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Ectomycorrhizal fungi diversity in a white sand forest in western Amazonia

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ABSTRACT

The genera *Dicymbe* and *Aldina* (Fabaceae) host ectomycorrhizal fungi (EcM) and are common in white sand forests (WSFs), a highly specialized habitat with a high level of plant endemism compared with *terra-firme* forests. In this study, we visited four times a 1-ha permanent plot established in a small patch of a WSF in the south of Colombia Amazonia. Forty-eight species of EcM fungi were recovered from sporocarps and 15 ITS species-level were detected from root tips. Seventeen species were new reports to Colombia and seven corresponded to undescribed species. These results confirm that this WSF supports a significant EcM fungal diversity. Most of the species found in this study have been previously reported to be associated with other legume and/or dipterocarp species from geographically distant forests. The long-distance occurrence combined with low host specificity, suggest the possibility of gene flow between geographically distant populations of EcM fungi in neotropical lowland rainforests.

1. Introduction

Mycorrhizal fungi are a diverse group of mutualistic root symbionts. Four major types of mycorrhizal interactions have been described based on their structure and function: ectomycorrhiza (EcM), arbuscular mycorrhiza, orchid mycorrhiza, and ericoid mycorrhiza. The EcM fungi form an external sheath enclosing the plant root, and the hyphae penetrate the spaces between the cortical and epidermal cells of the root, thus forming the Hartig network (Halling, 2001; Bonfante and Genre, 2008; Smith and Read, 2008). The EcM interaction improves the acquisition of nitrogen and phosphorus by the host plant through an increased absorptive surface area, and EcM fungi have been also shown to facilitate the acquisition of other nutrients. The hyphae that envelop the root tips act as a physical barrier to pathogens, and secondary metabolites produced by EcM fungi are toxic to pathogenic fungi, nematodes, and bacteria (Agerer, 2006; Smith and Read, 2008). In return, the EcM fungi receive organic compounds from their host plants, including glucose (Bonfante and Genre, 2008; Smith and Read, 2008). Estimates indicate that there are about 6000 species of EcM fungi (Ascomycota, Basidiomycota, and Mucoromycota-Endogonales) that are associated with 20,000–50,000 species of plants (Rinaldi et al., 2008; Tedersoo et al., 2010a). However, there are still many unknown species of several fungal genera occurring in tropical ecosystems, and thus more taxonomic efforts in those areas are needed to complete knowledge of the EcM fungal diversity. The distribution of EcM fungi was considered to be largely limited to

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temperate and Circumboreal regions with vegetation dominated by Pinaceae, Fagaceae, Betulaceae, and Salicaceae, and in Australia also the Myrtaceae subfamily Leptospermoideae (Henkel et al., 2002; Smith and Read, 2008). The arbuscular mycorrhizal fungi generally dominate the tropics, except in some areas where Holarctic plants such as *Quercus* species (Fagaceae) are dominant. However, recent studies provided evidence for the presence of EcM symbiosis in tropical ecosystems (Alexander, 2006). In neotropical lowland forests, the plant hosts identified are members of the families Dipterocarpaceae, Gnetaceae, Nyctaginaceae, Polygonaceae, and Fabaceae (subfamilies Caesalpinioideae and Papilionoideae) (Singer and Araujo, 1979; Singer et al., 1983; Henkel et al., 2002, 2012; Haug et al., 2005; Moyersoen, 2006, 2012; Tedersoo et al., 2010b; Smith et al., 2013; Sulzbacher et al., 2013; Roy et al., 2016; Vasco-Palacios et al., 2014; Vasco-Palacios, 2016). Singer and colleagues were the first to report the presence of EcM fungi from lowland white sand forests (WSFs) in Brazil (Singer and Araujo, 1979, 1986; Singer et al., 1983). They proposed that EcM fungi provide the host with an ecological advantage in areas with poor soils such as WSFs (Singer and Araujo, 1979).

In Guyana, long-term investigations have demonstrated a high diversity of EcM fungi associated with the dominant species *Dicymbe corymbosa, Dicymbe altsonii* (Fabaceae, subfamily Caesalpinioideae), and *Aldina insignis* (Fabaceae, subfamily Papilionoideae) in the Pakaraima mountains, and *Dicymbe jenmanii* and *Pakaraimaea dipterocarpacea* (Dipterocarpaceae) from savanna-fringing ecosystems (Henkel et al., 2002, 2012; Smith et al., 2011, 2013). A total of 174 species of EcM fungi have been reported from *Dicymbe*-, *Aldina*-, and *Pakaraimaea*-dominated forests in Guyana (Henkel et al., 2012; Henkel, 2015). A high diversity and heterogeneity of EcM fungal communities was also found in WSFs from Brazil and French Guyana (Roy et al., 2016); 62 morphospecies belonging to 23 genera were found from 10 plots of 200 m × 100 m (2 ha), in a 2-y survey. In general, the diversity of EcM fungi in mixed tropical rainforests (e.g., WSFs from Brazil and French Guyana) is lower than that of larger and monodominant patches with EcM host trees (e.g., Guyana) (Henkel et al., 2012; Smith et al., 2013; Roy et al., 2016).

The Amazon region likely harbors the highest biodiversity of organisms in the world (Soares-Filho et al., 2006; Hoorn et al., 2010; Ter Steege et al., 2013), and it comprises a mosaic of ecosystems, including the WSFs known as “varillal” (Peru and Colombia), “caatinga” (Venezuela), or “campina/campinarana” (Brazil). Extensive WSFs are present at the Guiana Shield and in eastern Amazonia, with small patches occurring in north and southwestern Amazonia (Peñuela-Mora, 2014). Soils in the WSFs consist almost exclusively of quartz sands and are characterized by a low nutrient exchange capacity, low phosphorus content, acidic pH, and low water-holding capacity (Fine et al., 2010; Peñuela-Mora, 2014; García-Villacorta et al., 2016). The vegetation on the WSFs is composed of small trees, the plant diversity is low when compared with that of *terra-firme* forests, and the trees tend to be endemic and highly specialized for WSF habitats (Janzen, 1974; Peñuela-Mora, 2014; García-Villacorta et al., 2016). Fabaceae, Clusiaceae, and Malvaceae are abundant families in these forests in western Amazonia (Fine et al., 2010; Calle-Rendón et al., 2011; Peñuela-Mora, 2014). The genera *Dicymbe* (Fabaceae, subfamily Caesalpinioideae) and *Aldina* (Fabaceae, subfamily Papilionoideae) have been reported as EcM hosts in Guyana, French Guyana, Venezuela, and Brazil (Singer and Araujo, 1979; Henkel et al., 2002, 2012; Smith et al., 2013; Roy et al., 2016). In Colombia, WSFs (Fig. 2C and G) are present in small patches, and species of the endemic *Dicymbe uaiparuensis* and a yet unknown *Aldina* species have been reported to be abundant (25% of all individuals) in a 1-ha plot in the Zafire Biological Station (ZBS) located in the south of the Colombian Amazon region (Peñuela-Mora, 2014). In Colombia, the knowledge of EcM fungi from tropical lowland forests is incipient, and only 20 species have been reported from forests with the dipterocarp *Pseudomonotes tropenbosii* and with leguminous hosts (López et al., 2002; Vasco-Palacios and Franco-Molano, 2013; Vasco-Palacios et al., 2014; Grupe et al., 2016; Vasco-Palacios, 2016). Studies on the diversity and ecology of tropical EcM fungi have provided new insights into the biogeographical patterns of species and their hosts at the regional and global scales, leading to the discovery of new taxa (e.g., Henkel et al., 2002, 2011, 2012; Moyersoen, 2006, 2012; Tedersoo et al., 2010a, 2014; Smith et al., 2011, 2013; Uehling et al., 2012a,b; Sulzbacher et al., 2013; Moyersoen and Weiss, 2014; Vasco-Palacios et al., 2014; Grupe et al., 2015, 2016; Roy et al., 2016; Vasco-Palacios, 2016). In this study we wanted to answer the following questions: how diverse is the composition of EcM fungi in WSF from Colombia?; do these forests host similar EcM fungal species compared with other ectotrophic forests in the neotropics?

2. Materials and methods

2.1. Study area

The fieldwork was performed in a previously established 1-ha plot in a WSF in the ZBS area, located in an undisturbed area in the southern Colombian Amazon (coordinates 4°0′21″ S, 69°53′55″ W) (Fig. 1). The total size of the WSF patch is ca. 10 ha. This region shows a mean monthly rainfall of 277 mm with a drier period from June to September (mean monthly rainfall of 189 mm), and a rainy season from October to May (mean monthly rainfall of 323 mm). The mean temperature is about 26 °C and does not fluctuate significantly during the year. The relative humidity is high, with an annual average of 86% (Jiménez et al., 2009; Peñuela-Mora, 2014). The WSF patch is small, above 10 ha, and is surrounded by a *terra-firme* mixed forest. Soils from the WSF at ZBS belong to the Terciario Superior Amazonico unit (Proradam, 1979; Herrera, 1997), which probably originated from the Guiana Shield (Hoorn, 1994; Hoorn et al., 2010), and are composed mainly of quartz. The terrain is flat and uniform, with a hard-pan at a 90–100 cm depth (Quesada et al., 2009). *D. uaiparuensis* (Fabaceae, subfamily Caesalpinioideae) and an unknown *Aldina* species (Fabaceae, subfamily Papilionoideae) are the most abundant trees species (25% of all individuals) in the patch (Peñuela-Mora, 2014). These two plant genera have been previously reported as EcM hosts in tropical ecosystems (Singer and Araujo, 1979, 1986; Henkel et al., 2012; Smith et al., 2013). The area was visited four times during the rainy season for 1 week each between March 2012 and November 2014.

2.2. Sample collection, description, and species accumulation curve

Sporocarps were collected with preference for those that belong to putative EcM fungal taxa. The specimens were photographed *in situ*, macromorphological characters were described from fresh material, and macrochemical tests were performed (Largent et al., 1977; Franco-Molano et al., 2005). Spore prints were obtained when possible. Color codes were designated according to the Methuen Handbook of Color (Kornerup and Wanscher, 1978). A small fragment of the fruit body was transferred to 2% cetyltrimethylammonium bromide (CTAB) for further molecular analysis. The collections were dried in the field using a sealed container with silica gel. The material was transported to the Fungi Taxonomy

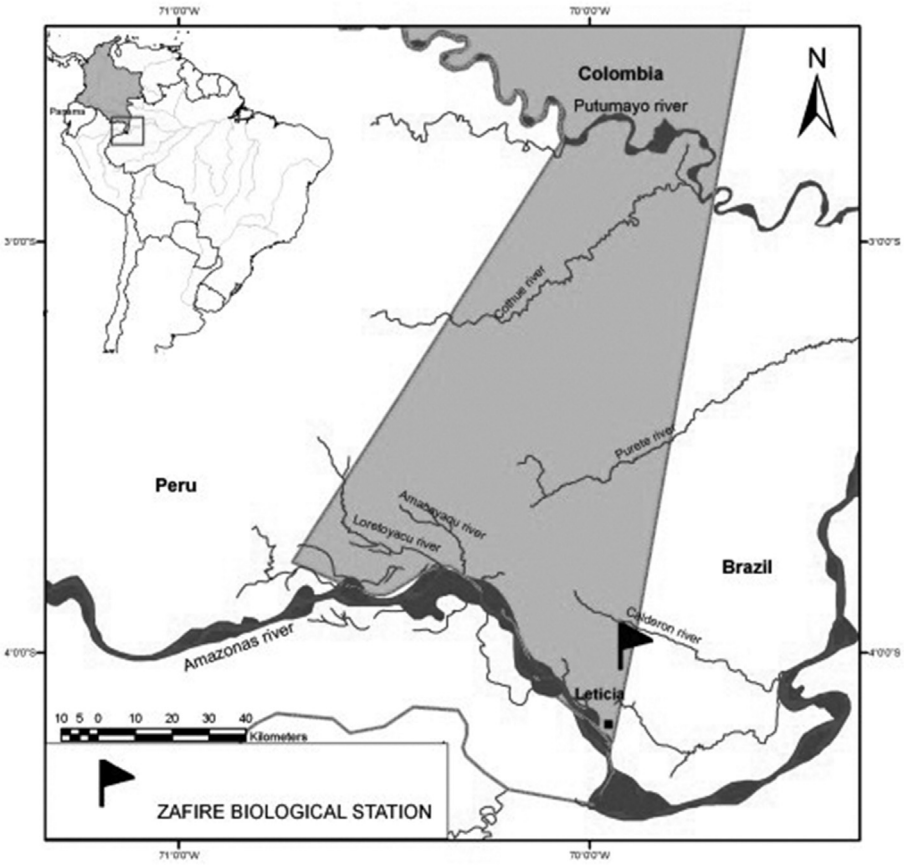

Fig. 1. Map showing the location of the El Zafire Biological Station (ZBS) in the Amazon Department in Colombia. It is bordered to the east by the Brazilian Amazon.

and Ecology lab (TEHO) at the University of Antioquia, Medellín, Colombia. The collections were deposited in the herbarium of the University of Antioquia (HUA). The online version of Index Fungorum (www.indexfungorum.org) was used to update the fungal nomenclature.

A rarefaction curve was constructed for diversity data of individual-based sporocarps collected during the four sampling events, using the function specaccum with 1000 permutations and a 95% confidence interval in the Vegan package of R (Oksanen et al., 2013; R Development Core Team, 2013).

2.3. Root samples and rDNA preservation

Soil samples were taken close to 12 trees of *D. uaiparuensis* and 8 trees of *Aldina* sp. from the 0–15-cm upper soil layer (Fig. 2A and B). The trees were selected in the 1-ha plot, and two soil cores were collected per tree. Owing to the sand soil structure that forms a hard-pan layer and the high density of roots, it was not possible to trace the roots to their trees of origin. This also hindered the collection of soil cores. The soil cores were air-dried and transported to TEHO. The roots were washed with tap water to remove soil residues and examined under a dissecting microscope to observe the presence of a fungal mantle or any other fungal structure. In some cases, the mantle was reduced and not clearly visible. Mycorrhizal root apices were separated according to morphotype (e.g., mantle structure, color of the hyphae, and structure and color of the root tips) and preserved in Eppendorf tubes with CTAB for further molecular analysis to identify the host plant and the EcM fungi (Smith et al., 2013).

2.4. Molecular analyses

DNA was extracted from pieces of the sporocarps using the MasterPure™ Yeast DNA Purification kit (Epicenter, Madison, WI, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS 1–2) regions and the D1/D2 regions of large subunit rDNA were PCR-amplified using the primers ITS1, ITS4, ITS5, and LR0R-LR7 (Vilgalys and Hester, 1990; White et al., 1990; Hopple and Vilgalys, 1994). The polymerase chain reaction 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 45 s at 72 °C; and a final extension cycle of 1 min at 72 °C. The amplicons were visualized on a 1% agarose gel stained with Gel Red (Biotium Inc., San Francisco, CA, USA).

The DNEasy plant mini kit (Qiagen, Crawley, UK) was used for DNA extraction from the mycorrhizal root tips according to the manufacturer's instructions. The ITS region was amplified as described above. In cases in which the entire fungal ITS region could not be amplified, the primers ITS2 and ITS3 were used in combination with ITS1 and ITS4 to amplify shorter ITS regions (1 or 2) for the fungi present in the root. This region was selected because sequence data from these loci are available for many fungal species. The amplicons were visualized Gel Red after separation on 1% agarose gels (Biotium Inc., San Francisco, CA, USA).

Amplicons were purified using a 96-well multiscreen HV plate (Millipore, Billerica, MA, USA) and Sephadex G-50 superfine columns (Amersham Biosciences, Roosendaal, the Netherlands). Sanger sequencing (Sanger and Coulson, 1975) of the purified PCR products was performed using Amplicons sequenced using the primers indicated above on an ABI Prism 3700 Genetic analyzer

Fig. 2. Images of the white sand forest (WSF) in El Zafire (A, B) and representative ectomycorrhizal fungi species collected in the WSF (C–G). Basidiomata of *Russula puiggarii* (Russulaceae) (C), *Clavulina amazonensis* (Clavulinaceae) (D), *Lactifluus subiculatus* (Russulaceae) (E), *Sarcodon rufogriseus* (Bankeraceae) (F), and *Craterellus atratoides* (Cantharellaceae) (G).

(Applied Biosystems, Foster City, CA, USA). Sequences were edited using the program SeqMan of the package DNAStar (Swindell and Plasterer, 1997). The sequences obtained from the sporocarps and roots were deposited in GenBank (Table 1).

2.5. Identification

Sporocarps were identified to species using the classical morphologically based taxonomy. Morphological characters allowed for identification at the genus level. Whenever possible, we identified the specimens to the species level based on morphology using keys and taxonomic descriptions (e.g., Corner, 1950; Bas, 1978; Pegler and Fiard, 1983; Singer et al., 1983; Simmons et al., 2002; Henkel et al., 2011, 2012; Uehling et al., 2012a, 2012b; Wilson et al., 2012). Due to the lack of literature documenting tropical fungal species and the high number of species that might be new to science, we also consulted taxonomic specialists (e.g., Leif Ryvarden, professor emeritus at University of Oslo, an expert on *Coltricia* and *Coltriciella*). In addition, phylogenetic boundaries of the species found were explored by sequence analysis of the ITS rDNA and D1/D2 regions of large subunit rDNA sequences obtained from the collected sporocarps. The sequences obtained were compared with the Basic Local Alignment Search Tool Nucleotide tool (BLASTN) against the sequences obtained in this study, the National Center for Biotechnology Information (NCBI) database, and the User-friendly Nordic ITS Ectomycorrhiza (UNITE) database (sh_general_release_s_30.12.2014; Abarenkov et al., 2010) using the global search tool of the USEARCH7 software program (Edgar, 2010). The ITS sequences were interpreted to represent a different species if they differed by <3% across the ITS region (i.e. 97% similarity) with sequences from the databases and sequences obtained in this study. Major limitations were that it was not possible to get good sequences from all sporocarps, as well as the fact that sequences from neotropical EcM fungi are not well-represented in the databases. Specimens without sequences and that could not be determined to species based on morphology, but were distinguished as being different species of the same genus, were treated as morphospecies (Table 1).

Taxa detected on the roots were identified as phylogenetic species using BLASTN comparisons against the sequence databases (NCBI and UNITE) and against sequences obtained from our

Table 1
Ectomycorrhizal fungi observed as sporocarps and detected on the root tips from the WSF in El Zafire in the Southern Colombian Amazon region. Species from sporocarps were identified based on morphology and ITS rDNA sequences, from the root tips based on ITS2 rDNA sequences.

Taxa	Occurrences sporocarps	Occurrences root-tips	Represent. Voucher	Accession number	Similar species, ITS sequences match with >97 % similarity	e-value	Distribution[1]
Amanitaceae							
Amanita campinaranae [a]	1		AMV1994a	–			BR, GUY
Amanita crebresulcata	1		JOH92	–			BR
Amanita lanivolva [a]	1		AMV1999	KT354671			GUY
Amanita xerocybe [a]	1		AMV1110	KT354672	*Amanita xerocybe* TH8930-KC155384	0	GUY, BR
Amanita sp. 1 sect. Vaginatae*	3		JOH12	KT354674, KT354673			
Amanita sp. 2 sect. Vaginatae*	4		AMV2021	KT354676	*Amanita* sp. 11 TH8165, TH8920-KT339246, KT339260	2E-152	
Amanita sp. 3 sect. Vaginatae	1		JOH73	–			
Amanita sp. 4 sect. Amanita*	1		JOH97	–			
Amanita sp. 5 subgen. Lepidella*	1		JOH91	–			
Bankeraceae							
Sarcodon rufogriseus	2		AMV1989	KT354755			
Boletaceae							
Singerocomus inundabilis [a,b]	3		AMV1843	KT354677	*Singerocomus inundabilis* TH10087-KT380014	0	GUY
Uncultured Boletaceae		2		KT354776*, KT354777*			
Cantharellaceae							
Craterellus atratoides [a]	7		AMV1870	KT354698, KT354699	*Craterellus atratoides* TH8243-KT339209	0	GUY
Craterellus atratus [a]	5		AMV1991A	–			GUY
Craterellus cinereofimbriatus [a]	1		AMV2245	KT354702	*Craterellus cinereofimbriatus*TH8999-JQ915104	0	GUY
Craterellus strigosus [a]	3		AMV1844	KT354701			GUY
Clavulinaceae							
Clavulina amazonensis [a, b]	6	2	AMV1877	KF937312, KT354683*	*Clavulina amazonensis* TH9191-HQ680356	0, 7e-85◦	GUY, BR
Clavulina cf. *effusa*	1		AMV2206	KT354680			GUY
Clavulina connata [a]	1		AMV2239	–			BR
Clavulina kunmudlutsa [a]	1		AMV1875	–			GUY
Clavulina sprucei [a]	2		AMV2002	KT354682	*Clavulina sprucei* MCA3989-HQ680345	0	GUY
Clavulina sp.	1		AMV2006	KF937317			
Cortinariaceae				–			
Cortinarius sp. 1	3	1	AMV2013	KT354695, KT354692*			
Cortinarius sp. 2	2		AMV2000	KT354697	Uncultured *Cortinarius* clone ECM37-1-KC155366	0	GUY
Hymenochaetaceae							
Coltricia cinnamomea	1		AMV2018	KT354685			GUY, BR
Coltricia hamata [a]	1		AMV1982	KT354687			BR, GUY, COL, VEN
Coltricia verrucata [a]	3		AMV2014	KT354688	*Coltricia verrucata* Dai 15120-KU360660	0	GUY
Coltricia sp. 1	1		AMV2212	–			
Coltriciella dependens [a]	3		AMV2217	KT354686			AUS, N-ZEA, SEY, C.A., USA
Coltriciella sp. nov. *	6		AMV2213	–			
Inocybaceae							
Uncultured *Inocybe*		1		KT354708*			
Russulaceae							
Lactarius brasiliensis	2	1	AMV1874, AMV1998	KT354738, KT354737*			GUY, BR
Lactarius sp. 1	1		AMV2028	–			
Lactarius sp. 2	3		JOH93	–			
Lactifluus annulifer [a, b]	2	2	JOH47	KT354739	*Lactarius* cf. *annulifer* TH9014-KC155376	1E-82	GUY, BR
Lactifluus subiculatus [a]	5		AMV2003, AMV 2004	KT354741	*Lactifluus subiculatus* SLM 10114-JQ405654	0	GUY
Uncultured *Lactifluus* sp. 1		16		KT354728*, KT354735*, KT354736*, KT354734*, KT354731*, KT354732*, KT354709*, KT354730*, KT354729*, KT354727*, KT354726*, KT354725*, KT354724*, KT354723*			

(*continued on next page*)

Table 1 (*continued*)

Taxa	Occurrences sporocarps	Occurrences root-tips	Represent. Voucher	Accession number	Similar species, ITS sequences match with >97 % similarity	e-value	Distribution[1]
Uncultured *Lactifluus* sp. 2		1		KT354722*			
Uncultured *Lactifluus* sp. 3		1		KT354720*			
Russula cf. *hygrophytica*	1		JOH9	–			IN, Lesser Antilles
Russula cf. *foetens*	1		AMV2200	KT354742			BR, G.B.
Russula cf. *rhizomorpha*	2		JOH100, JOH20	KT354743			BR
Russula cf. *rosea*	1		AMV1996	KT354744			G.B., IRE, USA
Russula puiggarii	4		AMV1995, JOH10	KT354746	Uncultured *Russula* ECM720-JN168739, *Russula puiggarii* G3197-KJ786696	0	GUY, BR
Russula sp. nov. 1 *	5		AMV2001, AMV2022	KT354748, KT354750			
Russula sp. nov. 2 *	2		JOH46	KT354754	*Russula* sp. TH9157-KT339230	0	GUY
Russula sp. nov. 3*	2	8	AMV2205	KT354751, KT354717*, KT354718*, KT354715*, KT354716*, KT354714*, KT354713*, KT354712*			
Russula sp. 2	2		AMV2209	KT354752			
Russula sp. 3	1		AMV1996	KT354744			
Russula sp. 4	1		JOH103	–			
Russula sp. 6	1		AMV2200a	–	*Russula* MCA4008-		
Russula sp. 7	1		AMV2208	–			
Uncultured *Russula*	1			KT354710*			
Sebacinaceae							
Sebacina sp. [b]	1		AMV2015a	KT354769	Uncultured *Sebacina* TUB 019406-HQ154314		
Uncultured *Sebacina* sp. 2		1		KT354757*			
Uncultured *Sebacina* sp. 3		7		KT354758*, KT354759*, KT354760*, KT354761*, 54763*, KT354762*, KT354767*			
Uncultured *Sebacina* sp. 4		3		KT354764*, KT354765*, KT354766*	Uncultured Sebacinales ECM828-JN168755, host *Dicymbe corymbosa*	2E-144	GUY
Uncultured Sebacinaceae		1		KT354768*			
Thelephoraceae							
Uncultured *Tomentella* sp. 1		4		KT354770*, KT354771*, KT354772*, KT354773*			
Uncultured *Tomentella* sp. 2		2		KT354774*, KT354775*	Uncultured *Tomentella* MES348-JN168772, host *Dicymbe corymbosa*	2E-154	GUY

In Taxa: "[a]" New record of species for Colombia, "[b]" New record of genera for Colombia, 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 not yet formally described. In column Distribution: Correspond to countries or regions where the species have been previously reported: Australia (AUS), Brazil (BR), Colombia (COL), French Guyana (FG), 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.).

sporocarps. The ITS sequences were considered to represent the same phylogenetic species, if they differed by <3% across the ITS region (i.e. 97% similarity), and for the genus level if they differed from 3% to 10% (Hughes et al., 2009).

3. Results

3.1. Diversity of EcM fungi in the WSF based on fruiting bodies

One hundred and five specimens of EcM fungi were recovered in a 1-ha WSF plot in the ZBS that corresponded with 48 species of the phylum Basidiomycota (Table 1). Russulaceae was the most diverse family with 18 species that belonged to the genera *Russula* (13), *Lactarius* (3), and *Lactifluus* (2). Hymenochaetaceae and Clavulinaceae followed with 6 species. The EcM genera *Clavulina*, *Lactifluus* and *Sebacina* were recorded for the first time in Colombia (Table 1). A total of 17 species are new reports for the country. Specimens of *Craterellus atratoides*, *Russula puiggarii* (Fig. 2C and G), and *Amanita* sp. 2 sect. Vaginatae were found in all four expeditions, and *Clavulina amazonensis*, *Craterellus strigosus,* and *Sarcodon rufogriseus* were identified in three out of four collection events (Table 1).

The species accumulation curve did not reach the asymptote (Fig. 3), indicating that not all EcM species occurring in the WSF from the ZBS were detected during our short-term research period and more sampling efforts are needed to recover the total diversity of EcM-forming fungi present in this WSF.

3.2. Diversity of EcM fungi in the WSF based on mycorrhizal roots

Molecular analysis was performed on 211 fragments of EcM roots but only 94 samples (44.5% success rate) yielded amplification of the ITS region. Fifteen phylogenetic ITS species of EcM fungi were recovered from the studied roots (>97% similarity of the ITS regions) (Table 1), and five sequences corresponded to four ITS genus-level (3–10% similarity) (Table 1). The most diverse families were Russulaceae (seven ITS-species) and Sebacinaceae (three ITS-species). Other species belonged to Thelephoraceae (two ITS-species), Clavulinaceae, Cortinariaceae, and Inocybaceae (one ITS-species each) (Table 1). The most common fungi detected at the root tips were uncultured *Lactifluus* sp. 1 (27.5%), *Russula* sp. 5 (13.8%), uncultured *Sebacina* (6.7%), and uncultured *Tomentella* (6.7%). Five of the 16 fungal ITS-species documented on the EcM

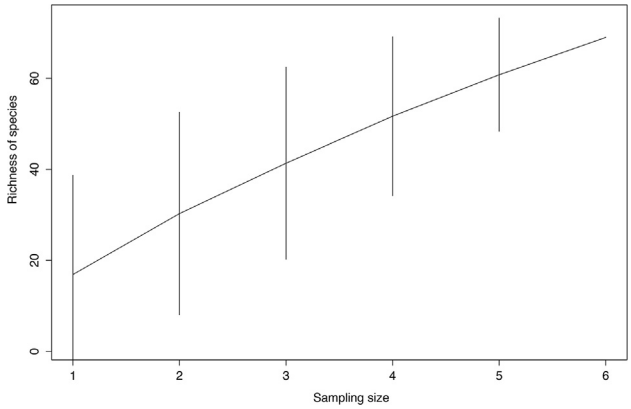

Fig. 3. Species accumulation curve for diversity data of individual-based sporocarps collected during the four sampling events, using the function specaccum with 1000 permutations and a 95% confidence interval in the Vegan package of R.

roots corresponded with formally described species and matched with vouchered specimens of *C. amazonensis*, *Cortinarius* sp. 1, *Lactarius brasiliensis*, *Lactifluus annulifer*, and *Russula* sp. 5 (>97% similarity).

It was not possible to identify the plant hosts in our study because the sequences obtained from the roots were of poor quality and did not allow taxonomic identification. However, the genera *Dicymbe* and *Aldina* that were abundantly present in the study plot have previously been reported as common EcM hosts in the neotropics (Smith et al., 2011, 2013; Henkel et al., 2012).

4. Discussion

This is the first study on the diversity of EcM fungi in a WSF in Colombia. A relatively high richness of EcM-forming fungal species was recovered during this short-term study in a 1-ha plot that contained the EcM host trees *D. uaiparuensis* and *Aldina* sp. Forty-three species of EcM fungi were represented by sporocarps, whereas 11 ITS-based species (similarity > 97%) were identified from the root tips, and five species were detected as ITS sequences at the root tips and from sporocarps. This result confirmed the advantage of combining searches for sporocarps with a molecularly based survey of root tips to best predict the diversity of EcM fungi. In general, the family Russulaceae presented the highest species richness followed by the families Amanitaceae, Clavulinaceae, and Hymenochaetaceae (Table 1). The families Inocybaceae and Tomentellaceae were recovered only by molecular means from the root tips, whereas Amanitaceae, Bankeraceae, Cantharellaceae, and Hymenochaetaceae were only observed as sporocarps (Table 1). Seventeen species were new records for Colombia, thus expanding the knowledge on fungal diversity in the country. *S. rufogriseus* is a species that was only recently described from specimens found in the WSF plot in El Zafire (Grupe et al., 2016).

Only five species were detected from both sporocarps and ITS sequences from the root tips, thus corroborating the incomplete recovery of EcM-forming fungal diversity. More sampling efforts are needed to recover the total diversity of EcM fungi present in this WSF plot (Fig. 3).

With 14 genera, the observed diversity of EcM fungi was relatively low in ZBS compared with studies on leguminous and dipterocarp hosts in Guyana where 49 genera were found (Henkel, 2015). Three main factors may have contributed to the lower number of species observed in our study forest when compared to those in Guyana on other species of *Dicymbe*. Firstly, the number of available individual host trees may be lower in ZBS, which may result in less suitable habitats for the EcM fungi. Secondly, the size of the patch may have influenced the results. The WSFs in western Amazonia are generally small (<10 ha), isolated, and surrounded by mixed forests (Peñuela-Mora, 2014; Adeney et al., 2016); thus, the lower density of host trees may limit the amount of fungal species that can be hosted. In Guyana, *Dicymbe* species is often monodominant (comprising ca. 43.6% of all individuals, Henkel, 2003), whereas in Colombia, *Dicymbe* and *Aldina* accounted for only 25% of all individuals. The third factor is that the sampling effort and time period has been more intense in Guyana with ca. 13-y of investigation compared with a 2-y in the present study.

The family Russulaceae was the dominant family among the EcM fungi in this WSF. At least 38% of the species recovered in this study corresponded to this family with species previously recorded from Guyana. The family was represented by the genera *Russula*, *Lactarius*, and *Lactifluus*. The genus *Russula* is taxonomically diverse in tropical forests (Buyck et al., 1996; Looney et al., 2016), and several of the specimens collected in this study remain to be identified to species level because of the taxonomic complexity of this group and the lack of taxonomic studies on neotropical species. The ITS rDNA sequences of four morphospecies of *Russula* were similar with ITS sequences of *Russula* from Guyana (>97% similarity). For example, the species *Russula* sp. nov. 3 (J. Duque, pers. comm. April 27, 2016) was similar to *Russula* sp. 5 (voucher TH9568, ITS rDNA sequences >97% similarity) that occurs in forests dominated by *P. dipterocarpacea* and *D. jenmanii* in Guyana (Smith et al., 2013). The recently redefined genus *Lactifluus* with a neotropical distribution (Verbeken et al., 2011) was highly represented on the mycorrhizal roots and sporocarps. *Lactifluus subiculatus* (Fig. 2E) and *L. annulifer* have also been found in Guyana in forests dominated by *D. corymbosa* and *D. altsonii* (Miller et al., 2012).

Hymenochaetaceae was also found to be a diverse family in the Colombian WSF. Specimens of *Coltricia* (four species) and *Coltriciella* (two species) were common in accumulated litter that covered tree trunks and stumps in the forests. This family was previously considered to be saprotrophic, but there is evidence that some species of *Coltricia* and *Coltriciella* form EcM associations with the host *Vateriopsis seychellarum* (Dipterocarpaceae), *Intsia bijuga* (Fabaceae), and *Eucalyptus robusta* (Myrtaceae) in African forests, and with *P. dipterocarpacea* (Dipterocarpaceae) and *Dicymbe* spp. (Fabaceae) in Guyana (Tedersoo et al., 2007; Henkel et al., 2012; Smith et al., 2013). One specimen of *Coltriciella* collected in the WSF was a new species (Ryvarden, pers. comm. October 6, 2015).

Sebacinaceae was the second-most diverse group of EcM fungi detected from root tips. The genus *Sebacina* represents one of the most common and species-rich groups of EcM fungi in temperate and tropical ecosystems and was recently detected as EcM symbionts in northeastern Ecuador and Venezuela (Tedersoo et al., 2010a, 2010b, 2014; Oberwinkler et al., 2013; Tedersoo and Smith, 2013; Moyersoen and Weiss, 2014). No species of *Sebacina* have been reported previously from Colombia (Vasco-Palacios and Franco-Molano, 2013). *Sebacina* EcM group A forms a symbiotic relationship with plants of the families Dipterocarpaceae, Fabaceae, Fagaceae, Malvaceae, Myrtaceae, Rosaceae, and Salicaceae, and is widely distributed from the Arctic to tropical zones (Tedersoo et al., 2010b; Oberwinkler et al., 2013). None of the sequences we obtained from *Sebacina* were related to sequences from neotropical specimens (>97% similarity). Three ITS sequences of *Sebacina* sp. 4 were similar to a sequence of *Sebacina* group A from Guyana (Table 1). Further exploration of this group is needed to improve our understanding of the ecology and evaluate the diversity of this lineage in Colombian ecosystems, especially in the Amazonian region.

The genus *Clavulina* is highly diverse in the neotropics (Henkel et al., 2012). In Guyana, 19 species of this genus have been reported with 15 representing newly described species (Henkel et al.,

2011; Uehling et al., 2012a, 2012b). We found six *Clavulina* species in the WSF and five of them also occurred in Guyana. *C. amazonensis* (Fig. 2D) was commonly observed as sporocarps and was also detected from root tips. *Sarcodon* is another interesting genus, with eight recently described species from the neotropical region (Grupe et al., 2015, 2016). *S. rufogriseus* (Fig. 2F) was described from abundantly occurring specimens during three out of four field surveys to WSF. These new data contribute to our knowledge of the distribution of the genus *Sarcodon*, and demonstrate that it has a wider distribution than previously thought (Grupe et al., 2015, 2016).

A high diversity was observed for families Entolomataceae and Tricholomataceae based on sporocarp counts. Some species of the genera *Entoloma* and *Tricholoma* have been reported as EcM (Agerer and Waller, 1993; Gehring et al., 1998; Walker et al., 2005; Matheny et al., 2006; Smith et al., 2007; Zeller et al., 2007; Henkel et al., 2012; Tedersoo and Smith, 2013), but we did not find evidence of EcM associations of these taxa on the root-tips.

Nearly 42% of the EcM-forming species found in the Colombian WSF have been previously recorded as EcM associated with host plants in Guyana, such as *D. altsonii*, *D. corymbosa*, *D. jenmanii*, *A. insignis* or *P. dipterocarpacea* (Dipterocarpaceae) (Henkel et al., 2002, 2011, 2012; Smith et al., 2011, 2013; Miller et al., 2012; Moyersoen, 2012; Uehling et al., 2012a). The genera *Craterellus* (Cantharellaceae, 4 species) and *Clavulina* (Clavulinaceae, 6 species) had the largest number of shared species between the WSFs in Colombia and Guyana. The species *Amanita campinaranae*, *Amanita xerocybe*, *C. amazonensis*, *Coltricia cinnamomea*, *L. brasiliensis, L. annulifer*, and *R. puiggarii* are associated with different tree hosts in Brazil, Guyana, and Colombia (Singer and Araujo, 1979; Singer et al., 1983; Henkel et al., 2002, 2012; Smith et al., 2013; Roy et al., 2016).

Host specificity of EcM fungi seems to vary among families of hosts and habitats, and it is difficult to draw general conclusions across environments. In the north of South America, highly specific host preferences of EcM fungi occur with *Coccoloba* (Polygonaceae), *Guapira*, and *Neea* (Nyctaginaceae) species (Tedersoo et al., 2010b). This is in contrast to the broader host preference of EcM symbionts with trees belonging to Fabaceae and Dipterocarpaceae (Singer et al., 1983; Henkel et al., 2012; Moyersoen, 2012; Smith et al., 2013; Roy et al., 2016).

Dispersion and isolation are important mechanisms that drive the distribution and diversification of EcM fungi (Moyersoen, 2012; Peay et al., 2012). It has been suggested that microorganisms disperse more widely than macroorganisms (Queloz et al., 2011). Long-distance dispersion has been proposed to explain the disjunct distribution of some taxa such as *Inocybe* between Australia and New Zealand (Matheny et al., 2006) and between trees of Dipterocarpaceae and Fabaceae in Venezuela (Moyersoen, 2012). Long-distance dispersal combined with low host specificity, may increase the possibility of gene flow between geographically distant populations of EcM fungi, including the small patches of ectotrophic forests in western Amazonia (Roy et al., 2008, 2013; Tedersoo et al., 2010b; Tedersoo and Nara, 2010; Moyersoen, 2012). These two mechanisms (the possibility of long-distance dispersal, and low host specificity) may result in a broad distribution of EcM fungi in the neotropics. Further phylogeographical studies of EcM fungal species occurring in tropical areas of Brazil, Colombia, French Guyana, Guyana, Peru, Suriname, and Venezuela are needed to assess the occurrence of gene flow between populations of EcM fungi across the greater Amazon region, and should take distance and associated plant hosts into account as well. A full assessment of the EcM fungal diversity of the WSFs in both Western and Eastern Amazonia will help to clarify the distribution patterns of the fungal symbionts and their host plants.

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