Ectomycorrhizal fungi in Amazonian tropical forests in Colombia

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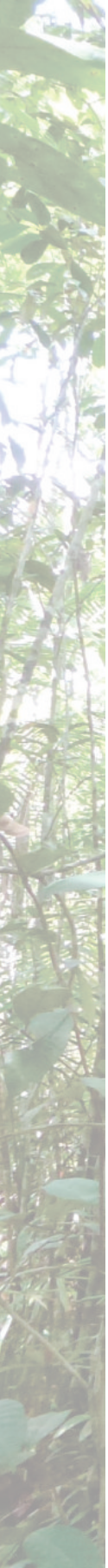


Ectomycorrhizal fungi in Amazonian tropical forests in Colombia

Ectomycorrhiza schimmels in tropische Amazone bossen in Colombia

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 26 september 2016 des middags te 6.00 uur

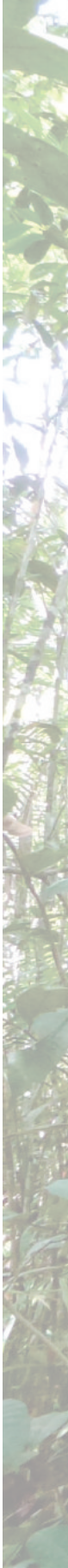
door

Aída Marcela Vasco-Palacios

geboren op 13 januari te Bogotá, Colombia

Promotoren: Prof. dr. H.A.B. Wösten
Prof. dr. T. Boekhout

To Gloria Galeano that will always
be present in my heart
and to my lovely family and friends



Contents

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

The Amazonian region encompasses an area of about 8 million km^2 that is located in the northern part of South America. It is home to the largest forests of the planet representing nearly 50 % of the global tropical rainforest area (Hoorn & Wesselingh 2010). The area includes two main ecoregions, the Amazon basin that holds the drainage basin for the Amazon River and its many tributaries, and the Guiana Shield (Eva et al. 2005). The Amazon basin can be found in Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Surinam, and Venezuela, while the Guiana Shield is located in Guyana, Surinam, French Guyana, and some parts of Venezuela, Colombia, and Brazil (Hoorn & Wesselingh 2010). The Amazon region holds a great diversity of terrestrial and aquatic organisms and plays a significant role in regulating the global climate, the hydrological cycle, and the carbon and nitrogen cycles (Hoorn & Wesselingh 2010; Phillips et al. 2009). The actual deforestation rates in the area are extremely high, ranging from 25,000 to 50,000 km^2 per year (Fearnside & Laurance 2003; Malhi et al. 2008; Shukla et al. 1990). This may result in the total disappearance of this tropical rainforest in a relatively short time (Malhi et al. 2008; Shukla et al. 1990). The overview of the biodiversity of Amazonia remains to be completed and biological processes underlying this diversity are poorly documented. While 90-95 % of plants, mammals, and bird species have been described, the vast majority of the other organisms, such as bacteria, insects, and fungi are virtually unknown (Behling et al. 2010).

Understanding Biodiversity in Amazonian Forests

Processes such as long-term landscape evolution and climate change have been identified as important drivers of speciation and extinction, thus shaping the current patterns of biodiversity in Amazonian forests (Hoorn et al. 2010). Four important historical events have strongly contributed to the current pattern of diversity in the Amazonia region: 1. a long period of isolation, 2. the Andean uplift, 3. the closing of the Panama isthmus, and 4. changes in the global climate (Burnham & Graham 1999; Hoorn et al. 2010). The different geology from East to West in combination with differences in rainfall resulted in a gradient of soil fertility, which also contributed to the pattern of biodiversity (Duque et al. 2002; Quesada et al. 2009; Sombroek 2000; ter Steege et al. 2006, 2010). Growth of plant species is restricted by a subset of environmental conditions that determines plant diversity (Fine et al. 2006). In particular, soil characteristics, such as water holding capacity, drainage, degree of flooding, soil structure, and nutrient contents affect plant growth, mortality, and levels of diversity (Duivenvoorden & Lips 1993; Ferry et al. 2010; Quesada et al. 2009). Northwestern Amazonia is typified by its relatively rich soils and has an exceptional high richness of plant diversity per unit area (Duivenvoorden & Duque 2010; Gentry 1988; ter Steege et al. 2006, 2010). In contrast, soils of Northeast Amazonia are relatively poor and this area presents a high dominance of Fabaceae (Henkel et al. 2012; Jimenez et al. 2009; McGuire 2007; ter Steege 2010). At a smaller scale, diversity and composition of plants are driven by factors such as local disturbance, predation, and soil nutrients (Augspurger 1983; Connell 1971, 1978; Duivenvoorden & Duque 2010; Fine et al. 2004; Janzen 1970; Stropp et al. 2009). Recent studies also

indicated a prominent role for interactions with soil microorganisms, in particular fungi, that structure the diversity and abundance of tropical rainforest trees (Augspurger 1983; Comita et al. 2010; Mangan et al. 2010; Packer & Clay 2000). Thus, understanding the fungal community structure is expected to have major implications for the development of ecological insights into the functioning of these tropical forests.

Importance of fungi in ecosystems

So far about 100,000 fungal species have been described but it has been estimated that there are between 1.5 to 5 million species (Blackwell 2011; Hawksworth 2012). This makes fungi the second most diverse group of Eukaryotes after insects (Blackwell 2011). They are found in aquatic and terrestrial environments and occur as unicellular yeasts, filamentous fungi, or are dimorphic. Fungi can be free-living, or establish parasitic, commensalistic, or mutualistic beneficial interactions (Gadd et al. 2007). These interactions shape the structure of communities of plants, animals, and microorganisms, such as bacteria, algae, and protozoa (Peay et al. 2008, 2013).

The decomposing activity of fungal saprotrophs plays important roles in the biogeochemical carbon and nitrogen cycles (van der Heijden et al. 2015). Fungi make up the largest pool of microbial biomass in soil and can contribute to maintenance of soil structure owing to their filamentous branching growth habit and exopolymer production (Gadd et al. 2007). The interactions of fungi with other organisms also have a major impact on ecosystems. Fungal endophytes that inhabit healthy plant tissues without causing disease produce secondary metabolites that protect the host from herbivory or promote plant fitness depending on environmental conditions or herbivore pressure (Arnold et al. 2003, 2007; Saikkonen et al. 2004). A high diversity of endophytic fungi is postulated and probably all living plants host them (Arnold 2003, 2007). Fungal plant pathogens cause serious economic losses to crops and shape the structure and composition of natural plant communities (Gilbert 2002). Mutual beneficial symbiosis also plays a major role in structuring communities. Lichens represent a mutually beneficial symbiosis between fungi and green algae and/or cyanobacteria (Lutzoni & Miadlikowska 2009). Lichens comprise thousands of species of ascomycetes and few basidiomycetes (Blackwell et al. 2000). They are widespread and particularly important in stressful abiotic environments, such as deserts and Arctic and Antarctic regions, where they contribute to biomass, nitrogen fixation, and mineral weathering (Blackwell et al. 2011; Will-Wolf et al. 2004). Other fungi form external mutualistic symbioses with insects, such as attine ants, termites, wood wasps, and ambrosia beetles (Mueller et al. 2001). These insects cultivate the fungus in gardens with a stable environment, free of pathogens. In return, the fungus provides nutrients for the insects (Currie et al. 2003; Hulcr et al. 2007; Mueller et al. 2001). The symbiosis between fungi and plant roots, known as mycorrhiza, is another example of a mutual beneficial interaction. In fact, it is one of the most ubiquitous mutualistic interactions in terrestrial ecosystems. The term “mycorrhiza” (from the Greek mykes: fungus and rhiza: root) was coined in 1885 by A.B. Frank (Kirk et al. 2008). Fungi acquire photosynthetically derived sugars from

the plants (van der Heijden et al. 2015; Wolfe et al. 2012), while the fungi contribute to mineral nutrition and water acquisition. The mycorrhizal symbiosis has a strong influence on plant growth and fitness. The absence of appropriate mycorrhizal fungi can significantly alter plant community structure (Weber et al. 2005). Mycorrhizal fungi also play an important role in the regulation of carbon and nitrogen cycles, and influence soil structure and ecosystem stability, and productivity (Bâ et al. 2014; van der Heijden et al. 2015). It has been estimated that about 50,000 fungal species form mycorrhizal association with approximately 250,000 vascular and non-vascular plant species (Smith & Read 2008; van der Heijden et al. 2015). Four major types of mycorrhizal interactions have been described based on their structure and function, namely ectomycorrhiza (EcM), arbuscular mycorrhiza (AM), orchid mycorrhiza, and ericoid mycorrhiza. The mycorrhizal symbiosis is considered ancient and seems to have evolved independently several times in the phyla Ascomycota, Basidiomycota, Glomeromycota, and Zygomycota (Rinaldi et al. 2008; Tedersoo et al. 2010a; van der Heijden et al. 2015). AM fossils of 400 million year-old have been found and it has been proposed that this interaction enabled plants to become terrestrial (Bonfante & Genre 2008; Heckman et al. 2001; Remy et al. 1994). Molecular evidence indicates that EcM-taxa of Agaricomycetes and families of Pezizales evolved about 200 and 150 million years ago, respectively (Berbee & Taylor 2001).

About 74 % of plant species are associated with Glomeromycota (AM), 2 % with EcM, and 1 % and 9 % form ericoid and orchid mycorrhizas, respectively (Brundrett 2009; van der Heijden et al. 2015). AM fungi tend to be host generalists and occur widespread in temperate and tropical ecosystems. Up to 300-1600 species have been estimated to exist based on molecular evidence, of which 244 species have been described (Oehl et al. 2011; Öpik et al. 2013; van der Heijden et al. 2015). It has been estimated that there are about 6000 species of EcM fungi (Ascomycota, Basidiomycota, and Zygomycota) that are associated with 20,000-50,000 species of plant lineages (Rinaldi et al. 2008; Tedersoo et al. 2010a). EcM fungi form an external network linked to the plant root. Some of the hyphae penetrate the spaces between the cortical and epidermal cells of the root, thus forming the Hartig network (Bonfante & Genre 2010; Carlisle et al. 1994; Halling 2001; Tedersoo et al. 2010a). EcM interactions improve the acquisition of nitrogen (N) and phosphorus (P) by the host plant by increasing the root surface area. They also protect the host against pathogens. The hyphae that envelop the root tips act as a physical barrier for these pathogens and evidence suggests that secondary metabolites of EcM fungi are toxic to pathogenic fungi, nematodes, and bacteria (Agerer 2006). In return, the EcM fungi receive organic compounds from the plants including glucose (Brearley 2012; Smith et al. 2013). EcM fungi play an important role in seedling establishment and tree growth in habitats across the globe. They are symbionts of the ecologically and economically most important forest trees belonging to Pinaceae, Fagaceae, Betulaceae, Nothofagaceae, Leptospermoideae of Myrtaceae, Dipterocarpaceae, and the Amhersteae of Caesalpiniaceae (Smith & Read 2008; Tedersoo et al. 2010a).

Ectomycorrhizal fungi in tropical ecosystems

The ectomycorrhizal symbiosis was previously assumed to be restricted to forests within temperate and boreal regions that are dominated by Pinaceae, Fagaceae, Betulaceae, and Salicaceae, as well as the Myrtaceae subfamily Leptospermoideae (Henkel et al. 2002; Smith & Read 2008). The tropics were supposed to be dominated by AM fungi with EcM interactions only present in tropical mountain ecosystems associated with Holarctic plant taxa such as *Quercus* and *Alnus* (Franco-Molano et al. 2000; González et al. 2006; Halling 1996; Mueller, 1996). The hypothesis of AM dominance in tropical lowland forests was based on a predominance of AM hosts in these forests and the lack of typical EcM fruiting bodies (Henkel et al. 2002). However, recent studies have provided evidence for the presence of EcM symbiosis in tropical lowland ecosystems (Bâ et al. 2012, 2014; Bas 1978; Brearley 2012; Diédhiou et al. 2010; Henkel et al. 2002, 2012; López-Quintero et al. 2012; Phosri et al. 2012; Smith et al. 2013; Singer & Araujo 1979; Singer et al. 1983; Tedersoo & Nara 2010; Tedersoo et al. 2010b, 2014). In fact, studies in the Paleotropics indicated a high diversity of EcM fungi associated with Dipterocarpaceae and Fagaceae (subfamily Caesalpinioideae) in Asia, Africa, and Madagascar (Brearley 2012; Corner & Bas 1962; Corner 1972; Henkel et al. 2002; Tedersoo et al. 2007; Watling & Lee 1995). The first reports of EcM in the Neotropical lowland forests were made by Singer and Araujo (1979; 1983) and Bas (1978). They described several species of EcM taxa from ectotrophic forests with *Aldina* (Fabaceae subfamily Papilionoideae) in Central Amazonia in Brazil. The recent discovery of EcM hosts in lowland forests contributed to a new understanding of EcM relationships and biogeography in the Neotropics (Henkel 2002; Moyersoen 2006). The unrelated plant genera *Aldina* and *Dicymbe* (Fabaceae subfamilies Papilionoideae and Caesalpinioideae, respectively), *Pakaraimaea* (Dipterocarpaceae subfamily Pakaraimoideae), *Pseudomonotes* (Dipterocarpaceae subfamily Monotoideae**),** *Gnetum* (Gnetaceae), the Nyctaginaceae genera *Pisonia*, *Neea*, and *Guapira,* and the genus *Coccoloba* (Polygonaceae) have independently evolved the ability to form EcM symbioses with fungi (Henkel et al. 2002, 2012; López-Quintero et al. 2012; Moyersoen 2006, 2012; Peay et al. 2010; Smith et al. 2013; Vasco-Palacios et al. 2014a, 2014b). These EcM plants present diverse distribution profiles. *Dicymbe*, *Aldina*, *Pakaraimaea,* and *Pseudomonotes* are canopy trees that tend to be co-dominant with a restricted distribution. *Pisonia*, *Neea*, *Guapira,* and *Coccoloba* species are widely distributed but are present in low densities in many forest types forming shrubs, small trees, or lianas (Singer & Araujo 1979; Tedersoo et al. 2010b). Although generally accepted as ecologically important, still little is known about macrofungal diversity, the implications of the EcM status for the host, fungal biogeography, and the ecological role of ectomycorrizha's in the structure and function of Neotropical rain forests.

Forests in Guyana that are dominated by *Dicymbe altsonii, D. corymbosa, D. jenmanii,* and *Aldina insignis* (Fabaceae) contain 174 species of EcM fungi with 54 species being recently discovered (Henkel et al. 2012; Smith et al. 2013). Approximately 60 species of EcM fungi have been identified in forests that are dominated by the dipterocarp

Pakaraimaea dipterocarpacea in Guyana and Venezuela (Moyersoen 2006; Smith et al. 2013). Studies of EcM fungi in Colombia were restricted to *Quercus*-dominated forests occurring in arboreal mountain ecosystems. Several new species have been described and the fungal community is quite well known (Franco-Molano et al. 2000; Halling 1996; Mueller 1996; Mueller & Wu, 1997; Singer 1963; Tullos et al. 1992; Tullos & Franco-Molano 2008; Vasco-Palacios et al. 2013). *Quercus humboldtii* is the only oak species that grows in Colombia, which represents the southern boundary of the geographic distribution of this important Holarctic lineage (Avella & Rangel 2014). The black-oak *Colombobalanus excelsa* (Fagaceae) is another ectotrophic element occurring in Neotropical montane forests. This endemic species belongs to the threatened category "vulnerable", mainly due to the conversion of these forests into agricultural fields (Cárdenas & Salinas 2006; Parra-Aldana et al. 2011). Not much is known about its EcM interactions (Tullos 2005). Pinaceae and *Eucalyptus* spp. (Myrtaceae) are also present in mountain areas but they are introduced species and the fungal symbionts were introduced together with these trees. As a result, species like *Amanita muscaria* and *Suillus luteus* are now part of the fungal diversity of Colombia (Vasco-Palacios & Franco-Molano 2013). In this country, 1239 species of macrofungi have been recorded (Vasco-Palacios & Franco-Molano 2013) of which only 20 % have been reported from the Amazon region and 14 belong to EcM fungi collected in forests with *Pseudomonotes tropenbosii* (López-Quintero et al. 2012; Vasco-Palacios & Franco-Molano 2013). Taken together, the last decade provided new insights in biogeographical patterns of fungal species and their hosts at global and regional scales, including the Neotropics, and resulted in the description of new taxa (Henkel et al. 2002, 2012, 2014; Moyersoen 2006; Smith et al. 2013; Tedersoo & Nara 2010; Tedersoo et al. 2014; Uehling et al. 2012a, 2012b).

Objectives

Flooded forests or várzea, non-flooded forests or terra-firme forests and white-sand forests (WSFs) represent the three main categories of forests in the Amazonian basin (Duivenvoorden & Duque 2010; Peñuela-Mora 2014). In this Thesis I focused on terra-firme forests and WSFs with the aim to document the diversity of ectomycorrhizal fungi associated with *P. tropenbosii* (Dipterocarpaceae) and Fabaceae occurring in tropical lowland forests of the Colombian Amazon region. Despite the ecological importance of Neotropical EcM symbioses, they have been poorly studied and relatively little is known about their biodiversity. This study provides a first approach into the taxonomic diversity, EcM status, and biogeographic patterns of EcM fungi in terra-firme forests with the endemic dipterocarp *P. tropenbosii* and in WSFs in Western Amazonia.

Terra-firme forest

The category terra-firme forest is used in several countries in the Amazon region referring to upland and non-flooded areas. This type of forest occurs on acidic sandy

to clayey soils that are well drained and that have a relatively high cation exchange capacity and a high amount of available phosphorus (Quesada et al. 2011; Peñuela-Mora 2014). Terra-firme forests cover about 80 % of the total area of Amazonia (ter Steege et al. 2000) and their canopy height ranges from 25 to 35 m. The understory is usually denser than that of WSFs and has many palms and tree buttresses (Riberiro et al. 1999). Notably, the terra-firme forests harbor one of world's most diverse tree communities, with few individuals of each species present (De Oliveira & Mori 1999; Duivenvoorden & Duque 2010; ter Steege et al. 2010). In Colombia, this forest is present in flat to undulating terrain with hills up to 40 m high (Parrado-Rosselli 2005). In some areas of Western Amazonia, *P. tropenbosii* (Dipterocarpaceae) constitutes an ecologically important species with an Importance Value Index (IVI) that ranges between 16-18 % (Appanah & Turnbull 1998; Londoño et al. 1995; Parrado-Rosselli 2005). Terra-firme forests with *P. tropenbosii* in Colombia are also co-dominated by species of Mimosaceae, Fabaceae (i.e. *Parkia* and *Monopteryx* species), Lecythidaceae, and Arecaceae (Parrado-Rosselli 2005). The legume trees *Parkia* and *Monopteryx* are known to establish relationships with endomycorrhizas (Moreira et al. 1992).

Pseudomonotes tropenbosii Londoño, Alvarez & Forero is an endemic member of Dipterocarpaceae that belongs to a monotypic genus described from the Colombian Amazon basin (Londoño et al. 1995). The family Dipterocarpaceae is widely distributed in Paleotropical regions and endemic species (*P. tropenbosii* and *Pk. dipterocarpacea* subsp. *dipterocarpacea* and *Pk. dipterocarpacea* subsp. *nitida)* have been described from the Neotropics (Appanah & Turnbull 1998; Londoño et al. 1995; Morton et al. 1999). *P. tropenbosii* is a member of the subfamily Monotoideae and the discovery of this species emphasized a phytogeographical link of the Colombian Amazon area with the Guiana Shield region (Duivenvoorden & Lips 1993) and even with Africa and Madagascar (Londoño et al. 1995; Morton et al. 1999). Recently, the Sarcolaenaceae, an endemic family from Madagascar, was shown to share an ancestor with Dipterocarpaceae and has been found to be ectomycorrhizal as well (Ducousso et al. 2004; Taylor & Alexander 2005). This suggests that this clade had already an EcM relationship at the Gondwana continent more than 88 million years ago (Moyersoen 2006, 2012; Taylor & Alexander 2005). Sixty-one species of EcM fungi have been found to be associated with *Pk. dipterocarpacea* (Moyersoen 2006; Smith et al. 2013) and at least 22 morphospecies of basidiomycetes of putative EcM-forming mushrooms have been collected in *P. tropenbosii* forests, but a physical link with these trees has not been proven yet (Franco-Molano et al. 2005; López-Quintero et al. 2012). Other ectomycorrhizal plant hosts, present in terra-firme forests that are dominated by Lecythidaceae, Leguminosae, Myristicaceae, Sapotaceae, and Moraceae (Duque et al. 2003) are *Coccoloba* (Polygonaceae), *Guapira* and *Neea* (Nyctaginaeae). They occur usually with a few and scattered individuals.

White-sand forests

White-sand forests (WSFs) are present in large extensions in Eastern Amazonia, with small patches occurring in North and Southwestern Amazonia (Peñuela-Mora 2014). WSFs are formed on white sandy soils or podzols that are extremely poor in nutrients, low in nutrient exchange capacity, and that are acidic and strongly leached. Usually they are water-logging in the raining season and dry in the dry seasons due to the low water retention capacity (Jiménez et al. 2009; Quesada et al. 2011). The vegetation on the sandy soils is composed of small trees with low tallness and low average diameter. Tree diversity is low when compared with terra-firme forests and they present a high specialization and high levels of plant endemism (Janzen 1974; Jiménez et al. 2009; Peñuela-Mora 2014). Fabaceae, Clusiaceae, and Malvaceae are dominant plant families in WSFs in Western Amazonia (Calle-Rendón et al. 2011; Fine et al. 2010; Peñuela-Mora 2014). Plants of the genera *Dicymbe* and *Aldina* (Fabaceae) have been recorded as EcM hosts in Guyana and Brazil (Henkel et al. 2002*,* 2012; Singer & Araujo 1979). Those genera are endemic for the Neotropics and some species form monodominant patches in the Guiana Shield region (Henkel et al. 2005; McGuire 2007; Peñuela-Mora 2014). Studies in forests dominated by *D. altsonii, D. corymbosa, D. jenmanii,* and *A. insignis* have shown a relatively high EcM fungal diversity and new taxa of fungi have been described from Guyana (Henkel et al. 2012; Smith et al. 2011). *D. uaiparuensis* and an *Aldina* species have been reported to be abundant (25 % of all individuals in a 1-ha plot) in a WSF in El Zafire Biological Station (ZBS) located in the south of the Colombian Amazon region (Peñuela-Mora 2014) (Fig. 1).

Thesis Outline

The first part of this Thesis focuses on the ectomycorrhizal status of the endemic Dipterocarpaceae *P. tropenbosii* and the Fabaceae *D. uaiparuensis* and *Aldina* sp. The fungal community composition and diversity was studied in a terra-firme mixed forest, a terra-firme forest with the Neotropical endemic dipterocarp *P. tropenbosii*, and WSFs dominated by members of the Fabaceae family within the Colombian Amazonia and other Neotropical lowland forests (Fig. 1).

The EcM status of *Dicymbe* and *Aldina* (Fabaceae) trees has been documented in Guyana and Brazil, showing a high diversity of EcM fungi. Species of these EcM host trees have been found in small patches of WSFs in Colombia Amazonia. In **Chapter 2**, the diversity and composition of EcM fungi associated with *D. uaiparuensis* and *Aldina* sp. was studied in a WSF in Colombia. Based on a fruiting body survey and molecular analysis of root-tips, 73 species of EcM fungi were identified. Of these, 7 genera and 16 species constitute new records for Colombia. Most of these EcM fungal species have been found in symbiotic associations with other legume and/or dipterocarp species in geographically distant forests.

The ectomycorrhizal symbiosis of species of the *Dipterocarpaceae* in the lowland

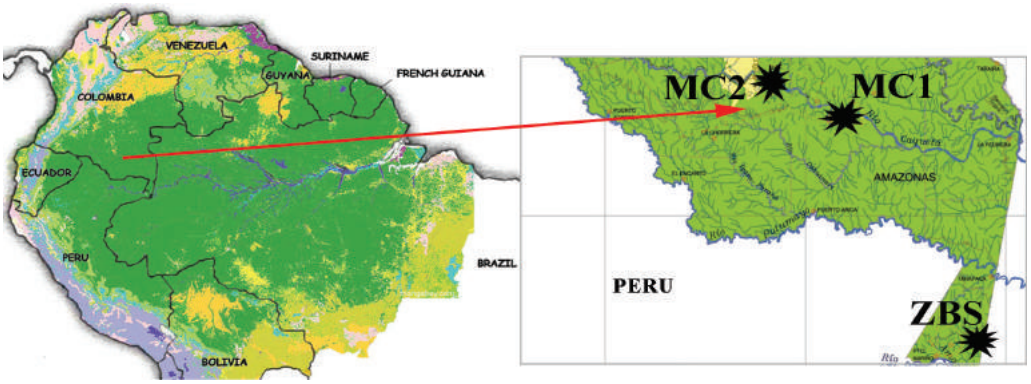


Figure 1. Location of the study sites. The biological station El Zafire (ZBS) in the Southeast Amazon Department in Colombia, and the Middle Colombian Amazon plots Peña Roja (MC1) and Puerto Santander (MC2). Map of South America (Eva *et al*. 1999), Colombia-Amazonia (IGAC 2003).

forests of Southeast Asia is well studied. However, relatively little is known about the EcM symbiosis of this plant family at other continents. The dipterocarp *P. tropenbosii* constitutes an ecologically important species in an unique lowland tropical rain forest in the Colombian Amazon region. Diversity and composition of EcM fungi associated with this endemic plant was studied in **Chapter 3** following a similar approach as used in **Chapter 2**. Eighty-three putative EcM fungal morphospecies were recovered that corresponded to commonly known EcM containing orders. Differences were observed in the composition of the EcM fungal communities in the three populations of *P. tropenbosii* studied.

Chapter 4 describes the fungal soil community structure across different sites in the Colombian Amazon region based on next-generation DNA sequencing. This study was done at the local scale in terra-firme forests with the EcM tree host *P. tropenbosii*, terra-firme mixed forests with a low abundance of the EcM hosts *Coccoloba* (Polygonaceae) or *Guapira*/*Neea* (Nyctaginaceae), and WSF with *D. uaiparuensis* and *Aldina* sp. (Fabaceae). A high diversity of soil fungi was observed in these Colombian Amazon forests. The WSF presented the most particular soil fungal community structure. The β-diversity was low across the terra-firme forests with and without *P. tropenbosii*. The fungal soil community composition correlated with forest types and soil factors, such as pH and the nitrogen/carbon ratio.

The second part of the Thesis describes several taxonomic novelties. Two boletes are described that frequently form fruiting bodies in *P. tropenbosii* forests of Colombia Amazonia (**Chapter 5**). One is a new species of *Austroboletus* described as *A. amazonicus* A.M. Vasco-Pal. & C. López-Quint. and the other one is *Fistulinella campinaranae* var. *scrobiculata* Singer, which is a new record for Colombia. Macromorphological, micromorphological, and habitat data for these species are provided as well as the DNA sequence data of the internal transcribed spacer (ITS) regions and the D1/D2 domains of the large subunit (LSU) ribosomal DNA.

In **Chapter 6,** the genera *Coltricia* and *Coltriciella* are revised based on data from the Neotropics. Despite the fact that recent phylogenetic analyses placed *Coltricia* and *Coltriciella* together in a strongly supported clade, the monophyly of each genus is still unresolved. Two new species of *Coltriciella* and one of *Coltricia* are described from Amazonian ecosystems in Colombia. Those new species, *Coltriciella cylindrospora* A.M. Vasco-Pal. & Ryvarden*, Coltriciella minuta* A.M. Vasco-Pal. & Ryvarden and *Coltricia dependella* A.M. Vasco-Pal. & Ryvarden occur in forests with the ectomycorrhizal (EcM) tree *P. tropenbosii* (Dipterocarpaceae). A key to *Coltricia* and *Coltriciella* species occurring in the Neotropics is provided.

Chapter 7 focuses on *Sarcodon*. This genus of toothed fungi appears to have a wider distribution and a broader host range than previously thought. Four species of *Sarcodon* that occur in forests dominated by the EcM trees *P. tropenbosii*, *D. uaiparuensis,* and *D. stipitata* are described as new to science. Molecular phylogenetic analysis corroborated the generic placement of the species, and, in combination with morphological characters, confirmed that they were undescribed species. Data on macromorphology, micromorphology, habitat, and DNA sequences from the nuclear rDNA internal transcribed spacer region (ITS) are provided. This is the first report of the presence of the genus *Sarcodon* in Colombia.

The results are summarized and discussed in **Chapter 8.**

SECTION 1

Diversity

CHAPTER 2

Diversity of ectomycorrhizal fungi from white-sand forests in the Colombian Amazonia

Aída M. Vasco-Palacios, Johnathan Hernández, María Cristina Peñuela-Mora, Ana E. Franco-Molano, Teun Boekhout

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Abstract

White-sand forest (WSF) ecosystems form part of the tropical rain forests in the lowlands of the Amazon region. In Western Amazonia these forests are dominated by trees of the families Clusiaceae, Malvaceae, and Fabaceae. Members of the family Fabaceae (*Dicymbe* and *Aldina*) are the main ectomycorrhizal (EcM) hosts in WSFs in Brazil, Guyana, French Guyana, and Venezuela. In WSFs in Southern Colombian Amazonia the endemic *Dicymbe uaiparuensis* and *Aldina* sp. are abundant species. In this study, we documented the EcM fungal diversity in a WSF in Colombia. We collected 117 specimens that corresponded to EcM fungi, confirming that the studied WSF hosts EcM fungi. Additionally, EcM fungal taxa were recorded from material taken from the root tips of the host trees. A total of 49 species were identified from the collected fruiting bodies, while 28 species were identified using the DNA extracted from roots. Most of the fungal species documented from the studied WSF have also been found in symbiotic associations with other legume and/or dipterocarp species from geographically distant forests located in Guyana, French Guyana, Venezuela, and Brazil. This result highlights the low specificity of EcM fungi in relation to their host plants in lowland Amazonian forests. The best-represented EcM fungi belonged to the families Russulaceae (18 species) and Amanitaceae (9 species). Seven genera and 16 species were new records for Colombia.

Introduction

The Amazon region is among the areas in the world with the highest biodiversity (Hoorn et al. 2010; Soares-Filho et al. 2006; ter Steege et al. 2013). It comprises a mosaic of ecosystems including the white-sand forests (WSFs) known as "varillal" (Peru and Colombia), "caatinga" (Venezuela), or "campina/campinarana" (Brazil). WSFs are present in large extensions in Eastern Amazonia with small patches occurring in North and Southwestern Amazonia (Peñuela-Mora 2014). WSFs are characterized by soils with low nutrient exchange capacity, low phosphorus content, acidic pH, and a low field capacity (Fine et al. 2010; Janzen 1974; Jiménez et al. 2009; Peñuela-Mora 2014). The vegetation on these sandy soils is composed of small trees with thin trunks and the tree diversity is low when compared with Amazonian terra-firme forests (Janzen 1974; Jiménez et al. 2009; Peñuela-Mora 2014). Fabaceae, Clusiaceae, and Malvaceae are dominant plant families in WSFs in the Western Amazon region (Calle-Rendón et al. 2011; Fine et al. 2010; Peñuela-Mora 2014). The genera *Dicymbe* and *Aldina* (Fabaceae) have been recorded as ectomycorrhizal (EcM) hosts in Guyana, French Guyana, Venezuela, and Brazil (Henkel et al. 2002, 2012; Roy et al. 2016; Singer & Araujo 1979, Smith et al. 2011; 2013). In Colombia, the endemic *D. uaipirensis* and an *Aldina* species have been reported to be abundant (25 % of all individuals in a 1-ha plot) in a WSF in the Zafire Biological Station (ZBS) located in the south of the Colombian Amazon region (Peñuela-Mora 2014).

Ectomycorrhizal fungi are a diverse group of mutualist root symbionts. EcM interactions improve the acquisition of nitrogen (N) and phosphorus (P) by the host plant by increasing the root surface area and they protect the hosts against pathogens. In return, the fungi receive organic compounds from the plants, including glucose (Brearley 2012; Smith et al. 2013). The distribution of EcM fungi was thought to be largely limited to temperate and boreal regions that are dominated by Pinaceae, Fagaceae, Betulaceae and Salicaceae, as well as the Myrtaceae subfamily Leptospermoideae in Australia (Henkel et al. 2002; Smith and Read 2008). The tropics were supposed to be dominated by arbuscular mycorrhizal (AM) fungi. However, recent studies have revealed that EcM fungi are also widely distributed in Neotropical lowland regions (Henkel et al. 2012; Moyersoen 2006, 2012; Roy et al. 2016; Singer & Araujo 1979; Smith et al. 2013). Data on tropical EcM hosts such as Dipterocarpaceae from Guyana, Venezuela and Colombia (López-Quintero et al. 2012; Moyersoen 2006; Smith et al. 2013; Chapter 5), Nyctaginaceae and Polygonaceae from Ecuador (Haug et al 2005; Petersen & Læssøe 2008; Tedersoo & Nara 2010), and Fabaceae in Brazil, French Guyana, and Guyana (Henkel et al. 2002, 2012; Singer & Araujo 1979; Singer et al. 1983; Roy et al. 2016) are now available. Singer was the first to report the presence of EcM fungi in lowland WSFs from Brazil (Singer & Araujo 1979; Singer et al. 1983). He proposed that EcM fungi provide the host the ability to acquire more nutrients than other plants and thus being more successful in areas with poor soils, such as white sandy soils (Singer & Araujo 1979). In Guyana, forests dominated by *Dicymbe altsonii, D. corymbosa, D. jenmanii* and *Aldina insignis* contain 174 species of EcM fungi, from which approximately 54 species were recently discovered (Henkel et al. 2012; Smith et al. 2013). Sixty-one species of EcM fungi have been identified in forests in Guyana and Venezuela that are dominated by the dipterocarp *Pakaraimaea dipterocarpacea* (Moyersoen 2006; Smith et al. 2013). In Colombia, 1239 species of macrofungi have been identified (Vasco-Palacios & Franco-Molano 2013), of which only 20 % have been reported from the Amazon region and 14 (1.1 %) belong to EcM fungi collected in forests with the dipterocarp *Pseudomonotes tropenbosii* (López-Quintero et al. 2012; Vasco-Palacios & Franco-Molano 2013). This knowledge obtained in the last decade has provided new insights in biogeographical patterns of species and their hosts at regional and global scales as well as the discovery of new taxa (e.g. Henkel et al. 2002, 2012, 2014; Moyersoen 2006; Smith et al. 2013; Tedersoo et al. 2010a, 2014; Uehling et al. 2012a; Chapters 5, 6, 7).

White-sand forests in Western Amazonia have been recently studied and they showed a high specialization and high levels of plant endemism. In Colombia, WSFs are present in small patches and are at least partially dominated by species of the genera *Dicymbe* and *Aldina* (Peñuela-Mora 2014). In this study, the diversity of EcM fungi associated with WSF was documented in a permanent plot in Colombia. Seventy-three species of EcM fungi were identified based on a survey of fruiting bodies and molecular detection on root-tips. Seven genera and 16 species represent new records

for the country, thus contributing to the knowledge of the mycota present in Colombian ecosystems. Most of the EcM fungal species found in the WSF in Colombia have been previously reported in symbiotic associations with other legume and/or dipterocarp species in geographically distant forests. We discuss how these results contribute to understand the distribution of EcM fungi and also address the low/high host specificity observed in these Neotropical ecosystems.

Materials and methods

Study area

The field work was performed in a previously established 1-ha plot in a WSF in the El Zafire biological research station area (ZBS), located in the Southern Colombian Amazon region (coordinates 4°00'21''S, 69°53'55''W) (Fig. 1 of Chapter 1). The approximate altitude is 80 m.a.s.l., the mean temperature is 26 °C, and the relative humidity is 86 % (Peñuela-Mora 2014). This zone has different types of soil with four main forest types: upland terra-firme, temporarily flooded, white-sand and transitional forests within the upland and flooded forests. All four types are primary forests with no evidence of human disturbance, except for occasional hunting (Balcázar-Vargas et al. 2012). The Fabaceae family members *Dicymbe uaiparuensis* (Subfamily Caesalpinioideae) and *Aldina* sp. (Subfamily Faboideae) are some of the most abundant tree species in the WSF of the ZBS area (25 % of all individuals) (Peñuela-Mora 2014). The area was visited four times between March 2012 and November 2014.

Sample collection, description and species accumulation curve

Fruiting bodies were collected in the WSF plot, with preference to those that belong to putative EcM fungal taxa. The specimens were macroscopically described in the field using techniques recommended for each taxon (Franco-Molano et al. 2005; Largent et al. 1977). The color was recorded with the Kornerup & Wanscher's Reinhold color atlas (1978). A small fragment of the fruiting body was stored in 2 % cetyltrimethylammonium bromide (CTAB) for molecular analysis. The remaining part of the material was dried in a sealed container with silica gel, stored in plastic boxes, and transported to the lab. Taxonomic identification of the fruiting bodies was performed by analyzing morphological characteristics and ribosomal DNA (rDNA) sequences. Morphological identification was performed using dichotomous keys (e.g. Bas 1978; Corner 1950; Henkel et al. 2011; Pegler & Fiard 1983; Simmons et al. 2002; Singer et al. 1983; Uehling 2012a; Wilson et al. 2012) and by consulting taxonomic specialists. The collections were deposited in the herbarium of the University of Antioquia (HUA), Medellín, Colombia. The online version of Index Fungorum (www.indexfungorum.org) was used to update fungal nomenclature.

Species accumulation curves were made using the function specaccum of the Vegan

package of *R* (Oksanen et al. 2013; *R* Development Core Team, 2013). To estimate the total richness of species, the Chao, Jackknife 1 and 2, and Bootstrap estimators were calculated with 1000 permutations.

Root samples and DNA preservation

Soil samples were collected at the site from the 0-15 cm upper soil layer close to 12 *D. uaiparuensis* and 8 *Aldina* spp. trees. They were air dried and transported to the laboratory (Taxonomía y Ecología de Hongos–TEHO, University of Antioquia). Roots were washed with water to remove soil residues. Mycorrhizal root apices were separated according to morphotype and preserved in Eppendorf tubes with CTAB for further molecular analyses (Smith et al. 2013).

Molecular analyses

DNA was extracted from the fruiting bodies using the MasterPure™ Yeast DNA Purification kit (Epicenter, Madison, WI, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region and the D1/D2 region of the large subunit (LSU) rDNA were amplified using primers ITS1, ITS4, ITS5, and LR0R-LR7 (Hopple & Vilgalys 1994; Vilgalys & Hester 1990; White et al. 1990). The Polymerase Chain Reaction (PCR) program consisted of 1 cycle of 5 min at 98 °C, 35 cycles of 45 s at 98 °C, 45 s at 52 °C and 20 s at 72 °C with a final extension cycle of 1 min at 72 °C.

The DNEasy plant mini kit (Qiagen, Crawley, UK) was used for DNA extraction from the mycorrhizal roots according to the manufacturer's instructions. The ITS region was amplified as described above. In the case that the entire fungal ITS region could not be amplified, primers ITS2 and ITS3 were used in combination with ITS1 and ITS4 to amplify shorter ITS regions of fungi present in the roots. To identify host plants, the chloroplast intron region trnL (UAA) was amplified using the combination of primers trnL e-f and trnL c-d (Taberlet et al. 1991). The same PCR program was used as described above with a final 1 min extension cycle at 56 °C. Amplicons were visualized with Gel Red (Biotium, San Francisco, CA, USA) after separation in 1 % agarose gels. They were purified using a 96-well multiscreen HV plate (Millipore, Billerica, MA) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, the Netherlands). Amplicons were sequenced (Sanger & Coulson 1975) using the previously listed primers and an ABI Prism 3700 Genetic analyzer (Applied Biosystems, Foster City, CA). Sequences were edited using the program SeqMan of the package DNAStar (Swindell & Plasterer 1997). Sequences obtained from the fruiting bodies and roots were deposited in GenBank (Table 1, Table 2).

Identification

Identification was based on morphological features and rDNA sequence information. Specimens were assigned to families and identified to genus and species level when

possible, and otherwise assigned to morphospecies. ITS sequences were blasted against the National Center for Biotechnology Information (NCBI) database and against the User-friendly Nordic ITS Ectomycorrhiza (UNITE) database (sh_general_release_s_30.12.2014, Abarenkof et al. 2010) using the global search tool of the USEARCH7 software program (Edgar 2010). Species were determined based on ≥ 97 % identity for operational taxonomic units (OTUs) and fruiting bodies. Identification was done at genus-level for sequences with an identity ≥ 90 % and < 97 %. Because Neotropical fungal species are not well represented in databases, ITS sequences from each OTU were also compared with ITS sequences from the collected fruiting bodies library using the global_search tool of USEARCH7 (Edgar 2010). Ectomycorrhizal taxa were assigned to the phylogenetically defined EcM fungal lineages recognized by Tedersoo et al. (2010a, 2013).

Phylogenetic analyses

ITS sequences of EcM taxa, particularly those from Guyana, were recovered from GenBank (Suppl. Material 1). Phylogenetic analyses were performed to facilitate the taxonomic placement of EcM specimens and mycorrhizal root-tips and to detect the phylogenetic relationships with other Neotropical taxa. Because of the short length of some of the sequences recovered in this study, only the ITS2 region was used for phylogenetic analysis. The ITS2 region was extracted using the fungal ITS extractor tool (ITSx) of the phylogenetic module UNITE (Bengtsson-Palme et al. 2013). Sequence alignment (Multiple Sequence Comparison by Log-Expectation-MUSCLE), phylogenetic analysis, and optimal evolutionary models were performed in MEGA6 (Tamura et al. 2013). The ITS2 sequence from the EcM ascomycete *Pseudotulostoma volvata* was used as an out-group for phylogenetic analysis of the fruiting bodies, whereas sequences of Helotiales found in root samples were used as out-group for the root analysis. The maximum likelihood estimate (MLE) was performed according to the best-fit model for each alignment and a bootstrap test with 1000 reiterations was included.

Results and Discussion

Ectomycorrhizal diversity

This is the first study of EcM fungi in a WSF in Colombia. Fourty-nine species of EcM fungi were collected as fruiting bodies from the WSF ecosystem in ZBS. They belonged to 12 genera and 9 families from the Agaricomycetes (Table 1, Fig. 1). The most diverse families were Russulaceae (18 species), Amanitaceae (9 species), and Hymenochaetaceae (7 species). Sixteen species were recorded for the first time in Colombia, enlarging the knowledge on fungal diversity in the country (e.g. 4 species of *Clavulina* and *Craterellus*, 3 species of *Amanita*, 2 species of *Lactifluus* and *Coltricia*, and 1 of *Coltriciella*) (Table 1). Seven species were identified as new species based

on molecular phylogenetic analysis in combination with morphological characters (Chapters 6, 7 and J. Duque, pers. comm. 27 January 2016). The new species belonged to the genera *Russula* (3 species), *Amanita* (2 species), *Coltriciella* (1 species), and *Sarcodon* (1 species).

Molecular analysis was performed on 211 root fragments with EcM that were selected microscopically. It should be noted that EcM fungi in the ZBS region generally did not form a well-defined mantle. Therefore, it was difficult to efficiently select roots with EcM in the soil samples. This explains why ITS regions were amplified from only 94 samples (44.5 %), of which 56 (59.5 %) corresponded to EcM fungi (Tedersoo et al. 2010a; 2013). Other ITS sequences corresponded to plants (15.9 %) and to non-EcM fungi (24.6 %). The 56 sequences of EcM fungi corresponded to 28 OTUs grouped in 8 lineages. Ascomycota was represented by the EcM lineage /helotiales (5 OTUs, 17.8 %) and Basidiomycota by 7 lineages (23 OTUs, 82.7 %) (Table 2). The most diverse lineages of Basidiomycota were /russula-lactarius (9 OTUs) and /sebacina (8 OTUs). The other OTUs belonged to /tomentella-thelephora (2 OTUs), /boletus (1 OTU), /clavulina (1 OTU), /cortinarius (1 OTU) and /inocybe (1 OTU) (Table 2). Unfortunately, the sequences obtained from plants had very poor quality and the taxonomic identification of the plant host was not possible.

Total biodiversity of EcM fungi comprised 73 species and from those 49 were collected as fruiting bodies and 28 detected from root tips. They belonged to 11 families and 14 genera of Ascomycota and Basidiomycota. The four species *Clavulina amazonensis*, *Lactarius* cf. *brasiliensis*, *Lactifluus annulifer,* and *Russula* sp. nov. 3 were recovered from both fruiting bodies and roots (Tables 1, 2). The 73 fungal species found in WSF belonged to 12 lineages of EcM fungi: /russula-lactarius (18 spp., 9 OTUs, 33 %), /amanita (9 spp., 12 %), /coltricia (7 spp., 10 %), /cortinarius (2 spp., 1 OTU, 4 %), /tomentella-thelephora (2 OTUs, 3 %), /clavulina (6 spp., 1 OTU, 8 %), /cantharellus (4 spp., 5 %), /sebacina (1 sp., 8 OTUs, 12 %), /helotiales (5 OTUs, 7 %), /boletus (1 sp., 1 OTU, 3 %), /inocybe (1 OTU, 1 %) and /hydnellum-sarcodon (1 sp., 1 %) (Tables 1, 2). Nearly 90 % of the identified species were shared with ecosystems dominated by *D. altsonii, D. corymbosa, D. jenmanii,* and *A. insignis,* or *Pk. dipterocarpacea* (Dipterocarpaceae) in Guyana (Tables 1, 2) (Henkel et al. 2002, 2012; Miller et al. 2012; Moyersoen 2006; Smith et al. 2013; Uehling et al. 2012a). These data corroborate previous findings that EcM fungi associated with lowland legume trees in the Neotropics do not show strong host preference (Smith et al. 2013).

The species accumulation curve has not reached the asymptote indicating that not all EcM species have been identified in the WSF of the ZBS area (Fig. 2). Studies in Guyana, mainly in leguminous hosts and that took > 13 years, revealed 174 EcM species (Henkel et al. 2012; Smith et al. 2011; 2013).The 73 species found in our study corresponded to approximately 42 % of the Guyana species. This suggests that more species remain to be discovered in the WSF in Colombia. However, it is important to

consider that the number of species may not reach the richness reported from Guyana due to two main factors: 1. the amount of individuals of host trees available and 2. the size of the patch. In Guyana *Dicymbe* species are monodominant (ca. 43.6 % of all individuals), while in Colombia *Dicymbe* and *Aldina* corresponded only to 25 % of all individuals and therefore less substrate is present and more competition may occur for EcM fungi. 2. WSFs in Western Amazonia are small (<10 ha) and they are generally isolated and surrounded by mixed-forests, implying that less symbiont trees are present with less interchange of species occurring between ectotrophic ecosystems (Adeney et al. 2016; Peñuela-Mora 2014).

As mentioned, Russulaceae contributed most to the diversity of ectomycorrhizal fungi with 3 identified species, 15 morphospecies (3 new species, Table 1) and 9 OTUs (Table 2). Although most of these species (41 %) were phylogenetically related to species recorded from Guyana (Figs 3, 4), several specimens remain to be identified because of the taxonomic complexity of the group, the absence of keys for species from Neotropical regions, and scarcity of expert taxonomists in this group. Thus, many of the specimens collected from WSF in the ZBS area may represent species that have not been identified previously in the region or they even may represent novel species. A similar situation holds for the other taxa (Table 1). For instance, *Russula* sp. nov. 3 (2209AMV) fruiting bodies were collected two times in the field and detected 8 times from the roots (Tables 1, 2). Its ITS sequence did not match with a significant level (≥ 97 %) with any sequence deposited in databases, neither did the morphology when compared to any described species. The most similar sequences to *Russula* sp. nov. 3 are the Uncultured fungus clone GUYSOILH05 from Guyana (95 % identity, 90 % Query Cover) and the Uncultured Russulaceae 8PdM1 from Venezuela (95 % identity, 64 % Query Cover).

The phylogenetic analyses positioned the representatives of Russulaceae within different clades (Figs 3, 4). The genus *Lactifluus*, which was recently redefined and determined to have a primarily Neotropical distribution (Verbeken et al. 2011), was highly represented at the mycorrhizal roots (Fig. 4). In general, several species found in this study were phylogenetically related to species that occur in similar ecosystems in Guyana (Table 1, Fig. 3). The phylogenetic analysis of fruiting bodies showed that *Lactifluus subiculatus* formed a well-supported clade (bootstrap of 99 %) with the specimen SLM10114 (Fig. 1). *Lf. annulifer* JOH47 (HUA 196929) formed a clade with the specimen TH9014, both known from Guyana (Fig. 3). Specimens of the *Russula puiggarii* complex (Fig. 1) were well represented in the forests. Morphologically, the description of Colombian specimens corresponded with the species description and specimens were grouped in a well-supported clade (bootstrap 99 %). Nevertheless specimens within this clade showed molecular differences (bootstrap 92 %) suggesting that they represent a species complex (Fig. 3). *Russula* sp. nov. 2 seem to be related to *Russula* sp. TH9503 (Fig. 3). This isolate has only been recorded from a dipterocarp

Figure 1. Representative ectomycorrhizal fungi (C-G) collected in WSF in El Zafire with *Dicymbe uaiparuensis* (Fabaceae) (A, B). Basidiomata of *Russula puiggarii* (Russulaceae) (C), *Clavulina amazonensis* (Clavulinaceae) (D), *Lactifluus subiculatus* (Russulaceae) (E), *Sarcodon rufogriseus* (Bankeraceae) (F), and *Craterellus atratoides* (Cantharellaceae) (G).

forest in Guyana and it may represent a new species. *Russula* sp. nov. 3 was located in the *Lactarius* clade (Fig. 3), but detailed morphological and molecular analysis assigned it as a new species of the genus *Russula* (J. Duque, pers. comm. 27 January 2016).

Table 1. Ectomycorrhizal fungi observed as fruiting bodies from WSF in El Zafire in the Southern Colombian Amazon region. Species were identified based on morphology and ITS rDNA sequences.

Lineage	Taxa	Occurrences	Represent. Voucher	Accession number	Similar species 97 %	Distribution[1]
/amanita	**Amanitaceae**					
	Amanita campinaranae [a]	1	HUA:186219	KT354670		BR, GUY
	Amanita crebresulcata	1	HUA:186220	--		BR
	Amanita lanivolva [a]	1	HUA:186221	KT354671		GUY
	Amanita xerocybe [a]	1	HUA:186222	KT354672		GUY, BR
	Amanita sp. 1 sect. Vaginatae	3	HUA:186223	KT354674, KT354673	*A. craseoderma* TH8907	GUY
	Amanita sp. 2 sect. Vaginatae	4	HUA:186226	KT354676, KT354675		
	Amanita sp. 3 sect. Vaginatae	1	HUA:186230	--		
	Amanita sp. 4 sect. Amanita	1	HUA:18623	--		
	Amanita sp. 5 subgen. Lepidella	1	HUA:186232	--		
/boletus	**Boletaceae**					
	Xerocomus sp. [b]	3	HUA:186234	KT354677, KT354678,	*Xerocomus* sp. TH8408	GUY
/cantharellus	**Cantharellaceae**					
	Craterellus atratoides [a]	7	HUA:186237	KT354698, KT354699,		GUY
	Craterellus atratus [a]	5	HUA:186242	--		GUY
	Craterellus cinereofimbriatus [a]	1	HUA:196886	KT354702		GUY
	Craterellus strigosus [a]	3	HUA:196887	KT354701		GUY
/clavulina	**Clavulinaceae**					
	Clavulina amazonensis [a, b]	6	HUA:196890,	KF937312, KT354681,		GUY, BR
	Clavulina cf. *effusa*	1	HUA:196895	KT354680		GUY

Lineage	Taxa	Occurrences	Represent. Voucher	Accession number	Similar species 97 %	Distribution[1]
	Clavulina connata [a]	1	HUA:196896	--		BR
	Clavulina kunmudlutsa [a]	1	HUA:196897	--		GUY
	Clavulina sprucei [a]	2	HUA:196898	KT354682		GUY
	Clavulina sp.	1	HUA:186179	KF937317	*Clavulina* sp. EcM1-1	
/cortinarius	**Cortinariaceae**			--		
	Cortinarius sp. 1	3	HUA:196900	KT354694, KT354695		
	Cortinarius sp. 2	2	HUA:196903	KT354696, KT354697	*Cort. galeriniformis* TH8546	GUY
/coltricia	**Hymenochaetaceae**					
	Coltricia cinnamomea	1	HUA:196905	KT354685		GUY, BR
	Coltricia hamata [a]	1	HUA:196909	KT354687		BR, Br-G, COL, VEN
	Coltricia verrucata [a]	3	HUA:196910	KT354688, KT354689		GUY
	Coltricia sp. 1	1	HUA:196914	--		
	Coltricia sp. 2	1	HUA:196918	--		
	Coltriciella dependens [a]	3	HUA:196906	KT354686		AUS, N-ZEA, SEY, C.A., USA
	Coltriciella minuta sp. nov. *	6	HUA:196913	KT354690		
/hydnellum-sarcodon	**Bankeraceae**					
	Sarcodon rufogriseus [b]*	2	HUA:186216	KR698937, KT354755		
	Russulaceae					
/russula-lactarius	*Lactarius brasiliensis*	2	HUA:186197,196924	KT354738, KT354737		GUY, BR
	Lactarius sp. 1	1	HUA:196925	--		

Lineage	Taxa	Occurrences	Represent. Voucher	Accession number	Similar species 97 %	Distribution[1]
	Lactarius sp. 2	3	HUA:196926	--		
	Lactifluus annulifer [a, b]	2	HUA:196929,196930,	KT354739		GUY, BR
	Lactifluus subiculatus [a]	5	HUA:186198,196931	KT354740, KT354741		GUY
	Russula cf. *hygrophytica*	1	HUA:196935	--		IN, lesser Antilles
	Russula cf. *foetens*	1	HUA:196936	KT354742		BR, G.B.,
	Russula cf. *rhizomorpha*	2	HUA:196937,196938	KT354743		BR
	Russula cf. *rosea*	1	HUA:196939	KT354744		G.B., IRE, USA
	Russula puiggarii	4	HUA:196940,196941	KT354745, KT354746		GUY, BR
	Russula sp. nov. 1 *	5	HUA:196944,196945	KT354748, KT354749	*Russula* MCA4008	
	Russula sp. nov. 2 *	2	HUA:196957,196958	KT354753, KT354754	*Russula* sp. TH9503	GUY
	Russula sp. nov. 3*	2	HUA:196953,196954	KT354752, KT354719		
	Russula sp. 2	1	HUA:196939	KT354744	Uncult. Russula ECM1094	
	Russula sp. 3	3	HUA:196949,196950	KT354751	*Russula* sp. 7 TH9568	GUY
	Russula sp. 4	1	HUA:196952	--		
	Russula sp. 6	1	HUA:196955	--	*Russula* MCA4008	
	Russula sp. 7	1	HUA:196956	--		
/sebacina	**Sebacinaceae**			--		
	Sebacina sp. [b]	1	HUA:196960	KT354769	*Sebacina incrustans* TUB020018	

[a] New record of species for Colombia. [b] New record of genera for Colombia. EcM lineages were identified according to Tedersoo et al. (2010; 2013). * New species. Taxa lacking epithets are morphologically distinct species level taxa and yet unidentified to species (morphospecies); taxa with epithets followed by "sp.nov." have been tentatively determined as new to science but are yet to be formally described. In column Accession number "--" means that no sequences were obtained. [1]Distribution: Correspond to countries or regions where the species have been previously reported: Australia (AUS), Brazil (BR), British Guiana (Br-G), Colombia (COL), Great Britain (G.B.), Guyana (GUY), India (IN), Ireland (IRE), New Zealand (N-ZEA), Seychelles (SEY), United State (USA), Venezuela (VEN); Central America (C.A.)

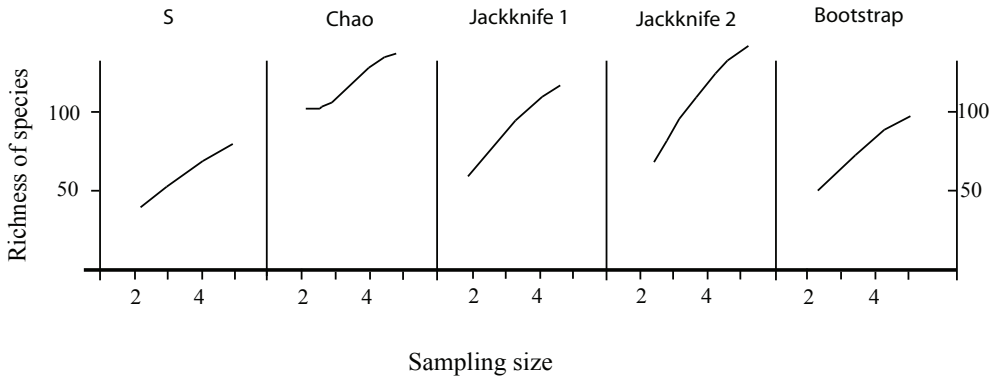

Figure 2. Rarefaction curve with the number of samples (x-axis) and number of ectomycorrhizal fungal species recovered from basidiomata (Y-axis) from a Colombian WSF. The curve represents the total number of fungal species with 95 % confidence interval, 1000 permutations and with first- and second-order jack-knife, Chao and Bootstrap estimates of species richness. S is the real observed data.

Hymenochaetaceae represented the family with the second highest number of species (7 species). This family was previously considered saprophytic, but evidence that some species of *Coltricia* and *Coltriciella* form EcM hosts with *Vateriopsis seychellarum* (Dipterocarpaceae), *Intsia bijuga* (Fabaceae), and *Eucalyptus robusta* (Myrtaceae) in African forests, and with *P. dipterocarpacea* (Dipterocarpaceae) and *Dicymbe* spp. (Fabaceae) in Guyana has been found (Tedersoo et al. 2007; Smith et al. 2013; Henkel et al. 2012; Chapter 3). Our phylogenetic analysis suggests the presence of six supported branches that could be interpreted as species (Fig. 3). Specimens of *Coltriciella oblectabilis* collected in the WSF of El Zafire were phylogenetically related to specimen TH9187 from Guyana (Fig. 3). The new species *Coltriciella minuta* sp. nov. described from *P. tropenbosii* forests, also occurs in the WSF (Chapter 6).

The only studies of Neotropical lowland taxa of the family Amanitaceae are those performed by Bas (1978) and Simmons et al. (2002). Phylogenetic analysis showed the presence of an *Amanita* clade that was not well-supported and comprised the collected species: *Amanita xerocybe*, *A. lanivolva*, *Amanita* sp. 1 sect. Vaginatae, and *Amanita* sp. 2 sect. Vaginatae (Fig. 3). Note that these species were located in well-supported branches (Fig. 3). The Vaginatae section was represented in the ZBS area with 4 species. Two of those species, *Amanita* sp. 1 sect. Vaginatae and *Amanita* sp. 2 sect. Vaginatae are morphologically different and have been collected three and four times, respectively (Table 1). These species were not related to species found in similar ecosystems in Guyana (Fig. 3) and are morphologically dissimilar to any species described until now.

The /sebacina lineage was the second-most diverse group of EcM fungi detected in roots after the /russula-lactarius lineage (Table 2). *Sebacina* represents one of the

most common and species-rich groups of EcM fungi from temperate and tropical ecosystems (Oberwinkler et al. 2013; Tedersoo et al. 2010a, 2010b 2014). It includes the genera *Sebacina* sensu stricto, *Tremellodendron,* and *Tremelloscypha.* These genera have been detected recently as EcM hosts in North Eastern Ecuador and Venezuela (Moyersoen & Weiss 2014; Tedersoo et al. 2010b; Tedersoo & Smith 2013). The Sebacina lineage forms a symbiotic relationship with plants of the families Malvaceae, Dipterocarpaceae, Myrtaceae, Fagaceae, Rosaceae, Fabaceae, and Salicaceae, and is widely distributed from the Artic to temperate and tropical zones (Oberwinkler et al. 2013; Tedersoo et al. 2010b). None of the specimens of *Sebacina* from the ZBS-WSF are related with species previously reported from the Western Amazon forests of Northern Ecuador or Venezuela (Moyersoen & Weiss 2014; Tedersoo et al. 2010b) (Figs 3, 5). A single fruiting body of this lineage was found in the studied area that was located in a well-supported clade (bootstrap 99) with EcM species from Ecuador and Germany (Oberwinkler et al. 2013; Tedersoo et al. 2010b) (Fig. 3). The 11 /sebacina sequences identified from roots formed a well-supported clade (bootstrap value of 99 %) with other sequences of Sebacina group A (EcM´s clade), including 3 from Guyana (Fig. 5). Internal branches of the /sebacina clade were not well-supported, so they cannot yet be interpreted as species. Further exploration of this group is needed to improve our understanding of the ecology and to evaluate the diversity of this lineage in Colombian ecosystems. Up to today, no species of *Sebacina* has been reported from Colombia (Vasco-Palacios & Franco-Molano 2013).

Species of the /clavulina lineage occur with high diversity in the Neotropics (Uehling et al. 2012b). In Guyana, 19 species of this lineage have been reported, of which 15 were newly recognized species (Henkel et al. 2011; Uehling et al. 2012a, 2012b). In the WSF 6 species were found, and from these five also occur in Guyana. Phylogenetic analysis from roots and fruiting bodies showed that the /clavulina lineage is a well-supported clade (99 bootstrap) with well-defined branches (Figs 3, 5). Most of the specimens collected in the ZBS area grouped with other specimens collected from WSFs in Guyana. The phylogenetic findings support morphology-based species identification, e.g. *Clavulina sprucei* (Fig. 3). *Clavulina amazonensis* (Fig. 1) was a common species that was detected both as mushrooms and from the roots. Due to short reads and low quality of the sequences obtained from the roots, in the MLE tree sequences from the root were found not to be closely related with sequences from specimen TH9191-Guyana of *C. amazonensis* (Bootstrap 89%, Fig. 5).

The phylogenetic analysis of Boletales did not result in a resolved clade (Fig. 3). Representatives of Boletales have an ITS region that is highly divergent and alignments may be ambiguous at best (Dentinger et al. 2010). Sequences of specimens collected of *Xerocomus* were related with sequences of specimens from Guyana TH8408 (Fig. 3). Evidence was found for the presence of species from the lineage /boletus at the mycorrhizal roots, but their taxonomic affiliations remained unclear because

Table 2. EcM species identified from root tips from WSF in El Zafire in the Southern Colombian Amazon region. OTUs were identified based on ITS 2 rDNA sequences

Lineage	Taxa	Occurrences-Sequences	Voucher	Accession number	Distribution[1]
/boletus	**Boletaceae**				
	Uncultured Boletales	2	JOH: 15z, 16z	KT354776, KT354777	
/clavulina	**Clavulinaceae**				
	Clavulina amazonensis [a]	2	JOH: 22z, 29z	KT354683	GUY, BR
/cortinarius	**Cortinariaceae**				
	Uncultured *Cortinarius* sp.	1	JOH: 30z	KT354692	
/inocybe	**Inocybaceae**				
	Uncultured *Inocybe*	1	JOH: 145z	KT354708	
/russula-lactarius	**Russulaceae**				
	Lactarius brasiliensis	1	JOH: 168z	KT354737	GUY
	Lactifluus annulifer [a]	2	JOH: 67z, 69z		GUY, BR
	Uncultured *Lactifluus* sp. 1	16	JOH: 48z, 66z, 68z, 71z, 77z, 80z, 84z, 90z, 94z, 96z, 98z, 100z, 102z, 104z, 113z, 121z	KT354735, KT354736, KT354734, KT354733, KT354731, KT354732, KT354709, KT354730, KT354729, KT354728, KT354727, KT354726, KT354725, KT354724, KT354723	
	Uncultured *Lactifluus* sp. 2	1	JOH: 42z	KT354722	
	Uncultured *Lactifluus* sp. 3	1	JOH: 105z	KT354720	
	Uncultured *Lactifluus* sp. 4	1	JOH: 106z	KT354721	
	Russula sp. nov. 3*	8	JOH: 46z, 85z, 86z, 92z, 93z, 97z, 112z, 119z	KT354717, KT354718, KT354715, KT354716, KT354714, KT354713, KT354712	
	Uncultured *Russula*	1	JOH: 188z	KT354710	
	Uncultured Russulaceae	1	JOH: 133z	KT354711	

Lineage	Taxa	Occurrences-Sequences	Voucher	Accession number	Distribution[1]
/sebacina	**Sebacinaceae**				
	Uncultured *Sebacina* sp. 1	2	JOH: 11z, 150z	KT354756	
	Uncultured *Sebacina* sp. 2	1	JOH: 32z	KT354757	
	Uncultured *Sebacina* sp. 3	4	JOH: 10z, 144z, 149z, 152z	KT354758, KT354759, KT354760, KT354761	
	Uncultured *Sebacina* sp. 4	2	JOH: 183z, 195z	KT354764, KT354765	
	Uncultured *Sebacina* sp. 5	2	JOH: 36z, 210z	KT354763, KT354762	
	Uncultured *Sebacina* sp. 6	1	JOH: 191z	KT354766	
	Uncultured Sebacinaceae	1	JOH: 33z	KT354767	
	Uncultured Sebacinaceae	1	JOH: 34z	KT354768	
/tomentella-thelephora	**Thelephoraceae**				
	Uncultured *Tomentella* sp. 1	4	JOH: 4z, 141z, 190z, 201z	KT354770, KT354771, KT354772, KT354773	
	Uncultured *Tomentella* sp. 2	2	JOH: 21z, 165z	KT354774, KT354775	
/helotiales	**Helotiales**				
	Uncultured Helotiales sp. 1	1	JOH: 159z	KT354703	
	Uncultured Helotiales sp. 2	1	JOH: 159za	KT354704	
	Uncultured Helotiales sp. 3	1	JOH: 197z	KT354707	
	Uncultured Helotiales sp. 4	1	JOH: 178z	KT354706	
	Uncultured Helotiales sp. 5	1	JOH: 198z	KT354705	

[a] Species found as fruiting bodies (Table 1). EcM lineages were identified according to Tedersoo et al. (2010; 2013). Taxa lacking epithets are morphologically distinct species level taxa as yet unidentified to species (morphospecies); taxa with epithets followed by "sp. nov." have been tentatively determined as new to science but are yet to be formally described. In column Accession number "--" means that no sequences were obtained. [1]Distribution: Correspond to countries where species have been previously reported: Brazil (BR), Guyana (GUY).

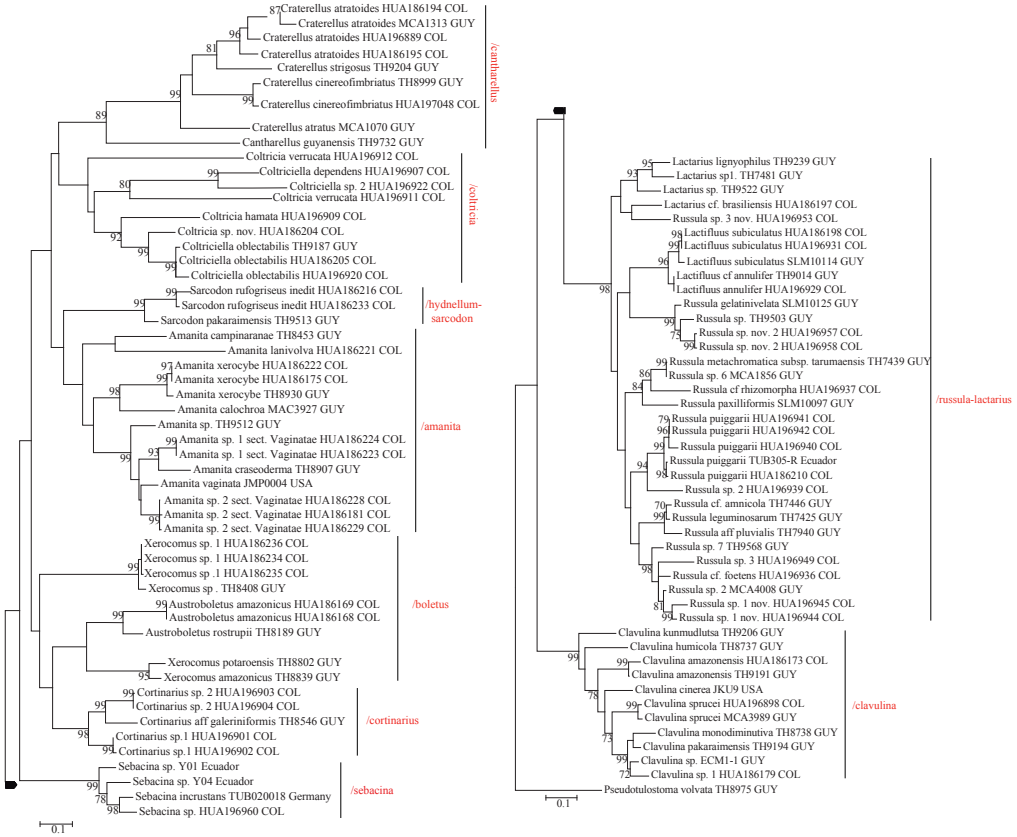

Figure 3. Phylogenetic maximum likelihood estimate (MLE) of sequences from the ITS region from fruiting body forming species collected from the WSF in Colombia. Significant bootstrap values (> 70) are indicated at the branches. The sequence of *Pseudotulostoma volvata* was used as outgroup.

the sequences were not related to those of previously identified genera or species (Fig. 5). In contrast, specimens of the lineages /cantharellus formed a well-supported group (99 bootstrap) that was represented by two species, *Craterellus atratoides* and *C. cinereofimbriatus* (Fig. 3). Despite the high number of basidiocarps of this lineage found in the ZBS area (16 in total) this group could not be detected from the roots. The /inocybe lineage was detected only from the mycorrhizal roots, whereas fruiting bodies of this group were not found during the study. Phylogenetic relationships indicated that it represents a species similar to *Inocybe amazoniensis* (Fig. 5). Specimens of found in the ZBS area (16 in total) this group could not be detected from the roots. The /inocybe lineage was detected only from the mycorrhizal roots, whereas fruiting bodies of this group were not found during the study. Phylogenetic relationships indicated that it represents a species similar to *Inocybe amazoniensis* (Fig. 5). Specimens of Fungi belonging to the Helotiales were detected from the mycorrhizal roots (Fig. 5). At the phylogenetic level, these species formed a well-defined clade despite their complex taxonomy (Tedersoo et al. 2010a) (Fig. 5). Further in-depth studies in Helotiales may

provide information about the real biodiversity of this group in tropical ecosystems and the EcM status of the species.

A great diversity was observed in mushroom-forming fungi of the families Entolomataceae and Tricholomataceae. Some species of the genera *Entoloma* and *Tricholoma* have been reported as EcM (Agerer et al. 1993; Gehring et al. 1998; Henkel et al. 2012; Matheny et al. 2006; Smith et al. 2007; Tedersoo & Smith 2013; Walker et al. 2005; Zeller et al. 2007). In our analysis, we did not find evidence of EcM associations of these fungi with the roots. A wide richness of saprophytic and parasitic fungi was observed in the WSF, possibly related to the large amount of organic matter present. As in other studies performed in the Amazon region we have demonstrated a large diversity of non-EcM fungi (Franco-Molano et al. 2005; Lopez-Quintero et al. 2012).

Most of the EcM fungal species documented from the ZBS area have been found in the genus *Cortinarius* were found as fruiting bodies and at the roots. The clade is not well-supported (40 % bootstrap), but sequences for each specimen are placed in well-supported branches (Figs 3, 5). Specimens of *Sarcodon rufogriseus* sp. nov.

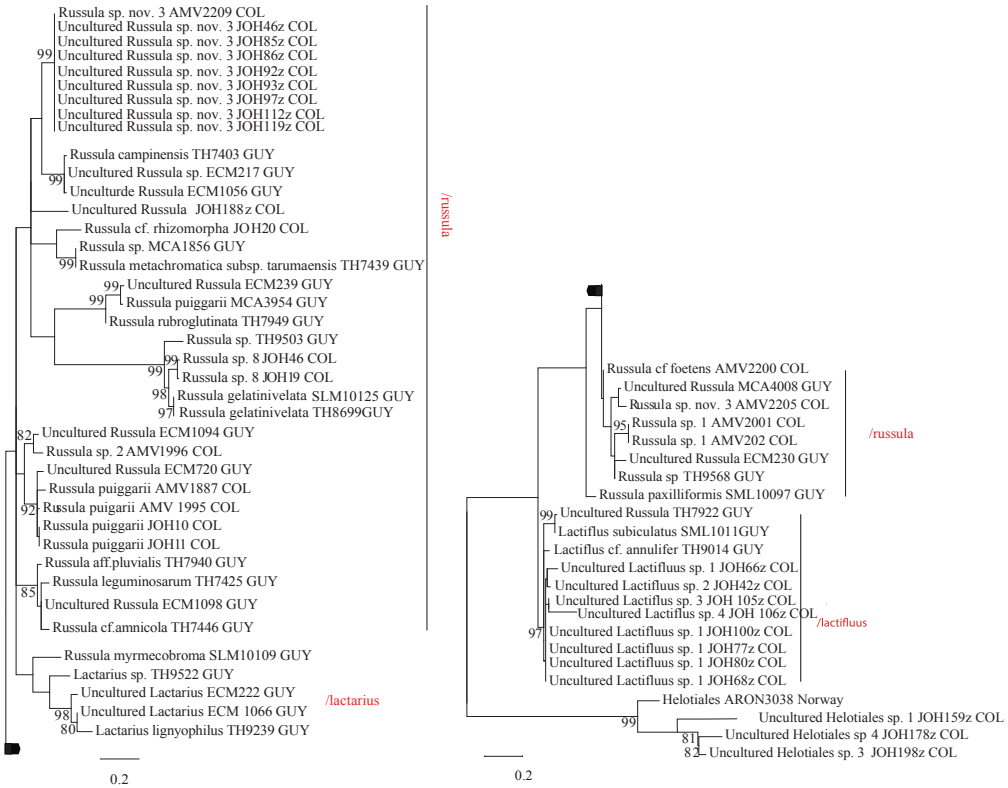

Figure 4. Phylogenetic maximum likelihood estimate (MLE) of sequences of the ITS 2 region from representatives of the Russulales clade obtained from the mycorrhized roots. Significant bootstrap values (> 70) are indicated at the branches. Sequences from Helotiales obtained in this study were used as outgroup.

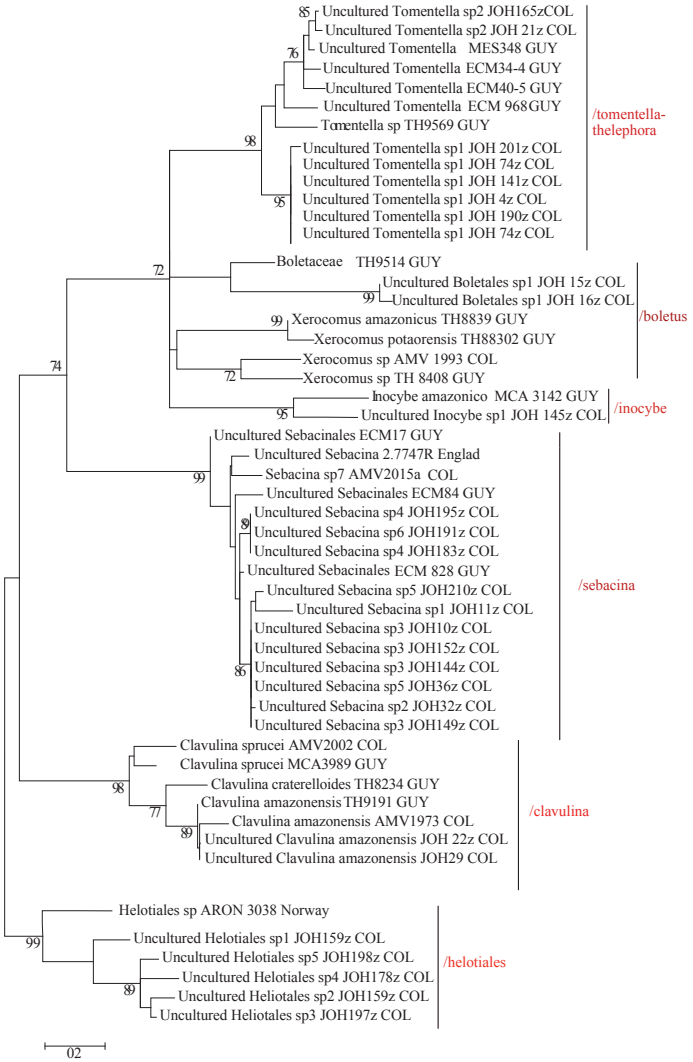

Figure 5. Phylogenetic maximum likelihood estimate (MLE) of ITS2 sequences from root-associated fungi, except the Russulaceae clade. Significant bootstrap values (> 70) are indicated at the branches. Sequences from Helotiales obtained in this study were used as outgroup.

(1989AMV and 2007AMV, Fig. 1; Chapter 7) grouped in a well-supported clade (99 % bootstrap) and were found to be related to *Sarcodon parakaimensis*, a species recently described from Guyana (Grupe et al. 2015) (Fig. 3). The lineage /tomentella-thelephora appeared as a strongly supported monophyletic group (99 % bootstrap). Sequences of this lineage were found in mycorrhizal roots and grouped into three clades/species that were phylogenetically related to specimens reported from Guyana, such as *Tomentella* sp. (TH9569) and an uncultured *Tomentella* (ECM34-4) (Smith et al. 2013) (Fig. 5). Basidiomata of *Tomentella* have not been collected in the ZBS area, which may be the result of lack of taxonomic knowledge of this group, similar

to the situation of a genus such as *Sebacina*. Fruit bodies of these genera can be easily overlooked as they occur erratic and are resupinate and/or cryptic (Moyersoen 2006). symbiotic associations with other legume and/or dipterocarp species in geographically distant forests located in Guyana. This indicates that the EcM relationship with the hosts may not be very specific. This concurs with other studies that reported that these fungi do not form highly specific symbiotic relationships with their hosts, but rather are generalists (Henkel et al. 2012; Moyersoen 2012; Smith et al. 2013). Studies have suggested that microorganisms disperse more widely than macroorganisms (Queloz et al. 2011). This combined with a low host specificity may imply a gene flow between distant populations of EcM fungi. Phylogeography studies of EcM species from tropical areas of Guyana, Venezuela, Colombia, and Brazil may be needed to elucidate the gene flow between populations of EcM fungi according to distance and plant hosts. Certainly a full assessment of the EcM fungal diversity of WSF in Western Amazonia will help to elucidate distribution patterns of the fungal symbionts and their plant hosts.

Populations of EcM plants in the Western Amazonia form small and isolated patches embedded in a larger areas of mixed anectotrophic forests. This contrasts with the large and monodominant forests of Fabaceae and Dipterocarpaceae trees occurring in Eastern Amazonia and Guyana (Peñuela-Mora 2014). However, trees that belong to the EcM host families Nyctaginaceae and Polygonaceae appear widely spread, but occurred in low abundance in the mixed forests and they may play a key role as bridges in the distribution of EcM fungi between populations.

Conclusions

Our study provided the first inside in EcM fungal diversity in a WSF in Western Amazonia in Colombia. We found 73 species of EcM fungi, of which 49 species were identified from fruiting bodies and 28 from roots. Sixteen species were newly recorded for Colombia and seven corresponded to non-described species, thus demonstrating the potential of these forests to support EcM fungal diversity. The results also suggest that more species remain to be discovered in this forest. However, it is important to consider that WSFs in Western Amazonia are small (< 10 ha), geographically isolated, and surrounded by mixed forests when compared to WSFs from Eastern Amazonia (Smith et al. 2013; Peñuela-Mora 2014). Thus, the availability of host individuals and competition between EcM fungi are factors that may affect the total species richness. The species composition was similar to that of other Fabaceae trees as well as phylogenetically distant hosts, such as Dipterocarpaceae, supporting previous data from other Neotropical forest ecosystems. Future research on the fungal diversity of WSFs in other areas of Colombia are needed as well as long lasting studies to fully characterize the diversity of EcM species and to assess whether the sizes of the patches correlate with fungal species richness. These will enhance our understanding of fungal

taxonomy, distribution patterns and ecology of this important group of fungi and their hosts at regional and global levels, also in the context of climate change.

Supplementary Material 1. List of reference specimens used in the phylogenetic analyses. For each specimen, the species name, voucher, accession number to Genbank, and country are indicated.

Uncultured Sebacina, 2.7747.3.R, EU668220, United Kingdom: England; *Clavulina sp.,* ECM1-1, JQ911751, Guyana; Uncultured Russula, ECM1056, JN168740, Guyana; Uncultured Lactarius, ECM1066, JN168729, Guyana; Uncultured Russula, ECM1094, JN168741, Guyana; Uncultured Russula, ECM1098, JN168742, Guyana; Uncultured Sebacinales, ECM17, JN168754, Guyana; Uncultured Russula, ECM217, KC155369, Guyana; Uncultured Lactarius, ECM222, JN168730, Guyana; Uncultured Russula, ECM230, JN168753, Guyana; Uncultured Russula, ECM239, JN168743, Guyana; Uncultured Tomentella, ECM34-4, KC155372, Guyana; Uncultured Tomentella, ECM40-5, KC155370, Guyana; *Clavulina* sp., ECM561, HQ680350, Guyana; Uncultured Tomentella, ECM698, JN168763, Guyana; Uncultured Russula, ECM720, JN168739, Guyana; Uncultured Sebacinales, ECM828, JN168755, Guyana; Uncultured Sebacinales, ECM84, JN168756, Guyana; *Clavulina cinerea*, JKU9, JN228228, USA; *Craterellus atratus*, MCA1070, JQ915092, Guyana; *Craterellus atratoides*, MCA1313, JQ915093, Guyana; *Russula* sp., MCA1856, JN168745, Guyana; *Inocybe amazoniensis*, MCA3142, JN642232, Guyana; *Amanita calochroa*, MCA3927, KC155375, Guyana; *Russula* aff. *Puiggarii*, MCA3954, JN168746, Guyana; *Clavulina sprucei*, MCA3989, HQ680352, Guyana; *Russula* sp. 2, MCA4008, JN168747, Guyana; Uncultured Tomentella, MES348, JN168772, Guyana; *Russula leguminosarum*, TH7425, KC155394, Guyana; *Russula metachromatica* subsp. *Tarumaensis*, TH7439, KC155393, Guyana; *Russula* cf *amnicola*, TH7446, JN168748, Guyana; *Lactarius* sp., TH7481, KC155400, Guyana; Uncultured Russula, TH7922, JN168749, Guyana; *Russula* aff *pluvialis*, TH7940, JN168750, Guyana; *Russula rubroglutinata*, TH7949, JN168751, Guyana; *Austroboletus rostrupii*, TH8189, JN168683, Guyana; *Clavulina craterelloides*, TH8234, JQ911749, Guyana; *Xerocomus* sp., TH8408, JN021114, Guyana; *Cortinarius* aff. *Galeriniformis*, TH8546, JN168714, Guyana; *Russula gelatinivelata*, TH8699, KC155395, Guyana; *Amanita craseoderma*, TH8907, KC155382, Guyana; *Clavulina humicola*, TH8737, DQ056368, Guyana; Clavulina monodiminutiva, TH8738, NR_119559, Guyana; *Xerocomus potaroensis*, TH8802, JN168784, Guyana; *Xerocomus amazonicus*, TH8839, JN168782, Guyana; *Amanita xerocybe*, TH8930, KC155384, Guyana; *Craterellus cinereofimbriatus*, TH8999, JQ91510, Guyana; *Lactarius* cf. *annulifer*, TH9014, KC155376, Guyana; *Coltriciella oblectabilis*, TH9187, KC155387, Guyana; *Clavulina amazonensis*, TH9191, HQ680356, Guyana; *Clavulina pakaraimensis*, TH9194, NR_121533, Guyana; *Craterellus strigosus*, TH9204, NR_120115, Guyana; *Clavulina kunmudlutsa*, TH9206, HQ680358, Guyana; *Lactarius lignyophilus*, TH9239, JN168732, Guyana; *Russula* sp., TH9503, KC155378, Guyana; *Amanita* sp., TH9512, KC155385, Guyana; *Boletaceae* sp., TH9514, KC155381, Guyana; *Lactarius* sp., TH9522, KC155399, Guyana; *Russula* sp., TH9568, KC155397, Guyana; *Tomentella* sp., TH9569, KC155401, Guyana; *Cantharellus guyanensis*, TH9732, KC878733, Guyana; *Russula campinensis*, TH7403, JN168738, Guyana; *Amanita campinaranae*, TH8453, KC155383, Guyana; *Pseudotulostoma volvata*, TH8975, JN168735, Guyana; *Sarcodon pakaraimensis*, TH9513, KC155390, Guyana; *Amanita vaginata*, JMP0004, EU819489, USA; *Helotiales* sp., ARON3038, AJ292197, Norway; *Sebacina incrustans*, TUB020018, KF000446, Germany; *Russula puiggarii*, TUB305-R, AY667425, Ecuador; *Russula paxilliformis*, SLM10097, JQ405656, Guyana; *Russula gelatinivelata*, SLM10125, JQ405655, Guyana; *Lactifluus subiculatus*, SLM10114, JQ405654, Guyana; *Russula myrmecobroma*, SLM10109, JQ405657, Guyana; Sebacina, Y01, UDB004249, Ecuador; Sebacina, Y04, UDB004252, Ecuador.

CHAPTER 3

Ectomycorrhizal fungal communities associated with the dipterocarp *Pseudomonotes tropenbosii* in Colombian Amazonia

Aída M. Vasco-Palacios, Ana E. Franco-Molano, Teun Boekhout

Abstract

The ectomycorrhizal (EcM) symbiosis of Asian species of the plant family *Dipterocarpaceae* is well studied. However, relatively little is known about EcM symbiosis of this plant family at other continents. Here, the EcM fungal biodiversity has been studied in three areas in Colombia with primary Amazonian rainforest dominated by *Pseudomonotes tropenbosii*, which is one of two species of the *Dipterocarpaceae* known from the Neotropics. Eighty-three putative EcM morphospecies were recovered from those forests that corresponded to commonly known EcM orders. The most diverse orders were Cantharellales (20 species), Boletales and Agaricales (17 species), and Russulales (12 species). Fifteen species represented new reports to Colombia. Five corresponded to new species that are being formally described and at least 13 other species may represent new species. The distribution of some fungal species that were previously considered restricted to the Guiana Shield was extended to *P. tropenbosii* forests in Colombia. Thirty-four OTUs at the species-level occurring in EcM root tips were identified using the Internal Transcribed Spacer (ITS) fungal barcode. Based on the number of species detected both from roots and fruiting bodies and the species accumulation curves, we predict that the number of EcM fungal species associated with *P. tropenbosii* will be between 125 and 150.

Introduction

The ectomycorrhizal (EcM) symbiosis was assumed to be restricted to forests within temperate regions that are dominated by EcM host plants. The tropics were supposed to be dominated by arbuscular mycorrhizal (AM) fungi with EcM only present in tropical mountain ecosystems associated with Holarctic plant taxa such as *Quercus* and *Alnus* (Franco-Molano et al. 2000; Gonzales et al. 2006; Halling 1996; Mueller 1996). However, recent studies provided evidence for the presence of EcM symbiosis in tropical lowland ecosystems as well (Bâ et al. 2012, 2014; Brearley 2012; Diedhiou et al. 2014; Henkel et al. 2002 2012; López–Quintero et al. 2012; Moyersoen 2006, 2012, 2014; Phosri et al. 2012; Singer & Araujo 1978; Smith et al. 2013; Tedersoo & Nara 2010; Tedersoo et al. 2010b, 2014).

The study of EcM fungi in Colombia has been restricted to *Quercus*-dominated forests in arboreal mountain ecosystems (Franco-Molano et al. 2000; Halling 1996; Mueller 1996; Mueller & Wu 1997; Singer 1963; Tullos & Franco-Molano 2008; Tullos et al. 1992; Vasco-Palacios & Franco-Molano 2013). *Quercus humboldtii* is the only oak species that grows in Colombia and this country represents the Southern boundary of the geographic distribution of this important Holarctic lineage (Avella & Rangel 2014). New fungal EcM species were found to be associated with *Q. humboldtii* belonging to *Amanita*, *Boletus*, *Cortinarius*, *Craterellus*, *Inocybe*, *Rozites*, *Lactarius*, *Leccinum*, *Russula,* and *Tylopilus* (Halling & Mueller 2005; Mueller & Wu 1997; Vasco-Palacios & Franco-Molano 2013). The black-oak *Colombobalanus*

excelsa (Fagaceae) is another ectotrophic element in Neotropical montane forests. This endemic species has the threatened category "vulnerable", mainly due to the conversion of these forests into agricultural fields (Cárdenas & Salinas 2006; Parra-Aldana et al. 2011). Not much is known about the EcM fungal community associated with *Col. excelsa*. Some potential ectomycorrhizal *Amanita* symbionts have been recorded (Tullos 2005). Pinaceae and *Eucalyptus* spp. (Myrtaceae) are also present in mountain areas but they are introduced and fungal symbionts were introduced together with these trees and, as a result, species like *Amanita muscaria* and *Suillus luteus* are part of the fungal diversity of Colombia (Vasco-Palacios & Franco-Molano 2013). Recent studies in the Amazonian region described the presence of EcM fungi in tropical rain forests associated with the endemic dipterocarp trees *Pseudomonotes tropenbosii* and the Fabaceae *Dicymbe uaiparuensis* and *Aldina* sp. (López-Quintero et al. 2012; Peñuela-Mora 2014; Vasco-Palacios et al. 2014a; Chapter 2).

Pseudomonotes tropenbosii Londoño, Alvarez & Forero is an endemic member of Dipterocarpaceae that belongs to a monotypic genus described from Colombian Amazonia. *P. tropenbosii* is member of the subfamily Monotoideae and the discovery of this species emphasizes a phytogeographical link of the Colombian Amazon area with the Guiana Shield region (Duivenvoorden & Lips 1993) and even with Africa and Madagascar (Londoño et al. 1995; Morton et al. 1999). Ectomycorrhizal symbiosis is an ecological feature of members of the Dipterocarpaceae that has been widely studied in Asian species (Brearley 2012; Lee 1990; Tata 2008). Recently, the Sarcolaenaceae, an endemic family from Madagascar, was shown to share an ancestor with Dipterocarpaceae and has been found to be ectomycorrhizal as well (Ducousso et al. 2004; Taylor & Alexander 2005). This suggests that this clade was already ectomycorrhizal at the Gondwana continent more than 88 million years ago (Moyersoen 2006 2012; Taylor & Alexander 2005). *Pakaraimaea dipterocarpacea* (Dipterocarpaceae) occurring in Guyana and Venezuela is also confirmed to have EcM symbionts. Sixty-one EcM species have been found to be associated with this tree based on ITS rDNA sequencing from root tips and basidiomata (Moyersoen 2006, 2012; Smith et al. 2013). This symbiosis originated before the separation between South America and Africa (Taylor & Alexander 2005) and thus exists more than 130 million years. Studies have also revealed the ectomycorrhizal status of *P. tropenbosii* (López-Quintero et al. 2012; Vasco-Palacios et al. 2005; Chapter 5). The Importance Value Index (IVI) of *P. tropenbosii* that ranges between 16-18 % and this species is therefore not considered as a monodominant species. Even so, *P. tropenbosii* constitutes an ecologically important species in an unique lowland tropical rain forest in the Colombian Amazon region (Appanah & Turnbull 1998; Londoño et al. 1995; Parrado-Rosselli 2005).

This study aims to document the fungal symbionts associated with *P. tropenbosii* by collecting fruit bodies and by using rDNA sequence analysis of root material for

in situ identification of both fungal and plant symbionts. We addressed whether the fungal EcM community associated with *P. tropenbosii* exhibited spatial differences and whether the community was similar with that of the other South American Dipterocarpaceae species *Pk. dipterocarpacea* and the leguminous trees occurring in the Guiana Shield and the white-sand forests (WSFs) in Colombia.

Materials and methods

Study area

Few populations of the Colombian endemic tree *P. tropenbosii* are known in terra-firme forests. Most populations have been identified in the Middle Colombian Amazon region (MC), while recently a population was found in the South of Colombia in El Zafire Biological Station (ZBS). Sampling of EcM root tips from *P. tropenbosii* and basidiomata of putative EcM species was performed at three sites in the Colombian Amazon. The first site is El Zafire Biological Station (ZBS), 4°00' S, 69°53' W, 27 km north of Leticia (Fig. 1 of Chapter 1). El Zafire is located in the South of the Amazon department close to the border with Brazil. This area belongs to the same upper and and lower Terciario superior Amazonico unit that probably originated from the Guiana Shield (Hoorn 2006; Jiménez et al. 2009). The mean temperature is about 26 °C and does not fluctuate much throughout the year. The relative humidity is about 86 % (Peñuela-Mora 2014) with a mean monthly precipitation of 280 mm. June to September is relatively dry (mean monthly precipitation 160 mm), while the rainy season spans from October to May (mean monthly precipitation 340 mm) (Jimenez et al. 2009). Soils are sandy and are mainly composed of quartz. The soils are well drained and strongly acidic (Jiménez et al. 2009). The terrain is slightly hilly with elevation ranging from 80 to 120 m. Four major types of forests are present in El Zafire, called floodplain forests, WSFs, transition forests, and terra-firme forests (Jimenez et al. 2009). They all represent primary forests with no evidence of human disturbance, except for hunting. The area was visited during the rainy season from March 2012 to November 2014. The two other sites were located in the Middle Colombian Amazon region (MC). This region is home to terra-firme forests, floodplain forests, WSFs and secondary forests (Parrado-Rosselli 2005). The communities of *P. tropenbosii* are found in terra-firme forests with a flat to undulating topography with valleys and hills of 20 to 40 m height. Soils in this area are well drained with low mineral nutrient content consisting of sands to clays of the Amazonian upper and lower Terciario superior Amazonico unit (Parrado-Rosselli 2005). The vegetation in this landscape unit is characterized by a high species richness. In some areas Mimosaceae, Fabaceae, Lecythidaceae, Arecaceae, and Dipterocarpaceae are dominant (Castaño-Arboleda & Betancur 2004; Parrado-Rosselli 2005). The first site in MC (MC1) is about 50 km downstream along the Rio Caquetá, near the locality of Peña Roja, 00°34' S, 79°08' W. The second site (MC2) is located close to the village of Puerto Santander, 00°39'

S, 72°23' W. Both sites are at 200–300 m elevation. Mean annual temperature is 26 °C and the average annual rainfall is 3060 mm (Duivenvoorden & Lips 1993). Although the region does not have a marked dry season, rainfall decreases between December and February. These localities were visited between 2010–2013. An extra site close to MC, anc called Meta, was visited ones at 00°45' S, 71°36' W.

Sample collection and preparation.

One plot of 0.1 ha was used per site. Fruiting bodies were collected from each plot. The specimens were photographed *in situ*, macromorphological characters were described from fresh material, and macrochemical tests were performed as described previously (Franco-Molano et al. 2005; Largent 1986; Lodge et al. 2004). Spore prints were obtained if possible. Color codes were designated according to the Methuen Handbook of Colour (Kornerup & Wanscher 1978). The collections were field-dried in plastic containers with silica gel. Small pieces were preserved in 2x CTAB buffer for molecular analysis (Schmit & Lodge 2005).

Roots were randomly collected within a few meters from the stem of Dipterocarpaceae hosts. Samples were air-dried and root tips were examined by light microscopy for the presence of a fungal mantle. Different EcM morphotypes that were detected at the roots were separately stored in 2x CTAB buffer for further molecular analyses. Young leaves of *P. tropenbosii* were collected from each site and dried with silica gel for molecular analysis.

Molecular analyses

DNA extraction and amplification, PCR, and sequencing were done according to Chapters 2 and 5. In brief, DNA from leaves of *P. tropenbosii* and mycorrhizal root tips was extracted using the DNEasy plant mini kit (Qiagen, Crawley, UK) following the manufacturer's recommendations. DNA from sporocarps was extracted from 20 mg of dried basidiocarp material using the PrepMan Ultra buffer (Applied Biosystems, Foster City, CA), followed by purification with JETquick general DNA clean up columns (Genomed, Löhne, Germany) according to the manufacturer's instructions. To confirm the identity of the plant host, the chloroplast trnL (UAA) intron and trnL-F spacer were amplified and sequenced using the primer combinations trnLc-trnLd and trnLe-trnLf, respectively (Taberlet et al. 2007; Chapter 2). For DNA extracted from fruiting bodies and root tips, internal transcribed spacer barcode (ITS1-2) regions of the ribosomal DNA (rDNA), including the 5.8S rDNA, were amplified and sequenced with the ITS1F, ITS5, ITS4 and ITS4B primers (Gardes & Bruns 1993; Tedersoo et al. 2007; White et al. 1990). In case amplification with the primers listed above was not successful, primers ITS 2 and ITS 3 were used in combination with primers ITS 5 and 4, respectively, which resulted in smaller amplicons (White et al. 1990). The D1/D2 domains of the large subunit (LSU) rDNA were also amplified from sporocarps with primers LR0 and LR7, and for sequencing the primers LR0, LR5 and LR7 were used

(Vilgalys & Sun; 1994). Sanger sequencing was performed using ABI Prism 3700 Genetic analyzer (Applied Biosystems, Foster City, CA).

Identification

Identification was based on morphological features and rDNA sequence information. Specimens were assigned to families and identified to genus and species when possible, and otherwise assigned to morphospecies. Specimens were deposited in the herbarium HUA, Antioquia University, Medellín, Colombia. Sequences of rDNA were edited and a consensus was obtained from forward and reverse sequences with the program SeqMan from the LaserGene package (v8.0, DNAstar, Inc.). ITS sequences from EcM root tips and fruiting bodies were compared using the BLASTn tool against the International Sequence Database (INSD) and the UNITE database (Abarenkov et al. 2010; sh_general_release_09.02.2014) by using the global-search tool, USEARCH7 (Edgar 2010). Internal transcribed spacer (ITS) sequences from the roots were considered to represent the same operational taxonomic unit (OTU), a proxy for species, if they presented an identity of ≥ 97 % of the ITS region, to genus at 90-97 % identity, or to family (85 % identity) or order level (80 % identity) (Hughes et al. 2009). The latter taxonomic units were used when the best matches were not informative, or when the quality of the root tips sequences was too low. As Neotropical fungal species are not well represented in the databases, we also compared the ITS sequences of the roots with the ones obtained from the collected fruiting bodies, employing the global-search tool, USEARCH7 (Edgar 2010). The D1/D2 LSU rDNA sequences generated from fruiting bodies, and sequences of the intron region of the trnL chloroplast (UAA) from the roots were matched against the NCBI database using the BLASTn algorithm. Ectomycorrhizal (EcM) taxa were assigned to the phylogenetically defined EcM fungal lineages recognized by Tedersoo et al. (2010a, 2013). Fungal taxonomy and names followed Index Fungorum (http://www.indexfungorum.org). All unique sequences were submitted to GenBank (Table 1).

Diversity analyses

To compare species accumulation and richness estimates among sites, rarefaction curves with 95 % confidence intervals, and 1000 permutation were used. The minimal species richness estimates Jackknife 1, 2, Chao2, and Bootstrap values were calculated using the Vegan package in R (Oksanen 2007). Distributions of species among sites were visualized in a Venn diagram using BioVenn (Hulsen et al. 2008).

Multivariate permutational analysis of variance implemented in the Adonis routine of the Vegan package (Oksanen et al. 2013) was used to address the effect of the site on fungal community composition. Adonis was calculated on a generated Bray-Curtis distance matrix based on the Hellinger-transformation of the abundance of species. Simple Mantel tests were run in Ecodist package of *R* (Goslee & Urban 2007) to determine correlations between studied forests and fungal community composition.

This was based on the abundance of all species per site. Dissimilarity matrices of fungal communities of raw abundance data were calculated using the Bray-Curtis index of similarity for all sets of data and also by type of forest and site. The significance of the Mantel statistic *P* was obtained after 9999 permutations.

Phylogenetic analyses

The ITS sequences belonging to the EcM taxa, particularly species recorded in similar forests in Guyana, were recovered from GenBank (Supp. data 1). The sequence alignment (Multiple Sequence Comparison by Log-Expectation - MUSCLE) and phylogenetic analysis were performed in MEGA 6 (Tamura et al. 2013). The optimal evolutionary model was determined for each group of analyzed sequences. Sequences of the ectomycorrhizal *Pseudotulostoma volvata* and EcM Helotiales were used as an outgroup. The maximum likelihood estimate (MLE) was performed according to the best-fit model for each alignment, and a bootstrap test with 1000 repeats was included. This analysis allowed us to cluster roots with known species and morphospecies of fungal species, while focusing on EcM taxa.

Results

Ectomycorrhizal fungal diversity and community structure

A total of 83 morphospecies of ectomycorrhizal fungi were recovered from the Colombian *P. tropenbosii* forests (Table 1). These taxa represented 16 families and 27 genera. At the family level, Boletaceae were the most diverse (7 genera; 13 species), followed by Clavulinaceae (13 *Clavulina* spp.) and Russulaceae (2 *Lactifluus* spp., 9 *Russula* spp., 1 *Lactarius* sp.). Additional taxa were found in the Hymenochaetaceae (7 *Coltricia* spp., 3 *Coltriciella* spp.), Amanitaceae (7 *Amanita* spp.), Cortinariaceae (7 *Cortinarius* spp.), Cantharellaceae (6 *Craterellus* spp.), Sebacinaceae (4 *Sebacina* spp., 1 *Tremellodendron* sp.), Thelephoraceae (2 *Thelephora* spp.), Inocybaceae (2 *Inocybe* spp.), Sclerodermataceae (2 *Scleroderma* spp.), Bankeraceae (1 *Sarcodon* sp.), Hydnaceae (1 *Sistotrema* sp.), Entolomataceae (1 *Entoloma* sp.), and Diplocystidiaceae (1 *Tremellogaster* sp.) (Fig 4.). The fruiting body of *Pseudotulostoma volvatum,* an ectomycorrhizal ascomycete described from *Dicymbe* forests in Guyana, was collected once (MC2) and was also detected from root tips (Tables 2, 3; Fig. 5). Fifteen species that were identified based on morphology and rDNA barcoding of ITS sequences constituted new reports for Colombia (Table 2). The 83 taxa represented 15 independent lineages of EcM fungi according to Tedersoo et al. (2010a; 2013) with the majority occurring in the /boletus and /clavulina (15.5 %), /coltricia-coltriciella (13.1 %) and /russula-lactarius (14.2 %) lineages (Fig. 1). At least 18 species found in the study area new to science. Five of those have recently been described as *Austroboletus amazonicus*, *Sarcodon colombiensis, Coltriciella cylindrospora Coltriciella minuta,* and *Coltricia dependella* (Chapters 5, 6, 7) and others belong to genera such as *Russula* and *Amanita*.

Table 1. Diversity of EcM fruiting bodies in *P. tropenbosii* forests. Species were identified based on morphology and ITS rDNA sequences

Taxa	Total Species	Taxa	Total Species
Agaricales	**17**	**Eurotiales**	**1**
Amanitaceae	7	Elaphomycetaceae	1
Cortinariaceae	7	**Hymenochaetales**	**10**
Entolomataceae	1	Hymenochaetaceae	10
Inocybaceae	2	**Russulales**	**12**
Boletales	**16**	Russulaceae	12
Boletaceae	13	**Sebacinales**	**4**
Diplocystidiaceae	1	Sebacinaceae	4
Sclerodermataceae	2	**Thelephorales**	**3**
Cantharellales	**20**	Bankeraceae	1
Cantharellaceae	6	Thelephoraceae	2
Clavulinaceae	13		
Hydnaceae	1		

The species accumulation curve indicated that the EcM fungal diversity was not fully recovered during sampling (Fig. 2). The estimated richness suggested that approximately 125-150 species (first-order and second-order jackknife/Chao, respectively) comprise the total biodiversity of EcM fungi in these *P. tropenbosii* forests (Fig. 2). Bootstrap estimates indicated that approximately 100 species are present, which is close to the number of 83 observed species.

Comparing the EcM fungal composition of the three sites revealed a non-uniform composition with 52 species recorded in MC1, 13 species in MC2, and 43 species in ZBS. These forests were dissimilar with respect to the fungal species composition (ANOVA, $P > 0.05$; Mantel $P > 0.05$); the EcM fungal communities were different also when compared by pairs (MC1-MC2, MC1-ZBS, MC2-ZBS; Mantel $P > 0.05$). The former two places (MC) were visited 6 times, while ZBS was visited 5 times. The number of shared species for plot pairs was 5 species for MC1-MC2-ZBS, 1 species for MC1-MC2, 14 species for MC1-ZBS, while no overlap in species was observed between MC2-ZBS (Figs 3A, B). Considering the Middle Colombian Amazon region as an unit, 59 species of EcM fungi were recovered and 19 species were shared with the geographically separated area ZBS (Fig. 3B). A different composition of EcM community was observed among sites when considering lineages (Figs 3A-C). The sites MC1 (12 lineages) and ZBS (12 lineages) differed by the lineage /hydnellum-sarcodon, represented by a new species of *Sarcodon* (Fig 4.). In the site MC2 only 7

lineages were represented. We expected a similar number as in MC1 because these two sites are relatively close in distance.

Nine species were found > 10 times, 9 species in MC1, 4 in MC2 and 7 in ZBS indicating that these species fructified abundantly during the study period. The most frequently encountered species was *Amanita* sp. 1 from section Vaginatae that was collected 17 times from MC1 and ZBS. This species was also found in WSFs with *D. uaiparuensis* (Fabaceae) in El Zafire (Chapter 2). *Amanita* sp. 2 sect. Vaginatae was collected 14 times from MC1, ZBS, and also from *Dicymbe* forests in Colombia and Guyana (Henkel et al. 2012; Chapter 2). These two species of *Amanita* sect. Vaginatae differ in pileus color and spore size. *Amanita* sp.1 has a brown pileus and ovoid spores of 7-9 x 12-15 µm, whereas the pileus of *Amanita* sp. 2 was greyish and its spores globose, 10-12 x 10-13 µm. The boletaceae *Fistulinella campinaranae* var. *scrobiculata* was collected 15 times but specimens were only found in MC1 (Chapter 5). *Austroboletus amazonicus* that was recently described from *P. tropenbosii* forests (Chapter 5) was collected 6 times in MC1 and 3 times in ZBS. Cantharellales were present with *Craterellus strigosus* and *Clavulina amazonensis* as abundant species. They were collected in all plots during all visits. Two *Clavulina* species described from *Dicymbe* forests, the particular shaped *Cla. craterelloides* and *Cla. guyanensis*, were frequently collected from MC sites, and the second species also from ZBS. *Russula* was an important group in the EcM fungal community in *P. tropenbosii* forests. Twelve species of Russulaceae belonged to *Russula* (9 species), *Lactarius* (1 species), and *Lactifluus* (2 species), respectively. *Russula puiggarii* and the purple colored *R. gelatinivelata* were abundant (Fig 5). *R. puiggarii* was found in MC, ZBS, and has been found hosted by Fabaceae and *Pakaraimaea* too (Smith et al. 2013). From the other taxa, *Entoloma* sp. 1 was relatively common in all plots and a different species, Entoloma sp. 839, was detected from a root tip but not from fruiting bodies in ZBS (Table 4).

Ectomycorrhizal roots

A total of 236 fragments of mycorrhizal roots tips were analyzed by DNA sequencing. 218 fragments showed fungal DNA sequences (92 %) and 177 of those (81 %) represented EcM fungi. Thirty-four ITS species-level operational taxonomic units (OTUs), 26 genus-level and 38 family-level OTUs were recovered from root tips (Table 3). Seven species-level OTUs and three genus-level OTUs were detected three or more times, 10 species-level and three genus-level OTUs were found twice, while 17 species-level and 20 genus-level OTUs were detected only once (Table 4). These EcM taxa represented 12 independent fungal lineages with the majority of taxa and abundances occurring in the /tomentella-telephora, /cortinarius, /russula-lactarius and /helotiales lineages (Table 3). MC1, MC2, and ZBS presented a similar representation of biodiversity recovered from the roots at species level with 8, 9 and 10 lineages respectively (Fig. 6). Nevertheless the site MC1 presented the highest richness with 18 OTUs, followed by MC2 and ZBS with 14 and 10 species, respectively.

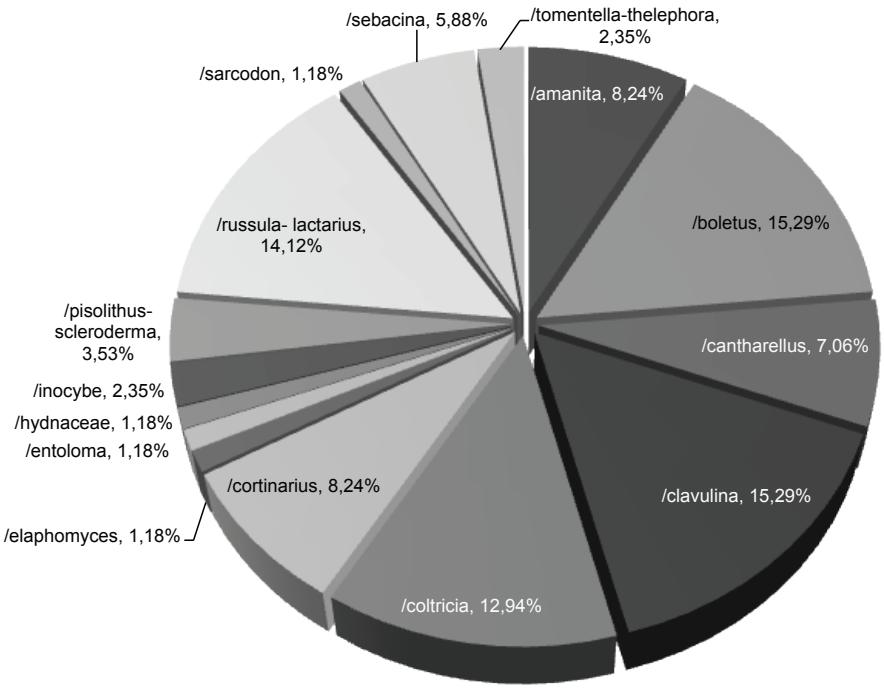

Figure 1. EcM fungal lineages recovered from fruiting bodies in *P. tropenbosii* forests. The EcM lineages were defined according to Tedersoo *et al*. (2010a, 2013).

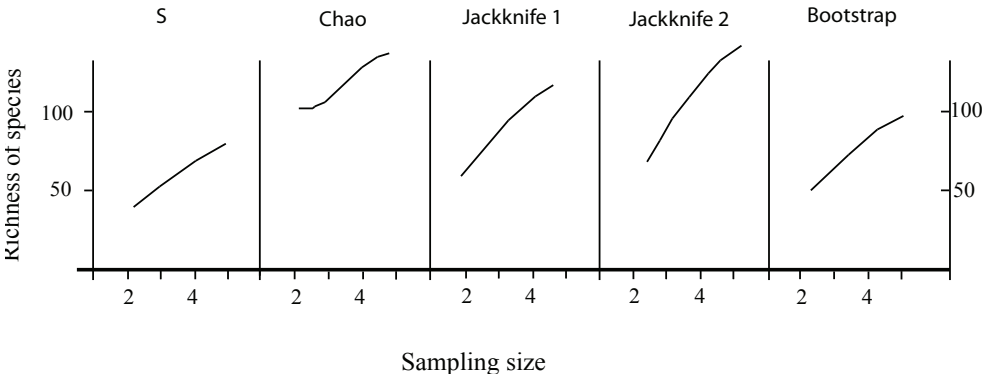

Figure 2. Rarefaction curve with the number of samples (x-axis) and number of ectomycorrhizal fungal species recovered from basidiomata (Y-axis) from Colombian *P. tropenbosii* forests. The curve represents the total number of fungal species with 95 % confidence interval, 1000 permutations and with first- and second-order jack-knife, Chao, and Bootstrap estimates of species richness. S is the real observed data.

Table 2. Ectomycorrhizal fruiting bodies from terra-firme forests with *P. tropenbosii*. Species were identified based on morphology and ITS rDNA sequences.

Lineage°	Taxonomy[1]	Representative Voucher[2]	Forest type[3]
	Ascomycota		
/elaphomyces	**Elaphomycetaceae**		
	Pseudotulostoma volvatum O.K. Mill. & Henkel*	HUA: 186187	1, 3
	Basidiomycota		
/amanita	**Amanitaceae**		
	Amanita campinaranae Bas	HUA: 196961, 196962	1, 2, 3
	Amanita sp. 1 sect. Vaginatae ined.	HUA: 186180, 186181, 196970, 196971, 196972, 196973, 196975	2
	Amanita sp. 2 sect. Vaginatae ined.	HUA: 196976, 196977, 196978, 196984, 196987, 196988, 196989	1, 2
	Amanita sp. 3 sect. Vaginatae	HUA: 196990, 196991	
	Amanita sp. 4 sect. Vaginatae	HUA: 196992	
	Amanita sp. 5 sect. Phalloideae	HUA: 196993	
	Amanita xerocybe Bas	HUA: 186174, 186175, 196994, 196995, , 196998, 196999, 197000	1, 2, 3
/boletus	**Boletaceae**		
	Aureoboletus sp. 1	HUA: 197001	
	Aureoboletus sp. 2	HUA: 197002	
	Austroboletus amazonicus A.M. Vasco-Pal. & C. López-Quint.[1]	HUA:186167, 186168, 186169, 197004, 197005, 197006, 197007	
	Austroboletus festivus (Singer) Wolfe *	HUA: 197008, 197009, 197010, 197011	1
	Austroboletus sp. 3	HUA: 197012	
	Boletellus ananas var. *minor* Singer *	HUA: 186186	
	Fistulinella campinaranae var. *scrobiculata* Singer	HUA: 186170, 186171, 197015, 197016, 197031, 197032	
	Rubinoboletus sp. 1	HUA: 197035	
	Tylopilus sp. 1	HUA: 197036	
	Tylopilus sp. 2	HUA: 197013	
	Xerocomus amazonicus Singer *	HUA: 197037	1, 3
	Xerocomus sp. 1	HUA: 197038, 197039	

Lineage°	Taxonomy[1]	Representative Voucher[2]	Forest type[3]
	Xerocomus/*Pyhlloporus* sp. 1	HUA: 197040	
/cantharellus	**Cantharellaceae**		
	Craterellus atratoides Henkel, Aime & Wilson	HUA: 197041, 197042, 197043, 197044	1, 2
	Craterellus atratus (Corner) Watling, Yomyart & Sihan	HUA: 186192, 197045, 197046, 197047	1, 2, 3
	Craterellus cinereofimbriatus Henkel & Wilson	HUA: 197048	
	Craterellus sp. 1	HUA: 197049, 197050, 197051, 197052	
	Craterellus sp. 2	HUA: 197053	
	Craterellus strigosus Henkel, Aime & Wilson	HUA: 186196, 197054, 197057, 197058, 197059, 197063, 197066	1, 2
/clavulina	**Clavulinaceae**		
	Clavulina amazonensis Corner	HUA: 186173, 197068, 197069, 197081, 197083, 197084, 197085	1, 2, 3
	Clavulina cinereoglebosa Uehling, Aime & Henkel *	HUA: 197086, 197087	1, 3
	Clavulina cirrhata (Berk.) Corner *	HUA: 197088	3
	Clavulina craterelloides Thacker & Henkel *	HUA: 186176, 186177, 197089, 197093, 197094, 197095, 197096	1, 3
	Clavulina effusa Uehling, Henkel & Aime	HUA: 197097, 197098, 197099	
	Clavulina griseohumicola Henkel, Meszaros & Aime *	HUA: 197100, 197101, 197102, 197103, 197104, 197105, 197106	1
	Clavulina guyanensis Uehling, Henkel & Aime *	HUA: 197107, 197112, 197113, 197114, 197115, 197116, 197117	1
	Clavulina kunmudlutsa Henkel & Aime	HUA: 197118	1, 2, 3
	Clavulina nigricans Thacker & Henkel*	HUA: 197119	1
	Clavulina rosiramea Uehling, Henkel & Aime *	HUA: 197120	1
	Clavulina sp. 1	HUA: 197121, 197122, 197123	
	Clavulina sprucei (Berk.) Corner complex	HUA: 197124, 197125, 197126, 197127	1, 2, 3
	Clavulina tepurumenga T.W.Henkel & Aime *	HUA: 186178, 197128, 197129, 197130, 197131	1
/coltricia	**Hymenochaetaceae**		
	Coltricia barbata Ryvarden & de Meijer *	HUA: 186204, 197132	
	Coltricia dependella sp. nov. [1]	HUA: 197146	

Lineage°	Taxonomy[1]	Representative Voucher[2]	Forest type[3]
	Coltricia hamata (Romell) Ryvarden	HUA: 197135, 197136, 197137, 197138, 197139	2
	Coltricia sp. 1	HUA: 197143	
	Coltricia sp. 2	HUA: 197144	
	Coltricia sp. 4	HUA: 197145	
	Coltriciella cylindrica sp. nov. [1]	HUA: 186205, 197134	
	Coltriciella dependens (Berk. & M.A. Curtis) Murrill *	HUA: 197147	3
	Coltriciella minuta sp. nov. [1]	HUA: 197145, 197148, 197149, 197150, 197151, 197152, 197153	
	Coltriciella oblectabilis (Lloyd) Ryvarden	HUA: 197140, 197141, 197142, 197133	1, 2, 3
/cortinarius	**Cortinariaceae**		
	Cortinarius sp. 1	HUA: 197154	
	Cortinarius sp. 2	HUA: 197155	2
	Cortinarius sp. 3 similar to TH8613	HUA: 197156	1, 2
	Cortinarius sp. 4 brown	HUA: 197157	2
	Cortinarius sp. 5 morado	HUA: 197158	2
	Cortinarius sp. 6 purple	HUA: 197159	
	Cortinarius sp. 7	HUA: 197160	
/entoloma	**Entolomataceae**		
	Entoloma sp. 1	HUA: 197161, 197162	
/hydnellum-sarcodon	**Hydnaceae**		
	Sistotrema sp.	HUA: 197067	
	Bankeraceae		
	Sarcodon colombiensis sp. nov.[1]	HUA: 186215	
/inocybe	**Inocybaceae**		
	Inocybe sp. 1	HUA: 197163, 197167, 197168, 197169, 197170, 197171, 197172	
	Inocybe sp. 2 gris	HUA: 197173, 197174, 197175	
/pisolithus-scleroderma	**Diplocystidiaceae**		
	Tremellogaster surinamensis E. Fisch	HUA: 197177	1
	Sclerodermataceae		
	Scleroderma sp. 1 yellow	HUA: 197178	

Lineage°	Taxonomy[1]	Representative Voucher[2]	Forest type[3]
	Scleroderma sp. 2	HUA: 197176	
/russula-lactarius	**Russulaceae**		
	Lactifluus sp. 1	HUA: 197179	1
	Lactifluus sp. 2	468 LQ, 474 LQ	
	Lactarius subgen. Plinthogalus sp. 5	HUA: 197209	
	Russula gelatinivelata S. Mill, Aime & Henkel *	HUA: 186207, 186208, , 197185, 197186, 197187, 197188, 197189	1
	Russula puiggarii complex Singer	HUA: 186210, 197190, 197201, 197202, 197203, 197204	1, 2, 3
	Russula sp. 2 brownish-viscid	HUA: 197206	
	Russula sp. 3	HUA: 197207	
	Russula sp. 4	HUA: 197208	
	Russula sp. 6 reddish	HUA: 197205	
	Russula sp. 7 whitish	HUA: 197180	
	Russula sp. 8 green	HUA: 197210	2
	Russula sp. 9	HUA: 197211	
/sebacina	**Sebacinaceae**		
	Sebacina sp. 1	HUA: 197212	
	Sebacina sp. 3	HUA: 197214	
	Tremellodendron sp. 1	HUA: 197216	
	Tremellodendron sp. 2	HUA: 197213	
/tomentella-thelephora	**Thelephoraceae**		
	Telephoral sp. 1	HUA: 197217, 197218	
	Thelephoral sp. 2 blackish	HUA: 197219, 197220	

°EcM lineages as identified by Tedersoo et al. (2010a, 2013). Taxa lacking epithets are morphologically distinct species level taxa (morphospecies) as yet unidentified to species; taxa with epithets followed by "ined." have been tentatively determined as new to science but are yet to be formally described. [2]LQ (Carlos López Quintero), FM (Ana Esperanza Franco-Molano) and HUA (Corresponds with numbers at Herbario de la Universidad de Antioquia). [3]Species previously reported from 1. Fabaceae-dominated forest in Guayana (Henkel et al. 2012), 2. WSFs with *D. uaiparuensis* in Colombia (Chapter 2), and *3. Pakaraimaea dipterocarpacea* Guyana-Venezuela (Moyersoen 2012; Smith et al. 2011, 2013). Novelty: Fifteen species constituted first report to Colombia * and 5 new species recently been described [1]

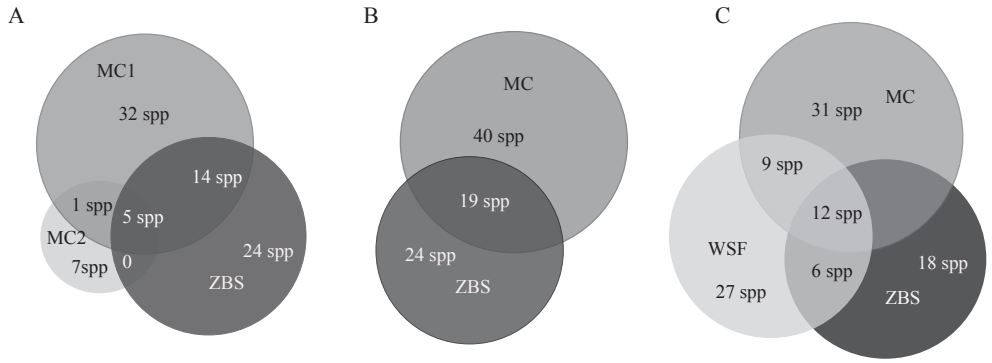

Figure 3. Venn diagram comparing EcM fungal diversity between Middle Caquetá region sites 1 (MC1) and 2 (MC2) and El Zafire (ZBS) (A), between MC sites and ZBS (B) and between *P. tropenbosii* forests in the MC region (MC), ZBS, and white-sand forests (WBS) in El Zafire.

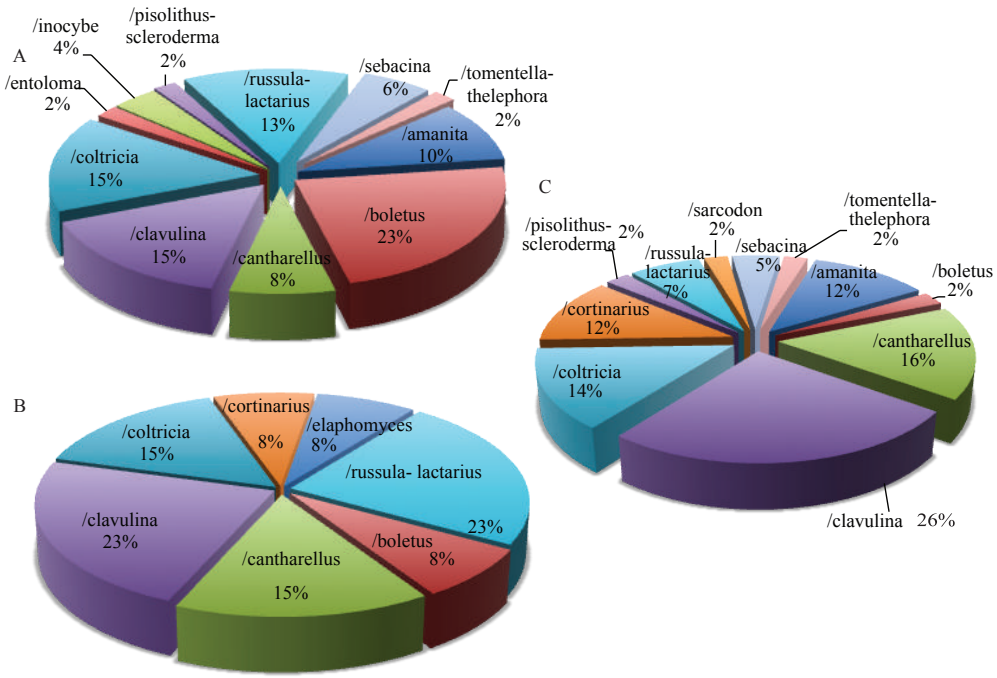

Figure 4. EcM fungal lineages recovered from fruiting bodies from the *P. tropenbosii* forests from the Middle Colombian Amazon region site 1 (MC1) (A) and 2 (MC2) (B) and El Zafire Biological Station (ZBS) (C). The EcM lineages were defined according to Tedersoo *et al*. (2010a, 2013).

The hosts of each of the 98 mycorrhizal roots that corresponded to EcM OTUs were identified as *P. tropenbosii* (83 detections), *Coccoloba* sp. (1 detection) and other plants not reported as EcM hosts (4 detections, e.g. *Brosimun* sp., *Erythroxylum* sp., *Ipomoea* sp., *Protium* sp.). Ten sequences were not informative and could not be used for the identification of the plant host.

Figure 5. Terra-firme forests with *Pseudomonotes tropenbsoii* (A) and representative ectomycorrhizal fungi collected in the *P. tropenbosii* forests (B-M). Fruiting bodies of *Pseudotulostoma volvatum* (B), *Coltricia hamata* (C), *Russula gelatinivelata* (D, E), *Lactarius* subgenera *Plinthogalus* sp. 5 (F-G), Badisidiospores of *Lactarius* subgenera *Plinthogalus* sp. 5 (H), *Austroboletus festivus* (I), *Boletellus ananas* var. *minor* (J), *Tremellodendron* sp. 1 (K), *Craterellus strigosus* (L), and *Clavulina griseohumicola* (M).

Overall, 4 OTUs (species- and genus-level) were detected on roots from the 3 sites, named *Amanita* sp. 2 sect. Vaginatae, *Craterellus cinereofimbriatus* and two Uncultured EcM Helotiales (155 root, 119 root). The site that showed most diversity was MC1 with 36 OTUs, followed by MC2 with 21 and ZBS with 14 OTUs. The most abundantly detected OTUs at the species level were *Cr. cinereofimbriatus* (8 detections), Uncultured *Cortinarius* 866root (10 detections), Uncultured *Cortinarius*

Table 3. EcM species identified from root tips. OTUs were identified based ITS rDNA sequences.

Lineage	OTUs-species level	OTUs-genera level	OTUs-family level	Total OTUs (spp. and genera level)	Abundance
/amanita	3	1	1	**4**	10
/boletal	4		1	**4**	7
/cantharellus	3	1		**4**	13
/clavulina	3		2	**3**	5
/coltricia			1		1
/cortinarius	5	3	4	**8**	31
/elaphomyces	2		1	**2**	3
/entoloma	1			**1**	1
/facultative biotrophic saprobe		1		**1**	1
/helotiales	3	3	3	**6**	19
/hydnellum-sarcodon	2	4		**6**	7
/inocybe			2		2
/russula-lactarius	5	2	5	**7**	17
/sebacina	2	4	3	**6**	13
/tomentella-thelephora	1	7	14	**8**	45
/xenasmatella			1		2
TOTAL	**34**	**26**	**38**	**98**	**177**

917root (8 detections) and Uncultured *Tomentella* 1452root (11 detections), Uncultured Helotiales 115root (6 detections) and Uncultured *Sebacina* sp. 8JOH 126root (5 detections) (Table 4).

Of the 34 ITS OTUs at the species-level detected on roots of *P. tropenbosii* 13 species have been found as fruiting bodies in *P. tropenbosii* forests and 12 have been detected from roots of *Pk. dipterocarpacea,* 16 in *Dicymbe* forests in Guyana, and 14 from WSFs with *D. uaiparuensis* in Colombia (Table 4). At the genus-level 8 OTUs gave a best match with samples previously reported from *Pk. dipterocarpacea,* 6 to genus level with *Dicymbe* forest in Guyana and 8 from *D. uaiparuensis* forests in Colombia (Table 4). Only six OTUs have not been detected before from Neotropical ecosystems (*Entoloma* 839root, EcM *Helotial* 95root, *Helotial* EcM 136root, *Sarcodon colombiensis* sp. nov. 2084AMV, *Sebacina* sp. 1 910root and *Sebacina* sp. 2 R4root).

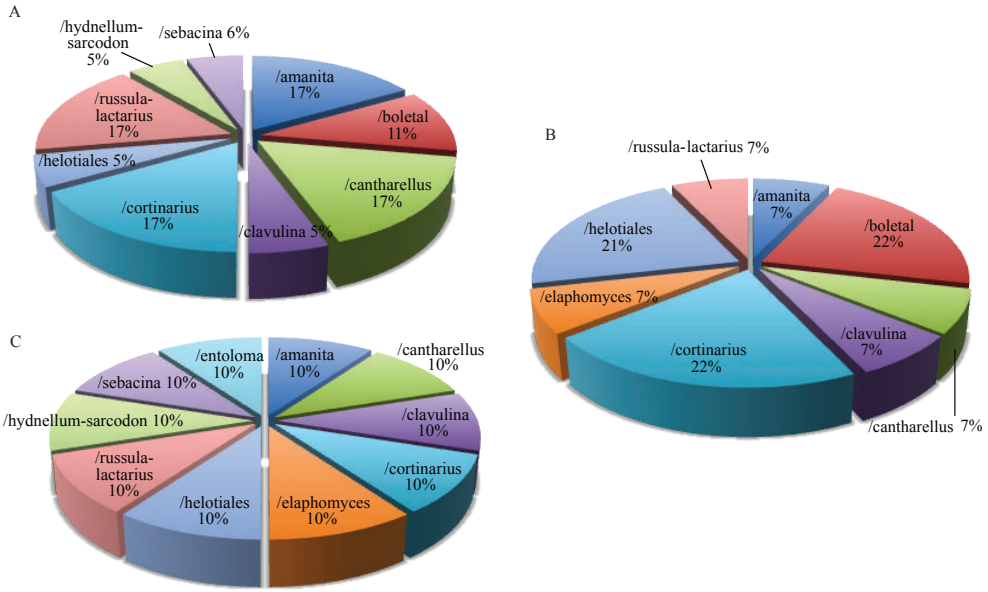

Figure 6. EcM fungal lineages recovered from root tips. The Middle Colombian Amazon region site MC1 (A) and MC2 (B) and El Zafire Biological Station (ZBS) (C). The EcM lineages were defined according to Tedersoo *et al.* (2010a, 2013).

Other taxa collected off of the studied plots

Twelve species of putative EcM fungi were recorded from a *P. tropenbosii* forest visited once at the south of MC1 in a place called Meta (Table 2). Ten species were shared between the MC and ZBS plots, but *Scleroderma* sp. 3 and *Cortinarius* sp. 7 were only observed from this site.

Discussion

This is the first in-depth study on the diversity of EcM fungi associated with *P. tropenbosii*. A relative high richness of EcM fungal species was found to be associated with this endemic host tree. Eighty-three morphospecies were collected and 34 and 26 species- and genus-level OTUs were detected from the roots, respectively. This study contributes to the knowledge of mycobiota of Colombia as 15 species were reported for the first time from the country (Table 2). The EcM fungal community associated with *P. tropenbosii* forests consists of taxa that are generally observed in tropical forests associated with Dipterocarpaceae in Asia and Fabaceae in Guyana and other biomes, such as those occurring in temperate areas (Brearley 2012; Henkel et al. 2012; Lopez-Quintero et al. 2012; Peay et al. 2010; Singer & Araujo 1978; Smith et al. 2011, 2013; Tedersoo et al. 2010a, 2011, 2013). Species-rich fungal orders included Agaricales, Cantharellales, Boletales, and Russulales and some taxa were found to be conspecific with those occurring in other EcM forests dominated by Fabaceae and the dipterocarp *Pk. dipterocarpacea* in tropical rainforests occurring at the Guiana Shield

(Henkel et al. 2012; Smith et al. 2013). At the lineage level, the diversity of EcM fungi associated with *P. tropenbosii* was similar to what has been documented from dipterocarps in Southeast Asia and Africa (Brearley 2012; Peay et al. 2010; Tedersoo et al. 2011).

The capability of some EcM fungi to form associations with Dipterocarpaceae and Fabaceae in the Neotropics was corroborated. A total of 37 species (43.5 %) of EcM fungi from *P. tropenbosii* forests has also been collected from Fabaceae-dominated forests in the Neotropics, 27 species (31,7 %) from forests in Guyana and 18 species (21 %) from Fabaceae forests in Colombia (Henkel el al. 2012; Smith et al. 2013; Chapter 2). It is important to note that in El Zafire *Dicymbe* and *Pseudomonotes* forests are only 5 kilometers apart. Likely, the number of shared species is higher than observed due to the fact that the Fabaceae forests were only briefly studied at this site (Chapter 2). Twelve species (12 %) were shared between *P. tropenbosii* and *Pk. dipterocarpacea* forests. *Amanita xerocybe*, *Craterellus atratus*, *Clavulina kunmudlutsa*, *Cl. sprucei*, *Coltricia oblectabilis* and *R. puiggarii* complex are species that have been detected from Fabaceae and Dipterocarpaceae forests in the Neotropics before (Henkel et al. 2012; Moyersoen 2006; Smith et al. 2013; Chapter 2).

The distribution of some fungal species that were previously considered to be restricted to the Guiana Shield region was extended in this study to the Southern areas of the Terciario superior Amazonico unit. It has been proposed that these Southern areas originated from the Guiana Shield (Hoorn 2006). Particularly relevant was the genus *Clavulina* that is globally distributed, but many species have been recorded in *Dicymbe*- and *Pakaraimaea*-dominated forests (Henkel et al. 2011, 2012; Smith et al. 2011, 2013). It has been proposed that the Guyana region might be a diversification spot for the *Clavulina* lineage (Uehling et al. 2012a). Eighteen species of this genus were collected from *P. tropenbosii* forests representing 72 % of the total of species reported from Guyana (Henkel et al. 2012). Three species were also detected from roots, namely *Clavulina amazonica*, *Cla. cinereoglobosa* and *Cla. rosiramea* (Table 4). *Cla. amazonensis* that was collected from all plots has been reported from *Dicymbe* forests in Colombia and Guyana (Henkel et al. 2012; Wilson et al. 2012; Chapter 2) and has been found also in central Brazil, Ecuador, and Venezuela (Corner 1970; Petersen 1988; Tedersoo et al. 2010b; Wartchow 2012; Chapter 2).

The family Russulaceae is globally distributed (Miller et al. 2006; Tedersoo et al. 2010a) and a recent phylogenetic study suggested ancient Paleo-Neotropical sister relationships, possibly resulting from Gondwana vicariance, with more recent diversification of taxa within the Neotropics (Hackel et al. 2014). We found 12 species in *P. tropenbosii* forests, which is a low number of species when compared with the species richness observed in Guyana, where 35 species have been reported from *Pakaraimaea* and Fabaceae forests after 2- and 13-years of sampling periods, respectively (Henkel et al. 2012; Smith et al. 2013) and 15 species from *D. uaiparuensis*

TTable 4. Ectomycorrhizal fungi detected at the roots of *Pseudomonotes tropenbosii* based on ITS rDNA sequences at species and genera level

ECM Lineage	ECM Taxon (OTU)	Voucher	Best aligned sequence voucher or accession # (% Match)	GenBank Number	Other host*	Richness	Site
/amanita	*Amanita* sp. 1 sect. Vaginatae	901r	*A*. sp.1 sect. Vaginatae 37JOH (99 %)	KT757687	*D. uaip.* (COL)	1	MC1
	Amanita sp. 2 sect. Vaginatae	803rs	*A*. sp. 2 sect. Vaginatae 36JOH (97%)		*D. uaip.* (COL)	3	MC1, ZBS, MC2
	Amanita xerocybe	29r, 1415r	*A. xerocybe* 1966a AMV (97-99%)	KT757688	*Pk., D. spp.* (GUY), *D. uaip* . (COL)	3	MC1, Meta
/boletal	*Fistulinella campinarana* var. *scrobiculata*	511r	*F. campinarana* var. *scrobiculata* 1784AMV KF937331 (97-100 %)	KT757689	Legu. (BR) (BR)	2	MC1
	Tylopilus pakaraimensis	140r	*T. pakaraimensis* JN168778 (98 %)	KT757690	*Pk., D. spp.* (GUY)	2	MC1, MC2
	Tylopilus vinaceipallidus	889r	*T. vinaceipallidus* JN168780 (98 %)		*Pk., D. spp.* (GUY)	1	MC2
	Xerocomus sp.	91r	Xerocomus TH8408 JN021114 (99 %)		*Pk., D. spp.* (GUY)	1	MC2
/cantharellus	*Craterellus atratoides*	308r	*Cr. atratoides* 14JOH (97-98 %)		*Pk, D. co.* (GUY), *D. uaip.* (COL)	2	MC1
	Craterellus cinereofimbriatus	806rs	*Cr. cinereofimbriatus* TH8999 JQ915104 (98 %)	KT757706	*Pk., D. spp.* (GUY), *D. uaip.* (COL)	8	MC1, MC2; ZBS
	Craterellus strigosus	802rs	*Cr. strigosus* 2245AMV (97)	KT757707	*Pk., D. co.* (GUY), *D. uaip.* (COL)	2	MC1

ECM Lineage	ECM Taxon (OTU)	Voucher	Best aligned sequence voucher or accession # (% Match)	GenBank Number	Other host*	Richness	Site
	Clavulina amazonensi	885r	*Cl. amazonensis* TH9191 HQ680356 (98%)	KT757708	*Pk.* (GUY, VEN), *D. spp.* and *Aldina insignis* (GUY), *D. uaip.* (COL); BR and VEN.	1	MC2
/clavulina	*Clavulina cinereoglebosa*	22r	*Cl. cinereoglebosa* JN228217(98 %)	KT757709	*Pk., D. spp.* (GUY)	1	ZBS
	Clavulina rosiramea	8r	*Cl. rosiramea* 1835 AMV (98 %)	KT757710	*Pk., D. spp.* (GUY)	1	MC1
/cortinarius	*Cortinarius amazonicus*	869r	*Co. amazonicus* AF389129 (98 %)	KT757693	Legu. (BR) (BR)	1	MC2
	Cortinarius sp. 3	886r	*Co.* sp. 3 2000AMV (99 %)	KT757694	*D. uai.* (COL)	1	MC2
	Uncult. *Cortinarius*	866r	Uncult. *Cortinarius* JN168712 (96-99%)	KT757695	*Pk., D. spp.* (GUY)	10	MC1, MC2
	Uncult. *Cortinarius*	917r	Uncult. *Cortinarius* KC155366 (99%)		*Pk. (*GUY)	8	MC1, ZBS
/elaphomyces	*Elaphomyces compleximurus*	845r	*E. compleximurus* TH8880 JN711441 (97%)		*Pk., D. spp.* (GUY)	1	ZBS
	Pseudolostoma volvata 1	124r	*Ps.* volvata TH8975 JN168735 (97%)		*Pk., D. spp.* (GUY)	1	MC2
/entoloma	*Entoloma* sp.	839r	Entoloma sp. (97%)			1	ZBS
/helotiales	Uncult. EcM Helotiales	95r	Uncult. EcM Helotiales UDB004225 (98%)			1	MC2
	Uncult. Helotiales	119r	Uncult. Helotiales sp. 5 198z (97%)	KT757696	*D. uaip.* (COL)	4	MC1, MC2, ZBS
	Uncult. Helotiales EcM	136r	Helotiales FM180476 (97%)	KT757697		2	MC2

ECM Lineage	ECM Taxon (OTU)	Voucher	Best aligned sequence voucher or accession # (% Match)	GenBank Number	Other host*	Richness	Site
	Sarcodon colombiensis sp.nov	662br	*S. colombiensis* 2084 AMV (98%)			1	ZBS
/hydnellum-sarcodon	*Sarcodon rufogriseus* sp. nov.	114r	*S. rufogriseus* 1989 AMV (98%)		*D. uaip.* (COL)	2	MC1
	Lactarius annulifer	899r	*L. annulifer* TH9014 KC155376 (99%)		*Pk., D. spp.* (GUY), *D. uaip.* (COL)	2	MC1
/russula-lactarius	*Russula puiggarii* 883r	883r	*R. puiggarii* complex 10JOH (97-98%)	KT757712	*D. uaip.* (COL), Legu. (BR)	2	MC2
	Russula sp. 8 verde	516r	*R.* sp. 8 verde 11JOH (98%)		*D. uaip.* (COL)	1	MC1
	Uncult. *Lactarius subiculata*	25r, 899	Uncult. *L. subiculata* JN168749 (99%)	KT757713,	*Pk., D. spp.* (GUY)	2	MC1
	Uncult. *Russula* sp. nov. 3	911r	Uncult. *R.* sp. nov. 3 85z (99%)	KT757714	*Pk.* (GUY), *D. uaip.* (COL)	1	ZBS
/sebacina	*Sebacina* sp. 1	910r	*Sebacina* sp. 1 38JOH (100%)		*D. uaip.* (COL)	1	ZBS
	Sebacina sp. 2	R4r	*Sebacina* sp. 2 8JOH (99%)	KT757715	*D. uaip.* (COL)	1	MC1
/tomentella-thelephora	Uncult. Tomentellaceae	1413ar	Uncult. *Tom.*ECM40 5 KC155370 (98%)	KT757723	*Pk., D.* spp. (GUY)	2	Meta
	Genera level						
/amanita	Uncult. *Amanita* sp. 2 sect. Vaginatae	837r	*A.* sp. 2 sect. Vaginatae 36JOH (91%)		*D. uaip.* (COL)	1	ZBS
/cantharellus	Uncult. *Craterellus atratus*	82r	*Cr. atratus* strain TH9203 JQ915107 (97%)		*Pk.* , D. spp. (GUY), *D. uaip.* (COL), Legu. (BR)	1	MC1
/cortinarius	Uncult. *Cortinarius* sp.	10r	*Cortinarius* TH8613 KC155377 (93%)	KT757698	*Pk.*, *D.* spp. (GUY)	1	MC1

ECM Lineage	ECM Taxon (OTU)	Voucher	Best aligned sequence voucher or accession # (% Match)	GenBank Number	Other host*	Richness	Site
	Uncult. *Cortinarius* sp. 1	258r	*Cortinarius* DQ481725 (91%)	KT757699		1	MC2
	Uncult. *Cortinarius* sp. 3	1r	*Cortinarius* sp. 3 2000AMV (90%)	KT757700	*Pk.* (GUY), *D. uaip.* (COL)	1	MC1, ZBS
/facultative biotrophic saprobe	Uncult. *Rhodocollybia turpis*	919r	*R. turpis* AF505749 (89-91%)	KT757722		1	MC2
/helotiales	Uncult. Helotiales	115r	Uncult. Helotiales sp. 3 197z (89-92%)		*D. uaip.* (COL)	6	MC1, MC2, ZBS
	Uncult. Helotiales	224r	Uncult. Helotiales sp. 5 198z (93%)		*D. uaip.* (COL)	2	MC1
	Uncult. Helotiales EcM	59r	Helotiales AF081443 (93%)	KT757701		1	MC1
/hydnellum-sarcodon	Uncult. *Sarcodon* sp.	921r	*Sarcodon* sp. (90%)			1	MC2
	Uncult. *Sarcodon* sp.	838r	Uncult. *Sarcodon* EU627606 (90%)	KT757701		1	ZBS
	Uncult. *Sarcodon* 2084	507r	Uncult. *S. colombiensis* 2084 AMV (90%)	KT757701		1	MC1
	Uncult. *Sarcodon* sp. nov.	801rs	Uncult. *S. rufogriseus* 1989AMV (90%)		*D. uaip.* (COL)	1	MC1
	Uncult. *Lactifluus* sp. 1	81r	Uncult. *Lactifluus* sp. 1 66Z (90%)	KT757716	*D. uaip.* (COL)	1	MC1
/russula-lactarius	Uncult. *Russula* sp. 8 verde	55r	Uncult. *Russula* 8 verde 11JOH (92%)	KT757717	*Pk.* (GUY), *D. uaip.* (COL)	2	MC1, Meta
/sebacina	Uncult. *Sebacina*	9r	Uncult. *Sebacina epigea* JQ665484 (90%)			1	MC1

ECM Lineage	ECM Taxon (OTU)	Voucher	Best aligned sequence voucher or accession # (% Match)	GenBank Number	Other host*	Richness	Site
	Uncult. *Sebacina*	Rr	Uncult. *Sebacina* (90% 5)			1	MC1
	Uncult. *Sebacina* 8JOH	126r	Uncult. *Sebacina* 8JOH (90%)		*Pk., D. spp.* (GUY)	5	MC1, MC2
	Uncult. *Sebacina* sp. 1	11r	*Sebacina* 38JOH (90% 4)			1	MC1
	Uncult. *Tom. umbrinospora*	89r	*Tom. umbrinospora* UDB016499 (90%)			1	MC2
/tomentella-thelephora	Uncult. *Telephora*	501r	*Theleph.* FR731345 (90%)	KT757724		1	MC1
	Uncult. *Thelephora*	122r	*Theleph.*UDB007920 (90%)			1	MC1
	Uncult. *Tomentella*	1411r	*Tom.* JQ975984 (90%)	KT757725		1	Meta
	Uncult. *Tomentella*	1452r	Uncult. *Tom.* ECM1111 JN168760 (93%)	KT757726	*Pk.* (GUY)	11	Meta, MC1, MC2
	Uncult. *Tomentella*	304r	Uncult. *Tom.* ECM12-6 KC238675 (90%)	KT757727	*Pk.* (GUY)	1	MC1
	Uncult. *Tomentella*	500r	Uncult. *Tom.* ECM755 JN168765 (92%)		*Pk., D. spp.* (GUY)	2	MC1

Species-level operational taxonomic units (OTUs) are defined as sequences that are 97 % similar across the ITS rDNA sequence region. Taxa defined as genera are 90 % similar and family 80 % . Taxa labeled with Latin binomials or voucher numbers (HUA) were identified based on ITS matches with sporocarps and with NCBI and UNITE databases. Uncult. is the abbreviation of Uncultured. All species are assigned to the EcM lineages defined in Tedersoo et al. (2010a, 2013). The column Sites are the Middle Colombian Amazon region 1 (MC1) and 2 (MC2), and El Zafire (ZBS). Other ecosystems indicates whether or not an OTU has been found previously on EcM roots or as sporocarps at other sites in Guyana (GUY), Venezuela (VEN), Brazil (BR) or Colombia (COL) (Henkel et al. 2012; Moyersoen 2012; Smith et al. 2011, 2013; Chapter 2). * Other host: *Pakaraimaea dipterocarpacea* (Pk), *Dicymbe uaiparuensis* (D. uai.), *Dicymbe corymbosa* (D. co.) and *Dicymbe spp.* (D. spp.) and Leguminous forests (Legu.)

forests in El Zafire after two years of collection (Chapter 2). *Russula gelatinivelata*, a recently described species from the greater Guiana Shield (Miller et al. 2012) was very abundant in both MC sites, but it was not found in the plot at the ZBS site. *R. puiggarii* that was observed in all plots is relatively common in tropical regions in South America and it may represent a species complex that needs further taxonomic study (Buyck & Ovrebo 2002; Jaeger & Neves 2013; Miller et al. 2000; Chapter 2).

While the majority of the taxa collected in this study are conspecific with species or morphospecies reported from Guyana (Henkel et al. 2012; Smith et al. 2013), 18 species found from *P. tropenbosii* seem to be new species. These include species of *Amanita*, *Autroboletus*, *Craterellus*, *Coltricia*, *Coltriciella, Cortinarius*, *Inocybe, Sarcodon*, *Scleroderma*, *Thelephora* and genera of Boletaceae and Russulaceae (Table 2). Taxonomic work on some of these potentially new species is ongoing (e.g. *Amanita*, *Coltricia, Coltriciella,* Russulaceae, and *Sarcodon*). Species of *Coltricia-Coltriciella* are especially distributed in tropical ecosystems and seven species were identified from *P. tropenbosii* forests and three of those correspond to new taxa (Chapter 6). Another genus, *Cortinarius*, is one of the most species rich taxa in Holarctic and Austral regions, and it is considered to be rare in tropical areas (Moyersoen 2012; Tedersoo et al. 2010a). In this study at least seven morphospecies of *Cortinarius* were identified from fruiting bodies and the same number was recorded by T. Henkel and collaborators from *Dicymbe*- and *Pakaraimaea*-dominated forests in Guyana, while 11 species were recorded by Moyersoen (2006) in Venezuela. Based on ITS sequences only, one species that was obtained as fruiting bodies and four obtained as OTUs at species level from roots were shared between *P. tropenbosii* and the Fabaceas and/or *Pakaraimaea*-hosts (Table 4). Thus, we assume that the diversity of this genus in the Neotropics may be considerable and is largely unknown. *Amanita* was represented by seven species, and four of those were found from *D. uaiparuensis* forests in El Zafire (Table 4; Chapter 2). At least four of the seven species seem to represent non-described species and three seem to be identical to specimens collected previously in Guyana (Table 2, T. Henkel pers. comm. 10 June 2015). The genus *Sarcodon*, also considered to be distributed in the Northern temperate Hemisphere, was recently discovered to occur in tropical regions with a high diversity (Grupe et al. 2015). *Sarcodon pakaraimensis* is a recently described species associated with *Pk. dipterocarpacea* and *Dicymbe* spp. in Guyana (Chapter 7). In this study we found specimens of a new *Sarcodon* species, *S. colombiensis,* that was also detected on root tips of *P. tropenbosii* (Tables 3, 4; Chapter 7).

Despite the high diversity of EcM fungi documented in forests of *P. tropenbosii*, some taxa were not well represented when compared to EcM fungal communities observed from other plants hosts in Guyana and Venezuela. This is the case for genera such as *Inocybe, Entoloma, Tomentella,* and *Sebacina.* Species of *Tomentella* and *Sebacina* have cryptic or atypical fruiting bodies that may have been overlooked. In Guyana

12 species of *Inocybe* have been reported with 9 of them representing new species (Henkel et al. 2012; Matheny et al. 2009), while in this study only two species were identified. Specimens of *Entolomataceae* were frequently collected in *P. tropenbosii* forests but only a small part of the genus, namely sections *Entoloma* and *Rhodopolia*, are EcM (Tedersoo et al. 2010a). Only *Entoloma* sp.1 was found as a putative EcM from the Colombian dipterocarp forests and a different *Entoloma* species was detected at the roots. Further studies in these groups are needed to assess the extent of their diversity in *P. tropenbosii* forests.

The diversity of the EcM fungal communities was not homogeneous among sites (Fig. 3). The fungal community composition presented a significant difference between sites. The site MC1 shared 14 species with ZBS, but only 5 with MC2 although the MC forests were separated by only 34 km and were visited during the same seasons. The studied forests, MC1, MC2 and ZBS, do not presented a significant similarity respect to the fungal community composition. In general, *P. tropenbosii* populations form relatively isolated stands surrounded by anectotrophic mixed forests. In MC1 the trees were located at the flatter part of the hills, while in MC2 the topography is more irregular and trees were located at steep slopes. This causes less litter to accumulate (Parrado-Rosselli 2005). In ZBS the density of the trees of *P. tropenbosii* is less than in MC and less litter accumulated above ground (M.C. Peñuela-Mora, pers. conversation). Yet, 43 species of EcM fungi were recovered from this site. The Northern MC area is separated from ZBS by approximately 420 km. No populations of *P. tropenbosii* are known to occur between these two areas, but it does not mean that they do not exist, as the Amazon region in Colombia is vast and has large areas that still remain to be explored.

Previous studies that examined the EcM fungal community associated with *Pk. dipterocarpacea* subspecies *nitidum* and *dipterocarpacea* in Venezuela and Guyana, respectively, provided evidence for the presence of 13 EcM fungal lineages (/ amanita, /boletus, /cantharellus, /clavulina, /coltricia, /cortinarius, /elaphomyces, / hysterangium, / hydnellum-sarcodon, /inocybe, /russula-lactarius, /sebacina, and / tomentella-thelephora) based on rDNA sequences from EcM roots and collected sporocarps (Moyersoen 2006, 2012; Smith et al. 2013). We discovered two additional well-established lineages of EcM fungi in forests of *P. tropenbosii*, namely /entoloma and /pisolithus-scleroderma, and two previously unknown groups from roots, namely /helotiales and /xenasmatella. The putative EcM family Xenamastaceae is a group of corticioid fungi that belongs to the order Polyporales. The lineage /xenamastella has been detected from roots of Dipterocarpaceae in Malaysia and *Dicymbe* and *Aldina* in Guyana (Peay et al. 2010; Tedersoo et al. 2013), and it was also detected by us from roots of *P. tropenbosii*. The EcM status of this lineage is still under discussion and authors ponder that it may represent an opportunistic fungus associated with other mycorrhizal species or it is present in the soil tightly mixed with the roots and

is detected during the amplification process (Tedersoo et al. 2013). Another taxon detected from roots was *Rhodocollybia turpis*. This species was frequently fruiting in the *P. tropenbosii* forests. *Rhodocollybia* together with *Trechispora telephora*, another commonly fruiting species in *P. tropenbosii* forests, are considered to be facultative biotrophic saprobes and were recovered from roots tips in several studies (Tedersoo et al. 2010a, 2013). However, contradictory evidence from a phylogenetic placement of EcM lineages made by Tedersoo and Smith (2013) did not find any specific clade of *Rhodocollybia* or *Trechispora* with EcM isolates. Hence, we consider *Rhodocollybia* as a saprotrophic root colonizer and not as a true EcM symbiont.

Given that our sampling of *P. tropenbosii* associated fungi remained below saturation (Fig. 2), the cumulative data suggested that the diversity of mycorrhizal fungi associated to *P. tropenbosii* is high and probably similar to the number of 174 species found to be associated with leguminous hosts plants in Guyana as observed over a 13-year sampling period (Henkel et al. 2002, 2011, 2012; Smith et al. 2011). Incomplete recovery of EcM fungal diversity was corroborated by the notion that 20 OTUs species-level of putative or confirmed EcM detected from the roots have not been found as fruiting bodies and 71 species collected as fruiting bodies were not detected from the roots of *P. tropenbosii*. If we take into consideration the number of species represented by fruiting bodies and roots, the fungal biodiversity recovered from *P. tropenbos*ii forests in this study was 99 species. It is important to note that *P. tropenbosii* does not form monodominant patches or large populations in the Middle Colombian Amazon region. Despite *P. tropenbosii* is one of the most important canopy species, the relative abundance of this tree is not more than 18 % and its populations do not occupy more than a couple of hectares (Appanah & Turnbull 1998; Londoño et al. 1995; Parrado-Rosselli 2005). Henkel et al. (2012) considered that the high α fungal diversity in forests dominated by *Dicymbe corymbosa* in Guyana is due to the fact that this tree maintains a relative abundance of 60-90 % with the presence of high numbers of seedlings and saplings in the Guyana plots, which results in an ample availability of substrate for EcM fungi. Considering that *P. tropenbosii* occurs less abundantly in the forests, less substrate is available for EcM fungi to colonize and therefore more competition is at stake resulting in a lower species richness compared with other tropical ectotrophic ecosystems.

Conclusions

Differences were observed in the composition of the EcM fungal community for 3 populations of *P. tropenbosii* in Colombia Amazonia. Several factors, including the landscape, forest structure, size of the host plants, host distribution and dominance within the community may be important drivers of EcM fungal diversity. Soil composition has been found to explain the structure of fungal communities (Tedersoo et al. 2014; Chapter 4), and this factor should be considered in the future to understand

how this influences the EcM communities associated to *P. tropenbosii* forests.

No evidence was observed at the higher taxonomic levels (i.e. genus level and higher) that the EcM fungal communities associated to *P. tropenbosii* and the nearby or remote Fabaceae forests were greatly different. Furthermore, we did not find evidence that the EcM fungal community structure at species level was more similar within the Neotropical hosts of the family Dipterocarpaceae *P. tropenbosii* and *Pk. dipterocarpacea* subspecies *nitidum* and subspecies *dipterocarpacea* (Moyersoen 2006, 2012; Smith et al. 2013). In general, similarities in the EcM fungal community structure suggested that fungal dispersion is an important radiation mechanism for EcM in the region. Species have a wide distribution at the regional level when they have multiple hosts, including phylogenetically unrelated trees (Moyersoen 2012; Smith et al. 2013). *Dipterocarpaceae* and *Fabaceae* represent two distantly related plant lineages within the angiosperms that have separately evolved the ability to form the EcM symbiosis (Wang & Qiu 2006). *P. tropenbosii* presents a high richness of EcM symbionts despite the fact that the trees do not form large canopy forests in dense stands, as occur in Guyana with Fabaceae and *Pk. dipterocarpacea*. Landscape, forest structure, and host dominance may govern the EcM fungal diversity. Further studies to address these ecological questions about EcM community structure in Neotropical lowland forests and host specificity are needed.

CHAPTER 4

Forest type and soil chemistry are important drivers that structure the highly diverse fungal communities in lowland tropical rain forests in Colombia

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Abstract

Studies on fungal diversity in the Amazon region are limited even though recent studies have suggested that fungi play a prominent role in structuring the diversity and abundance of plants in tropical rainforests. In this study, we characterized the soil fungal communities in a terra-firme mixed forest with Nyctaginaceae or Polygonaceae (MF), a terra-firme forest with the dipterocarp *Pseudomonotes tropenbosii* (PtF), and a white-sand forest (WSF) dominated by members of the Fabaceae family in the lowlands of Colombian Amazonia. It was hypothesized that these forests that occur on different soils would harbor an unique fungal soil community. WSFs were expected to have the most particular fungal composition due to the presence of a high plant species endemicity related to adaptations to drought and to white sandy soils. The fungal diversity was analyzed by 454 pyrosequencing of the ITS2 region of the ribosomal DNA. A high fungal diversity was detected from soil samples of all three lowland tropical forests in Colombia Amazonia. MF and PtF shared a large number of OTUs, while the WSF presented the most unique fungal community composition. The most dominant functional groups were saprotrophs, plant pathogens, and ectomycorrhizal symbionts. Forest type, soil pH, and C/N ratio were found to be important drivers that structure the highly diverse fungal communities. Our data highlight the high diversity and heterogeneity of fungal soil community composition in mixed forests and WSFs in Colombian Amazonia.

Introduction

Amazonia has the largest and continuous tropical forest coverage in the world (Hoorn & Wesselingh 2010) and is composed of complex and diverse (micro-) habitats that harbor a high diversity of plants and animals (Gentry 1988; Hoorn & Wesselingh 2010; Hoorn et al. 2010; ter Steege et al. 2000, 2010). These forests play a significant role in the regulation of the global climate and together with their soils they contain approximately 10 % of the global terrestrial carbon pool (ter Steege et al. 2010). The high plant biodiversity of such habitats has been explained mostly by the paleoclimatic history that resulted in important differences in soil fertility and rainfall seasonality (Duivenvoorden & Duque 2010; Quesada et al. 2009; Sombroek 2000; ter Steege et al. 2006, 2010). Marked differences do exist between Eastern and Western Amazonia. Soils in the Northeast are poor, acid, and of Paleozoic formation, whereas Western Amazonian has more fertile soils of Cenozoic formation (Quesada et al. 2009; ter Steege et al. 2010). The α-diversity of trees is highest in Northwest Amazonia and decreases Eastwards and towards the South (ter Steege et al. 2006).

The biodiversity of insects and microorganisms in Amazonia remains poorly documented (Behling et al. 2010). Soil microorganisms and in particular fungi play a prominent role in structuring the diversity and abundance of trees in the tropical

rainforest (Augspurger 1983; Comita et al. 2010; McGuire 2007; Peay et al. 2013; Peh et al. 2011; Peñuela-Mora 2014; Torti et al. 2001). The poor soil/high-biodiversity paradox in Amazonia is explained in part by rapid recycling of organic matter facilitated by fungi and bacteria and a greater nutrient uptake due to fungal-plant interactions known as mycorrhiza (Adeney et al. 2016; Brearley 2012; Jimenez et al. 2009; Quesada et al. 2009; van der Heijden et al. 2015).

Microorganisms, including fungi, were long assumed to be cosmopolitan ("everything is everywhere"; Baas-Becking 1934) but emerging evidence suggests that many microorganisms have a restricted distribution (Bahram et al. 2015; Burns et al. 2015; Hanson et al. 2012). The interaction of selection, drift, dispersal, and mutation has been proposed as main drivers of the biogeography of organisms including fungi (Hanson et al. 2012). Recent studies highlight the importance of climatic factors followed by edaphic and spatial patterns as the best predictors of soil fungal richness and community composition from local to global levels (Geml et al. 2014; Peay et al. 2013; Talbot et al. 2014; Tedersoo et al. 2010a, 2010b, 2014).

Recent studies on fungal communities in Amazonia (Henkel et al. 2012; López-Quintero et al. 2012; Lucheta et al. 2015; McGuire et al. 2010; Moyersoen 2006, 2012, 2014; Peay et al. 2013; Smith et al. 2013; Tedersoo et al. 2010c; Vasco-Palacios et al. 2014a; Chapters 2, 3) have shown that fungal diversity is high in tropical soils. A strong association was shown between plant species composition and the fungal community in the Amazon basin (Mueller et al. 2014; Peay et al. 2013). The tropics were supposed to be dominated by arbuscular mycorrhizal fungus (AM) due to a predominance of AM hosts in these forests and the lack of typical fruiting bodies of ectomycorrhizal (EcM) fungi (Henkel et al. 2002). However, recent studies have provided evidence for the presence of EcM symbiosis in tropical lowland ecosystems (Bâ et al. 2012, 2014; Bas 1978; Brearley 2012; Diédhiou et al. 2010; Henkel et al. 2002, 2012; López–Quintero et al. 2012; Moyersoen 2006, 2012, 2014; Phosri et al. 2012; Roy et al. 2016; Singer & Araujo 1979; Singer et al. 1983; Smith et al. 2013; Tedersoo & Nara 2010; Tedersoo et al. 2010a, 2014; Chapters 2, 3). EcM fungal communities associated with tropical trees show a high species richness with a weak host preference mostly for Fabaceae and Dipterocarpaceae (Smith et al. 2013; Chapters 2, 3). However, a strong host specificity of EcM fungi was described for the trees *Coccoloba* (Polygonaceae), *Guapira* and *Neea* (Nyctaginaceae) in tropical lowland forests in Ecuador (Tedersoo et al. 2010b). The EcM association may partly explain the dominance of EcM host trees occurring on nutrient poor soils in Amazonia (McGuire 2007; Peh et al. 2011; Singer & Araujo 1979;). Recent studies indicated that mycorrhizal fungi play a key role in the cycling of carbon (C), nitrogen (N), and phosphorus (P) in terrestrial ecosystems and that C storage may increment in ecosystems dominated by EcM fungi versus those dominated by AM fungi (Averill et al. 2014; Clemmensen et al. 2013; Ekblad et al. 2013; Talbot et al. 2013). Evidence

suggests that some tropical trees may rely on EcM-mediated N acquisition, particularly in monodominant forests with high soil organic matter and low N availability (Mayor et al. 2015). These processes are important in C cycles and C sequestration in forests, and may affect model predictions of the global carbon balance (McGuire et al. 2010; van der Heijden et al. 2015). Despite these important roles, little is known about the relation between plant and microbial communities and their associations.

Here the fungal community was studied of soils of three types of forests from three different sites in the Colombian Amazon basin using next-generation DNA sequencing. Based on previous studies we hypothesized that the fungal community composition corresponds to plant community composition and edaphic factors (Lucheta et al. 2015; Peay et al. 2013; Taylor 2008; Tedersoo et al 2014). The effect of plant diversity and edaphic factors on fungal composition at the local scale was also addressed. This study highlights the importance of forest type and soil chemistry (pH and C/N ratio) as important drivers that structure fungal communities in lowland tropical forests in Colombia, more specific of terra-firme mixed forests and WSFs.

Materials and methods

Study sites and sampling

Two types of terra-firme mixed forests and a WSF were studied in the Amazon area in Colombia (Fig. 1 of Chapter 1). The forests present different types and densities of EcM plants. The terra-firme mixed forests (MF) are characterized by a high richness of trees represented by few individuals with species of the EcM trees *Coccoloba* (ca. Important Value Index Polygonaceae, IVI 0.477), *Guapira* or *Neea* (Nyctaginaceae, IVI-family 0.845). In some regions of Colombian Amazonia, the ectomycorrhizal tree *Pseudomonotes tropenbosii* is one of the most important canopy species of the MF (IVI 16-18 %) (Duivenvoorden & Lips 2005; Londoño et al. 1995; Parrado-Rosselli 2005) and therefore this type of forest is called PtF. WSFs on the other hand are home to EcM trees such as *Dicymbe uaiparuensis* (IVI 25 %) and *Aldina* sp. The three types of forests were studied at three different sites. The first site was located 27 km north of Leticia in the Amazon department in the Zafire Biological Station area (ZBS) at 4°00' S, 69°53' W (Fig. 1 of Chapter 1). This zone has different types of soil with four main forest types: upland terra-firme, temporarily flooded, white-sand and transitional forests within upland and flooded forests. The landscape of ZBS includes upland terraces with elevations ranging from 80 to 120 m. Soils are sandy, mainly composed of quartz and the parental material belongs to the Terciario superior Amazonico unit that probably originated from the Guiana Shield (Jimenez et al. 2009). The mean annual temperature is about 26 °C and does not fluctuate much through the year. The relative humidity is about 86 % with a mean annual rainfall of 3250 mm. The period from June to September is generally drier (mean monthly precipitation of 160 mm)

compared to the rainy season from October to May (mean monthly precipitation of 340 mm) (Jimenez et al. 2009; Peñuela-Mora 2014). The other two sites were located in Northern Colombian Amazonia, in the Middle Colombian Amazon region (MC) (Fig. 1 of Chapter 1). This area is separated from ZBS by approximately 420 km. This region is home to terra-firme forests, floodplain forests, WSFs, and some areas with secondary forests (Parrado-Rosselli 2005). Mean annual temperature is 26°C, the relative humidity is about 86-92 % and the average annual rainfall is 3100 mm (Duivenvoorden & Lips 1993; Peñuela-Mora 2014). Although the region does not have a marked dry season, rainfall decreases between December and February. In MC two sites were sampled that are 34 km apart, one is located close to Peña Roja (MC1), 00°34' S, 79°08' W, and the other close to the village of Puerto Santander (MC2), 00°39' S, 72°23' W. Sites are at 100–300 m elevation with a flat to undulating topography with valleys and hills of 20 to 40 m altitude (Parrado-Rosselli 2005).

Sampling design

Nine plots were analyzed in PtFs (3 in MC1, 3 in MC2 and 3 in ZBS); six plots in MFs (3 in ZBS and 3 in MC) and four in WSFs (all 4 in ZBS). In the Middle Colombian Amazon region, only one pooled sample was collected in WSFs due to the fact that this place had an external intervention by a herd of wild pigs that disturbed the soil and the litter layer some days before we visited the area. Two extra plots were established in two small patches of PtF, one close to MC1 and the other close to ZBS (Suppl. Table 1).

Sampling design followed protocols of Tedersoo et al. (2014). Circular plots with a diameter of 20 m were established. In each plot of WSF and PtF 20 trees of the dominant EcM hosts were selected. Two lateral soil cores were collected at 2 meters from the trunk of each selected trees. In MF, 40 soil cores were taken randomly inside the circular plot. The 40 core soils were pooled per plot, after removal of coarse roots and stones. A subset of each pooled soil sample was air-dried, preserved in silica gel, and transported to the laboratory of Taxonomy and Ecology of fungi (TEHO) of the Antioquia University, Medellín, for further analysis. Samples could not be cooled due to the remote locations of the field sites.

Molecular analysis

DNA was extracted from 2.0 g of soil using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA). The ITS 2 region was amplified by polymerase chain reaction (PCR) using a mixture of six forward primers (in equimolar concentration) analogous to ITS3 and a degenerate reverse primer analogous to ITS4 (Tedersoo et al. 2014). The ITS4 primer was tagged with a 10-12 base identifier tag at the 5′ end (at least 4 differences to each other). The 20 µl PCR mix consisted of template DNA, 20 pmol of each primers, and HOT FIREPol Blend Master Mix (Solis Biodyne, Tartu, Estonia). PCR was performed in four replicates using an initial incubation at 95 °C for 15 min,

followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final cycle at 72 °C for 10 min (Tedersoo et al. 2014). PCR products were pooled and their relative quantity was estimated by running on a 1 % agarose gel. Amplicons were treated with Exonuclease I and FastAP thermosensitive alkaline phosphatase (Thermo Scientific, Pittsburgh, PA USA) and normalized using a SequalprepTM Normalization Plate Kit (Invitrogen, Carlsbad, CA). Normalized amplicons were subjected to 454-adaptor ligation, emulsion PCR, and 454 pyrosequencing by using the Roche GS FLX+ platform and titanium chemistry (Beckman Coulter Genomics, Danvers, MA).

Edaphic properties

The values of the edaphic properties used in the analysis were taken from (Tedersoo et al. 2014) (Suppl. Table 2).

Bioinformatics and statistical analyses

Pyrosequences were curated based on quality information provided by the sequencing company. Adapters were removed using ACACIA 1.52 (Bragg et al. 2012). To exclude short and low quality sequences, sequences were trimmed in Mothur 1.32.2 (Schloss et al. 2009) using parameters: minlength = 200; maxambig = 0; maxhomo*P* = 10; qwindo-waverage = 35; qwindowsize = 50; and bdiffs = 1. Sequences were demultiplexed based on the MID tags and primers. Putative chimeras were identified and removed with UCHIME (Edgar 2010; Edgar et al. 2011). After these filtering steps, the remaining sequences were pooled and clustered into operational taxonomic units (OTUs) at 97 % identity threshold using UCLUSTER 3.05 (Edgar 2010). The longest sequences obtained were selected to assign taxonomic affiliation to each OTU. These representative sequences were blasted (BLASTn) against UNITE and INSD databases (Abarenkov et al. 2010; species hypothesis, h refs qiime ver6 99 13.05.2014.fasta) and a CBS database (CBS proprietary sequencing data gathered from a large scale DNA-barcoding sequencing project of the CBS collection). For each query, the 10 best BLASTn hits were annotated as detailed as possible. Species were determined based on ≥ 97 % identity for operational taxonomic units (OTUs). We typically relied on 90, 85, 80, and 70 % sequence identity for assigning names to a genus, family, order, and class, respectively (Tedersoo et al. 2014). Fungal OTUs identified to lower taxonomic levels were based on Index Fungorum as featured in UNITE. Functional categories were assigned for each genus, family or order based on Tedersoo et al. (2014). Ectomycorrhizal (EcM) taxa were considered if they matched best with any sequence considered to represent EcM lineages (Tedersoo et al. 2010a; Tedersoo & Smith 2013). All singletons were removed for the analysis, because nearly half of them were suggested to be artifactual (Tedersoo et al. 2010c) and to reduce the effect of rare OTUs in the analysis.

Statistical analysis

Rarefaction curves of OTUs (S) were calculated using the function specaccum with

1000 permutations in the Vegan package of *R* (Oksanen et al. 2013; *R* Development Core Team 2012). The distribution of OTUs in the three types of forests was visualized in a Venn diagram using BioVenn (Hulsen et al. 2008). Species richness in each sample was standardized to the minimum number of sequences (individuals) using Rarefaction in Vegan (Oksanen et al. 2013) to compare samples.

Cluster analysis was performed to analyze similarity in species composition of plots. A furthest-neighbor joining Cluster analysis was done from a distance matrix calculated with a Jaccard index of similarity in hclust in Vegan (Oksanen et al. 2013).

Simple Mantel tests were run in Ecodist package of *R* (Goslee & Urban 2007) to determine correlations between geographic distance and fungal community composition and also between plots, types of forest and sites. This was based on the relative abundance of all OTUs. Dissimilarity matrices of fungal communities of raw presence/absence data were calculated using the Bray–Curtis index of similarity for all sets of data and also by type of forest and site. Similarity matrices for linear coordinates, edaphic factors (pH, N, C, K, M, Ca, and C/N ratio), and the most representative functional fungal groups (EcM, AM, saprotrophs, and plant pathogens) were generated by calculating Euclidean distances. The significance of the Mantel statistic *P* was obtained after 9999 permutations.

Multivariate permutational analysis of variance implemented in the Adonis routine of the Vegan package (Oksanen et al. 2013) was used to address the effect of environmental and soil variables on fungal community composition. Adonis was calculated on a generated Bray-Curtis distance matrix based on the Hellinger-transformation of the presence/absence of OTUs. This analysis represented a nonparametric multivariate analysis of variance (MANOVA) that allows the simultaneous testing of multiple factors and covariates based on permutation tests, and provide their partial coefficients of determination. We used one-way ANOVA to compare the differences in fungal composition among types of forest. Using the same metrics, differences in the structure of fungal communities were visualized using a nonmetric multidimensional scaling (NMDS) ordination as implemented in the Ecodist package. The NMDS was run on a presence/absence matrix of sites by OTUs and by environmental and fungal community variables. To determine whether type of forest and edaphic characteristics contribute to maintaining the fungal community structure of the soil, we performed an NMDS analysis. To assess the relative importance of environmental components (soil parameters) on soil fungal communities, vectors of principal coordinates of neighbor matrices (PCNM) were constructed using the envfit function in the Vegan package (Oksanen et al. 2013). All PCNM vectors with a significance of $P > 0.001$ were included in the subsequent analysis. The surface of the gradient of change of each of the most significant variables was plotted using the function ordisurf and ordihull in Vegan (Oksanen et al. 2013).

Results

Fungal diversity

Pyrosequencing of ITS2 rDNA from 22 pooled soil samples of tropical lowland forests from Colombian Amazonia recovered 74,177 reads. Curation resulted in 65,275 high quality sequences. After removal of chimeras, 56,128 reads were obtained and clustered in 3929 OTUs, of which 3630 (48,004 sequences) were classified as Fungi. Sixty-four OTUs (2272 sequences, 4 %) either had no blastn hit or had a low e-value (< 1e100). The fungal sequences were assembled into 3145 non-singleton OTUs (47,519 sequences) and 485 singletons (13 %). Out of the total of 48,004 fungal sequences, 5276 sequences (21.5 %) corresponding to 677 OTUs (11.1 %) could not be classified to order level, and 16,377 (34.5 %) sequences corresponding to 1668 OTUs (53.0 %) not at family level. These results highlight the current lack of data on fungal diversity from tropical ecosystems in databases.

The number of OTUs per sample ranged between 131 (WSF-ZBS) and 624 (PtF-ZBS). The soil sampling revealed representatives of all major phyla and classes of fungi (Fig. 1A). The phylum Ascomycota (59.2 % of OTUs and 46.7 % of sequences) was the most diverse followed by Basidiomycota (36.9 % of OTUs, 37.1 % of sequences), Zygomycota-Mucoromycotina (1.7 % of OTUs and 14.7 % of sequences), and Cryptomycota (1.1 % of OTUs, 0.3 % of sequences) (Fig. 1A). Archaeorhizomycetes, a recently described phylum from temperate regions (Rosling et al. 2011) was relatively diverse and comprised 6.6 % of the fungal OTUs (4.1 % of sequences). Fungal OTUs were assigned to 68 orders, 144 families, and 304 genera. The orders Helotiales (255 OTUs), Hypocreales (228 OTUs), Chaetothyriales (215 OTUs) of Ascomycota, and Agaricales of Basidiomycota (171 OTUs) were found to be the most diverse.

Overall patterns of taxonomic representation were fairly consistent across the different forest types but the proportions varied (Fig. 2). In general, WSF presented the most unique composition when compared to the other two forest types. WSF presented less diversity of Agaricomycetes and had high number of OTUs belonging to Eurotiomycetes (e.g. *Aspergillus* and *Penicillium*) and Leotiomycetes which comprises many plant pathogens (Fig. 2).

The species accumulation curve did not reach an asymptote, indicating that the number of fungal species in the Amazon region in Colombia is higher than the 3630 OTUs found (Figs 3A, B). In general MF and PtF soils presented a higher fungal diversity than WSFs (Fig. 3A). Despite differences in the number of soil samples by forest type, the rarefied number of species per type of forest set at 12,803 sequences showed a similar trend, with the highest OTUs fungal diversity present in PtF, followed by MF and WSF (Fig. 3B). Chao 1 estimates 21 % more richness in the fungal diversity than the 3630 OTUs found in this study (i.e. 400 species) (Fig. 3C).

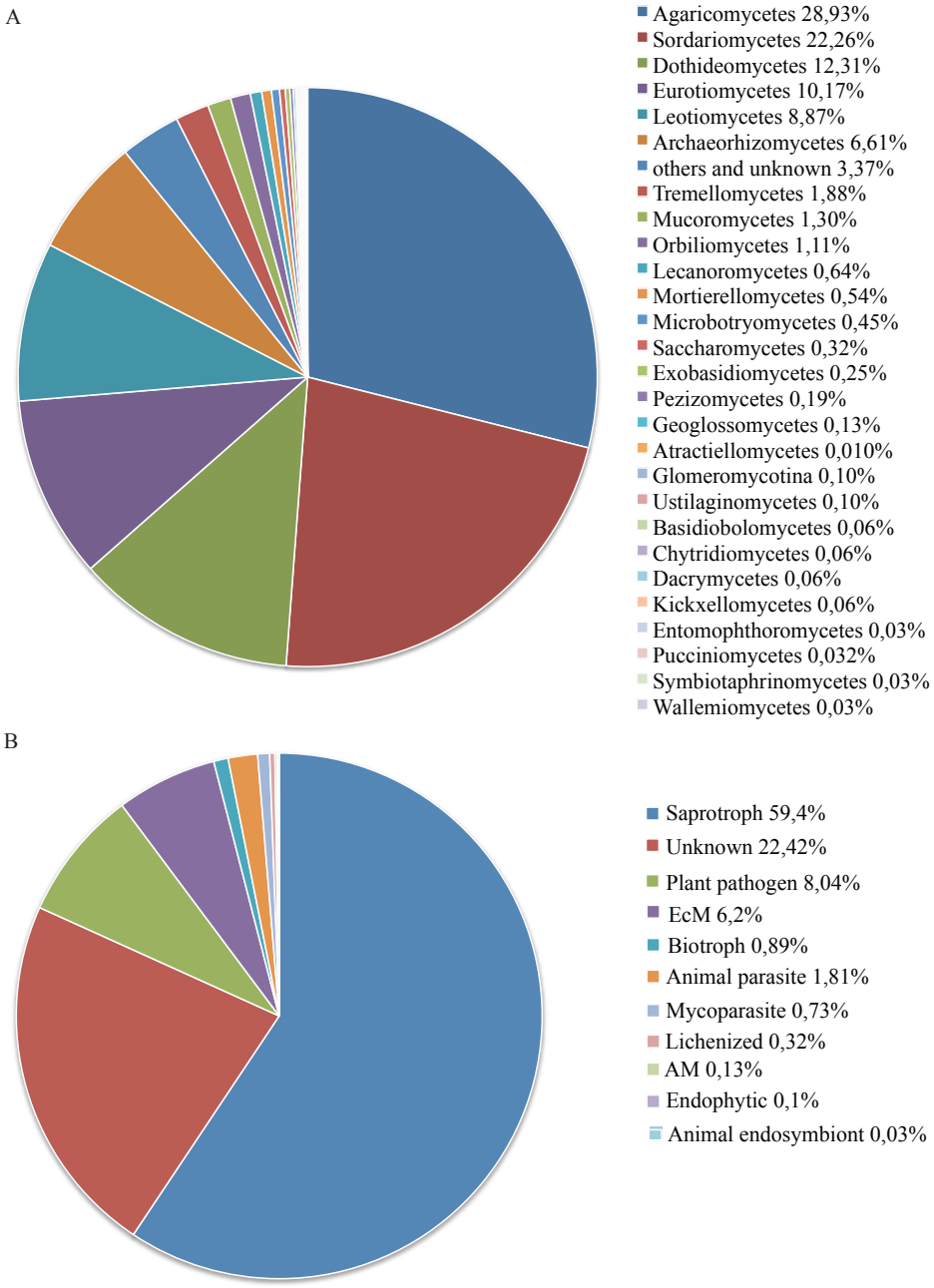


Figure 1. Distribution of operational taxonomic units (OTUs) within major taxonomic (A) and functional groups (B)

The main phylogenetic and functional groups of fungi were present at all sites (Figs 1A-B, 2). Saprotrophic fungi (59.5 %) were found to be the most diverse, followed by plant-pathogens (7.4 %) and EcM fungi (6.2 %) (Fig. 1B). Other trophic categories,

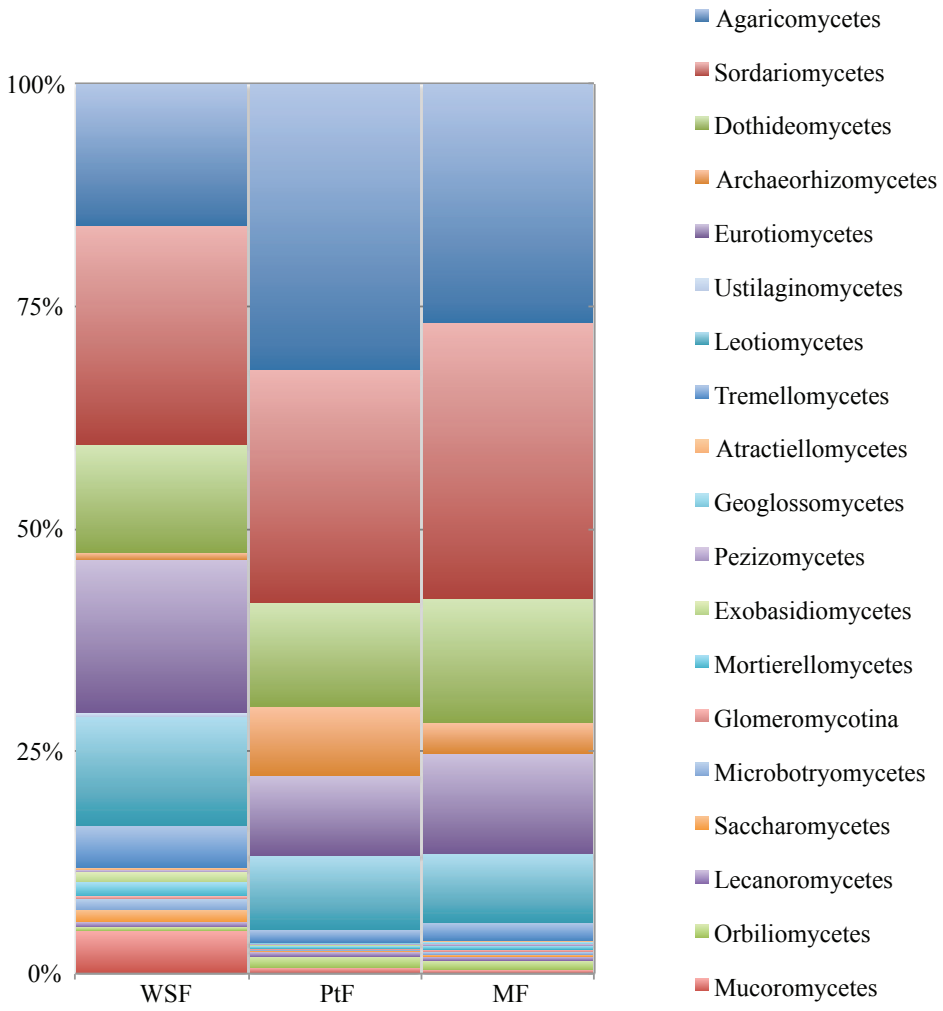


Figure 2. Taxonomic composition of fungal communities from soils of MT, PtF, and WSF from the Amazon basin in Colombia. Lineages with less than five occurrences across all samples (Atractiellomycetes, Basidiobolomycetes, Chrytridiomycetes, Dacrymycetes, Entomophthoromycetes, Incertae sedis, Kickxellomycetes, Pucciniomycetes, Taphrinomycetes) were grouped with the unknown and others for clarity. In total, 3145 fungal OTUs from 68 orders, 144 families, and 304 genera were detected. Despite differences in community structure, phylogenetic representation at class level appears largely similar between the forest types.

such as lichens, endophytes, mycoparasites, animal pathogens, animal endosymbionts, and arbuscular mycorrhizae (AM) represented less than 5 % of the fungal OTUs (Fig. 1B), while 22.4 % of the OTUs could not be classified. These OTUs were grouped in the category "unknown" because they could not be assigned to any trophic group due to lack of taxonomic resolution. The richness of plant pathogens positively correlated with the fungal community structure by forest type (Mantel $P < 0.05$) and presented

a similar proportion to that reported in tropical lowland forests (Peay et al. 2013; Tedersoo et al. 2014). The arbuscular mycorrhizae were not well represented in any of the ecosystems studied (less than 5 %). EcM fungi comprised 195 OTUs (6.2 %), 141, 74, and 56 of which were present in PtF, MF and WSF, respectively. Most diverse EcM fungal lineages were /tomentella-thelephora (53 OTUs, 27.2 %), /russula-lactarius (24 OTUs, 12.3 %), /clavulina (23 OTUs, 11.8 %), /cortinarius (12 OTUs, 6.2 %), and / pisolithus-scleroderma and /boletus (10 OTUs, 5.1 %) (Table 1).

Fungal diversity in different forest types

The most rich fungal community was PtF with 2139 OTUs followed by MF with 1766 OTUs. WSF showed a remarkably lower diversity with 848 OTUs (ANOVA, WSF-MF $P = 0.023$, WSF-PtF $P = 0.005$) (Fig. 4). MF and PtF soils did not present a significant difference in the fungal community composition ($P > 0.05$). OTUs richness per type of forest was supported by the normalized and rarefied number of OTUs. From 12,803 sequences, 1895 fungal OTUs (+12.89) occurred in PtF, 17,66 OTUs in MF, and 833 OTUs (+ 3.69) in WSF (Fig. 3B; Suppl. Table 3).

Regarding the fungal community from PtF, the highest richness was recovered from plots in ZBS (1325 OTUs), followed by plots from MC1 (1097 OTUs) and MC2 (975 OTUs). The three sites shared 336 OTUs and a high number of common OTUs were detected across the PtF forests (Mantel $P < 0.05$). However, the fungal soil communities were more similar between PtF-ZBS and PtF-MC1 despite the geographical distance (Mantel $P < 0.05$). Those two fungal soil communities shared 591 OTUs that corresponded with 31 % of all OTUs from PtF. A Venn diagram (Fig. 5) shows that 34.5 % of the OTUs (1084 out of 3145) were shared between PtF and the MF and a positive correlation between the fungal communities composition was found (Mantel $P < 0.05$). The MF fungal communities from ZBS and MC highly correlated (Mantel $P < 0.05$) and shared 312 OTUs (20.0 %). The WSF presented the lowest soil fungal community richness (848 OTUs), which was significant different from the PtF and MF fungal communities (Mantel $P > 0.05$).

Fungal community composition between types of forest

In general, soil fungal community composition was found to be more similar in samples taken from the same type of forest (Suppl. Table 4; Fig. 6). Cluster analysis showed that WSF had the most unique fungal soil community composition (Fig. 6), being most similar with a nearby plot from MF. WSF exhibited relatively less diversity and shared 6 % of its OTUs with the other forest types. The fungal community compositions were relatively similar between the WSF plots that are separated < 100 km. In general, fungal β-diversity was high between the WSF and the other ecosystems as the Mantel test was statistically not significant ($P > 0.5$). The soil fungal community from WSF seemed to be similar only with the fungal community from plot PtF-MC1 (Suppl. Table 4). The other major cluster included samples from MF and PtF (Fig. 6). The fungal

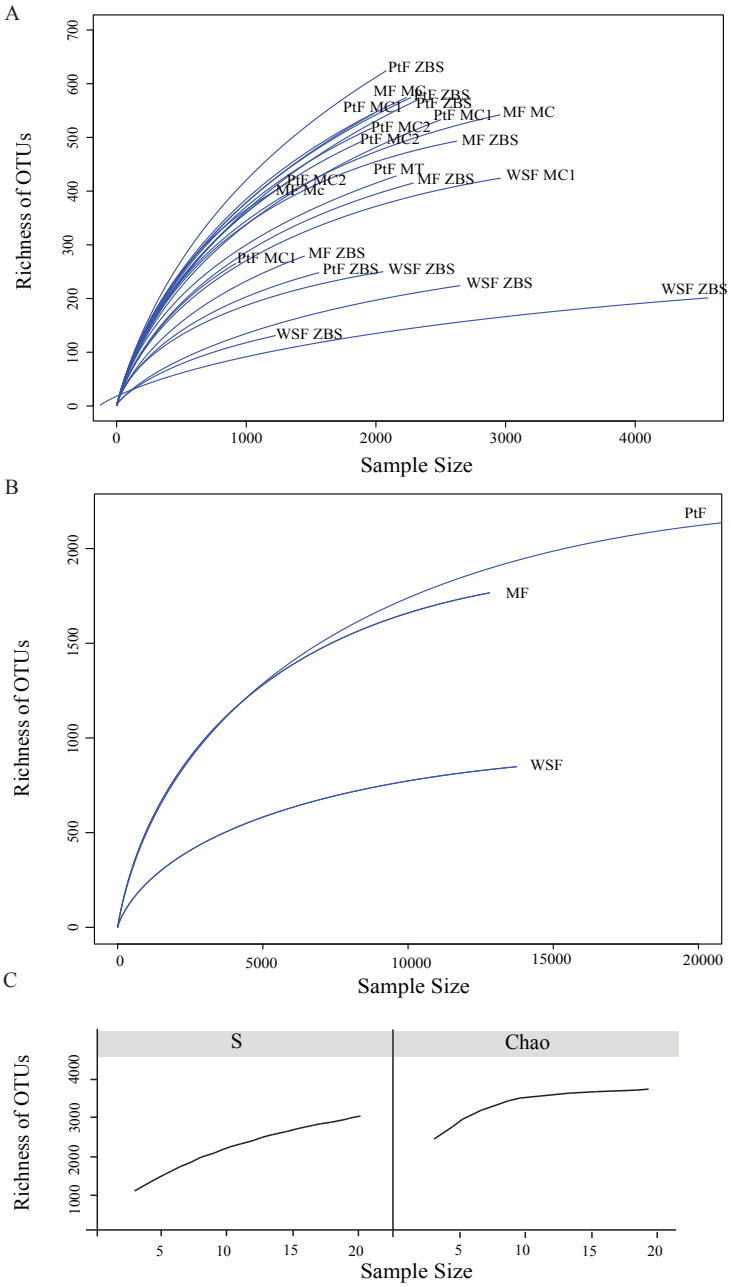


Figure 3. Rarefaction curve of the total number of fungal OTUs per plot (A). Rarefied number of species per type of forest at 12,803 sequences. Note the differences in number of plots by types of forests; MF = 6 plots, PtF = 11 plots and WSF 5 plots (B). Rarefaction curves of the total number of OTUs species with 95 % confidence interval, 1000 permutations and with Chao estimates of species richness of OTUs recorded in tropical rain forests in Colombia Amazonia. S curve shows the real data excluding the WSF from MC (C).

Table 1. Distribution of operational taxonomic units (OTUs) belonging to ectomycorrhizal lineages as recovered in the current and previous studies on EcM of Neotropical ecosystems (Henkel *et al.* 2012; Moyersoen 2006, 2014; Smith *et al.* 2011; Smith *et al.* 2013; Chapters 2, 3). The first row (*) corresponds with the total of OTUs/species reported by each study. Values in the other rows are the percentage of OTUs/species found within the particular fungal lineage (lineages follow Tedersoo *et al.* 2010a; 2013) with values ≥ 10 % highlighted in bold. Note that the different studies used different methodological approaches and different primers to amplify fungal DNA.

Lineage*	This study	*P. tropenbosii* and *D. uaiparuensis* Colombia[1]		*Aldina insignis, D. corymbosa, and D. altsonii,* Guyana[2]	*Pk dipterocarpacea and D. jenmanii,* Guyana[3]		*Dicymbe and Pakaraimaea,* Guyana[4]	*Pk dipterocarpacea,* Venezuela[5]
	OTUs 454-pyrosec.	EcM° species	OTUs Root-tips	OTUs Root-tips	OTUs Root-tips	EcM species	EcM species	OTUs Root-tips
Total OTUs/species reported*	195	119	55	115	52	164	170	77
/tricholoma				1.8	1.9	1.2	1.2	
/albatrellus	0.5							
/amanita	3.1	9.2		4.3		**12.2**	**14.1**	**14.3**
/atheliales1	0.5			0.9	3.8			
/atheliales2	0.5							
/boletus	5.1	**11.8**	5.5	**15.5**	**26.9**	**18.3**	**17.6**	
/cantharellus	2.6	5.9		4.3		6.1	4.7	
/cenococcum	1.5							
/clavulina	**11.8**	**12.6**		**18.1**	**15.4**	**12.2**	**11.2**	**28.6**
/coltricia	3.1	**14.3**		4.3	1.9	4.9	3.5	
/cortinarius	6.2	6.7	**12.7**	5.2	**11.5**	8.5	4.1	**14.3**
/descolea								

Lineage*	This study	*P. tropenbosii* and *D. uaiparuensis* Colombia[1]		*Aldina insignis, D. corymbosa, and D. altsonii,* Guyana[2]	*Pk dipterocarpacea and D. jenmanii,* Guyana[3]		*Dicymbe and Pakaraimaea,* Guyana[4]	*Pk dipterocarpacea,* Venezuela[5]
	OTUs 454-pyrosec.	EcM° species	OTUs Root-tips	OTUs Root-tips	OTUs Root-tips	EcM species	EcM species	OTUs Root-tips
/entoloma	0.5	0.8	1.8				3.5	
/elaphomyces	1.5	0.8	1.8	1.7	1.9	4.9	1.8	
/helotiales*			**14.5**	0.9				
/hydropus	0.5							
/hygrophorus	1.5							
/hysterangium					1.9	1.2	1.2	
/inocybe	4.6	1.7	1.8	6.9		2.4	7.1	**28.6**
/pisolithus-scleroderma	5.1	2.5		0.9			2.9	
/polyporales1					1.9			
/russula-lactarius	**12.3**	**23.5**	**12.7**	**16.4**	**19.2**	**24.4**	**20.6**	
/sarcodon	3.1	3.4	5.5			1.2	0.6	
/sebacina	3.6	5.0	**25.5**	4.3			2.4	**14.3**
/sordariales1	3.6			0.9				
/terfezia-peziza depressa	1.5							
/tomentella-thelephora	**27.2**	1.7	**18.2**	**13.3**	**13.5**	2.4	3.5	
Other				0.9				

[1] Chapter 2; Chapter 3; [2]Smith et al. 2011; [3]Smith et al. 2013, [4]Henkel et al. 2012; [5]Moyersoen 2006, 2014 *EcM lineages of Helotiales (Tedersoo et al. 2010a) were grouped in /helotiales. °Species richness based on fruiting body survey.

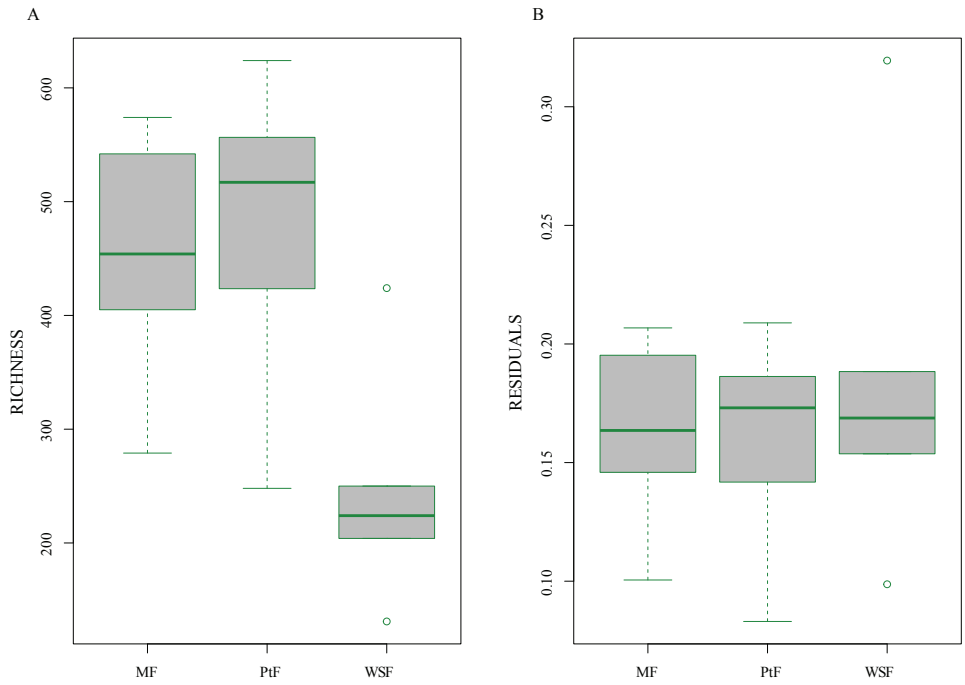


Figure 4. Differences in species richness between MF, PtF, and WSF in Colombia (A). Species richness residuals and standard deviation of species richness recovered from rainforest soils in the Amazon basin in Colombia (B). The internal green bar within the boxes is the standard deviation. The external green bars indicated the lowest and highest data of species richness.

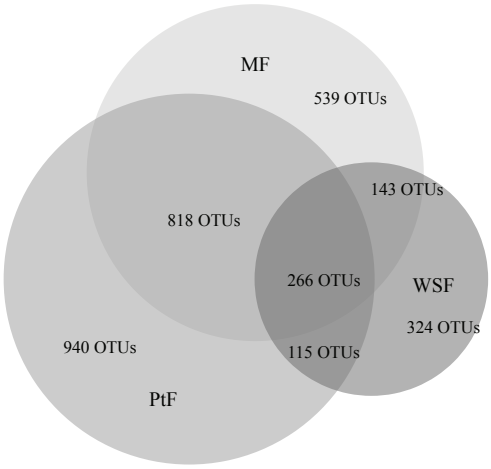


Figure 5. Venn diagram showing overlap between OTUs in MF, PtF, and WSF. PtF was the most OTUs rich forest sharing 34.5 % of the OTUs with MF and 12.1 % with WSF. WSF shared 13 % of the OTUs with MC. The diagram was built based on OTUs richness data from WSF (5 plots), PtF (11 plots) and MF (6 plots).

communities at MF and PtF from the same site tended to be more similar to each other (Figs 6A-C). However, in some cases the fungal composition was significantly more similar in samples from distant places (Figs 6D, E; Mantel P < 0.5; Suppl. Table 4).

Factors that shape the fungal communities

The NMDS analysis revealed that the type of forest plays a fundamental role in driving the fungal community structure. The soil fungal community composition seemed to be more similar in samples from the same type of forests ($F_{2,19}$ = 1.48; P = 0.001). As observed before in the cluster analysis (Fig. 6) the community composition from MF and PtF was in general more similar in samples taken from short geographical distances (Site $F_{6,21}$ = 2.41 ; P = 0.004) (Figs 6, 7A-B; Suppl. Table 4). However, there was no significant correlation between the type of forest and geographical distance suggesting that distance is not impacting fungal species composition (Suppl. Tables 4, 5).

Soil environmental variables showed a significant relationship with fungal OTUs richness. It was found that fungal community composition was significantly affected by soil pH ($F_{1,20}$ = 3.42, P = 0.001), and soil carbon/nitrogen ratio (C/N) ($F_{1,20}$ = 2.72, P = 0.001), which explained 14 % and 12 % of fungal variation, respectively (Figs 8A-B; Suppl. Table 5). The fungal communities were influenced by the increment of the C/N ratio that presented values ranging from 20 to 22.5. The pH and C/N ratio also correlated with EcM, saprotrophic and plant pathogen fungal communities (Suppl. Tables 4, 5).

Discussion

Fungal diversity of Colombian Amazon forests

This study showed the presence of a high diversity of soil fungi in the lowland rain forests of the Colombian Amazon region. We identified 3145 OTUs, belonging to 68 orders, 144 families, and 304 genera. A previous metabarcoding study from similar ecosystems in Western Amazonia revealed 1776 fungal OTUs (Peay et al. 2013). Differences in the diversity with our study may be due to sampling design.

Overall the taxonomic compositions were fairly consistent across the MFs, PtFs, and WSFs in the Colombian Amazon. The main phylogenetic and functional groups of fungi were present in all types of forests and the taxonomic profile was comparable with previous findings from Amazonia (Lucheta et al. 2015; Peay et al. 2013; Tedersoo et al. 2014). The cryptic fungal groups, such as Archaeorhizomycetes and Cryptomycota, were relatively abundant in our samples (6.6 % of OTUs and 4.1 % of sequences, 1.1 % of OTUs and 0.3 % of sequences respectively), indicating that both taxa are highly diverse in the Neotropics. In agreement, similar observations have

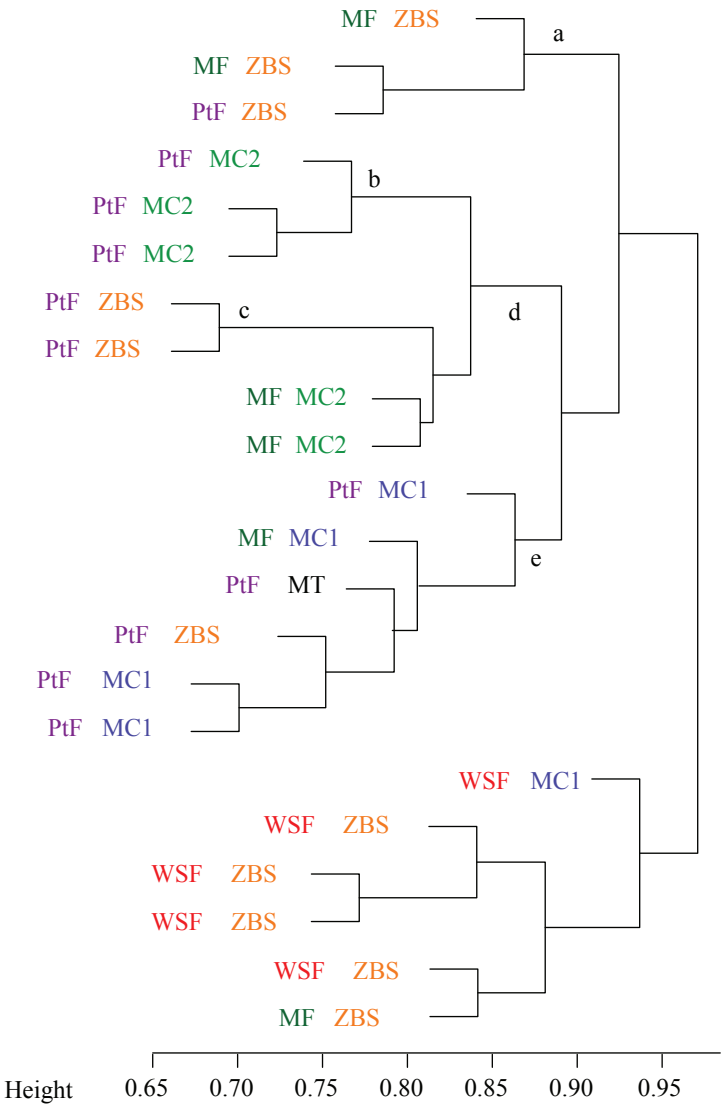


Figure 6. Cluster analysis showing similarity values of the fungal community compositions between plots based on the Jaccard index. Scale 0-1, with 1 indicating maximal similarity. Color red represents plots from WSFs, purple from PtFs and green from MFs. Plots were established in three localities, the biological station El Zafire in orange color, and in the Middle Colombian Amazon region, the localities of Peña Roja in blue color and Puerto Santander in green color.

been made in other tropical and subtropical ecosystems in South America (Geml et al. 2014; Gleason et al. 2012; Tedersoo et al. 2014). The lack of a complete understanding of the diversity of fungi in the tropics was reflected in the number of OTUs (22.4 % of

the total) that could not be identified at lower taxonomic levels.

The main fungal functional groups, such as saprotrophs, plant-pathogens, EcM, AM, and endophytes were present in all types of forest, but their relative proportion varied across samples. The saprotrophic fungi were most abundant and evenly distributed in the three forest types, although the species composition varied between plots and forests. The EcM and plant pathogenic fungi were also evenly distributed in the tropical lowland forests and occurred in similar proportions previously found in global fungal data from soils (Tedersoo et al. 2014). AM OTUs were rare in our study (0.07 % of sequences), which was also found in soils in the North of South America and Panama (Lucheta et al. 2015; McGuire et al. 2012; Peay et al. 2013; Tedersoo et al. 2014).

A total of 195 fungal EcM OTUs were found. This number is in good agreement with the 172 species of EcM fungi reported from WSFs and PtFs in Colombia based on fruiting bodies and root tips (Chapters 2, 3) and the 174 EcM species from forests in Guyana that are dominated by EcM hosts of the Fabaceae and the dipterocarp *Pakaraimaea dipterocarpacea* (Henkel et al. 2012; Smith et al. 2011, 2013). We expect that the number of EcM species may increase in future studies. This is strengthened by the fact that lineages such as /amanita, /boletus, /cortinarius, and /sebacina were not well represented in this study and that a higher richness of OTUs have been previously reported. It is important to note that even though next generation sequencing provides a deeper insight in fungal diversity compared with the traditional methods, this method does not reflect the complete species composition that occurs in a sample (Tedersoo et al. 2010c). A comparison of the soil fungal composition with other ecosystems is difficult as there are still relatively few studies with NGS data from Neotropical ecosystems (Lucheta et al. 2015; McGuire et al. 2012; Peay et al. 2013; Tedersoo et al. 2014). The EcM lineages /tomentella-thelephora and /russula-lactarius were most abundant in the Colombian lowland forests. These lineages are known to be associated with all major plant host taxa in a variety of ecosystems (Brearley 2012; Tedersoo et al. 2010a). Russulaceae has been found to be an important group in the EcM fungal community in PtF and WSF in previous studies based on rDNA sequences from EcM roots and collected sporocarps (Roy et al. 2016; Chapters 2, 3). In contrast, the lineage /tomentella-thelephora is widely unknown in many tropical countries. Species of *Tomentella* have cryptic or atypical fruiting bodies that may be easily overlooked in diversity research.

Our data showed that PtF presented the highest number of EcM fungal OTUs with 123 OTUs, followed by MF and WSF with 74 and 53 OTUs, respectively. We expected a higher richness of EcM fungi in forests with a high abundance of EcM trees, such as WSF and PtF, and not in MF where the EcM trees occurred scattered with low abundance. We also found that the EcM fungal community composition was most

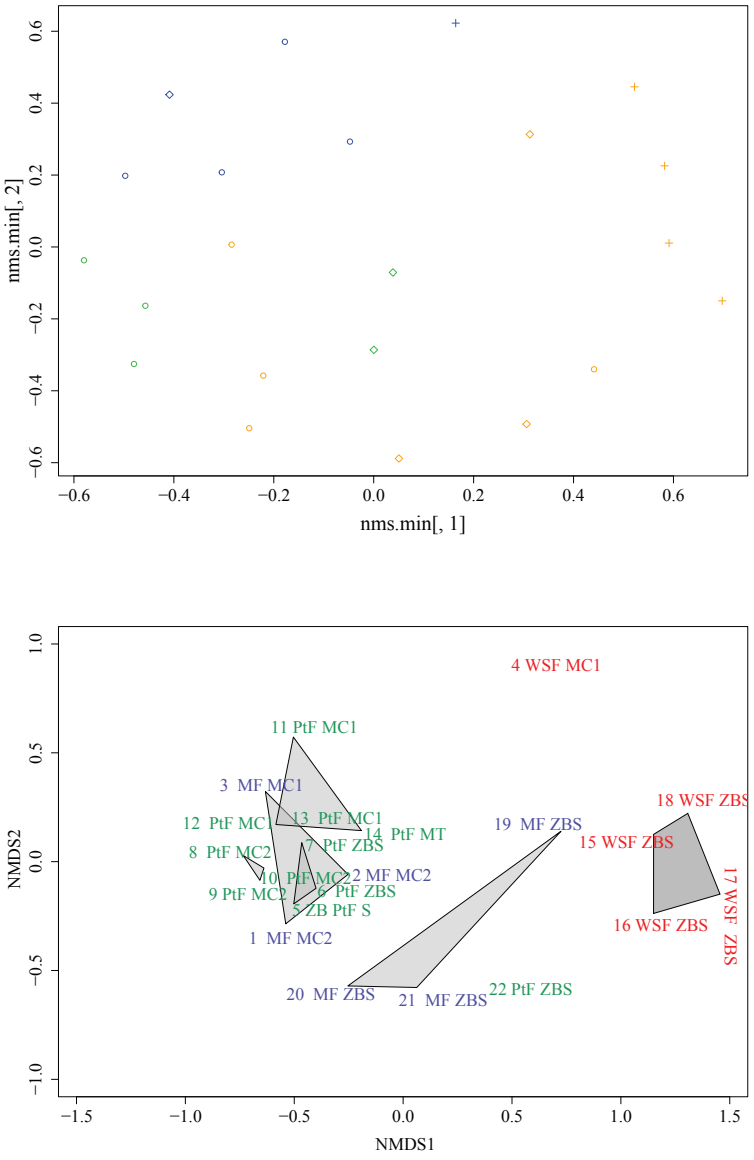


Figure 7. Nonmetric multidimensional scaling (NMDS) plot representing similarity of the fungal soil community in different forest types in Colombia Amazonia. Stress value = 0.1141; R2 = 0.986. In A) a circle represents a fungal community recovered from PtFs; rhombus from MFs and cross from WSFs. Colors represent different sites; MC1 in blue, MC2 in green, and ZBS in orange (A). NMDS plots grouped according to soil fungal community composition. Colors represent different fungal communities recovered from PtFs (green); MFs (blue) and WSFs (red). Samples from WSFs grouped together and samples from PtF and MF correlated based on the fungal composition despite geographic distance (B).

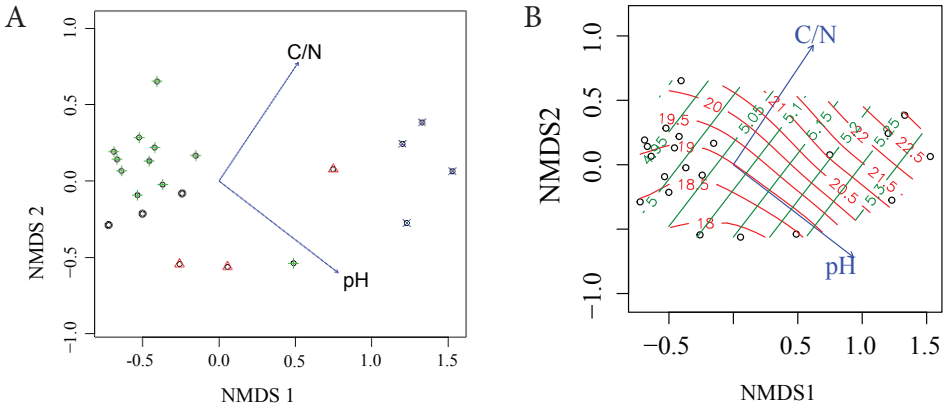


Figure 8. Nonmetric multidimensional scaling (NMDS) ordination plots representing similarity of the fungal soil community in different forest types in Colombian Amazonia and the effect of pH and C/N ratio in the fungal communities. In (A) green circle represent PtF plots, red triangles represented MF plots from ZBS, blue crosses plots of WSFs, and grey circles MF plots from MC (A). NMDS plot showing intensity of the gradient of variation for pH and C/N ratio (B).

similar between PtFs and MFs. This may be explained by the fact that species of EcM fungi can be symbionts with several species within the families of Fabaceae and Dipterocarpaceae (Moyersoen 2012; Smith et al. 2013; Chapter 3). However, some host specificity has been reported in Ecuador, where Tedersoo et al. (2010b) detected a strong relationship between specific species of EcM fungi and trees of the genera *Coccoloba*, *Guapira* and *Neea*.

Determinants of fungal communities

Fungal communities in Colombian Amazonia were mainly found to be affected by forest types. The cluster analysis showed that the composition of OTUs was relatively similar between MF and PtF plots, particularly those that are located nearby, although in most of the cases the similarity was independent of the geographical distance. *P. tropenbosii* is abundant in PtFs, while MFs consisted of members of Lecythidaceae, Leguminosae, Myristicaceae, Sapotaceae and Moraceae with species of *Coccoloba*, *Guapira* or *Neea* being present in low abundance (Duque et al. 2003; Londoño 2011; Parrado-Rosselli 2005). The soil fungal community in WSF was the most unique. In contrast with terra-firme MFs and PtFs, WSFs occur on extremely poor, acidic and strongly leached soils (Quesada et al. 2011). As a result, plant diversity in WSFs is poor and commonly dominated by members of Fabaceae (*Dicymbe* and *Aldina*), Clusiaceae, and Arecaceae presenting many endemic plant species (Fine et al. 2010; Fine & Baratolo 2016; Peñuela-Mora 2014). Fungal species richness was shown to correlate with tree species richness in other tropical and subtropical studies and was shown to be more predictive when compared to geographic distance or environmental

variation (Burns et al. 2015; Geml et al. 2014; Peay et al. 2013). Specific associations between plant species and soil fungi likely contribute to the strong plant-soil feedbacks observed in many ecosystems with important implications for co-existence and community assembly (Bever et al. 2010; Burns et al. 2015). Fungi, for example, have a strong role in structuring the diversity and abundance of tropical trees (Augspurger 1983; Comita et al. 2010; McGuire 2007; Peay et al. 2013; Peh et al. 2011; Peñuela-Mora 2014; Torti et al. 2001). Plant species composition may impact fungal species composition through selection resulting from host-specific symbiosis, root structure, the production of root exudates, and the presence of recalcitrant leaves (Burns et al. 2015; Hanson et al. 2012; McGuire et al. 2010). In our study, a strong correlation was observed between the plant community and plant pathogenic fungi. This was not unexpected since plant pathogens often present host specificity (Gilbert & Webb 2007). Peay et al. (2013) found evidence of putative lineages of pathogenic fungi in white-sand, flooded and terra firme forests, with very low overlap in OTUs across habitats. The number of species of pathogenic fungi increases in the tropics and plays an important role in maintaining tree species diversity (Augspurger 1983; Tedersoo et al. 2014).

Microbial species differ in their nutrient preferences and soil chemistry niches (Burns et al. 2015). The pH was the best predictor of the fungal community composition in the soil followed by the C/N ratio. Variations of the pH had a great influence on fungal communities in WSFs, due to the fact that this type of forest develops on more acidic soils than terra-firme forests. A significant impact of soil pH on the fungal dominance of microbial communities has been reported before at local and global scales (Burns et al. 2015; Geml et al. 2014; Högberg et al. 2007; Lucheta et al. 2015; Tedersoo et al. 2014). The C/N ratio strongly shapes fungal decomposer communities in natural ecosystems and forest plantations (Högberg et al 2007; Peršoh et al. 2015; Schneider et al. 2012; Strickland et al. 2009; Strickland & Rousk 2010). The optimum C/N ratio for a maximum decomposition is 20-25 since a favorable soil environment is created to bring about equilibrium between mineralization and immobilization processes. This was the range of values observed in our study.

Nutrients such as P, Ca, and Al have been reported as important predictors of fungal richness (López-Quintero et al. 2012; Lucheta et al. 2015; Tedersoo et al. 2014). Although a number of studies have shown clear relationships between edaphic variables and the fungal community composition, other studies indicated that in general, soil type has no direct impact on fungal communities (Henkel et al. 2002; Hovatter et al. 2011; Peay et al. 2013). It is important to note that plant composition in Amazonian forests results from, at least in part, environmental conditions, like soil structure, soil nutrient content, soil drainage, and water holding capacity (Fine et al. 2006; Quesada et al. 2009). The data resulting from our study were not sufficient

to determine whether the effect on the community of fungi was a response to the plant community structure or to edaphic conditions, or to both. In order to address this question, long-term observations on plant and fungal biodiversity together with assessment of other biotic and abiotic factors are needed.

Conclusion

Our study provided documentation on fungal communities in three forest types in Western Amazonia in Colombia. The species composition was more similar between MF and PtF and these two types of forest shared a high number of species. WSFs presented the most unique fungal composition. This study highlights the importance of forest types and soil chemistry (pH and C/N ratio) as important drivers that structure fungal communities in lowland tropical forests in Colombia. Despite the fact that the EcM host trees are rare and scattered in terra-firme mixed forests, they associate with a large number of EcM fungal taxa. These host trees may act as connecting bridge for EcM fungi between PtF patches, thus facilitating the distribution of EcM fungi in various types of terra-firme forests, and probably also with WSFs. Further studies about the main factors that shape the fungal communities, and the role of fungal communities in structuring plant communities and how they facilitate nutrient cycling in Amazonian ecosystems, are needed. A better understanding of these fungi-plant associations may show light on the fungal contribution to plant community ecology of tropical lowland rain forests in the light of climate change.

Supplementary Table 1. Information of the 22 plots used in this study. MF, PtF, and WSF comprised 6, 11, and 5 plots, respectively, from the Zafire Biological Station area (ZBS) and the Middle Colombian Amazon region (MC). IVI: Important Value Index; ND: No data available.

# plots		Forests	Site	EcM host	Host tree IVI
2	MF	Mixed terra-firme forests	MC2	*Coccoloba* (Polygonaceae) or *Guapira*/*Neea* (Nyctaginaceae)	*Coccoloba* 0.477 %, Nyctaginaceae 0.845 %
1	MF	Mixed terra-firme forests	MC1	*Coccoloba* (Polygonaceae) or *Guapira*/*Neea* (Nyctaginaceae)	*Coccoloba* 0.477 %, Nyctaginaceae 0.845 %
3	MF	Mixed terra-firme forests	ZBS	*Coccoloba* (Polygonaceae) or *Guapira*/*Neea* (Nyctaginaceae)	ND*
3	PtF	Mixed terra-firme forests	MC1	*Pseudomonotes tropenbosii*	16-18 %
3	PtF	Mixed terra-firme forests	MC2	*Pseudomonotes tropenbosii*	16-18 %
3	PtF	Mixed terra-firme forests	ZBS	*Pseudomonotes tropenbosii*	ND*
1	PtF	Mixed terra-firme forests	Meta-nearby to MC1	*Pseudomonotes tropenbosii*	ND*
1	PtF	Mixed terra-firme forests	Km18 nearby to ZBS	*Pseudomonotes tropenbosii*	ND*
1	WSF	White-sand forests	MC1	*Dicymbe* spp.	ND*
4	WSF	White-sand forests	ZBS	*Dicymbe uaiparuensis*	*D. uaiparuensis* 25 %

Supplementary Table 2. Soil physic-chemical characteristics of the plots studied

SITIO	pH	C %	N %	C/N	N15	C13	LogP	LogK	LogMg	LogCa
Mix MC	4.93	5.60	0.74	19.13	1.67	-30.65	1.08	2.25	1.65	2.05
Mix MC	4.93	5.60	1.50	19.29	2.07	-31.14	1.67	2.73	1.93	1.98
Mix MC	4.93	5.60	1.58	18.44	3.39	-30.55	1.21	1.95	1.43	2.08
WSF MC	4.93	5.60	0.71	22.92	-0.45	-30.45	1.73	2.51	2.14	2.27
PtF ZBS	5.03	6.89	1.76	19.12	3.01	-30.28	1.82	2.89	2.24	2.20
PtF ZBS	5.28	7.46	1.96	18.04	2.90	-30.67	1.93	2.94	2.29	2.20
PtF ZBS	4.94	7.35	2.17	19.69	1.70	-30.59	1.94	2.85	2.12	1.99
PtF MC2	4.93	5.60	1.47	19.92	0.14	-30.34	1.59	2.63	1.91	1.91
PtF MC2	4.93	5.60	0.99	19.38	2.08	-30.51	1.37	2.57	1.76	2.09
PtF MC2	4.93	5.60	0.68	16.88	2.14	-30.99	1.13	2.32	1.58	2.08
PtF MC1	4.93	5.60	1.97	20.36	3.11	-31.20	1.81	2.66	1.96	1.95
PtF MC1	4.93	5.60	1.99	19.66	2.23	-30.40	1.74	2.64	1.84	2.09
PtF MC1	4.93	5.60	1.15	18.82	1.98	-30.4	1.72	2.61	2.02	2.09
Meta	4.93	6.21	1.24	17.17	3.93	-29.9	1.36	2.36	1.60	1.97
WSF ZBS	5.28	7.46	1.63	21.57	-0.26	-30.38	1.96	2.79	2.40	2.46
WSF ZBS	5.28	7.46	0.65	20.99	1.10	-29.87	1.67	2.30	1.80	1.98
WSF ZBS	5.28	7.46	1.34	22.94	-0.46	-29.96	2.10	2.83	2.48	2.47
Mix ZBS	5.28	7.46	1.44	22.80	-0.14	-30.74	1.73	2.82	2.32	2.42
Mix ZBS	5.03	6.89	0.93	18.60	4.62	-29.85	1.34	2.25	1.57	1.98
Mix ZBS	5.28	7.46	1.69	16.79	3.15	-30.17	1.65	2.20	1.60	1.83

Supplementary Table 3. Distribution of OTUs among each type of forest at the different sites recovered with 454-pyrosequencing.

Site	OTUs	Rarefied	Se
***PtF*-ZBS**	1325	1118.35	11.33
***PtF*- MC1**	1097	958.66	9.65
***PtF* -MC2**	975	975	0
WSFZBS	767	407.52	8.59
MF-ZBS	674	931.64	6.77
MF-MC	1162	1086.23	7.65
PtF total	2139	1895	12.89
MF total	1766	1766	0
WSF total	848	833	3.49

Supplementary Table 4. Next page.

Supplementary Table 5. Adonis test analyses to evaluate the effect of different factors on the fungal community structure.

	Factor	Df	Sums of Sqs	Pr>F
All types of forests	Type of forests	2/19	1.48	**0.001**
	Plot	6/21	2.41	**0.004**
	pH	1/20	3.42	**0.001**
	C/N	1/20	2.72	**0.001**
	Propor AM	14/21	4.64	0.189
	Porpor EcM	21/21	6.79	1
	Factor	Df	Sums of Sqs	Pr>F
White-sand forests	Plot	4/4	1.20	1
	pH	1/4	0.44	1
	N	2/4	0.47	1
	C	1/4	0.29	1
	Factor	Df	Sums of Sqs	Pr>F
***Pseudomonotes tropenbosii* forests**	Site	10/10	2.58	1
	pH	4/10	1.06	1
	N	6/10	1.51	1
	Factor	Df	Sums of Sqs	Pr>F
Terra-firme forests	Site	4/5	1.25	1
	pH	2/5	0.65	1
	N	3/5	0.89	1

$P < 0.05$ was used to indicate significant differences, those values are in bold.

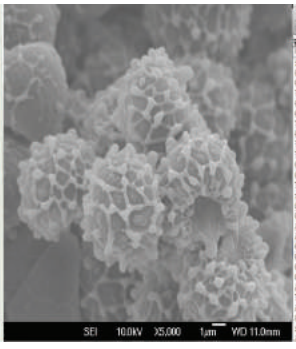
Supplementary Table 4. Mantel test analyses for the association between fungal community structure, geographical distance, edaphic factors, and functional groups in lowland rain forests in in the area of the biological station El Zafire (ZBS) Peña Roja (MC1) and Puerto Santander (MC2) in the Middle Colombian Amazon region.

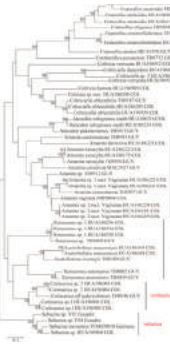
Parameter	Parameter	Mantel	
Control for		r	P
Sample*	Geographical distance	0.1575	**0.0194**
Forests-Site	Geographical distance	0.0864	0.2017
Pseudomonotes tropenbosii forest	Geographical distance	0.2843	0.0619
Mixed terra-firme forest	Geographical distance	0.1790	0.1602
White-sand forest	Geographical distance	0.8705	0.1318
El Zafire (ZBS)	Geographical distance	-0.0362	0.5266
Middle Colombian Amazon region (MC)	Geographical distance	0.0175	0.3096
Endomycorrhizae (AM)*	Distance Proportion	0.1653	0.4329
EcM*	Distance Proportion	0.0585	0.2754
Saprotrophic*	Distance Proportion	0.2090	0.0526
Plant Pathogenic*	Distance Proportion	0.2489	**0.0321**
Ph.*	Distance	0.0616	0.2574
Carbon*	Distance	-0.0339	0.6283
Carbon-Nitrogen*	Distance	0.4423	**0.0008**
Nitrogen*	Distance	0.0102	0.4269
ZBS-MC	Distance	0.1029	0.1974
MF MC/MF ZBS	Distance	0.9889	**0.0441**
MF MC/PtF ZBS	Distance	0.9995	**0.0112**
MF MC/PtF MC2	Distance	0.9999	**0.0110**
MF MC/ PtF MC1	Distance	0.9717	**0.0452**
MF MC/WSF ZBS	Distance	0.8853	0.0549
MF ZBS/ PtF ZBS	Distance	0.9906	**0.0440**
PtF ZBS /PtF MC2	Distance	0.9993	**0.0108**
PtF ZBS/PtF MC1	Distance	0.9735	**0.0445**
PtF MC2/ PtF MC1	Distance	0.9713	**0.0446**
PtF MC2/MF ZBS	Distance	0.9884	**0.0448**
PtF MC1/MF ZBS	Distance	0.9953	**0.0110**
WSF ZBS /PtF ZBS	Distance	0.8983	0.0541
WSF ZBS /PtF MC2	Distance	0.8828	0.0545
WSF ZBS/PtF MC1	Distance	0.8635	**0.0441**
WSF ZBS /MF ZBS	Distance	0.8912	0.0560
PtF ZBS/PtFMC2/PtFMC1**	Distance	0.9891	**0.0126**
WSF ZBS/PtF ZBS/MC ZBS**	Distance	0.249	0.1633
EcM/ Carbon-Nitrogen	Distance	0.4387	**0.0002**
EcM/Coordinates	Geographical distance	0.2862	**0.0012**
EcM/Edaphic	Distance	0.2862	**0.0011**
EcM/pH	Distance	0.4693	**0.0002**
Plant Pathogenic /Coordinates	Geographical distance	0.1379	**0.0105**
Plant Pathogenic/ Carbon-Nitrogen	Distance	0.5081	**0.0002**
Plant Pathogenic/ pH	Distance	0.4162	**0.0003**
Saprotrophic /Coordinates	Geographical distance	0.1379	**0.0090**
Saprotrophic/ Carbon-Nitrogen	Distance	0.5081	**0.0002**
Saprotrophic/ pH	Distance	0.4162	**0.0002**

SECTION 2

Taxonomic Novelties

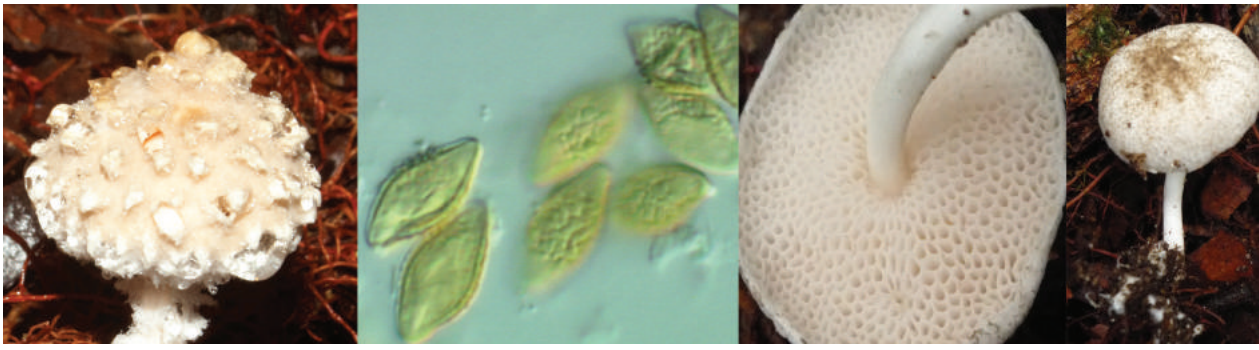




CHAPTER 5

Austroboletus amazonicus sp. nov. and *Fistulinella campinaranae* var. scrobiculata, two common boletes from a forest dominated by the dipterocarp *Pseudomonotes tropenbosii* in Colombian Amazonia



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Abstract

Two boletes that frequently form fruiting bodies in *Pseudomonotes tropenbosii* forests are described from Colombian Amazonia. One is a new species of *Austroboletus* here described as *A. amazonicus* and the other one is *Fistulinella campinaranae* var. *scrobiculata* Singer, which is a new record for Colombia. Macromorphological, micromorphological and habitat data for these species are provided as well as DNA sequence data of the internal transcribed spacer (ITS) and the D1/D2 domains of the large subunit (LSU) ribosomal DNA.

Introduction

Colombia is the world's fourth most biodiverse country and the most megadiverse per square kilometer (Profepa 2002). So far, 22,840 flowering plant species have been recorded in the country (Bernal et al. 2015). Because of the intimate ecological interactions between plants and fungi, a high diversity of fungi is expected in the country as well (ca. 137,000 species). Unfortunately, a fungal inventory is still far from complete. Vasco-Palacios and Franco-Molano (2013) listed 1239 species of macrofungi reported from Colombia until 2011, but this number is low when compared with the world's estimate of existing fungal species, which ranges from 611,000 (Mora et al. 2011) to 5.1 million species (Taylor et al. 2010).

Recent studies in the tropics, mainly from lowland tropical rain forests, reported a high biodiversity of macrofungi in diverse ecosystems and a high number of new species (Aime & Brearley 2012; Blackwell 2011; Brearley 2012; Henkel et al. 2012; Smith et al. 2013; Chapters 2, 3). In Colombia, López-Quintero et al. (2012) found 403 macrofungal morphospecies in successional forests of different stages in two geographical areas in the Amazon region. Although 52 % of species could not be named and remained as a morphospecies, the number of species found by these authors is high compared with the 248 species reported by Vasco-Palacios and Franco-Molano from this region (2013).

In Colombia 34 species of Boletaceae have been reported. Thirty-one came from the Andean region where they were collected mainly from oak forests (*Quercus humboldtii*) and three from the Amazon area (Vasco-Palacios & Franco-Molano 2013). Only three species of *Austroboletus* have been observed in Colombia. *Austroboletus subvirens* (Hongo) Wolfe is associated with *Quercus humboldtii* (Bonpl.) in Andean ecosystems (Vasco-Palacios & Franco-Molano 2013), while *A. subflavidus* (Murrill) Wolfe is represented by a specimen collected in an oak forest in Nariño department that is deposited in the collection of the New York Botanical Garden (viz. R. Halling 6110 NY, 20 Nov 1988). The third species is described here and was reported by López-Quintero et al. (2012) as *Austroboletus* sp1. No members of *Fistulinella* have

been reported from Colombia until now. *Fistulinella* contains 27 species mostly with a tropical distribution (Fulgenzi et al. 2010; Pegler & Young 1981; Pegler 1983; Singer et al. 1983; Watling & Gregory 1989; Watling 2008).

A large number of ectomycorrhizal (EcM) boletes is known to be associated with Tropical Dipterocarpaceae (Brearley 2012; Halling et al. 2007). For instance, 15 species were found to be associated with *Pakaraimaea dipterocarpacea* in Guyana (Smith et al. 2013). Attempts have been made to document the diversity of ectomycorrhizal fungi associated with *Pseudomonotes tropenbosii* (López-Quintero et al. 2012; Vasco-Palacios et al. 2005). This endemic and dominant dipterocarp occurs terra-firme forests on the Western Amazonia basin in Colombia (Londoño et al. 1995; Parrado-Rosselli 2005). Eighty-three putative ectomycorrhizal morphospecies have been collected in these forests and they all belong to commonly known families of EcM fungi (Chapter 3). In these *Pseudomonotes* forests the family Boletaceae (Boletales, Agaricomycetes, Basidiomycota) is well represented with 13 morphospecies (Chapter 3). Here we describe *Austroboletus amazonicus* sp. nov. A.M. Vasco-Pal. & C- López-Q. and make a new distribution record for Colombia of *Fistulinella campinaranae* var. *scrobiculata* Singer. Macromorphological, micromorphological, habitat and DNA sequence data are provided for both species.

Materials and methods

Study site

Fieldwork was conducted in the Colombian Amazon region, in the El Zafire Biological Station (ZBS) and in the Middle Colombian Amazon region (MC) in forests with *P. tropenbosii* (Dipterocarpaceae). *P. tropenbosii* constitutes an ecologically important species in an unique lowland tropical rain forest in the Colombian Amazonia region (Appanah & Turnbull 1998; Londoño et al. 1995; Parrado-Rosselli 2005). The ZBS is situated in the southern part of Colombian, 4°00′S, 69°53′W, 27 km north of Leticia. Relative humidity is about 86 % with a mean temperature of about 26 ºC that does not fluctuate much throughout the year. Mean monthly precipitation is 280 mm with a drier period from June to September (mean monthly precipitation of 160 mm) and a rainy season from October to May (mean monthly precipitation of 340 mm) (Jimenez et al. 2009). Soils are sandy and mainly composed of quartz. The parental material belongs to the Terciario superior Amazonico unit that probably originated from the Guiana Shield (Jimenez et al. 2009). The terrain is slightly hilly and situated 80-120 m above sea level. Four major types of forests are present in El Zafire, namely floodplain forest, white-sand forest (WSF), a transition forest and a terra-firme forest where *P. tropenbosii* is dominant (PtF) (Jimenez et al. 2009). All are well developed forests with no evidence of human disturbance, except for hunting. The area was visited during the rainy season between March 2012 to March 2013.

Two places were studied in the Middle Colombian Amazon region. The first (MC1) is about 50 km downstream along the Rio Caquetá, near Peña Roja, 00°34′S, 79°08′W, and the second (MC2) is close to the village of Puerto Santander, 00°39′S, 72°23′W, both at 200-300 m above sea level. This region consists of terra-firme forest, food plain forest, WSF and secondary forest (Parrado-Rosselli 2005). Mean annual temperature is 25.7 °C with an average annual rainfall of 3060 mm (Duivenvoorden & Lips 1993). Although the region does not have a marked dry season, rainfall decreases during December to February.

Sample collection and preparation

The specimens were photographed in situ, macromorphological characters were described from fresh material and macrochemical tests were performed (Franco-Molano et al. 2005; Largent 1986; Lodge et al. 2004). Spore prints were obtained where possible. Color codes were designated according to the Methuen Handbook of Color (Kornerup & Wanscher 1978). The collections were field-dried at 40 ºC with a fruit dehydrator (SIGG Dörrex), and pieces of the samples were preserved in 2 × CTAB buffer for further molecular analysis (Schmit & Lodge 2005). The material was transported to the laboratory of Taxonomy and Ecology of Fungi (TEHO) at Antioquia University, Medellín.

Dried material was studied with traditional mycological methods (Largent et al. 1977). All measurements of anatomical features were made in mounts of 3 % KOH, Congo red and Melzer's reagent. Length and width of basidiospores were measured and the Q parameter was calculated (Q = length/width ratio from *n* basidiospores, *n* = number of basidiospores measured). Line drawings were made with a drawing tube. Basidiospores ornamentation was examined with a Jeol JSM-5410 LV scanning electron microscope operating at 7 kV. To prepare samples for SEM the methods of Lopez and Rios-Velazquez (2005) were applied. Morphological identification of specimens was carried out with identification keys and taxonomic revisions (Fulgenzi et al. 2010; Horak 1988; Singer et al. 1983; Watling 2008; Wolfe 1979; Wolfe et al. 1988). The Dictionary of the Fungi (10^{th} ed.) was used to update fungal nomenclature (Kirk et al. 2008). Specimens were deposited in the herbarium HUA, Antioquia University, Medellín, Colombia.

For molecular analysis, EcM root tips were randomly collected within a few meters of the stem of the *Pseudomonotes* host in the Araracuara and Puerto Santander plots. Samples were air-dried and transported to the TEHO laboratory for further study. Root tips were examined under magnification for the presence of a fungal mantle. Ectomycorrhizal root tips morphotypes were stored in CTAB buffer for molecular analyses. Young leaves of *P. tropenbosii* were collected and dried with silica gel for molecular analysis.

Molecular analyses

Fragments of basidiomata and root tips that had been preserved in CTAB were transferred to 2 mL collection tubes and dried 2 h in vacuum (DNA speed Van 110, SAVANAT). DNA was extracted from 20 mg dried basidioma material with the PrepMan Ultra buffer (Applied Biosystems, Foster City, California), followed by purification with JETquick general DNA clean-up columns (Genomed, Löhne, Germany) according to the manufacturer's instructions. The ribosomal DNA (rDNA) internal transcribed spacer (ITS1-2) regions, including the 5.8S rDNA, were amplified with the ITS1F and ITS4 primers, and primers ITS1F, ITS5 and ITS4 were used for sequencing (Gardes & Bruns 1993; Tedersoo et al. 2007; White et al. 1990). The D1/D2 domains of the large subunit (LSU), rDNA were amplified with primers LR0 and LR7, and primers LR0, LR5, and LR7 were used to sequence the DNA (Vilgalys & Sun 1994). The PCR conditions consisted of 40 cycles of 45 s at 96 ºC, 45 s at 52 ºC and 2 min at 70 ºC, with one initial step of 5 min at 96 ºC for initial denaturation and a final step of 7 min at 72 ºC for final extension. PCR products were quantified on 1 % agarose gels with GelRed (Biotium Inc.).

DNA from EcM root tips and from leaves of *P. tropenbosii* was extracted with the DNEasy plant mini kit (Qiagen, Crawley, UK) following the manufacturer's protocol. The fungal ITS region was amplified as described above. To confirm the identity of the plant host the chloroplast trnL (UAA) intron and trnL-F spacer were amplified and sequenced with the primer combination trnLc-trnLd and trnLe-trnLf, respectively (Taberlet et al. 2007). Reference sequences of *P. tropenbosii* were generated with the above primers. The PCR program consisted of 40 cycles of 1 min at 94 ºC, 1 min at 56 ºC, and 2 min at 70 ºC for extension, with one step of 5 min at 98 °C for initial denaturation and a final step of 7 min at 72 ºC for final extension. Dimethyl sulfoxide 2 % (DMSO) was used in the PCR mix protocol (GoTaq DNA Polymerase, Promega, Madison, Wisconsin) to optimize DNA amplification. PCR products were purified with a 96-well multiscreen HV plate (Millipore, Billerica, Massachusetts) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, the Netherlands). Fragments were sequenced with an ABI Prism 3700 genetic analyzer (Applied Biosystems, Foster City, California). Sequences were edited and contigs assembled with SeqMan from the LaserGene package (8.0, DNAstar Inc., Madison, Wisconsin). Fungal and host taxa were identified by running BLAST queries against GenBank and against the sequences generated by the authors. All unique sequences were submitted to GenBank (Table 1).

Phylogenetic analyses

ITS and LSU sequences from *Austroboletus* and *Fistulinella* species were retrieved from GenBank (Suppl. Table 1) for phylogenetic analysis. Additional sequences from other representative species of Boletaceae from Guyana or belonging to genera with a pink hymenophore, such as *Zangia* and *Tylopilus*, also were included (Suppl. Table

1). *Russula* sp. and *Clavulina amazonensis* were selected as outgroup sequences. Sequences were aligned and phylogenetic analyses were performed in MEGA 5.2 (Tamura et al. 2011). The optimal model of sequence evolution determined in MEGA 5.2 was the Kimura-2 model for LSU and the Tamura-3 parameter for ITS. Both models with gamma distribution were used to perform a maximum likelihood (ML) analysis, including a bootstrap test with 1000 replications.

Results

Molecular identification

ITS BLASTn queries of the Colombian specimens of *Austroboletus* and *Fistulinella* indicated the highest matches with species of *Austroboletus* and *Fistulinella,* respectively, but none of the searches resulted in more than 95 % similarity. Unfortunately, both genera are not yet well represented in the GenBank database. Only four *Austroboletus* species were represented with data on ITS and 11 on LSU and only one *Fistulinella* species with ITS data and three with LSU sequences. No sequences of *F. campinaranae* were present. The ITS and LSU sequences of *A. amazonicus* was most similar to those of *A. rostrupii* (92 % query cover, 86 % similarity for ITS, 100 % query cover, 95 % similarity for LSU), followed by those of *A. novae-zelandiae* (67 % query cover, 91 % identity for ITS, 100 % query cover, 93 % identity) and *A. gracilis* (93 % query cover, 92 % identity for LSU). Therefore these BLAST results corroborated the placement of the new species in genus *Austroboletus* based on morphological grounds. With respect to *Fistulinella* the highest similarity values for the ITS and LSU sequences were found with those of *Fistulinella gloeocarpa* (86 and 85 % similarity, respectively). The ITS from one out of 65 root tips showed a match with that of *F. gloeocarpa* (85 % identity, 33 % cover). Using TrnL gene sequence analysis the host tree belonged to *P. tropenbosii* (99 % query cover and 99 % identity against *P. tropenbosii*, BLAST TrnL accession number KF878351).

Phylogenetic analyses

Our analysis showed a well-supported position of *A. amazonicus* (bootstrap 100 for LSU) within a clade that includes most of the *Austroboletus* species present in the analysis (bootstrap 70 for LSU) (Fig. 1). The *Austroboletus* clade included a sequence of a representative specimen of the type species *Austroboletus dictyotus*, but this was not based on the voucher of the type specimen. The closest related species to *A. amazonicus* were *A. rostrupii* described from Guyana (bootstrap 99 for ITS) (Fig. 2) and *A. mucosus* (bootstrap 99 for LSU) (Fig. 1). The phylogenetic relationship based on the ITS sequence did not show a clear grouping for the *Austroboletus* or the *Fistulinella* species. The ITS region is known to be highly variable in Boletales and was used here only to show the closest related species. *F. campinaranae* var. *scrobiculata* is closely related to *F. cinereoalba*, a species described from Guyana (bootstrap 98 for LSU). The ITS branch that included the Colombian *Fistulinella* species consisted of

species belonging to of genera like *Zangia*, *Xerocumus,* and *Boletellus* (Fig. 2). The Uncultured 511root isolated from *P. tropenbosii* in the MC (Chapter 3) is similar to that of *F. gloeocarpa* (bootstrap 98 for ITS), a species that has not been observed in Colombia.

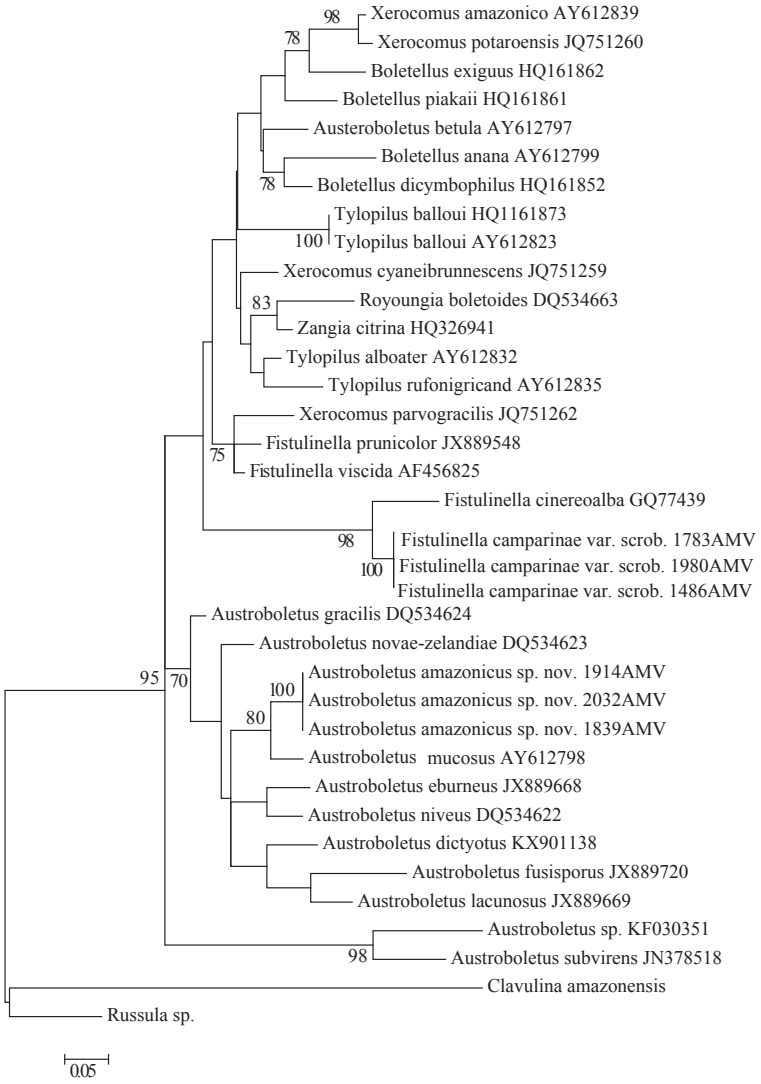


Figure 1. Phylogenetic maximum likelihood analysis of sequences of the LSU rDNA region from representatives of *Fistulinella* and *Austroboletus* species and from other representatives of Boletales. Significant bootstrap values (>70) are indicated at the branches (1000 replications). *Clavulina amazonensis* and *Russula* sp. were used as out-group.

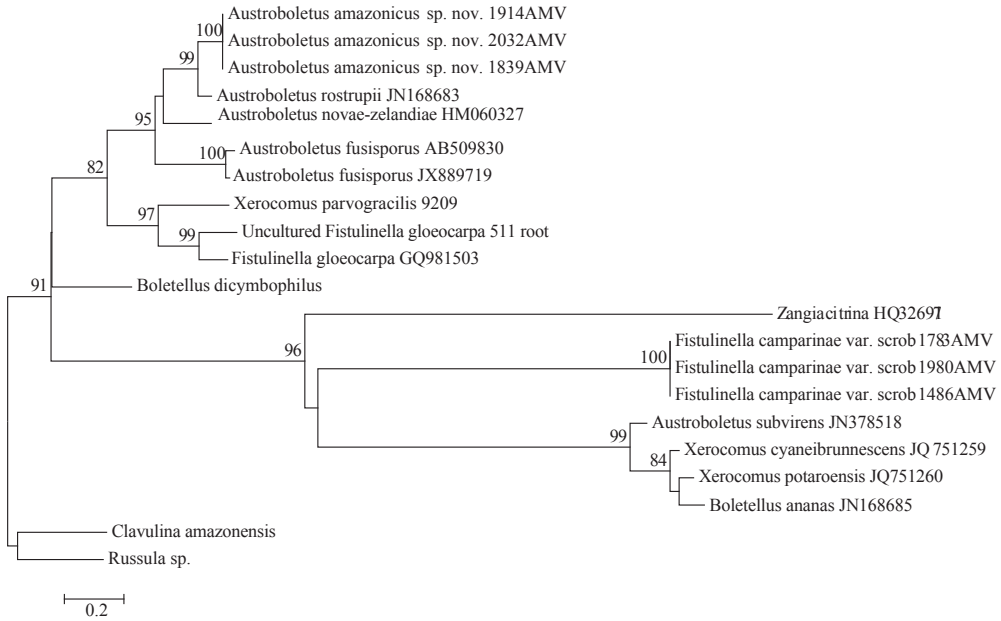


Figure 2. Phylogenetic maximum likelihood analysis of sequences of the ITS region from representatives of *Fistulinella* and *Austroboletus* and from other representatives of Boletales. Significant bootstrap values (>70) are indicated at the branches (1000 replications). *Clavulina amazonensis* and *Russula* sp. were used as out-group.

Taxonomy

Austroboletus amazonicus A.M. Vasco-Pal. & C. López-Quint., sp. nov. (Figs 3, 4). MycoBank MB805436, *Holotype: 2032 AMV (*HUA).
Etymology: Named after the Amazon region, where the specimens were found.

Pileus 5-15 mm broad, conical when young, becoming campanulate, with an acute umbo; surface dry, woolly to finely fibrillose, beige to pale yellow (4A2, 4B3) at the margin and pale pink or orange grayish (5A2, 5B3) at the center, with large, grayish to whitish (4A1, 4A2), pyramidal and fibrillose scales that are lifted at the tips and cover the surface; scales extending to the partial veil and stipe initially, at maturity concentrated over the center; marginal veil entire and clasping the stipe, later separating into fibrillose appendiculate remnants at the margin of the pileus and the stipe surface, beige to whitish; context less than 0.3 cm thick, solid, white, and not changing when exposed to air (Fig. 4A-D). Hymenophore poroid-tubulose; tubes up to 2.5 mm long, depressed around the stipe, white when young and turning pale rose to pale red (7A3, 9A3) when mature; pores concolorous with the tubes, round or angular, 2-3 pores per mm. Stipe 1.8-50 × 1.5 mm, central, subcylindrical, tapering slightly toward apex to 1 mm wide; surface whitish to pinkish white (4A2, 5A2), cottony, lacunose, with whitish fibrillose scales some like remnants of the veil. Partial

veil white, fibrillose, not forming a conspicuous ring but breaking into fibrillose appendages and leaving remnants at the edge of the pileus or over the surface of the stipe. Basal mycelium whitish, almost absent to well developed; white, slender hyphal cords are often present. KOH and NH_4OH macrochemical reactions were negative on the pileus, stipe and context. Odor and flavor indistinctive.

Basidiospores (10-) 12-14 (-18) × (4-) 6-8 µm, Q range = 13.6 × 6.9 (Q mean = 1.8, *n* = 40), subfusiform to amygdaliform, slightly angular; surface ornamented with peg-like cylindrical warts up to 1 µm tall over the central region, becoming shorter to nearly smooth toward the both poles, yellow in KOH, inamyloid, non-metachromatic, uniguttulate; hilar appendage 0.1-0.3 µm long; spore deposit not obtained (Fig. 3A, 4E). Basidia 30-46 × 12-18 µm, clavate to sphaeropedunculate, thin-walled, hyaline, inamyloid, occasionally with granular content, with four sterigmata, 4-5 µm long (Fig. 3B). Pleurocystidia 54-67 × 8-11 µm, cylindrical to subventricose, projecting 20-30 µm above hymenial palisade; thin-walled, occasionally with one transverse septum in upper part (Fig. 3C); hyaline, inamyloid. Hymenophoral trama divergent from a mediostratum, 30-60 µm wide, gelatinized, formed by thin-walled hyphae up to 5 µm wide, hyaline to slightly colored, inamyloid; lateral strata composed by hyaline, cylindrical to slightly inflated hyphae up to 14 µm, subcompact to loosely disposed in an oblique manner; subhymenium cellular, composed of subglobose cells forming a palisade and giving rise to the hymenial elements. Pileipellis a mixed cutis composed of slightly interwoven repent hyphae that are 3-8 (-12) µm wide, septate, thin-walled and hyaline, inamyloid and with obtuse tips and sparse tuffs of erect to suberect long peg-like structures. Stipitipellis similar to pileipellis. Clamp connections absent.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS: Municipio de Leticia. Area de Reserva Forestal El Zafire, 4°0′S, 69°53′W, 200-300 m, on soil, litter at the base of trees and palms in tropical rain forests dominated by *P. tropenbosii*; Megaparcela, 10 Jan 2012, *2032 AMV* (HOLOTYPE HUA, 186168), ITS GenBank KF937309, LSU GenBank KF714510; Megaparcela, 17 Mar 2012, *1838 AMV* (HUA); Megaparcela, 17 Mar 2012, *1839 AMV* (HUA), GenBank ITS KF937307, LSU GenBank KF714508. DEPARTMENT AMAZONAS: Corregimiento de Puerto Santander, Peña Roja, 0°34′S, 79°08′W, 200-300 m, on soil, litter or at the base of trees and palms in tropical rainforests dominated by *P. tropenbosii*; Tropenbos plot, 2 Jun 2002, *Lopez-Q. 32* (HUA); Tropenbos plot, 2 Mar 2002 *Lopez-Q. 102* (HUA); Tropenbos plot, Feb 1999, *Franco-Mol. 1706* (HUA); 3 km East of Peña Roja houses, 23 Jun 2001, *1348 AMV* (HUA); 2 km East of Peña Roja houses, on forests dominated by *P. tropenbosii*, 27 Jun 2001, *1469 AMV* (HUA); 1 km East of Peña Roja houses on a forest dominated by *P. tropenbosii*, 1 Aug 2001, *1514 AMV* (HUA); Tropenbos plot, 6 Jul 2001, *1914 AMV* (HUA), ITS GenBank KF937308, LSU GenBank KF714509.

Habit, habitat and distribution: Solitary on litter and root mat of the forest floor in stands dominated by *P. tropenbosii* in Colombian Amazonia. Known from ZBS, the

type locality, and elsewhere 470 km north, in the Peña Roja region.

Other specimens examined: Austroboletus subflavidus (Murrill) Wolfe: COLOMBIA. DEPARTMENT NARIÑO: Pasto, "La Josefina", km 17 on the road from Pasto to Chachagüí, in *Quercus humboldti* forests, 20 Nov 1988, *R. Halling 6110* (NY). *Austroboletus rostrupii* (Syd. & P. Syd.) E. Horak: GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, 710-750 m; in *Dicymbe* plot, 3, 5 May 2001, *Henkel 8032* (NY).

Commentary: *Austroboletus amazonicus* can be easily distinguished from other tropical species of *Austroboletus* by its dry basidioma with colors ranging from pale yellow to pale pink, with a woolly pileus covered by large triangular scales. Young specimens possess a fibrillose whitish partial veil, which remains as fibrillose appendages at the pileus margin and the stipe (Figs 3A-C). Micromorphologically *A. amazonicus* is characterized by subfusiform to amygdaliform basidiospores that are slightly angular and ornamented with peg-like cylindrical warts that are up to 1 μm tall at the middle and becoming shorter toward the apex of the basidiospores, and by septate cystidia (Figs 4D-F).

Other Neotropical species of *Austroboletus* that occur in lowland forests can be differentiated from *A. amazonicus* by the color of their basidiomatas. *Austroboletus olivaceus* has olive tones while *A. festivus*, *A. mucosus* and *A. rionegrensis* are red-brown. The phylogenetically closest species, *A. rostrupii* (Figs 1, 2) differs from *A. amazonicus* by its smooth pileus covered by a glutinous pellicle (Fulgenzi et al. 2010; Wolfe 1979). The ornamentation of the basidiospores is a useful character to distinguish the Neotropical species as well. The basidiospores of *A. olivaceus* appear smooth (Wolfe et al. 1988) (Figs 24-27) and those of *A. festivus* are finely reticulate (Fulgenzi et al. 2010). The basidiospore ornamentation of *A. rionegrensis* is similar to that of *A. amazonicus*, but the basidiospores of the former are bigger, namely 14.5-17.7 (-19.5) × 7-9 (-10.4) μm (Singer et al. 1983; Wolfe et al. 1988). Septate cystidia occur in the Neotropical species *A. amazonicus, A. graciliaffinis,* and *A. rostrupii,* but these species differ by the pileus color and pileipellis structure. Moreover *A. graciliaffinis* possesses setoid hymenial cystidia (Wolfe et al. 1988) that are not present in the other *Austroboletus* species.

Fistulinella campinaranae var. scrobiculata Singer 1978 (Figs 3, 4).

Pileus 10-40 mm broad, hemispherical to convex, fleshy; surface viscid, finely rugulose, pruinose mainly at the center, grayish brown, dark brown or paler (6D2-4-5, 7E4, 5C4-5) toward the margin, overall brownish (5B5, 5C5) with age, on a white to beige background; cuticle easily removable from the context; margin entire, slightly incurved and embracing the stipe when young but rapidly separating into membranous appendages forming a fugacious whitish ring (Fig. 4H). Context fleshy, up to 3 mm

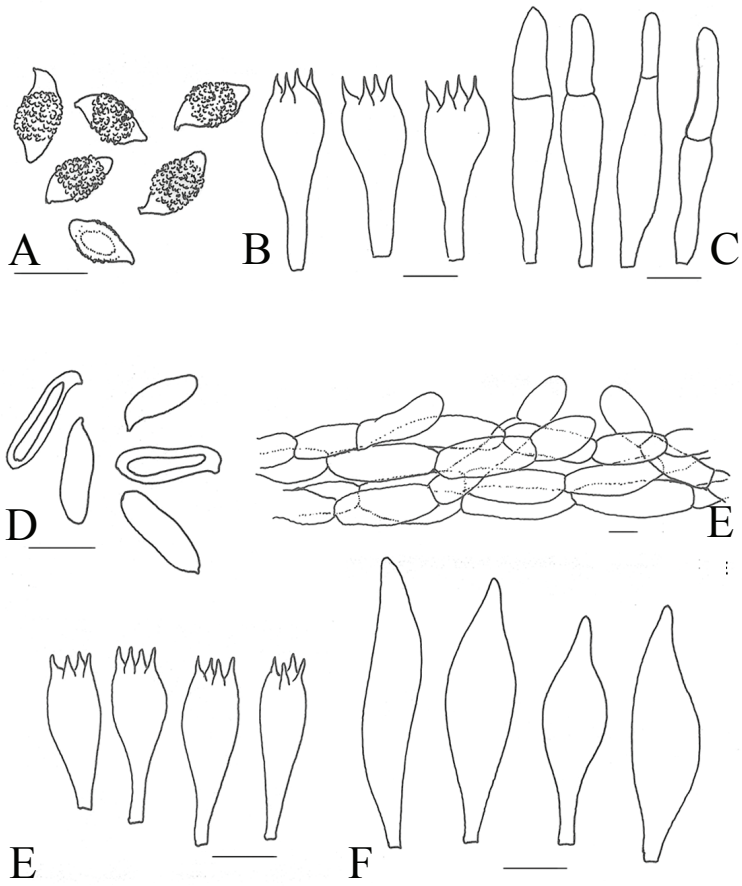


Figure 3. Microscopical structures of *Austroboletus amazonicus* (A-C) and *Fistulinella campinaranae* var. *scrobiculata* (D-G) showing spores (A), basidia (B), pleurocystidia (C), spores (D), pileipellis (E), basidia (F) and pleurocystidia (G). Bar for microstructures = 10 µm.

thick, whitish, not changing when exposed to air. Hymenophore poroid-tubulose; tubes easily detached from the context; depressed around stipe, up to 5-12 mm long, white when young, pinkish when mature (7A2, 11A2); pores concolorous with the tubes, 1-3 per mm, round to angular. Stipe 40-80 × 2-4 mm diam., central to slightly eccentric, cylindrical to subequal; surface white, fibrillose, with fine erect scales embedded in a thick gelatinous pellicle (Fig. 4I). Basal mycelium white, cottony, and slender hyphal cords often present. Macrochemical reactions in KOH and NH_4OH absent. Odor mildly fungoid; flavor indistinctive. Basidiospores (12-) 13-15 (-17) × (3-) 4-5 µm, Q range = 14.2 × 4.03, (Q mean = 3.3, *n* = 70), subcylindrical, elongate fusoid to subfusiform, pale yellowish brown, smooth-walled with one or two guttulaes, with a suprahilar depression, dextrinoid, not metachromatic; deposit not obtained (Fig. 3D-G, 4J).

Figure 4. Morphology of *Austroboletus amazonicus* (A-G) and *Fistulinella campinaranae* var. *scrobiculata* (H-J), detail veil and pileus (A-D), basidiospores (E-G), basidiomata (H-I), and basidiospores. Bar for microstructures = 1 µm.

Specimens examined: COLOMBIA. DEPARTAMENTO AMAZONAS: Corregimiento de Puerto Santander, Peña Roja, 0°34′S, 79°08′W, 200-300 m, on litter in terra-firme forests dominated by *P. tropenbosii*, 1 Jul 2001, *330 AMV* (HUA); 23 Jun 2001, *1328 AMV* (HUA); 1 km East of Peña Roja houses, 23 Jun 2001, *1354 AMV* (HUA); 1 km East of Peña Roja houses, 25 Jun 2001, *1356 AMV (*HUA); 1 km East of Peña Roja houses, 27 Jul 2001, *1470 AMV* (HUA); Tropenbos plot, 31 Jul 2001, *1486 AMV* (HUA); Tropenbos plot, 1 Aug 2001, *1489 AMV* (HUA); 3 km East of Peña Roja houses, 2 Aug 2001, *1539 AMV* (HUA); 1 km East of Peña Roja houses, 2 Sep 2001, *1783 AMV* (HUA), ITS GenBank KJ195891, LSU GenBank KJ195892; 1 km East of Peña Roja houses, 5 Jul 2001, *1979 AMV* (HUA); 1 km East of Peña Roja houses, 17 Jan 2002, *1980 AMV* (HUA), ITS GenBank KF937331, LSU GenBank KF714520. DEPARTAMENTO AMAZONAS: Municipio de Leticia, meta river tropical rainforests dominated by *Pseudomonotes tropenbosii*, 00°54′S, 71°35′W, on soil, 28 Jun 2010, *1364 AMV* (HUA).

Other specimens examined: Fistulinella cinereoalba Fulgenzi & TW Henkel 2010. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, 710-750 m; vicinity of Potaro base camp, 17 Jun 2002, Henkel 8471 (ISOTYPE: NY).

Commentary: F. campinaranae var. *scrobiculata* is morphologically and molecularly (LSU rDNA) similar to *Fistulinella cinereoalba*, a species described from Guyana (Fulgenzi et al. 2010). Both species share a glutinous pileus, a glutinous and white stipe, and have similar basidiospores (Fulgenzi et al. 2010). The hymenophore of *F. campinaranae,* however, does not change when exposed to air and its cystidia are clavate, lageniform to fusoid, whereas the hymenophore of *F. cinereoalba* becomes brownish under pressure and its hymenial cystidia are aciculate to cylindrical (Fig. 4H). Singer and co-workers (1983) separated *F. campinaranae* into two varieties, *F. campinaranae* var. *scrobiculata* and *F. campinaranae* var. *campinaranae* due to differences in the pileus ornamentation. The specimens of *F. campinaranae* from Colombia have a distinct scrobiculate pileus, so they belong to var. *scrobiculata* (Fig. 4H). Both varieties of *F. campinaranae* differ from *F. mexicana* by having larger basidiospores without a germ pore and a more distinct pseudoamyloid reaction (Singer et al. 1983). Both varieties of *F. campinaranae* were reported by Singer from forests on white sandy soil (i.e. Caatinga and Campinarana vegetation) where they occurred on rotting wood, mostly on stumps, growing solitary or forming small groups of basidiomata that fruited early or late in the rainy season in the state of Amazonas in Brazil (Singer et al. 1983).

Discussion

This study reports two species of boletes with a pink hymenophore that frequently have been collected in tropical lowland forests dominated by the EcM dipterocarp *P. tropenbosii* in the Colombian Amazon region. *A. amazonicus* and *F. campinaranae* var. *scrobiculata* can be distinguished based on morphological characters and molecular rDNA sequences for the ITS and the LSU regions. Unfortunately, sequences from species belonging to these two genera are poorly represented in the GenBank database so a reliable phylogenetic inference of both species could not be made. We did not generate new sequences from other species of *Austroboletus* and *Fistulinella* because that was beyond the scope of our study. A phylogenetic analysis performed with the information available showed that *A. amazonicus* and *F. campinaranae* var. *scrobiculata* belong to the genera *Austroboletus* and *Fistulinella*, respectively, and that they are not similar to any of the species of these genera represented in GenBank. It is important to consider that genera within Boletales are well represented in this database (Binder & Hibbet 2006; Dentinger et al. 2010; Drehmel et al. 2008; Halling et al. 2012; Li et al. 2011) and the LSU rDNA has been shown to be useful for the delimitation of groups in Boletales (Neves et al. 2012). In our LSU rDNA phylogenetic analysis the *Fistulinella* species formed two groups. One included the Neotropical species *F. campinaranae* var. *scrobiculata* and *F. cinereoalba*, which shared a branch with species from other genera such as *Xerocomus*, *Tylopilus* and *Boletellus*. The second is an isolated and not well supported group that included the Australasian species *F. pruinicolor* and *F. gracilis.* In our analysis 9 out of 11 species of *Austroboletus* formed a well-supported group (bootstrap value 81), whereas 2 species occurred with low bootstrap values at another position in the tree. Based on these data it seems likely that the genera *Fistulinella* and *Austroboletus* are both polyphyletic.

Austroboletus amazonicus was found in 10 out of 18 visits to ZBS and MC, and a total of 13 basidiomata were collected. *F. campinaranae* var. *scrobiculata* was found only from the northern populations of *P. tropenbosii* in MC. Here 40 basidiomata were collected in 15 out of 18 visits. No basidioma of either species have been observed in our study outside the *P. tropenbosii* forests. A first evidence of the presence of *Fistulinella gloeocarpa* at the root tips of *P. tropenbosii* was detected when analyzing ITS data (Chapter 3), thus an ectotrophical relation with this tree species is strongly suggested.

Studies in Neotropical ecosystems revealed a high macrofungal diversity with many new species to be described (Henkel et al. 2012; López-Quintero et al. 2012; Smith et al. 2013). Species belonging to *Austroboletus* and *Fistulinella* have been found in tropical lowland rainforests. In Brazil three species of *Austroboletus* and four species of *Fistulinella* were collected by Singer and co-workers from WSFs near Manaus (Singer & Araujo 1979; Singer et al. 1983). Henkel and collaborators (2012)

described two species of *Austroboletus*, *A. rostrupii,* and *A. festivus*, and one species of *Fistulinella*, *F. cinereoalba,* in a *Dicymbe*-dominated forest in Guyana. *Austroboletus mucosus* also was reported from this area (Drehmel et al. 2008). Recent studies from forests dominated by *Pakaraimaea dipterocarpacea* and *Dicymbe jenmanii* (Fabaceae subfamily Caesalpinioideae) in Guyana revealed a diversity of 52 EcM species identified from root tips and basidiomata (Smith et al. 2013). *A. rostrupii* was detected from root tips and basidiomata but no *Fistulinella* species was found (Smith et al. 2013).

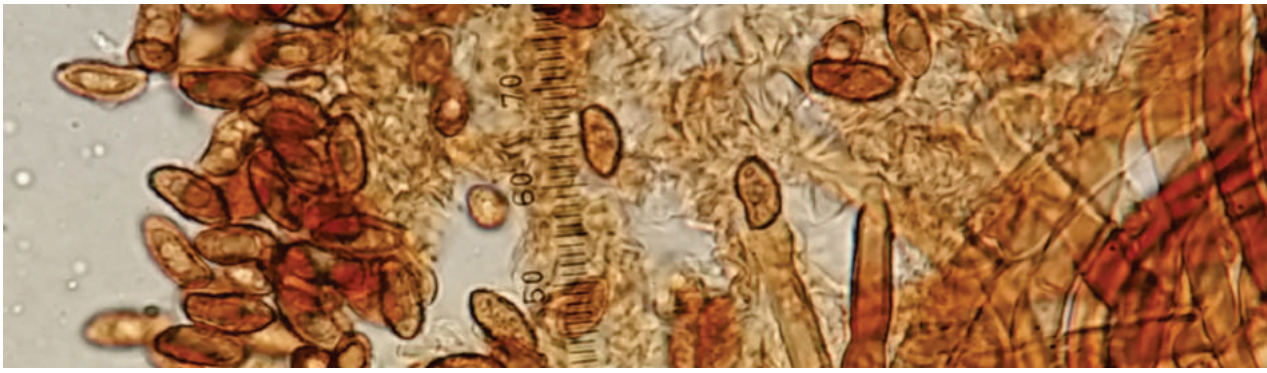
Data on the macrofungal diversity in Neotropical Dipterocarpaceae forests are scarce. It is likely that the fungal community present in EcM *P. tropenbosii* forests share fungal species with other ectotrophic lowland rainforests, like the ones from Guyana.

Supplementary Table 1. Material included in the phylogenetic analysis with their respective GenBank accession numbers for LSU and ITS sequences and the voucher numbers. "--" represents not voucher information or a sequence in GenBank

Species	Origin	Gen Bank accession #		Collection
		LSU	ITS	number
Austroboletus amazonicus	Colombia	KF714508	KF937307	AMV1839
Austroboletus amazonicus	Colombia	KF714509	KF937308	AMV1914
Austroboletus amazonicus	Colombia	KF714510	KF937309	AMV2032
Austroboletus betula	USA	AY612797	--	DD9852
Austroboletus dictyotus	China	JX901138	--	HKAS59804
Austroboletus eburneus	Australia	JX889668	--	REH9487
Austroboletus fusisporus	China	JX889720	JX889719	HKAS75207
Austroboletus fusisporus	Japan	--	AB509830	--
Austroboletus gracilis	USA	DQ534624	--	strain 112/96
Austroboletus lacunosus	Australia	JX889669	--	REH9146
Austroboletus mucosus	Guyana	AY612798	--	TH6300
Austroboletus niveus	New Zealand	DQ534622	--	--
Austroboletus novae-zelandiae	New Zealand	DQ534623	HM060327	--
Austroboletus rostrupii	Guyana	--	JN168683	TH8189
Austroboletus sp.	--	KF030351	--	--
Austroboletus subvirens	Japan	JN378518	JN378518	KPM-NC-0017836
Boletellus ananas	Guyana	AY612799	JN168685	TH6264
Boletellus dicymbophilus	Guyana	HQ161852	KC155373	TH8840
Boletellus exiguus	Guyana	HQ161862	--	TH8809
Boletellus piakaii	Guyana	HQ161861	--	TH8077
Clavulina amazonensis	Colombia	KF714513	KF937313	AMV1830
Fistulinella camparinae var. scrobiculata	Colombia	FUNCO083-13, KF714520	FUNCO083, KF937331	AMV1783, AMV1486
Fistulinella cinereoalba	Guyana	GQ477439	--	TH8471
Fistulinella gloeocarpa	Guyana	--	GQ981503	KM162946
Uncult. Fistulinella gloeocarpa	Colombia	--	KF878352	AMV511root
Fistulinella prunicolor	Australia	JX889648	--	REH9502
Fistulinella viscida	New Zealand	AF456826	--	--
Royoungia boletoides	Australia	DQ534663	GQ981525	ACW4137, KM125531
Russula sp .	Colombia	KF714534	KF937358	AMV1980
Tylopilus alboater	Durham Co. NC	AY612832		TH6941
Tylopilus balloui	Guyana, Japan	HQ161873, AY612823	TH8409, TH6385	--
Tylopilus rufonigricans	Guyana	AY612835	--	TH6376
Xerocomus amazonicus	Guyana	AY612839	--	TH6304
Xerocomus cyaneibrunnescens	Guyana	JQ751259	JQ751259	TH9197
Xerocomus parvogracilis	Guyana	JQ751262	JQ751261	TH9209
Xerocomus potaroensis	Guyana	JQ751260	JQ751260	TH9260
Zangia citrina	China	HQ326941	HQ326917	HKAS52684

CHAPTER 6

Synopsis of Coltricia and Coltriciella in the Neotropics, new species from Amazonian ecosystems in Colombia



Aída M. Vasco-Palacios, Gerardo Lucio Robledo, Leif Ryvarden

Abstract

In Amazonian ecosystems, species of *Coltricia* and *Coltriciella* have been found in terra-firme forests with the ectomycorrhizal (EcM) trees *Pseudomonotes tropenbosii* (Dipterocarpaceae) or in white-sand forests with the EcM tree *Dicymbe uaiparuensis* or *D. stipitata* (Fabaceae). *Coltriciella cylindrospora* A.M. Vasco-Pal. & Ryvarden, *Coltriciella minuta* A.M. Vasco-Pal. & Ryvarden and *Coltricia dependella* A.M. Vasco-Pal. & Ryvarden are described as new to science. DNA of the new species *Coltriciella minuta* was detected at the roots of *P. tropenbosii* and *Dicymbe corymbosa* suggesting that this species forms EcM with that tree. Macromorphological, micromorphological, and habitat data are provided for each of the new species. A key to the Neotropical species of *Coltricia* and *Coltriciella* species is provided in order to facilitate the morphology-based identification of these taxa in future studies.

Introduction

The genus *Coltricia*, described by S. F. Gray in 1821, is a cosmopolitan genus of Hymenochaetales (*Coltricia*-clade, Basidiomycota). The genus is characterized by terrestrial, poroid and usually centrally stipitate basidiocarps with a homogeneous context composed by a monomitic hyphal system lacking clamp connections. The basidiospores are ellipsoid to cylindrical, usually pigmented, and variably dextrinoid (Ryvarden 2004). *Coltriciella* Murrill 1904 is a closely related genus that differs by its verrucose spores, which is a unique character within Hymenochaetaceae (Larsson et al. 2006; Ryvarden 2004). The geographic distribution and taxonomic diversity of these genera may be underestimated since basidiocarps of many species (mainly in *Coltriciella*) are small and they are commonly found in not easy to observe habitats, like woody debris, the lower side of fallen trunks, or in trunk cavities, making them difficult to find (Aime et al. 2003). Currently 16 species of *Coltricia* and four of *Coltriciella* have been reported from Neotropical ecosystems (Baltazar & Gibertoni 2009; Baltazar et al. 2010; Bian & Dai 2015; IndexFungorum 2015; Ryvarden 2004; Valenzuela et al 2010). So far, seven species of *Coltricia* and four of *Coltriciella* were known from Colombia, mainly from the Amazon region (Table 1; Vasco-Palacios & Franco-Molano 2013; Chapters 2, 3). Here we presented data about the biodiversity of these genera in Colombia, including three new species that we described here.

Species of *Coltricia* and *Coltriciella* are usually terrestrial and associated with decayed wood, but the trophic relationships and nutritional strategy of most species are incompletely known. Recent studies demonstrated that based on both morphological and molecular data several species of the two genera are ectomycorrhizal (Tedersoo et al. 2007; 2010a; Chapter 3). Among Hymenochaetales, this character is known only for a few genera (Tedersoo et al. 2007; 2010a). Recent phylogenetic analyses using the Internal Transcribed Spacers 1 and 2 (ITS 1-2) and the D1/D2 domains of the

large subunit ribosomal DNA (LSU rDNA) placed *Coltricia* and *Coltriciella* together in a strongly supported clade (Larsson et al. 2006; Tedersoo et al. 2007). Based on these phylogenetic analyses, Larsson et al. (2006) concluded that the phylogenetic analysis gave no support for the distinction of the two genera. However, based on the limited available phylogenetic data (Bian & Dai 2014; Larsson et al. 2006; Tedersoo et al. 2007) and the phylogenetic information collected in this study we consider that merging both genera may be premature. Moreover, as a full taxonomic and phylogenetic revision is beyond the scope of this study we prefer to maintain both genera in this study. A further phylogenetic analysis using improved taxon sampling and sampling of additional genes is a prerequisite to solve this taxonomic dilemma.

The aim of this work is to review the diversity of the genera *Coltricia* and *Coltriciella* from Amazonian ecosystems in Colombia. Three new species, namely *Coltriciella cylindrospora* A.M. Vasco-Pal. & Ryvarden, *Coltriciella minuta* A.M. Vasco-Pal. & Ryvarden and *Coltricia dependella* A.M. Vasco-Pal. & Ryvarden were described from forests dominated by the ectomycorrhizal tree *Pseudomonotes tropenbosii* (Dipterocarpaceae). Species of *Coltricia*-*Coltriciella* have been also found from white-sand forests with the Fabaceae trees *Dicymbe stipitata* and *D. uaiparuensis.*

Materials and methods

Specimens of *Coltricia* were collected in Amazonian areas of Colombia that are dominated by the ectomycorrhizal trees *P. tropenbosii* (Dipterocarpaceae), *D. stipitata* and *D. uaiparuensis* (Fabaceae). The specimens have been deposited in the Herbario de la Universidad de Antioquia (HUA) and the Herbarium of the Natural Museum of Oslo (O). The specimens were photographed *in situ*, macromorphological characters were made from fresh material and macrochemical tests were performed as described (Largent 1986; Franco-Mol. et al. 2005). Basidiocarps were cut by hand for microscopic study. To this end, sections were mounted in 3 % KOH with 1 % aqueous phloxine solution or Melzer's reagent (Singer 1986). At least 10 and a maximum of 30 hyphae, setal hyphae, basidia and basidiospores were measured. Line drawings were made with a camera lucida. Herbarium designations followed Holmgren and Holmgren (1998).

DNA extraction and amplification from dried specimens followed Chapter 5. Generated sequences of the D1/D2 domains of the LSU rDNA and ITS 1+2 of rDNA were deposited in Genbank and are listed in Suppl. Material 1 together with the fungal taxa used in the molecular analysis. Additional D1/D2 and ITS sequences from *Coltricia* and *Coltriciella* and uncultured fungi isolated from mycorrhizal root tips of these taxa were selected from Genbank (Suppl. Material 1) for phylogenetic analysis. Sequences were aligned with Mafft (Katoh & Standley 2013). The aligned ITS and LSU were analysed using Maximum likelihood (ML). The best fit-model for ML was selected

based on the Akaike Information Criterion (AIC) calculated in MEGA6 (Tamura et al. 2013). Bootstrap (BT) analysis used 1000 replicates (Felsenstein 1985). Branches that received BT values ≥ 75 % were considered significantly supported.

Results and Discussion

Molecular phylogeny

Amplification of both ITS 1+2 regions and the D1/D2 regions of the LSU rDNA of *Coltricia* and *Coltriciella* was difficult using the primers listed. Unfortunately, it was not possible to obtain LSU sequences of the new species *Coltriciella cylindrospora* and *Coltricia dependella*. The LSU dataset included 34 sequences representing 25 species, 19 of them belonging to *Coltricia* and *Coltriciella* obtained from Genbank and from specimens collected by us (Fig. 1). The phylogenetic analysis of the LSU region placed *Coltriciella* nested within the genus *Coltricia*, but with weak bootstrap support (less 70 %) (Fig. 1). Species of *Coltriciella* formed a clade with moderate bootstrap support (76 %). Some species of *Coltricia* clustered into three well-supported clades, *C. cinnamomea* group 1, group 2 and representatives of the type species *C. perennis,* whereas other species occurred in single species lineages (Fig. 1). Within *Coltriciella*, a clade by *Cl. minuta* specimens was well-supported. The sequence voucher ECM710 isolated from a root-tip of *Dicymbe corymbosa* in Guyana corresponded to *Cl. minuta* 1925AMV with 99 % identity. This *Coltriciella* species was collected from forests with *P. tropenbosii* and *D. uaiparuensis* in Colombia and has also been detected on roots of *P. tropenbosii*, which suggests that it likely is an EcM symbiont of those plants. Specimens of *C. cinnamomea* and *Cl. dependens* occurred at different positions in the LSU tree (Fig. 1), suggesting that they may represent species complexes. These two species complexes are supposed to be cosmopolitan, but our analysis suggest that some specimens represent a new species. In a Bayesian analysis of the LSU rDNA, Tedersoo et al. (2007) found that *Coltricia* aff. *oblectans* (AM412246) occurred in a cluster with *C. cinnamomea*, whereas sequences from these two species clustered separately in our analysis (Fig. 1). The ITS fragment of the sequence of *Coltricia* aff. *oblectans* (AM412246) formed a clade with *Coltriciella minuta* in the ITS analysis.

The ITS analysis included sequences of the three new species from Colombia (Fig. 2). The analysis of the ITS showed a well-supported lineage of *Cl. minuta* with *C.* aff. *oblectans* (79 % bootstrap). *Cl. cylindrospora* grouped with *C. hamata* (< 70 % bootstrap) and *C. dependella* with *Cl. globosa* and *C. confluens* but with a not significant support (Fig. 2). The analysis also showed a well-supported clade with *Coltriciella* species (82 % bootstrap, Fig. 2). The new species *Cl. cylindrospora* was placed within *Coltricia* species (bootstrap less than 70%), despite its finely ornamented spores that characterize the genus *Coltriciella*. The majority of the *Coltricia* species formed non-supported single species lineages, except two sequences of *C. hamata* that formed a highly supported cluster (Fig. 2).

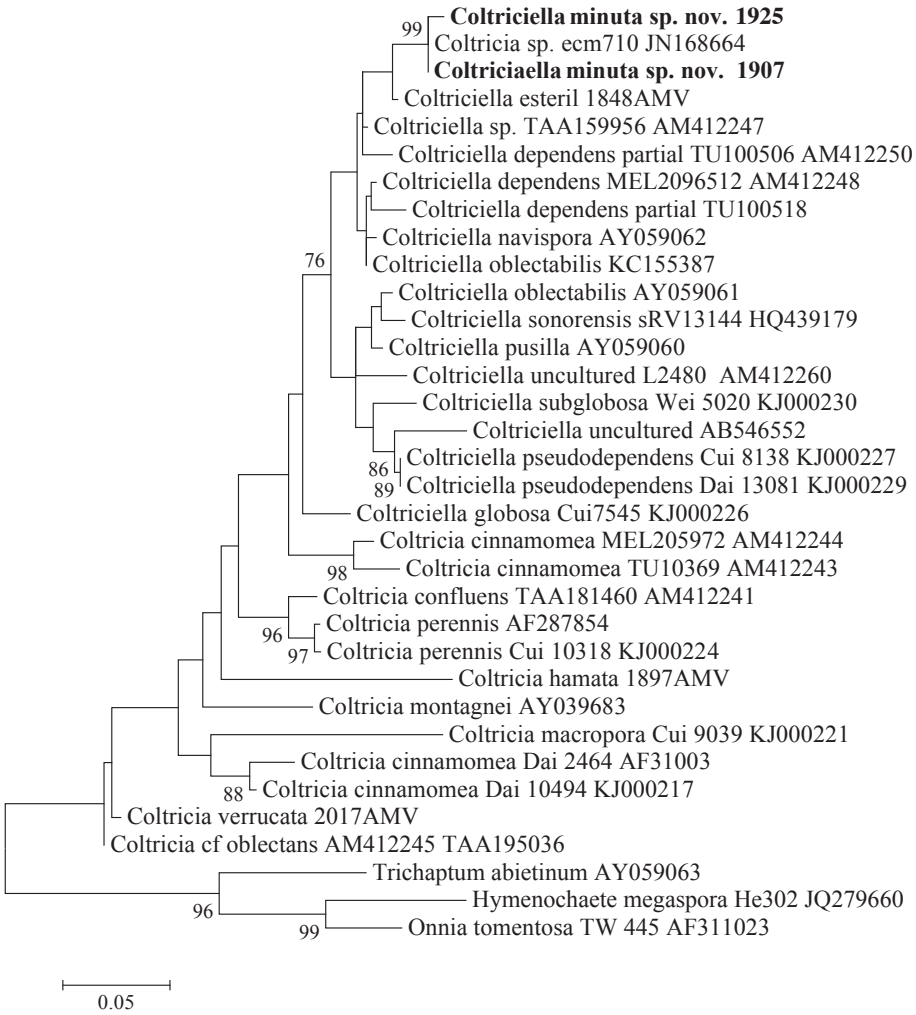


Figure 1. Phylogenetic analysis of *Coltricia* and *Coltriciella* species. Maximum likelihood tree based on the LSU rDNA region showing the closest relatives as present in Genbank. Bootstrap values (> 70 %) are indicated on the branches (1000 replications). The sequence from *Trichaptum abietinum*, *Onnia tomentosa* and *Hymenochaete* megaspore were used as outgroup. In bold sequences from the new species described in this work.

The ITS and LSU analysis presented here revealed that the species of *Coltriciella* and *Coltricia* largely formed different lineages. The *Coltriciella* clade was supported with ITS (82 % bootstrap) and with LSU (76% bootstrap) (Figs 1, 2). However, species with finely verrucose spores, such as *Cl. cylindrospora*, *Cl. oblectabilis*, and *Cl. globosa*, were located close to *Coltricia* species, but without a bootstrap support in the ITS analysis (Fig 2). A more complete analysis including more species and the inclusion of other regions of the genome like protein-coding genes (RPB1, RPB2, TEF1-alpha) is recommended to elucidate the phylogenetic relationship of the *Coltricia* and *Coltriciella* species. Note that such a study should ideally include sequence data

obtained from the type specimen of the type species of the genera considered. Because of the above considerations, we keep the genera *Coltricia* and *Coltriciella* in this work using the only distinctive morphological character, i.e. the finely verruculose spores present in *Coltriciella* species.

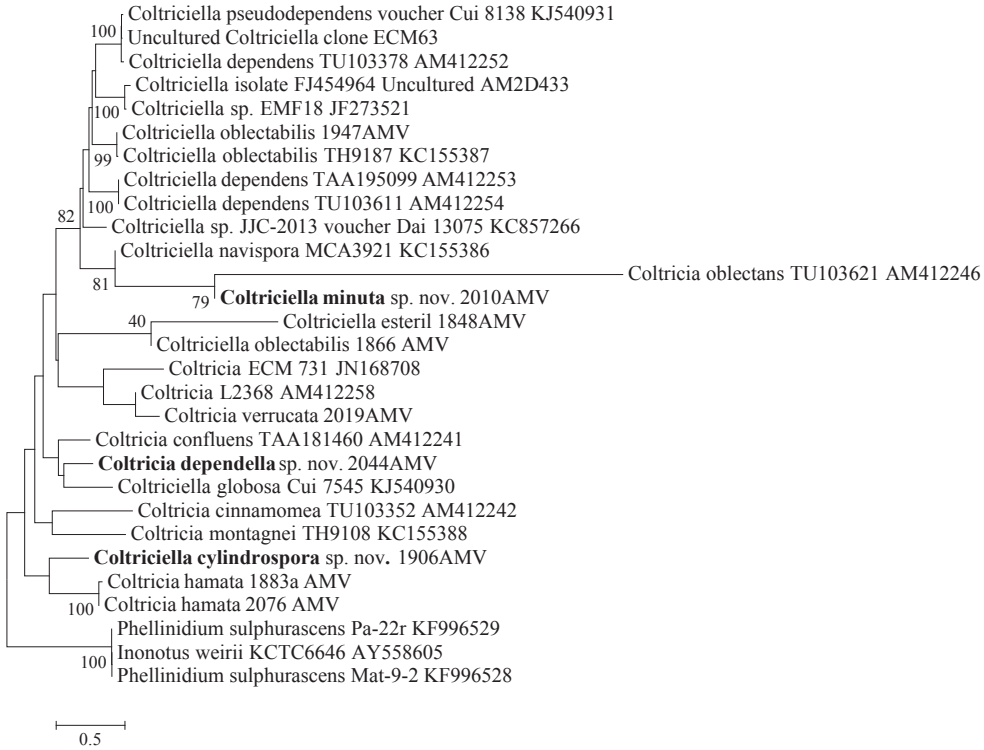


Figure 2. Phylogenetic analysis of *Coltricia* and *Coltriciella* species. Maximum likelihood tree based on ITS rDNA region showing the closest relatives as present in Genbank. Bootstrap values (> 70 %) are indicated on the branches (1000 replications). The sequence from *Phellinidium sulphurascens*, *Inonotus weirii* were used as outgroup. In bold, sequences from the new species described in this work.

Descriptions of the new species

Coltricia dependella A.M. Vasco-Pal. & Ryvarden nov. sp. (Figs 3 B, E)
Index Fung. 551094.
Etymology: *Dependella*: referring to its similarity to *Cl. dependens.*

Basidiocarps pendant from a distinct stipe. Pileus usually circular, 0.3-0.8 cm in diam., up to 1 mm thick, soft and brittle or fragile when dry, light in weight; surface glabrous, pale rusty brown. Hymenophore poroid; pore surface reddish brown, applanate, pores angular, 3-4 per mm and with fimbriate dissepiments; tubes up to 0.6 mm deep. Context rusty brown, soft and 0.4 mm deep. Stipe up to 0.3 cm long and 1-2 mm wide, dull, glabrous pale rusty brown. Basidiospores 5-6 µm in diam., subglobose, yellowish,

smooth and without reaction in Melzer's reagent. Basidia 10-15 x 5-7 µm, broadly clavate with simple septate base. Hyphal system monomitic; generative hyphae with simple septa, 5-8 µm wide, in context and trama thick-walled, yellow to rusty brown.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS; El Zafire Biological Station (ZBS), 4°00' S, 69°53' W, 27 km north of Leticia, 10 April 2013, on dead hard wood log, 2044 AMV, (HOLOTYPE HUA 197146; ISOTYPE O).

Habit, habitat and distribution: Gregarious in accumulated litter covering tree trunks and stumps in forests of *P. tropenbosii*. Known only from the type locality, but most likely overlooked due to its small size of the basidiocarps and habitat, i.e. hanging on the lower side of dead logs. It was collected two times from *P. tropenbosii* located in the biological station El Zafire in the south of Colombia (Chapter 3).

Commentary: The species has tiny, rusty brown, pendant basidiocarps. It can be confused with *C. dependens*, which has ornamented, larger spores.

Coltriciella cylindrospora A.M. Vasco-Pal. & Ryvarden nov. sp. (Figs 3 A, D)
Index Fung. 551093.
Etymology: *Cylindrospora* (Latin): referring to the cylindrical basidiospores.

Basidiocarps annual, laterally to eccentrically stipitate, consistence soft and flexible. Pileus more or less circular, slight depressed centrally, 1-2 cm in diam., up to 2 mm thick in centre, margin thin, slightly incised, deflexed when dry; pileus slightly shiny, glabrous and smooth, dark cinnamon to pale rusty brown, weakly concentrically zonate. Hymenophore poroid; pore surface greyish brown to rusty brown with age, pores angular, shallow, 1-2(-3) per mm; tubes concolorous, up to 1 mm deep. Context rusty brown, homogeneous, up to 250 µm thick. Stipe up to 3 cm long, 1-3 mm in diam., finely velutinate, cinnamon to rusty brown. Basidiospores 8-10 x 4 µm, cylindrical, golden yellow to light rusty brown, finely verrucose and somewhat thick-walled and inamyloid. Basidia barrel to rounded shaped, 12-15 x 4-8 µm, tetrasterigmatic with a simple septum at the base. Hyphal system monomitic; generative hyphae, golden yellow to light rusty brown, on the pileus 5-8 µm wide with distinct wide lumen, in the trama 3-6 µm, thick-walled, moderately branched, septate without clamp connections.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS, Pena Roja, 00°34' S, 79°08' W, Parcela Nico, 4 July 2001, on the ground, Leg. 1906 AMV (HOLOTYPE HUA 197134; ISOTYPE O).

Habit, habitat and distribution: Gregarious in accumulated litter covering tree trunks and stumps in forests with *P. tropenbosii*. Known only from Colombia, collected two times in a forest with *P. tropenbosii* in the Middle Colombian Amazon region from two different areas, but may occur also elsewhere in the Amazonas basin. Due to its small basidiocarps size it is easily overlooked.

Commentary: The cylindrical spores characterize this species and separate it from *Cl. oblectabilis*, which has distinctly ellipsoid spores. In a phylogenetical analysis of the D1/D2 domain of the LSU, the species was placed with *C. hamata* (but without boostrap support). The verrucose spores vs smooth spores, however, clearly differentiates between the two species (Fig. 2). The latter species has basidiocarps of 3-8 cm in diam. With a context that is up to 2 mm thick, has smooth and broadly ellipsoid basidiospores, and setal hyphae.

Coltriciella minuta A.M. Vasco-Pal. & Ryvarden nov. sp. (Figs 3 C,F)
Index Fung. No 551095.
Etymology: *Minuta* (Latin): referring to the small and tiny basidiocarps.

Basidiocarps annual, laterally to eccentrically stipitate, soft and flexible. Pileus more or less circular, slight depressed centrally, 1-3 cm in diam., up to 2 mm thick in centre, margin thin, slightly incised to dentate, deflexed when dry; pileus surface slightly shiny, first glabrous and smooth the becoming radially fibrous with tufted and raised hyphae in the centre, dark cinnamon to deep rusty brown, weakly concentrically zonate, a few zones becoming almost black. Hymenophore poroid; pore surface rusty brown, pores angular, shallow and with wavy walls, 1-2(-3) per mm; tubes concolorous, up to 1 mm deep. Context rusty brown, homogeneous, up to 250 µm thick. Stipe up to 3 cm long, 1-4 mm in diam., finely velutinate, cinnamon to rusty brown. Basidiospores 6-8 x 4-5 µm, broadly ellipsoid, golden yellow to light rusty brown, finely verrucose and somewhat thick-walled and without reaction in Melzer's reagent. Basidia barrel to round shaped, 12-15 x 4-8 µm, with four sterigmata and septate at the base. Hyphal system monomitic; with simple septate generative hyphae, golden yellow to light rusty brown, those on the pileus 5-8 µm wide with a distinct wide lumen, in the trama 3-6 µm wide, thick-walled, and moderately branched.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS, El Zafire, 4°00' S, 69°53' W, 27 km north of Leticia, 5 April 2013, on the ground, *Dicymbe uaiparuensis* forests, AMV. 2012, (HOLOTYPE HUA 196922; ISOTYPE in O). Pena Roja, Parcela Nico, 4 June 2001, on the ground, 1907AMV (HUA 197149, O).

Habit, habitat and distribution: Scattered to gregarious on the ground in hardwood forests with *D. uaiparuensis* and *P. tropenbosii*. *Cl. minuta* is only known from Colombia, but most likely it will be found elsewhere in the Amazon basin. Because of the small size of the basidiocarps, the species is easily overlooked. This species was commonly found in forests with *P. tropenbosii* (four specimens) and white-sand forests with *D. uaiparuensis* (four specimens). A D1/D2 domain of the LSU sequence of *Coltricia* sp. isolate from a root tip of *D. corymbosa* root in Guyana (*Coltricia* sp. ECM 710) showed a good blast score with *Cl. minuta* (99 % Identity, 99 Query cover). This suggests that *Cl. minuta* is an EcM symbiont of this Fabaceae tree and possibly of other species of *Dicymbe* and *P. tropenbosii* as well.

Commentary: The relative short ellipsoid spores characterize this species and separate it from *Cl. oblectabilis*, which has distinctly larger spores, i.e. 7-10 x 4-5 µm. Phylogenetically, the species is close to *C. navispora* (Fig. 2; 79 % identity-ITS). The two species can be distinguish by the basidiospores, being broadly ellipsoid and 6-8 x 4-5 µm in *Cl. minuta* when compared with the navicular spores, 10-12 x 4-5 µm, in *C. navispora*. It is possible that *Cl. minuta* may be present in *D. corymbosa* forests since its ITS sequences were similar to a sequence isolated from a root of this tree from Guyana (Fig. 2).

The genera *Coltricia* and *Coltriciella* in Colombia

Previously, three species of *Coltricia* were reported to occur in Colombia, whereas no species of *Coltriciella* was known from the country (Vasco-Palacios & Franco- Molano 2013). During our investigations of ectomycorrhizal fungi in Colombian Amazonia, specimens belonging to eight species of *Coltricia* and *Coltriciella* were found in forests dominated by *P. tropenbosii* or *Dicymbe* spp. (Table 1). Three corresponded to the new species *Cl. cylindrospora, Cl. minuta, and C. dependella* (Table 1). The number of species of *Coltricia* registered for the country is eight from 42 species described up today and three species of *Coltriciella* occur in Colombia from 15 species known (Baltazar & Gibertoni 2009; Baltazar et al. 2010; IndexFungorum 02-07-2015). In mountainous areas in Colombia only three species have been reported. *C. cinnamomea* and *C. focicola* were found in *Quercus humboldtii* (Fagaceae) forests, whereas *C. perennis* was found in a forest with an introduced *Pinus* spp. (Pinaceae) (Table 1). We consider *C. perennis* as an exotic species that was introduced in Colombia with plantations of *Pinus,* similar to what has been observed in Brazil (Gomes-Silva et al. 2009; Ryvarden & de Meijer 2002). The species of *Coltricia* and *Coltriciella* from the Amazon region in Colombia were found in forests with EcM trees. Specimens of *C. hamata*, *Cl. minuta* and *Cl. oblectabilis* were frequently found in forests with *P. tropenbosii* and in white-sand forests with *D. uaiparuensis*. Those fungal species are gregarious and *Cl. minuta* and *Cl. oblectabilis* grow on the ground or in accumulated litter that cover tree trunks and stumps in the forests. Species such as *Cl. navispora, Cl. sonorensis* and *C. velutina* are probably present in the country, but may have been overlooked because of their tiny sizes. The sequences of the D1/D2 domain of LSU rDNA sequence of the new species *Cl. minuta* gave a blast score with a fungal sequence (*Coltricia* sp. ECM 710) obtained from roots of *D. corymbosa* (98 % Identity, 99 Query cover). This match provides molecular evidence of the likely EcM lifestyle of *Cl. minuta*. Probably most of the species of *Coltricia* and *Coltriciella* collected from forests with EcM hosts are also symbionts with these plants. Further research should elucidate the diversity within the genera *Coltricia* and *Coltriciella* in Colombian ecosystems and in the Neotropics in general. This knowledge will contribute to our understanding of the distribution of *Coltricia* and to establish the EcM status of each

species of this genus. A morphological description of the *Coltricia* and *Coltriciella* species found from Colombia is provided below.

COLTRICIA

Coltricia cinnamomea (Jacq.) Murrill, Bull. Torrey Bot. Club 31:343, 1904. - *Boletus cinnamomeus* Jacq. Collect. Bot. 1:116, 1787. - *Polyporus cinnamomeus* (Jacq.) Pers., Mycol. Europ. 2:41, 1825.

Basidiocarps annual, more or less stipitate. *Pileus* circular, flat to infundibuliform, rarely above 3-4 cm in diam., in the tropics up to 12 cm in diam., up to 5 mm thick in centre, margin lobed, incised to entire, often fused with adjacent basidiocarps, sharp and mostly deflexed when dry; pileus surface finely velutinate, shiny to glossy, with numerous distinct to indistinct concentric zones, brown to deep reddish brown. *Hymenophore poroid*; pore surface reddish brown, pores thin-walled and angular, 2-4 per mm. *Context* thin, up to 1 mm thick, fibrous and rusty to reddish brown; tube layer up to 2 mm thick, more or less concolorous with the pore surface. *Stipe* cylindrical to flattened, mostly expanded towards the base, finely velutinate, ochraceous rusty to deep reddish brown, up to 3-4 cm long and 2-6 mm in diam. Basidiospores 6.5-8 x 5-6 µm, oblong to broadly ellipsoid, smooth, thin to distinctly thick-walled, golden yellow, cyanophilous, weakly dextrinoid in Melzer's reagent. Basidia clavate, 2- to 4-sterigmate, 18-30 x 5-7 µm, simple-septate at the base. Hyphal system monomitic; generative hyphae with simple septa, at first thin-walled and hyaline (best seen in the subhymenium), later more thick-walled and golden to light rusty brown, septation frequent in hymenium and subhymenium, more scattered in the context where the hyphae are longer and straighter, not branched to the same degree as in the hymenium, branching at right or wide angles, in the hymenium 2-5 µm in diam., in the context of pileus and stipe up to 10 µm in diam. and sometimes very thick-walled. Setae or other sterile hymenial elements absent.

Habit, habitat and distribution: Solitary to gregarious on the ground in hardwood or mixed forests, rarely in coniferous ones. Apparently mycorrhizal, at least facultatively, and possibly also saprobic (Tedersoo et al. 2007). It has been collected from white-sand forests in Brazil. Cosmopolitan species. In Colombia it has been reported from mountain forests dominated by *Quercus humboldtii*.

Commentary: The species is rather easy to recognize by its shiny deep warm colours, its terrestrial habitat and the large pores.

Coltricia focicola (Berk. & M.A. Curtis) Murrill, N. Am. Fl. 9:92, 1908. - *Polyporus focicola* Berk. & M.A. Curtis, J. Linn. Soc. Bot. 10:305, 1868.

Basidiocarps annual, stipitate. Pileus circular, centrally depressed, 1-4 cm in diam., margin often incised or lobed, tough and coriaceous when fresh, more brittle when dry, upper surface first rusty brown to cinnamon, becoming more greyish from the centre and with age brownish grey, tomentose, multizonate, in dry condition usually with radial wrinkled ridges. Hymenophore poroid; pore surface cinnamon to rusty brown, pores angular, thin-walled, in age pore-mouths becoming lacerated and more irregular, usually 1-2 per mm and not decurrent on the stipe, but often larger pores do occur near the stipe and then somewhat radially elongated, up to 2 mm in longest dimension; tubes rusty brown, up to 5 mm deep. Context cinnamon, 1-2 mm thick. Stipe cinnamon to rusty brown, finely tomentose to velvety, 1.5-5 cm long and 2-5 mm in diam. Basidiospores cylindrical to cylindrical-ellipsoid, hyaline to golden yellow, weakly dextrinoid, 8-11 x 4-5 µm. Basidia clavate, 18-23 x 7-8 µm, 2 to 4-sterigmate, simple septate at the base. Hyphal system monomitic with two types of generative hyphae, a) straight to sparingly branched, 4-8 µm wide, and with a wide lumen, yellow to pale rusty brown and with numerous simple septa, this type is predominant in the trama and the context, b) strongly branched and twisted, narrow, 2-4.5 µm wide, hyphae with few simple septa, hyaline to pale golden yellow and is most common in the stipe-context, but only scattered in the pileus context; the latter hyphae simulate binding hyphae and make the stipe harder than the context in the pileus. Setae none.

Habit, habitat and distribution: On the ground, usually on burnt soil, or around old campfire sites, also known from sandy soil and among dead wood debris and litter on the ground. *C. focicola* is apparently a rare fungus. In Central America the species is known from Costa Rica and in the north of South America from Colombia, where it has been collected from oak forests (*Quercus humboldtii*). Widespread in Eastern North America.

Commentary: This species is characterized by its large, robust basidiocarps with large pores and broadly ellipsoid to almost cylindrical and thick-walled basidiospores. Microscopically the cylindrical spores distinguish *C. focicola* from the other species described here. Like *C. pyrophila* it almost always grows on burnt wood or at fireplaces, but this species has much shorter basidiospores and is a tropical lowland rain forest species.

Coltricia hamata (Rom.) Ryvarden, Sv. Bot. Tidskr. 68:276, 1974. - *Pelloporus hamatus* Rom. K. Sv. Vetensk. Akad. Handl. 26:26, 1901 (Fig. 3 G).

Basidiocarps centrally stipitate with a circular and infundibuliform pileus. Pileus of 3-8 cm in diam., up to 2 mm thick in centre, rigid and brittle; surface rusty to snuff-brown, finely tomentose to adpressed velutinate in concentric zones, becoming glabrous and darker with age, margin thin and deflexed when dry. Hymenophore poroid; pore surface snuff-brown, pores entire, round to slightly angular, 2-3 mm. Context thin,

cinnamon to snuff-brown, up to 1 mm thick, in old specimens the upper part of the context becomes darker as the hyphae agglutinate and then the context appear subduplex in structure (strong lens), but this agglutination appears zone wise and not as a distinct dark line below an upper tomentum. Stipe more or less circular in section, often somewhat swollen towards the base, 5-13 cm high, 3-15 mm in diam., dark fulvous to snuff-brown, finely velutinate, in section weakly duplex with an outer softer and slightly spongy layer covering a more dense core, but no zones or lines separate the two layers, of which the inner is darker than the outer. Basidiospores broadly ellipsoid, smooth slightly thick-walled, light yellowish golden-brownish, 8-10 x 5.5-6.5 µm. Basidia not seen. Hyphal system monomitic; generative hyphae subhyaline to rusty brown with simple septa, on the pileus broad and with wide lumen, sparingly branched, 6-10 µm in diam. in the trama more thick-walled and more branched, the hyphae on the stipe are as on the pileus, those of the core as in the lower context and trama. Setal hyphae present in lower context and trama, partly embedded, partly projecting into the hymenium with a hooked tip, thick-walled and dark brown, 6-14 µm wide, up to 400 µm long.

Habit, habitat and distribution: Scattered to gregarious on the ground in lowland tropical rain forests, also in accumulated litter covering tree trunks and stumps in the forests. *C. hamata* is a South American species known from Brazil, British Guiana, Colombia and Venezuela. Most likely it occurs scattered throughout the rain forests in the Amazonian area. In Colombia it has been found four times in forests with the ectomycorrhizal host *P. tropenbosii* in the middle Caquetá region and twice in a white-sand forest with *D. uaiparuensis* in the south of the country.

Commentary: The shiny zonate pileus and, above all, the dark, hooked setal hyphae make this a distinctive species.

Coltricia perennis (L.) Murrill, J. Mycol. 9(2): 91, 1903. - Boletus perennis L. Sp. pl. 2: 1177, 1753.

Basidiocarps annual, stipitate. Pileus more or less circular, often confluent with adjacent specimens when growing in groups, up to 10 cm in diam. 2-5 mm thick al center, tough and coriaceous when fresh, brittle and hard when dry, upper surface velvety tomentose, pale cinnamon to deep brown becoming greyish with age, usually densely zonate, often with slightly different tomentum from one zone to another, reflecting changing growth conditions, margin thin and wavy, bent down in dry specimens. Hymenophore porose, pores angular, thin walled, 2-4 per mm, often slightly decurrent on the stipe and pores with age sometimes slightly incised or dentate, tubes up to 3 mm deep, cinnamon to rusty-brown, context 1-2 mm thick, rusty brown and dense, paler toward the pileus. Basidiospores ellipsoid to cylindric-ellipsoid, pale yellowish brown, smooth, slightly dextrinoid, 6-9 (-10) x 3.5-5 (-5.5) µm. Basidia clavate, 4-sterigmate, 15-25 x 5-7 µm, simple- septate at the base. Hyphal system monomitic with two types of generative

hyphae, both with simple septa, a) predominantly straight and sparingly branched, rusty brown 4-8 µm wide, in most sections with numerous septa, b) twisted, rusty yellowish and narrow wide and with few septa, these hyphae are especially common in the context and the central part of the stem; hyphae on pileus surface thick walled, erect, with distinctive dichotomous branching, pale yellowish brown, 3-8 µm in diam.

Habit, habitat and distribution: Gregarious, on the ground. This species is widely distributed in the conifer zone and rather common.

Commentary: In Colombia this species was reported from a *Pinus* plantation, but a revision of the taxonomical identification of the specimen is recommended. Due to the known distribution pattern of this species and because we could not study the Colombian specimen identified as C. perennis, we considered this species as a non tropical species and, hence, it was not included in the key.

Coltricia verrucata Aime, Henkel & Ryvarden, Mycologia 95:617, 2003.

Basidiocarps annual, centrally stipitate. Pileus circular, infundibuliform, 1.0-1.5 cm in diam., deep reddish brown, striate with bundles of hyphae, erect in the centre, more flattened towards the margin, each bundle up to 600 µm long, margin thin, partly incised, deflexed when dry. Hymenophore poroid; pore surface reddish brown, pores thin-walled, angular, 4-5 per mm. Context reddish brown, fibrous, up to 400 um thick. Stipe 10-30 x 1-2 mm, reddish brown and velutinous. Basidiospores ellipsoid, smooth, thick-walled, golden yellow and without reaction in Melzer's reagent, 5-6 µm in diam. Basidia barrel-shaped 18-20 x 7-10 µm with 4 sterigmata. Setae or other sterile hymenial elements absent. Hyphal system monomitic; generative hyphae with simple septa, thin-walled to thick-walled, golden to rusty brown, strongly pruinate by numerous small tubercles throughout the basidiocarp, (5-) 8-13 (-16) µm in diam.

Habit, habitat and distribution: Scattered to gregarious in accumulated litter covering tree trunks and stumps in forests with *D. stipitata* and *D. uaiparuensis*. *C. verrucata* was described from Guyana. In Colombia specimens of *D. verrucata* have been collected from white-sand forests dominated by *D. stipitata* or *D. uaiparuensis*. The species is probably widely distributed in the Amazon basin, but overlooked in many places because of its tiny size of the basidiocarps.

Commentary: The species is conspicuous by its strongly striate pileus with short erect hairs in the depressed centre but towards the margin with adpressed hairs that reminded combed hairs. The pruinate ornamentation of the hyphae is highly characteristic being present on almost each hypha, except those that are close to the basidia.

COLTRICIELLA

Coltriciella dependens (Berk. & M. A. Curtis: Imazeki) - *Polyporus dependens* Berk. & M. A. Curtis, Ann. Mag. Nat. Hist. Ser. 2, Vol. 12:431, 1853. (Fig. 3 I)

Basidiocarp pendant from a distinct stipe or more contracted vertex. Pileus usually circular, 0.3-2 cm wide, 2-8 mm thick, soft and brittle or fragile when dry, light in weight, pileus rusty-brown in variable shades, first finely velutinate, with age the adpressed tomentum becomes agglutinated and the pileus glabrous with some faint radial striate, margin vertical. Hymenophore poroid; pore surface rusty-brown, applanate, pores angular, 2-3 per mm; tubes up to 6 mm deep. Context rusty-brown, soft and 2-5 mm deep. Stipe up to 1 cm long and some mm wide. Basidiospores ellipsoid to slightly pip shaped, yellowish, finely verruculose, 7-10 x 4-6 µm. Hyphal system monomitic; generative hyphae with simple septa, 2-8 µm wide, in context and trama thick-walled and yellowish, in the subhymenium hyaline and narrower, moderately branched, often at right angles.

Habit, habitat and distribution: On deciduous trees, often on burnt wood. In the Amazon region it was found gregarious in accumulated litter covering tree trunks and stumps in forests with *P. tropenbosii* and *D. uaiparuensis*. Specimens of *Cl. dependens* have been found in Central America and USA. The species is widely distributed in tropical and subtropical zones, but and easily overlooked. In Colombia it was recently reported from the Amazon, collected from a *P. tropenbosii* forest in the Middle Colombian Amazon region and from a white-sand forest with *D. uaiparuensis* in the South of Colombia.

Commentary: The species is usually easy to recognize because of the small, rusty-brown, pendant basidiocarps and microscopically by the finely verruculose spores.

Coltriciella oblectabilis (Lloyd) Kotlaba, Pouzar & Ryvarden - *Polystictus oblectabilis* Lloyd, Lloyd Mycol. Writ. 3:164, 1912. - *Coltricia pseudocinnamomea* Burds., Mycologia 61:647, 1969. (Figs 3 J)

Basidiocarps annual, centrally stipitate. Pileus infundibuliform, 1-3 cm in diam., up to 2 mm thick in centre, margin thin, slightly lobed to incised, deflexed when dry; pileus slightly shiny, adpressed tomentose, dark cinnamon to rusty-brown, weakly concentrically zonate, no cuticle below the tomentum. Hymenophore poroid; pore surface greyish brown to rusty-brown with age, pores angular, shallow, 1-2(-3) per mm; tubes concolorous, up to 1 mm deep. Context rusty-brown, homogeneous, about 1 mm thick. Stipe up to 3 cm long, 1-3 mm in diam., finely velutinate, cinnamon to rusty-brown. Basidiospores oblong ellipsoid with rounded base and somewhat tapering at the other end, 7-10 x 4-5 µm. Some spores slightly oblique at the tapering end, golden-yellowish to light rusty-brown, finely verrucose and somewhat thick-walled. Hyphal system monomitic; with simple septate generative hyphae, golden-yellow to light rusty-brown, on the pileus 5-8 µm wide with distinct wide lumen, in the trama 3.5-6.5 µm with slightly thicker walls, moderately branched.

Habit, habitat and distribution: On the ground or scattered to gregarious in accumulated

litter covering tree trunks and stumps in ectotrophic forests with *P. tropenbosii*, *D. corymbosa* and *D. uaiparuensis*. *Cl. oblectabilis* is known from Brazil, Guyana, Colombia and South-Eastern United States. This species has been found frequently in forests with *P. tropenbosii* in the middle Caquetá region and El Zafire biological station, and also from white-sand forests with *D. uaiparuensis*. In Brazil, the species was reported from white-sand forests with *Aldina heterophylla.*

Commentary: As remarked by Burdsall (1969) the species is morphologically similar to *C. cinnamomea*, but can be separated by the conical ornamented spores, while those of *C. cinnamomea* are smooth and broadly ellipsoid

Key to Neotropical species of *Coltricia* and *Coltriciella*

Main Key

1. Spores ornamented	***Coltriciella***
1. Spores smooth	***Coltricia***

Key to *Coltriciella* species

1. Basidiocarps pendant	***Cl. dependens***
1. Basidiocarps stipitate	**2**
2. Basidiocarps centrally stipitate	***Cl. oblectabilis***
2. Basidiocarps lateral to eccentrically stipitate	**3**
3. Spores mainly navicular, longer than 10 µm	***Cl. navispora***
3. Spores ellipsoid, or oblong to cylindrical, shorter than10 µm	**4**
4. Spores broadly ellipsoid, 6-8 µm long	***Cl. minuta***
4. Spores oblong to cylindrical, 8-10 µm long	**5**
5. Spores navicular to oblong to cylindrical, pileus surface finely velutinate to slightly fibrillose	***Cl. sonorensis***
5. Spores cylindrical, pileus surface glabrous	***Cl. cylindrospora***

Key to *Coltricia* species

1. Setae or setal hyphae present	***C. hamata***
1. Setae or setal hyphae absent	**2**
2. Basidiospores longer than 8 µm	**3**
2. Basidiospores shorter than 8 µm	**9**

3. Hymenophore with large pores that split to form concentric lamellae, basidiospores 9-14 µm long. ***C. montagnei***
3. Hymenophore porose, pores not split, basidiospores 8-11 µm long. **4**

4. Basidiospores cylindrical, basidiocarps on burnt wood or fireplaces ***C. focicola***
4. Basidiospores, globose or ellipsoid, on the ground **5**

5. Basidiospores globose ***C. globispora***
5. Basidiospores ellipsoid **6**

6. Basidiospores 10-12 µm long ***C. fibrosa***
6. Basidiospores 8-10 µm long **7**

7. Basidiocarps laterally stipitate ***C. duportii***
7. Basidiocarps centrally stipitate **8**

8. Basidiocarps large, up to 120 mm wide, 2-4 pores per mm ***C. cinnamomea***
8. Basidiocarps small of 30 mm wide, 1 or less pores per mm ***C. permollis***

9. Basidia with an elongate narrow stipe like stalk, basidiocarps large, up to 100 mm wide ***C. cylindrospora***
9. Basidia not with an elongate narrow stipe, basidiocarps small **10**

10. Basidiospores globose to subglobose **11**
10. Basidiospores ellipsoid **13**

11.Basidiocarps pendant ***C. dependella***
11. Basidiocarps non pendant, centrally to laterally stipitate **12**

12. Pileus glabrous, spores non dextrinoid ***C. barbata***
12. Pileus velutinate to tomentose with erect hairs in centre, spores dextrinoid ***C. velutina***
13. Pores large 2-4 per mm, basidiocarps up to 85 mm diam. ***C. pyrophila***
13. Pores small 4-8 per mm, basidiocarps less than 50 mm diam. **14**

14. Context duplex, pores 6-8 per mm ***C. fonsecoensis***
14. Context homogenous, pores 4-6 per mm **15**

15. Hyphae strongly pruinate, basidiocarps less than 2 cm wide ***C. verrucata***
15. hyphae non pruinate, basidiocarps more than 2 cm wide ***C. stuckertiana***

Table 1. List of species of *Coltricia* from Colombia.

Taxa	Altitude m.a.s.l.	Ecosystem	Reference
Coltricia			
Coltricia barbata Ryvarden & de Meijer	100-200	PtF	Chapter 3
Coltricia cinnamomea (Jacq.) Murrill	100-200, 2430	OakF	Vasco-Palacios & Franco-Molano 2013
Coltricia dependella A.M. Vasco-Pal. & Ryvarden*	100-200	PtF	
Coltricia focicola (Berk. & M.A. Curtis) Murrill	2100-2600	OakF	Vasco-Palacios & Franco-Molano 2013
Coltricia hamata (Romell) Ryvarden	100-200	PtF and WSF	Chapter 2; Chapter 3
Coltricia perennis (L.) Murrill	2100-2430	Plantation of *Pinus* spp.	Henao-M LG. 1989.
Coltricia verrucata Aime, T.W. Henkel & Ryvarden	100-200	WSF and WSF *D. stipitata*	Chapter 2
Coltriciella			
Coltriciella cylindrospora A.M. Vasco-Pal. & Ryvarden *	100-200	PtF	Chapter 3
Coltriciella dependens (Berk. & M. A. Curtis) Imazeki	100-200	PtF	Chapter 3
Coltriciella minuta A.M. Vasco-Pal. & Ryvarden *	100-200	PtF and WSF	Chapter 3
Coltriciella oblectabilis (Lloyd) Ryvarden	100-200	PtF and WSF	Chapter 2; Chapter 3

Taxa with * corresponds to new species. In Ecosystem, PtF Corresponds with forests with the dipterocarp tree *P. tropenbosii;* WSF, corresponds with a white sand forests with *Dicymbe uaiparuensis*, and OakF corresponds with a forests with *Quercus humboldtii*

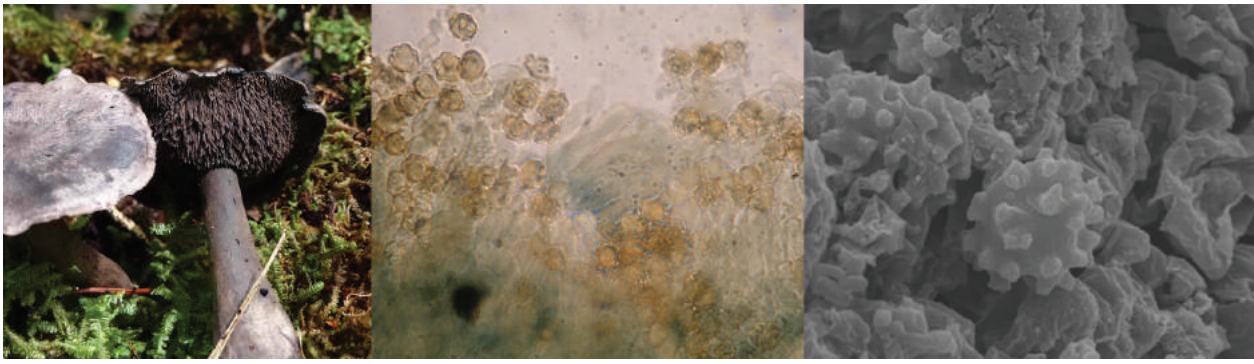
Figure 3. Morphology of fruiting bodies of *Coltriciella cylindrospora* sp. nov. (A), *Coltricia dependella* sp. nov. (B), *Cl. minuta* sp. nov. (C), basidiospores of *Cl. cylindrospora* sp. nov. (D), *C. dependella* sp. nov. (E) and *Cl. minuta* sp. nov. (F), morphology of fruiting bodies of *C. hamata* (G), *C. verrucata* (H), *Cl. dependens* (I) and *Cl. oblectabilis* (J).

Supplementary Material 1. List of specimens used in the phylogenetic analyses with species names and accession number to Genbank.

Coltricia barbata 1925AMV LSU; *Coltricia cinnamomea* KJ000217, AF31003, AM412243, AM412244 LSU, AM412242 ITS; *Coltricia confluens* KC152084 LSU, AM412241 ITS; *Coltriciella cylindrospora* 1906AMV ITS; *Coltricia dependella* 2044AMV ITS; *Coltricia* ECM731 JN168708 ITS; *Coltricia hamata* 1883aAMV, 2076AMV ITS; *Coltricia macropora* KJ000221 LSU; *Coltricia minuta* 1938AMV LSU; *Coltricia montagnei* AY039683, 1897AMV LSU, KC155388 ITS; *Coltricia oblectans* AM412245 LSU, AM412246 ITS; *Coltricia perennis* KJ000224, AF287854 LSU; Coltricia uncultured ECM710 JN168664 LSU; *Coltricia* uncultured L2368 AM412258 ITS; *Coltricia* isolate L2480 AM412260 LSU; *Coltricia verrucata* 2017AMV LSU, KT354689 ITS; *Coltriciella baoshanensis* KC857266 ITS; *Coltriciella dependens* AM412250, AM412251, AM412248 LSU, AM412252, AM412253, AM412254 ITS; *Coltriciella* esteril 1848AMV LSU, 1848AMV ITS; *Coltriciella globosa* KJ000226 LSU, KJ540930 ITS; *Coltriciella minuta* KT354691 ITS; *Coltriciella navispora* KC155386 ITS; *Coltriciella navisporus* AY059062 LSU; *Coltriciella oblectabilis* AY059061, KC155387 LSU, KC155387, 1947 AMV ITS; *Coltriciella pseudodependens* KJ000227, KJ000229 LSU, KJ540931 ITS; *Coltriciella pusilla* AY059060 LSU; *Coltriciella sonorensis* HQ439179 LSU; *Coltriciella* sp AM412247 LSU; *Coltriciella* sp. EMF18 EMF18JF273521 ITS; *Coltriciella subglobosa* KJ000230 LSU; *Coltriciella* uncultured AM2D433 FJ454964 ITS; *Coltriciella* uncultured ECM63 JQ991688 ITS; *Coltriciella* uncultured sp1 AB546552 LSU; *Hymenochaete megaspora* JQ279660 LSU; *Onnia tomentosa* AF311023 LSU; *Trichaptum abietinum* AY059063 LSU; uncultured Helotiales 114root 114root ITS; Uncultured Sordariales KJ827601 ITS.

CHAPTER 7

Sarcodon in the Neotropics: four new species from Colombia and a key to selected species



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Abstract

This work reports on four species of the ectomycorrhizal (EcM) tooth fungus genus *Sarcodon* (Bankeraceae, Thelephorales, Basidiomycota) recently discovered in the Colombian Amazon region. *Sarcodon colombiensis* sp. nov., *Sarcodon rufobrunneus* sp. nov., *Sarcodon pallidogriseus* sp. nov., and *Sarcodon bairdii* sp. nov. are described as new to science. These fungi occur in forests dominated by EcM trees of the genera *Pseudomonotes* (Dipterocarpaceae), *Dicymbe* (Fabaceae subfam. Caesalpinioideae) and *Aldina* (Fabaceae subfam. Papilionoideae). These records bring the number of *Sarcodon* species known from the Neotropics to 10, and demonstrate that, while being less diverse in the tropics relative to temperate and boreal regions, the genus has a wider distribution and putative host range than previously thought. Each of the new species possesses the accepted diagnostic characters for the genus. They have a pileate-stipitate stature, a dentate hymenophore, determinate basidiomata development, fleshy, non-zonate context, and brown, tuberculate basidiospores. Molecular phylogenetic analysis corroborated the generic placement of the species, and, in combination with morphological characters, confirmed that they are new to science. Macromorphological, micromorphological, habitat, and DNA sequence data from the nuclear rDNA internal transcribed spacer region (ITS) are provided for each of the new species. A key is provided that allows identification of all known Neotropical *Sarcodon* species and similar extralimital taxa.

Introduction

Grupe et al. (2015) summarized the current knowledge of the ectomycorrhizal (EcM) fungal genus *Sarcodon* Quél. ex P. Karst. (Bankeraceae, Thelephorales, Basidiomycota) in the Neotropics and described four new species from Belize, Guyana, and Puerto Rico. These new *Sarcodon* species were associated with a diverse assemblage of putative EcM broadleaf host tree species in the genera *Dicymbe* (Fabaceae subfam. Caesalpinioideae), *Pakaraimaea* (Dipterocarpaceae), and *Quercus* (Fagaceae), and brought the number of species known from the Neotropics to six. Neotropical species diversity for this largely Nearctic, conifer-associated genus is still low, given that > 91 *Sarcodon* species have been identified worldwide.

Recent macrofungal field studies in Amazonian Colombia have revealed a diverse set of putatively EcM fungi (e.g. Chapters 2, 3). Here we describe *Sarcodon colombiensis* sp. nov. from forests dominated by the EcM trees *Pseudomonotes tropenbosii* A.C. Londoño, E. Alvarez & Forero (Dipterocarpaceae) and *Sarcodon rufobrunneus* sp. nov. from forests dominated by the EcM trees *Dicymbe uaiparuensis* R.S. Cowan (Fabaceae subfam. Caesalpinioideae) and *Aldina* sp. (Fabaceae subfam. Papilionoideae), from El Zafire, Department of Amazonas. *Sarcodon pallidogriseus* sp. nov. and *Sarcodon*

bairdii sp. nov. are described from a white sand forest dominated by *Dicymbe stipitata* R.S. Cowan in the Middle Colombian Amazon region, Amazonas. These new species also constitute the first records of *Sarcodon* for Colombia (Vasco-Palacios & Franco-Molano 2013).

MATERIALS AND METHODS

Collections

Collections of *S. colombiensis* and *S. rufobrunneus* were obtained in 2012 from El Zafire in Dept. Amazonas, Colombia at 4˚00`69”S; 69˚53’97”W; elevation ~180–220 m, along a trail in a mixed forest dominated by *P. tropenbosii* (PtF) and white sand forests (WSFs) dominated by *D. uaiparuensis* and *Aldina* sp. Collections of *S. pallidogriseus* and *S. bairdii* were made in May 2001 from Resguardo de Monochoa, Comunidad de Chukiki, Puerto Santander, Dept. Amazonas, 0˚40’S; 72˚31’W; elevation ~150 m, in forests where the putative EcM host is *D. stipitata* in white sand forests (WSF).

Macroscopic features of freshly collected basidiomata were described in the field. Colors were described subjectively and coded according to Kornerup and Wanscher (1978), with color plates noted in parentheses. Collections of fresh basidiomata were dried using silica gel desiccant beads. For *S. colombiensis*, a minimal macromorphological description is provided and a macroscopic image is lacking, as it was collected while travelling from the i El Zafire Biological Station (ZBS) to the city of Leticia. Micromorphological features of dried specimens were examined with an Olympus BX51 microscope with light and phase contrast optics and 1000-fold maximum magnification. Separate mounts of fungal tissue were made in H_2O, 3 % potassium hydroxide (KOH), and Melzer’s solution. At least 20 individual basidiospores, basidia, and other structures were measured per collection. The dimension of the basidiospores included ornamentation. Range and mean quotients of basidiospore length divided by width (Q) were calculated. Outlying measurements observed in less than 5 % of the population are indicated in parentheses. Specimens were deposited at the University of Antioquia Herbarium and the Humboldt State University (Index Herbariorum: http://sweetgum.nybg.org/science/ih/).

DNA extraction, amplification, sequencing, and phylogenetic analyses

DNA extraction, polymerase chain reactions (PCR), cloning and sequencing followed the standard protocols of Gardes and Bruns (1993) as modified by Grupe et al. (2015). Bidirectional sequencing was performed with primers ITS1F and ITS4 by the University of Florida ICBR sequencing center (www.biotech.ufl.edu). Sequences were edited using CodonCode Aligner 3.5.7 (CodonCode Corp., Centerville, MA) and aligned using Mesquite V3.04 (Maddison & Maddison 2015).

New sequences were compiled and aligned with sequences from GenBank to

generate a final alignment of 882 characters. The phylogenetic analyses included 21 sequences of *Sarcodon* species with one sequence from a *Hydnellum* sp. (AF351871) (Thelephorales) as the outgroup. Maximum Parsimony (MP) analysis was completed with default settings in PAUP* 4.0 (Swofford 2003). A Maximum Likelihood (ML) search was run in RAxML (Stamatakis 2014) with the GTR+G model on the CIPRES Science Gateway (Miller et al. 2010). Support for phylogenetic relationships was assessed based on 500 bootstrap replicates in PAUP (for MP) and RAxML (for ML). The alignment is available at TreeBase at: http://purl.org/phylo/treebase/phylows/study/TB2:S18131.

Results and Discussion

BlastN queries and phylogenetic analysis

BlastN queries for ITS sequences of *S. colombiensis*, *S. rufobrunneus*, *S. pallidogriseus*, and *S. bairdii* produced best matches to species of *Sarcodon* but none exceeded 95 % similarity. The best ML tree (Fig. 1, likelihood score = -ln 5816.46) differed slightly from the topology of the MP tree (1168 steps) in that the placement of some of the *Sarcodon* species outside the *S. atroviridis* clade had little to no bootstrap support in the MP tree (data not shown). The phylogenetic analyses demonstrated that *S. colombiensis*, *S. rufobrunneus*, *S. pallidogriseus*, and *S. bairdii* are phylogenetically distinct from other recently described Neotropical *Sarcodon* species. Our analysis focused on the placement of *S. colombiensis*, *S. rufobrunneus*, *S. pallidogriseus*, and *S. bairdii* and could not resolve other phylogenetic relationships within the genus, nor did we address the position of *Sarcodon* within the Bankeraceae or Thelephorales. *S. colombiensis* had the closest identity with *S. pakaraimensis*, with the two species occurring in the well-supported clade A along with *S. rufobrunneus* and *S. umbilicatus* (Fig. 1). *Sarcodon bairdii* and *S. pallidogriseus* occurred at a basal position of clades A and B, with low confidence with respect to the actual position of these species (Fig. 1).

The ectomycorrhizal (EcM) tooth fungus genus *Sarcodon* is well represented in the Northern temperate Hemisphere and to a lesser extent in Paleotropical forests, with 91 species currently being described. However, this genus is poorly known from the lowland Neotropics. This situation is changing rapidly with the analysis of new collections from Guyana, Puerto Rico, Belize, and Colombia. *S. colombiensis*, *S. rufogriseus*, *S pallidogriseus* and *S. bairdii* are the first report of the genus in Colombia (Vasco-Palacios & Franco-Molano 2013) and brought the number of species known from the Neotropics to 10 (Baltazar & Gibertoni 2009; Grupe et al. 2015). One of these Neotropical species is *S. atroviride*. This species has been reported from several places in Brazil, including ecosystems with Fabaceae in the Amazon region (Baltazar & Gibertoni 2009; Komura et al. 2015; Singer et al. 1983). The four new species that are described here from Colombia were associated with a diverse set of putative EcM broadleaf host tree species in the genera *Dicymbe* (Fabaceae subfam. Caesalpinioideae)

and *Pseudomonotes* (Dipterocarpaceae). So far, *Sarcodon* is known to be associated with woody angiosperms and gymnosperms, particularly within Fagaceae and Pinaceae (Vizzini et al 2013). Together, these data indicate that *Sarcodon* has a much broader host range than previously described.

Each of the four new species is morphologically similar to, but distinguishable from, a group of previously described *Sarcodon* species characterized by their overall somber colors and KOH-soluble bluish green pigments (Maas Geesteranus 1971). These species include *S. atroviridis* (Morgan) Banker from temperate North America, Europe, and East Asia, *S. thwaitesii* (Berk. & Br.) Maas G. from the Asian tropics, and *S. bambusinus* (Baker & Dale) Maas G. from the Neotropics (Berkeley & Broome 1873; Baker & Dale 1951; Maas Geesteranus 1964, 1974a, 1975; Morgan 1895). Specimens putatively identified as *S. atroviridis* sensu lato were also recently reported from Brazil but without any molecular data (Komura et al. 2015; Magnago et al. 2015).

The recently described Neotropical species *S. pakaraimensis* A. Grupe & T.W. Henkel, *S. portoricensis* A. Grupe & T.J. Baroni, *S. quercophilus* A. Grupe & D.J. Lodge, and *S. umbilicatus* A. Grupe, T.J. Baroni & D.J. Lodge, also fall into this rather narrow morphological group of tropical species (Table 1, Grupe et al. 2015). Nonetheless, variation in key morphological characteristics corroborated by molecular phylogenetic analysis, supports that *S. colombiensis*, *S. rufobrunneus*, *S. pallidogriseus*, and *S. bairdii* are new species. *Sarcodon colombiensis* and *S. rufogriseus* were detected from root-tips of *P. tropenbosii*, thus confirming that both are forming EcM symbiosis with this dipterocarp tree (Chapter 3). Macromorphological, micromorphological, habitat, and ITS ribosomal DNA (rDNA) sequence data are provided for these fungal species. A key is provided that allows identification of all known Neotropical *Sarcodon* species and similar extralimital taxa.

Taxonomy

Sarcodon rufobrunneus A.M. Vasco-Pal. & A. Grupe, sp. nov. (Figs 2A-F)
MycoBank MB813077
Etymology: Rufus (Latin adj. A) = adjectival prefix indicating reddish, and -brunneus (Latin adj. A) = adjectival suffix indicating brown, referring to the reddish brown coloration of the fresh pileus.
Diagnosis: Sarcodon rufobrunneus differs from other species of *Sarcodon* in its combination of dark reddish brown (7F8-8F8), umbonate pileus with a smooth to fibrillose pileus surface, unchanging pileus trama, grayish brown (7F3-7F4) stipe with initially reddish brown trama, and unique ITS sequence.

Pileus conical when young, parabolic to plane with age, 12-70 mm broad, 2-3 mm thick, dark reddish brown (7F8-8F8), hygrophanous; surface smooth to fibrillose, umbonate; margin eroded; trama not staining upon exposure (Fig. 2A). Hymenophore

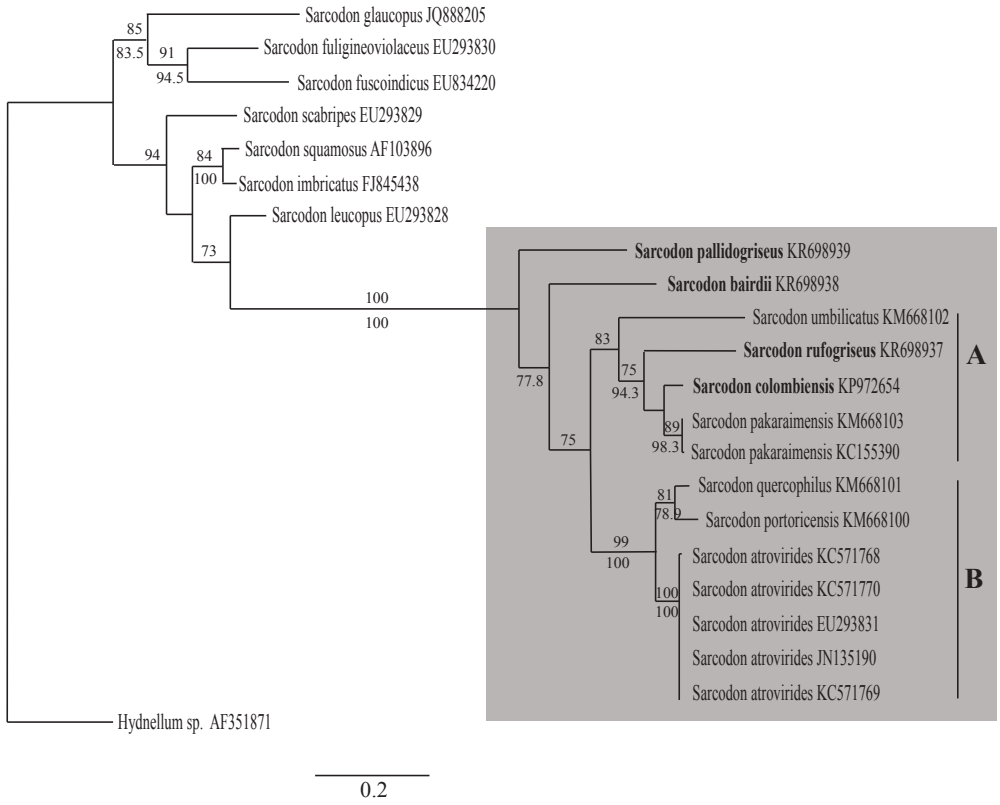

Figure 1. Maximum likelihood phylogram based on internal transcribed spacer (ITS) ribosomal DNA sequences of *Sarcodon* species from Colombia. Support values above the nodes are maximum likelihood bootstrap values. Support values below the nodes are maximum parsimony bootstrap values. Nodes with bootstrap support values < 70 are not shown. The gray box demarcates the *S. atroviridis* clade and the well-supported clade A and clade B are shown with thick black boxes. New species in bold.

dentate; teeth 2-4 mm long, apices sharply conical, concolorous with pileus, delicate, easily removed. Stipe subequal, 30–50 × 3-6 mm, cylindrical to slightly clavate, grayish brown (7F3-7F4); surface glabrous; interior trama initially reddish brown, turning black with damage or age; basal mycelium a low grayish tomentum. Odor not distinguishable; taste bitter. Macrochemical reactions: KOH black on all surfaces of the fresh basidioma.

Basidiospores 5-7 × 7-9 µm including ornamentation (mean = 5.75 × 7.7 µm; n = 20), Q range = 0.62–0.857, Q mean = 0.75, suboblate, tuberculate, gray-tan in H_2O, tan in KOH, inamyloid; tubercles prominent in polar view, less so in side view, predominantly exsculpate; hilar appendage 1-2 µm (Fig. 2C). Basidia (25-) 30-38 (-42) × (9-) 11-13 µm wide apically, 8-9 µm wide centrally, 3-4 µm at basal septum, clavate, gray in H_2O and KOH; basal septum with clamp connection; sterigmata four, curved, 5-7 µm long (Fig. 2B). Hymenial cystidia absent. Hymenophoral trama parallel, predominantly

gray in mass in H_2O, bright blue-green in KOH; individual hyphae gray in H_2O, light gray or light blue-green in KOH, 3-5 μm wide, with copious granular dark gray-blue pigment bodies, these soluble in KOH. Pileipellis a cutis of repent hyphae, gray-blue in mass in H_2O, light tan or blue-green in KOH, with copious granular dark gray-blue pigment bodies, these soluble in KOH; individual hyphae gray to light tan in H_2O, light gray in KOH, 4-6 μm wide, cylindrical; terminal cells undifferentiated. Pileus trama tan to brown-orange in mass in H_2O, tan to light tan or bright blue-green where pigment bodies have dissolved in KOH, with copious granular dark gray-blue pigment bodies, these soluble in KOH; individual hyphae light gray to light tan in H_2O, gray or light blue in KOH, frequently terminating in bifurcating tips of unequal lengths, others cylindrical and unbranched, (3-) 6-10 μm wide (Figs 2D, E). Stipitipellis a cutis of repent hyphae, in mass brown-orange or dark blue where pigment bodies are dense in H_2O, tan or bright blue-green in KOH, with copious granular dark gray-blue pigment bodies, these soluble in KOH; individual hyphae light tan or light gray in H_2O, light gray or light blue-green in KOH, cylindrical, 2-4 μm wide; terminal cells undifferentiated. Stipe trama brown-orange in mass in H_2O, tan or blue-green in KOH, copious granular dark gray-blue pigment bodies present, soluble in KOH; individual hyphae color light tan in H_2O, light tan or light gray in KOH, frequently terminating in bifurcated tips of unequal lengths, others cylindrical and unbranched, 4-8 (-10) μm wide (Fig. 2F). Clamp connections abundant on hyphae of all tissues.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS; Municipio de Leticia. Area de Reserva Forestal El Zafire at 4°00`69''S; 69°53'968''W; elevation ~180-220 m; along trail in white-sand forests dominated by *Dicymbe uaiparuensis*, 9 Jan 2012, *Vasco 1989* (HOLOTYPE HUA 186216; ISOTYPE HSC G1164). GenBank accession: ITS KR698937.

Habit, habitat and distribution: Gregarious on soil in forests with *D. uaiparuensis* and *Aldina* sp., known only from the type locality in El Zafire, Colombia.

Commentary: S. rufobrunneus is recognized in the field by its dark reddish brown, umbonate pileus with a smooth to fibrillose surface, unchanging pileus trama, and grayish brown stipe with initially reddish brown trama. *S. rufobrunneus* can be differentiated from each of the other Neotropical species described in Grupe et al. (2015) and here based on pileus color and surface features, lack of a pileus staining reaction, and the morphology of the pileus trama and stipe trama terminal cells (Table 1). *S. rufobrunneus* and the Neotropical *S. bambusinus* have a similar pileus shape, non-decurrent teeth, and similar tooth, stipe, and basidium lengths, but *S. rufobrunneus* differs from *S. bambusinus* in its dark reddish brown pileus color (vs. vinaceous drab, becoming fuscous or fuliginous with age), maximum pileus size (70 vs. 50 mm), smooth to fibrillose pileus (vs. villose to subfurfuraceous), and shorter basidiospores (5-7 vs. 6.5-9 μm) (Baker & Dale 1951; Maas Geesteranus 1974a). *S. rufobrunneus*

resembles the Paleotropical *S. thwaitesii* in pileus shape, stipe length, surface texture of the stipe, and basidium lengths. *S. thwaitesii* is distinguished by its grayish lilac to dark purple colors, tomentose pileus, and taller basidiospores (7.6-9.4 vs. 5-7 μm) (Berkeley & Broome 1873; Maas Geesteranus 1964, 1971, 1974b).

Among extratropical species, *S. rufobrunneus* is most similar to the north temperate *S. atroviridis* in that both have a similar range of pileus sizes and shapes, stipe surface texture, and basidium sizes (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). *S. rufobrunneus* can be distinguished from *S. atroviridis* in its combination of a reddish brown pileus and grayish brown stipe colors (vs. brownish gray, grayish violet, or black), unchanging pileus trama (vs. lilac, later bluish gray), shorter teeth (1-5 vs. 1-16 mm), shorter basidiospores (5-7 vs. 8-9 μm), and bifurcate pileus and stipe tramal hyphae (vs. unbranching) (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). Additionally, the ITS sequence of *S. rufobrunneus* is only ~82 % similar to sequences of *S. atroviridis* from the Southeastern USA and these two species are distinct in the phylogenetic analysis (Fig. 1).

Sarcodon pallidogriseus A. Grupe & A.M. Vasco-Pal., sp. nov. (Figs 2G-J).
MycoBank MB813076
Etymology: Pallidus (Latin adj. A) = adjectival prefix indicating pale coloration, and -griseus (Latin adj. B) = adjectival suffix indicating gray, referring to the pale gray color of the fresh basidiomata.

Diagnosis: Sarcodon pallidogriseus is distinct from all other described *Sarcodon* species in its combination of pale gray colors, campanulate, fibrillose, centrally scabrous pileus, unchanging pileus trama, velutinous stipe with unchanging trama, and unique ITS sequence.

Pileus campanulate, 8–16 mm broad, 4 mm tall, gray (24D1, 24B1-24C1), drying to darker gray with orange or green tones, hygrophanous; surface slightly rugose, overall interwoven fibrillose, center with scales; margin entire, gray with orange tones (5C4); trama gray (24D1), unchanging, soft (Fig. 2G). Hymenophore dentate, adnate; teeth 2 mm broad, smaller toward the margin, conical, acute apex, surface pale orange (6A3) to grayish orange when mature (5B2-5B3), drying to dark brown. Stipe subequal, 30-45 mm long, 2-5 mm broad, cylindrical, fragile, pale gray (24D1), bruising black; surface fibrillose; trama subsolid throughout development, cream-colored (2A2), unchanging; basal mycelium white. Odor none; taste bitter. Macrochemical reactions: KOH black on all surfaces of the fresh basidioma.

Basidiospores 5-6 (-7) × (6-) 7-8 μm including ornamentation (mean = 5.65 × 7.15 μm; n = 20), Q range = 0.71-0.85 (-1.0) μm, Q mean = 0.79 μm, suboblate, tuberculate, greenish brown in H_2O, pale golden brown in KOH, inamyloid; tubercles mostly prominent and short; apices rounded, infrequently exsculpate, rarely pointed;

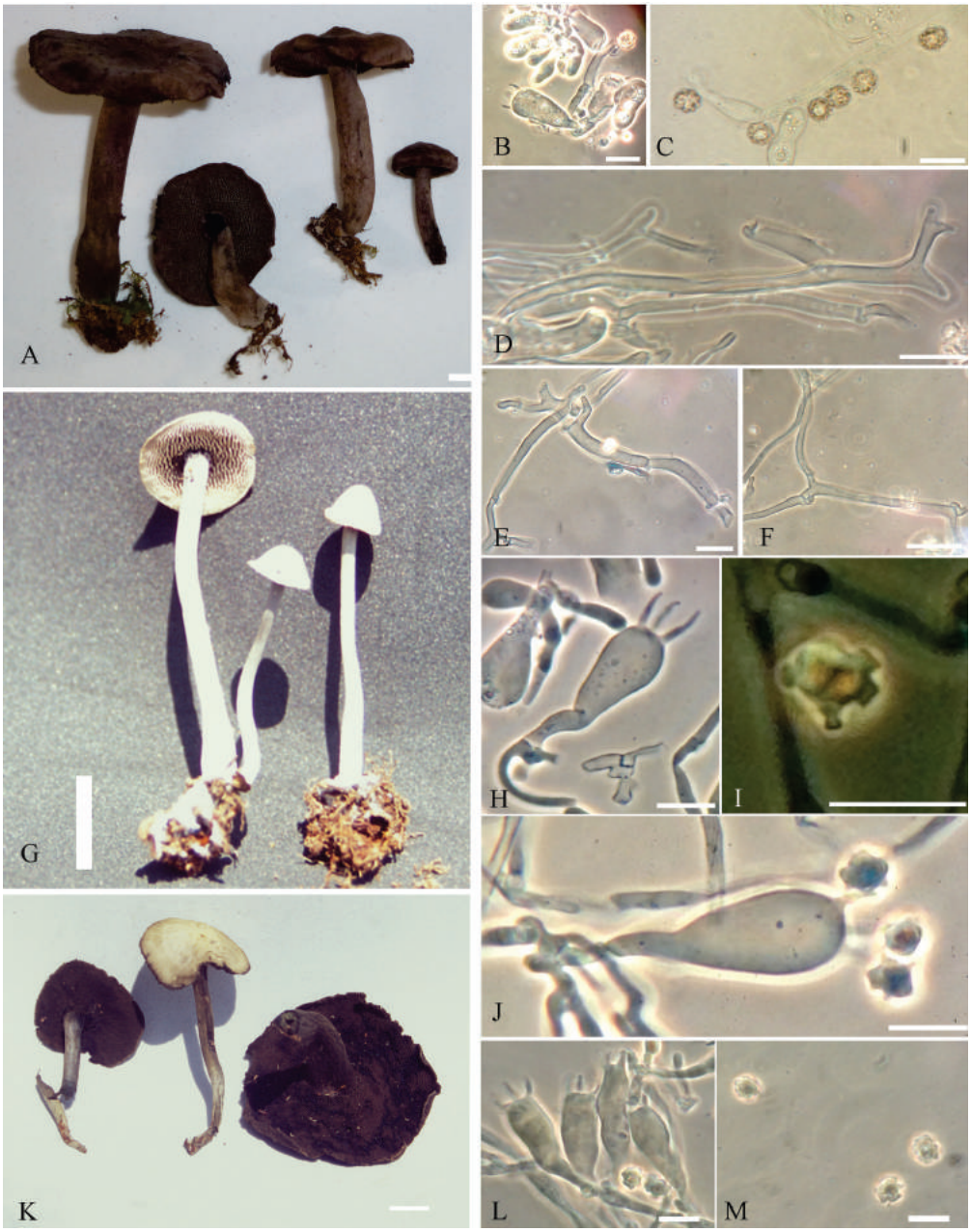

Figure 2. Morphology of *Sarcodon rufobrunneus* sp. nov. (A-F); fruiting bodies (A), basidia (B), polar view of basidiospores (C), terminal cells of the pileus (D, E) and stipe trama (F). Morphology of *Sarcodon pallidogriseus* sp. nov. (G-J); fruiting bodies (G), basidium (H), basidiospores (I), and basidium (J). Morphology of *S. bairdii* sp. nov. (K-M); fruiting bodies (K), basidia (L) and basidiospores (M). Bar for fruiting bodies = 10 mm and bar for microstructures = 10 μm

hilar appendage 1–2 μm long (Fig. 2I). Basidia (30-) 34-41 (-44) × 10-13 (-15) μm wide apically, (3-) 5-8 μm wide centrally, 2-4 μm at basal septum, clavate, hyaline in H_2O and KOH; basal septum with clamp connection; sterigmata four, curved, 6-8 μm long (Figs 2H, J). Hymenial cystidia absent. Hymenophoral trama interwoven, faint tan or light blue-green in mass in H_2O and KOH; individual hyphae light gray or pale blue-green in H_2O and KOH, 3-5 μm wide, with dense clusters of small, bluish green extracellular pigment bodies scattered throughout. Pileipellis a cutis of strongly repent hyphae, in mass light gray-green in H_2O, dark gray-blue in KOH, with dense clusters of small, bluish green extracellular pigment bodies scattered throughout, these dark blue-gray in H_2O, bright blue-green in KOH and eventually dissolving and leaching into solution; individual hyphae light gray-green in H_2O, faintly gray or gray-brown in KOH, 3-5 μm wide, cylindrical; terminal cells undifferentiated. Pileus trama in mass gray-brown to orange-brown in H_2O, light gray-tan in KOH, with highly scattered, extracellular, dark bluish green to nearly black granular pigment bodies in H_2O, these bluish green in KOH; individual hyphae light gray-brown in H_2O, light gray in KOH, cylindrical, (3-) 4-7 μm wide. Stipitipellis a cutis of repent hyphae, in mass orange-brown to dark blue where pigment bodies are dense and copious in H_2O, in KOH light orange-brown or light blue-green; individual hyphae light gray in H_2O, faint gray to faint gray-blue in KOH, cylindrical, 3-4 μm wide; terminal cells undifferentiated. Stipe trama in mass orange-brown in H_2O, light brown-orange to light blue-green where pigment bodies have dissolved in KOH; individual hyphae faint gray to faint tan in H_2O, light gray in KOH, cylindrical, 5-8 (-10) μm wide. Clamp connections abundant on hyphae of all tissues.

Specimens examined: COLOMBIA, DEPARTMENT CAQUETA: Puerto Santander, Vereda Puerto Fresco, Chukiki, 0˚40`S, 72˚31`W, elevation 150 m, in white-sand forests with *D. stipitata*, 23 Sep 2005, *Vasco 989* (HOLOTYPE HUA 186217; ISOTYPE HSC G1165). GenBank accession: ITS KR698939.

Habit, habitat and distribution: Gregarious on sandy soil under *D. stipitata*; known only from the type locality in the Middle Colombian Amazon region.

Commentary: S. pallidogriseus is recognized in the field by its combination of pale gray colors, a pileus that is campanulate, fibrillose, centrally scabrous, the unchanging pileus trama, and a velutinous stipe with unchanging trama. *S. pallidogriseus* can be differentiated from each of the other Neotropical species described in Grupe et al. (2015) and here based on its pale gray basidioma color, shape and surface features of the pileus, lack of a tramal staining reaction, and surface features of the stipe (Table 1). *S. pallidogriseus* and the Neotropical *S. bambusinus* have a similar pileus shape, non-decurrent teeth of equal lengths, stipe lengths, and basidium lengths, but *S. pallidogriseus* differs from *S. bambusinus* in its campanulate pileus shape (vs. orbicular, conical to plano-convex), pale gray basidioma color (vs. vinaceous drab,

becoming fuscous or fuliginous with age), rugulose, fibrillose, centrally scabrous pileus surface (vs. villose to subfurfuraceous), and shorter basidiospores (5-6 vs. 6.5-9 μm) (Baker & Dale 1951; Maas Geesteranus 1974a). *S. pallidogriseus* resembles the Paleotropical *S. thwaitesii* in its black surface staining reaction, lack of a tramal staining reaction, stipe length, and basidium lengths. However, *S. thwaitesii* is distinguished by its grayish lilac to dark purple colors, tomentose pileus, and longer basidiospores (7.6-9.4 vs. 5-6 μm) (Berkeley & Broome 1873; Maas Geesteranus 1964, 1971, 1974b). Among extratropical species, *S. pallidogriseus* is most similar to the north temperate *S. atroviridis* in that both have basidiomata that exhibit some shade of gray, stipe surface that stains black upon pressure, but lack of a stipe trama staining reaction, and basidium sizes (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). *S. pallidogriseus* can be distinguished from *S. atroviridis* in its lack of a pileus trama staining reaction (vs. lilac, later bluish gray), orangish teeth (vs. white, yellowish, brown), rugulose, fibrillose, centrally scabrous pileus surface (vs. tomentose to felted or glabrous), fibrillose stipe surface (vs. felted, but predominantly glabrous), cream colored stipe trama (vs. lilac to dark violet) and shorter basidiospores (5-6 vs. 8-9 μm) (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). Additionally, the ITS sequence of *S. pallidogriseus* is only ~86 % similar to sequences of *S. atroviridis* from the southeastern USA and these two species are distinct in the phylogenetic analysis (Fig. 1).

Sarcodon bairdii A. Grupe & A.M. Vasco-Pal., sp. nov. (Figs 2K-M).
MycoBank MB812925
Etymology: The species is named after Dr. Richard E. Baird, a world authority on hydnoid fungi of the Thelephorales.

Diagnosis: Sarcodon bairdii differs from all other described species of *Sarcodon* in its combination of yellow-gray, convex to plane, subumbilicate pileus that has a fibrillose and centrally squamulose surface, yellow-gray, unchanging pileus trama, brownish gray, fibrillose stipe, and unique ITS sequence.

Pileus broadly convex to plane, subumbilicate, 26-45 mm broad, yellowish gray (4B4-4B5), slightly hygrophanous; fibrillose, centrally squamulose; margin eroded, rimose, with olive tones (4E4-4E3); trama 2 mm wide, spongy, yellow-gray (4B2), unchanging. Hymenophore dentate, adnate, teeth 1-3 mm long, tapered, with acute apices, grayish brown (5F2), teeth shorter at margin (Fig. 2K). Stipe equal, 30–50 mm long, 3-11 mm wide, cylindrical, tapering gradually toward the base, brittle, brownish gray (5D2) towards apex, darker towards the base (5E3-5D3); surface fibrillose, bruising black; trama solid to substuffed, spongy, yellow-gray (4B2), bruising dark blue. Odor non-distinguishable; taste bitter. Macrochemical reactions: KOH black on all surfaces of the fresh basidioma.

Basidiospores (5-) 6-7 × 7-8 (-9) µm including ornamentation (mean = 6.2 × 7.8 µm; n = 20), Q range = 0.66-0.88, Q mean = 0.79, oblate, tuberculate, dark gray to light tan in H_2O, light tan in KOH, inamyloid; tubercles short, commonly rounded, infrequently flat topped, rarely exsculpate; hilar appendage 1-2 µm (Fig. 2M). Basidia (28-) 33-46 × 10-12 (-14) µm wide apically, (2-) 4-9 (-11) µm wide centrally, 2-3 µm at basal septum, clavate, light gray in H_2O, light gray to light tan in KOH; basal septum with clamp connection; sterigmata four, curved, 5-7 µm long (Fig. 2L). Hymenial cystidia absent. Hymenophoral trama parallel, in mass greenish brown in H_2O, orange-brown to dark gray-blue in KOH; individual hyphae light gray in H_2O, light tan or light gray in KOH, 5-7 (-10) µm wide, with copious granular dark grayish blue pigment bodies, these soluble in KOH. Pileipellis a cutis of repent hyphae, dark grayish green to brown-green in mass in H_2O, orange-brown or dark grayish blue in KOH, with copious granular dark grayish blue pigment bodies, these soluble in KOH; individual hyphae light gray in H_2O, light gray to light tan in KOH, 6-9 µm wide, cylindrical; terminal cells undifferentiated. Pileus trama dark grayish green to brown-green in mass in H_2O, orange-brown or dark grayish blue in KOH, with copious granular dark gray-blue pigment bodies, these soluble in KOH; individual hyphae light gray in H_2O, light gray to light tan in KOH, cylindrical, 7-10 (-12) µm wide. Stipitipellis a cutis of repent hyphae, in mass dark orange in H_2O, dark orange to darkest blue in KOH, with copious granular dark grayish blue pigment bodies, these soluble in KOH; individual hyphae light gray in H_2O, light gray to light tan in KOH, more or less cylindrical, with irregular bulges and constrictions, 4-7 µm wide; terminal cells undifferentiated. Stipe trama brownish green in mass in H_2O, light tan or dull blue-green in KOH, with copious granular dark grayish blue pigment bodies, these soluble in KOH; individual hyphae light brown-green in H_2O, light gray to light tan in KOH, 10-16 µm wide. Clamp connections abundant on hyphae of all tissues.

Specimens examined: COLOMBIA, DEPARTMENT CAQUETA: Puerto Santander, Vereda Puerto Fresco, Chukiki, white-sand soil forests with *Dicymbe stipitata*, 0˚40`S, 72˚31`W, elevation 150 m, 23 Sep 2005, *Vasco 990* (HOLOTYPE HUA 186218; ISOTYPE HSC G1166). GenBank accession: ITS KR698938.

Habit, habitat and distribution: Gregarious on white sandy soil in forest with *D. stipitata*, known only from the type locality in the Middle Colombian Amazon region.

Commentary: S. bairdii is recognized in the field by its yellow-gray pileus that is convex to plane, subumbilicate, fibrillose, and centrally squamulose, yellow-gray, unchanging pileus trama, a brownish gray, fibrillose stipe, and stipe trama that bruises blue. *S. bairdii* can be differentiated from each of the other Neotropical species described in Grupe et al. (2015) and here based on overall basidioma color, shape and surface features of the pileus, lack of a pileus tramal staining reaction, and surface features of the stipe (Table 1). *S. bairdii* and the Neotropical *S. bambusinus* have a

similar pileus shape, non-decurrent teeth of equal lengths, stipe lengths, and basidium lengths, but *S. bairdii* differs from *S. bambusinus* in its pileus shape being convex to plane, subumbilicate in the center (vs. orbicular, conical to plano-convex), basidioma color of yellowish gray (vs. vinaceous drab, becoming fuscous or fuliginous with age), pileus surface that is fibrillose with interspersed fibers and centrally squamulose (vs. villose to subfurfuraceous), and basidiospores that are both shorter and wider (6-7 × 7-8 vs. 6.5-9 × 5-7 μm) (Baker & Dale 1951; Maas Geesteranus 1974a). *S. bairdii* resembles the Paleotropical *S. thwaitesii* in its non-staining pileus trama, stipe lengths, and basidium lengths. *S. thwaitesii* is distinguished by its grayish lilac to dark purple colors, tomentose pileus, and basidiospore dimensions (7.6-9.4 × 5.4-7.2 vs. 6-7 × 7-8 μm) (Berkeley & Broome 1873; Maas Geesteranus 1964, 1971, 1974b). Among extratropical species, *S. bairdii* is most similar to the north temperate *S. atroviridis* in that both have basidiomata that exhibit some shade of gray, stipes that bruise black, comparable basidiospore widths, and basidium sizes (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). *S. bairdii* can be distinguished from *S. atroviridis* in its pileus surface being fibrillose and centrally squamulose (vs. tomentose to felted or glabrous), unchanging pileus trama (vs. lilac, later bluish gray), fibrillose stipe surface (vs. felted to pubescent or glabrous), stipe trama that is yellow-gray before bruising a dark blue (vs. lilac to dark violet), and shorter basidiospores (6-7 vs. 7-9 μm) (Baird et al. 2013; Banker 1906; Coker & Beers 1951; Morgan 1895). Additionally, the ITS sequence of *S. bairdii* is only ~86 % similar to sequences of *S. atroviridis* from the southeastern USA and these two species are distinct in the phylogenetic analysis (Fig. 1).

Sarcodon colombiensis A.M. Vasco-Pal. & A. Grupe, sp. nov. (Figs 3A-E).
MycoBank MB811919
Etymology: Colombiensis (-ensis Latin adj. B) = adjectival suffix indicating origin or place, referring to the type locality of the country of origin, Colombia.

Diagnosis: Sarcodon colombiensis differs from other species of *Sarcodon* in its overall dark gray to black basidioma colors, umbonate pileus, and unique ITS sequence.

Pileus umbonate, up to 30 mm broad, hygrophanous, dark gray to nearly black; pileus staining reactions, surface features, and trama characteristics not recorded. Hymenophore dentate, adnate, teeth up to 3 mm long, sharp, yellowish. Stipe equal, up to 50 mm long, 3-4 mm wide, smooth surface, concolorous with the pileus, hygrophanous. Odor and taste not recorded. Macrochemical reactions: KOH dark blue to black on all tissues of the dried basidioma.

Basidiospores 5-6 (-7) × (6-) 7-8 μm including ornamentation (mean = 5.95 × 7.45 μm; n = 20), Q range = 0.75-0.86, mean Q = 0.80, oblate, tuberculate, light brown in H_2O, light tan in KOH, inamyloid; tubercle apices variable in height, blunt-rounded, exsculpate; hilar appendage 1 μm long (Fig. 3C). Basidia (25-) 28-37 (-41) × 10-12

μm apically, 6-8 (-10) μm centrally, 2-4 μm at basal septum, clavate, occasionally with a central constriction, light tan to light gray in H_2O, faint gray in KOH; basal septum with clamp connection; sterigmata four, curved, 4-5 (-7) μm long (Figs 3A, B). Hymenial cystidia absent. Hymenophoral trama subparallel to slightly divergent, in mass light reddish brown to light gray in H_2O, bright green-blue in KOH; individual hyphae light tan to light gray in H_2O, faint gray to faint green-blue in KOH, (3-) 5 (-10) μm wide, with copious dark blue, nearly black pigment bodies (Figs 3D, E). Pileipellis a cutis of repent hyphae, in mass light tan to light gray in H_2O, light tan to light blue-green in KOH, with dark blue, nearly black pigment bodies scarce compared to other tissues, these eventually dissolving and leaching into solution; individual hyphae light tan to faint gray in H_2O, light gray in KOH, 4-6 (-10) μm wide, infrequently branching near the basal clamp connection; terminal cells undifferentiated. Pileus trama in mass brown-orange in H_2O, green-blue or light tan in KOH, pigment bodies present, of two types, some small, clustered, dark blue, nearly black, dissolving to bluish green and leaching into solution in KOH, others relatively large and copious, dark yellow-orange in H_2O, more or less soluble in KOH; individual hyphae frequently terminating in bifurcating tips of unequal lengths, others cylindrical and unbranched, (4-) 8-11 (-12) μm wide. Stipitipellis a cutis of repent hyphae, in mass gray or tan in H_2O, light tan in KOH, with scattered clusters of irregularly-shaped, extracellular, granular pigment bodies, these dark blue, nearly black in H_2O, bluish green in KOH and eventually dissolving and leaching into solution; individual hyphae light gray to faint tan in H_2O, gray in KOH, cylindrical, (2-) 3-4 (-5) μm wide; terminal cells undifferentiated. Stipe trama in mass brown-orange or faint tan in H_2O, light tan to light green-blue in KOH, with scattered extracellular granular pigment bodies, these darkest blue to nearly black in H_2O, dark bluish green in KOH; individual hyphae light gray to faint tan in H_2O, light gray to light green-blue in KOH, more or less cylindrical, (5-) 8-11 (-15) μm wide. Clamp connections abundant on hyphae of all tissues.

Specimens examined: COLOMBIA, DEPARTMENT AMAZONAS: El Zafire, 4˚00`69”S; 69˚53’968”W, elevation ~180-220 m; along trail in forest under *Pseudomonotes tropenbosii*, 9 Jan 2013, *AMV 2084* (HOLOTYPE HUA 186215; ISOTYPE HSC G1167). GenBank accession: ITS KP972654.

Habit, habitat and distribution: Solitary, on soil in forest with *P. tropenbosii*; known only from the type locality in the Biological Station El Zafire, Colombia.

Commentary: S. colombiensis is recognized in the field by its overall basidioma colors of dark gray to black, and umbonate pileus. *S. colombiensis* can be differentiated from each of the four Neotropical species recently described in Grupe et al. (2015) and here based on key features of basidioma color and terminal cells of the pileus trama hyphae (Table 1). *S. colombiensis* and the Neotropical *S. bambusinus* have a similar pileus shape, non-decurrent teeth, similar tooth lengths, stipe lengths, and basidium lengths,

but *S. colombiensis* differs from *S. bambusinus* in its dark gray to black (vs. vinaceous drab, becoming fuscous or fuliginous with age) basidioma colors, yellowish teeth (vs. pallid fuliginous to ochraceous), and shorter basidiospores (5-6 vs. 6.5-9 μm) (Baker & Dale 1951; Maas Geesteranus 1974a). *S. colombiensis* resembles the Paleotropical *S. thwaitesii* in pileus shape, stipe length, surface texture of the stipe, and basidium lengths. *S. thwaitesii* is distinguished from *S. colombiensis* by its grayish lilac to dark purple colors, white, grayish, or brown teeth (vs. yellowish), and shorter basidiospores (5-6 vs. 7.6-9.4 μm) (Berkeley & Broome 1873; Maas Geesteranus 1964, 1971, 1974b). Among extratropical species, *S. colombiensis* is most similar to the north temperate *S. atroviridis* in that both have a similar stipe surface texture, and basidium sizes (Banker 1906; Baird et al. 2013; Coker & Beers 1951; Morgan 1895). *S. colombiensis* can be distinguished from *S. atroviridis* in its umbonate pileus (vs. convex to planar), shorter teeth (≤ 3 vs. 1-16 mm), shorter basidiospores (5-6 vs. 8-9 μm), and bifurcate pileus trama hyphae (vs. unbranching) (Banker 1906; Baird et al. 2013; Coker & Beers 1951; Morgan 1895). Additionally, the ITS sequence of *S. colombiensis* is only ~85 % similar to sequences of *S. atroviridis* from the southeastern USA and these two species are distinct in the phylogenetic analysis (Fig. 1).

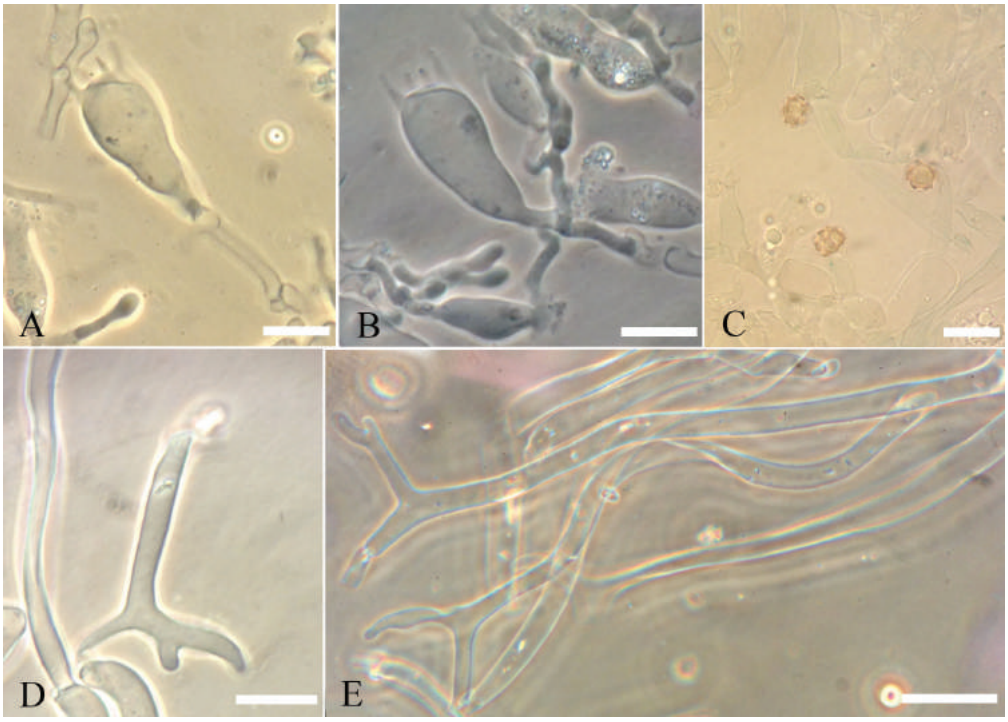

Figure 3. Morphology of basidia of *Sarcodon colombiensis* sp. nov. (A, B), basidiospores (C) and terminal cells of the pileus trama of *S. colombiensis* sp. nov. (D, E).

Key to Neotropical species of *Sarcodon* with selected extralimital taxa

1. Pileus color pinkish gray (7B2-7C2 KW), with irregular darker purplish spots, with age developing irregular black auto-oxidative patches, or grayish lilac or dark purple when young **2**

1. Pileus color not as above; some shade of grayish, orangish, yellowish brown, or with reddish tones **4**

2. Pileus surface irregularly pitted; a densely interwoven mat under hand lens; trama slowly staining pink (11A2-11B2) upon exposure; Guyana, in *Pakaraimaea* (Dipterocarpaceae) ***S. pakaraimensis***

2. Pileus surface not as above; trama unchanging or not staining as above **3**

3. Pileus surface appressed, felt-like; substriate at extreme margin, basidia predominately 22-30 (–36) × 10-16 µm; stipitipellis hyphae terminal cells with 2-8 serpentine undulations; Belize, in montane *Quercus* forests ***S. quercophilus***

3. Pileus surface a fine erect tomentum or velutinous; eventually areolate before collapsing to glabrous; stipitipellis terminal cells undifferentiated; Malaysian Archipelago and New Guinea (Dipterocarpaceae and Fagaceae), New Zealand in *Nothofagus* forests ***S. thwaitesii***

4. Pileus surface tomentose, felted or pubescent, eventually glabrous; basidioma some shade of orange white (5A2), orange grey (5B2), pale orange (5B3), reddish blond (5C3), teeth up to 16 mm long; whole basidioma drying olivaceous; Eastern North America, Europe, Eastern Asia ***S. atroviridis***

4. Pileus surface not as above, or not with those combinations; basidioma some shade of gray, grayish brown, yellowish gray (4B2), dark reddish brown (7F8-8F8), or fuscous; teeth not exceeding 6 mm, basidioma not drying olivaceous **5**

5. Pileus regularly with a deep, prominent umbilicus; Belize, in montante *Quercus* forests ***S. umbilicatus***

5. Pileus without an umbilicus, or not deep and prominent **6**

6. Pileus surface matted fibrillose throughout, with age finely areolate over disk; all tissues staining black upon pressure or exposure; Puerto Rico, in lower montane wet forests ***S. portoricensis***

6. Pileus surface not as above, or not becoming areolate, not all tissues staining black upon pressure or exposure **7**

7. Pileus with an umbo; hyphae of the pileus commonly branching dichotomously at septa **8**

7. Pileus without an umbo; hyphae of the pileus trama not as above **9**

8. Pileus dark reddish brown (7F8-8F8); surface smooth to fibrillose, stipe grayish brown, trama initially reddish brown before turning black; hyphae of both the pileus and stipe commonly branching dichotomously at septa; Colombia, in *Pseudomonotes* forests ***S. rufobrunneus***

8. Pileus dark gray to nearly black, without reddish tones; surface as above or not; stipe trama not initially reddish brown; hyphae of the stipe not as above; Colombia, in *Pseudomonotes* forests ***S. colombiensis***

9. Pileus surface smooth, villose or subfurfuraceous, more repent fibrillose near margin; stipe staining slowly fuliginous or fuscous with contact or age; Trinidad, Brazil, in lowland tropical rain forests ***S. bambusinus***

9. Pileus surface smooth to fibrillose, or slightly rugose, overall interwoven fibrillose with scales centrally, or centrally squamulose, and lacking a fuliginous or fuscous staining reaction **10**

10. Pileus broadly convex to plane, yellowish gray, centrally squamulose; stipe bruising black; trama yellow-gray, bruising dark blue; Colombia, in *Dicymbe* forests ***S. bairdii***

10. Pileus campanulate, gray, drying to darker gray with orange or green tones, surface with scales centrally; stipe bruising black; trama cream colored, not bruising; Colombia, in *Dicymbe* forests ***S. pallidogriseus***

Table 1. Diagnostic morphological characters of Neotropical *Sarcodon* species.

Species	Pileus surface	Pileus staining	Terminal cells of pileus and stipe trama	Tramal staining	Basidiospore ornamentation	Basidia	Stipitipellis terminal cells	Hymenophore attachment
S. colombiensis	not recorded	not data	(Pileus) terminating in bifurcating tips of unequal lengths	not recorded	exsculpate; apices variable in height, blunt-rounded	clavate 28-37 × 10-12 µm	Undifferentiated 3–4	adnate
S. rufobrunneus	smooth to fibrillose, umbonate	none	(Pileus and stipe) terminating in bifurcating tips of unequal lengths	absent	prominent to low, predominately exsculpate	clavate 30-38 × 11-13 µm	Undifferentiated, 3–4 µm wide	adnate
S. pallidogriseus	slightly rugose, overall interwoven fibrillose, center with scales	none	undifferentiated	absent	apices rounded, frequently prominent and short, uncommonly exsculpate	clavate 34-41 × 10-13 µm	Undifferentiated 3-4 µm wide	adnate
S. bairdii	fibrillose, centrally squamulose	none	undifferentiated	absent	short, commonly rounded, infrequently flat topped, rarely exsculpate	clavate 33-46 × 10-12 µm	Undifferentiated 4-7 µm wide	adnate
S. pakaraimensis	glabrous, pitted	black	undifferentiated	pink	rounded, rarely exsculpate	clavate 27-47 × 5-7 µm	Undifferentiated, 3-9 µm wide	adnate
S. portoricensis	fibrillose, areolate over disc	black	undifferentiated	black	exsculpate, rounded corners	clavate 33-44 × 7-12 µm	subclavate to subcapitate, 2-3 µm wide	adnate
S. quercophilus	appressed felted, substriate at margin	none	undifferentiated	absent	exsculpate, outward projecting corners	short-clavate 22-30 × 5-10 µm	serpentine, 2-4 µm wide	adnate
S. umbilicatus	matted fibrillose, rugulose, umbilicate	dark brown	undifferentiated	grayish brown	rounded, rarely exsculpate	clavate 32-38 × 5-7 µm	Undifferentiated, subcapitate, or subclavate, 2-5 µm wide	adnexed

CHAPTER 8

General Discussion and Summary

Aída M. Vasco-Palacios

Introduction

The symbiosis between fungi and plant roots, known as mycorrhiza, is one of the most ubiquitous mutualistic interactions occurring in terrestrial ecosystems. It has been estimated that about 50,000 fungal species form mycorrhizal associations with approximately 250,000 vascular and non-vascular plant species (Smith & Read 2008; van der Heijden et al. 2015). The types of mycorrhizal interactions are known as ectomycorrhiza (EcM), arbuscular mycorrhiza (AM), orchid mycorrhiza, and ericoid mycorrhiza. The AM fungi associate with about 74 % of plant species while EcM, ericoid mycorrhiza, and orchid mycorrhiza fungi interact with about 2 % , 1 % and 9 % of plant species, respectively (Brundrett 2009; van der Heijden et al. 2015). It has been estimated that there are about 6000 species of EcM fungi belonging to Ascomycota, Basidiomycota, and Zygomycota that associate with 20,000-50,000 species of plant lineages (Rinaldi et al. 2008; Tedersoo et al. 2010c). EcM fungi help to mitigate plant stress and enhance seedling establishment and growth. Studies suggest that they even promote local dominance of EcM tropical trees and decrease negative effects caused by pathogens (McGuire 2007; Rodriguez et al. 2004). The ectomycorrhizal symbiosis was previously assumed to be restricted to forests within temperate and boreal regions that are dominated by Pinaceae, Fagaceae, Betulaceae, and Salicaceae, as well as the subfamily Leptospermoideae of Myrtaceae (Henkel et al. 2002; Smith & Read 2008). The tropics were supposed to be dominated by AM fungi due to a predominance of AM hosts in these forests and the lack of fruiting bodies of typical EcM fungi (Henkel et al. 2002). However, recent studies showed the presence of EcM symbiosis in tropical ecosystems (Bâ et al. 2012, 2014; Bas 1978; Brearley 2012; Diedhiou et al. 2010; Henkel et al. 2002, 2012; Lopez et al. 2012; Moyersoen 2006, 2012; Phosri et al. 2012; Roy et al. 2016; Singer & Araujo 1979; Singer et al. 1983; Smith et al. 2013; Tedersoo and Nara 2010a; Tedersoo et al. 2010b, 2014). The EcM hosts in tropical ecosystems mainly belong to the Fabaceae (subfamilies Papilionoideae and Caesalpinioideae), Gnetaceae, Nyctaginaceae, Polygonaceae, and Dipterocarpaceae. Soil microorganisms, particularly fungi, are known to play a prominent role in structuring the diversity and abundance of trees in the tropical rainforest (Augspurger 1983; Comita et al. 2010; McGuire 2007; Peh et al. 2011; Peay et al. 2013; Peñuela-Mora 2014; Torti et al. 2001). It is thus tempting to speculate that EcM fungi also contribute to these ecological roles.

The Amazon region is a biodiversity hotspot and comprises a mosaic of ecosystems. Colombia is the fourth world's most biodiverse country, and the most megadiverse per square kilometer (IUCN 2009). So far, 27,881 flowering plant species have been listed for the country (Stuessy 2007) and, because of the close ecological relationships between plants and fungi, it is expected that it harbors a high mycological diversity of fungi as well. Little is known about fungal diversity in these ecosystems. Until

2013, 1239 species of macrofungi had been recorded in Colombia (Vasco-Palacios & Franco-Molano 2013) of which only 20 % occurred in the Amazon region. There is a major knowledge gap about EcM fungi that are associated with white-sand forests (WSF) in Western Amazonia and terra-firme forests with the endemic dipterocarp *Pseudomonotes tropenbosii* (PtF). For instance, only 14 EcM fungal species have been collected from PtF (López-Quintero et al. 2012; Vasco-Palacios & Franco-Molano 2013). Fungal diversity was studied in this Thesis project in WSF, PtF and terra-firme mixed forests (MF) in the lowland of the Colombian Amazonian basin. WSF are home to EcM Fabaceae trees such as *Dicymbe uaiparuensis* and *Aldina* sp. (ca. Important Value Index, IVI, 25 %). MF is characterized by a high diversity of trees represented by few individuals of EcM forming *Coccoloba* (Polygonaceae, IVI 0.477 %), *Guapira* or *Neea* (Nyctaginaceae, IVI-family 0.845 %) species. PtF is a terra firme forest with the ectomycorrhizal tree *P. tropenbosii* as one of the most important canopy species (IVI, 16-18 %) (Duivenvoorden & Lips 2005; Parrado-Rosselli 2005).

Fungal diversity

Fungal diversity in the Colombian Amazon basin was studied using traditional morphology-based techniques as well as molecular techniques (Chapters 2, 3, 4). Sequences of the internal transcribed spacer (ITS) regions and the D1/D2 region of the large subunit ribosomal DNA (LSU rDNA) were used to identify fungal species from fruiting bodies and root tips, while the chloroplast intron region trnL (UAA) was used to identify the host plants (Chapters 2, 3). High-throughput sequencing methods adopted in Chapter 4 enabled deep molecular sampling of the fungal communities in the different forest types and enabled us to address the effect of plant diversity and edaphic factors on fungal richness at the local scale. A total of 114 species of EcM macrofungi were identified based on morphology-based techniques (Chapters 2, 3), of which 83 were identified in PtF (Chapter 3) and 49 in WSF (Chapter 2). The most abundant families were *Russulaceae* (28 species), *Clavulinaceae* (15 species), *Hymenochaetaceae* and *Boletaceae* (14 species), *Amanitaceae* (12 species), and *Cantharellaceae* (6 species). Thirty-one species (27.1 %) found during this study were not reported before in Colombia, and twenty-four corresponded to undescribed species (21.0 %) (Chapters 2, 3, 5, 6, 7). The species accumulation curves did not reach the asymptote, indicating that more species remain to be discovered in these forests (Chapters 2, 3). It was estimated that 150 and 100 EcM fungi occur in PtF and WSF, respectively. Incomplete recovery of EcM fungal diversity was corroborated by the notion that 24 and 21 species-level OTUs of putative or confirmed EcM fungi were detected at the root surface but had not been found as fruiting bodies in WSF and PtF respectively. Conversely, 70 species collected as fruiting bodies were not detected from the roots of *P. tropenbosii* (Chapter 3). A similar situation was found in WSF (Chapter 2).

Fourty-nine and 28 EcM fungi were identified in WSF based on mushroom typing and root analysis, respectively. The mushrooms belonged to 12 genera and 9 Agaricomycetes families (Chapter 2). The most diverse families were Russulaceae (18 species), Amanitaceae (9 species) and Hymenochaetaceae (7 species). The most diverse lineages identified from root-tips were /russula-lactarius (9 OTUs), /sebacina (8 OTUs), /helotiales (5 OTUs), and /tomentella-thelephora (2 OTUs). WSF are known to host plant species that are restricted to white sandy soils (Anderson 1981, Damasco et al. 2013). Some of them, mainly from the family of Fabaceae, are ectomycorrhizal (Peay et al. 2013). For instance, the genera *Dicymbe* and *Aldina* that are present in WSF in Colombia have been recorded as ectomycorrhizal hosts in Guyana (Henkel et al. 2002, 2012). Recently, 64 species of EcM fungi were identified in 10 plots of WSF in Brazil with Russulaceae and Amanitaceae as the most diverse families (Roy et al. 2016). Heterogeneity in the EcM composition in the WSF from French Guyana and Brazil was found not to be significant (Roy et al. 2016).

The ectomycorrhizal symbiosis of Dipterocarpaceae has been well studied in Asian species (Brearley, 2012; Lee, 1990; Tata, 2008). Little is known about EcM interactions of this family in the Amazon region. *Pakaraimaea dipterocarpacea* associates with 61 EcM fungi in Guyana and Venezuela (Morton et al., 1999; Londoño, 1995; Moyoerson, 2006; Smith et al., 2013). Chapter 3 studied the diversity of EcM fungi related to the dipterocarp *P. tropenbosii* that is found in small patches in Colombian Amazonia (Morton et al., 1999; Londoño, 1995). A total of 83 EcM species were found corresponding to the commonly known EcM orders, including 16 families and 27 genera. The most diverse families were Clavulinaceae and Boletaceae (13 species), Russulaceae (12 species), Hymenochaetaceae (10 species), and Amanitaceae (7 species). Based on molecular analysis of EcM root tip, 34 OTUs were identified at species-level, 26 at genus-level and 38 at family-level. These EcM taxa represented 12 independent fungal lineages with the majority of taxa belonging to /tomentella-thelephora and /cortinarius (8 OTUs each), /russula-lactarius (7 OTUs), and /sebacina, /hydnellum-sarcodon and /helotiales (6 OTUs each). A large number of these species are widely distributed in the Amazon region in Venezuela, Colombia, and in Guyana with EcM hosts of the families of Fabaceae (43.5 %) and Dipterocarpaceae (12 %) (Chapter 3). These data corroborate previous findings that EcM fungi associated with lowland legume trees do not show a strong host preference (Smith et al. 2013). In general, the studied PtF from MC1, MC2 and ZBS (Chapters 2, 3, 4) do not presented significant differences in the EcM fungal community composition. Data suggest that tropical ecosystems are variable in terms of EcM symbiosis. Ecosystems with a dominance of EcM host trees are relatively rich in EcM fungal diversity with a low level of fungal host preference (Henkel et al 2012; Smith et al. 2013). In contrast, tropical forests with a low number and scattered EcM hosts show low EcM fungal diversity with a certain extent of host preference (Tedersoo et al. 2010b).

Some EcM taxa, such as *Inocybe, Entoloma, Tomentella,* and *Sebacina* spp., were not well represented in the morphospecies data presented in Chapters 2 and 3 when compared to EcM fungal communities in Guyana and Venezuela. This may in part be explained by overlooking cryptic or atypical fruiting bodies of *Tomentella* and *Sebacina*. Russulaceae represented by the genera *Lactarius*, *Lactifluus,* and *Russula* was the most diverse in the studies of Chapters 2 and 3. Russulaceae represents a diverse family with a worldwide distribution and interactions with several plant families. The distribution patterns differ between genera. For instance, *Russula* is the most widespread genus, while *Lactarius* and *Lactifluus* are mainly found in temperate and tropical regions, respectively (van de Putte et al. 2012). A recent phylogenetic study suggested ancient Paleo-Neotropical sister relationships, possibly resulting from Gondwana vicariance, with more recent diversification of taxa within the Neotropics (Hackel et al., 2014). We found 12 species of Russulaceae in PtF and 18 species in WSF. This number is relatively low when compared with the diversity observed in Guyana, where 35 species have been reported from *Pakaraimaea* and Fabaceae forests after 2- and 13-years periods of sampling, respectively (Henkel et al., 2012; Smith et al., 2011, 2013) (Chapter 4). Twenty-four species of Russulaceae have been reported from terrestrial Amazonian ecosystems in Brazil with 7 species from WSF (Jaeger 2013; Roy et al 2016; Sa et al. 2013).

Sebacina represented a major lineage in the root-tip analysis (Chapters 2, 3). It is one of the most common and species-rich groups of EcM fungi from temperate and tropical ecosystems (Oberwinkler et al. 2013; Moyersoen & Weiss 2014; Tedersoo et al. 2010a, 2010b 2014). The genera *Sebacina* sensu stricto, *Tremellodendron* and *Tremelloscypha* have previously been found to form ectomycorrhizae in North Eastern Ecuador and Venezuela (Moyersoen & Weiss 2014; Tedersoo et al. 2010b; Tedersoo and Smith 2013). However, species of *Sebacina* and *Tremellodendron* detected in this study were different from those found in Ecuador and Venezuela and they may represent new species (Moyersoen & Weiss 2014; Tedersoo et al. 2010b).

Fungal communities of *Pseudomonotes tropenbosii* forests were analyzed in the Middle Colombian Amazon region MC1 and MC2 and Zafire Biological Station (ZBS) (Chapter 3). The MC sites and ZBS are separated by approximately 420 km and no populations of *P. tropenbosii* are known to occur between these two areas. Yet, they may be present as the Amazon region in Colombia has large areas that remain to be explored. Differences between the EcM fungal communities of MC1, MC2, and ZBS were not significant based on fruiting body - and root tip analysis (Chapter 3). Fruiting bodies of 52, 13, and 43 species were collected in MC1, MC2, and ZBS. Taking Middle Colombian Amazon region as a unit (i.e. MC1 + MC2), 59 EcM species were identified of which 18 species were shared with ZBS. Root tip analysis showed the highest biodiversity for MC1 (17 OTUs), followed by MC2 and ZBS with 14 and 10 OTUs, respectively. The three sites shared one species-level OTU. From

the 34 species-level ITS OTUs detected on roots of *P. tropenbosii* 13 species have been found as fruiting bodies in PtF, while 12 have been detected from roots of *Pk. dipterocarpacea,* 16 in *Dicymbe* forests in Guyana and 14 from WSF from Colombia (Chapter 3). Together, it is concluded that EcM fungal community is diverse in the Colombian lowland Amazon forests. Landscape, forest structure, and host dominance may govern the EcM fungal diversity. Indeed, soil composition and vegetation type have explained the structure of fungal communities in Western Amazonian forests in Peru and Colombia (Peay et al. 2013; Chapter 4).

Chapter 4 describes the first deep study addressing fungal diversity in soils of rain forests in the Colombian Amazon region using a high throughput sequencing method. The fungal sequences obtained from 22 plots of WSF, MF, and PtF soils were assembled into 3145 non-singleton OTUs (47,519 sequences). A previous metabarcoding study from similar ecosystems in Western Amazonia revealed 1776 fungal OTUs at the species-level (Peay et al. 2013). Differences in the diversity between our study and that of Peay et al. (2013) may be due to sampling design. The phylum Ascomycota (59.2 % of OTUs, 46.7 % of sequences) was the most diverse followed by Basidiomycota (36.9 % of OTUs, 37.1 % of sequences), Zygomycota-Mucoromycotina (1.7 % of OTUs, 14.7 % of sequences), and Cryptomycota (1.1 % of OTUs, 0.3 % of sequences). The most diverse fungal community was found in PtF with 2139 fungal OTUs, followed by MF that represented 1766 OTUs. WSF showed a remarkably lower diversity with 848 OTUs. The nonmetric multidimensional scaling (NMDS) analysis revealed that the type of forest plays a fundamental role in driving the fungal community structure. This was supported by Mantel analysis showing that soil fungal community composition is more similar in samples from the same type of forests and thus overrules distance between plots as a determining factor. In general, WSF soils showed the most unique composition and the fungal communities from MF were similar to the ones from PtF ($P > 0.05$) (Chapter 4). A cluster analysis also showed that more OTUs were shared between fungal communities from MF and PtF plots, particularly those that were nearest, although in some cases the similarity was independent from the distance (Chapter 4).

Forest type explained 21 % of the fungal variation. Our data showed that EcM fungal diversity was low compared to the Paleotropics or monodominant EcM forests occurring in Guyana. Nevertheless, a relatively large number of EcM taxa were found in MF that presented a high abundance of AM host plants and a low abundance of EcM host plants belonging to *Coccoloba*, *Guapira* or *Neea*. These EcM host trees may act as bridges for EcM fungi between geographically separated PtF patches, facilitating the dispersal of EcM fungi between different types of terra-firme forest, and probably even with WSF. The EcM fungi seem to share their hosts belonging to different lineages within Fabaceae, Dipterocarpaceae, Polygonaceae, and Nyctaginaceae. Soil chemistry (pH and C/N ratio) was also found to be an important driver to structure

fungal communities in lowland tropical forests in Colombia. Soil pH and C/N ratio explained 14 % and 12 % of fungal variation, respectively (Chapter 4). Variations of pH impacted mainly WSF fungal communities because these forests occur on more acidic soils when compared to terra firme forests.

Taxonomic Novelties

Twenty-four new species have been identified during the course of this project (Chapters 2, 3, 5, 6, 7). Thirteen species of Boletaceae were identified and nine of them remain as unidentified species, mainly due to lack of monographs on the taxonomy on tropical boletes. Abundant specimens of a pink boletes were frequently found in PtF from the MC region and ZBS. Based on molecular and morphological data, it was identified as a new species named *Austroboletus amazonicus* A.M. Vasco-Pal. & C. López-Quint. (Chapter 5). Two other species of the genus, *A. festivus* and *Austroboletus* sp. 3, were found in PtF in MC1. Basidiocarps of *Fistulinella campinaranae* var. *scrobiculata* were also common in forests dominated by *P. tropenbosii* and it was first reported for Colombia in Chapter 5.

Recent phylogenetic analyses placed *Coltricia* and *Coltriciella* together in a single clade. Morphologically, the two genera only differ in the verrucose spores of *Coltriciella*. Because our phylogenetic analyses suggested that the two genera may not be congeneric we tentatively kept them separate in our treatment (Chapter 6). Neotropical species of *Coltricia* and *Coltriciella* were presented in Chapter 6. Sixteen species of *Coltricia* and four of *Coltriciella* have been reported from Neotropical ecosystems (Baltazar & Baptiste 2009; Baltazar et al. 2010; Bian & Dai 2015; IndexFungorum 2015; Ryvarden 2004; Valenzuela et al 2010). *Coltricia dependella* sp. nov.*, Coltriciella minuta* sp. nov. and *Coltriciella cylindrospora* sp. nov. are new species described from forests with *P. tropenbosii.* The fact that an uncultured *Coltriciella* was identified from root tips of *P. tropenbosii* and *Dicymbe* supports the EcM status of this genus (Chapter 3). The true distribution and diversity of these genera may be underestimated since the basidiocarps, in particular those of *Coltriciella,* are often small and commonly occur in habitats like woody debris, the lower side of fallen trunks, or in trunk cavities, making them difficult to find (Aime et al. 2003).

An major contribution to the knowledge of macrofungi in the Neotropics was made for the ectomycorrhizal genus *Sarcodon* (Bankeraceae, Thelephorales, Basidiomycota). The genus is well represented in the Northern temperate Hemisphere and to a lesser extent occurs in Paleotropical forests, with ~87 described species. *Sarcodon*, however, is poorly known from the Neotropics. This situation is changing rapidly through analysis of new collections from Belize, Colombia, Guyana, and Puerto Rico. Chapter 7 reports four new species of *Sarcodon* associated with a diverse group of EcM trees. *Sarcodon colombiensis* and *S. rufogriseus* were detected from root-tips

of *P. tropenbosii*, thus confirming that both are forming EcM symbiosis with this dipterocarp tree (Chapter 3).

Highlights about EcM symbiosis in the Amazonian region

A high fungal diversity was found in the studied ecosystems in the Colombian Amazon region. However, our results agree with previous findings that EcM fungal richness is low when compared with forests in the Paleotropics or monodominant forests in Guyana (Henkel et al. 2012; Smith et al 2013; Tedersoo et al. 2014). This is explained by the fact that temperate forests are dominated by EcM host tree lineages, like Fagales, Pinaceae, and Salicaceae. In contrast, tropical forests are rich in AM-plants, except for monodominant Fabaceae trees in forests occurring at the Guiana Shield. Ectomycorrhizal host lineages such as the Dipterocarpaceae or certain groups of Fabaceae can be found in high or low abundance in tropical ecosystems. In this study, it was found that PtF was more diverse in EcM fungi than MF or WSF. In all cases, the diversity was lower than that has been observed in Guyana. In the case of PtF this may be explained by the fact that the patches with *P. tropenbosii* are small and the trees have a lower abundance (Importance Value Index, IVI 17-18 %) in the forests. In the case of WSF this may be explained by the fact that the abundance of *Dicymbe* and the size of the forest-patches (>10ha) were considerably smaller than those in Guyana. The Colombian WSF presented the most particular EcM community reflecting the unique flora of this type of forest. Similarly, the fungal species in WSF in Peru showed a positive correlation with tree species composition (Peay et al. 2013). An unexpected diversity of EcM fungi was found in terra-firme forests (74 OTUs) despite the low abundance of EcM hosts. In Ecuador, 38 species of EcM fungi were recorded from this type of forest with trees and lianas of *Coccoloba*, *Guapira* or *Neea* (Tedersoo & Nara 2010b). The EcM hosts in terra-firme forests may act as connecting bridges for EcM fungi across the Amazon, thus facilitating the distribution of EcM fungi between various types of terra-firme forests, and probably also within WSF. Further studies should uncover the complete fungal community in the lowland forests of Colombia in order to reveal a better understanding of the distribution of EcM fungi, their host specificity, and the edaphic factors that influence the EcM distribution in different types of forests in Western Amazonia. Studies in permanent plant plots are recommended to link fungal diversity to vegetation and forest dynamics. This may facilitate an integrative analysis to understand the role of fungi in structuring the diversity and abundance of trees in the tropical rainforest, as well as the influence of plants on the EcM fungal diversity distribution across Amazonian region. A better understanding of this fungus-plant association will reveal the impact of fungi on the ecology of Neotropical lowland rain forests, especially in the light of climate change.

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Samenvatting

Een mycorrhiza is een wederzijds-voordelige interactie tussen schimmels en planten. Deze symbiose is zeer veel voorkomend in de natuur. Men denkt dat ongeveer 50.000 schimmelsoorten en 250.000 plantensoorten mycorrhiza's vormen. Er worden verschillende vormen van mycorrhiza's onderscheiden, namelijk de ectomycorrhiza's (EcM), de arbuscular mycorrhiza's (AM), de orchid mycorrhiza's en de ericoid mycorrhiza's. De AM schimmels gaan met ongeveer 74% van de landplanten een interactie aan, terwijl dit voor de andere groepen veel lager ligt. Zo denkt men dat er ongeveer 6000 soorten EcM schimmels zijn die met 20.000-50.000 planten een interactie aangaan. Deze EcM schimmels bevorderen groei van de plant, helpen de plant bij stress, en verhogen de kans dat zaailingen zich kunnen vestigen. Daarnaast zouden zij betrokken kunnen zijn bij het abundant worden van bepaalde bomen in bossen en zouden zij ziekte door schimmels helpen voorkomen. Er werd altijd gedacht dat de EcM interactie met name voor zou komen in koude en gematigde klimaatzones, terwijl in tropische bossen met name interacties zouden voorkomen met AM schimmels. Echter, recent is duidelijk geworden dat EcM schimmels ook relatief actief zijn in de tropen, waarbij zij interacties aangaan met planten van de families Fabaceae, Gnetaceae, Nyctaginaceae, Polygonaceae en Dipterocarpaceae. Het is bekend dat bodem micro-organismen een belangrijke rol spelen in de samenstelling van een tropisch bos. De EcM schimmels zouden deze rol ook kunnen vervullen en daarom is het belangrijk deze groep schimmels te bestuderen.

Colombia is een hotspot van biodiversiteit. Tot dus ver zijn 27,881 bloemplanten in dit land geïdentificeerd. Gezien de abundantie van mycorrhiza interacties is het waarschijnlijk dat Colombia dus ook veel schimmeldiversiteit herbergt. Echter, er waren nog maar 1239 paddenstoelvormende soorten tot 2013 geïdentificeerd, waarvan maar 20% in het Amazonegebied. Er was met name een lacune in de kennis van EcM schimmels in zogenaamde "white-sand forests" (WSF) waar Fabaceae bomen voorkomen, zoals *Dicymbe uaiparuensis* en *Aldina* spp. en in bossen waar de dipterocarp *Pseudomonotes tropenbosii* (PtF) voorkomt. Zo waren er maar 14 soorten EcM schimmels beschreven in laatstgenoemde bossen. In dit proefschrift wordt de diversiteit van EcM schimmels beschreven in WSF, PtF en zogenaamde "gemengde bossen" (MF) waar een hoge diversiteit bomen voorkomt elk in geringe hoeveelheden. Zo komen in deze bossen EcM vormende *Coccoloba*, *Guapira* en *Neea* soorten voor. De diversiteit werd bestudeerd gebruikmakend van traditioneel morfologische en moleculaire technieken.

Er werden in totaal 114 EcM schimmels geïdentificeerd op grond van morfologische en moleculaire kenmerken, waarvan 83 in PtFs (Hoofdstuk 3) en 49 in WSFs (Hoofdstuk 2). De meest voorkomende families waren *Russulaceae*, *Clavulinaceae,*

Boletaceae, *Hymenochaetaceae*, *Amanitaceae*, en *Cantharellaceae*. Van deze paddenstoelvormende schimmels waren 31 soorten niet eerder beschreven in Colombia en 24 soorten waren zelfs wereldwijd niet eerder beschreven. De asymptoten werden niet bereikt in de soortenaccumulatiecurves. Dit impliceert dat er meer EcM soorten aanwezig moeten zijn in de bestudeerde WSFs en PTFs. De totale aantallen zouden op grond van de berekeningen uitkomen op respectievelijk 100 en 150 soorten. Het feit dat er 24 soorten werden geïdentificeerd op wortelmateriaal die niet als paddenstoel waren gevonden bevestigt dat de soortenrijkdom in de bossen hoger is dan die gevonden werd middels de morfologische analyse.

Naast de 49 en 83 soorten die op grond van morfologie waren geïdentificeerd in de WSFs en de PtFs werden er respectievelijk 28 en 34 soorten EcM schimmels gevonden middels DNA analyse van wortelmateriaal. De paddenstoelen in de WSFs behoorden tot 12 genera en 9 families (Hoofdstuk 2), waarvan de Russulaceae, Hymenochaetaceae en Amanitaceae het meest divers waren. De meest diverse taxa binnen het wortelmateriaal van de WSFs waren /russula-lactarius, /sebacina, /helotiales, en /tomentella-thelephora. De paddenstoelen van de PtFs behoorden tot 27 genera en 16 families. De meest diverse paddenstoelen families waren Clavulinaceae, Boletaceae, Hymenochaetaceae, Russulaceae, en Amanitaceae, terwijl /tomentella-thelephora en /cortinarius, /russula-lactarius, /sebacina, /hydnellum-sarcodon, and /helotiales de meest diverse taxa waren binnen het wortelmateriaal. Deze en andere resultaten laten zien dat EcM schimmels in de WSFs en PtFs geen sterke gastheervoorkeur hebben.

Hoofdstuk 4 beschrijft de eerste diversiteitsstudie aan schimmels in de laagland bossen in de Amazone van Colombia waar gebruikt gemaakt werd van “high throughput sequencing”. Er werden 3145 Operational Taxonomic Unit (OTUs) gevonden die meer dan één keer werden gevonden in bodems van WSF, MF, and PtF. Ascomyceten waren het meest divers (59.2 %), gevolgd door de basidiomyceten (36.9 % van de OTUs). Met 2139 OTUs waren PtFs het meest divers, gevolgd door MFs (1766 OTUs) en WSFs (848 OTUs). Analyse liet zien dat het type bos een belangrijke factor is die de schimmelsamenstelling bepaalt, waarbij de bodem van WSFs de meest unieke schimmelsamenstelling had. Het was opmerkelijk dat een relatief groot aantal EcM schimmels werd gevonden in bossen die een hoge dichtheid hadden aan AM bomen. De weinige EcM gastheren behorend tot *Coccoloba*, *Guapira* en *Neea* zouden een brugfunctie kunnen vervullen tussen geografisch verspreide PtF bossen, en dit zou ook de overeenkomst in schimmelsamenstelling tussen deze bossen verklaren. De zuurgraad en de C/N ratio bleken ook de schimmelsamenstelling te bepalen in de laagland bossen van Colombia. De pH was met name van belang voor de samenstelling in WSF. Deze bossen komen namelijk voor op bodems die relatief zuur zijn.

Dit promotieonderzoek heeft geresulteerd in 24 nieuwe soorten fungi (Hoofdstukken 2, 3, 5, 6 en 7) waarvan 8 zijn beschreven in dit proefschrift. Zo werd veelvuldig een

roze boleet gevonden in PtFs in de MC regio en in ZBS. Gebaseerd op moleculaire en morfologische data werd geconcludeerd dat dit een nieuwe soort betreft die *Austroboletus amazonicus* A.M. Vasco-Pal. & C. López-Quint. werd genoemd (Hoofdstuk 5). Vruchtlichamen van *Fistulinella campinaranae* var. *scrobiculata* waren ook algemeen voorkomend in de PtFs. Deze vinding is uniek voor Colombia. Daarnaast werden er 4 nieuwe *Sarcodon* soorten gevonden bij een diverse groep van EcM gastheren (Hoofdstuk 7). Dit genus komt algemeen voor in noordelijke gematigde gebieden en, in mindere mate, in Afrika en Azie. Nu blijkt dat *Sarcodon* ook in Neotropische bossen voorkomt. Tenslotte werden drie nieuwe soorten beschreven van de genera *Coltricia* en *Coltriciella* (Hoofdstuk 6). Er zijn nu 11 soorten van dit genus gevonden in Colombia. De werkelijke verspreiding en diversiteit kan veel groter zijn omdat de paddenstoelen van dit genus vaak klein zijn en voorkomen in houtachtig afval en aan de onderzijde van gevallen bomen, waardoor ze moeilijk te vinden zijn. Het feit dat DNA van *Coltriciella minuta* werd gedetecteerd op wortelmateriaal van *Dicymbe* en *Pseudomonotes* (Hoofdstuk 3) ondersteunt de EcM status van dit genus.

Samenvattend kan worden geconcludeerd dat de MF, PtF en WSF ecosystemen in Colombia divers zijn aan EcM schimmels. Het is echter zo dat deze diversiteit lager is dan waargenomen in bossen in gematigd klimaat, uit delen van Afrika en Azie en in mono-dominante bossen in Guyana waar Fabaceae voorkomen. De relatieve diversiteit aan EcM schimmels in de MFs en PtFs is opmerkelijk gezien de relatief lage aantallen aan EcM gastheerbomen. Dit kan komen omdat deze bomen een brugfunctie vervullen voor bossen waarin EcM gastheren veel meer voorkomen. Toekomstige studies moeten de gehele biodiversiteit aan EcM schimmels in het Amazone gebied ontrafelen alsmede hun rol in het functioneren van dit gebied met zijn brede spectrum aan ecosystemen.

Resumen

Las micorrizas constituyen un grupo de hongos que crecen asociados con las raíces de las plantas, siendo esta una de las interacciones simbióticas más ubicuas en los ecosistemas naturales. Se estima que alrededor de 50,000 especies de hongos forman asociaciones micorrízicas con aproximadamente 250,000 especies de plantas vasculares y no vasculares (Smith y Lee 2008; van der Heijden et al. 2015). Existen diferentes tipos de micorrizas como son micorrizas arbusculares (AM), ectomycorrhizas (EcM), orquideo-micorrizas y micorrizas ericoides. Los hongos AM se asocian con aproximadamente el 74% de las especies de plantas, mientras que las EcM, micorrizas ericoides y micorrizas de orquídeas interactúan con el 2%, 1% y el 9% de las especies de plantas, respectivamente (Brundrett 2009; van der Heijden et al. 2015). Los hongos EcM promueven el crecimiento vegetal, mitigan el estrés y mejoran el establecimiento de las plántulas; estudios sugieren que además promueven la dominancia de ciertas especies de árboles y disminuyen los efectos negativos causados por patógenos en los trópicos (McGuire 2007; Rodríguez et al. 2004). Se piensa que alrededor de 6000 especies de hongos EcM - pertenecientes a los phyla Ascomycota, Basidiomycota y Zygomycota- que interactúan con 20,000-50,000 especies de linajes específicos de plantas (Rinaldi et al. 2008; Tedersoo et al. 2010c). La simbiosis ectomicorrízica se suponía restringida a bosques de regiones templadas y boreales que están dominados por árboles de las familias Pinaceae, Fagaceae, Betulaceae, y Salicaceae, así como la subfamilia Leptospermoideae de Myrtaceae (Henkel et al. 2002; Smith y Lee 2008). Por otro lado, los trópicos se suponían dominados por hongos AM debido a la predominancia de plantas hospederas de estos hongos simbiontes y a la ausencia de cuerpos fructíferos de hongos EcM en los bosques tropicales (Henkel et al., 2002). Sin embargo, estudios recientes hicieron evidente la presencia de hongos EcM en los trópicos, asociados a plantas de las familia Fabaceae (subfamilias Papilionoideae y Caesalpinioidea), Gnetaceae, Nyctaginaceae, Polygonaceae y Dipterocarpaceae (ca. Henkel et al. 2012; López-Quintero et al. 2012; Moyersoen 2006; Roy et al. 2016; Singer & Araujo 1979; Singer et al. 1983; Smith et al. 2011, 2013; Tedersoo & Nara 2010).

Colombia es considerado el cuarto país más biodiverso del mundo y el más megadiverso por kilómetro cuadrado. Se han registrado 36.600 especies de plantas en el país, y por la asociación entre éstas y los hongos, se espera que el número de especies de hongos supere las 156.000. Sin embargo, hasta el 2013 tan solo 1239 especies de macrohongos (Ascomycota y Basidiomycota) se habían registrado para el país (Vasco-Palacios et al. 2013), además, no existe un registro del número de especies de otros grupos taxonómicos, lo que significa que no conocemos ni siquiera el 10% de la diversidad total de hongos de Colombia. Del total de macrohongos, tan solo un 20% se había registrado para la región Amazónica a pesar de que dicha región se considera un "hotspot" de biodiversidad (Vasco-Palacios y Franco-Molano 2013).

En Colombia los estudios sobre la diversidad de hongos EcM se han restringido principalmente a bosques montanos dominados por robles (*Quercus humboldtii*), y un importante número de nuevas especies de hongos de los géneros *Amanita, Boletus, Cortinarius, Craterellus, Inocybe, Rozites, Lactarius, Leccinum, Russula y Tylopilu*s han sido descritas de este ecosistema (Franco-Molano et al. 2000; Halling 1996; Halling & Mueller 2005; Mueller 1996; Mueller & Wu 1997; Singer 1963; Tullos & Molano 2008; Tullos et al. 1992; Vasco-P. et al. 2013). Hasta el 2010, únicamente 14 especies de hongos EcM habían sido reportadas para la Amazonía colombiana, colectadas en bosques de tierra-firme en los que crece *Pseudomonotes tropen*bosii (PtF), un árbol endémico de la familia Dipterocarpaceae, cuyos miembros asiáticos son conocidos por formar simbiosis ectomicorrízica con hongos (López-Quintero et al. 2012; Vasco-Palacios y Franco-Molano 2013). Otros posibles simbiontes de hongos EcM han sido registrados para bosques de tierra-firme y bosques de arenas blancas en la Amazonía colombiana. El presente trabajo tuvo como objetivo principal estudiar la diversidad de hongos EcM en diferentes ecosistemas de la región Amazónica colombiana. Los ecosistemas escogidos fueron bosques de arenas blancas (WSF en inglés) y bosques de tierra firme (TF). Los bosques de arenas blancas se encuentran únicamente hacia la parte norte de la Amazonía, desarrollándose sobre suelos de arenas blancas cuarcíticas, con muy baja capacidad de intercambiar nutrientes y deficientes en fósforo, estos suelos son ácidos y poseen una baja capacidad de retención de agua (Fine et al. 2010; Janzen 1974; Jiménez et al. 2009; Peñuela-Mora 2014). Las formaciones vegetales desarrolladas en estos suelos corresponden con mosaicos de selvas bajas, con árboles de troncos delgados en los que crecen especies pero abundantes individuos por especie, en comparación con los bosques de tierra-firme (Janzen 1974; Jiménez et al. 2009; Peñuela-Mora 2014). Las familias Fabaceae, Clusiaceae y Malvaceae son dominantes en estos bosques (Calle-Rendón et al. 2011; Fine et al. 2010; Peñuela-Mora 2014) y los géneros de Fabaceae, *Dicymbe* y *Aldina*, han sido registrados como formadores de ectomicorrizas en bosques en Guyana, Guyana Francesa, Brasil y Venezuela (Singer 1979; Henkel et al. 2002*,* 2012; Roy et al. 2016).

Por el contrario, los bosques de tierra-firme se desarrollan en suelos arenosos o arcillosos bien drenados, con una alta capacidad de intercambio catiónico y de fósforo disponible (Quesada et al 2011; Peñuela-Mora 2014). Este tipo de bosque cubre alrededor del 80% de la superficie total de la Amazonia (ter Steege et al., 2000) y la altura del dosel varía de 25 a 35 m. El sotobosque suele ser más denso que el de los bosques de arenas blancas y el bosque alberga una de las más diversas comunidades de plantas del mundo, con pocos individuos por especie (De Oliveira y Mori 1999; Duivenvoorden y Duque 2010; Ter Steege et al. 2010). En este tipo de bosques, se encuentran representantes de los géneros ectomicorrizicos *Coccoloba* (Polygonaceae), *Guapira* y *Neea* (Nyctaginaceae) en muy bajas densidades. En algunas zonas de la Amazonía occidental, el árbol *Pseudomonotes tropenbosii* (Dipterocarpaceae) constituye una especie de importancia ecológica con un Indice de Importancia Valor

(IVI) que oscila entre el 16-18%, el cual es endémico de la región y se presume ectomicorrízico (Appanah y Turnbull 1998; Londoño et al 1995. Parrado-Rosselli 2005).

DIVERSIDAD DE HONGOS

La diversidad de hongos ectomicorrízicos presentes en bosques de arenas blancas y en el monte firme con presencia de *P. tropenbosii* se caracterizó mediante la recolección e identificación de cuerpos fructíferos y el estudio de las raíces micorrizadas. Los hongos EcM recolectados durante el trabajo de campo se identificaron con base en el estudio de los caracteres macro y microscópicos de los cuerpos fructíferos colectados, así como por el análisis de las secuencias obtenidas de las regiones del espaciador interno (ITS) del ADN ribosomal (rDNA) y la región D1/D2 de la subunidad grande del rDNA (LSU-rDNA) (Capítulos 2, 3, 5, 6, 7). Las raíces micorrizadas se estudiaron con técnicas moleculares, se extrajo ADN de los ápices de las raíces micorrizadas y para la identificación del hongos simbionte y de la planta hospedera se amplificaron regiones específicas de ADN (la región ITS 1 y 2 del rDNA y la región del intrón trnL (UAA) del cloroplasto, respectivamente) (Capítulos 2 y 3). Además, con el fin de caracterizar la comunidad de hongos del suelo en los dos bosques descritos anteriormente, y de bosques de tierra-firme con presencia de los géneros ectomicorrizicos *Coccoloba* (Polygonaceae), *Guapira* y *Neea* se utilizó secuenciación de alto rendimiento (454-pirosecuenciación) y se analizó el efecto del tipo de bosque y de los factores edáficos sobre la composición y la riqueza de especies de hongos del suelo de estos tres tipos de bosque a escala local (Capítulo 4).

En general se encontró una importante diversidad de hongos micorrízicos en los ecosistemas estudiados en la Amazonía colombiana. Se identificaron un total de 114 especies de hongos EcM con base en cuerpos fructíferos, incluyendo 83 especies colectadas en PtF (Capítulo 3) y 49 especies en WSF (Capítulo 2). Las familias más abundantes fueron Russulaceae (28 especies), Clavulinaceae y Boletaceae (15 especies), Hymenochaetaceae (14 especies), Amanitaceae (11 especies) y Cantharellaceae (7 especies). Treinta y un especies de macrohongos se registraron por primera vez para Colombia, y 24 representaron nuevas especies. A pesar de la alta diversidad de hongos EcM encontrados, la curva de acumulación de especies indicó que la diversidad de hongos EcM no se recuperó totalmente durante el muestreo. Los estimadores de riqueza sugirieron que la riqueza de especies para PtF es de aproximadamente 125-150 especies y 100 para WSF. Además, 24 unidades taxonómicas identificadas partir de las raíces micorrizadas no se encontraron formando cuerpos fructíferos, lo que puede indicar que la riqueza de especies EcM en los bosques estudiados es mayor. Nuestros resultados concuerdan con los hallazgos previos, según los cuales la riqueza de hongos EcM encontrado es baja si se compara con el número de especies registrado para bosques de las zonas templadas. Esto se explica por el hecho de que los bosques

templados están dominados por árboles de linajes EcM tales como fagales, Pinaceae y salicáceas. Por el contrario, los bosques tropicales son ricos en AM-plantas a excepción los bosques monodominantes en la zona del escudo guyanés y algunos bosques que presentan dominancia o abundancias importantes de plantas reconocidas por ser hospederas de hongos ectomicorrízicos (Henkel et al. 2012; Moyersoen 2006; Tedersoo et al. 2014; Smith et al. 2011; 2013).

El capítulo 4 describe el primer estudio profundo de la diversidad de hongos del suelo en tres bosques húmedos tropicales en la región Amazónica de Colombia. Utilizando un método de secuenciación de alto rendimiento se obtuvieron 3145 unidades taxonómicas operativas (UTOs) para los bosques de PtF, WSF y TF. El phylum Ascomycota (59.22% de las UTOs que representa el 46.7% de las secuencias) fue el más diverso, seguido de Basidiomycota (36.9% de las UTOs y el 37.1% de las secuencias), Zygomycota-mucoromycotina (1.68% de las UTOs y el 14.7% de las secuencias), y Cryptomycota (1,11% de las UTOs y el 0.3% de las secuencias). La comunidad de hongos de PtF fue la más diversa con 2139 UTOs, seguidos de MF con 1766 UTOs y WSF con 848 UTOs. El análisis de escalamiento multidimensional no métrico (NMDS) reveló que el tipo de bosque juega un papel fundamental en la estructura de la comunidad de hongos; estos resultados fueron apoyados por el test de Mantel que mostró que la composición de la comunidad fúngica del suelo es más similar en muestras del mismo tipo de bosques, sin importar la distancia entre las parcelas. Es de destacar que en este estudio se encontró un número relativamente alto de hongos EcM en los bosques de tierra-firme que presentan una alta densidad de árboles de AM, y en que se encuentran árboles hospederos de los géneros *Coccoloba*, *Guapira* y/o *Neea* en bajas densidades de individuos. Especies de hongos EcM se compartieron entre los tres tipos de bosques estudiados y surgió la hipótesis de que a pesar de la baja dominancia, los géneros *Coccoloba*, *Guapira* y/o *Neea* estarían sirviendo de puentes al facilitar la dispersión y distribución de los hongos EcM entre áreas y entre parches de bosques con mayor abundancia de plantas hospedantes separadas geográficamente, como es el caso de bosques de tierra firme con *P. tropenbosii* e incluso con parches de WSF. También se encontró que el pH y la relación C/N en el suelo son determinantes de la composición de los hongos en los bosques de tierras bajas en Colombia, el pH fue de particular importancia en WSF, ya que estos bosques se desarrollan en suelos muy pobres y ácidos y presentaron la composición de la comunidad de hongos más particular de los tres tipos de bosques estudiados.

Durante el desarrollo de la investigación, se identificaron 24 nuevas especies de hongos (capítulos 2, 3, 5, 6 y 7) de las cuales 8 se describen en este documento. Dos especies de boletales con poros rosados fueron particularmente abundantes en los bosques con *Pseudomonotes tropenbosii,* con base en datos moleculares y morfológicos se estableció que se trataba de la nueva especie descrita en esta Tesis, *Austroboletus amazonicus* Vasco-Pal. & C. López-Quint. sp nov. y de *Fistulinella campinaranae* var.

scrobiculata un nuevo registro para Colombia (Capítulo 5). En total se encontraron 14 especies de Boletaceae, de las cuales 10 permanecen sin identificar a nivel de especie, debido principalmente a la falta de monografías específicas para muchos de los géneros de boletales tropicales. Dos especies más del género *Austroboletus*, A. festivus y *Austroboletus* sp. 3 fueron registradas para PtF.

En los ecosistemas amazónicos, especies de *Coltricia* y *Coltriciella* se encontraron en bosques con *P. tropenbosii* y WSF. A pesar de que las especies de estos géneros suelen ser terrestres y encontrarse asociadas con la madera en descomposición, las relaciones tróficas y la estrategia nutricional de la mayoría de las especies son desconocidas aún. Estudios recientes, basados en datos morfológicos y moleculares, han demostrado que varias especies de los dos géneros son ectomicorrízas. Una nueva especie de *Coltricia* y dos de *Coltriciella* se describen en el capítulo 6, aumentando el número de especies conocidas para el país a 7 y 4 respectivamente. La distribución y diversidad de estos géneros se supone mayor a la conocida, debido a que muchas especies forman cuerpos fructíferos pequeños que se desarrollan en residuos leñosos y en la parte inferior de árboles caídos, lo que los hace difíciles de encontrar. La nueva especie *Coltriciella minuta* se encontró en raíces micorrizadas de *Dicymbe* y *Pseudomonotes* (Capítulo 3) confirmando el estatus EcM de este género.

Una importante contribución al conocimiento de macrohongos en el Neotrópico se hizo para el género EcM *Sarcodon* (Bankeraceae, Thelephorales, Basidiomycota). Especímenes de *Sarcodon* fueron registrados por primera vez para Colombia de bosques con *Pseudomonotes* y de bosques de arenas blancas, los cuales representan cuatro nuevas especies para la ciencia. Estos registros elevan el número de especies conocidas de *Sarcodon* para el Neotrópico a 10, demostrando que el género tiene una distribución y gama de huéspedes más amplia que lo que se consideraba anteriormente, ya que el género se consideraba restringido, principalmente, a las regiones templadas y boreales (Capítulo 7). *Sarcodon colombiensis* sp. nov. y *S. rufogriseus* sp. nov. se detectaron en raíces micorrizadas de *P. tropenbosii,* probando la relación simbiótica con este árbol endémico (Capítulo 3).

Este estudio resalta la importancia de investigar la diversidad de hongos en Colombia, campo aún incipiente para muchas regiones y ecosistemas del país. El veintisiete por ciento de las especies encontradas en este trabajo corresponden con nuevos registros para el país y el 21% con nuevas especies para la ciencia. En general, TF, PtF y WSF presentan una alta diversidad de especies de hongos EcM. Sin embargo, esta diversidad es menor a la encontrada en bosques templados, o en algunos bosques tropicales en África, Asia y bosques monodominados por especies de árboles de la familia Fabaceae en Guyana. En el área occidental de la región Amazónica, los árboles hospederos de hongos EcM son menos abundantes y en algunos casos se presentan en bajas densidades (Ej. *Neea*, *Coccoloba* o *Nea*), además. se considera

que estos árboles sirven como puentes de dispersión entre bosques donde los árboles simbiontes EcM son más abundantes (Ej. bosques con P. *tropenbosii* o WSF). Estudios futuros deben profundizar en la caracterización de la diversidad de hongos EcM en ecosistemas amazónicos, con el fin de revelar una mejor comprensión de la distribución de los hongos EcM, su especificidad de huésped, y los factores que influyen en la distribución EcM en diferentes tipos de bosques. Se recomienda realizar estudios en parcelas permanentes de plantas que permitan hacer análisis integrativos, vinculando la composición de la comunidad de hongos a la dinámica de la vegetación, los factores edáficos y el tipo de bosques. Esto puede facilitar la comprensión del papel de los hongos en la estructuración de la diversidad y la abundancia de árboles de la selva tropical, así como la influencia de las plantas sobre la distribución de la diversidad de hongos EcM a través de la región Amazónica. Una mejor comprensión de esta asociación hongo-planta revelará el impacto de los hongos en la ecología de los bosques húmedos neotropicales de tierras bajas, especialmente a la luz del cambio climático.

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Curriculum Vitae

Aída Marcela Vasco Palacios was born in Bogotá, Colombia. She received her BSc degree in Biology at the National University in 2003. After her graduation, she participated in the project "Microthesauri related to traditional knowledge associated with biodiversity in seven tribes in the Andean region" at the Institute Alexander von Humboldt. She was also teaching at the Javeriana University. In 2004, Aída moved to Medellín to start her M.Sc. and she obtained her diploma in September 2007. During her M.Sc., she studied the ethnomycology knowledge of three indigenous groups in Amazonia, under the supervision of Dra Ana Esperanza Franco at the Antioquia University. In 2008-2010 Aída developed the project "Macrofungi from Amazonian ecosystems" at Antioquia University. She was awarded a KLARF grant (2008, Latin America Research Fellowships Programme) to visit the fungal collection at Kew Botanical Garden, UK. Aída started her PhD in 2010 within the Yeast and Basidiomycota Group at CBS-KNAW Fungal Biodiversity Centre under the supervision of Dr. Teun Boekhout and the Molecular Microbiology group of the Department of Biology at Utrecht University under the supervision of Prof. Dr. H.A.B. Wösten. Her project "Ectomycorrhizal fungi in Amazonian tropical forests in Colombia" was financially supported during this period by NUFFIC (2010-2015), the Faculty for the Future - Schlumberger Foundation (FFTF, 2011-2013), the International Science Foundation (IFS Grant D/5052-1, 401 2011-2-13f) and the University of Utrecht (2014-2015) and the results are described in this thesis. As a member of the board of the Latin American Mycological Association (ALM, 2011-2015), Aída participated as organizer and coordinator of the VIII Latin American Mycological Congress (CLAM) held in Medellín in 2014. She was also co-editor of the conference proceedings. Aída has been interested in forming new generations of local mycologist, she has participated as a trainer and organized several courses on taxonomy and ecology of fungi (Javeriana University, Antioquia University, CLAM VII and VIII). She is part of the directive board of the Fundación Biodiversa Colombia, an NGO that promotes and developes research and conservation in the biological, social and cultural fields and in other areas of knowledge that contribute to the preservation of the biological and cultural heritage of Colombia. Her main interests are to contribute to the knowledge of the diversity of fungi in Colombia and to understand the ecological role of EcM fungi in Amazonian forests, in particular to help to conserve these invaluable ecosystems.

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