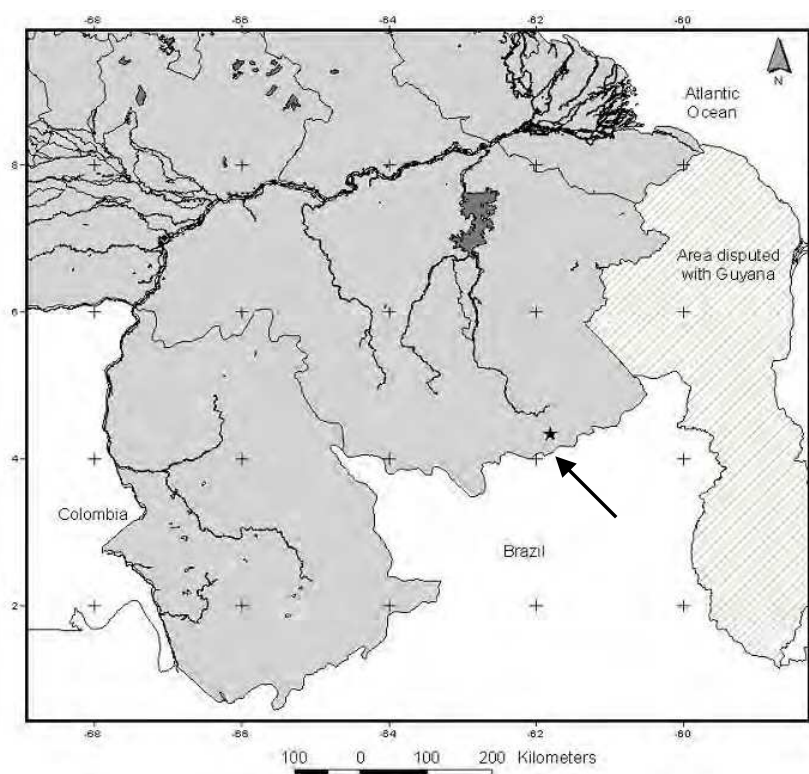


Fig. 1. Location of the field site

### 2.2 Fruit body and fine root sampling

All terricolous fruit bodies belonging to different morphotypes as well as resupinate fruit bodies growing under dead trunks and branches were collected inside the 20 X 20 m plot in 2007 and 2008 (Fig. 2-b, c, d). A brief description from fresh material was made in the field for each collection and a piece of mycelium from the cap was taken and fixed in cetyltrimethylammonium bromide (CTAB) for further molecular study. Time lapse between

collection and fixation was less than one day. Microscopic observations of 3% KOH rehydrated material was carried out using a light microscope (Leica DM 2500) . Fruit body collections were assigned to families or genus using general keys and assigned to morphospecies. Fungi were considered to be putatively EcM on the basis of identification (e.g. Rinaldi et al., 2008; Singer, 1986; Smith & Read, 2008).

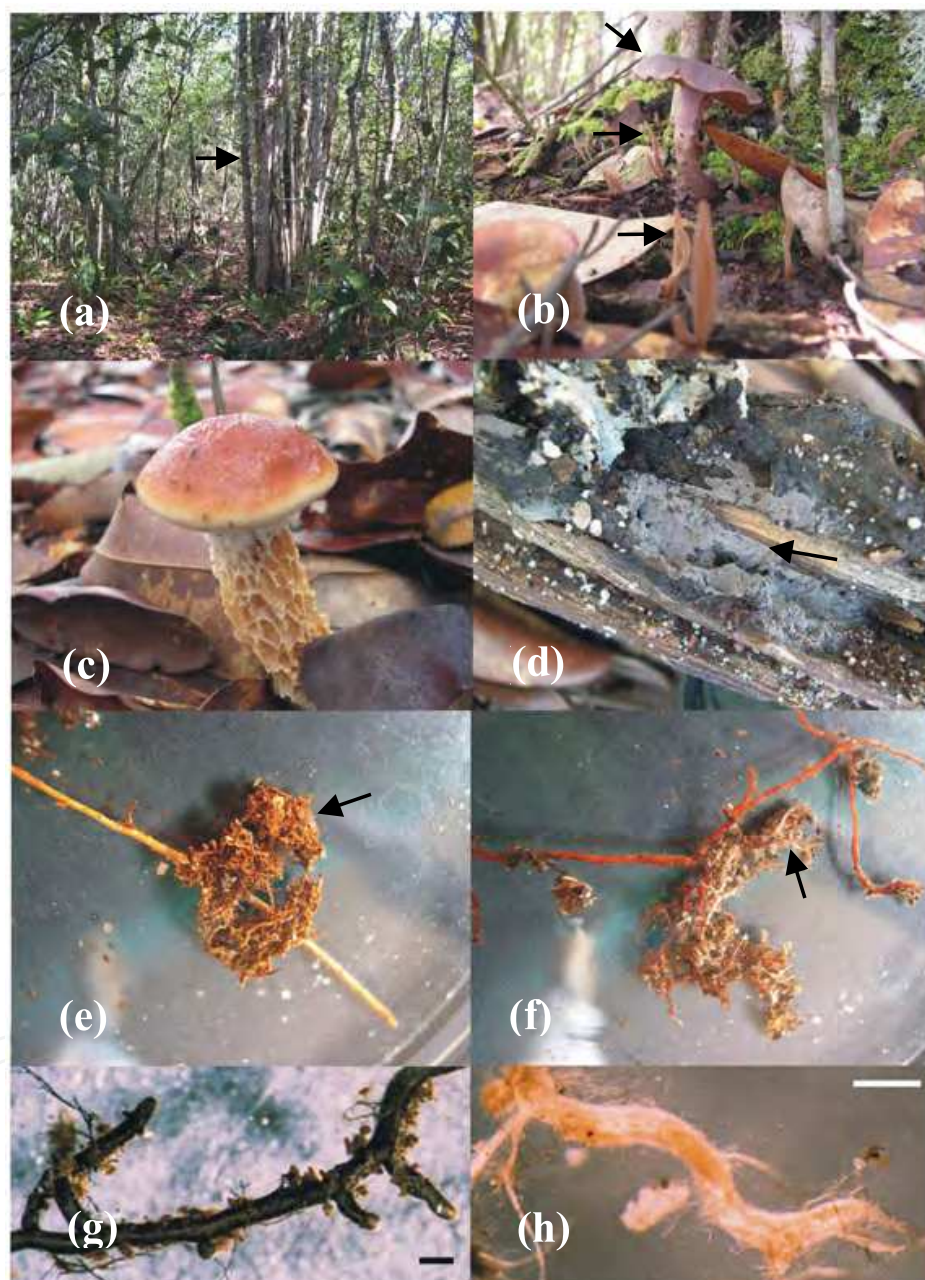


Fig. 2. Ectomycorrhizal (EcM) elements in *Pakaraimaea dipterocarpacea* forest plot. (a) Clump of *P. dipterocarpacea*; (b) *Cortinarius* sp. (BM08C30) surrounded by clavate *Clavulina* sp. (cf. BM07C9) fruit bodies; (c) *Austroboletus rostrupii* fruit body; (d) Resupinate *Tomentella* sp. (BM07C26, matching PD2\_3) fruit body under dead trunk; (e) EcM system attached to *Aldina* sp. "B" secondary root (f) EcM system attached to *P. dipterocarpacea* secondary root ; (g) *Tomentella* sp. EcM (BM03M8, matching PD2\_3) on *Aldina* sp.; *Cortinarius* sp. EcM (BM03M4, matching BM08C20) on *P. dipterocarpacea*. Bar, 0,4 mm.

Fine roots were sampled in each sampling expedition. They were traced from identified *P. dipterocarpaceae* trees in the first three surveys and sampled in nine 10 X 10 X 5 cm deep soil cores scattered in the plot in the last harvest. The soil cores were located either in places where *P. dipterocarpaceae* and *Aldina* sp trees occurred at < 2 m distance from each other (5 cores) or in random locations (4 cores).

Traced root samples were morphotyped using a field dissecting microscope (Novex AP-7) or a 10X magnifying glass before fixation in CTAB. Time lapse between tracing and root fixation was always up to one day. Morphology of traced *P. dipterocarpaceae* and *Aldina* sp. fresh secondary roots was characterized for subsequent sorting in the soil cores. Color was rust brown to yellowish and silvery creamish in *P. dipterocarpaceae* and *Aldina* sp., respectively (Fig. 2- e, f). Frequent longitudinal peels of bark were often observed on *P. dipterocarpaceae* roots.

A 5 X 5 X 5 cm<sup>3</sup> subsample including top organic soil was taken from each soil core and most fine roots attached to secondary roots as well as loose root tips were carefully washed in the field Lab. EcM and non-EcM root systems were separated using a 10X magnifying glass. EcM roots were subsequently separated on *P. dipterocarpaceae* and *Aldina* root systems using color and morphological features. Loose EcM tips were grouped into a third “unclassified” category. All roots from the three categories were fixed in CTAB for further morpho-anatomical study in the laboratory in Belgium. Time lapse between root sampling and fixation was less than one day.

For each soil core, all fine roots belonging to *P. dipterocarpaceae*, *Aldina* and “unclassified” categories were screened separately under a dissecting microscope to assign them to EcM morphological categories (Fig. 2-g, h). Each morphological category, including several tips, was cross checked using anatomical features on mantle peels following Agerer’s (1991) method with some modifications. Different morphotypes from each *P. dipterocarpaceae*, *Aldina* and “unclassified” root category were stored separately in CTAB and kept as a representative sample for further molecular analysis.

## 2.3 DNA protocols

Genomic DNA was extracted using QIAGEN Dneasy™ Plant Mini Kit (Qiagen S.A. Courtaboeuf, France) from fruit body morphospecies, traced ECM and morphotypes from the soil cores. To test the accuracy of morphotypes in the soil cores, DNA was extracted from several replicates (between two and 6) in EcM morphotypes including more than one sample. Fungal internal transcript spacer (ITS) and partial large subunit (LSU, 25-28S) nuclear rDNA were PCR-amplified with forward primer ITS1f in combination with LR6 (Gardes & Bruns, 1993; Vilgalys & Hester, 1990). If multiple or no PCR products were obtained, DNA was extracted from another EcM tip or the same extract was reamplified using the following primers in different combinations: ITS1f, ITS4b, ITS4, 5,8SR, LR21, LROR, LR6 (Gardes & Bruns, 1993; Vilgalys & Hester, 1990; White et al., 1990; R Vilgalys Lab <http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The PCR protocols were as described in Moyersoen (2006) with some modifications in cycling parameters for different primers. To check host identity of traced roots and morphotypes from the soil cores, *rbcL* DNA was amplified from 7 EcM samples of *P. dipterocarpaceae* and *Aldina* sp. using the primers *rbcLN* and *rbcLR* (Käss & Wink, 1997), following the same PCR protocols as in Moyersoen (2006). ITS-LSU and *rbcL* amplification products were electrophoresed in

1% agarose gels stained with ethidium bromide and visualized under UV light. 1kb + DNA ladder (Invitrogen) was used as a marker. Controls with no DNA were included in every set of PCR amplifications. PCR products were purified using QIAquick protocol (Qiagen) or 96-well filtration system (Multiscreen-PCR plate, Millipore Corporation, MA, USA). Sequencing was performed by Genotranscriptomics Platform, GIGA, University of Liège, using the same primers as for PCR. Sequence editing was done using SEQUENCHER, version 4.0 (Gene Codes Corporation). ITS sequences of EcM specimens have been deposited at the National Center for Biotechnology Information (NCBI, GenBank: <http://www.ncbi.nlm.nih.gov>) under accession numbers JQ063044-JQ063063. Sequences of new fruit body species will be published separately.

## 2.4 Sequence analysis and phylogenetic analysis of *Inocybe*

ITS sequences were assigned to molecular species on the basis of arbitrary 3% similarity cut-off value (Nilsson et al., 2008). Sequence similarities were determined using the BLASTN sequence similarity tool (Altschul et al., 1997) in GenBank together with BLAST comparisons with recently published ITS sequences from a *Dicymbe* forest kindly supplied by M Smith (Smith et al., 2011).

A preliminary phylogenetic analysis was performed to place *Inocybe* sequences in Inocybaceae clades (Matheny et al., 2009). 5' end nLSU sequences of two *Inocybe* species from *P. dipterocarpacea* forest were aligned using Clustal X (V. 2.0.9) to Matheny et al. (2009) global Inocybaceae sequences data set kindly supplied by the first author. Maximum likelihood (ML) analysis was implemented in the program RAxML V. 7.0.6, using GTRCAT approximation. Taxon sampling was made on the basis of this analysis. All strains in *Inocybe* subgenus *Inocybe* sequences and a strain from *Nothocybe* lineage were selected in Matheny et al. (2009) data base together with sequences from recently published tropical strains Y01 (UDB 004238), Y02 (UDB004239) (Tedersoo et al., 2010a) and *I. tauensis* (GU97711, GU977212, GU977213) (Kropp & Albee-Scott, 2010). In total, the data set included 77 RNA polymerase II (RPB1), 68 RPB2 and 114 LSU sequences. The RPB1, RPB2 and nLSU sequences were aligned using Clustal X. For consistency, the same criteria as Matheny et al. (2005, 2009) were used for manual alignment using Bioedit V. Introns 1 of RPB1 and the intron of RPB2 were excluded from the analysis. Intron boundaries were inferred both by sequence comparison using published *I. lilacina* RPB1 intron 1 (AY351834), *I. sindonia* RPB1 intron 2 (AY351839) and by insertion between conserved amino acid and the canonical guanine-thymine and adenosine-guanine splice sites. Other positions, too ambiguous to align, were removed from the data set. RPB1 exon, RPB1 intron 2, RPB2 and nLSU were then concatenated in Bioedit. Data set partitioning was done following Matheny (2005). To test the phylogenetic relationships of *Inocybe* strains from *P. dipterocarpacea* forest with *Inocybe* data set, a ML Rapid Bootstrapping algorithm was implemented for 1000 replicates in RAxML, using GTRCAT approximation.

## 3. Results

### 3.1 Above and below ground EcM richness

A total of 64 EcM fruitbody samples were collected in the plot (Table 2). From these samples, 41 specimens (including replicates) were selected for molecular analysis and 38 (93%) were successfully sequenced. These sequences belonged to 26 molecular species. Descriptions of new fruit body species will be published separately.

From 15 adult trees and the soil cores, 150 EcM samples were recovered (Table 2). A total of 113 EcM samples were selected for molecular analysis and 97 EcM tips (86%) were successfully amplified and sequenced. These sequences belonged to 27 EcM fungal species. Only 12 of these species matched DNA from fruitbody surveys.

	Samples	PCR	Total sequences*	Success rate (%)	<i>Inocybe</i> sequences	Total species**	<i>Inocybe</i> species
Fruitbodies	64	41	38	0.93	2	26	1
EcM	150	113	97	0.86	8	27	2
Total	214	154	135	0.88	10	38***	2

\*Including ITS1, 5.8S, ITS2, LSU DNA regions depending on species.  
\*\*Species were defined using 97% sequence similarity cutoff across the ITS1, 5.8S, ITS2 region as well as phylogenetic analysis of LSU on selected fungal groups.  
\*\*\*This figure does not include one unsequenced *Russula* and *Scleroderma* species.

Table 2. Observed fruitbody and EcM species in 400m<sup>2</sup> plot dominated by *Pakaraimaea dipterocarpacea*

EcM morphotypes accuracy was tested before further measurement of species density. A total of 96 EcM samples from soil cores were classified in 28 preliminary morphological categories. From these samples, 64 EcM were subject to molecular analysis, resulting in 56 (83%) successful sequences and 22 species. Some preliminary categories were lumped together and 20 morphotypes were defined after cross molecular analysis. Only two morphotypes belonging to *Cortinarius* and *Tomentella* were accurate at genus level. The remaining morphotypes were accurate at the species level. Between one and 12 different morphotypes could be retrieved from a single 125 cm<sup>3</sup> soil core (Table 3). Species density per soil core was similar or above values reported in an highly diverse temperate *Picea abies* and *Tilia cordata* dominated EcM community where similar sampling strategy was used (Table 3).

Overall, 40 fungal species were recovered in the plot. Comparison with surveys in similar plot size in other tropical regions or in temperate regions showed that observed species richness was great and similar to or greater than values both in temperate and tropical areas (Table 4).

3.2 Host identity of ectomycorrhizal species

Host identity of all 7 *P. dipterocarpacea* EcM samples including traced roots (three samples) and morphotypes from the soil cores was confirmed after molecular cross checking (305/308, 99% similarity with *P. dipterocarpacea*, DQ406587). Among the 7 *Aldina* EcM samples (from soil cores), 5 tips matched *A. latifolia*, U74252 (99%, 594/600) *rbcL*. The remaining two samples belonged to *P. dipterocarpacea*. *Aldina* leaf morphology corresponded to “species B” in Flora of Venezuelan Guayana (1995) (G Aymard, pers. com.). Sequenced EcM singletons observed on “unclassified” roots belonged to *P. dipterocarpacea*.

In total, 22 EcM fungal species were associated with *P. dipterocarpacea*; 11 (50 %) of these species belonging to 7 fungal groups were also putative or molecularly confirmed associates of *Aldina*, and three singletons were associated with *Aldina* only (Table 5).

3.3 EcM community composition and comparisons with other tropical forest communities

The 40 fungal species were distributed in 7 fungal orders including Agaricales (11 species), Thelephorales (6 species), Cantharellales (6 species), Boletales (6 species), Russulales (5 species), Hymenochaetales (3 species) and Sebacinales (3 species) (Table 5). Agaricales were the richest fungal order including *Cortinarius* species (5 species), Amanitaceae (3 species), Inocybaceae (2 species) and one Tricholomataceae species (Table 5).

Eighty percent (80%) of the best aligned sequences were from the tropical areas including both the Paleotropics and the Neotropics (Table 5). Only 13 (33%) species matched ( $\geq 97\%$  similarity) the published ITS sequences. These species belonged to *Amanita*, *Inocybe*, Boletaceae, *Clavulina*, Sebacinales and *Tomentella*. All but one of these species were reported from a *Dicymbe* dominated forest situated ca 240 kms apart in the Guayana region (Guyana, Upper Potaro River Basin). Confirmed tree hosts included *Dicymbe* and *Aldina* species (Smith *et al.* 2011).

	This study	Morris et al. (2009)	Tedersoo et al. (2010a)	Smith et al. (2011)	Tedersoo et al. (2003)	Morris et al. (2008)
Climate	Humid tropical	Humid subtropical	Humid tropical	Humid tropical	Temperate	Mediterranean
Host species	<i>P. dipterocarpacea</i> , <i>Aldina</i> sp.	<i>Quercus crassifolia</i> , <i>Quercus laurina</i>	<i>Coccoloba</i> sp., <i>Guapira</i> sp., <i>Neea</i> sp.	<i>Dicymbe</i> sp., <i>Aldina</i> sp.	<i>Picea abies</i> , <i>Tilia cordata</i> , <i>Betula pendula</i> , <i>Populus tremula</i>	<i>Quercus douglasii</i> , <i>Quercus wislizeni</i>
Soil core volume (cm <sup>3</sup> )	125	942	2250	1000	125	900
Number of root tips processed/ soil core	All root tips in the core	100	All root tips in the core	20	All root tips in the core	100
Replicates	9	80	60	57	108	64
EcM tips sampling strategy	Morphotyping*	Random selection**	Morphotyping	Morphotyping	Morphotyping	Random selection
EcM species density (species in soil core)	1- 6.6 $\pm$ 3.4 (M $\pm$ STD) - 12	6.2 (M)	1.42 (M)	9 - 18	3.65 $\pm$ 1.7 (M $\pm$ STD)	6.5 (M)

\*root sorting in morphotypes before molecular analysis.  
\*\*molecular analysis of root tips randomly selected in soil core.  
m=mean, STD= standard deviation

Table 3. Comparison of observed EcM species density between *Pakaraimaea dipterocarpacea* forest and recent tropical and temperate surveys

Stand type	Area	Survey	Species richness	Source
Tropical				
Pakaraimaea/ Aldina	400 m <sup>2</sup>	Mixed*	40	This study
Mixed Dipterocarpaceae forest in Borneo	400 m <sup>2</sup>	EcM	4-26	Peay et al. (2010)
Temperate				
Arctostaphylos/Douglas fir	625 m <sup>2</sup>	Mixed	> 40	From Horton & Bruns (2001)
Norway spruce	500 m <sup>2</sup>	Mixed	25	
Bishop pine	625 m <sup>2</sup>	EcM	20	
Bishop pine	625 m <sup>2</sup>	EcM	7	

\*mixed fruitbody and EcM survey.

Table 4. Observed EcM fungi richness in similar sized tropical and temperate forest plots

Fungal species (accession No) (host plant) <sup>s</sup>	Best aligned sequence (Accession No.) (overlapping base pairs/ total aligned bases)	Geographic origin	Host family (when tropical)
Agaricales			
<i>Amanita</i> BM08C22 (Pd*)	EcM 780 (JN168679) (627/628, 99%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Amanita</i> BM07C21	<i>Amanita vaginata</i> (AB458889) (433/508, 85%)	Paleotropical, Thailand	Dipterocarpaceae
<i>Amanita</i> BM07C1	<i>Amanita populiphila</i> † (HQ539724) (719/751, 96%)	North temperate	
<i>Cortinarius</i> BM08C20 (Pd*, A <sup>6</sup> )	<i>Cortinarius limonius</i> (GQ159869) (364/412, 88%)	North temperate	
<i>Cortinarius</i> BM07C2 (Pd*)	Uncultured ectomycorrhizal fungus L7789_Cort MAD02 (FR731470) (697/736, 95%)	Paleotropical, Madagascar	Not given
<i>Cortinarius</i> BM08C11 (Pd*)	Uncultured ectomycorrhizal fungus L7789_Cort MAD02 (FR731470) (724/761, 95%)	Paleotropical, Madagascar	Not given
<i>Cortinarius</i> BM08C30	Uncultured ectomycorrhiza clone SDL13 (FJ769528) (506/548, 92%)	North temperate	
<i>Cortinarius</i> 1PdM4 (JQ063044) (Pd°)	Uncultured ectomycorrhizal fungus isolate L5110_Cort_Cam02†	Paleotropical , Cameroon	Fabaceae (Caesalpinaceae), Phyllantaceae

Fungal species (accession No) (host plant) <sup>s</sup>	Best aligned sequence (Accession No.) (overlapping base pairs/ total aligned bases)	Geographic origin	Host family (when tropical)
	(FR731783) (590/623, 95%)		
<i>Inocybe</i> BM08C27 (JQ063045) (Pd°, A°)	<i>Inocybe</i> EcM 434 (JN168723) (586/669, 88%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Inocybe</i> BM03M2 (JQ063046) (Pd°, A°)	<i>Inocybe</i> EcM 1091 (JN168720) (372/381, 98%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
Tricholomataceae BM08C2	<i>Catathelasma ventricosum</i> † (AM946418) (981/1088, 90%)	North temperate	
Boletales			
Boletaceae BM06M3 (JQ063047) (Pd*)	<i>Boletellus ananas</i> voucher TH6264 (JN168685) (198/198, 100%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
Boletaceae BM08C29	<i>Tylopilus ballouii</i> voucher R.E. Halling 8187† (EU430732) (647/673, 96%)	Neotropical, Costa Rica	Fagaceae
Boletaceae BM08C31	<i>Austroboletus rostrupii</i> TH8189 (JN168683) (537/537, 100%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
Boletaceae BM08C8	<i>Tylopilus felleus</i> † (AY586723) (734/772, 95%)	North temperate	
Boletaceae 7MM7 (JQ063048) (Pd°)	<i>Boletus</i> sp. MHM075 (EU569236) (252/280, 90%)	Neotropical, Mexico	Fagaceae
Scleroderma BM08C9	Unsequenced		
Cantharellales			
<i>Cantharellus</i> BM07C6	<i>Cantharellus cibarius</i> (AB453024) (236/251, 94%)	Paleotropical , Thailand	Dipterocarpaceae
<i>Clavulina</i> BM03sp1 (JQ063049) (Pd°, A°)	<i>Clavulina</i> voucher MCA 4022 (MCA4022) (541/545, 99%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae)
<i>Clavulina</i> BM07C10	<i>Clavulina amazonensis</i> isolate TH9191 (HQ680356) (596/650, 92%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae)
<i>Clavulina</i> BM07C9 (Pd°)	<i>Clavulina humicola</i> (DQ056368) (555/590, 94%)	Neotropical, Guyana	Fabaceae (Papilionoideae)

Fungal species (accession No) (host plant) <sup>s</sup>	Best aligned sequence (Accession No.) (overlapping base pairs/ total aligned bases)	Geographic origin	Host family (when tropical)
<i>Clavulina</i> BM08C24	<i>Clavulina monodiminutiva</i> (DQ056365) (570/643, 89%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Hydnum</i> BM03M19 (JQ063050) (Pd*)	<i>Hydnum repandum</i> (AY817136) (762/762, 100%)	North temperate	
Hymenochaetales			
Hymenochaetales 2PdM2 (JQ063051) (Pd <sup>δ</sup> , A <sup>δ</sup> )	<i>Coltriciella dependens</i> specimen voucher TU103611 (AM412254) (735/802, 92%)	Paleotropical , Seychelles	Fabaceae (Caesalpinaceae), Dipterocarpaceae
Hymenochaetales 1MM1 (JQ063052)	<i>Coltriciella dependens</i> specimen voucher TU 103611 (AM412254) (715/799, 89%)	Paleotropical , Seychelles	Dipterocarpaceae
Hymenochaetales PD6.3 (JQ063053) (Pd*)	Uncultured <i>Coltricia</i> voucher EcM 731 (JN168708) (442/567, 78%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae)
Russulales			
<i>Russula</i> BM07C7	Uncultured ectomycorrhizal fungus isolate L6171_Russ_Y04 (FN557554) (550/570, 96%)	Neotropical, Ecuador	Nyctaginaceae
<i>Russula</i> BM07C18 (Pd*, A*)	Uncultured ectomycorrhiza voucher 403 (AY667427) (556/583, 95%)	Neotropical, Ecuador	Nyctaginaceae
Russulaceae 8PdM1 (JQ063054) (Pd°, A°)	Uncultured ectomycorrhizal fungus isolate L7612_Russ MAD41 (FR731334) (236/261, 90%)	Paleotropical , Madagascar	Not given
Russulaceae 1PdM3 (JQ063055) (Pd°)	Uncultured <i>Russula</i> voucher EcM1094 (JN168741) (225/245, 92%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Russula</i> “lilac” BM07C20	Unsequenced		
Sebacinales			
Sebacinales BM03M3 (JQ063056) (Pd°, A°)	Sebacinales EcM 17 (JN168754) (532/549, 97%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)

Fungal species (accession No) (host plant) <sup>s</sup>	Best aligned sequence (Accession No.) (overlapping base pairs/ total aligned bases)	Geographic origin	Host family (when tropical)
Sebacinales PD10 (JQ063057) (Pd*, A <sup>o</sup> )	Uncultured ectomycorrhizal isolate L7604_Seb MAD02 (FR731328) (518/568, 91%)	Paleotropical , Madagascar	Not given
Sebacinales 6MM2 (JQ063058) (Pd°, A°)	Sebacinales voucher EcM84 (JN168756) (251/258, 97%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae)
Thelephorales <i>Tomentella</i> 7AM7 (JQ063059) (A°)	<i>Tomentella</i> EcM 963 (JN168770) (342/358, 96%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae)
<i>Tomentella</i> PD2_3 JQ063060 (Pd*, A°)	<i>Tomentella</i> EcM 963 (JN168770) (593/602, 99%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Tomentella</i> PD11 (JQ063061) (A°)	<i>Tomentella</i> EcM 712 (JN168764) (600/602, 99%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Tomentella</i> PD9_2 (JQ063062) (A°)	<i>Tomentella</i> EcM712 (JN168764) (587/605, 97%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Tomentella</i> BM07C39	<i>Tomentella</i> MES348 (JN168772) (170/174, 98%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)
<i>Tomentella</i> 2PdM6 (JQ063063) (Pd <sup>o</sup> )	<i>Tomentella</i> EcM 698 (JN168763) (318/329, 97%)	Neotropical, Guyana	Fabaceae (Caesalpinaceae, Papilionoideae)

†LSU sequence. Unmarked aligned sequences include ITS region.  
<sup>s</sup>Host for each fungal OTU was identified from EcM after *rbcL* sequencing<sup>o</sup>, root tracing\* or root morphology<sup>o</sup>. Fungal OTUs with no identified host were collected only from the fruiting bodies. Pd : *P. dipterocarpacea* ; A : *Aldina* sp. B.

Table 5. Sequence matches of ectomycorrhizas and ectomycorrhizal fruit bodies collected in *Pakaraimaea dipterocarpacea* forest

3.4 *Inocybe* EcM frequency and occurrence on host plants in the plot

Two *Inocybe* species were recovered in the plot. *Inocybe* EcM were characteristic and could be distinguished from other fungal genera on the basis of morphology and anatomy. They were both characterized by a smooth and whitish mantle with frequent rhizomorphs and a pinkish color at the apex (Fig. 2a). Hyphae in the rhizomorphs were uniformly shaped and agglutinated (type B in Agerer 2006). EcM habit corresponded to medium-distance smooth exploration type in Agerer’s (2001) EcM mycelial system. Typical *Inocybe* prominent clamp connections (Fig. 2b) were observed in the rhizomorphs.

Despite the low number of *Inocybe* species in the total EcM fungal diversity, *Inocybe* EcMs were frequent and were observed in 55 % of the soil cores. Two *Inocybe* species were

recovered from traced roots of *P. dipterocarpacea* and the host identity was confirmed molecularly. They were also recovered from *Aldina* sp. roots in soil cores and *Aldina rbcL* could be sequenced for one *Inocybe* EcM species.

### 3.5 Phylogeny and biogeography of *Inocybe* species associated with *P. dipterocarpacea*

One *Inocybe* species from *P. dipterocarpacea* forest (BM03M2) matched (372/381, 98% similarity) *Inocybe* EcM1091 ITS1/5.8S/ITS2 recovered from *Aldina insignis* and *Dicymbe alstonii* (Table 5). Phylogenetic analysis including LSU, together with fruit bodies' morphological comparisons, are necessary to confirm that EcM1091 and BM03M2 are conspecific. No significant match could be found for the second *Inocybe* species (Table 5). Best match with 25S LSU in GenBank was with *I. epidendron* (EU569840) for both species. Preliminary alignment of the two *Inocybe* species to Matheny et al. (2009) global Inocybaceae data set confirmed their position in *Inocybe* subgenus *Inocybe* (data not shown). These results were consistent with the characteristic *Inocybe* s. str. nodulose spores and pleurocystidia observed on *Inocybe* BM08C27 fruitbody species. LSU alignments with Matheny et al. (2009) global *Inocybe* subgenus *Inocybe* data set demonstrated that the two species were phylogenetically close to neotropical *Inocybe* species associated with *Dicymbe* and *Aldina* sp. in Guyana (Fig. 3). These species clustered within a tropical only, basal clade. A weakly supported node suggested a sister relationship with an African strain from Zambia. This clade was sister with a couple of related species from Zambia and Guyana.

## 4. Discussion

### 4.1 EcM community diversity and structure in *P. dipterocarpacea* forest

This is the first above and below ground EcM fungal diversity survey in a Neotropical forest dominated by a Dipterocarpaceae. Only few pioneering surveys have been reported on EcM fungal communities in Neotropical monospecific Dipterocarpaceae genera *Pseudomonotes* and *Pakaraimaea* (Franco-Molano et al., 2005; Moyersoen, 2006; Vasco-Palacios et al., 2005). Moyersoen (2006) study mostly focused on *P. dipterocarpacea* EcM status and only a small proportion of EcM community was described. The present study was necessary to get a more precise EcM diversity estimate in *Pakaraimaea* forest.

Despite the relatively small plot size, a great number of EcM fungal species was recorded. Total above and below ground species richness and species density in soil cores was comparable to highly diverse EcM temperate forest communities. It has been suggested that EcM diversity in tropical forests is lower than in temperate forests (Tedersoo & Nara, 2010; Tedersoo et al., 2011). In contrast, with boreal forests, where a niche differentiation of EcM fungi is observed at the level of soil horizons (Rosling et al., 2003; Tedersoo et al., 2003), a study by Tedersoo et al. (2011) suggested that EcM communities are homogeneous across soil horizons in the African forests. Recent studies indicate that trends in EcM fungal biodiversity are not simply driven by a latitudinal gradient (Smith et al., 2011). Biodiversity depends on the forest type under study (Brearly, 2011; Smith et al., 2011) and is also influenced by host identity (Haug et al., 2005; Tedersoo et al., 2010a). Amongst the EcM surveys in Dipterocarpaceae vegetation (see Brearly, 2011 review and references therein), comparisons could be made with a SE Asian Mixed-dipterocarp forest (Peay et al., 2010), where diversity was measured in plot size identical to this study. EcM

richness (Table 2) was at the top end of observed values (Table 4) in SE Asia and total above and below ground richness (Table 4) was within the range of estimated richness (9.3-60.4) in Mixed-dipterocarp forest. This comparison still needs to be taken with caution since root sampling strategy differed between the two studies. Contrary to the relatively low species density observed on Neotropical *Pisonieae* (Tedersoo et al., 2010a), density in *P. dipterocarpacea* was within the range or greater than in the temperate forests (Table 3). Both total fungal diversity and species density indicated that EcM fungi occur at a fine scale in *P. dipterocarpacea* forest such as in boreal forests. A greater EcM fungal diversity might be expected by sampling both organic and mineral soil layers in *P. dipterocarpacea* forest. This study extends the findings of a previous survey in a Neotropical Fabaceae forest where great levels of EcM fungi biodiversity were observed (Smith et al. 2011).

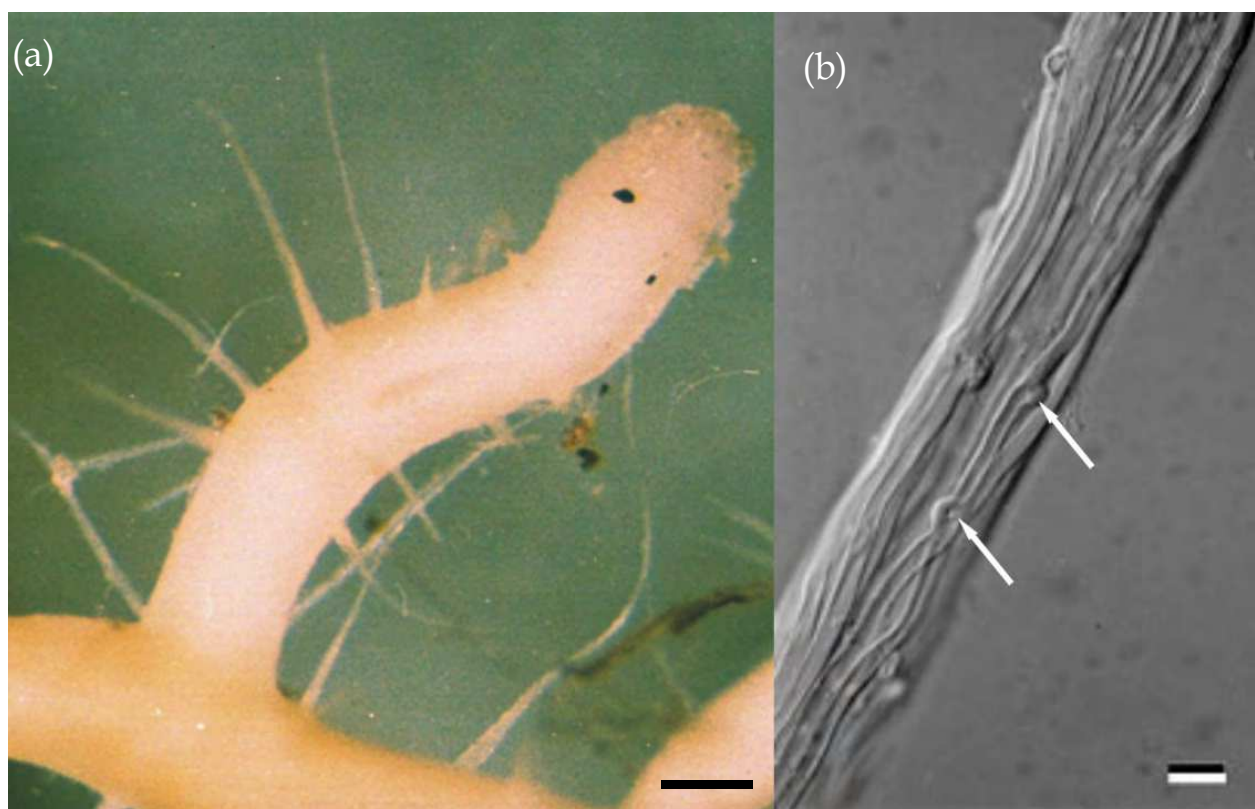


Fig. 2. (a): Habit of *Inocybe* sp. (BM03M2) ectomycorrhiza. Bar, 0.2 mm. (b): Detail of rhizomorph with clamp connections. Bar, 10 $\mu$ m.

EcM fungi in the *P. dipterocarpacea* forest belong to fungal orders generally observed in the tropical forests and elsewhere (e.g. Dickie & Moyersoen, 2008; Diédhiou et al., 2010; Lee et al., 2003; Peay et al., 2010; Riviere et al., 2007; Smith et al., 2011; Tedersoo et al., 2011). Species rich fungal orders included Agaricales, Thelephorales, Cantharellales, Boletales and Russulales. Particularly relevant was the number of *Clavulina* species. Many *Clavulina* species were also observed in *Dicymbe* forests and Smith et al. (2011) hypothesized that the Guayana region might be a centre of diversification for the *Clavulina* lineage. *Cortinarius* is usually considered to be rare in the tropical environments (Tedersoo et al., 2010b) and the great number of species identified in this study was striking. Interestingly, Peay et al. (2010) observed a greater number of *Cortinarius* in Mixed-dipterocarp forests on sandy soils. *P. dipterocarpacea* typically grows on

white sands (Maguire & Steyermark, 1981). Whether preference for sandy soils is an important ecological character in these tropical *Cortinarius* species is worth investigating. Tricholomataceae fruit body species in this study did not match any RPB2/LSU/ITS sequences either deposited in GenBank or from a recent *Dicymbe* forest EcM survey. This fruit body species is currently under further investigation and is potentially a new endemic species. *Cortinarius*, *Amanita* and *Russula* are typical “late stage” fungi characteristic of undisturbed forest stands in the temperate forests (Deacon & Fleming, 1992; Nara et al., 2003).

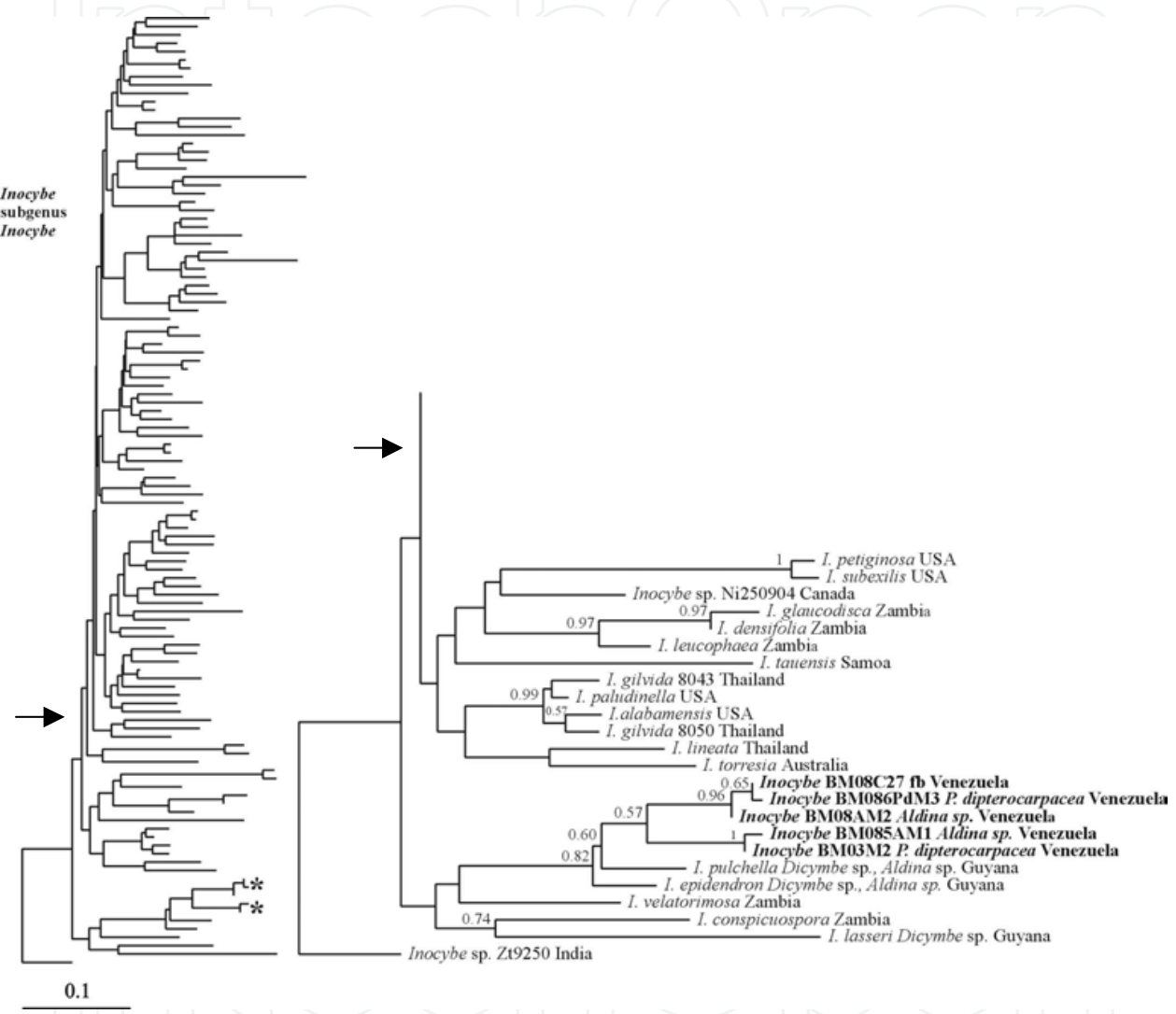


Fig. 3. Phylogenetic placement of two *P. dipterocarpacea* forest *Inocybe* species (\*). Best ML tree based on combined analysis of RPB1, RPB2 and LSU sequences, resulting from 1000 replicates Rapid Bootstrapping algorithm and ML tree search in RAxML (v. 7.2.6) using GTRCAT approximation. The tree was rooted with *Inocybe* sp. ZT 9250. Bootstrap values < 50 % are not shown.

The great total diversity, together with the abundance of these late stage fungi and the presence of a possible novel endemic species, indicated that the plot is a well preserved *P. dipterocarpacea* stand despite human activities in the region. This study also indicated that Neotropical *Pakaraimaea* and *Dicymbe* forests show similarities in EcM fungal community structure.

## 4.2 EcM fungal communities comparison between *P. dipterocarpacea* and other tropical tree host species

Until recently, biodiversity information on Neotropical lowland EcM fungi mostly relied on traditional fruit body surveys (e.g. Franco-Molano et al., 2005; Henkel et al., 2002, 2005, 2011; Matheny et al., 2003; Moyersoen, 1993; Singer et al., 1983; Vasco-Palacios et al., 2005) with inherent difficulties to confirm the host plant identity. Relatively few EcM surveys have included molecular host identifications (Haug et al., 2004; Moyersoen, 2006; Smith et al., 2011; Tedersoo et al., 2010a). This study in *P. dipterocarpacea* forest provides the first report of multi-host EcM fungal species associated both with a Dipterocarpaceae and EcM Fabaceae in the Neotropics.

With the exception of *Pisonieae* and *Gnetum* sp. where patterns of host specificity were reported (Bechem & Alexander, 2011; Haug et al., 2005; Tedersoo et al., 2010a), recent tropical EcM biodiversity surveys have shown that co-occurring (sympatric) tree species including different tree families can be colonized by identical dominant EcM fungi (Diédhiou et al., 2010; Smith et al., 2011; Tedersoo et al., 2011). These sympatric tree families included the Dipterocarpaceae and Caesalpiniaceae in the Paleotropics (Tedersoo et al., 2007, 2011). In addition to host overlap in the same forest, Tedersoo et al. (2011) also observed that some EcM fungal species are broadly distributed in tropical Africa. In contrast, host identity influences EcM fungal communities in the temperate forests (Ishida et al., 2007; Tedersoo et al., 2008). This study in *P. dipterocarpacea* forest indicated that several fungi from different fungal groups can colonise both *P. dipterocarpacea* and a sympatric Papilionoideae. Since EcM fractional colonisation and root length were not measured, sampling design did not allow to evaluate whether or not shared fungal species were true generalists (i.e. if they equally colonized the root system of both tree hosts).

The capacity of some EcM fungi to associate with *Pakaraimaea* and EcM Fabaceae was further demonstrated by the comparisons between *Pakaraimaea* forest fungal community and *Dicymbe* dominated forests. Around 15% of total species in *P. dipterocarpacea* forest (including undescribed *Amanita* sp. BM08C22, *Boletellus ananas*, *Austroboletus rostrupii*, *Clavulina* MCA4022 and two undescribed *Tomentella* species) were conspecific ( $\geq 99\%$  ITS similarity) with specimens collected in *Dicymbe* forest 240 km apart from the study site. *Cantharellus guianensis* might be an additional example of broadly distributed species but this hypothesis should be tested with complementary morphological and molecular study. Interestingly, *C. guianensis* was also described in *P. tropenbosii* dominated forest (Franco-Molano et al., 2005). Phylogenetics and morphological comparisons are necessary to confirm that the remaining 6 presumed shared species between *P. dipterocarpacea* and *Dicymbe* forests are conspecific. This study demonstrated that a proportion of EcM fungi associated with *P. dipterocarpacea* can also associate with sympatric and allopatric tree species belonging to EcM Fabaceae in the same region.

## 4.3 *Inocybe* ecology and phylogeography

Matheny et al. (2009) suggested that *Inocybe* flora in the Neotropics resulted from multiple recent immigration events in South America. The lower *Inocybe* diversity in the Neotropics than in the Paleotropics was attributed (apart from sampling effort) to a combination of factors including competition or extinction and paucity of EcM tree hosts.

The low proportion of *Inocybe* species in *P. dipterocarpacea* forest EcM fungal community was consistent with the general trend in the Neotropics. At least three different *Inocybe* lineages were observed in *Dicymbe* forests (Matheny et al., 2003; Matheny et al., 2009). *P. dipterocarpacea* survey needs to be extended to test whether this tree species also associates with several *Inocybe* lineages.

Despite the few species recorded, the frequent occurrence of *Inocybe* EcM suggested that this fungal group is successful in *P. dipterocarpacea* plot. An interesting feature of morphotypes observed on two *P. dipterocarpacea* *Inocybe* species was the presence of rhizomorphs with agglutinated hyphae and ramified distal end in close contact with soil particles. A small proportion of *Inocybe* EcM morphotypes have been formally described (Agerer, 2006) and only one of them (*I. avellana* in association with *Shorea*, Dipterocarpaceae) (Ingleby, 1999) was tropical. One of the patterns in described morphotypes was the presence of emanating hyphae only as soil exploration structures (Agerer, 2006). Ryberg et al. (2010) demonstrated that soil preference reflects the evolutionary history of Inocybaceae. Preference for poor soil prevail in some phylogenetic groups. Agerer (2001) highlighted the possible ecological importance of rhizomorphs for soil exploration and plant nutrition. Peay et al. (2011) also suggested that differences in EcM exploration type influence EcM fungal dispersion ability. Rhizomorphs might contribute to the success of *Inocybe* in poor sandy soils associated with *P. dipterocarpacea* forest. Whether this morphological character is a conserved morphological characteristic in *Inocybe* subgenus *Inocybe* basal clade is worth investigating.

In contrast with the hypothesis that *Inocybe* species associated with *P. dipterocarpacea* represent an isolated immigration event (Matheny et al., 2009), both BLASTN and phylogenetic analysis demonstrated that *P. dipterocarpacea* shared identical or phylogenetically close *Inocybe* species with EcM Fabaceae. The capacity of two *Inocybe* species to associate with both *P. dipterocarpacea* and co-occurring *Aldina* species B is consistent with the host generalist status of Inocybaceae within angiosperms in the temperate forests (Ryberg et al., 2010; Kuyper, 1986). This host sharing ability was demonstrated for several *Inocybe* species on *Dicymbe* and co-occurring *Aldina* in the Guayana region (Smith et al., 2011). Most interestingly, *Inocybe* species in *P. dipterocarpacea* forest were phylogenetically close to species in *Dicymbe* dominated forest. Singer et al. (1983) described *I. amazoniensis* from Campinarana vegetation near Manaus, Northern Brazil. They did not identify *I. amazoniensis* host plant and putative EcM hosts in Campinarana include EcM Fabaceae (*Aldina*), Pisonieae and Coccolobeae. Confirmation of *I. amazoniensis* phylogenetic position which is morphologically close to *I. epidendron* (Matheny et al., 2003) and *Inocybe* sp. BM08C27 (unpublished data) would further support the evidence that several closely related *Inocybe* species associate with diverse tree hosts including Dipterocarpaceae and EcM Fabaceae across the Guayana region.

The broad distribution of phylogenetically close *Inocybe* species might be the result of an efficient dispersion ability, the capacity to associate with different tree hosts and a long evolutionary history in the Guayana Region. A possible explanation for the disjunct distribution of *Inocybe* related species in the region is that extant forests with a dominance or codominance of one or several Neotropical EcM tree families including Dipterocarpaceae (Franco-Molano et al., 2005; Moyersoen, 2006; Vasco Palacios et al., 2005) and EcM Fabaceae (Henkel et al., 2002; Singer & Araujo, 1979, 1986) are remnants of more extensive EcM forests in the past. This hypothesis is difficult to evaluate since knowledge on Guayanian Region

vegetation history is scanty (O Huber, pers. com). *Inocybe* is often mentioned in the literature as a pioneer and an effective coloniser by spore dispersal (Smith and Read, 2008). Although they are often scattered in AM dominated forests (Béreau et al., 1997; Lodge, 1987; Singer & Araujo, 1979; St John, 1980; Tedersoo et al., 2010a), species belonging to confirmed EcM tree hosts are diverse and widely distributed in the Guayana region (Table 1). These non-dominant Neotropical EcM plants might have contributed to the dispersion of EcM fungal groups such as *Inocybe* subgenus *Inocybe* by co-migration (Halling, 2001) or by providing “EcM tree islands” for fungus colonization (Peay et al., 2007). *Inocybe* species belonging to different clades in *Inocybe* subgenus *Inocybe* (data not shown) have been found on *Guapira* and *Neea* sp. from Ecuador, Belize and US Virgin Islands (Matheny et al., 2009; Tedersoo et al., 2010a) as well as on *Coccoloba* species in Ecuador (Tedersoo et al., 2010a). Fruit bodies belonging to 8 EcM lineages including Inocybaceae were reported by Moyersoen (1993) in Bana vegetation, Southern Venezuela, where Pisonieae (*Neea* and *Guapira*) are particularly conspicuous together with *A. kunhardtiana* and *C. excelsa*. Additional surveys are necessary to test the importance of non-dominant EcM tree hosts on EcM fungi dispersion and diversity in Guayana Region. Phylogeographic analyses similar to this *Inocybe* study should also be undertaken on more diverse fungal lineages such as *Clavulina* that are associated with different confirmed tree hosts from different families across the Guayana region (Henkel et al., 2005, 2011; Smith et al. 2011).

*P. dipterocarpacea* *Inocybe* clustering within a basal clade found only in the tropics demonstrated their tropical origin. The results are consistent with Matheny et al. (2009) in the species composition of the *Inocybe* subgenus *Inocybe* basal clade (tree node 84). These basal species were not included in the phylogenetic study by Kropp and Albee-Scott (2010), who selected a subset of Neotropical and Paleotropical *Inocybe* strains. According to molecular clock phylogeny, the estimated age of tree node 84 was 65 Myr (41-89 Myr, 95% CI) (Matheny et al. 2009). *Inocybe* species associated with *P. dipterocarpacea* are the recorded oldest origin on a Dipterocarpaceae. The present study highlights the need to get a more exhaustive Dipterocarpaceae *Inocybe* ITS/LSU/RPB1/RPB2 sequence data set across the tropics. Comparisons of EcM flora between *P. dipterocarpacea* and *P. tropenbosii* as well as Dipterocarpaceae from Africa or the related EcM family Sarcolanaceae (Ducousso et al., 2004) from Madagascar would be particularly appropriate. Basal clade topology fits with a vicariance hypothesis, but Matheny et al. (2009) excluded this hypothesis on the basis of an estimated divergence date. An alternative boreotropical migration route (Pennington & Dick, 2004) was proposed (Matheny et al., 2009) but this hypothesis is not reflected in the topology of *Inocybe* subgenus *Inocybe* phylogeny. Long distance dispersal between Africa and South America would be another alternative hypothesis. There is no clear cut biogeographic interpretation for the basal clade including Paleotropical and Neotropical *Inocybe* subgenus *Inocybe* strains. Observation of two of these basal species on *P. dipterocarpacea*, a tree host with possible Gondwanic ancestors, is intriguing.

## 5. Conclusion

This study demonstrated the importance of fungal dispersion and host sharing in understanding of the EcM community associated with *P. dipterocarpacea*. Host sharing between sympatric tree species belonging to different lineages within Fabaceae had already been described in a *Dicymbe* dominated forest (Smith et al. 2011). This study highlighted that

EcM fungal species can associate with allopatric tree hosts belonging to two typical EcM tree families in the Guayana region, Dipterocarpaceae and EcM Fabaceae. There is a need to extend *Inocybe* phylogeographic study to other fungal groups and to include non-dominant tree species including Pisoniae and Coccolobeae in the surveys in Guayana Region. These studies are necessary to evaluate the importance of host overlap in the area and the possible importance of non-dominant host tree species in EcM fungal dispersion and diversity.

The hypothesized origin of Inocybaceae is Paleotropical (Matheny et al., 2009). The old origin of *Inocybe* strains associated both with *P. dipterocarpacea* and EcM Fabaceae and their tropical root suggest a long evolutionary history of EcM symbiosis in the Neotropics. Present knowledge of Inocybaceae molecular clock phylogeny is insufficient to say with certainty which EcM radiation scenario could explain the disjunct distribution of the basal clade of *Inocybe* subgenus *Inocybe*. A more complete ITS/LSU/RPB1/RPB2 sequence data set including *Inocybe* strains associated with Dipterocarpaceae across the tropics is needed to improve the understanding of Dipterocarpaceae associated *Inocybe* phylogeography.

This study confirmed that forests dominated by typical EcM tree families host EcM rich fungal communities in the Guayana Region. EcM fungal species density was great and further studies are needed to evaluate the effect of soil substrate on EcM fungal communities. The presence of a possible tropical endemic Tricholomataceae species in the *P. dipterocarpaceae* forest suggests that EcM flora could include old relictual species, therefore confirming the great biodiversity value of EcM flora in the Guayana Region (Smith et al., 2011).

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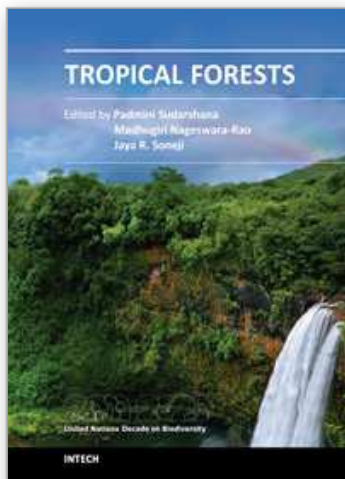
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The astounding richness and biodiversity of tropical forests is rapidly dwindling. This has severely altered the vital biogeochemical cycles of carbon, phosphorus, nitrogen etc. and has led to the change in global climate and pristine natural ecosystems. In this elegant book, we have defined "Tropical Forests" broadly, into five different themes: (1) tropical forest structure, synergy, synthesis, (2) tropical forest fragmentation, (3) impact of anthropogenic pressure, (4) Geographic Information System and remote sensing, and (5) tropical forest protection and process. The cutting-edge synthesis, detailed current reviews, several original data-rich case studies, recent experiments/experiences from leading scientists across the world are presented as unique chapters. Though, the chapters differ noticeably in the geographic focus, diverse ecosystems, time and approach, they share these five important themes and help in understanding, educating, and creating awareness on the role of "Tropical Forests" for the very survival of mankind, climate change, and the diversity of biota across the globe. This book will be of great use to the students, scientists, ecologists, population and conservation biologists, and forest managers across the globe.

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