STUDY OF THE GENUS *AMANITA* AND SOCIAL ASPECTS IN THE CONSERVATION OF MACROFUNGI IN COLOMBIA

BY

NATALIA VARGAS ESTUPIÑÁN

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ADVISOR: SILVIA RESTREPO
LABORATORY OF MYCOLOGY AND PLANT PATHOLOGY

UNIVERSIDAD DE LOS ANDES
BOGOTÁ, COLOMBIA

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List of manuscripts

This thesis is based on the following manuscripts. The Roman numbers are indicated along the document.

Chapter 1:

I. Vargas-Estupiñán N, Pardo de la Hoz C., Franco-Molano, A.E., Jimenez, P. Restrepo, S., Grajales, A. Defining the phylogenetic position of Amanita species in Colombia. Accepted for publication in *Mycologia*

II. Vargas-Estupiñán N, Pardo de la Hoz C., Crawford A,. Restrepo, S. Phylogeography of *Amanita* spp., collected in Neotropical montane oak *Quercus humboldtii* Bonpl. in Colombia. In preparation.

Chapter 2:

III. Vargas-Estupiñán N, Goncalves S., Franco-Molano A.E, Restrepo S., Pringle A. In Colombia the Eurasian fungus *Amanita muscaria* (Amanitaceae) is expanding its Range into Native, Tropical *Quercus humboldtii* Forests. Submmited to *Biological Invasions.*

IV. Vargas-Estupiñán N, Gonzalez L.N., Restrepo S. Population genetics of the invasive species *Amanita muscaria* in the native oak forest (*Quercus humboldtii)* in Colombia. In preparation.

Chapter 3:

V. Vargas-Estupiñán N, and Restrepo S. Diversity of ectomycorrhizal mushrooms associated with *Quercus humboldtii* in Colombia. Chapter submmited to the Book IWEMM 9 (International Workshop on Edible Mycorrhizal Mushrooms, 2017).

VI. Vargas-Estupiñán N, Mesa L., Diaz M.A., Gutiérrez C., Restrepo S, and Velasco N. Oyster mushroom cultivation as an economic and nutritive alternative for rural low-income women in Villapinzón (Colombia). Submmited to *Rural Studies.*

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INTRODUCTION

Colombia is one of the most diverse countries worldwide, exhibiting a wide variety of ecosystems and species (Andrade 2011, CDB 2014). However, its diverse ecosystems are highly vulnerable due to the high demands generated by agriculture, roads, mining, energy, among others (*Plan Nacional de Desarrollo*-PND 2010-2014, 2014-2018). Thus, the protection and management of this diversity has become a priority for the government and research institutions.

According to the NGO *Fundación Natura* (2007) nearly 10,500 hectares of natural ecosystems have been replaced by miscellaneous forests and to a lesser extent by areas designated for agricultural activities. Among these, are the native oak (*Quercus humboldtii*) forests. These forests are distributed in the three cordilleras of Colombia (Cárdenas & Salinas, 2006) and occupy small discontinuous relicts in the Colombian departments of Antioquia, Boyacá, Cauca, Chocó, Cundinamarca, Huila, Nariño, Quindío, Santander, and Tolima (Pulido *et al.* 2006). Fragmentation, has caused one of the major threats to the biodiversity associated to oak forests, rendering oak a vulnerable (VU) species since it is considered a source for charcoal production and tanning processes (Barrios *et al*. 2006; Cardenas & Salinas, 2006).

Oak forests establish symbiotic ecological relationships with **EC**to**M**ycorrhizal Fungi (ECM), throughout a mutually beneficial ecological interaction between plant roots and fungal mycelium. This ecological interaction plays an essential role in the dynamics of forest ecosystems: it allows the exchange of nutrients especially phosphorus from the plant to fungi, allows the exchange of carbohydrates from the host to the fungi, and constitutes an overall communication system between several trees by facilitating the translocation of nutrients.

Justification

Most studies that have been carried out in relation to macrofungi have been conducted on oak forests (*Quercus humboldtii*), given that fungal diversity in this ecosystem is very high (Franco *et al*. 2000). However, in Colombia the ECM fungal diversity has not been taken into account by any National biodiversity report (CDB 2010, 2014) nor is reported in a global database for biodiversity. All biodiversity surveys and conservation efforts made so far in this ecosystem, are based on the characterization of several higher taxonomic groups (birds, woody plants, insects, and fish) (Chavez *et al*. 2007).

In Colombia, the National Policy on Biodiversity (*Política Nacional de Biodiversidad*-PNB) was adopted in 1995 and subsequently actions related to the management of the nation's biodiversity were implemented. The three objectives adopted by the PNB are: to *know*, to *conserve,* and to *use* biodiversity. This research project is in line with these three objectives: *i*) To *know*: the taxonomy and phylogeography of the species in the genus *Amanita,* as well as the history of introductions and genetic diversity of populations of *Amanita muscaria* in native oak forests; *ii)* To *conserve:* through research, provide decision-making tools based on knowledge on ECM diversity and by providing recommendations to help protect macrofungi in oak forests to people living near these areas; *iii)* To *use*: the edibility potential of these fungal species, makes them a nutritive and economic alternative to low-income rural women.

Objectives

General: Investigate fungi associated to oak forests (*Quercus humboldtii*) from a

phylogenetics, conservational, and social perspective.

Specific Objectives:

Biological

1) To assess the taxonomic and phylogenetic relationships among *Amanita* spp. in Colombia.
2) To infer the origin of *Amanita* spp. in the montane forests located in the northeastern cordillera in the Colombian Andes.
3) To reconstruct the history and origin of the invasive *Amanita muscaria* species in Colombia.
4) To investigate the population genetics structure of *A. muscaria* associated to oak and pine tree plantations in Colombia.

Conservational and social

5) To investigate the current state-of-the-art of ECM fungi associated to *Quercus humboldtii* in Colombia.
6) To elaborate an illustrated booklet with recommendations for sustainable collection and management of invasive species.
7) To include information of macrofungi in Colombia, in the Barcode of Life Database system.
8) To integrate the cultivation of *Pleurotus ostreatus* as an economic and nutritive

income alternative for rural communities in Villapinzón, a locality in Cundinamarca, Colombia.

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CHAPTER 1

WHICH IS THE ORIGIN AND PHYLOGEOGRAPHIC HISTORY OF THE AMANITA SPP. PRESENT IN NATIVE OAK FORESTS?

OBJETIVES

1) To assess the taxonomic and phylogenetic relationships among *Amanita* spp. in Colombia.

2) To infer the origin of *Amanita* spp. in the montane forests located in the northeastern cordillera in the Colombian Andes.

I. Defining the phylogenetic position of *Amanita* species in Colombia

Natalia Vargas-Estupiñán, Carlos José Pardo-de La Hoz, Giovanna Danies, Silvia Restrepo

Laboratorio de Micología y Fitopatología Universidad de Los Andes, Bogotá, Colombia

Ana Esperanza Franco-Molano

Laboratorio de Taxonomía y Ecología de Hongos, Universidad de Antioquia, Medellín, Colombia

Pedro Jiménez

Laboratorio de Fitopatología, Universidad Militar Nueva Granada, Bogotá, Colombia

Alejandro Grajales

Laboratorio de Micología y Fitopatología Universidad de Los Andes, Bogotá, Colombia

Abstract*:* *Amanita* is a worldwide-distributed fungal genus, with ca. 600 species known. The species representing this genus are mostly EctoMycorrhizal (ECM), and some are

saprotrophic. A total of 17 species have been reported for Colombia based on morphological descriptions. In this study we conducted the first comprehensive phylogeny of ECM species in the genus collected in native Colombian *Quercus humboldtii* and in introduced *Pinus patula* forests. We included eight (*A. arocheae*, *A. colombiana, A. flavoconia, A. fuligineodisca, A. muscaria, A. rubescens, A. sororcula* and *A. xylinivolva*) out of 17 species present in the country, a first report of *A. citrina*, and the description of a new species: *Amanita capillensis*. Morphological taxonomic keys together with a phylogenetic approach using three nuclear gene regions: the rDNA 28S nuclear ribosomal Large SubUnit rRNA (nLSU) partial sequence, the Internal Transcribed Spacers ITS1 and ITS2, and a portion of the gene for translation elongation factor 1 (*tef 1a*), were used to classify the specimens. Several highly supported clades were obtained from the phylogenetic hypotheses inferred by Bayesian inference and maximum likelihood approaches, allowing us to position the Colombian collections in a coherent infrageneric level, and to contribute to the *Amanita* local diversity knowledge.

Key words: *Amanita,* molecular systematics, rDNA, translation elongation factor 1 alpha gene, acrophysalids, infrageneric classification.

Introduction

Fungi in the genus *Amanita* Pers.: Hooker are morphologically characterized by producing a whitish spore print, gills that are free to sub-free from the stipe, and by the presence of morphologically diverse universal veils. Microscopic characteristics include a divergent hymenophoral trama and smooth, amyloid or inamyloid, spores. In the past, several classification systems have been proposed for this genus, based on a combination of morphological characteristics. Corner and Bas (1962) and Bas (1969) initially separated the species of *Amanita* into two widely accepted subgenera: those belonging to the subgenus *Amanita*, characterized by a striate to plicate pileus margin and inamyloid spores, and those belonging to the subgenus *Lepidella* characterized for having an even pileus margin and amyloid spores. More precisely, according to Bas (1969), the variation in the universal veil traits and whether or not the pileus margin has an appendiculate aspect, were used to classify species into six sections: *Amanita* and *Vaginatae* in the subgenus *Amanita,* and *Amidella, Lepidella, Phalloideae,* and *Validae* in the subgenus *Lepidella*. Based on this primary classification, Singer (1986) added four sections: *Caesareae, Mappae, Ovigerae,* and *Roanokenses*, to separate species according to specific morphological differences such as the presence or absence of an annulus, subtle variations in the volva, as well as microscopic features.

Over the last 15 years, different rearrangements of infrageneric sections for the genus have been hypothesized, based on molecular phylogenetic analyses of nuc rDNA sequences, and the traditional classification systems (Bas, 1969; Singer, 1986). Although no clear split of the two major subgenera has been highly supported in most of the studies, Weiss *et al.* (1998) confirmed the monophyly of most of the sections. Later, a subdivision of three sections within the subgenus *Amanita* and a modification of the taxonomic treatments at sectional

level in the subgenus *Lepidella* were proposed by Oda *et al*. (1999). Drehmel *et al*. (1999) proposed a classification using partial 28S rDNA sequence (28S), providing a rearrangement into seven subsections (*Amanita*, *Amidella*, *Caesareae*, *Ovigerae*, *Phalloideae*, *Vaginatae,* and *Validae*), and two series (*Mappae* and *Validae*). A study by Zhang *et al*. (2004) established the relationships among Eastern Asian species, based on a phylogenetic hypothesis of nuc rDNA ITS1-5.8S-ITS2 (ITS) and partial 28S sequences. They obtained highly supported clades for sections *Amidella* and *Validae* when analyzed by distance-based methods (neighbor joining), and for sections *Amanita*, *Vaginatae,* and *Validae*, when using maximum parsimony analysis; however, the initial division of the genus into two subgenera *Lepidella* and *Amanita,* was not strongly supported in any of their analyses.

To date, a total of 16 species of *Amanita* are reported in Colombia (Singer 1963, Nasi 1977, Pulido 1983, Tullos *et al*. 1992, Franco-Molano and Uribe-Calle, 2000, Franco-Molano *et al*. 2000, Halling and Mueller 2005, Vasco-Palacios and Franco-Molano 2013). Singer (1963) reported *Amanita inaurata* Secr. and *A. humboldtii* Sing*; A. rubescens* was reported initially by Nasi (1977) and *A. muscaria* was initially reported by Pulido (1983)*;* new species were described by Tulloss *et al*. (1992): *A. advena, A. arocheae, A. aureomonile*, *A. brunneolocularis, A. colombiana, A. fuligineodisca, A. picea, A. sororcula* and *A. xylinivolva; A. gemmata* was initially reported by Guzman and Varela (1978); *A. savannae* by Tulloss and Franco-Molano (2008); and *A. flavoconia* has been reported by Tulloss *et al*. (1992), Saldarriaga *et al*. (1988), Franco-Molano *et al*. (2000), Franco-Molano and Uribe-Calle (2000), Halling and Mueller (2005) and Vasco-Palacio and Franco-Molano (2013). Sixteen taxa represent the sections *Amanita*, *Phalloideae* and *Vaginatae*, and the species *Amanita advena,* represents the section *Lepidella*. Most of these species are reported to be associated with the species *Quercus humboldtii*, which is a tree species endemic to northern South

America and in the Darién region of Panamá (Orwa *et al.* 2009), and distributed in the three cordilleras of Colombia in an altitudinal range from 1,100 to 3,200 m; on the contrary two of the *Amanita* spp., *A. muscaria* and *A. rubescens* are commonly associated with *Pinus* spp. introduced to Colombia. No species has been reported in section *Caesareae* in Colombia; these species are mainly distributed in temperate regions and other tropical regions different from the South American neotropics (Sanchez-Ramirez *et al*., 2015).

The Andean montane forests support a high biodiversity of organisms (Bush *et al*. 2011), and it is estimated that the diversity of fungi in *Quercus* spp. forests is high (Franco-Molano *et al*. 2000). Although a high number of Agaricales in Colombia have been described according to morphological traits (Halling and Ovrebo, 1987; Halling 1989a; Halling 1989b; Horak and Halling, 1991; Singer *et al.* 1995; Franco-Molano, 1999), the phylogenetic relationships of Agaricomycotina has not been explored yet in Colombia. In this study, specimens representing ten species of *Amanita* collected in Colombia were identified based on morphological characteristics, and positioned in a global phylogenetic context using maximum likelihood (ML) and Bayesian inference approaches, allowing infrageneric level classification. Based on the combined results of the morphological analysis and their position in the phylogeny, a new species, a new variety and a new record are proposed, and some taxonomic notes on the infrageneric ranks of the genus are discussed.

Materials and Methods

Fungal collection.

77 fruiting bodies of the genus *Amanita* were collected between Mar 2012 and Jun 2014 in the North Eastern cordillera of the Colombian Andes: Departamento de Boyacá, Municipio de Villa de Leyva, Vereda Capilla 5°39’ 26.78’’ N, 73°30’ 46.41’’ W; Departamento de

Boyacá, Municipio de Arcabuco, Vereda Piedras Blancas, 05°48.546" N, 73°28.751" W; Departamento de Boyacá, Municipio de Arcabuco, 5°45' 35.38'' N, 73°26' 47.10'' W; Departamento de Santander, Municipio de Belén, vereda San José de La Montaña, 06°02' 29.82'' N, 73°00' 02.8''W. A herbarium collection AFM1812 of the species *A. fuligineodisca* collected in Departamento de Antioquia, Corregimiento de Santa Elena, Estación biológica Piedras Blancas, and stored in HUA (Herbario de la Universidad de Antioquia, Medellín, Colombia) was included in the molecular analysis. The fungi were collected in *Quercus humboldtii* and in *Pinus patula* forests. Macroscopic features were described, color designations were assessed according to Kornerup and Wansher (1978) (given within parentheses); microscopic measurements were made in samples treated with 3% KOH, and other cross-sections were treated with Melzer's reagent. Lengths (L) and widths (W) of 30 spores and 30 basidia were measured, and the Q' value (average ratio of length/width for all spores measured for all specimens of a species) was calculated (Tulloss, 2005b). Macroscopic test with KOH 3% was carried out on the pileus and stipe context. Once the specimens were described, they were dried and packaged in plastic bags, stored at the ANDES Herbarium (Universidad de los Andes, Bogotá, Colombia), and registered in the SPECIFY database. Based on their macro and microscopical characters, specimens were assigned to a putative species according to Bas (1969), Singer (1986), Jenkins (1986), Tulloss *et al.* (1992), Halling and Mueller (2005), Franco-Molano *et al*. (2000), Tulloss and Possiel (2005), and Tulloss (2002, 2005a, 2008, 2009a, 2009b).

DNA extraction, amplification, and sequencing.

DNA was obtained from 24 dried fruiting bodies using the protocol reported by Zolan and

Pukkila (1986), with the following modifications: the lysis buffer consisted of 2.5% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris, pH 8, 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 1% polyvinyl pyrrolidone (PVPP) 40000 and 1% PVPP 360000 (Zolan and Pukkila, 1986).

Primers ITS4 and ITS5 (White *et al.* 1990) were used to amplify the ITS region. PCR was performed with a Peltier thermal cycler (Bio-Rad) in 25 µL reaction mixtures containing double distilled H_2O, 1 µL of DNA template, 0.5 µL of each 10 µM primer, 2.5 µL of Taq 10x buffer, 0.5 µL of 10 mM dNTP mix, 2 µL of 25 mM $MgCl_2$, and 1 µL of 5 U/µL *Taq* polymerase. Cycling parameters were as follows: initial denaturation at 94 ºC for 1 min, followed by 35 cycles of denaturation at 96 ºC for 2 min, annealing at 55 ºC for 1 min, extension at 72 ºC for 2 min, and a final extension at 72 ºC for 10 min. PCR amplification of nLSU was carried out with primers LROR and LR7 (Vilgalys and Hester, 1990), using the reaction conditions mentioned above with the following thermal cycling parameters: Initial denaturation at 94 ºC for 4 min, followed by 35 cycles of denaturation at 94 ºC for 30 sec, annealing at 54.5 ºC for 1 min, extension at 72 ºC for 1 min, and a final extension at 72 ºC for 5 min. In addition to the nuclear ribosomal regions, which have proven to be informative in reconstructing the phylogenetic relationships within *Amanita* and other closely related taxa (Drehmel *et al*. 1999, Zhang *et al*. 2004, Justo *et al*. 2010), and commonly used as phylogenetic fungal barcodes (Schoch *et al*. 2012), we used the *tef 1a* protein-coding gene, which is informative in low-level phylogenetics approaches (Rehner 2001). PCR amplification of *tef 1a* gene was carried out with the primers 983F and 1567R following the PCR conditions by Rehner and Buckley (2005). Amplified PCR products were visualized by gel electrophoresis on a 1% agarose gel. PCR products were sequenced using a 3730xl DNA analyzer (PE Applied Biosystems, USA). Forward and reverse sequences were assembled

using Geneious Basic 4.8.5 (Biomatters) and blasted again the nucleotide base of GenBank and UNITE (Kõljalg *et al*. 2013, http://unite.ut.ee/cite.php) to confirm the sequence obtained corresponded to the target organism.

Phylogenetic analyses and species delimitation

A total of 151 ITS, 114 28S, and 32 *tef 1a* gene sequences from ECtoMycorrhizal, (ECM) *Amanita* species, were used to construct the consensus phylogeny (SUPPLEMENTARY TABLE I). A data set containing the concatenated alignments of the ITS, 28S, and *tef 1a* alignment, was generated. This data set consisted of a matrix that resembles the phylogenetic approach presented by Sanderson *et. al.* (1998) as there was missing data. An independent phylogenetic tree for each gene was constructed by using Bayesian inference (SUPPLEMENTARY FIGURES 1-3). The alignments were performed by using MUSCLE (Edgar, 2004), implemented in the CLC DNA Workbench and curated using Gblocks (Castresana, 2000) using default parameters. Sequence statistics of each gene and concatenated alignments were calculated with MEGA 5 (Tamura *et al*., 2011). The alignment file can be accessed on TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S17914). Bayesian inference was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Fifty million MCMCMC generations were run, using a sample frequency of 1000 and a burnin of 25% of the total, specifying a different model of substitution per gene using jModelTest (Posada, 2008). Two runs using four chains each, one cold and three heated chains, were performed and the results of each chain were summarized to obtain the majority consensus trees from the total run, collapsing all branches with posterior values less than 0.5 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Each run was examined using Tracer 1.5 (Rambaut and Drummond, 2009) to determine if the burnin procedures were correctly

assumed, and to determine if there was convergence between the chains and the runs. A maximum likelihood (ML) analysis was performed with RAxML (Stamatakis *et al.* 2008) using the GTRCAT substitution model with 1000 bootstrap iterations to assess clade support. We used closer related species to the genus *Amanita*: *Pluteus romelli* and *Limacella glioderma* as outgroups. Phylogenetic species and monophyletic groups recognition was performed by the identification of highly supported clades on the phylogeny (i.e ML > 75% boostrap support, BS, or > 0.95 Bayesian posterior probability, PP) Dettman *et al.* (2003).

Results

A total of 77 sporocarps were collected and classified in 10 species, among which 24 specimens were included in the phylogeny of the genus (Table 1). A total of 297 sequences from the three genes were used to construct the phylogenetic trees, which corresponds to 104 species (SUPPLEMENTARY TABLE 1). The ITS, nLSU, *tef 1a* and the combined data sets consisted of: 821, 577, 522, and 1920 characters (including gaps); 451, 210, 214, and 903 parsimony-informative characters; 569, 266, 245, and 1104 variable sites; and 236, 273, 260, and 752 conserved sites, respectively.

Both ML and Bayesian-based reconstruction hypotheses yielded similar phylogenies, grouping five species (*A. colombiana* Tulloss, Ovrebo and Halling*, A. fuligineodisca* Tulloss, Ovrebo and Halling*, A. muscaria* (L.)*, A. sororcula* Tulloss, Ovrebo and Halling*,* and *A. xylinivolva* Tulloss, Ovrebo and Halling) within the subgenus *Amanita,* and five species (*A. citrina* Pers.*, A. flavoconia* var. *inquinata* Tulloss, Ovrebo and Halling*, A. arocheae* var. nov. *alba*, *A. rubescens* Pers., and *A. capillensis* sp. nov.) in the subgenus *Lepidella*. FIGURE 1 shows some of the species' fruiting bodies, spanning the sections *Amanita*, *Phalloideae,* and *Vaginatae.*

Subgenus *Amanita*

An initial morphological classification of 49 specimens in the subgenus *Amanita* was determined using taxonomic keys (Jenkins, 1986; Tullos *et al*. 1992; Tulloss 2000, 2008; Franco-Molano *et al.* 2000; Halling and Mueller 2005). Of these, 20 specimens were classified in the section *Vaginatae* (Table 1), all of them without an annulus: 17 specimens determined as *A. fuligineodisca* (Fig. 1A), 3 as *A. colombiana* (Fig. 1B), and 1 as *A. sororcula* (Fig. 1C).

Although there was no high support by ML, the division of the subgenus *Amanita* into two sections *Amanita* and *Vaginatae*, is further explained by the traditional classifications system, supported by morphological structures. Species in section *Amanita* are mainly differentiated from those in section *Vaginatae* by having bulbous stipe bases and non-saccate volva (Jenkins, 1986; Singer 1986; Drehmel *et al*. 1999). According to the phylogeny, within subsection *Vaginatae sensu* Drehmel *et al*. (1999) two highly supported clades were separated, corresponding with the series *Ceciliae* (clade g, Fig. 2) and series *Fulvae* (clade d, Fig. 2) proposed by Tulloss and Yang (2012a, b). *Amanita colombiana,* (Fig. 1B), a species that is characterized by its red volva, and *A. sororcula* (Fig. 1C) characterized by its submembranous, pale gray volva, grouped within the series *Ceciliae* (Fig. 2 clade g). *Amanita fuligineodisca* clearly clustered together with *A. fulva* Fr., in stirps *Fulva* (Tulloss and Yang, 2012c) (clade f, Fig. 2).

29 specimens were determined in the section *Amanita,* grouping 17 in the species *A. muscaria* and 12 in the species *A. xylinivolva* (Table 1). The sporocarps were mainly diagnosed according to differences in the pileus and volva. Sporocarps classified as *A. xylinivolva* (Fig. 1D) were characterized by having pale yellow to pale gray color, pileus surface with non-uniform volval remnants, a thin membranous ring that disappears in mature

specimens, a limbate volva, and inamyloid globose to subglobose spores. Following Tulloss 2009a, *A. farinosa* differs from this species by having brownish gray pileus and a pulverulent volva, among others. According to the phylogeny, these samples were closely related to the species *A. stranella*, which is characterized by citron straw-colored to cream pileus, membranous ring and volva with marginal limb (Tulloss and Yang, 2015a). On the other hand, sporocarps of *A. muscaria*, were characterized by polished surface, red to orange sometimes blood-red, with white to yellowish pale patches of volva, and a volva formed by superficial membranous or scaly warts at the base of stipe.

Subgenus Lepidella

The division of the subgenus *Lepidella* into two sections *Lepidella* and *Phalloideae*, was supported in the phylogeny by ML analysis (Fig. 2). This division is supported morphologically by the presence of a prominent basal bulb and appendiculate margin of the pileus in species within section *Lepidella*, differing from species in Section *Phalloideae* which lack these two characteristics in the stipe and the pileus, respectively (Jenkins 1986; Drehmel *et al*. 1999).

Following the taxonomic keys (Tulloss *et al*. 1992; Franco-Molano *et al.* 2000; Tulloss, 2002, 2008, 2009a; Halling and Mueller, 2005), 27 sporocarps were classified within subgenus *Lepidella*: 24 in section *Validae*, and 3 specimens in section *Phalloideae.* Individuals classified within section *Validae*, were recognized for having even and non-appendiculate margin in the pileus, globose to ellipsoid spores, and membranous to friable volva, which according to Bas (1969), corresponds microscopically to the presence of terminal inflated cells in the universal veil. Thirteen sporocarps were determined to be *A. flavoconia* var. *inquinata* (Fig. 1E), characterized by their yellow-orange pileus, yellowish

not bruising stipe, and a mean spore Q' = 1.21. Three samples represented this species in the phylogeny, which grouped within a cluster of species which lack a red bruising reaction on the stipe (clade j, Fig. 2). In contrast, 9 specimens were classified in the rubsecent group *A. rubescens* and *A. capillensis* sp. nov., (Fig. 1F). Colombian collections of *A. rubescens* were classified according to the tabular key of rubescent taxa (Tulloss, 2002) by having brown pileus with pale margins, annulus cream and spores L' = 8.75 µm with Q' = 1.43. Furthermore, following the taxonomic key of Tulloss (2005a) ,2 sporocarps were classified as *A. citrina* in the section *Validae*. The new variety *A. arocheae* var. *alba,* was represented by 3 sporocarps, and it was the only species classified in the section *Phalloideae*.

In the following TAXONOMY section records for Colombia are described. The term "undifferentiated hyphae", following Tulloss *et al*. (1992), means the presence of thin-walled hyphae lacking refractive contents. A detailed description of the species, *A. colombiana*, *A. flavoconia*, *A. fuligineodisca*, *A. muscaria*, *A. rubescens*, *A. sororcula*, and *A. xylinivolva* is provided by Tulloss *et al*. (1992), Halling and Mueller (2005) and Franco-Molano *et al*. (2000).

Taxonomy

Amanita capillensis **sp. nov**. N. Vargas, A.E Franco-Molano and S. Restrepo Figs. 1F, 2 clade k, and 3

MycoBank MB812882

Typification: COLOMBIA, BOYACÁ/MUNICIPIO VILLA DE LEYVA/ VEREDA CAPILLA 5°39' 26.78'' N, 73°30' 46.41'' W, on soil in *Quercus humboldtii* forests, 24 Apr 2007, *Isabel Pulgarín* no. 24 (**holotype** HUA).

Etymology: named after the collecting site, vereda Capilla, a zone abundant in *Quercus*

humboldtii forests in the department of Boyacá.

Pileus: 50–60 mm wide, conical to convex; surface even to finely fibrillose, greyish yellow (4B6) becoming light yellow (4A4) to light orange (5A4) to pale greyish yellow (4B4) toward margin; *margin* nonstriate, nonappendiculate; universal veil sometimes present over the surface of the pileus as irregular, thin, yellowish white (4A2) to orange white (5A2) or orange grey (6B2) patches. *Context*: 2–4 mm thick, white, unchanging. *Lamellae*: 4 mm wide, free, close, white; fimbriate margin; *lamellulae* showing different lengths. *Stipe*: 125–135 × 14–18 mm, central; surface fibrillose, white at apex, pale orange (6A3) to orange white (5A2); with subglobose bulb; context not noted. *Annulus*: superior, membranous, thin, skirt-like, pale orange (6A3), striate above, not totally detached from pileus margin. *Volva*: pale orange (6A3), to pinkish white (7A2) sometimes with dull red (8B4) traces towards base, friable, leaving patches and crumbling remnants about the stalk base. Odor: non distinctive. KOH negative in pileus and stipe contexts. *Pileipellis*: light yellow-green in 3% KOH, as cutis of filamentous hyphae 7–15 μm wide, subradially arranged, some branching, hyphae gelatinized. *Pileus context*: interwoven, undifferentiated hyphae often short, inflated, with yellowish walls; *Lamellar trama*: bilateral, with distinct width of the central stratum (wcs = 35–50 μm); formed by 8–10 μm wide, filamentous, undifferentiated, branching hyphae. *Subhymenium*: composed of inflated-ramose cells 10 μm × 10 μm between central stratum and base of basidia, broadly fusiform, with basidia arising from inflated cells, with a distance from an outer margin of the central stratum to the nearest base of a basidium (wst-near) = 10–13 μm and with a distance from an outer margin of the central stratum to the farthest base of a basidium (wst-far) = 18–30 μm. *Lamellar edge cells*: globose as inflated cells or sphaerocytes, 17–38 μm × 17–30 μm, with oleiferous hyphae at lower end of trama with

resinous material which turns yellow in 3% KOH. *Basidia*: (37-40)–45 × 8 –(11–15), four-sterigmate, with some basidioles; clamps not observed. Stipe context: longitudinally arranged; filamentous, undifferentiated hyphae 5–10 μm wide, with walls thin to slightly thickened, acrophysalids 50–110 × 20–26; vascular hyphae 6–20 μm wide. *Stipitipellis*: cutis with filamentous hyphae. *Universal veil*: formed by 80–140 μm long × 40–60 μm wide, inflated and sometimes piriform hyphae, presence of vascular hyphae 5–8 μm wide, with yellowish walls. *Partial veil*: formed by 45–60 × 10–20 μm, inflated, filamentous, branching hyphae, some with yellow content. *Basidiospores*: white in mass, [90/3/3] 7.0–10.0 (11.5) × 7.0–9.0 μm, (L = 8.1–8.8 μm; L' = 8.5 μm; W = 7.4–8.2 μm; W' = 7.8 μm; Q = 1.05–1.3; Q' = 1.1), hyaline, thin-walled, smooth, amyloid, subglobose to broadly ellipsoid; contents multiguttulate.

Other material examined: COLOMBIA: BOYACÁ/MUNICIPIO DE ARCABUCO/VEREDA PEÑAS BLANCAS/ 05°48.546" N, 73°28.751" W, on soil in *Quercus humboldtii* forests, 27 Apr 2014, *Natalia Vargas Estupiñán* no. 698; ANDES F2201. BOYACÁ/MUNICIPIO DE ARCABUCO, 5°45' 35.38'' N, 73°26' 47.10'' W, on soil in *Quercus humboldtii* forests,15 Dec 2013, *Natalia Vargas Estupiñán* no. 624, ANDES F2126.

Notes: Some species to be compared to *A. capillensis* are *A. brunneolocularis*, *A. rubescens* var. *alba* Coker, *A. orsonii* Ash. Kumar and T.N. Lakh., and *A. novinupta* Tulloss and J. Lindgr. Brown cells are present in the universal veil of *A. brunneolocularis*, while in *A. capillensis* they are yellowish inflated to piriform; the spores in *A. brunneolocularis* are shorter and broadly ellipsoid, the stipe present white fibrils that become reddish, to grey and black over a pallid ground, while in *A. capillensis* the fibrils are pale cream to pinkish. Spores of *A. rubescens* var. *alba* differ from those of *A. capillensis* because they are more ellipsoid

(Q’ = 1.6), and the universal veil becomes partially gray when ageing, while *A. capillensis* has a pale yellowish pileus with bronze stains, and microscopically globose to slightly subglobose spores (Q’ = 1.05–1.1). The species *A. orsonii* differs from *A. capillensis* by having a pileus that stains orange (Tulloss 2009c), turning to shades of red or brown with handling or ageing. At last, *A. novinupta* differs from *A. capillensis* for presenting subpyramidal warts at the universal veil, with narrower inflated cells, and wider inflated hyphae at the partial veil (Tulloss and Lindgren, 1994). According to the classification of rubescent taxa (Tulloss, 2002), the new species differed from the taxa in the rubscent group by a smaller Q’ = 1.1, which corresponds to more subglobose spores, than in other rubescent taxa with larger Q’ values. The BLAST of the sequences IP24 and NVE698 in the NCBI and the UNITE databases, showed a 98% of identity with a maximum score of 869 with the species *A. rubescens* voucher LE241998 (Acc. Number JF313652). In the consensus tree, the position of *A. capillensis* specimens renders the clade of *A. rubescens* paraphyletic, it also includes a specimen of *A. novinupta* (Fig. 2). We consider this fact raises the question of whether more than one species have been incorrectly assigned to *A. rubescens*, rather than challenging the recognition of *A. capillensis* as a new species. In fact, two different groups are formed within the ‘*A. rubescens’* clade: The first clade (Fig. 2-ka) show relatively high support (BS > 75%) and includes the specimens of this study collected in *Pinus patula* plantations, morphologically assigned to *A.rubescens*. A second clade (Fig. 2-kb) includes two *A. rubescens* specimens from Japan and Turkey, *A. capillensis* collected in *Q. humboldtii* and a species assigned to *A.novinupta* from Mexico. A detailed revision of the specimens of the second clade would allow testing if they could be assigned to new taxa, in light of these results. Single gene trees recovered *A. capillensis* as a monophyletic clade and confirmed their close relationship to *A. rubescens* (SUPPLEMENTARY FIGURES 1 AND 2).

Amanita citrina Pers. (Figs. 1G, 2 clade m)

COLOMBIA, BOYACÁ/MUNICIPIO DE ARCABUCO 5°45’ 35.38’’ N, 73°26’ 47.10’’ W, on soil in *Quercus humboldtii* forests, 15 Dec 2013, Natalia Vargas Estupiñán 600 ANDES_F2101, and Natalia Vargas Estupiñán 616 ANDES_F2117.

Pileus: 48–52 mm wide, convex to hemispheric; surface dry, bright, smooth, with some patches of pale brown membranous universal veil, pastel green (30A4) to pale green (30A3); margin even. *Context*: 6–7 mm thick, white, unchanging. *Lamellae*: 4.2–4.5 mm broad, free to slightly adnexed, close, pale green (30A3). *Stipe*: 70–80 mm long × 11 mm wide at the upper half and 40 mm in the bulbose base, fibrillose to slightly rugose in the upper half to fibrillose opaque at the lower half, compact with thin hollow line, upper half concolorous with the pileus, the lower half pale green with traces of light orange (5A4), abruptly bulbous, and marginate basal bulb; context cream. *Annulus*: pale green (30A3), pendant, skirt-like ring. *Volva*: limbate to membranous, pale orange (5A49 to greyish orange (5B4), becoming gelatinous and putrescent dark with age. Odor: potato-like. KOH negative in pileus and stipe contexts. *Pileipellis* formed by filamentous, inflated and periclinally arranged hyphae. *Hymenophoral trama*: bilateral, with distinct central stratum (wcs = 93–107 μm); formed by 7–10 μm wide, filamentous, undifferentiated, branching hyphae, hyaline, inamyloid. *Universal veil:* formed by 5–8 μm wide narrow hyphae, occasionally 85–100 μm long x 40–65 μm wide inflated hyphae; and vascular hyphae. *Basidiospores* pale cream in mass [60/2/1]: white in mass, 6–10 (–11) µm long, 8–11 µm broad, mean Q’ = 1.3, subglobose, smooth, slightly amyloid. *Basidia*: (30–) 42–46 µm long, (5–) 7–10 µm broad, four-

sterigmate basidia.

Notes: Collections of *A. citrina* (Fig. 1F) were classified following the taxonomic keys corresponding to North American and Canadian species (Tulloss, 2005a). The key provided for species from Costa Rica and neighboring regions (mostly associated to neotropical *Quercus* spp.), was not used since it does not include this species (Tulloss, 2009a). Collections NVE 600 and 616 (Fig. 1G) were classified based on the pale yellowish pileus, marginate basal bulb, a distinct potato odor, strongly limbate volva, and broadly ellipsoid spores (Q´ = 1.3) (Tulloss, 2005a). Individuals of *A. citrina* from Colombia were grouped phylogenetically closer to worldwide collections of *A. citrina*, in the group *Mappae,* where species are distinguished by having limbate volva, with marginate to submarginate basal bulb (Singer 1986; Drehmel *et al.* 1999) (clade m, Fig. 2). These collections of *A. citrina* associated to *Q. humboldtii,* constitute the first report for Colombia of a species allocated in stirps *Bulbosa*.

Amanita arocheae **var. nov.** *alba* N. Vargas, A.E Franco-Molano and S. Restrepo (Figs. 1H, 2 clade n, and 4)

Typification: COLOMBIA, BOYACÁ/ MUNICIPIO VILLA DE LEYVA/VEREDA CAPILLA/ 5°39’ 26.78’’ N, 73°30’ 46.41’’ W; on soil in *Q. humboldtii* forest/ Collection Natalia Vargas Estupiñán 74 ANDES_F 239.

Etymology: Named after its white pileus.

Pileus: 50–80 mm wide, convex to hemispheric; surface dry, smooth, white (1A1) with yellowish white (1A2), membranous universal veil patches, white (1A1) towards the margin; margin smooth to slightly appendiculate. *Context*: 4 mm thick, white, unchanging. *Lamellae*: 3.5 mm, broad, free to adnexed, close, and yellowish white (3A2). *Stipe*: 90 mm long × 6 mm

wide at the upper half and 28 mm at the lower half, fibrillose, hollow, white towards the apex to yellowish white (1A2) towards the base, abruptly bulbous; context white. *Annulus*: subapical, skirt-like, thin. *Volva*: limbate to membranous, white. KOH negative in the context of pileus and stipe. *Pileipellis* suprapellis formed by filamentous, periclinally arranged, very thin hyphae, 2–3 µm wide, with thin wall, vascular hyphae of 5–6 µm wide; subpellis with inflated hyphae. *Hymenophoral trama*: divergent, hyaline, inamyloid. *Universal veil:* formed by thin walled, narrow, occasionally slightly inflated hyphae. *Basidiospores*: white in mass, [90/3/3], 7–10 (–11) µm long, 8–10 (–11) µm broad, mean Q' = 1.03, globose, smooth, slightly amyloid. *Basidia*: 36–44 (–47) µm long, (11–) 14–16 µm broad, four-sterigmate basidia.

Other material examined: BOYACÁ/MUNICIPIO DE ARCABUCO/VEREDA PEÑAS BLANCAS/ 05°48.546" N, 73°28.751" W; on soil in *Q. humboldtii* forest/ Collection NVE410a ANDES_F2272. BOYACÁ, MUNICIPIO DE SABOYÁ, GARAVITO/ 05°42.006" N, 73°46.170" W; on soil in *Q. humboldtii* forest/ Collection NVE473 ANDES_F973. MYCOBANK: MB813038.

Notes: The collections NVE74, NVE475, NVE410a are similar in the anatomy to the species *A. arocheae*, described by Tulloss *et al*. (1992) but differ in its white pileus. The species *A. arocheae* has been previously reported to have a very wide range of colors in the pileus (Tulloss *et al*. 1992). The collection NVE74 was previously included in the study of lethal *Amanita* spp., by Cai *et al.* (2014) interpreting the collection (Acc. number FJ890028), as *A. "bisporigera"*. However, characters such as the four-sterigmate basidia (Fig. 4A) and globose spores (Q = 1.03–1.1), suggest that do not classify as *A. "bisporigera"*. On the other hand, in a previous study (Vargas *et al.* 2011) the collection NVE74 was determined as *A. virosa,* associated to the group of lethal *Amanita* with a high concentration of phallotoxins.

However, the species *A. virosa* are mainly reported in Palearctic regions–Europe and North-eastern Asia (Zhang *et al.* 2010; Cai *et al.* 2014) and presents a positive KOH reaction of the pileus and stipe contexts, which in the species *A. arocheae* is negative. The collections representing this taxon clearly grouped closer to the Costa Rican collection of *A. arocheae* (SUPPLEMENTARY FIGURES 2 and 3) within subsection *Phalloideae sensu* Drehmel *et al.* (1999) (Fig. 2, clade n).

Discussion

Previous phylogenetic studies assessed by ML and BI methods have shown the monophyly of two subgenera *Amanita* and *Lepidella* (Drehmel *et al.* 1999; Justo *et al.* 2010; Wolfe *et al.* 2012; Cai *et al.* 2014; Hosen *et al.* 2015), while other phylogenetic hypotheses assessed by distance-based methods or parsimony have failed to support this major division (Oda *et al.* 1999; Weiss *et al.* 1998; Zhang *et al.* 2004). Our results were not conclusive on the monophyly of each subgenus, each one being supported by a specific optimization criteria (with high support values from either ML or BI) but not both.

The infra-subgeneric classification varies among studies regarding morphological characters and molecular markers (Oda *et al.* 1999; Weiss *et al.* 1998; Zhang *et al.* 2004). A previous phylogenetic study by Oda *et al*. (1999) using ITS and nLSU, showed no clear grouping of species in section *Amanita* within subgenus *Amanita*. More recently Zhang *et al.* (2004) recovered section *Caesareae* and section *Vaginatae* as sister groups, with higher support values after using the ITS region but not with the nLSU region. So far, our phylogenetic hypotheses using ML and BI corroborate that, within subgenus *Amanita,* two sections are delimitated: *Amanita* and *Vaginatae* (Fig. 2 clades c and d). Furthermore, the section *Vaginatae* was divided into two subsections, which agrees with the infrageneric

classification system *sensu* Drehmel *et al.* (1999). The main morphological character supporting the split of these two subsections is the presence of an inconspicuous basal bulb and normally the absence of a ring in subsection *Vaginatae*. Besides that, the rank of subsection for the *Caesareae* group is consistently shown in our phylogenetic hypothesis (Fig. 2).

Our results support the monophyly of sections *Phalloideae* and *Lepidella sensu* Drehmel *et al.* (1999) (Fig. 2) within the subgenus *Lepidella*. Subsections *Phalloideae,* and *Validae*, are the sister group of subsection *Amidella*, agreeing with Weiss *et al.* (1998). The monophyly of subsection *Amidella* in our phylogenetic tree was well supported, and grouped species characterized by having a thick and firm saccate volva, and elongated to cylindrical spores (Singer 1986), as opposed to species with saccate to membranous volva and not elongated spores, grouped in subsection *Phalloideae,* and to those with a poorly developed volva in subsection *Validae*. In addition, the division of species in subsection *Amidella* could be further supported by the presence of the appendiculate character of the pileus margin, considered a more basal character than even or non-striate margins of the pileus (Bas, 1969).

Determining taxon ranks associated to particular names, i.e. species names, will normally differ between traditional classification systems and phylogenetic hypotheses (Cantino and de Queiros, 2010). The phylogenetic framework proposed by Drehmel *et al.* (1999) provides a classification system, which agrees with the three-gene phylogenetic approach in the present study. However, taxonomic keys used in this study propose ranks that differ from some the ranks displayed in the phylogeny. For comparison purposes, we compared different classification sources at the infra-generic rank (Table 1).

Our phylogenetic approach allows us going further in the classification below subsection level, since not many taxonomic keys treat the ranks below this level. Some series as well as

stirps in the monographic treatment for the family Amanitaceae in www.amanitaceae.org, can be assigned to clades in our proposed phylogeny. Two well-supported clades (clades g and d, Fig. 2) within subsection *Vaginatae,* correspond to the series *Ceciliae* and *Fulvae* (Tulloss & Yang, 2012a, b). The *A. fuligineodisca* group share features with the species *A. fulva* such as the Q' value of 1.03 obtained in *A. fuligineodisca* specimens, closer to that in *A. fulva* (Jenkins, 1986), but they differ in a darker color of the pileus in the disc to pale brown margins and a lower angle of split of the inflated cells of the hymenophoral trama in *A. fuligineodisca* (Tullos *et al*. 1992). This species also differed from the species *A. humboldtii* because of the dark brown to blackish brown center to pale brown margin of the pileus.

Moreover, a more specific division separates the specimens classified as *A. colombiana* (clade h, Fig. 2). Following the taxonomic key provided in Tulloss *et al*. (1992), 3 specimens were characterized by a pulverulent reddish volva, which were determined as *A. colombiana*, and differed from specimens characterized by a membranous volva in shades of gray (clade i), including the species *A. sorocula* characterized by the submembranous grayish volva, tick smoky grey membranous remnants over the pileus surface, and densely sulcate pilues margin (Fig. 1C). Subsection *Validae*, series *Validae* is composed of two clades, clades j and k. Clade j, consisted of species characterized by having non-rubescent color in the stipe, and shades in yellow at the pileus. Clade k, contained rubescent taxa, characterized by fuscous, to brown pileus in shades of red color over the disc and pale brown towards the margin; the new species *A. capillensis* was positioned in series *Validae* within clade k. Within the series *Mappae* two distinct clades were well supported, corresponding to the stirps *Bulbosa* (Tulloss and Yang, 2015b; Fig. 2, clade m) and stirps *Brunnescens* (Tulloss and Yang, 2015b; Fig. 2, clade l).

Finally, it is worth noting that the similarity parameter, proposed by Tulloss (2005b), between species present in Colombia and those in other regions such as Costa Rica, or North America will tend to change as studies of local macrofungal diversity increase. For instance, the similarity parameter is likely to increase after adding *A. rubescens*, *A. citrina*, and *A. capillensis* sp. nov., to the Colombian species list in subsection *Validae* , when compared to countries such as Costa Rica and the United States.

In addition to the 17 previously reported species, two new reports, *A. citrina* and *A. capillensis* are included in this study for a total of 19 species reported in Colombia. Whilst the phylogeny of 10 species was assessed in the present study, from specimens collected between 2012 and 2014 in the North Eastern Andean forests of Colombia, further phylogenetic analysis, should include the remaining nine species, by analyzing early herbaria collections and/or sampling more areas across the Andean montane forests in Colombia. Moreover, in order to have a broader panorama of the diversity of the genus in Colombia, it will be important to include species that are distributed in lowland tropical forests, where a high diversity of *Amanita* spp. is expected (Simmons *et al*. 2001, Henkel *et al*. 2012),

In the present study, the phylogenetic relationships of *Amanita* species found in Colombia was assessed by combining classical morphological and molecular methods with character-based methods such as ML and Bayesian inference, and compared to a wide number of species distributed world-wide. This study led to the description of a new species, a new variety, and a new record, together with an analysis of phylogenetic relationship of species that were not previously included in the *Amanita* phylogeny. Furthermore, it allowed the classification at a lower rank (*e.g.* stirps), permitting a better understanding of system classifications of the collected species. This combined morphological and phylogenetic approach can be useful to avoid ambiguous classifications of the taxa when these

classifications are based just on one criterion of analysis.

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TABLES

Table 1. Rank categories below section level of the collected species according to four classification systems.

Species	**Collected sporocarps/ Collections/ Samples in the phylogeny/ Host/ Distribution***	**Classification according to**			
		Drehmel *et al*. (1999)	Taxonomic keys Jenkins (1986) and Tulloss**	Amanitaceae.org	This Study
A. arocheae	3/NVE74, 410a, 473/3/*Q. humboldtii*/ANT, **BOY**, VAL	Sect. *Phalloideae*, Subsect. *Phalloideae*	Sect. *Phalloideae*	Sect. *Phalloideae*	Sect. *Phalloideae*, Subsect. *Phalloideae*, clade n
A. capillensis	3/NVE698, NVE624, IP24/2/*Q. humboldtii*/ **BOY**	Sect. *Phalloideae*, Subsect. *Validae*, Series *Validae*	Sect. *Validae*	Sect. *Validae*	Sect. *Phalloideae,* Subsect. *Validae, Series Validae,* clade k
A. citrina	2/NVE600, 616/2/*Q. humboldtii*/ **BOY**	Sect. *Phalloideae*, Subsect. *Validae*, Series *Mappae*	Sect. *Validae*	Sect. *Validae*, Series *Mappae*, Stirps *Bulbosa*	Sect. *Phalloideae*, Subsect. *Validae*, Series *Mappae*, Stirps *Bulbosa*
A. colombiana	3/NVE410, Penagos3, DS7/3/*Q. humboldtii*/ANT, **BOY**	Sect. *Vaginatae*, Subsect. *Vaginatae*	Sect. *Vaginatae*	Sect. *Vaginatae*, Series *Ceciliae*, Stirps *Ceciliae*	Sect. *Vaginatae*, Subsect. *Vaginatae*, Series *Ceciliae*, clade h
A. flavoconia	13/NVE242, 314, 351, 354, 397, 411, 454b, 521, 533, 563, 627, 746, CV3/3/*Q. humboldtii*/ANT, **BOY**, CUN, NAR	Sect. *Phalloideae*, Subsect. *Validae*, Series *Validae*	Sect. *Validae*	Sect. *Validae* infrasp. taxa of *flavoconia*	Sect. *Phalloideae*, Subsect. *Validae*, Series *Validae*, clade j
A. fuligineodisca	18/NVE 286b, 240, 304, 324, 343, 455, 505, 522, 567, 569, 630, 644, 665b, 692, 743, 748, AFM1812/2/*Q. humboldtii*/ANT, BOY, CUN, **SAN**, NAR	Sect. *Vaginatae* Subsect. *Vaginatae*	Sect. *Vaginatae*	Sect. *Vaginatae*, Series *Fulvae*, Stirps *Fulva*	Sect. *Vaginatae*, Subsect. *Vaginatae*, Series *Fulvae*, Stirps *Fulva*
A. muscaria	17/NVE277, 157, 488, 500, 588, 590, 636, 649, 662, 664, 674, 680, 719, 725, 726, 750, 760/3/*Pinus patula*/ ANT, BOY, CAL, CUN, **SAN**	Sect. *Amanita*	Sect. *Amanita*	Sect. *Amanita*, Subsect. *Amanita*, Series *Amanita*, Stirps *Muscaria*	Sect. *Amanita*, clade b
A. rubescens	10/NVE160, 285, 599, 613, 618, 624/2/*Pinus* spp./ANT, CUN, **BOY**	Sect. *Phalloideae* Subsect. *Validae*, Series *Validae*	Sect. *Validae*	Sect. *Validae* infrasp. taxa of *rubescens*	Sect. *Phalloideae*, Subsect. *Validae*, Series *Validae*, clade k
A. sororcula	1/NVE587/1/*Q. humboldtii*/ANT, BOY	Sect. *Vaginatae* Subsect. *Vaginatae*	Sect. *Vaginatae*	Sect. *Vaginatae*, Series *Ceciliae*, Stirps *Sororcula*	Sect. *Vaginatae*, Subsect. *Vaginatae*, Series *Ceciliae*, clade g
A. xylinivolva	12/NVE56, 126, 490, 491, 504, 511, 535, 670, 671, 672, 735, 747/ 3/*Q. humboldtii*/ANT, **BOY**, CAU, CUN, NAR, **SAN**	Sect. *Amanita*	Sect. *Amanita*	Sect. *Amanita*, Subsect. *Gemmatae*	Sect. *Amanita*, clade a
Total	# of collected sporocarps=77; # of specimens in the phylogeny=24				

*Departments: Antioquia (ANT), Boyacá (BOY), Caldas (CAL), Cundinamarca (CUN), Nariño (NAR), Santander (SAN), Valle del Cauca (VAL). Ref.: Nassi (1977), Tulloss *et al*. (1992), Franco-Molano *et al*. (2000), Vasco-Palacios and Franco-Molano (2013). In **bold** are new distributions according to this study. ** Ref.: Tulloss *et al*. (1992), Tulloss (2002), Tulloss (2005) or Tulloss (2009a).

Figures

Figure 1. Fruiting bodies of a subset of species analyzed in the present study. A) *Amanita fuligineodisca;* B) *Amanita colombiana;* C) *Amanita sororcula;* D) *Amanita xylinivolva;* E) *Amanita flavoconia;* F) *Amanita capillensis* sp. nov*;* G) *Amanita citrina*, and H) *Amanita arocheae* var. nov. *alba*

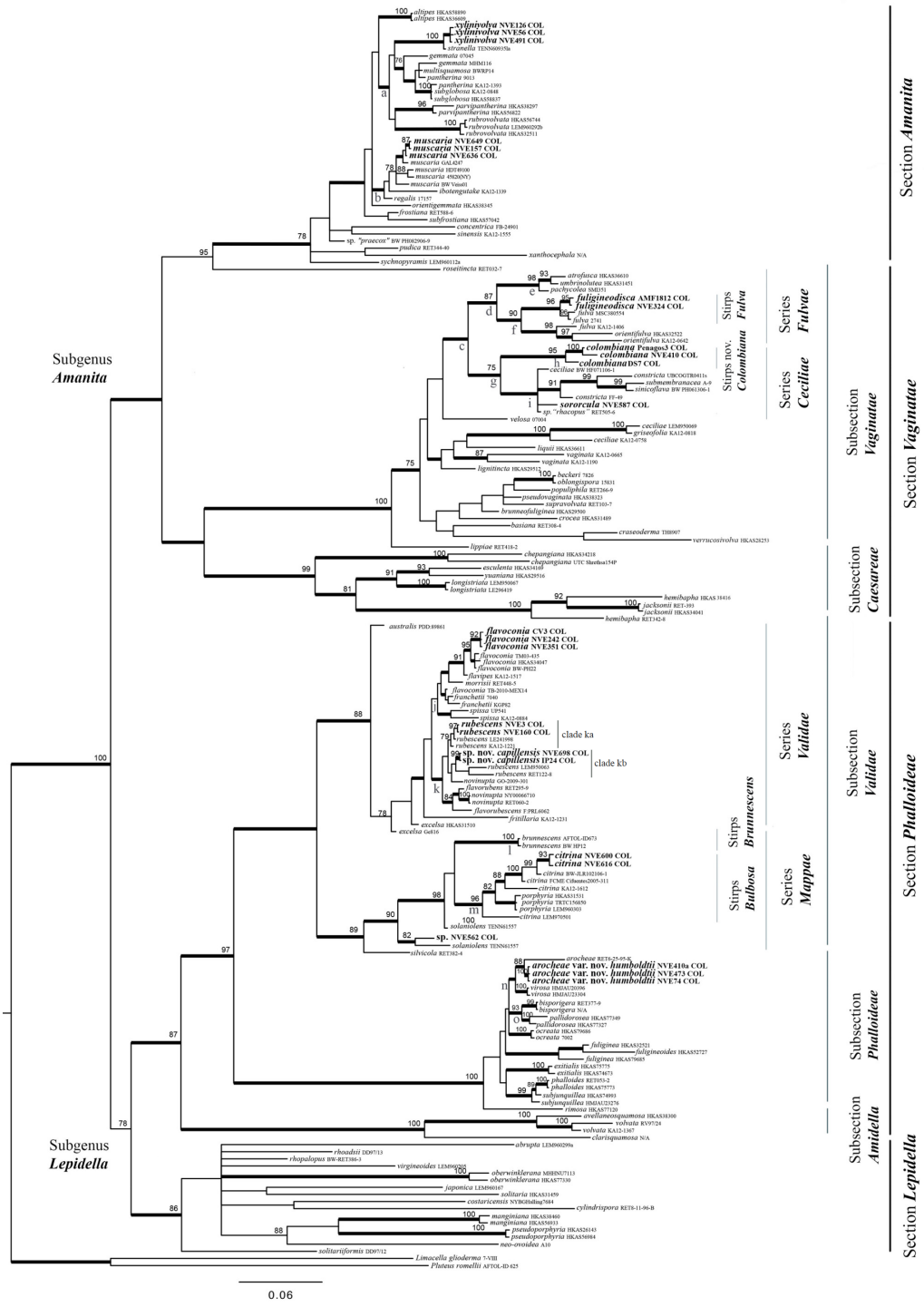


Figure 2. Phylogenetic reconstruction of three concatenated loci: nLSU, ITS, and EF1, by using Bayesian and maximum likelihood inferences. Posterior probability (PP) values are shown as thick black branches, indicating a Bayesian posterior probability (PP) > 0.95. Maximum likelihood bootstrap values (BS) > 75% are indicated above each branch. *ITS and nLSU sequences from collections belonging to the same country but different collector, were

concatenated.

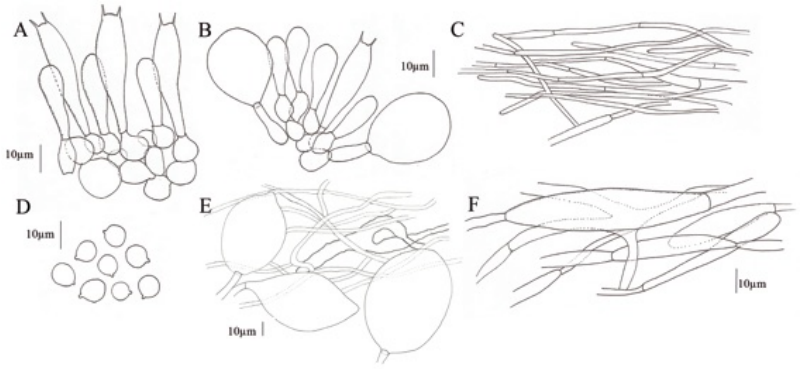


Figure 3. Microscopy of *Amanita arocheae* var. nov. *alba.* A) Basidia; B) Electronic microscopy of the hymenial layer showing four-sterigmate basidia; C) Spores; D) Volval elements, and E) Suprapellis.

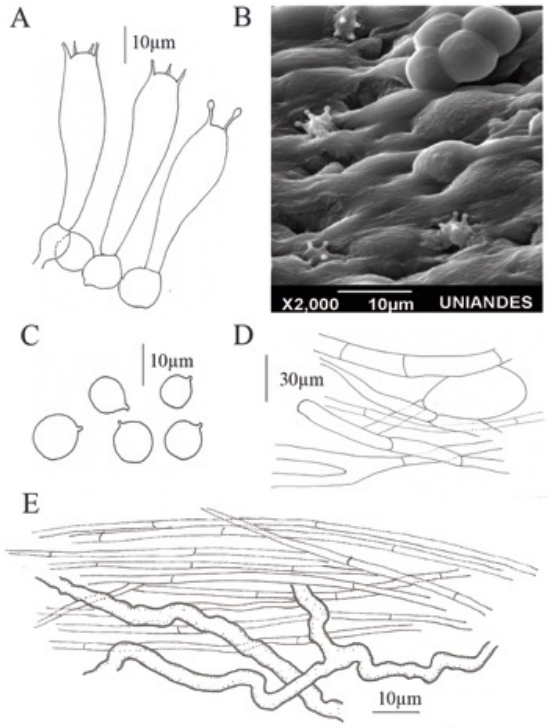
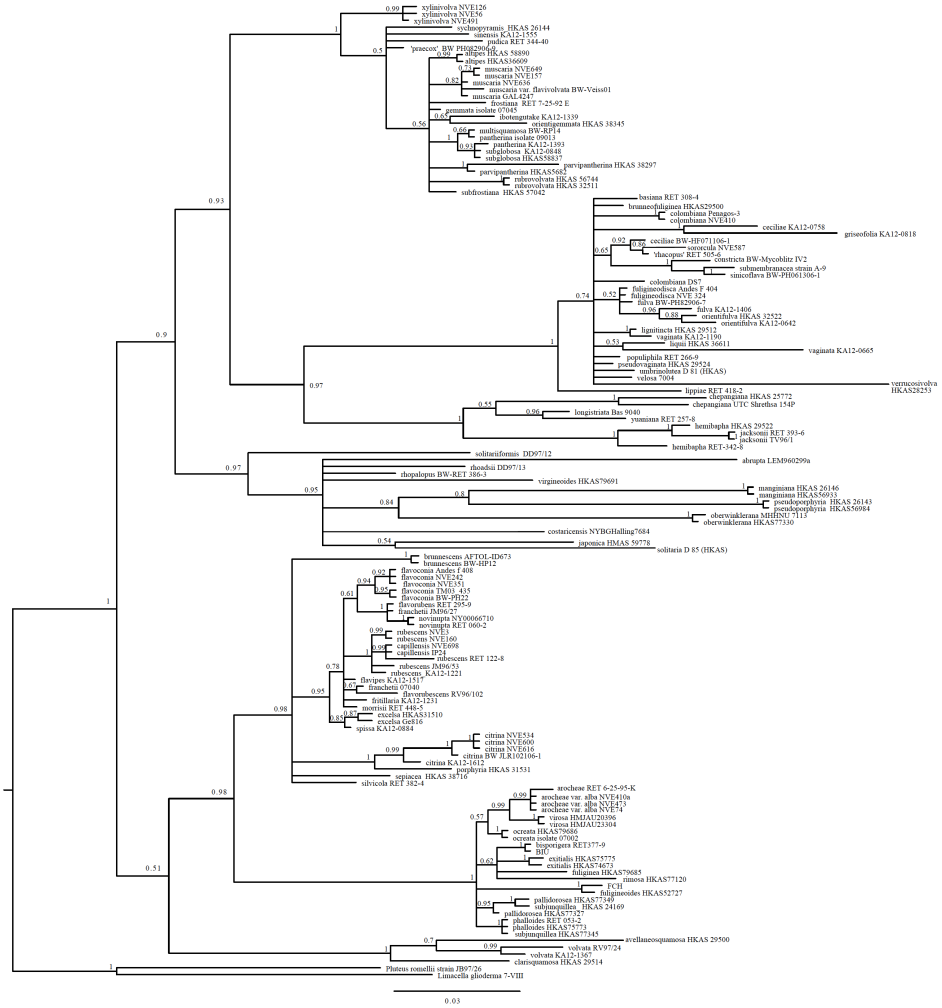


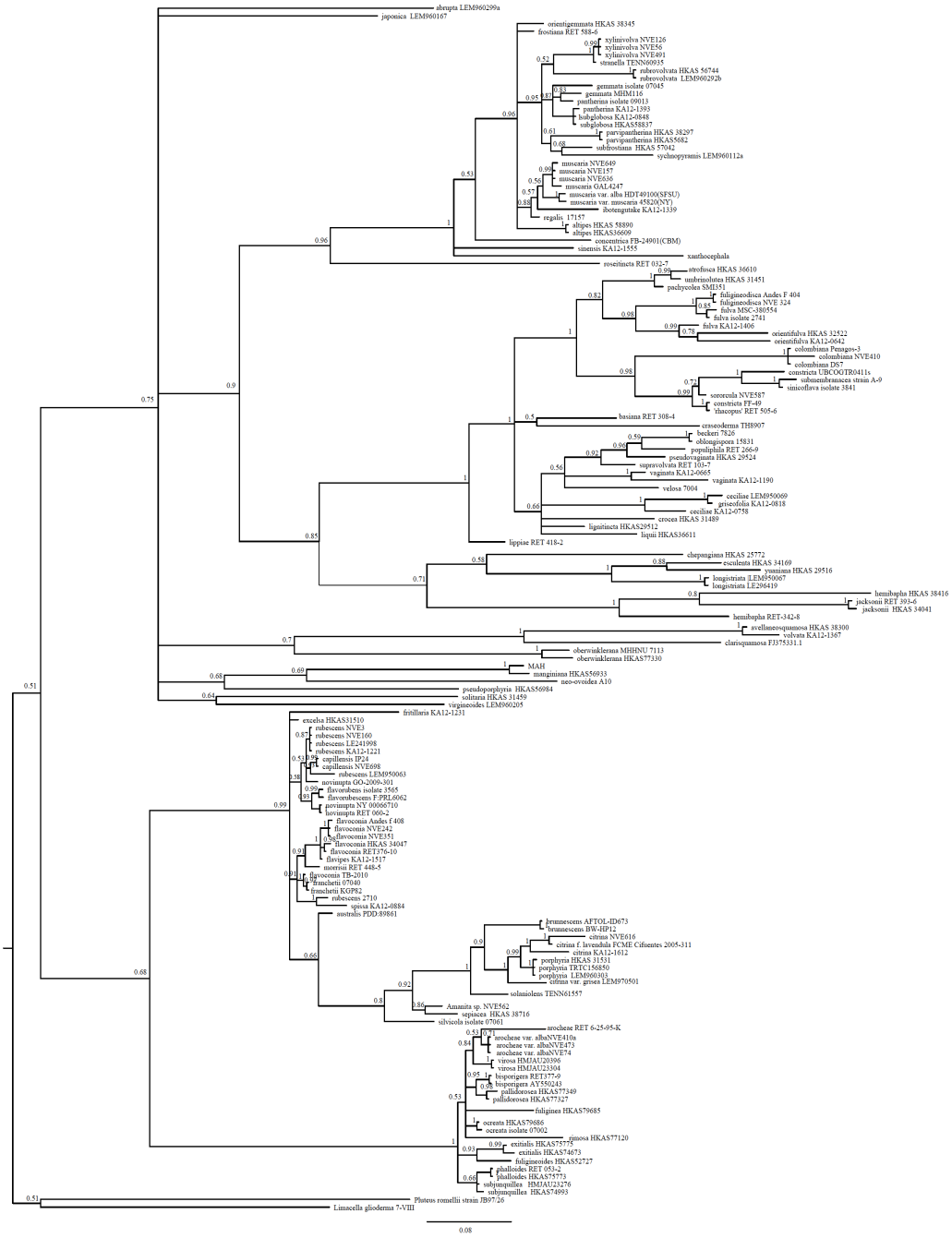
Figure 4. Microscopy of *Amanita capillensis* sp. nov. A) Basidia; B) Cheilocystidia; C)

Suprapellis; D) Spores; E) Volval elements, and F) Subpellis.

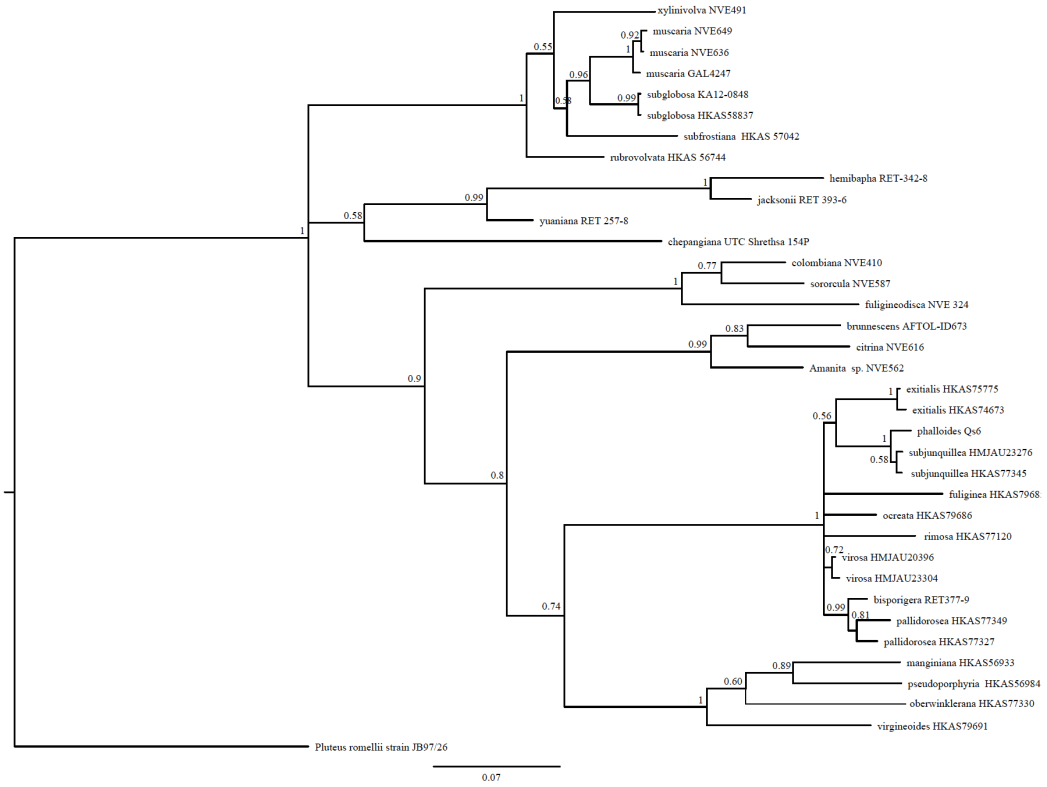
Supplementary information



Supplementary Figure 1. Phylogenetic reconstruction of the nLSU region by using Bayesian inference. Bayesian posterior probability (PP) is indicated above each branch.



Supplementary Figure 2. Phylogenetic reconstruction of the ITS region by using Bayesian inference. Bayesian posterior probability (PP) is indicated above each branch.



Supplementary Figure 3. Phylogenetic reconstruction of the *ef1* region by using Bayesian inference. Bayesian posterior probability (PP) is indicated above each branch.

Supplementary Table 1. Species used in the phylogenetic tree reconstruction, including their voucher number, country of origin, and the accession number for the gene fragment used per specimen. In bold we highlighted the specimens collected in Colombia.

Species	Voucher	Country	ITS	nLSU	EF1
A. abrupta	LEM960299a	Japan	AB015685.1	NA	NA
A. altipes	HKAS 36609	China	AY436445.1	AY436487.1	NA
A. altipes	HKAS 58890	China	JN943174.1	JN941159.1	NA
A. atrofusca	HKAS 36610	China	AY436446.1	NA	NA
A. australis	PDD:89861	New Zealand	GU222314.1	NA	NA
A. avellaneosquamosa	HKAS 38300	China	AY436447.1	NA	NA
A. avellaneosquamosa	NA	Germany	NA	AF024441.1	
A. arocheae* var. *alba	NVE74	**Colombia**	FJ890028.1	FJ890040.1	NA
A. arocheae* var. *alba	NVE410a	**Colombia**	KT008022	KT008036	NA

A. arocheae* var. *alba	NVE473	**Colombia**	KT008027	KT008042	NA
A. arocheae	RET 6-25-95-K	Costa Rica	AY325832.1	AY325879.1	NA
A. basiana	RET 308-4	Italy	KP258986.1	KP258987.1	NA
A. beckeri	7826	Italy	JF907758.1	NA	NA
A. bisporigera	RET377-9	USA	KJ466374.1	KJ466434.1	KJ481936.1
A. bisporigera	NA	USA	AY550243.1	NA	NA
A. brunnescens	BW_HP12	USA	HQ539780.1	HQ539674.1	NA
A. brunnescens	AFTOL-ID673	USA	AY789079.1	NA	AY881021.1
A. ceciliae	BW_HF071106-1	USA	NA	HQ539990.1	NA
A. ceciliae	LEM950069	Japan	AB015694.1	NA	NA
A. ceciliae	KA12-0758	South Korea	KF017929.1	KF021668.1	NA
A. chepangiana	HKAS 34218	China	AY436450.1	NA	NA
A. chepangiana	UTC Shrethsa 154P	Nepal	NA	KF877220.1	KF877113.1
A. capillensis	IP24	**Colombia**	FJ890032.1	FJ890047.1	NA
A. capillensis	NVE698	**Colombia**	KT008021	KT008035	NA
A. citrina	NVE600	**Colombia**	NA	KT008031	NA
A. citrina	NVE616	**Colombia**	KT008018	KT008032	KT008014
A. citrina	LEM970501	Japan	AB015680.1	NA	NA
A. citrina	KA12-1612	South Korea	KF245909.1	KF245893.1	NA
A. citrina	BW JLR 102106-1	USA	NA	HQ539679.1	NA
A. citrina	FCME Cifuentes 2005-311	USA	JF313662.1	NA	NA
A. clarisquamosa	NA	China	FJ375331.1	NA	NA
A. clarisquamosa	HKAS 29514	**NA**	NA	AF024448.1	NA
A. colombiana	NVE410	**Colombia**	KT008027	KT008041	KT008012
A. colombiana	Penagos3	**Colombia**	KT008023	KT008037	NA
A. colombiana	DS7	**Colombia**	FJ890045.1	NA	NA
A. concentrica	FB-24901(CBM)	Japan	AB080783.1	NA	NA
A. constricta	UBCOGTR0411s	Canada	EU597073.1	NA	NA
A. constricta	BW-Mycoblitz IV2	USA	NA	HQ539684.1	NA
A. constricta	FF-49	USA	KF007934.1	NA	NA
A. costaricensis	NYBGHalling7684	NA	NA	HQ539685.1	NA
A. craseoderma	TH8907	Guyana	KC155382.1	NA	NA
A. crocea	HKAS 31489	Germany	AY436484.1	NA	NA
A. esculenta	HKAS 34169	China	AY436451.1	NA	NA
A. excelsa	HKAS 31510	Germany	AY436453.1	AY436491.1	NA
A. excelsa	Ge 816	China	NA	HQ539691.1	NA

A. exitialis	HKAS74673	China	KJ466375.1	KJ466435.1	KJ481937.1
A. exitialis	HKAS 75775	China	JX998026.1	JX998053.1	JX998002.1
A. flavipes	KA12-1517	South Korea	KF245912.1	KF245896.1	NA
A. flavoconia	TM03-435	Canada	NA	EU522816.1	NA
A. flavoconia	CV3	**Colombia**	FJ890029	FJ890041	NA
A. flavoconia	NVE242	**Colombia**	KT008024	KT008038	NA
A. flavoconia	NVE351	**Colombia**	KT008026	KT008040	KT008007
A. flavoconia	TB-2010-MEX 14	Mexico	KC152064.1	NA	NA
A. flavoconia	HKAS 34047	USA	AY436456.1	NA	NA
A. flavoconia	BW_PH22	USA	NA	HQ539693.1	NA
A. flavoconia	RET376-10	USA	KC152064.1	NA	NA
A. flavorubens	isolate 3565	Canada	KJ638285.1	NA	NA
A. flavorubens	RET 295-9	USA	NA	HQ539694.1	NA
A. flavorubescens	F:PRL6062	USA	GQ166902.1	NA	NA
A. flavorubescens	RV96/102	USA	NA	AF097380	NA
A. franchetii	7040	USA	GQ250398.1	GQ250413.1	NA
A. franchetii	JM96/27	USA	NA	AF097381.1	NA
A. franchetii	KGP82	USA	DQ822790.1	NA	NA
A. fritillaria	KA12-1231	South Korea	KF245913.1	KF245897.1	NA
A. frostiana	RET 588-6	USA	KP313583.1	NA	NA
A. frostiana	RET 7-25-92 E	NA	NA	AF024453.1	NA
A. fuliginea	HKAS32521	NA	NA	AF024454.1	NA
A. fuliginea	HKAS79685	China	KJ466377.1	KJ466437.1	KJ481938.1
A. fuligineodisca	AFM1812	**Colombia**	FJ890027	FJ890039	NA
A. fuligineodisca	NVE324	**Colombia**	KT008025	KT008039	KT008011
A. fuligineoides	HKAS 52727	China	JX998024.1	JX998047.1	NA
A. fulva	BW_PH82906-7	USA	NA	HQ539697.1	NA
A. fulva	MSC 380554	USA	AY325844.1	AY325868.1	NA
A. fulva	isolate 2741	Canada	KJ638270.1	NA	NA
A. fulva	KA12-1406	South Korea	KF017933.1	KF021672.1	NA
A. gemmata	isolate 07045	USA	GQ250399.1	GQ250414.1	NA
A. gemmata	MHM116	Mexico	EU569282.1	NA	NA
A. griseofolia	KA12-0818	South Korea	KF017934.1	KF021673.1	NA
A. hemibapha	HKAS 38416	China	AY436460.1	NA	NA
A. hemibapha	RET-342-8	India	JX844716.1	KF877233.1	KF877124.1
A. hemibapha	HKAS 29522	China	NA	AF024458.1	NA

A. ibotengutake	KA12-1339	South Korea	KF017937.1	KF021676.1	NA
A. jacksonii	RET-393-6	USA	JX844725.1	KF877251.1	KF877140.1
A. jacksonii	HKAS 34041	USA	AY436461.1	NA	NA
A. jacksonii	TV96/1	USA	NA	AF097376.1	NA
A. japonica	LEM960167	Japan	AB015684.1	NA	NA
A. lignitincta	HKAS 29512	NA	JN182880.1	AF024461 2	NA
A. lippiae	RET 418-2	Brazil	KP258991.1	KP258992.1	NA
A. liquii	HKAS 36611	China	AY436462.1	AY436493.1	NA
A. longistriata	LE 296419	Russia	KJ739810.1	NA	NA
A. longistriata	LEM950067	Japan	AB015678.1	NA	NA
A. longistriata	Bas 9040	NA	NA	AF024462.1	NA
A. manginiana	HKAS 56933	China	KJ466378.1	KJ466438.1	HKAS56933
A. manginiana	HKAS 38460	China	AY436463.1	NA	NA
A. manginiana	HKAS 26146		NA	AF024463.1	NA
A. morrisii	RET 448-5	USA	KP284300.1	KP284301.1	NA
A. multisquamosa	BW_RP14	USA	NA	HQ539710.1	NA
A. muscaria	NVE649	**Colombia**	KT008020	KT008034	KT008010
A. muscaria	NVE636	**Colombia**	KT008019	KT008033	KT008009
A. muscaria	NVE157	**Colombia**	FJ890026	FJ890038	NA
A. muscaria	45820(NY)	USA	AB080790.1	NA	NA
A. muscaria	GAL4247	USA	DQ060894.1	DQ060874.1	EU071870.1
A. muscaria	HDT49100	NA	AB080793.1	NA	NA
A. muscaria	45820(NY)	USA	AB080790.1	NA	NA
A. muscaria var. *flavivolvata*	BW_Veiss_01	USA	NA	HQ539712.1	NA
A. neo-ovoidea	A10	China	FJ441040.1	NA	NA
A. novinupta	NY 00066710	USA	KJ535437.1	NA	NA
A. novinupta	GO-2009-301	Mexico	KC152067.1	NA	NA
A. novinupta	RET 060-2	USA	KF561974.1	KF561978.1	NA
A. oberwinklerana	HKAS 77330	China	KJ466380.1	KJ466441.1	KJ481946.1
A. oberwinklerana	MHHNU 7113	China	FJ176725.1	NA	NA
A. oblongispora	15831	Italy	JF907762.1	NA	NA
A. ocreata	7002	USA	GQ250404.1	GQ250419.1	NA
A. ocreata	HKAS 79686	USA	KJ466381.1	KJ466442.1	KJ481947.1
A. orientifulva	HKAS 32522	China	AY436464.1	AY436496.1	NA
A. orientifulva	KA12-0642	South Korea	KF017940.1	KF021679.1	NA
A. orientigemmata	HKAS 38345	China	AY436465.1	AY436497.1	NA

A. pachycolea	SMI351	Canada	HQ650725.1	NA	NA
A. pallidorosea	HKAS 77327	China	KJ466386.1	KJ466446.1	KJ481948.1
A. pallidorosea	HKAS 77349	China	KJ466389.1	KJ466449.1	KJ481961.1
A. pantherina	9013	USA	GQ401354.1	GQ401355.1	NA
A. pantherina	KA12-1393_	South Korea	KF017944.1	KF021683.1	NA
A. parvipantherina	HKAS 56822	China	JN943170.1	JN941163.1	NA
A. parvipantherina	HKAS 38297	China	AY436467.1	AY436499.1	NA
A. phalloides	RET 053-2	USA	KF561975.1	KF561979.1	NA
A. phalloides	HKAS 75773	China	JX998031.1	JX998060.1	NA
A. phalloides	Qs6	France	NA	NA	EU886739.1
A. porphyria	LEM960303	Japan	AB015677.1	NA	NA
A. porphyria	TRTC156850	Canada	JN020970.1	NA	NA
A. porphyria	HKAS 31531	China	AY436471.1	AY436500.1	NA
A. populiphila	RET 266-9	USA	KP224323.1	KP224346.1	NA
A. sp. *"praecox"*	BW_PH082906-9	USA	NA	HQ539725.1	NA
A. pseudoporphyria	HKAS 26143	China	NA	AF024471.1	NA
A. pseudoporphyria	HKAS 56984	China	KC429050.1	KC429047.1	KJ481953.1
A. pseudovaginata	HKAS 38323	China	AY436470.1	NA	NA
A. pudica	RET 344-40	Zambia	NA	HQ539730.1	NA
A. regalis	17157	Finland	JF907764.1	NA	NA
A. rimosa	HKAS 77120	China	KF479044.1	KJ466453.1	HKAS77120
A. sp. *rhacopus*	RET 505-6	Canada	KP224338.1	KP221313.1	NA
A. rhoadsii	DD97/13	USA	NA	AF097391.1	NA
A. rhopalopus	BW_RET 386-3	USA	NA	HQ539733.1	NA
A. roseitincta	RET 032-7	USA	KC855226.2	NA	NA
A. rubescens	NVE3	**Colombia**	FJ890030.1	FJ890042.1	NA
A. rubescens	NVE160	**Colombia**	FJ890031.1	FJ890043.1	NA
A. rubescens	LEM950063	Japan	AB015682.1	NA	NA
A. rubescens	KA12-1221	South Korea	KF245919.1	KF245903.1	NA
A. rubescens	LE241998	Russia	JF313651.1	NA	NA
A. rubescens	JM96/53	NA	NA	AF097382.1	NA
A. rubescens	isolate 2710	Canada	KJ638284.1	NA	NA
A. rubescens	RET 122-8	Turkey	NA	HQ539735.1	NA
A. rubrovolvata	HKAS 56744	China	JN943181.1	JN941156.1	KJ482002.1
A. rubrovolvata	LEM960292b	Japan	AB015689.1	NA	NA
A. rubrovolvata	HKAS 32511	NA	NA	AF024473 2	NA

A. sepiacea	HKAS 38716	China	AY436473.1	AY436501.1	NA
A. silvicola	RET 382-4	USA	NA	HQ539737.1	NA
A. silvicola	isolate 07061	USA	GQ250408.1	NA	NA
A. sinensis	KA12-1555	South Korea	KF017946.1	KF021685.1	NA
A. sinicoflava	isolate 3841	Canada	KJ638263.1	NA	NA
A. sinicoflava	BW_PH061306-1	USA	NA	HQ539739.1	NA
A. solaniolens	TENN61557	USA	JF313659.1	NA	NA
A. solitaria	HKAS 31459	Germany	AY436475.1	NA	NA
A. solitaria	Yang D 85 (HKAS)	NA	NA	AF024475.1	NA
A. solitariiformis	DD97/12	USA	NA	AF097390.1 2	NA
A. sororcula	NVE587	**Colombia**	KT008017	KT008030	KT008013
A. spissa	KA12-0884	South Korea	KF245910.1	KF245894.1	NA
A. spissa	UP541	Sweden	EF493270.1	NA	NA
A. stranella	TENN60935	USA	FJ596814.1	NA	NA
A. subglobosa	HKAS58837	China	JN943177.1	JN941152.1	KJ482004.1
A. subfrostiana	HKAS 57042	China	JN943173.1	JN941162.1	KJ482003.1
A. subglobosa	KA12-0848	South Korea	KF017947.1	KF021686.1	KJ482004.1
A. subjunquillea	HKAS74993	China	KJ466424.1	NA	NA
A. subjunquillea	HKAS77345	China	NA	KJ466491.1	KJ481989.1
A. subjunquillea	HKAS 24169	China	NA	AF024479.1	NA
A. subjunquillea	HMJAU23276	China	KJ466428.1	NA	KJ481991.1
A. submembranacea	strain A-9	Czech Republic	FJ705275.1	FJ705276.1	NA
A. supravolvata	RET 103-7	France	KP258995.1	NA	NA
A. sychnopyramis	LEM960112a	Germany	AB015690.1	NA	NA
A. sychnopyramis	HKAS 26144	NA	NA	AF024480.1	NA
A. umbrinolutea	HKAS 31451	Germany	AY436478.1	NA	NA
A. vaginata	KA12-1190	South Korea	KF017949.1	KF021688.1	NA
A. vaginata	KA12-0665	South Korea	KF017948.1	KF021689.1	NA
A. velosa	7004	USA	GQ250409.1	GQ250424.1	NA
A. verrucosivolva	HKAS 28253	NA	NA	AF024483	NA
A. virgineoides	HKAS79691	China	NA	KJ466495.1	KJ481996.1
A. virgineoides	LEM960205	Japan	AB015686.1	NA	NA
A. virosa	HMJAU23304	China	KJ466431.1	KJ466498.1	KJ481999.1
A. virosa	HMJAU20396	China	JX998029.1	JX998059.1	JX998008.1
A. volvata	RV97/24	USA	NA	AF097388.1	NA
A. volvata	KA12-1367	South Korea	KF245923.1	KF245907.1	NA

A. xanthocephala	NA	Australia	AY194982.1	NA	NA
A. xylinivolva	NVE56	**Colombia**	FJ890024	FJ890036	NA
A. xylinivolva	NVE126	**Colombia**	FJ890022	FJ890034	NA
A. xylinivolva	NVE491	**Colombia**	KT008029	KT008043	KT008008
A. yuaniana	RET 257-8	China	NA	KF877306.1	KF877188.1
A. yuaniana	HKAS 29516	China	AY436479.1	NA	NA
Limacella glioderma	7-VIII	NA	AY176453.1	DQ071728.2	NA
Pluteus romellii	strain JB97/26	NA	NA	AF261575.1	NA
Pluteus romellii	AFTOL-ID 625	NA	AY854065.1	NA	AY883433.1
***A.* sp**	NVE562	**Colombia**	KT008016	NA	KT008015

NA: Not Available

II. Phylogeography of *Amanita* spp., collected in Neotropical montane oak *Quercus humboldtii* Bonpl. in Colombia

INTRODUCTION

Numerous organisms with disjunct geographical distributions especially plants, have been the subject of studies supported by the boreotropic hypothesis of species distribution. This hypothesis is based on the biogeographic evidence of temperate deciduous plants having evergreen relatives in the tropics (Axelrod 1966, Wolfe 1975, Burnham and Graham 1999, Hughes and Eastwood 2006). This finding indicates that the biota that dominated the northern hemisphere during the early Cenozoic migrated to the southern hemisphere during the Miocene and Pliocene (Axelrod 1966, Wolfe 1975, Lavin and Luckow 1993).

Particularly, the genus *Quercus* (family Fagaceae) which comprises ~500 species (Dosmann and Aiello 2013, Stein *et al*. 2003), has a broad distribution in the American continent. It shows a high diversity of species in North America, expanding southwards with a tendency to decrease in species number when reaching lower latitudes (Pulido *et al*. 2006): 161 spp. in Mexico (Valencia-Ávalos 2004) 12-17 in Costa Rica (Müller 1942, Burger 1975), 10 in Panama (Müller 1942) and 1 in Colombia (Pulido *et al*. 2006)

For a long period of time, Central America was reported as the southernmost border in the distribution of *Quercus* (Hooghiemstra 2006). However, according to terrestrial pollen records found in the north Andean cordillera in Colombia, the recent immigrant species *Quercus humboldtii* Bonpl. (section *Lobatae*) expanded this range south during the Pleistocene (Kapelle 2006, Hooghiemstra 2006). The species is distributed from the Darien region in Panama (8º N) to the southern montane cordilleras in the Colombian Andes (1º N) (Pulido *et al*. 2006, Orwa *et al.* 2009). Some geological events that could have shaped the

diversification and evolution of neotropical species including *Q. humboldtii* are the Andean uplift, with an estimated development during the Miocene and early Pliocene (Burnham and Graham 1999; Pirie *et al*. 2006; Antonelly *et al*. 2009), the gradual closing of the Panama Isthmus during the Pliocene (5-3.5 My before present) (Burnham and Graham 1999; Hooghiemstra 2006, Pirie *et al*. 2006), among others.

As many plant taxa distributed in temperate/boreal (Halling 2001, Alexander 2006) or tropical regions (Lee 1998, Alexander 1989, Moyersoen 2006), *Q. humboldtii* establishes symbiotic relationship between cortical tissue of its actively growing rootlets and the fungal mycelium of **EC**to**M**ycorrhizal (ECM) species (Harley and Smith, 1983). Biogeographic studies suggest a co-migration of neotropical ECM Agaricales with theirs hosts having a north temperate ancestor (Halling 2001; Kennedy *et al*. 2011). Particularly, by comparing ECM species in northern and southern latitudes, a north/south distribution is claimed for some ectomycorrhizal species: *Cortinarius iodes, Lactarius indigo, L. atroviridis, Laccaria amethystina,* and *Strobylomyces confusus* (Halling 2001), which are present in plants distributed in North America and with *Q. humboldtii* in Colombia, as well.

A widely known and globally distributed ECM taxa is the genus *Amanita*, having approximately 500-1000 spp., distributed in temperate and tropical climates (Tulloss 2005, Cai *et al*. 2014, Sánchez-Ramírez *et al*. 2015). All species in the subgenera *Lepidella* and *Amanita* are ECM mutualists (Wolfe *et al*. 2012), whose global distribution consequently appears to be determined by their host distribution partners. Recent biogeographic analysis in the *Caesareae* group (subgenus *Amanita*) supported the boreotropic hypothesis, showing disjunct distributions of sister taxa with Palaeotropical and Neotropical distribution (Sánchez-Ramírez *et al*. 2015). Similarly, Cai *et al*. (2014) showed a North American origin in some

lethal taxa of the genus *Amanita,* extending southwards into Central America with their host plants oaks. In Colombia the majority of the reported *Amanita* species belong to either the subgenus *Lepidella* or subgenus *Amanita (*Vargas *et al*. unpub., Chapter 1, previous article). Both subgenera contain representative symbiotic species collected in *Q. humboldtii* forests (Franco-Molano *et al*. 2000).

The geological history related to the formation of the high-altitude montane forests located in the northern part of the Andes show that these areas are interesting hotspots to study the biogeography and neotropical diversification, since these type of forests have been considered continental islands with potential ecological niches for species diversification (Kenneth *et al*. 2002, Hughes and Eastwood 2006). In Colombia a high number of species of macrofungi have been reported in *Q. humboldtii* (Singer 1963, Guzman and Varela 1978, Franco-Molano *et al*. 2000, Halling and Mueller 2005). However neither ECM taxa in montane forests nor their Andean oak host species have been subjected to biogeographic analysis. Furthermore, the time and place of origin of most fungal species remains unknown (Lumbsch *et al*., 2008; Sánchez-Ramírez *et al*. 2015). In this study we used a phylogenetic approach to understand the biogeographic history and distribution of *Amanita* species as well as the neotropical *Quercus humboldtii* host, by using divergence dating and ancestral areas reconstructions. We seek to answer the following questions: When did *Amanita* species in Colombia begin to separate from their closest relatives? Do the fungal and oak species distribution fit to the boreotropical hypothesis?

MATERIALS AND METHODS

Oak collection–Leaves of *Q. humboldtii* were collected in San José de La Montaña, Municipio de Belén, Santander (06°02' 29.82'' N, 73°00' 02.8''W), at 3300 masl. The leaves were dried at 52 ºC.

DNA extraction, amplification, and sequencing– Amplification of the plant genes ITS and rbcL (Large subunit of the Photosynthetic enzyme Rubisco) were carried out in *Q. humboldtii* DNA. ITS is a nuclear gene with a high copy number; rbcL is a universal gene for plants, with a slow rate of change (Judd *et al*. 2008). Primers ITS 17F/26 were used in the ITS reaction. PCR was performed with a Peltier thermal cycler (Bio-Rad) in 25 µL reaction mixtures containing double distilled H2O, 2 µL of DNA template, 1 µL of each 10 µM primer, 2.5 µL of Taq 10x buffer, 0.5 µL of 10 mM dNTP mix, 3 µL of 25 mM MgCl2, 0.5 µL of 10x BSA and 0.2 µL of 5U/µL Taq polymerase. Cycling parameters were as follows: initial denaturation at 94ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 30 sec, annealing at 48 ºC for 30 sec, extension at 72 ºC for 2 min, and a final extension at 72ºC for 10 min. PCR amplification of *rbcL* was carried out with primers rbcL 1F/724R, using 25 µL reaction mixtures containing double distilled H2O, 1 µL of DNA template, 1.25 µL of each 10 µM primer, 2.5 µL of Taq 10x buffer, 1 µL of 10 mM dNTP mix, 2.5 µL of 25 mM MgCl2, 0.5 µL of 10x BSA and 0.2 µL of 5U/µL Taq polymerase. Cycling parameters were as follows: initial denaturation at 95 ºC for 4 min, followed by 5 cycles of denaturation at 94 ºC for 30 sec, annealing at 55 ºC for 1 min, extension at 72 ºC for 1 min, and 30 cycles of denaturation at 94 ºC for 30 sec, annealing at 54 ºC for 1 min, extension at 72 ºC for 1 min a final extension at 72 ºC for 10 min. Amplified PCR products were visualized by gel electrophoresis on a 1% agarose gel. Single band PCR products were sequenced using a

3730xl DNA analyzer (PE Applied Biosystems, USA). Sequences from *Amanita* spp. collected in Colombia were those previously used in the phylogeny in Chapter 1 (Vargas *et al*. unpub.).

Dated phylogenies of the fungi and *Amanita*– To confirm the secondary calibration point of the divergence in the genus *Amanita,* previously reported by Cai *et al*. (2014), we constructed a dated phylogeny of the fungi by using a dataset of sequences from the nuclear Large Subunit nLSU (Supplementary Table 1). Three fossil node calibrations were used, (in parenthesis are the parameters used following Hibbett *et al*. 1997 and Feng *et al*. 2012): (i) the divergence between Ascomycota and Basidiomycota (a normal distribution was applied by setting the mean to 582.5 My and the standard deviation to 20), (ii) the initial diversification of the mushroom forming fungi (logmean = 2.5, logstdev = 0.5, and offset = 90.0); and (iii) the divergence between Hymenochaetaceae and Fomitopsidaceae (Polyporoid fungi) (log- mean = 2.0, logstdev = 0.5, and offset = 125.0).

To construct the dated phylogeny of the genus *Amanita*, we used a dataset with the ITS, nLSU and *ef 1a* gene sequences from *Amanita* used previously in Chapter 1 (Vargas *et al*. unpub.) and, additionally, for each species represented in Colombia we included all the sequences available in GenBank from different countries (Supplementary Table 2). The secondary calibration point for the divergence of the genus *Amanita,* obtained from the dated phylogeny of the fungi, was used to estimate the divergence times within the species of the genus *Amanita*. The divergence times were estimated with Beast 1.8 (Drummond and Rambaunt, 2007) using an uncorrelated normally distributed clock model, mean=151.88 Myr [HPD 95% 105.527–247.318 Mya, SD 4], a value close to the secondary calibration obtained

in Cai *et al*. (2014). XML files were constructed with BEAUti 1.8 by importing separate NEXUS files of each gene partition. The gene partitions were set to be unlinked for substitution model and linked (concatenated) for molecular-clock model and tree-model prior (Yule speciation model). The substitution model K2+G was used for ITS dataset, K2 + G for nLSU and K2 + G +I for *ef1*. Four independent Markov chain Monte Carlo (MCMC) chains of 100,000,000 generations and sampling frequency of 1000 were conducted for each dataset. Convergence was assessed by comparing log files in Tracer 1.6. Per-clade posterior probabilities were summarized using TreeAnnotator 1.8, with 10% burn-in, and confirming a minimum value of 200 corresponding to the effective sample size (ESS).

Dated phylogenny of the hosts– We searched for ITS and rbcL sequences belonging to species in section *Lobatae, Quercus, Cerris,* and the subgenus *Cyclobalanopsis* in GenBank (Supplementary Table 3). Most of the data set for *Quercus* was based on previous sequences used by Manos *et al.* (2001). To calibrate the tree, we used fossil dates at three nodes following previously used parameters by Cavender-Bares *et al*. (2015): the first node corresponding to the white oak clade (*Quercus* section *Quercus*) was assigned an informative lognormal prior with a median age of 31.3 My with 95% of the distribution between 28 and 38.2 My. The second node corresponds to the American oak clade, which includes the red (*Quercus* section *Lobatae*), white (*Quercus* section *Quercus*) and golden oaks (*Quercus* section *Protobalanus*) in which an informative lognormal prior with a median of 40 million years ago and 95% of the distribution within 37.25 and 44.0 My is based on dates for the earliest documented *Quercus* macrofossils in the Americas, and a third node corresponding to the root node for the genus *Quercus*, was assigned an informative exponential prior with a

median of 50.4 My and 95% of the distribution within 40.8 and 85 My. The substitution model K2 + G was used for ITS dataset and JC for rbcL. Four independent Markov chain Monte Carlo (MCMC) chains of 100,000,000 generations and sampling frequency of 1000 were conducted for each dataset. Convergence was assessed by comparing log files in Tracer 1.6. Per-clade posterior probabilities were summarized using TreeAnnotator 1.8, with 10% burn-in, and confirming a minimum value of 200 corresponding to effective sample size (ESS) values.

Biogeographic analysis – The ancestral area reconstruction was based on the posterior distribution of the dated ITS phylogeny estimated from BEAST, using a Bayesian Binary MCMC analysis conducted in RASP (Yu *et al*. 2005). Nine specific geographic regions were assigned for each *Amanita* taxon based on the country information available in GenBank (Supplementary Table 2): East Asia (A), Europe (B), Russia (C), India (D), Africa (E), Oceania (F), North America (G), Central America (H) South America (I). Five regions were used for *Quercus* taxa: Asia (A), Europe (b), North America (C), Central America (D) and South America (E). The Bayesian Binary MCMC analysis was by setting generations to 1000 and other parameters were kept at the default setting. The boreotropical hypothesis was tested by using the following models according to the angiosperm distribution (Wolfe 1975, Sánchez-Ramírez *et al*. 2015): a) 34-15 Mya (Oligocene and early Miocene) dispersal among Paleotropical areas (Africa, East Asia and India), b) 15-5Mys (Late Miocene), among Paleotropical and temperate Europe and c) 5–0 Mya allowing a high dispersal between North America, Central America and South America (Fig. 1). Three null models were used based on high, moderate or restrictive dispersal rates. To test if the distribution and origin of the

Colombian collections follow the Boreotropical hypothesis we generate scripts using the Lagrange configurator (www.reelab.net/lagrange) with the dispersal models and extinction set as constant over time and across lineages (Cai *et al*. 2014).

RESULTS

Dated phylogeny of *Amanita* collected in Colombia. The dataset used to construct the dated phylogeny in the genus *Amanita* consisted of ITS, nLSU and *ef1* regions: 652, 519 and 511 characters (including gaps); 356, 181 and 250 parsimony-informative characters; 456, 234 and 423 variable sites; 150, 275, and 87 conserved sites, respectively.

Based on the secondary calibration for the genus *Amanita* obtained from the dated phylogeny for fungi (Supplementary Fig. 1), the estimated divergence for the genus was 155.93 Mya (95% HPD 105.527–247.318) (Fig. 2) coinciding with previous studies (Cai *et al.* 2014) where the authors estimated a 95% HPD interval (116.48 –193.63 Mya). Within the subgenus *Amanita* section *Amanita* the species *A. xylinivolva* was separated from its closest relative 5.29 Mya (Figs. 2 and 3) and has a North American origin (Fig. 3). The cognate species *A. stranella* is described from Pine and Eastern Hemlock forests of New York (Tulloss and Yang 2015a). The species *A. muscaria* collected in ectotrophic forest of *Pinus patula*, grouped close to southern hemisphere collections (Australia and New Zealand) together within samples of Eurasian collections (Fig. 2 and 3). In the section *Vaginatae* (subsection *Vaginatae*) *A. fuligineodisca* diverged from a clade of North American *A. fulva* individuals 12.17 Mya (95% HPD [4.29–23.42]) (Fig. 2, Table 1), and from the European clade during early Miocene (Fig. 2). *A. craseoderma* a Brasilian species and the Colombian species *A. sororcula* diverged 13.57 Mya during the mid-Miocene.

On the other hand, within subgenus *Lepidella, A. citrina (*subsection *Validae*, series *Mappae*) a species originally described from Europe (Supplementary table 2) showed relatives in Colombia. According to the ancestral reconstruction *A. citrina* was derived from North America collections and its most recent common ancestor inhabited North America, and following a dispersal event (probability of 0.85) the two populations split 8.07 Mya during the late Miocene (Fig. 2).

Within the subsection *Validae* series *Validae,* four species were analyzed. *Amanita flavoconia* is a widely dispersed species in the Colombian cordilleras, specifically the variety *inquinata* is distributed from Mexico, Costa Rica and Colombia (Halling and Mueller 2005). The species separated from the North American populations 12.08 Mya in the mid-Miocene (Fig. 2) whose most recent common ancestor inhabited North America. *Amanita capillensis* a newly described species was more closely related with rubescent species from Asia (Fig. 3) and the ancestral population inhabited Asia 8.8 Mya during the mid Mycocene. On the other hand *A. rubescens* a species collected in the ectotrophic species *Pinus patula* was closely related with individuals from Russia, Europe and Asia and separated from the Russian collection 0.28 Mya in the Pleistocene. Furthermore, the collections *Amanita* sp. NVE562 (Supplementary Fig. 3) diverged from the species *A. silvicola* 7.35 Mya (Fig. 2) which originally was described from Oregon –USA– (Tulloss and Yang 2015b) (Supplementary Table 2).

The toxic species *Amanita arocheae*, diverged from the Costa Rican *A. arocheae* collection during the Miocene (9.36 Mya) and early in the Miocene these species separated from the North American *A. virosa* collections 16 Mya (Fig. 2 and 3).

The boreotropic hypothesis shown to be the most probable model for distribution of all species associated to *Q. humboldtii* (Table 3), indicating that these neotropical species were initially derived from North American populations. The two *Amanita* spp., *A. muscaria* and *A. rubescens*, associated to exotic host species, appeared to fit to models of moderate to high dispersion among geographic regions. None of the Colombian collections studied here were explained by any of the Gondwana dispersal models.

Phylogeography of *Q. humboldtii*– The species clearly grouped within the monophyletic section *Lobatae* (red oak) (Fig. 4). It shares a common ancestor with a group of species distributed in North America. The species diverged approximately 16 Mya (HPD 95% 10.32–22.58 Mya) during the mid-Miocene (Table 2). The ancestral reconstruction showed a North American-South American origin for *Q. humbodltii* (Fig. 5).

DISCUSSION

Molecular dating results showed that the divergence ages of most of the *Amanita* species in Colombia from their sister species fall roughly within the same divergence time than their host *Q. humboldtii* from its sister clade — distributed in North America (Figs. 2 and 4). Most of the Colombian *Amanita* species collected in *Quercus humboldtii* have diverged from the temperate North American populations during the middle Miocene. This similarity in the divergence age among *Q. humboldtii* and *Amanita* spp. and North American origin fits with the hypothesis that ectomycorrhizal fungi in the Northern Hemisphere dispersed following their host dispersion and divergence, and that many Neotropical taxa are immigrant from north temperate zone (Halling 2001, Matheny *et al*. 2009, Wilson *et al*. 2012), rather than a fungal inter-populational gene exchange via long-distance dispersal of spores (Halling *et al.*

2008).

The results obtained in this study confirmed the North American origin of Neotropical *Amanita* taxa collected in Colombia, having a temperate relative including *A. flavoconia, A. citrina, A. fuligineodisca, A. sororcula, A. arocheae* and *Amanita* sp. NVE562 (Tedersoo & Nara 2010). Moreover the probable event that could have taken place during the divergences for most of the *Amanita* species collected in Colombia, was vicariance (Fig. 3). Our results suggest that the lineages in South America (*Amanita* and *Querqus* host) diverged from the north temperate ones during the Miocene, coinciding with an epoch of recurrent events of dispersal during the late Miocene and Pliocene (Sánchez-Ramírez *et al*. 2015). It is important to highlight that several geological events occurred contemporary to the divergence time from northern *Amanita* and oak species, such as the uplifting of the Andes. Recent phylogenetic studies have shown that the Andean uplift acted as a dispersal route for boreotropical lineages (Pirie *et al*. 2006).

An interesting result comes from the observation of species whose host is not *Quercus humboldtii*. These species including *A. rubescens* and *A. muscaria* had more relatedeness with individuals from Eurasia or Oceania, respectively. In this case, the node ages separating the Colombian collections from other clades, do not necessary reflect their arrivals into Colombia, since it is known that the introduction of *Pinus* spp. to Colombia (Chapter 2) was carried out during the 1950s. Probably these species diverged from a common ancestor in Eurasia during the Miocene (Fig. 2 and 3).

Phylogeography of the genus *Amanita* The genus *Amanita* could originate in the

Paleotropics, where East Asia can be considered as the ancestral area where the genus started to diversify and disperse. Asia had the major quantity of dispersal events from and within the region, 33 and 74, respectively (Table 2). Previously in other ancestral reconstructions, Cai *et al*. (2014) demonstrated Asia as the ancestral area for lethal *Amanita* spp. Furthermore, Sánchez-Ramírez *et al*. (2015) test the boretropical hypothesis, conciliating that the species in section *Caesareae* were Paleotropical (Africa) in origin. Similarly, early members of the ectomycorrhizal family Inocybaceae have been tested as out-of-Paleotropics (Matheny *et al*. 2009).

Future perspectives

The information we provided here serve as starting point to know the history of the ECM species in Colombia which have a northern hemisphere origin and diverged during the Miocene. Central American samples will be necessary to complete a comprehensive history divergence and migration of *Amanita* species throughout Central America and Colombia. Specifically molecular data from both *Amanita* and *Quercus*, extending from Mexico to Costa Rica will be necessary to corroborate later divergence times (e.g. during the Pliocene) among Colombian collections and Central American collections, that could be related to geological events such as the landbridge connecting North America and South America, occurring about 3.5 Mya (Lomolino *et al*. 2006). This later would be a plausible hypothesis that will support the recent colonization of *Q. humboldtii* to the Colombian Andes according to fossil record, dating approximately to 0.47 Mya during the early Pleistocene (Hooghiemstra and Sarmiento 1991, Van´t and Hooghiemstra 2000, Hooghiemstra 2006).

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Tables

Table 1. Divergence times of *Amanita* spp. collected in *Q. humboldtii*

Species	Divergence node (Mya)	95% HPD	Origin of the closest species in Fig. 2	Vicariance or Dispersal event (probability)
Subgenus *Amanita*				
A. xylinivolva	4.5	[0.85–11.52]	North Eastern USA	Vicariance (1.0)
A. muscaria	6.23	[0.27–6.74]	New Zealand- Australia	Vicariance and dispersal (0.52)
A. fuligineodisca	12.17	[4.29–23.42]	North America	Vicariance (1.0)
A. colombiana	30.85	[16.34–47.09]	North America	Vicariance (0.81)
A. sororcula	13.57	[3.30–27.04]		
Subgenus *Lepidella*				
A. flavoconia	12.08	[5.40–20.46]	North America	Vicariance (1.0)
A. rubescens	0.28	[0.01–1.46	Russia and Europe	Vicariance (1.0)
A. capillensis	8.54	[3.18–15.05	East Asia	Vicariance (1.0)
A. citrina	8.18	[2.34–17.02]	North America - East	Vicariance (1.0)
A. arocheae	9.36	[2.40–17.91]	North America	Vicariance (1.0)
Amanita sp. NVE562	7.35	[0–26.25]	USA	Vicariance (1.0)
Section *Lobatae*				
Q. humboldtii	15.95	[10.32–22.58]	Pacific North West USA	Vicariance (1.0)

Table 2. Dispersal events from, to, within and among areas

Area	Dispersal			Speciation Within Areas
	from	*to*	*within*	
East Asia (A)	33.5	5	74	74
Europe (B)	5.5	19	15	15
Russia (C)	0.5	5	0	–
India (D)	0.5	1	0	–
Africa (E)	1.5	1	0	–
Oceania (F)	0	3	2	2
North America (G)	19	15	60	60
Central America (H)	0	4	2	2
South America (I)	3.5	11	18	18

Dispersal events among Areas

A->B:12
A->C:2.5
A->D:1
A->F:2.5
A->G:13
A->H:0.5
A->I:2
B->A:0.5
B->C:2
B->E:0.5
B->G:1
B->I:1.5
C->I:0.5
D->G:0.5
E->A:0.5
E->C:0.5
E->I:0.5
G->A:2.5
G->B:6.5
G->E:0.5
G->H:3
G->I:6.5
I->A:1.5
I->B:0.5
I->F:0.5
I->G:0.5
I->H:0.5

Table 3. Likelihood-ratio test comparison of dispersal models within clades of the studied *Amanita* spp. in Colombia

Models	ln-Likelihood
A. fuligineodisca	
Boreotropic D	-60.31
Boreotropic A	-77.34
Boreotropic C	-79.78
Boreotropic B	-80.12
Restrictive	-99.12
Gondwana A	-129.8
Gondwana B	-130.65
Highly permisive	-134.65
Moderate permisive	-144.09
A. colombiana	
Boreotropic D	-82.01
Boreotropic A	-86.64
Boreotropic C	-90.43
Boreotropic B	-100.35
Gondwana A	-100.93
Gondwana B	-103.76
Highly permisive	-104.65
Restrictive	-154.12
Moderate permisive	-184.34
A.xylinivolva	
Boreotropic D	-82.09
Boreotropic B	-90.59
Boreotropic C	-94.01
Gondwana A	-102.67
Boreotropic A	-109.64
Highly permisive	-114.29
Restrictive	-115.73
Gondwana B	-120.78
Moderate permisive	-187.99
A. citrina	
Boreotropic D	-65.24
Boreotropic C	-74.91
Boreotropic B	-75.83
Gondwana A	-101.48
Boreotropic A	-103.81
Gondwana B	-104.12
Highly permisive	-112.61
Moderate permisive	-116.29
Restrictive	-124.05

A. flavoconia	
Boreotropic D	-104.67
Boreotropic B	-130.76
Boreotropic C	-132.09
Boreotropic A	-145.86
Moderate permisive	-158.37
Gondwana A	-165.98
Highly permisive	-182.41
Restrictive	-189.01
Gondwana B	-190.26
A. capillensis	
Boreotropic C	-82.67
Moderate permisive	-95.3
Gondwana B	-100.32
Boreotropic D	-107.45
Highly permisive	-120.09
Gondwana A	-127.39
Boreotropic B	-129.76
Restrictive	-138.42
Boreotropic A	-156.66
A. arocheae var. *alba*	
Boreotropic D	-98.56
Gondwana B	-100.32
Boreotropic A	-106.05
Highly permisive	-109.65
Gondwana A	-120.12
Boreotropic C	-120.34
Restrictive	-128.07
Moderate permisive	-163.78
Boreotropic B	-183.09
A. rubescens	
Highly permisive	-82.81
Moderate permisive	-99.36
Boreotropic D	-111.35
Boreotropic A	-126.66
Boreotropic B	-134.72
Boreotropic C	-154.98
Gondwana B	-165.32
Gondwana A	-187.52
Restrictive	-198.42
A. muscaria	
Highly permisive	-92.26
Moderate permisive	-100.88
Boreotropic C	-123.84

Restrictive	-124.75
Boreotropic B	-125.98
Gondwana B	-130.42
Boreotropic D	-139.03
Gondwana A	-150.76
Boreotropic A	-178.25

Supplementary Tables

Supplementary Table 1. Accession numbers of fungal species used in the fossil calibrated tree

SPECIES	nLSU	nLSU VOUCHER	TAXONOMY
Taphrina deformans	DQ470973	CBS 356.35	Ascomycota; Taphrinomycotina; Taphrinomycetes; Taphrinales
Hygrocybe conica	DQ071739.2	FO 46714	Basidiomycota; Agaricomycotina, Agaricomycetes; Agaricomycetidae; Agaricales
Laccaria bicolor	DQ071773.1	TUB 011581	Basidiomycota; Agaricomycotina, Agaricomycetes; Agaricomycetidae; Agaricales
Mycena pura	AF291347.1	FO 46623	Basidiomycota; Agaricomycotina, Agaricomycetes; Agaricomycetidae; Agaricales
Lactarius deliciosus	EU522812.1	TM03_430	Basidiomycota; Agaricomycotina, Agaricomycetes; Agaricomycetidae; Russulales
Stereum hirsutum	AY039330.1	strain TJV93_161	Basidiomycota; Agaricomycotina, Agaricomycetes; Agaricomycetidae; Russulales
Clavaria flavostellifera	KC759468.1	BRACR15924	Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Agaricales; Clavariaceae
Marasmius alliaceus	AY207234.1	HKI ST 22292	Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Agaricales; Marasmiaceae
Hygrophoropsis aurantiaca	AF042007.1	Ha1	Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Boletales
Coltricia perennis	KJ000223.1	Cui 9282	Basidiomycota; Agaricomycotina; Agaricomycetes; Hymenochaetales
Ramaria rubella	KC345747.1	OSC:110599	Basidiomycota; Agaricomycotina; Agaricomycetes; Phallomycetidae; Gomphales
Gautieria otthii	AF336249.1	636	Basidiomycota; Agaricomycotina; Agaricomycetes; Phallomycetidae; Gomphales
Fomitopsis pinicola	EU232290.1	TFRI 513	Basidiomycota; Agaricomycotina; Agaricomycetes; Polyporales
Calocera cornea	AB472738.1	CBS 124.84	Basidiomycota; Agaricomycotina; Dacrymycetes; Dacrymycetales
Dacryopinax spathularia	AF291312.1	FO 42687	Basidiomycota; Agaricomycotina; Dacrymycetes; Dacrymycetales
Cryptococcus neoformans	FJ914894.1	ATCC MYA-4567	Basidiomycota; Agaricomycotina; Tremellomycetes; Tremellales
Tremella moriformis	AF291374.1	MW 335	Basidiomycota; Agaricomycotina; Tremellomycetes; Tremellales
Sporobolomyces corallinus	AB638335.1	MAFF 654003	Basidiomycota; Pucciniomycotina; Agaricostilbomycetes
Puccinia sparganioi dis	*GU058027.1*	BPI 879285A	Basidiomycota; Pucciniomycotina; Pucciniomycetes
Ustilago maydis	KP132875.1	UOA/HCPF 14759	Basidiomycota; Ustilaginomycotina; Ustilaginomycetes; Ustilaginales
Rhizopus oryzae	KM491901.1	taxon:64495	Mucoromycotina; Mucorales

Supplementary Table 2. *Amanita* sequences retrieved from GenBank

Species	Country GenBank	Origin – Amanitaceae.org	Region of origin	ITS2	nLSU
altipes1	China	China	EAS	AY436445.1	AY436487.1
altipes2	China	China	EAS	JN943174.1	JN941159.1
arocheae1	Costa Rica	Mexico	CAM	AY325832.1	AY325879.1
arocheae2	Colombia	Mexico	CAM		
arocheae3	Colombia	Mexico	CAM		
arocheae4	Colombia	Mexico	CAM		
atrofusca	China	China	EAS	AY436446.1	
australis	New Zealand	New Zealand	OCE	GU222314.1	
avellanoesquamosa	China	Japan	EAS	AY436447.1	
basiana	Italy	France	EUR	KP258986.1	KP258987.1
beckeri	Italy	France	EUR	JF907758.1	
bisporigera1	USA	USA	NAM	EU819411.1	
bisporigera2	USA	USA	NAM	KJ466374.1	KJ466434.1
brunnescens1	USA	USA	NAM	AY789079.1	
brunnescens2	USA	USA	NAM	HQ539780.1	HQ539674.1
capillensis1	Colombia	Colombia	SAM		
capillensis2	Colombia	Colombia	SAM		
chepangiana1	China	China	EAS	AY436450.1	
citrina1	Korea	Europe	EUR	KP004945.1	
citrina2	Japan	Europe	EUR	AB972821.1	
citrina3	South Korea	Europe	EUR	AF085489.1	
citrina4	USA	Europe	EUR	JF313662.1	
citrina5	China	Europe	EUR	JF273504.1	
citrina6	South Korea	Europe	EUR	AF085483	
citrina7	South Korea	Europe	EUR	KF245908.1	KF245892.1
citrina8	South Korea	Europe	EUR	KF245909.1	KF245893.1
citrina9	Denmark	Europe	EUR	AJ889919.1	
citrina10	Denmark	Europe	EUR	AJ889920.1	
citrina11	Japan	Europe	EUR	AB015679.1	
citrina12	Japan	Europe	EUR	AB015680.1	
citrina13	USA	Europe	EUR	AY325846.1	AY325862.1
citrina14	Colombia	Europe	EUR		
citrina15	Colombia	Europe	EUR		
citrina16	Colombia	Europe	EUR		
citrina17	Poland	Europe	EUR	JN235140.1	
citrina18	USA	Europe	EUR	FJ596864.1	
clarisquamosa	China	Japan	EAS	FJ375331.1	

colombiana1	Colombia	Colombia	SAM		
colombiana2	Colombia	Colombia	SAM		
colombiana3	Colombia	Colombia	SAM		
colombiana4	Colombia	Colombia	SAM		
constricta	USA	USA	NAM	KF007934.1	
craseoderma	Guyana	Brazil	SAM	KC155382.1	
crocea	Germany		EUR	AY436484.1	
cylindrispora	USA	USA	NAM	AY325839.1	
esculenta	China	Japan	EAS	AY436451.1	
excelsa	Germany	Sweden	EUR	AY436453.1	AY436491.1
exitialis1	China	China	EAS	KJ466375.1	KJ466435.1
exitialis2	China	China	EAS	JX998026.1	JX998053.1
flavipes	South Korea	Japan	EAS	KF245912.1	KF245896.1
flavoconia1	USA	Eastern USA	NAM	GQ415317.1	
flavoconia2	Colombia	Eastern USA	NAM		
flavoconia3	USA_NE	Eastern USA	NAM	AY436456.1	
flavoconia4	Canada	Eastern USA	NAM	KJ638283.1	
flavoconia5	Canada	Eastern USA	NAM	KJ638280.1	
flavoconia6	Canada	Eastern USA	NAM	KJ638281.1	
flavoconia7	Canada	Eastern USA	NAM	KJ638282.1	
flavoconia8	Canada	Eastern USA	NAM	KJ638278.1	
flavoconia9	Canada	Eastern USA	NAM	KJ638279.1	
flavoconia10	USA	Eastern USA	NAM	KM373250.1	
flavoconia11	USA	Eastern USA	NAM	EU819463.1	
flavoconia12	USA	Eastern USA	NAM	JF313656.1	
flavoconia13	USA	Eastern USA	NAM	GQ452058.1	
flavoconia14	USA	Eastern USA	NAM	AY325847.1	AY325863.1
flavoconia15	Colombia	Eastern USA	NAM		
flavoconia16	Colombia	Eastern USA	NAM		
flavoconia17	Colombia	Eastern USA	NAM		
flavoconia18	USA	Eastern USA	NAM	JF313657.1	
flavoconia19	Mexico	Eastern USA	NAM	KC152064.1	
flavoconia20	USA	Eastern USA	NAM	JF313655.1	
flavoconia21	Canada	Eastern USA	NAM	JN020967.1	
flavoconia22	Canada	Eastern USA	NAM	JN020966.1	
flavorubens2	USA	USA-east	NAM		HQ539694.1
flavorubens1	USA	USA-east	NAM	GQ166902.1	AF097380
franchetii1	USA	France	EUR	GQ250398.1	GQ250413.1
franchetii2	Bulgaria	France	EUR	JX515561.1	
franchetii3	Bulgaria	France	EUR	JX515562.1	
fritillaria	South Korea	India	MEA	KF245913.1	KF245897.1
frostiana	USA	USA-East	NAM	KP313583.1	
fuliginea	China	Japan	EAS	KJ466377.1	KJ466437.1

fuligineodisca 1	Colombia	Honduras-Colombia	SAM		
fuligineodisca 2	Colombia		SAM		
fuligineoides	China	China	EAS	JX998024.1	JX998047.1
fulva1	Russia	Europe	EUR	AY237177.1	
fulva2	UK	Europe	EUR	JQ888151.1	
fulva3	Canada	Europe	EUR	KJ638269.1	
fulva4	Canada	Europe	EUR	KJ638270.1	
fulva5	Finland	Europe	EUR	KM517238.1	
fulva6	South Korea	Europe	EUR	KF017933.1	KF021672.1
fulva7	Japan	Europe	EUR	AB015692.1	
fulva8	USA	Europe	EUR	|AY325844.1	AY325868.1
fulva9	Poland	Europe	EUR	KM085407.1	
fulva10	Canada	Europe	EUR	JN020968.1	
fuscosquamosa	Australia	Australia	OCE	AY194974.1	
gemmata1	Canada	Europe	EUR	JF899545.1	
gemmata2	Canada	Europe	EUR	HQ604823.1	
gemmata3	Canada	Europe	EUR	KJ146699.1	
gemmata4	USA	Europe	EUR	GQ250399.1	GQ250414.1
gemmata5	Mexico	Europe	EUR	EU569282.1	
gemmata6	Canada	Europe	EUR	HQ604824.1	
griseofolia	SouthK	China	EAS	KF017934.1	KF021673.1
hemibapha1	China	Srilanka	MEA	AY436460.1	
hemibapha2	India	Srilanka	MEA	JX844716.1	KF877233.1
ibotengutake	SouthK	Asia	EAS	KF017937.1	KF021676.1
jacksonii1	USA	Canada-Mexico	NAM	JX844725.1	KF877251.1
jacksonii2	USA	Canada-Mexico	NAM	AY436461.1	
japonica	Japan	Japan	EAS	AB015684.1	
lignitincta	China	China	EAS		AF024461 2
lippiae	Brasil	Brazil	SAM	KP258991.1	KP258992.1
liquii	China	China	EAS	AY436462.1	AY436493.1
longistriata1	Japan	Japan	EAS	AB015678.1	
longistriata2	Russia	Japan	EAS	KJ739810.1	
manginiana1	China	Vietnam	EAS	KJ466378.1	KJ466438.1
manginiana2	China	Vietnam	EAS	AY436463.1	
morrisii	USA	USA-east	NAM	KP284300.1	KP284301.1
muscaria1	USA	Alaska-USA	NAM	AB080980.1	
muscaria2	Germany	Alaska-USA	NAM	AJ549964.1	
muscaria3	USA	Alaska-USA	NAM		HQ539712.1
muscaria4	Australia	Alaska-USA	NAM	EU236711.1	
muscaria5	Japan	Alaska-USA	NAM		
muscaria6	USA	Alaska-USA	NAM	DQ060905.1	DQ060885.1
muscaria7	USA	Alaska-USA	NAM	DQ060909.1	DQ060889.1

muscaria8	New Zealand	Alaska-USA	NAM	GQ267468.1	
muscaria9	Russia	Alaska-USA	NAM	EU071913.1	
muscaria10	Colombia	Alaska-USA	NAM		
muscaria11	Colombia	Alaska-USA	NAM		
muscaria12	Colombia	Alaska-USA	NAM		
muscaria13	USA	Alaska-USA	NAM	EU071887.1	EU071964.1
muscaria14	Mexico	Alaska-USA	NAM	EU071913.1	EU071986.1
muscaria15	USA	Alaska-USA	NAM	EU071892.1	EU071969.1
multisquamosa	USA	USA-east	NAM		HQ539710.1
neoovoidea	China	Japan	EAS	FJ441040.1	
novinupta1	Mexico	USA-Western	NAM	KC152066.1	
novinupta2	Mexico	USA-Western	NAM	KC152067.1	
novinupta3	Mexico	USA-Western	NAM	KC152065.1	
novinupta4	USA	USA-Western	NAM	GQ250403.1	
novinupta5	USA	USA-Western	NAM	KJ535437.1	
novinupta6	USA	USA-Western	NAM	KF561974.1	KF561978.1
novinupta7	USA	USA-Western	NAM	DQ974690.1	
oberwinkleriana1	China	Japan	EAS	KJ466380.1	KJ466441.1
oberwinkleriana2	China	Japan	EAS	FJ176725.1	
oblongispora	Italy	Italy	EUR	JF907762.1	
ocreata1	USA	USA	NAM	GQ250404.1	GQ250419.1
ocreata2	USA	USA	NAM	KJ466381.1	KJ466442.1
orientifulva1	China	China	EAS	AY436464.1	AY436496.1
orientifulva2	South Korea	China	EAS	KF017940.1	KF021679.1
orientigemmata	China	China	EAS	AY436465.1	AY436497.1
pachycolea	Canada	USA-Western	NAM	HQ650725.1	
pallidorosea1	China	China	EAS	KJ466386.1	KJ466446.1
pallidorosea2	China	China	EAS	KJ466389.1	KJ466449.1
pantherina	Poland	France-Germany	EUR	KM085405.1	
parvipantherina1	China	China	EAS	JN943170.1	JN941163.1
parvipantherina2	China	China	EAS	AY436467.1	AY436499.1
phalloides1	Portugal	Europe	EUR	FM203300.1	
phalloides2	USA	Europe	EUR	KF561975.1	KF561979.1
phalloides3	China	Europe	EUR	JX998031.1	JX998060.1
Pluteus	na			AY854065.1	AF261575.1
populiphila	USA	USA-western	NAM	KP224323.1	KP224346.1
porphyria1	China	Germany	EUR	AY436471.1	AY436500.1
porphyria2	Canada	Germany	EUR	JN020970.1	
porphyria3	Japan	Germany	EUR	AB015677.1	
porphyria4	Norway	Germany	EUR	KP866177.1	
praecox	USA	USA-east	NAM		HQ539725.1

pseudoporphyria1	China	Japan	EAS		AF024471.1
pseudoporphyria2	China	Japan	EAS	KC429050.1	KC429047.1
pseudovaginata	China	Japan	EAS	AY436470.1	
pudica	Zambia	Africa	AFR		HQ539730.1
regalis	Finland		EUR	JF907764.1	
rhacopus	Canada	USA-east	NAM	KP224338.1	KP221313.1
rhoadsii	USA	USA	NAM		AF097391.1
rhopalopus	USA	USA-east	NAM		HQ539733.1
rimosa	China	China	EAS	KF479044.1	KJ466453.1
roseitincta	USA	USA-east	NAM	KC855226.2	
rubescens1	Germany	Europe	EUR	AF438593.2	
rubescens2	South Korea	Europe	EUR	KM052530.1	
rubescens3	South Korea	Europe	EUR	KM052535.1	
rubescens4	Czeck Republic	Europe	EUR	JX679369.1	
rubescens5	China	Europe	EUR	JF273507.1	
rubescens6	South Korea	Europe	EUR	AF085484.1	
rubescens7	USA	Europe	EUR		
rubescens8	South Korea	Europe	EUR	KF245918.1	
rubescens9	South Korea	Europe	EUR	KF245919.1	KF245903.1
rubescens10	Russia	Europe	EUR	JF313651.1	
rubescens11	Russia	Europe	EUR	JF313652.1	
rubescens12	Japan	Europe	EUR	AB015682.1	
rubescens13	Germany	Europe	EUR	EU346872.1	
rubescens14	Denmark	Europe	EUR	AJ889922.1	
rubescens15	Colombia	Europe	EUR		
rubescens16	Colombia	Europe	EUR		
rubescens17	Poland	Europe	EUR	KM409441.1	
rubescens18	Denmark	Europe	EUR	AJ889923.1	
rubescens19	Denmark	Europe	EUR	AM087243.1	
rubrovolvata1	China	JAPAN	EAS	JN943181.1	JN941156.1
rubrovolvata2	Japan	JAPAN	EAS	AB015689.1	
rubrovolvata3	na	JAPAN	EAS		AF024473 2
sepiacea	China	Japan	EAS	AY436473.1	AY436501.1
silvicola	USA	USA	NAM		HQ539737.1
sinensis	South Korea	China	EAS	KF017946.1	KF021685.1
sinicoflava	USA	USA	NAM		HQ539739.1
solaniolens	USA	USA-Costa Rica	NAM	JF313659.1	
solitaria	Germany	Europe-Mediterranean Africa	EUR	AY436475.1	
sororcula	Colombia	Colombia	SAM		
spissa	South Korea	Asia	EAS	KF245910.1	KF245894.1

spNVE562COL	Colombia	Colombia	SAM		
stranella7	USA	USA-east	NAM	FJ596814.1	
subfrostiana	China	China	EAS	JN943173.1	JN941162.1
subglobosa1	SouthK	China	EAS	KF017947.1	KF021686.1
subglobosa2	China	China	EAS	JN943177.1	JN941152.1
subjunquillea1	China	Asia	EAS	KJ466424.1	
subjunquillea2	China	Asia	EAS	KJ466428.1	
submembranacea	Czeck Republic	France	EUR	FJ705275.1	FJ705276.1
supravolvata	France	France-Germany	EUR	KP258995.1	
umbrinolutea	Germany	Europe	EUR	AY436478.1	
vaginata1	SouthK	USA	NAM	KF017949.1	KF021688.1
vaginata2	USA	USA	NAM	EU819489.1	
velosa	USA	USA-Western	NAM	GQ250409.1	GQ250424.1
verrucosivolva	na	China	EAS		AF024483
virgineoides	Japan	Japan	EAS	AB015686.1	
virosa1	China	Sweden-Eastern Aisa	EUR	EF493032.1	
virosa2	Finland	Sweden-Eastern Aisa	EUR	GU373492.1	
virosa3	China	Sweden-Eastern Aisa	EUR	FJ176737.1	
virosa4	CheckR	Sweden-Eastern Aisa	EUR	FJ755188.1	FJ755189.1
virosa5	China	Sweden-Eastern Aisa	EUR	JX998030.1	JX998058.1
virosa6	Japan	Sweden-Eastern Aisa	EUR	KJ466429.1	
virosa7	China	Sweden-Eastern Aisa	EUR	JX998028.1	
virosa8	China	Sweden-Eastern Aisa	EUR	JX998029.1	JX998059.1
virosa9	China	Sweden-Eastern Aisa	EUR	KJ466430.1	
virosa10	China	Sweden-Eastern Aisa	EUR	KJ466431.1	KJ466498.1
virosa11	Canada	Sweden-Eastern Aisa	EUR	KJ638291.1	
virosa12	Canada	Sweden-Eastern Aisa	EUR	KJ638289.1	
virosa14	USA	Sweden-Eastern Aisa	EUR	KM373251.1	
virosa15	France	Sweden-Eastern Aisa	EUR	AY325829.1	
virosa16	USA	Sweden-Eastern Aisa	EUR	EU909449.1	
volvata	USA	USA-east	NAM		AF097388.1
xanthocephala	Australia	Australia	OCE	AY194982.1	
xylinivolva1	Colombia	southwestern USA-CAM-Colombia	SAM		
xylinivolva2	Colombia	southwestern USA-CAM-Colombia	SAM		
xylinivolva3	Colombia	southwestern USA-CAM-Colombia	SAM		
yuaniana	China	China	EAS	AY436479.1	
				AY176453.1	

Supplementary Table 3. Sequences retrieved from GenBank

Section	*Species*_Region*	Origen–Country	Origen_State/City
Lobatae	*agrifolia*_NAM	USA	California
Quercus	*alba*_NAM	USA	Eastern-Central
Quercus	*austrina*_NAM	USA	Southeastern North America
Cyclobalanopsis	*austroglauca*_NAM	China	
Quercus	*berberidifolia*_NAM	USA	California
Quercus	*bicolor*_NAM	USA	Eastern-Midwestern North America
Lobatae	*buckleyi*_NAM	USA	South Central North America
Lobatae	*candicans*_NAM	Mexico	
Protobalanus	*cedrosensis*_NAM	USA	California- Baja California
Cerris	*cerris*_EUR	Europe	Southern Europe, Southwestern Asia
Quercus	*chapmanii*_NAM	USA	Southeastern North America
Protobalanus	*chrysolepis*_NAM	USA	Southwestern North America
Lobatae	*coccinea*_NAM	USA	Eastern North America
Quercus	*cornelius-mulleri*_NAM	USA	Southwestern North America
Lobatae	*crassipes*_NAM	Mexico	
Quercus	*dalechampii*_EUR	Europe	Southeastern Europe
Quercus	*dumosa*_NAM	USA	Southern California, Baja California, Arizona
Quercus	*engelmannii*_NAM	USA	Southern California, Baja California
Lobatae	*falcata*_NAM	USA	Southeastern North America
Cerris	*franchetii*_EAS	China	Eastern Asia
Quercus	*fusiformis*_NAM	USA	south central North America
Quercus	*geminata*_NAM	USA	Southeastern United States
Cyclobalanopsis	*gilva*_EAS	Japan, Taiwan, China	
Cyclobalanopsis	*glauca*_EAS	China	
Lobatae	*gravesii*_NAM	USA-Mexico	Mexico, southwestern North America (Texas)
Lobatae	*grisea*_NAM	Mexico	
Lobatae	*hemisphaerica*_NAM	USA	southeastern North America
Lobatae	*humboldtii*_SAM	Colombia	Northern South America
Lobatae	*incana*_NAM	USA	Southeastern North America
Cerris	*infectoria*_EUR	Europe-Asia	Southern Europe, Southwestern Asia
Quercus	*insignis*_NAM	Mexico, Belize, Costa Rica, Guatemala, Panama	
Cerris	*ithaburensis*_EUR	Europe-Asia	southeastern Europe, southwestern Asia
Lobatae	*kelloggii*_NAM	USA	California, southwestern Oregon
Lobatae	*laeta*_NAM	Mexico	
Lobatae	*laevis*_NAM	USA	Southeastern North America
Lobatae	*laurifolia*_NAM	USA	Southeastern North America
Quercus	*lobata*_NAM	USA	California
Cyclobalanopsis	*merrillii*_EAS	Malaysia	
Lobatae	*mexicana*_NAM	Mexico	
Quercus	*michauxii*_NAM	USA	Eastern North America
Quercus	*minima*_NAM	USA	Southeastern North America

Quercus	*mohriana*_NAM	USA	Southwestern North America
Quercus	*montana*_NAM	USA	Eastern North America
Quercus	*muehlenbergii*_NAM	USA	Eastern, central, and southwestern US
Lobatae	*myrtifolia*_NAM	USA	Southeastern North America
Lobatae	*nigra*_NAM	USA	eastern North America
Quercus	*oblongifolia*_NAM	USA-Mexico	Southwestern U.S., northwestern Mexico
Quercus	*oleoides*_CAM	Costa Rica-Mexico	
	*delavayi*_outgroupC	China	
	*pumila*_outgroupC_	USA	
	*sativa*_outgroupC	USA	
Protobalanus	*palmeri*_NAM	USA	Oregon, California, western Arizona
Lobatae	*palustris*_NAM	USA	eastern North America
Quercus	*petraea*_EUR		Europe, Anatolia
Quercus	*prinoides*_NAM	USA	Eastern North America
Quercus	*pubescens*_EUR		Europe, Anatolia
Quercus	*pungens*_NAM	Mexico	Southwestern U.S., Mexico
Quercus	*robur*_E		Europe, West Asia
Lobatae	*rubra*_NAM	USA	Eastern North America
Lobatae	*rugosa*_NAM	USA-Mexico	Southwestern U.S., northwestern Mexico
Quercus	*sadleriana*_NAM	USA	Southwestern Oregon, northern California
Cyclobalanopsis	*schottkyana*_EAS	China	
Quercus	*serrata*_EAS	China, Taiwan, Japan, Korea	
Lobatae	*shumardii*_NAM	USA	Eastern North America
Quercus	*sinuata*_NAM	USA	Southern North America
Quercus	*stellata*_NAM	USA	Eastern North America
Cerris	*suber*_EUR	Europe	Southwestern Europe, northwestern Africa
Protobalanus	*tomentella*_NAM	USA	Offshore islands of California-Baja California
Cerris	*trojana*_EUR		Southeastern Europe
Cerris	*variabilis*_EAS		Eastern Asia
Lobatae	*velutina*_NAM	USA	Eastern North America
Quercus	*virginiana*_NAM	USA	Southeastern North America
Lobatae	*wislizeni*_NAM	USA	California

*Region: Asia (EAS), Europe (EUR), North America (NAM), Central America (CAM), South America (SAM)

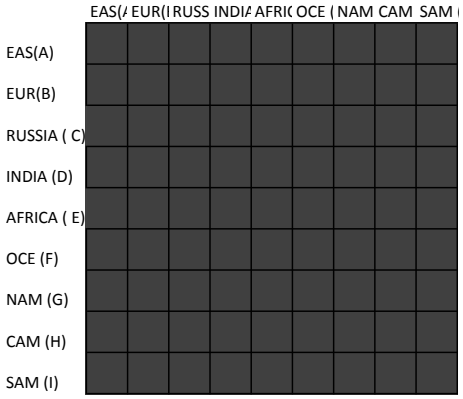
Supplementary Table 4. Summary statistics of estimated divergence times in the dated phylogeny of *Amanita* and *Quercus*

Summary Statistic	**tmrca(genus *Amanita*)**	**tmrca(subgenus *manita*)**	**tmrca(genus *Quercus*)**	**tmrca(Section *Quercus*)**	**tmrca(American oaks)**
mean	155.93	76.41	50.89	31.16	50.57
stderr of mean	0.02	0.03	0.00	0.01	0.01
stdev	2.48	1.97	0.49	0.91	1.18
variance	6.16	3.88	0.24	0.82	1.40
median	155.99	76.42	50.75	31.05	50.68
mode	n/a	n/a	n/a	n/a	n/a
geometric mean	155.91	76.39	50.89	31.15	50.55
95% HPD Interval	[150.96, 160.71]	[72.49, 80.24]	[50.4, 51.86]	[29.56, 32.96]	[47.93, 52.59]
auto-correlation time (ACT)	5395.29	14521.62	1354.91	3313.28	3701.19
effective sample size (ESS)	16681.40	6197.73	66425.76	27163.74	24316.77

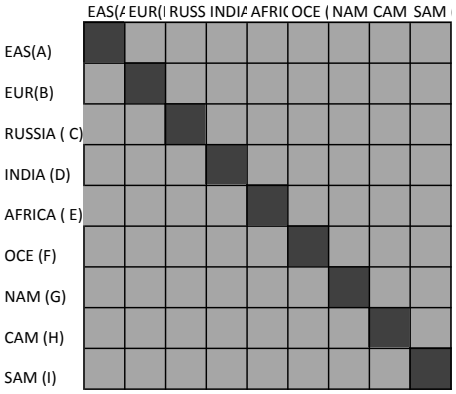
Figures

NULL MODELS

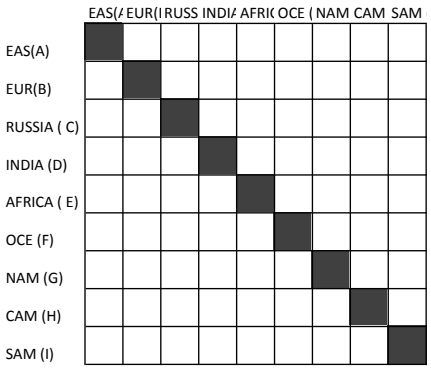
PERMISSIVE MODEL (HIGH)



PERMISSIVE MODEL (MODERATE)

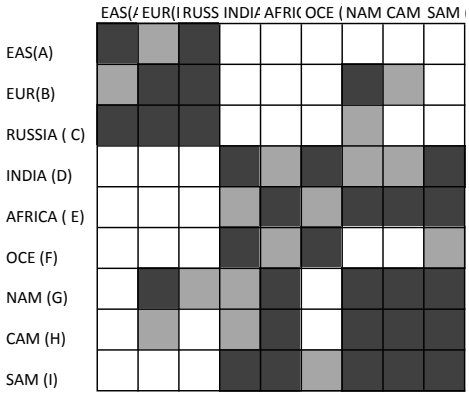


STRICT MODEL

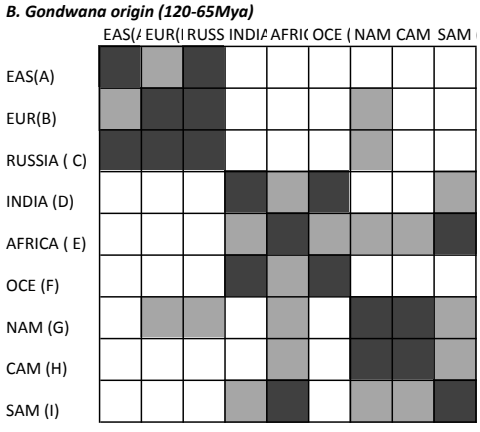


GONDWANA ORIGIN MODELS

A. Gondwana origin (160-120Mya)



B. Gondwana origin (120-65Mya)



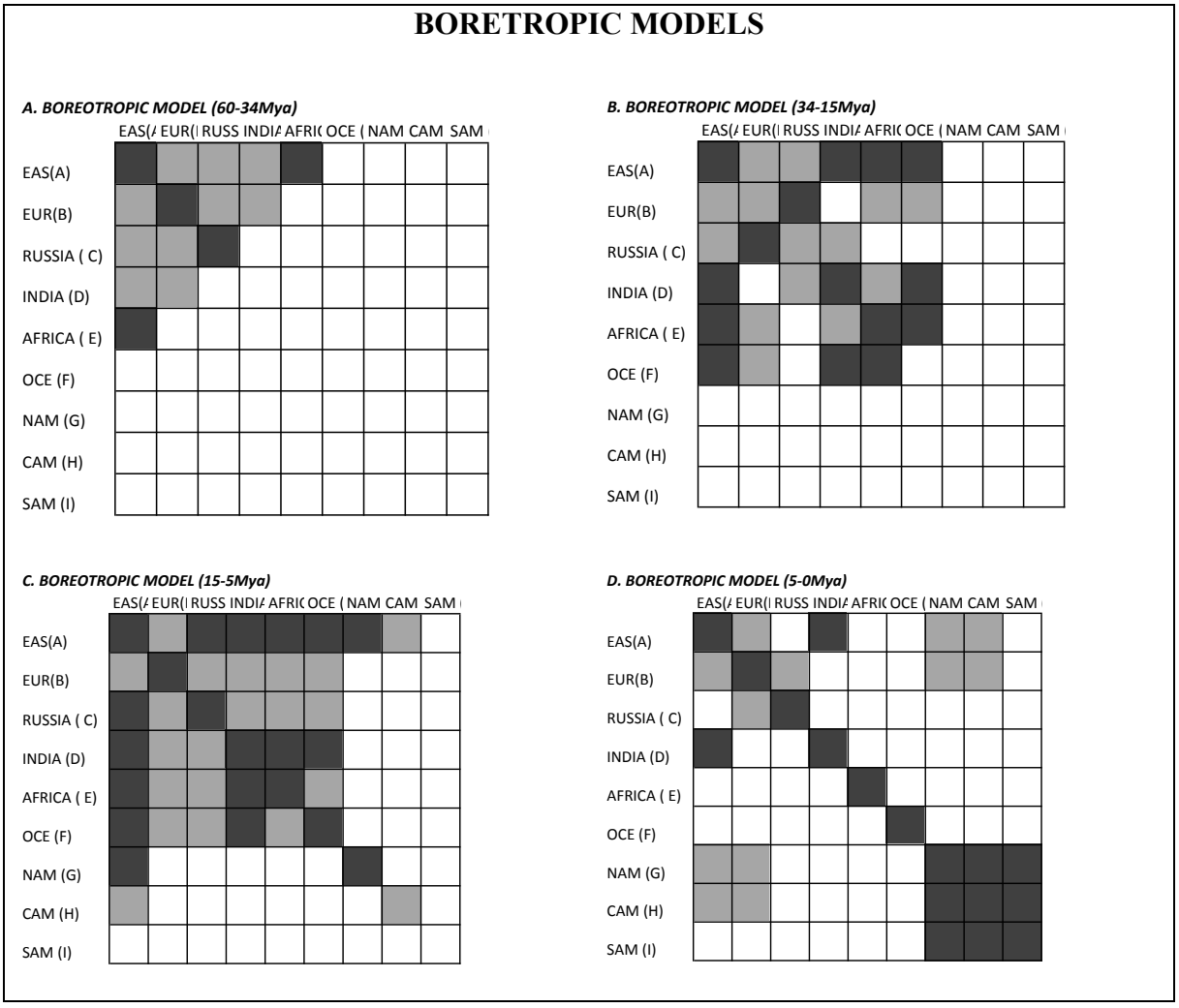


Figure 1. Dispersal events priors for models and their corresponding area-dispersal rate matrices. Dispersal rates: Dark grey=1; Pale grey= 0.5; White= 0.1. East Asia (EAS-A), Europe (EUR-B), Russia (C), India (D), Africa (E), Oceania (OCE-F), North America (NAM-G),Central America (CAM-H), South America (SAM-I).

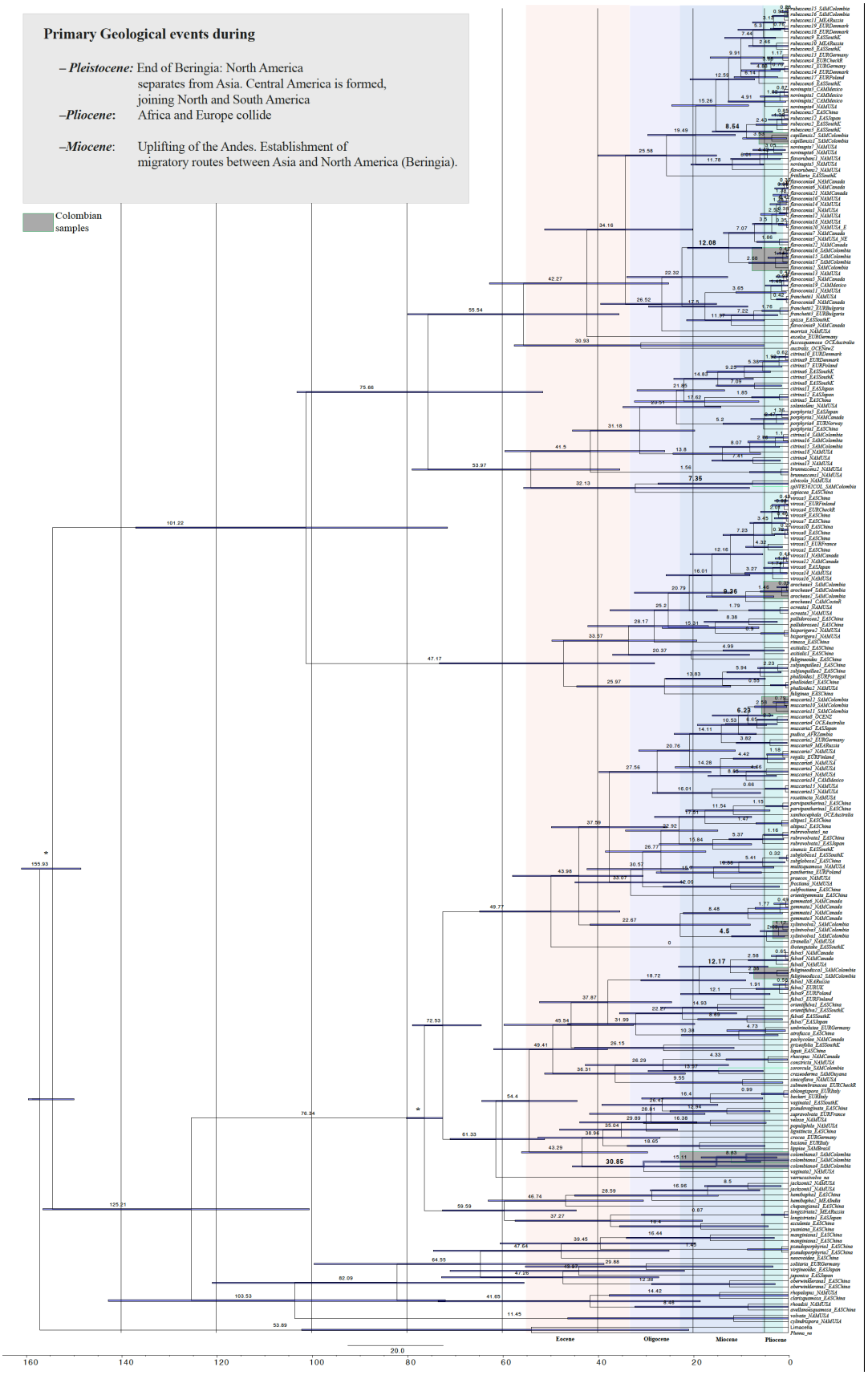
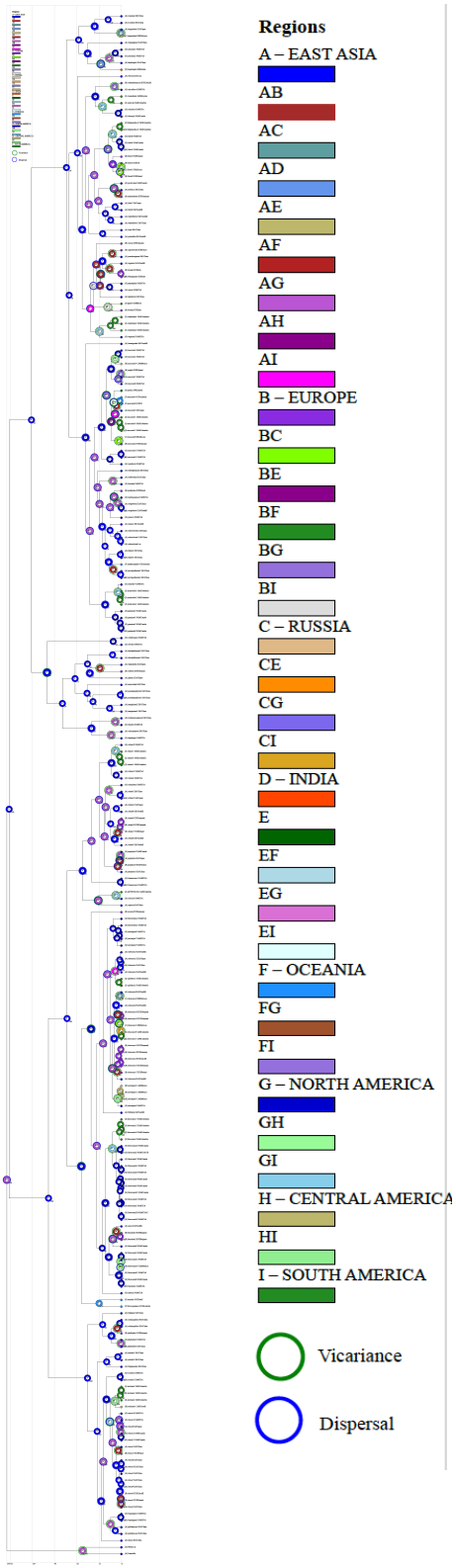
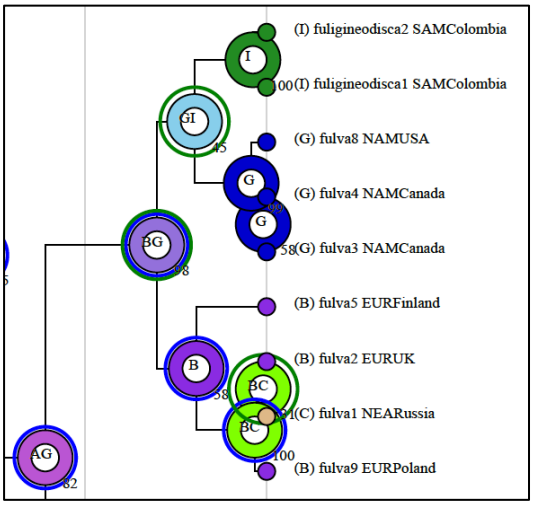


Figure 2. Divergence time estimations in the genus *Amanita*. Numbers above branches

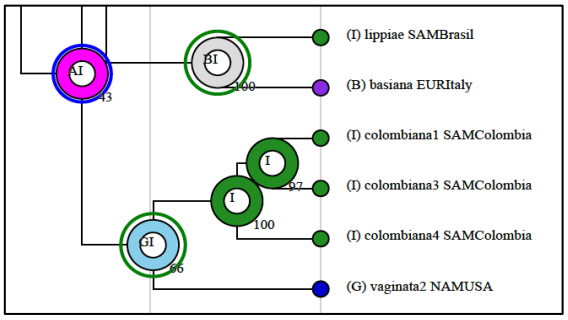
represent mean ages in Millions of years ago (Mya) of each node, and bars represent 95% highest posterior densities (HPDs). East Asia (EAS), Europe (EUR), Oceania (OCE), North America (NAM), Central America (CAM), South America (SAM).


Regions
A – EAST ASIA
AB
AC
AD
AE
AF
AG
AH
AI
B – EUROPE
BC
BE
BF
BG
BI
C – RUSSIA
CE
CG
CI
D – INDIA
E
EF
EG
EI
F – OCEANIA
FG
FI
G – NORTH AMERICA
GH
GI
H – CENTRAL AMERICA
HI
I – SOUTH AMERICA
Vicariance
Dispersal

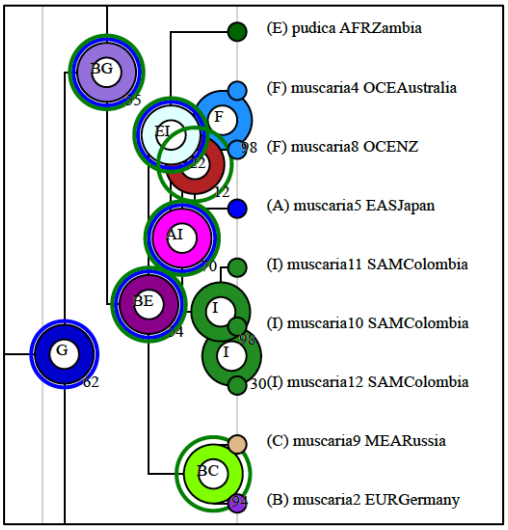
Amanita fuligineodisca



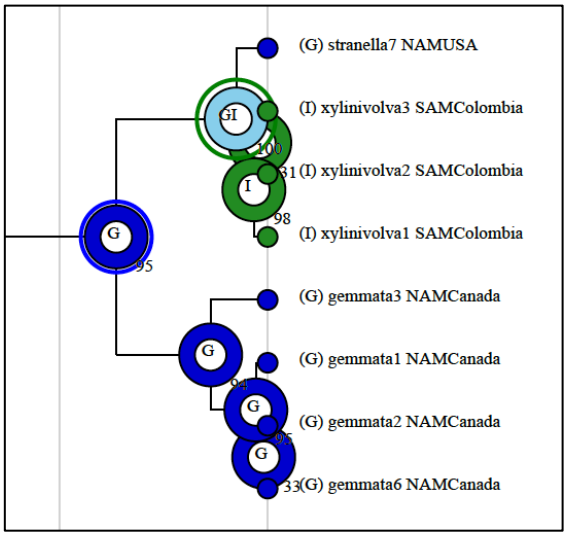
A. colombiana



A. muscaria

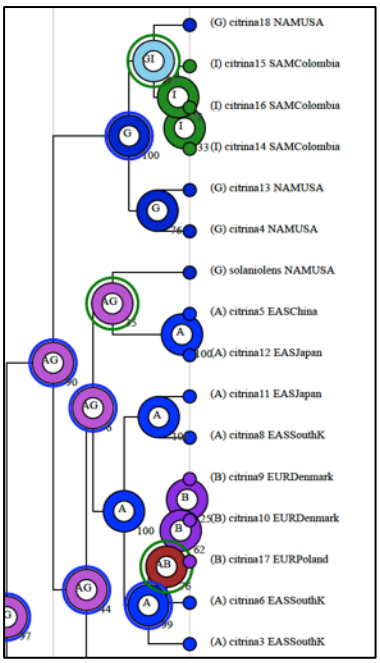


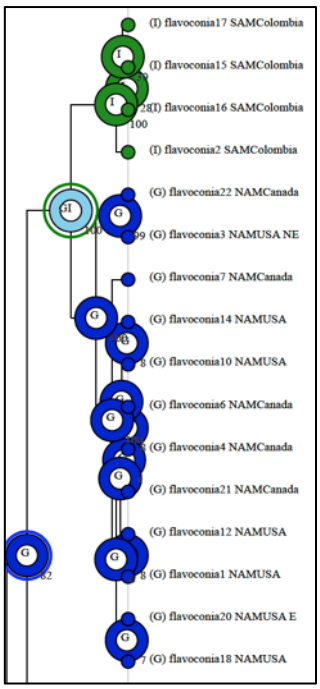
A. xylinivolva



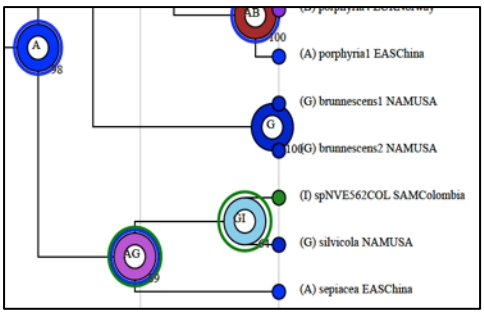
A. citrina

A. flavoconia

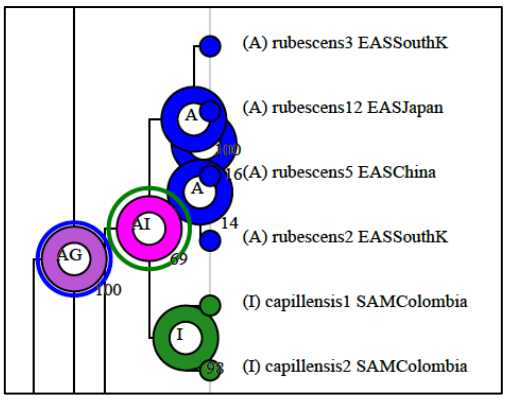




Amanita sp. NVE562



A. capillensis



A. rubescens **** ***A. arocheae* var. *alba***

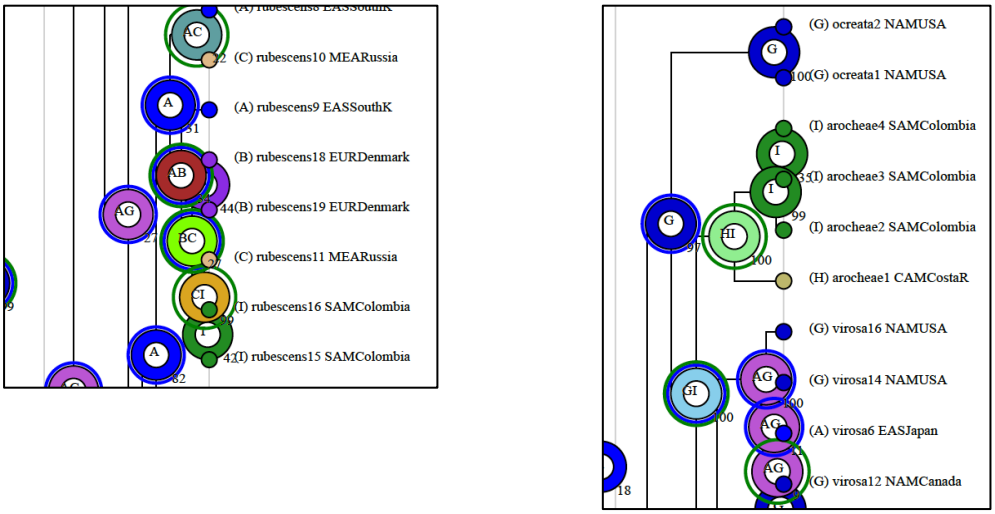


Figure 3. Sections of the chronogram representing the species of the genus *Amanita* collected in Colombia. The chronogram was obtained from molecular clock analysis by using BEAST. Pie chart in each node indicates ancestral distributions inferred from Bayesian Binary MCMC analysis (BBM) implemented in RASP; characters inside each node identify the probable ancestral distribution estimated by maximum likelihood-based program LAGRANGE. Blue and green circles around pie charts indicate possible dispersal and vicariance events, respectively, as suggested by BBM analysis. Characters before species names indicate current distribution area of each species: East Asia (A), Europe (B), Russia (C), India (D), Oceania (F), North America (G), Central America (H), South America (I).

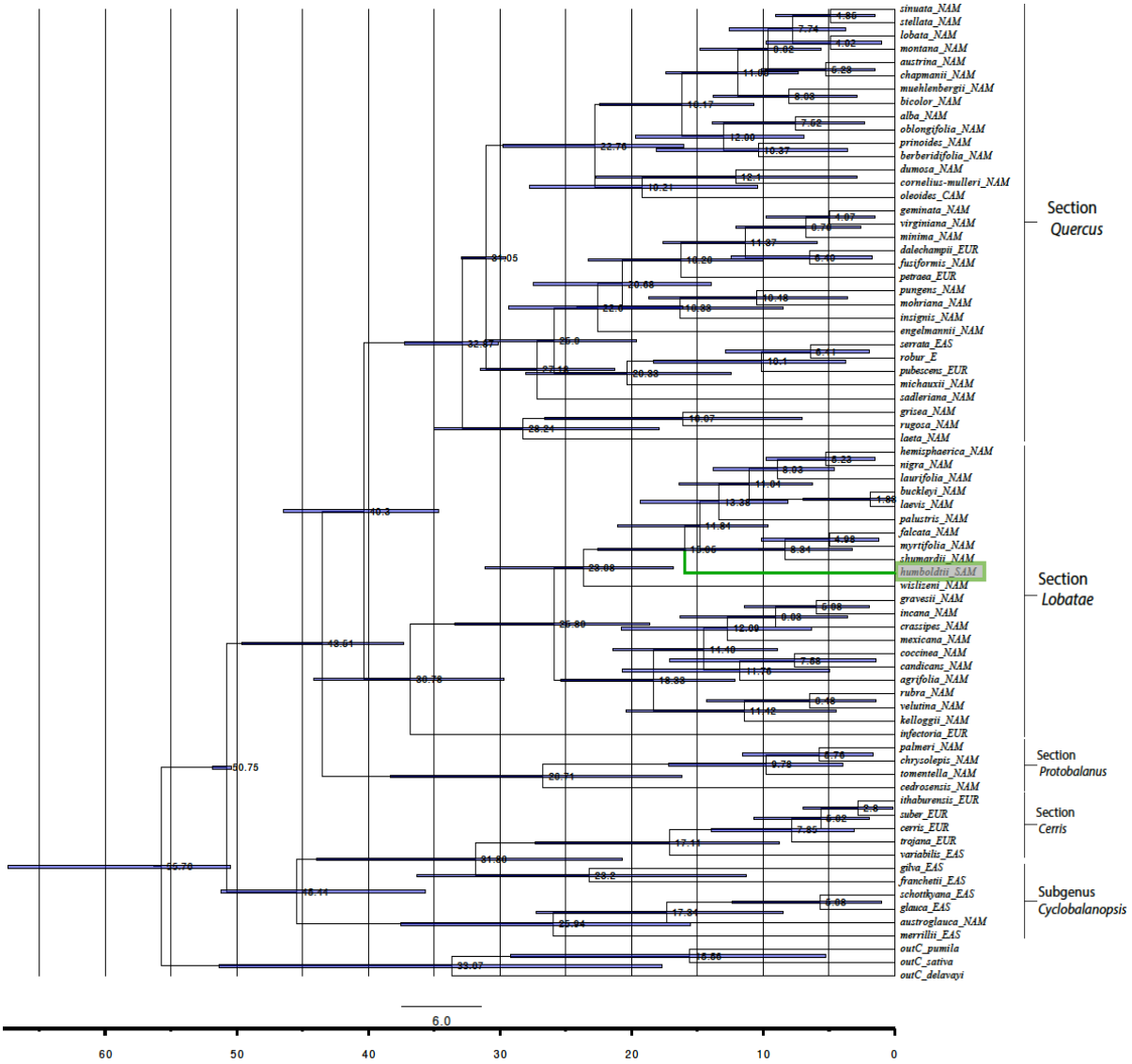
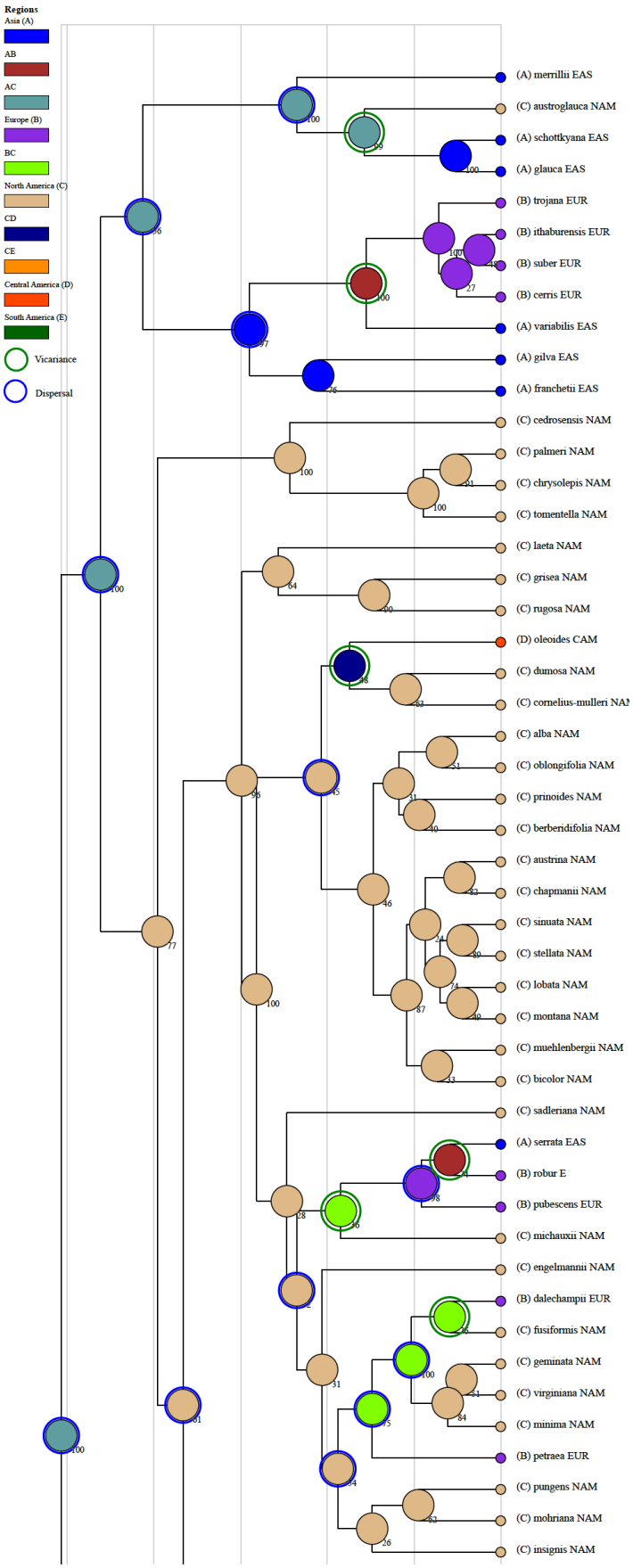


Figure 4. Divergence time estimations in the genus *Quercus*. Numbers above branches represent mean ages in Millions of years ago (Mya) of each node, and bars represent 95% highest posterior densities (HPDs).


Regions
Asia (A)
AB
AC
Europe (B)
BC
North America (C)
CD
CE
Central America (D)
South America (E)
Vicariance
Dispersal
(A) merrillii EAS
(C) austroglauca NAM
(A) schottkyana EAS
(A) glauca EAS
(B) trojana EUR
(B) ithaburensis EUR
(B) suber EUR
(B) cerris EUR
(A) variabilis EAS
(A) gilva EAS
(A) franchetii EAS
(C) cedrosensis NAM
(C) palmeri NAM
(C) chrysolepis NAM
(C) tomentella NAM
(C) laeta NAM
(C) grisea NAM
(C) rugosa NAM
(D) oleoides CAM
(C) dumosa NAM
(C) cornelius-mulleri NAM
(C) alba NAM
(C) oblongifolia NAM
(C) prinoides NAM
(C) berberidifolia NAM
(C) austrina NAM
(C) chapmanii NAM
(C) sinuata NAM
(C) stellata NAM
(C) lobata NAM
(C) montana NAM
(C) muehlenbergii NAM
(C) bicolor NAM
(C) sadleriana NAM
(A) serrata EAS
(B) robur E
(B) pubescens EUR
(C) michauxii NAM
(C) engelmannii NAM
(B) dalechampii EUR
(C) fusiformis NAM
(C) geminata NAM
(C) virginiana NAM
(C) minima NAM
(B) petraea EUR
(C) pungens NAM
(C) mohriana NAM
(C) insignis NAM

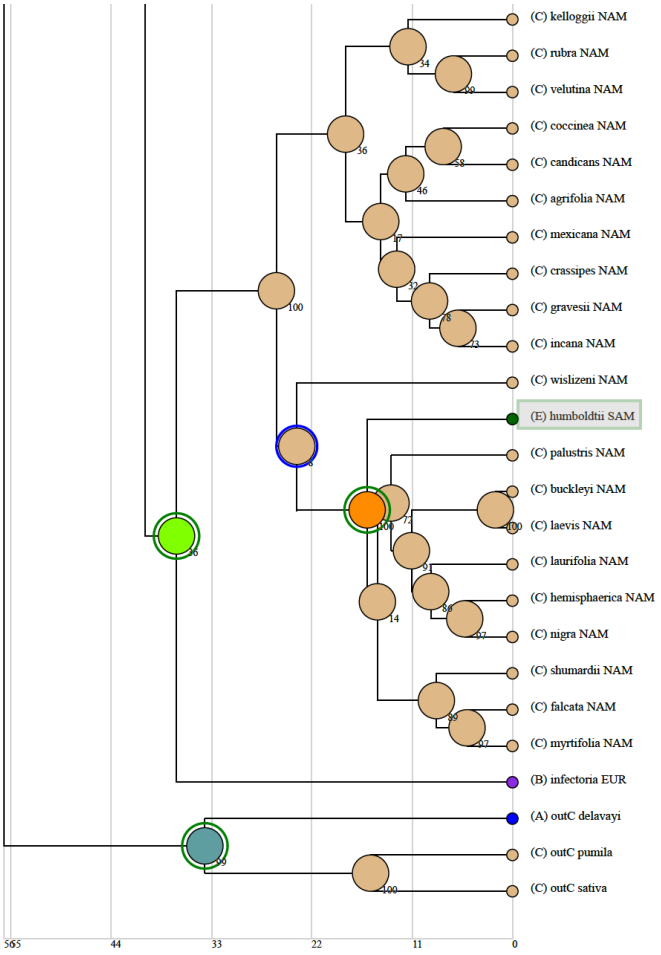
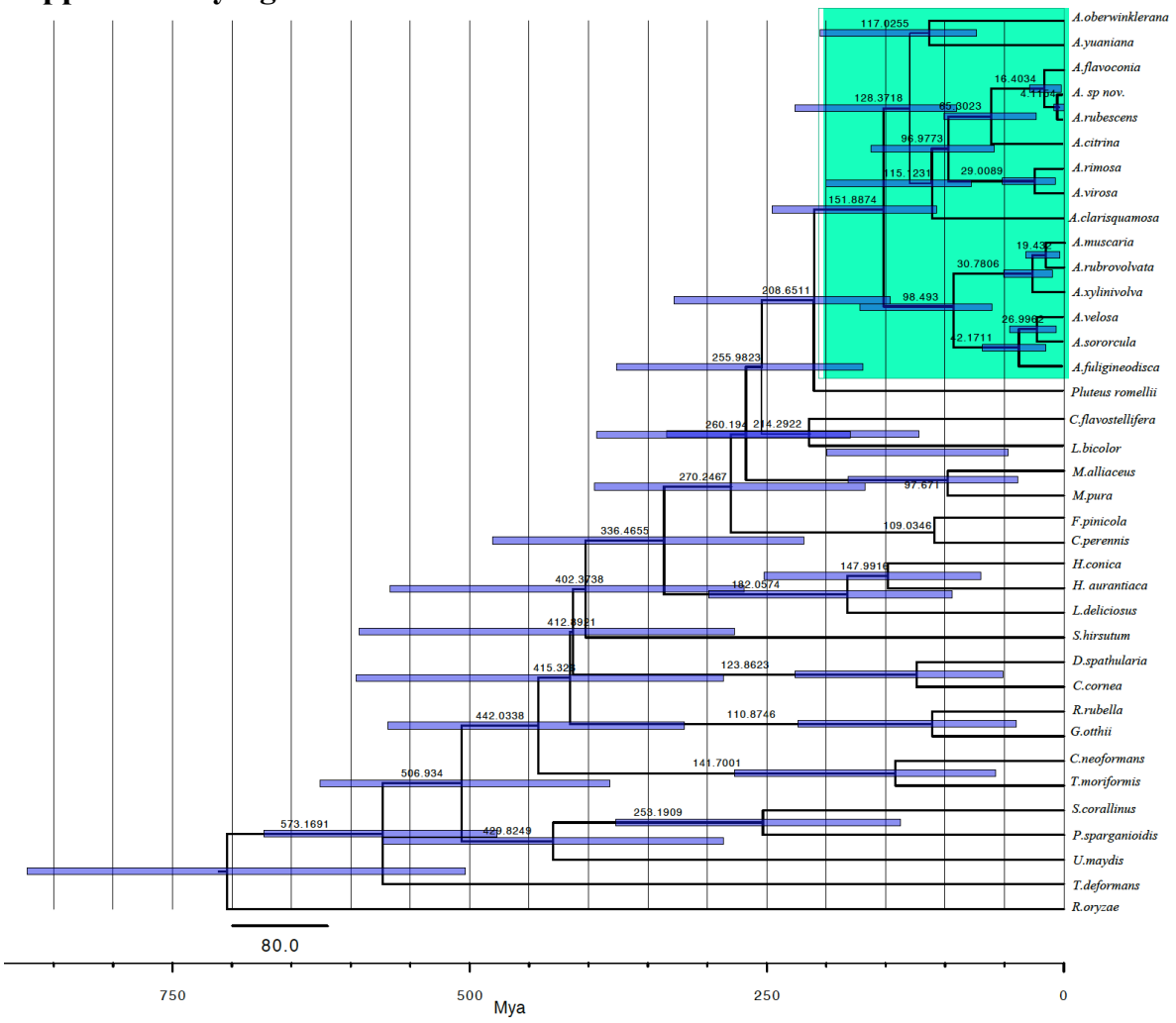
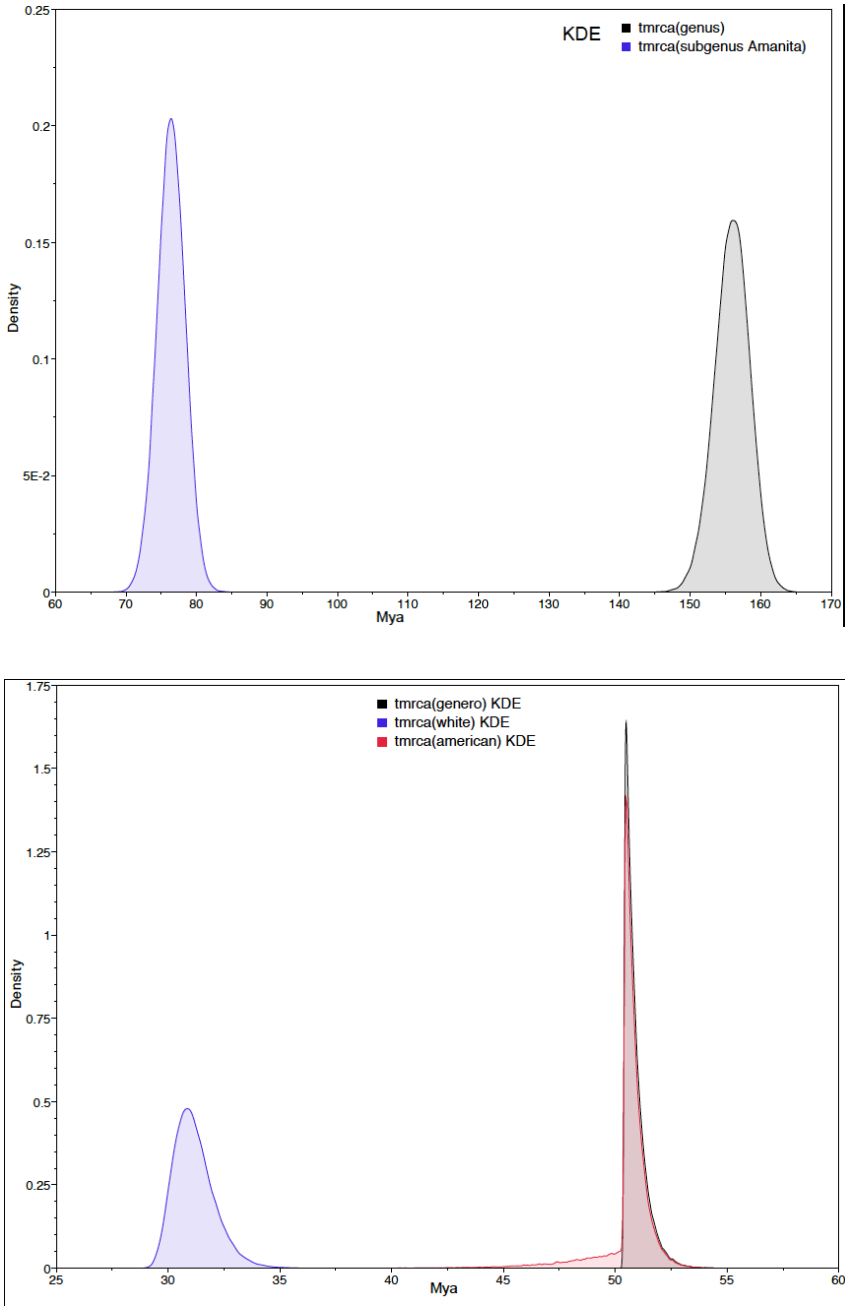


Figure 5. A chronogram of the genus *Quercus* obtained from molecular clock analysis by using BEAST. Pie chart in each node indicates ancestral distributions inferred from Bayesian Binary MCMC analysis (BBM) implemented in RASP; the probable ancestral distribution estimated by maximum likelihood-based program LAGRANGE is shown by color according to the regions. Blue and green circles around pie charts indicate possible dispersal and vicariance events, respectively, as suggested by BBM analysis. Characters before species names indicate current distribution area of each species: Asia (A), Europe (B), North America (C), Central America (D), South America (E).

Supplementary figures



Supplementary Figure 1. Chronogram and estimated divergence times of the genus *Amanita* generated from molecular clock analysis using nLSU data. Chronogram obtained using the Ascomycota – Basidiomycota divergence time of 582 Mya as the calibration point is shown in panel A. The calibration point and objects of this study are marked in the chronogram. The geological time scale is in millions of years ago (Mya).



Supplementary Figure 2. Marginal probability distribution in the dated phylogeny of the genera *Amanita* and *Quercus*

Supplementary Figure 3. Fruiting body of the collection *Amanita* sp. NVE 562

CHAPTER 2

WHICH IS THE HISTORY OF INTRODUCTION AND THE POPULATION GENETICS OF THE INVASIVE *AMANITA MUSCARIA* IN NATIVE OAK FOREST *Q. HUMBOLDTII*?

OBJETIVES

1) To reconstruct the history and origin of the invasive *A. muscaria* species in Colombia
2) To investigate the population genetics structure of *A. muscaria* associated to oak and pine tree plantations in Colombia.

III. In Colombia the Eurasian fungus *Amanita muscaria* (Amanitaceae) is expanding its Range into Native, Tropical *Quercus humboldtii* Forests

Natalia Vargas-Estupiñán, Silvia Restrepo

Laboratory of Mycology and Plant Pathology, Universidad de Los Andes, Bogotá, Colombia

Susana Gonçalves,

Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

Ana Esperanza Franco-Molano

Laboratorio de Taxonomía y Ecología de Hongos, Universidad de Antioquia, Medellín, Colombia

Anne Pringle

Departments of Botany and Bacteriology, University of Wisconsin-Madison, Madison, WI, U.S.A

Abstract

To meet a global demand for timber, tree plantations were established in South America during the first half of the 20th C. Extensive plantations are found in countries including Brazil, Chile, Argentina and Uruguay. In Colombia, miscellaneous plantations were established during the 1950s, when industrial activities were at their height, and policies to limit deforestation in native *Quercus humboldtii* forests were established. One unforeseen consequence of planting non-native trees was the simultaneous introduction and subsequent persistence of fungal symbionts, including ectomycorrhizal fungi in the genus *Amanita*. We

sought to document the origins of the *Amanita muscaria* growing in Colombian plantations of the Mexican species *Pinus patula*, North American *P. taeda*, and Australian *Acacia melanoxylon* and *Eucalyptus globulus*. The fungus is now spreading into native *Q. humboldtii* forests. According to a Bayesian phylogeny and haplotype analysis based on the nuclear ribosomal Internal Transcribed Spacer (ITS) region, *A. muscaria* individuals associated with the four exotic plant species, and those colonizing *Q. humboldtii* forests, are Eurasian in origin, belonging to two Eurasian haplotypes. This is the first time the spread of an introduced mutualist fungus is reported into native *Q. humboldtii* forests of Colombia. We suggest the use of local inocula including native fungi, rather than introduced fungi, as a strategy to help decrease the spreading of *A. muscaria.*

RESUMEN

Con el fin de suplir la demanda de madera, desde mitades del siglo XX, plantaciones de árboles han sido establecidas en varios países Suramericanos tales como Brasil, Chile, Argentina y Uruguay. En Colombia, las plantaciones de árboles se han establecido desde los años 1950s cuando las actividades industriales estaban en auge y se propusieran políticas para proteger los bosques nativos tales como el roble *Quercus humboldtii* (fuente mayor de carbón). Una consecuencia inesperada del establecimiento de nuevas plantaciones es la introducción de hongos simbiontes exóticos. En este estudio revisamos las posibles fuentes de la introducción de *Amanita muscaria* en Colombia en plantaciones de *Pinus patula* originario de México, *Pinus taeda* de Norte América, y *Eucalyptus globulus*. y *Acacia melanoxylon* de Australia. Esta especie fúngica está expandiendo su rango en bosque de roble nativo, *Q. humboldtii*. Según los análisis filogenéticos Bayesianos y de haplotipos, basados en secuencias moleculares de la región Espaciadora Transcrita Interna (ITS por sus siglas en

inglés), los individuos de la especie *A. muscaria* asociados tanto a plantaciones exóticas como a la especie nativa *Q. humboldtii*, son de origen Euro-Asiático, perteneciendo a dos haplotipos dentro del clado Eurasia. En este estudio se realiza el primer reporte de la expansión de un hongo mutualista en bosque nativo de roble *Q. humboltii* en Colombia. Finalmente, sugerimos que en programas enfocados en conservación se deba promover el uso de hongos nativos en plantaciones de árboles antes que especies de hongos introducidos.

Key words: ITS; montane forests; ectomycorrhizal fungi*;* ectotrophic forests; exotic trees.

Introduction

In the southern hemisphere, many plantations of exotic trees, including species of *Pinus*, *Eucalyptus* and *Acacia* have been established to make up for local timber shortfalls. Harvested trees are primarily used in industrial activities, for example pulping, and for timber (Le Maitre 1998, Overbeek *et al*. 2012). In the late 17th century, exotic tree plantations were first established in southern Africa and New Zealand (Mirov 1967, Richardson & Higgins 1998). Eventually plantations were established in other countries of the global south, and by the 18th century, plantation species had become invasive in Australia and some South American countries, including Chile, Argentina, Uruguay, and Brazil (Mirov 1967, Kral 1993, Sawyer 1993, Richardson *et al*. 2008, Pauchard *et al*. 2010).

In Colombia, most plantations of pines, as well as species of *Eucalyptus* and *Cupressus*, started in the 1950s–1960s (Cavelier & Tobler 1998, Ramírez *et al.* 2014), when environmental concerns motivated the creation of controls on the deforestation of native

forests. Deforestation activities were concentrated in native montane cloud forests, and particularly affected *Quercus humboldtii* (Ramírez *et al.* 2014), a native species distributed in the Colombian Andes and reaching its greatest extent of about 171 thousand hectares in the departments of Boyacá and Santander (Fundación Natura 2007, Orwa *et al*. 2009). After controls were in place to protect *Q. humboldtii*, demand for wood was met by establishing exotic tree plantations (Ramírez 2009). Whilst fulfilling the national need for wood, the tree plantations may have caused other problems. An extensive literature documents the problems associated with plantations elsewhere in the global south, and generally problems concern conflicts with native ecosystem services (Dickie *et al*. 2014), and negative abiotic (Nullvalue 1996, Richardson & Higgins 1998, Le Maitre *et al*. 2000, Céspedes-Payret *et al*. 2009), biotic (Moran *et al*. 1999, Simberloff *et al*. 2010), and social and environmental factors (Overbeek *et al*. 2012).

Another generally unforeseen consequence of tree plantations is the simultaneous introduction of mutualistic mycorrhizal fungi, which typically grow with the roots of plants. The mycorrhizal interaction benefits planted trees by enhancing access to nutrients and increasing survival and growth (Harley & Smith 1983, Read 1998). Commercial tree plantations do not thrive in introduced ranges without mycorrhizal fungi (Nuñez *et al*. 2009, Nuñez & Dickie 2014), and distributions of mycorrhizal fungi are often shaped by the distributions of their hosts (Geml *et al.* 2010). Once a tree and its associated fungi are introduced, the fungi can establish within plantations or disperse to native forests, occasionally establishing novel symbioses with native plant species (Pringle *et al*. 2011, Dickie *et al*. 2010).

In fact, a systematization of the available data (Vellinga *et al.* 2009) on global ECtoMycorrhizal (ECM) introductions suggests most exotic fungi are concentrated in the southern hemisphere, and associated with plantations. The ECM species *Amanita muscaria* (Fig. 1) illustrates this pattern. The fungus is native to boreal and temperate forests in the northern hemisphere (Geml *et al*. 2006). Introductions of this species have been reported as a concern in New Zealand, where introduced forestry species include *Pinus radiata* and *Pseudotsuga menziesii,* as well as in Australia (Shepherd & Totterdell 1988, Sawyer *et al*. 2001, Dickie & Johnston 2008). In both countries, *A. muscaria* now grows with native *Fuscospora* spp. and *Nothofagus* spp. (Orlovich & Cairney 2004, Bagley & Orlovich 2004, Dickie & Johnston 2008, Fuhrer & Robinson 1992, Bougher 1996, Robinson 2010). The fungus has also been reported from pine plantations (Marais & Kotzé 1977, Lundquist 1986, Van der Westhuizen & Eicker 1987, Reid & Eicker 1991) and eucalypt plantations (Ducousso *et al.* 2012) in Africa. In South American countries, where tree plantations are relatively recent, compared to plantations in Australasia and southern Africa, the presence of *A. muscaria* within exotic tree plantations is rarely reported (Nasi 1977, Pulido 1983, Garrido 1986, Stijve & De Meijer 1993, De Meijer 2001, Giachini *et al*. 2000, Malvárez *et al*. 1997, Franco-Molano *et al*. 2000, Vellinga *et al*. 2009), and no associations between *A. muscaria* and native trees have been reported.

In Colombia, conserving biodiversity and protecting ecosystems are becoming priority tasks for the government and research institutions. Invasion biology is an emerging field of research, and fauna and flora are already research targets, but generally fungal invasions are ignored. However, during the course of our research it became evident that *A. muscaria* is establishing in native forests in the northeastern Andes, near to plantations (Fig. 1). Therefore we aimed to document the potential origins of introductions of *A. muscaria* to

Colombia, and record its current distribution in both plantations and native forests. We first reviewed historical and modern literature, then mapped collections of the fungus, and finally used molecular techniques to relate Colombian *A. muscaria* to global populations. Recently published phylogenies suggests *A. muscaria* is a species complex (Geml *et al.* 2006, Geml *et al*. 2008), encompassing multiple, geographically distinct clades, and we sought to understand if Colombian *A. muscaria* comprise multiple clades, and identify which clades are in Colombia. Finally, we explored the historical events in a global context related to the introduction of this fungus to Colombia.

Methods

History: reports of *A. muscaria* and literature on Colombian tree plantations.— To determine the earliest dates and initial distribution of *A. muscaria* in Colombia, we explored the fungal collections of two herbaria of Colombia: Colombia´s National Herbarium (COL) and the Herbarium of the Antioquia University (HUA). We also looked for reports of *A. muscaria* in local newspapers, using online search portals and typing the words "*eucalipto*", "*pino*", "*Amanita*" and/or "*hongo*". We searched the database of fungal introductions published by Vellinga *et al*. (2009) with four search terms: "*Amanita muscaria*", "*Pinus*", "*Eucalyptus* ", and "Colombia". We also searched a recently published checklist (Vasco-Palacios & Franco-Molano 2013) of Colombian macrofungi for references to *A. muscaria*. To document additional reports of this species made after Vellinga *et al.* (2009), we searched within the ISI Web of Knowledge using the same search criteria. An exhaustive survey of the literature on global tree plantations was complemented with a literature search related to Colombian plantations based in the Federación Nacional de

Maderas (FEDEMADERAS) library.

New fungal collections.—Sporocarps were collected between March 2007 and June 2015 at sites along trails, roads, and forest edges from twenty-four localities in Colombia (Table 1; Table S1). Collections are stored in the ANDES_F collection in the Natural History Museum (Universidad de los Andes) and registered in the SPECIFY database. We estimated the sizes of plantations and forests where sporocarps were found, and the distances among *Q. humboldtii* forests and *P. patula* plantations, by using Google Earth Pro 7.1.5.1557 (Google Inc. May 2015) and ArcMap version 10.3.1 (ESRI Inc. May 2015).

DNA extraction, amplification and sequencing.—DNA was obtained from dried sporocarps using the DNeasy Plant Mini kit (Qiagen, USA). Template DNA for sequencing was obtained by polymerase chain reaction (PCR), using primers ITS4 and ITS5 (White *et al*. 1990). PCR was performed with a Peltier thermal cycler (Bio-Rad) in 25 µL reaction mixtures containing double distilled H_2O, 1 µL of 200 ng DNA template, 0.5 µL of each 10 µM primer, 2.5 µL of Taq 10x buffer, 0.5 µL of 10 mM dNTP mix, 2 µL of 25 mM $MgCl_2$ and 1 µL of 5U/µL Taq polymerase. Cycling parameters were as follows: initial denaturation at 94ºC for 1 min, followed by 35 cycles of denaturation at 96ºC for 2 min, annealing at 55ºC for 1 min, extension at 72ºC for 2 min, and a final extension at 72ºC for 10 min. Amplified PCR products were visualized by gel electrophoresis on a 1% agarose gel. Reverse and forward PCR products were sequenced using a 3730xl DNA analyzer (PE Applied Biosystems, U.S.A) and were assembled using Geneious Basic 4.8.5 (Biomatters Ltd. April 2010).

Phylogenetic analysis.—A total of 150 *A. muscaria* ITS sequences were used to construct a phylogeny (Table S2), with *Amanita pantherina* used as an outgroup. The total alignment encompassed 25 sequences from Colombian sporocarps and 125 retrieved from GenBank and previously published (Oda *et al.* 2004, Geml *et al.* 2006, Geml *et al.* 2008). The ITS dataset was aligned with MUSCLE (Edgar, 2004), using default parameters. Bayesian inference was performed using MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001) in CIPRES Science Gateway version 3.3 (Miller *et al.* 2010). Ten million MCMCMC generations were run, using a sample frequency of 1000 and a burnin of 25%. The selected substitution model was Kimura 2-parameter + Gamma, estimated with jModelTest (Posada 2008). Two runs using four chains each, one cold and three heated chains, were performed (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck 2003). Each run was examined using Tracer 1.5 (Rambaut & Drummond 2009) to determine if the burnin procedures were correctly assumed, and to determine if there was convergence between the chains and the runs. Recognition of monophyletic groups was based on the identification of highly supported clades on the phylogeny (i.e > 0.95 Bayesian Posterior Probability, PP) (Dettman *et al.* 2003). Individual sequences of the ITS set containing 656 bp and 97 sequences in clade II, were collapsed into unique haplotypes by using SNAP Map (Price & Carbone 2005, Monacell & Carbone 2014), recoding indels as unique integers and exclude infinite-sites violation (Geml *et al*. 2010).

Results

Early collections and literature. — Colombian herbarium collections of *A. muscaria* dating up to and including 1995 are constrained to the central and eastern cordilleras of the Colombian Andes. The first collection of *A.muscaria* dates to 1968 (Idrobo 6268) (Fig. 2),

and was made on the National University campus in Bogota near *Pinus radiata*. This date coincides with the start of intensive tree plantations, and a decade after, collections of *A. muscaria* begin to be more frequent (Fig. 2). No collections are reported outside of plantations.

The literature describing *A. muscaria* in Colombia is scarce. The earliest reports were made by Nasi (1977) from conifer forests in Bogota, in *Embalse del Muña*, *El Neusa* and *La Regadera,* and along roads via the cities of Villavicencio and Medellin. Two reports were found in the database of fungal invasions provided by Vellinga *et al*. (2009): the earliest report was made by Pulido (1983), describing *A. muscaria* associated with introduced *Pinus* plantations in Colombia and found in the Departments of Antioquia and Cundinamarca; a second report by Franco-Molano *et al*. (2000) described the species as commonly associated with introduced pines in the whole country. Additional searches revealed two additional localities reported in the literature, in *Pinus patula* forests in the department of Antioquia, municipality of Jardin (Montoya *et al.* 2005), and in the department of Cundinamarca, municipality of Cota (Vargas *et al*. 2011). Google searches of local media identified one additional putative locality, Sumapaz (Cundinamarca) where *A. muscaria* sporocarps were observed growing in a pine plantation (Shroomery 2015).

Current Distribution.—Sporocarps were found associating either with one of the four exotic tree species: *P. patula, P. taeda, Eucalyptus globulus*., and *A. melanoxylon*, or one native host: *Quercus humboldtii* (Fig. 1C). Most sporocarps were found in *P. patula* plantations (Table S1), at sites within departments distributed in the Colombian Andes (Antioquia, Boyacá, Cundinamarca, Santander, Quindío, Tolima and Valle del Cauca) (Table

1), where plantations of *P. patula* are common (Lopez *et al*. 2010). Site elevations range from 2100-3400 asl (Table S1). So far, associations between *A. muscaria* and native *Q. humboldtii* are restricted to a single locality in the department of Santander (Fig. 1B), within 3 forest patches totaling approximately 3.77 ha (Table S1). A road divides two of the patches, and the third is approximately 950 m away. The estimated distances between the *Q. humboldtii* forests where *A. muscaria* is found, and local *P. patula* forests, range from 851 m to 2423 m.

Origins of Colombian *A. muscaria*.— A Bayesian phylogeny reconstructed the clades of *A. muscaria* documented by Geml *et al.* (2008) with great statistical support (PP > 0.95) (Fig. 3A). The Colombian samples from the five hosts (*P. patula, P. taeda, A. melanoxylon, Eucalyptus globulus* and *Q. humboltii*) group together within the monophyletic Eurasia clade II *sensu* Geml *et al*. (2008) (Fig. 3A). Colombian *A. muscaria* do not comprise multiple clades, instead, the Colombian *A. muscaria* group as a single clade with samples from European and Asian countries, including Germany, Switzerland, Japan, Russia and Scotland. Nevertheless, Colombian samples are genetically heterogeneous, and belong to two haplotypes, A and J *sensu* Geml *et al*. (2010) (Fig. 3A and 3C) which are distributed along the eastern cordillera in the departments of Santander, Boyacá and Cundinamarca. The majority of Colombian samples are haplotype A, currently found in Europe, Africa, Asia, and Oceania, but 10 are haplotype J, which is found in Poland, Germany and England (Fig. 3B and C).

Literature on the history of *P. patula* and other exotic plantations.—A considerable amount of literature describes the history of tree plantations in Colombia, and in the global south, a contrast to the limited literature describing *A. muscaria*. A review

(Overbeek *et al.* 2012) suggests global southern tree plantations are a recent phenomenon dating to the 1960s and 1970s. In Colombia, the species most often planted for commercial purposes are *P. patula* and *E. grandis*, typically in the Andean montane forests (Sicard & Suarez, 1998; Von Christen *et al.* 1998, Ospina *et al*. 2011), and these species were introduced during the 50s and 60s, for both pulp and timber (Wright *et al*. 1996, Ramírez 2009, Caro *et al*. 2012) (Fig. 2). Going backwards in time, as people began to make use of forests in the early 1900s, there was a simultaneous concern to establish forest preserves and protect native forests from destruction by creating protective laws (Fig. 2). However, despite these laws, a contradictory effort to empower forestry concessions and exploit wood was also developing, causing a shortfall in native wood sources (Ramírez 2009). The start of national regulations and conservation programs to protect native forests coincided with a promotion of the logging industry (Ramírez 2009) (Fig. 2).

History of Colombian hosts.— Between 1956 and 1958, government research based near Bogota, at sites in *El embalse del Neusa*, aimed to identify *Pinus* species for high wood production capable of adapting to montane forests (3000-3300 asl), and identified *P. patula* as a promising species (Ramírez 2009). *P patula* occurs naturally in the Northeast and Southeast of Mexico and is one of the most intensively planted conifer species in tropical South America and southern Africa (Richardson & Rundel 1998). It is very important to commercial reforestation in Colombia, and is mainly planted in the Colombian Andes. Seeds are selected to enable fast growth and durability (Sicard & Suarez 1998). According to Ladrach & Lambeth (1991) and Ospina *et al*. (2011), seeds are primarily imported from places such as South Africa, Malawi, Zimbabwe and Transvaal-South Africa, where advanced genetic improvement programs exist. Globally, South Africa and Zimbabwe are the

countries that provide the best genetically improved seed source (Nyoka 2002). In the literature, ECM spp. used to inoculate the soil of *P. patula* plantations are documented as *Boletus* sp., *Rhizopogon roseolus* and *Pisolithus tinctorius* (Sicard & Suarez 1998, Rivera *et al.* 1998). But personal communications suggest *A. muscaria* is also used, for example, Smurfit Kappa is a global business providing paper-based packaging to world markets, and in Colombia *A. muscaria* sporocarps from their plantations are ground and mixed with soils in nurseries to promote seedling growth (Norman Parra, Smurfit Kappa, pers. comm.)

Other species planted in Colombia are *P. taeda, E. grandis*, *E. globulus,* and *A. melanoxylon.* Loblolly pine (*P. taeda*) comes from the southeast U.S.A (Richardson & Rundel, 1998), where it is an important commercial species. It is widely used in South Africa and Zimbabwe, and has been used in South America for pulpwood (Peterson 2001). The other three species, *E. grandis*, *E. globulus,* and *A. melanoxylon,* are native to Australia (Richardson *et al*. 1997). The species *E. grandis* occurs naturally along the coasts of Queensland and South New Wales, in Australia. It has been widely planted in southern Africa for timber, paper and fiber board (Turnbull & Pryor 1984). In Colombia, it is the most common planted species of *Eucalyptus*; it was introduced in the late 19th century, and its use in Colombia began in the 1960s, (Sicard & Suarez 1998) (Fig. 2). *E. globulus* comes from the southeast and west coast of Tasmania (Hall *et al.* 1975) and it is extensively planted in southern hemisphere countries because it grows rapidly (Turnbull & Pryor 1984). *A. melanoxylon* is also a species native to South East Australia (Cowan & Maslin 2001), and is reported as invasive in Colombia (Camelo *et al*. 2012).

Discussion

We identified a considerable amount of literature and information about introduced ectotrophic trees, especially about the history of their introductions, a contrast to the scanty literature on introduced ECM fungi, and complete lack of literature related to the history of introductions of ECM fungi to Colombia. Ours is the first effort to decipher the origin of *A. muscaria* introduced to Colombia, focused on herbarium collections, ITS sequencing of Colombian collections and phylogenetic analyses, and a local and global literature review of hosts associations of *A. muscaria*. Evidence shows *A. muscaria* was first found in the 1960s (Fig. 2). Nowadays, it is found in tree plantations distributed along the three cordilleras (Fig. 1A), and it is actively spreading into a new host range in the northeastern cordillera, in association with native *Q. humboldtii.*

The phylogenetic analysis of newly sequenced collections and other *A. muscaria* collections suggests that: i) *A. muscaria* fungi associated with different hosts in Colombia have a common Eurasian origin, ii) *A. muscaria* sporocarps in native forests have the same origin as sporocarps in introduced plantations, and iii) *A. muscaria* collected in Colombia are genetically diverse, comprising at least two Eurasian haplotypes (A and J; *sensu* Geml *et al.* 2010) (Fig. 3). The association between *A. muscaria* and *P. patula* in Colombia reveals the extent to which novel symbioses are created by global markets. Neither *P. patula* nor *A. muscaria* are native to Colombia, and the plant and fungus have different origins: i) the species *P. patula* is Mexican but ii) seeds of *P. patula* in Colombia are mainly imported from southern African countries (Ladrach & Lambeth 1991, Ospina *et al*. 2011), and iii) the *A. muscaria* individuals associated with *P. patula* tree plantations are Eurasian in origin, not North American (Fig. 3).

A logical hypothesis for the Eurasian origin of Colombian *A. muscaria* would link the extensive movement of plant material among southern African countries (Deacon 1986, Wells 1986, Read 1998, Richardson *et al*. 1997, Richardson *et al*. 2014) with inoculations of soils from Europe (Mikola 1970), and the subsequent introduction of both to Colombia (Fig. 2). Efforts to track the provenance of the exotic plantations of *P. patula* in Africa showed that seeds were introduced from Mexico to South Africa during 1907-1928 (Poyton 1961). Although the specific region of origin of plants exported from Mexico remains unclear, the majority of plantations in Africa started from seeds grown in South Africa (Wormald 1975). On the other hand, in an attempt to track the movement of soil used to inoculate plants with ECM fungi, including *P. patula,* Mikola (1970) demonstrated that soil was repeatedly moved between Europe and Africa and among southern African countries. Moreover, *Amanita* spp. were commonly used to inoculate trees in African plantations (Wormald 1975).

***A. muscaria* is invasive in Colombian oak forest.**— The term invasive is used differently by different authors (Table 2) (Mooney & Drake 1989, Richardson *et al.* 2000, Rejmánek *et al*. 2002, Richardson and Rejmánek 2004, Diéz 2005, Keller *et al.* 2011, Simberloff *et al.* 2012), but often an invasive species is defined as a species that spreads, even if the spread does not cause any economic or environment impact (Richardson *et al*. 2000). According to Nuñez & Dickie (2014), ECM fungi can be considered invasive even if a species is constrained to associate with introduced or invasive plants, although ECM fungi forming novel associations with native plants may present especially interesting ecological dynamics. By any of these definitions *A. muscaria* is invasive, and although we do not yet know if *A. muscaria* is causing environmental harm, the Colombian *A. muscaria* is clearly an introduced species. The fungus is now associating with a native host, *Q. humboldtii,* and the

minimum distance between *A. muscaria* associated with that host and *A. muscaria* within a plantation (of *P. patula*) is 851 m, suggesting it has colonized a novel host at a considerable distance from the parent source (Richardson & Rejmánek 2004). During the last 8 consecutive years, the fungus has persisted, survived, and produced fruiting bodies in association with the Colombian species *Q. humboldtii* (Vargas personal observation); the fungus has clearly overcome both geographic and host barriers.

The larger the planted areas in South American countries the higher the number of reports on fungal introductions.— A strong correlation between trade and the number of exotic species is hypothesized (Levine & D'Antonio 2003, Nuñez & Pauchard 2010). Perhaps regions highly impacted by humans will receive more species introductions than other regions, and perhaps also there will be a correlation between human activities and the number of publications about invasive species. We tested these ideas using published data for South America, and in fact discerned a significant positive correlation between countries with the largest areas (ha) of introduced plantations (FAO 2010, FRA 2010), and the number of reports of fungal introductions retrieved from a database of ECM fungal introductions (Vellinga *et al.* 2009) (Fig. 4) (r (12)= 0.9682, p<0.001). But very little of the existing literature on fungal introductions was found about *A. muscaria* (Fig. 4): Brazil (4.2%), Chile (4.9%) and Argentina (2.2%) (Fig. 4). In spite of its conspicuous red fruiting body, reports of the species in South America remain scarce, and ours is the first report of a shift to a native host.

Concluding remarks.—In Colombia, as in many countries in the southern hemisphere, the panorama of introductions and establishments of exotic organisms (Lee *et al*.

2006) depend on human-assisted transportation. A constant increase in industry continues to encourage planting, and more lands are expected to be converted to plantations in the future, both in Colombia and in the southern hemisphere in general (Pauchard *et al*. 2010). The southern hemisphere has emerged as the strategic focus for commercial forestry operations and exports (Le Maitre 1998, Richardson *et al*. 2008) (Fig. 5).

Tree plantations have unintended consequences, although the consequences are not well documented in Colombia. So far, there is evidence that two haplotypes of *A. muscaria* both with a Eurasian origin are present in the Colombian Andes, and that *A. muscaria* has spread from tree plantations and shifted to a new, native Colombian host. To prevent additional invasions by other fungi, limiting additional introductions may be more effective than using strategies to get rid of an already established invasive, as the costs of eliminating an invasive species may be prohibitive (Keller *et al*. 2011). However, picking the fruiting bodies of this invasive species could gradually reduce the population year by year, as we can learn from other experiences related to the harvesting of native, edible fungi in countries where the use is extensive (Pilz *et al*. 2003), because collecting a species' reproductive structures may cause declines in populations (Pilz and Molina 2002).

Exotic tree plantations should be planted apart from native forests (Jairus *et al*. 2011) to limit potential dispersal to native forest (although exact distances will depend on the dispersal abilities of the target fungi). It would also be useful to focus on developing local inocula, rather than using inocula of introduced fungi (Schwartz *et al.* 2006). It is quite likely that a native fungus would provide the required benefits to planted trees.

Finally, the task of designing policies to reduce the movement and release of non-native species, and to manage those already established (Keller *et al.* 2011), must be a target of future programs related to macrofungal diversity and native forests conservation in Colombia.

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Tables

TABLE 1. *Localities of* Amanita muscaria *collections*

Department[1]	Locality Name	Host	Elevation (masl)
ANT	Estación experimental Piedras Blancas, Municipality of Santa Elena	*Pinus* sp.	2460
ANT	El Chaquiro, Municipality of Santa Rosa de Osos	*Pinus* sp.	2663
ANT	Municipality El Retiro,	NA	2225
ANT	Rio Grande dam, Municipality of San Pedro de los Milagros	*Pinus* sp.	2313
ANT	Llanos de Cuivá, Municipality of Yarumal	NA	2764
BOY	Via Paipa-Tunja	*Pinus taeda*	2670
BOY	Via Arcabuco-Moniquirá	*Pinus patula*	2517
BOY	Vereda Capilla 1-Municipality Villa de Leyva	*Pinus patula*	2504
BOY	Via Villa de Leyva-Gachantiva	*Pinus patula*	2422
BOY	Via Gachantiva-Arcabuco	*Pinus patula*	2458
BOY	Via Belén-San José de la Montaña, Municipality of Belén	*Eucalyptus* sp.	3394
BOY	Via Belén-San José de la Montaña, Municipality of Belén	*Pinus patula*	2911
BOY	Via Arcabuco-Paipa, Municipality of Arcabuco	*Pinus patula*	2958
CUN	Vereda Chiquira, Muncipalty of Villapinzón	*Pinus patula*	2930
CUN	Embalse del Neusa, Municipality of Cogua	*Pinus patula*	2986
CUN	Vereda la Moya, Municipality of Cota	*Pinus* sp.	2762
CUN	Bogotá, Municipality of Bogotá	*Pinus* sp.	2906
CUN	Vía Bogotá-Choachí, Km 2	*Pinus* sp.	2100
CUN	Vía Bogotá-La Calera	*Pinus* sp.	2702
QUI	Municipality of Salento	*Pinus* sp.	1987
SAN	Vereda San Jose de la Montaña-Municipality of Belén	*Quercus humboldtii*	3214
SAN	Vereda San Jose de la Montaña-Municipality of Belén	*Pinus patula* and *Acacia melanoxylon*	2905
TOL	Municipality of Murillo	Mixed forests	2980
VAL	Dapa/Municiality of Cali	*Pinus patula*	2000

1 Abbreviation: Antioquia (ANT), Boyacá (BOY), Cundinamarca (CUN), Quindio (QUI) Santander (SAN), Tolima (TOL), Valle del Cauca (VAL)

[2] SISBEN is an instrument that obtains socioeconomic information on specific groups in the country. It is the main instrument for targeting social programs to poor and vulnerable groups.

TABLE 2. Definitions of the term invasive used by some authors

Invasive definition	**Reference**
"A species that spreads widely and causes measurable environmental, economic, or human health impacts."	Keller *et al*. (2011)
"The characteristic of an invader is related to the act of colonize and persist in an ecosystem in which has never been before."	Mooney & Drake (1989).
"Introduced plants produce reproductive off- spring in areas distant from sites of introduction (approximate scales: > 100 m over < 50 years for taxa spreading by seeds and other propagules; > 6 m/3 years for taxa spreading by roots, rhizomes, stolons or creeping stems)."	Richardson *et al*. (2000)
" 'Alien plants': are taxa clearly outside their natural range, although not necessarily in a different country". 'Naturalized': if they reproduce consistently and sustain populations over several life cycles. 'Invasive': are those alien plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants and thus have the potential to spread over a considerable area (we find 100 m a useful rule of thumb in this regard)"	Richardson and Rejmánek (2004)
"Invasive species recruit seedlings, often in very large numbers, at long distances from parent plants (often more than 100 m). Only some of the naturalized plants become invasive, producing important environmental or economical damages."	Diéz (2005)
"Invasive include any nonindigenous species that has spread and become abundant in a new geographic location regardless of the actual or perceived ecological impact"	Rejmánek *et al*. (2002).
"A species entering natural or semi-natural habitats and having some effect on the resident species"	Zimberloff *et al*. (2012)

Figures

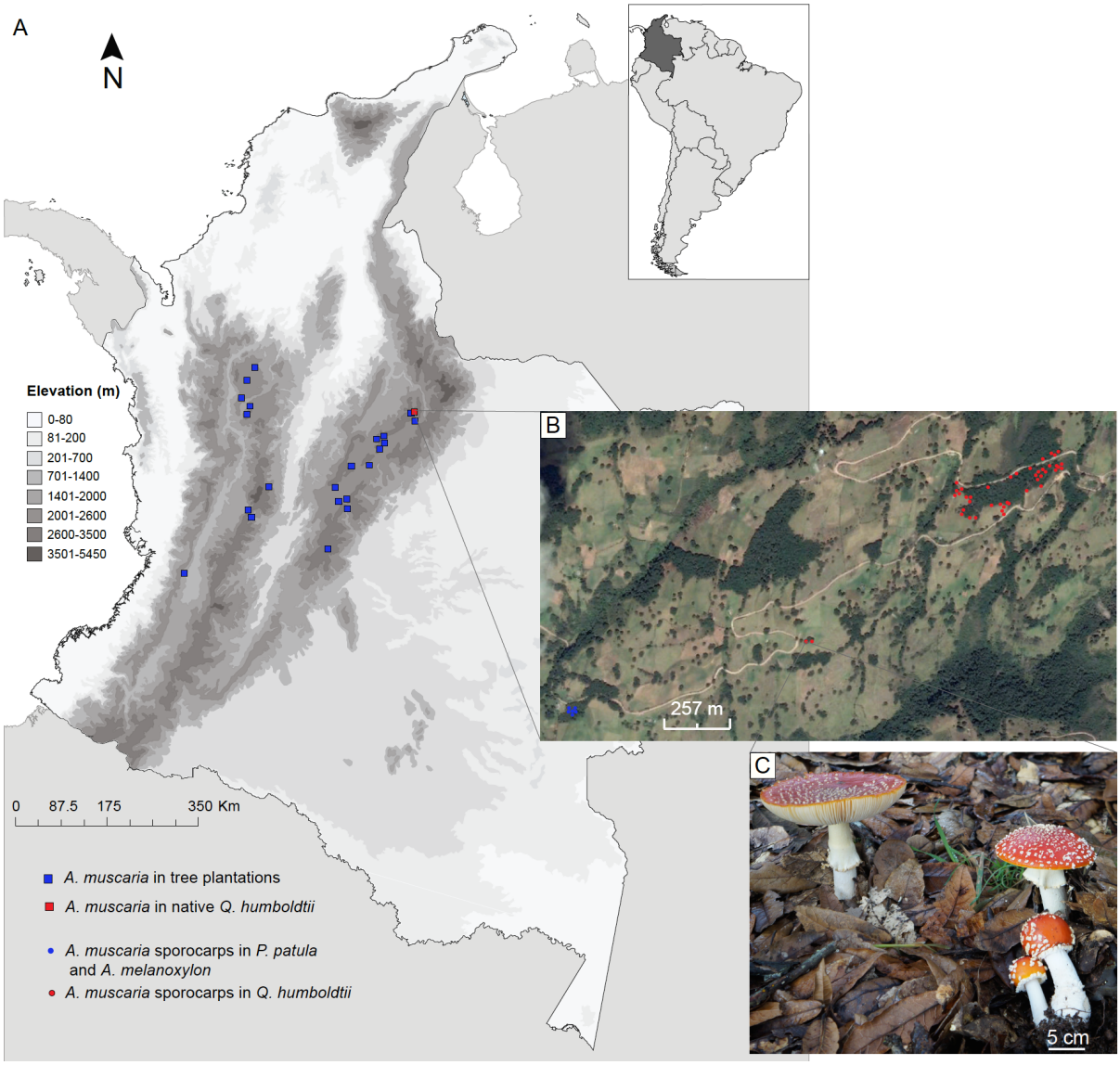

FIGURE 1. A) Distribution of collections of *Amanita muscaria* in tree plantations (blue squares) and *Q. humboldtii* forests (red squares). B) Sporocarps of *A. muscaria* in *Q. humbo*ldtii forests (red circles) and *P. patula* plantations (blue circles). C) Young and mature sporocarps of *A. muscaria* growing in *Q. humboldtii.*

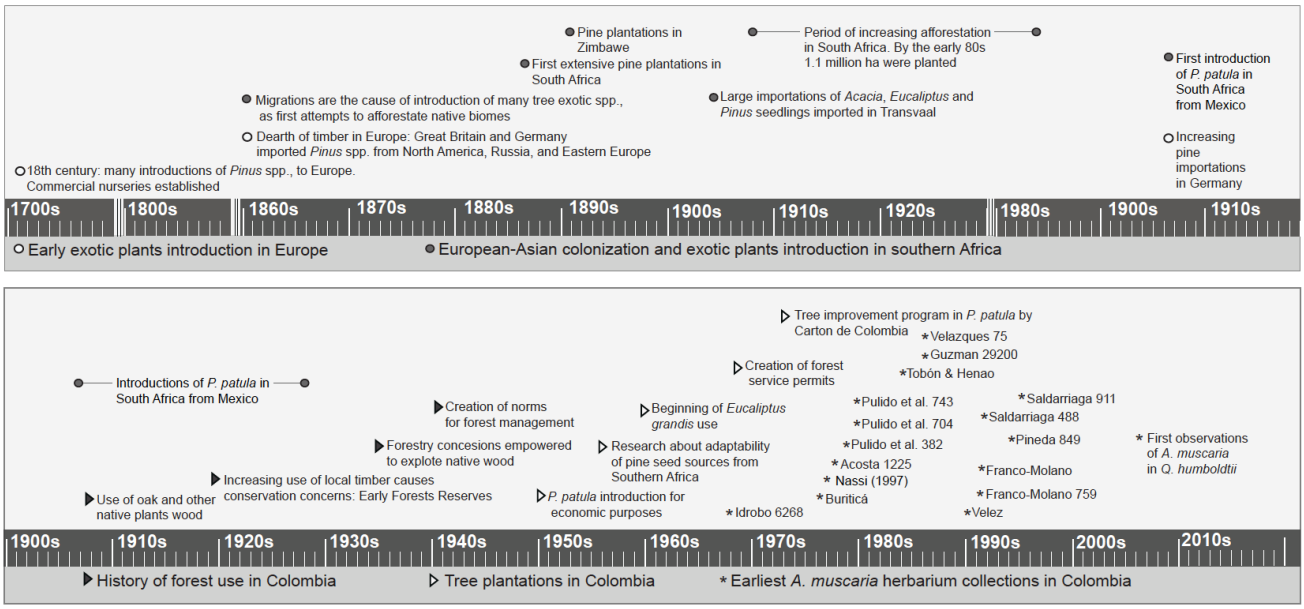

FIGURE 2. Chronology of events related to the introduction of tree plantations to Colombia. Historical events are described in Cavelier & Tobler (1998), Deacon (1986), Keuffel & Krott (1997), Le Maitre (1998), Ospina *et al*. (2011), Poyton (1961), Ramírez (2009), Richardson *et al.* (1997), Richardson & Rundel (1998), Sicard & Suarez (1998), Wells (1986), and Wormald (1975).

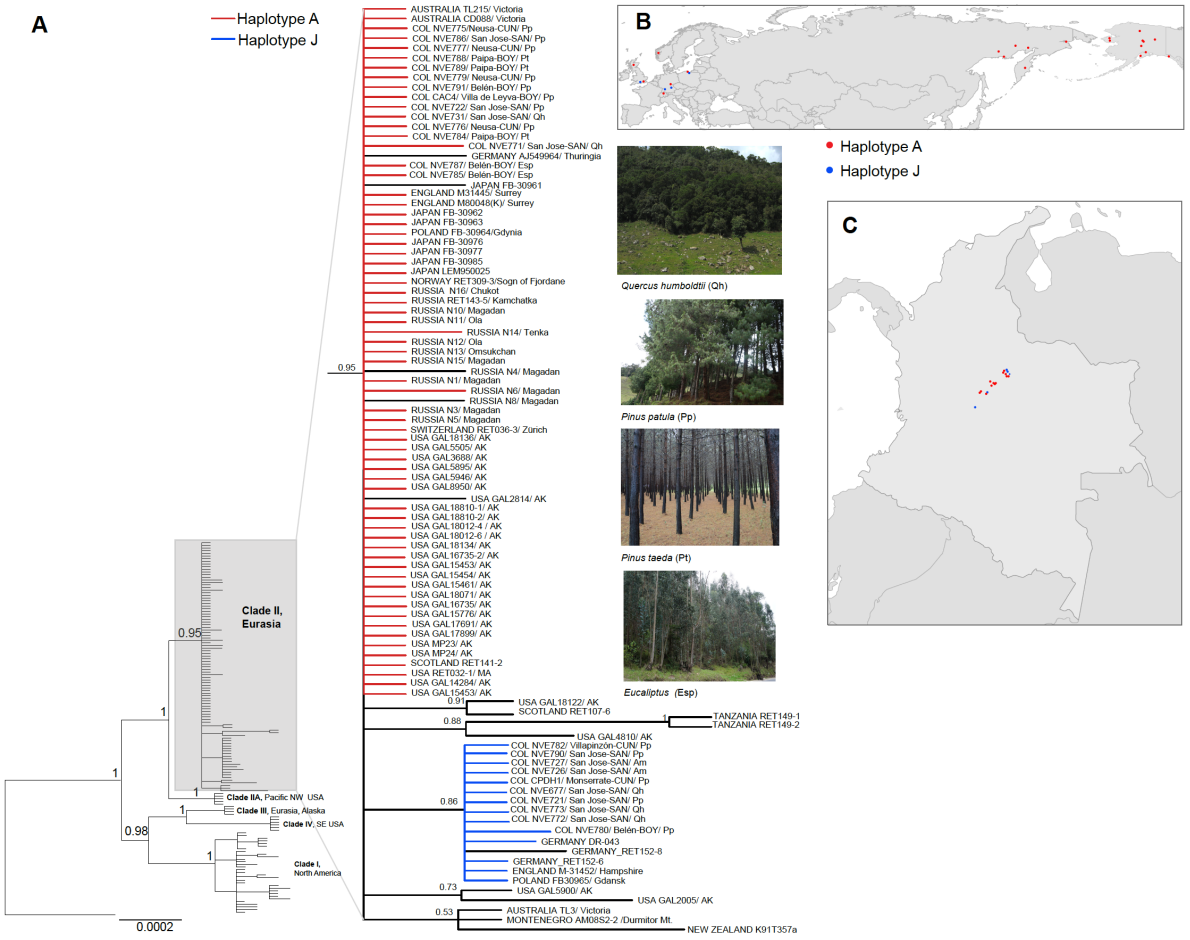

FIGURE 3. A) Bayesian phylogenetic reconstruction using ITS. Posterior probability (PP) values are shown next to the nodes. Colombian collections are named with the following information: COL (Colombia), NVE (collector's name and number)/ Locality-Department: CUN (Cundinamarca), BOY (Boyacá), SAN (Santander)/ Abbreviation of the host species. Other collections in the phylogeny were originally described in Oda *et al.* (2004) and Geml *et al.* (2006, 2008). Haplotypes A and J are highlighted in red and blue, respectively. B) Distribution of haplotypes A (red circles) and J (blue circles) in the northern hemisphere. D) Distribution of haplotypes A (red circles) and J (blue circles) in Colombia.

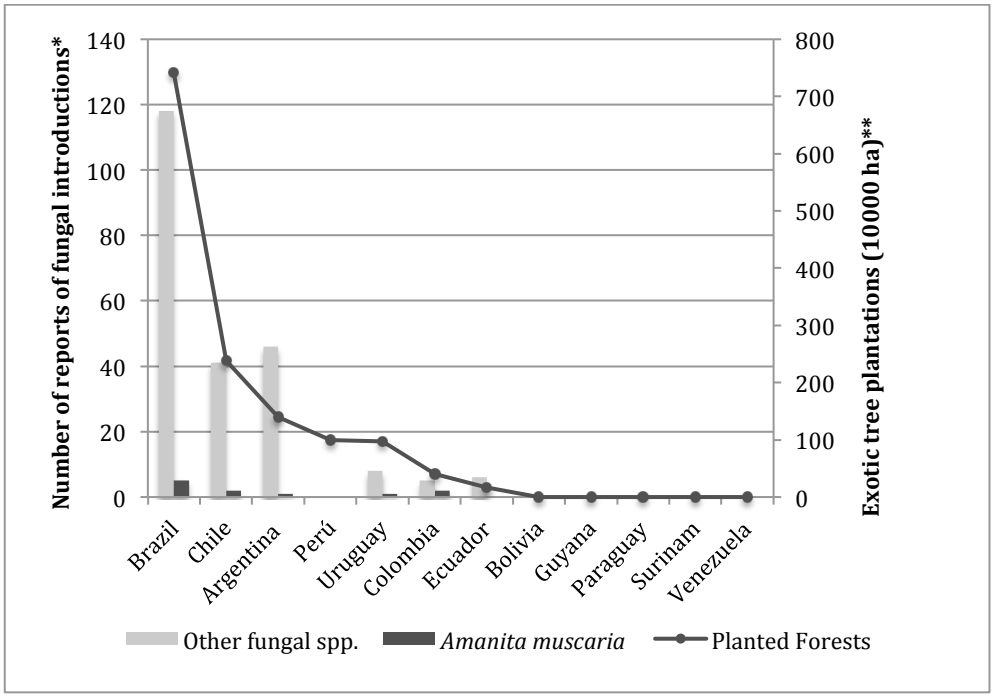

FIGURE 4. Correlation between the area of tree plantations and the number of fungal introductions reported in South America. Sources: * Vellinga *et al*. (2009), **FRA (2010). r (12) = 0.9682, p<0.001.

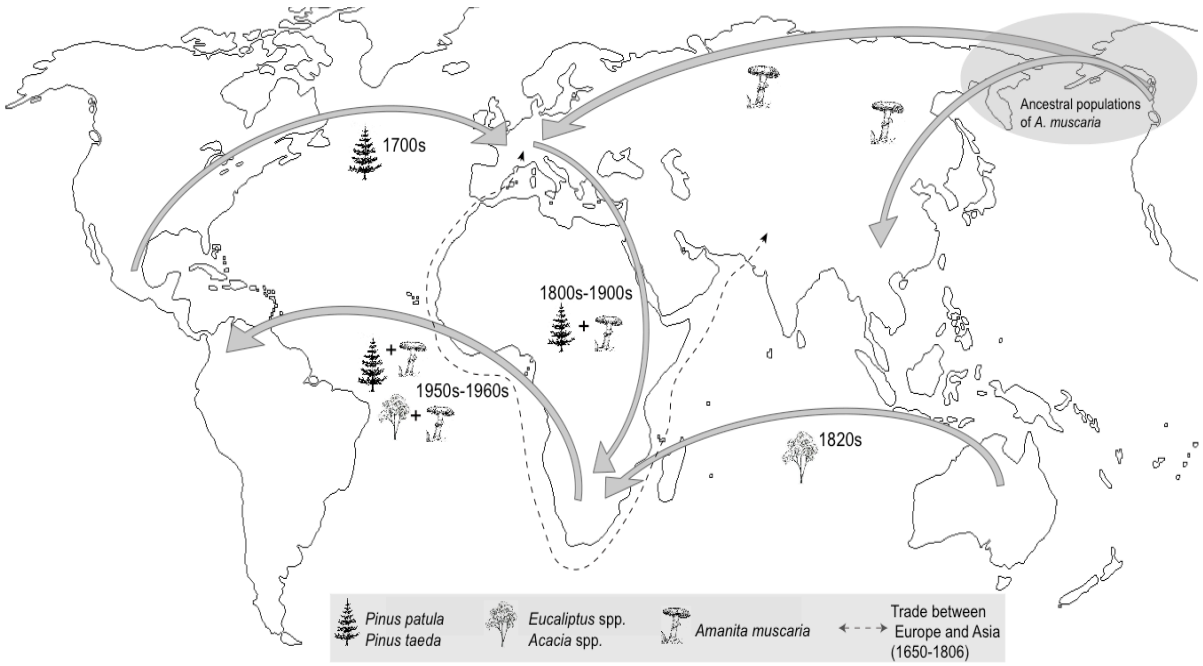

FIGURE 5. A summary of events related to the introduction of *A. muscaria* in Colombia

Supplementary Information (SI)

TABLE S1. Information data of *Amanita muscaria* collections used in this study

Department *	**Locality**	**Host**	**Coll.**	**Herbarium or Reference**	**Collecting Year**	**Elevation (masl)**	**Latitude**	**Longitude**	**Forest area (ha)**	**Forest perimeter (m)**
CUN	National University, Municipalty of Bogotá	*P. radiata*	J.M Idrobo 6268	COL	1968	2560	4°38'11.82"N **	74° 5'0.77"W **		
CUN	Vía Bogotá-Choachí, Km 2	*Pinus* sp.	P. Buriticá	COL	1976	1927	4°31'43.17"N **	73°55'22.68"O **		
CUN	Usaquén, Municipalty of Bogotá	*Pinus* sp.	Acosta 1225	COL	1977	2663	4°41'35.05"N **	74° 1'30.40"O **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	*Pinus* sp.	Pulido *et al*. 382	COL	1978	2460	6°16'56.64"N **	75°29'26.32"W **		
CUN	Embalse del Neusa, Municipalty of Cogua	*Pinus* spp.	Pulido *et al*. 743	COL	1979	2986	5°10'7.41"N **	73°55'59.00"W **		
CUN	Vía Bogotá-La Calera	*Pinus* sp.	Pulido *et al*. 704	Pulido (1983)	1979	2702	4°42'55.66"N **	73°58'19.35" W**		

ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	*P. radiata*	L.E Tobón & L.G Henao	COL	1984	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	Pine and oak forest	Guzman 29200	HUA	1986	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Velazques	HUA	1986	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Pineda	HUA	1986	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Velazques 75	HUA	1986	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Embalse La Fe, Municipalty El Retiro	NA	Velez, H.	HUA	1990	2170	6° 6'29.79"N **	75°29'45.71"W **		
ANT	Corregimiento de Llanos de Cuivá,	NA	Saldarriaga 488	HUA	1991	2764	6°48'21.14"N**	75°29'26.25"W**		

ANT	El Chaquiro, Municipalty of Santa Rosa de Osos	*Pinus* sp.	A.E. Franco-Molano 759	HUA	1991	2663	6°43'0.16"N **	75°32'23.27"W **		
CUN	Embalse del Neusa, Municipalty of Cogua	*P. patula*	A.E. Franco-Molano	COL	1991	2986	5°10'7.41"N **	73°55'59.00"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Pineda 849	HUA	1994	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Saldarriaga 911	HUA	1995	2460	6°16'56.64"N **	75°29'26.32"W **		
ANT	Embalse Rio Grande, Municipalty of San Pedro de los Milagros	*Pinus* sp.	Reyes, H. 2	HUA	2004	2313	6°30'9.67"N **	75°32'47.54"W **		
ANT	Vereda Carrizales, Municipalty El Retiro	NA	Gomez, N. 55	HUA	2004	2225	6°7'28.34"N **	75°30'52.82"W **		
ANT	Estación experimental Piedras blancas, Municipalty of Santa Elena	NA	Alba Cecilia O.	HUA	2005	2460	6°16'56.64"N **	75°29'26.32"W **		

TOL	Vereda Las Novillas,Municipalty of Murillo	*P. patula*	Medina 8	HUA	2006	3085	4°52'26.45"N **	75°11'3.15"W **		
CUN	Vereda la Moya, Municipalty of Cota	*Pinus* sp.	NVE 157	ANDES_F	2008	2762	4°49'58.35"N	74° 6'5.00"W	1.39	518
QUI	Via Armenia-Salento, Municipality of Salento	*P. patula*	Observation NVE	ANDES_F	2008	1987	4°39'5.45"N	75°36'10.37"O		
TOL	Vereda Albania, Municipalty of Murillo	Mixed forests	Leon Hoyos 3	HUA	2012	2966	4°52'22.12"N **	75° 9'50.88"W **		
BOY	Entrada Iguaque, Vereda Capilla 1-Municipality of Villa of Leyva	*P. patula*	NVE 719	ANDES_F	2013	2577	5°43'1.81"N	73°28'25.38"W	0.04	145.7
BOY	Via Gachantiva-Arcabuco	*P. patula*	NVE 761	ANDES_F	2013	2458	5°45'24.29"N	73°28'58.68"W	0.47	847
CUN	Monserrate, Municipalty of Bogotá	*Pinus* sp.	CPDH 1	ANDES_F	2014	2906	4°36'32.68"N	74° 3'29.15"W		
BOY	Vía Arcabuco-Paipa, Municipality of Arcabuco	*P. patula*	NVE 780a	ANDES_F	2014	2958	5°43'29.74"N	73°22'22.67"W	10.93	1387
BOY	Vereda Capilla 1-Municipality Villa de Leyva	*P. patula*	NVE 720	ANDES_F	2014	2504	5°42'22.83"N	73°28'56.41"W	0.27	450

CUN	Vereda Chiquira, Muncipalty Villapinzón	*P. patula*	NVE 782	ANDES_F	2014	2930	5°12'45.04"N	73°34'31.91"W	3.52	1213
SAN	Vereda San Jose de la Montaña, Municipality of Belén	*P. patula*	NVE 721, 790, 786, 722.	ANDES_F	2014	2905	6° 2'6.88"N	73° 1'12.64" W	0.2	201
SAN	Vereda San Jose de la Montaña, Municipality of Belén	Mixed forest *A.melanoxylon* and *P. patula*	NVE 726, 727	ANDES_F	2014	2290	6° 2'5.85"N	73° 0'53.22" W	0.51	375
BOY	Via Arcabuco-Moniquirá	*P. patula*	NVE	ANDES_F	2014	2517	5°45'52.17"N	73°26'57.80"W	0.12	310
BOY	Via Belén-San José de la Montaña, Municipality of Belén	*Eucalyptus* sp.	NVE 785, 787	ANDES_F	2014	3394	6° 1'5.80"N	72°56'52.46"W	0.09	178
BOY	Via Belén-San José de la Montaña, Municipality of Belén	*P. patula*	NVE 791	ANDES_F	2014	2911	6° 0'32.64" N	72°55'50.70"W	0	72
BOY	Via Paipa-Tunja	*P. taeda*	NVE 788, 789	ANDES_F	2014	2670	5°38'14.75"N	73°17'29.94"W	5.06	1249

BOY	Via Villa de Leyva-Gachantiva	*P. patula*	NVE 761, 762	ANDES_F	2014	2422	5°43'8.80"N	73°30'54.79"W	0.16	378
CUN	Embalse del Neusa, Municipalty of Cogua	*P. patula*	NVE 775, 776, 777, 779	ANDES_F	2015	2986	5°10'7.41"N	73°55'59.00"W	0.81	434
SAN	Vereda San Jose de la Montaña, Municipality of Belén	*Q. humboldtii*	NVE 677, 731, 771, 772, 773	ANDES_F	2007-2015	3238	6° 2'33.38"N	72°59'59.11"W	1.31	518

*Abbreviation: Antioquia (ANT), Boyacá (BOY), Cundinamarca (CUN), Quindío (QUI) Santander (SAN), Tolima (TOL), Valle del Cauca (VAL)

** Latitude and longitude are approximate data (estimated in Google Earth Pro) corresponding to the locality information provided in the physical collection.

TABLE S2. Sequences retrieved from GenBank

Country/State or Region/Locality	Voucher	ITS
Australia/Victoria	CD088	EU236711.1
Australia/Victoria	TL215	EU236712.1
Australia/Victoria	TL3	GQ914932.1
Canada/Newfoundland	RET383-3	EU071893.1
	NVE780	KU693333
Colombia/Boyacá/Belén	ANDES_F2290	
	NVE785	KU693315
Colombia/Boyacá/Belén	ANDES_F2295	

	NVE787	KU693314
Colombia/Boyacá/Belén	ANDES_F2297	
	NVE791	KU693325
Colombia/Boyacá/Belén	ANDES_F2301	
	NVE784	KU693316
Colombia/Boyacá/Paipa-Tunja Hwy	ANDES_F2294	
	NVE788	KU693317
Colombia/Boyacá/Paipa-Tunja Hwy	ANDES_F2298	
	NVE789	KU693318
Colombia/Boyacá/Paipa-Tunja Hwy	ANDES_F2299	
	CAC4	KU693326
Colombia/Boyacá/Villa de Leyva	ANDES_F2280	
	CPDH1	KU693327
Colombia/Cundinamarca/Bogotá	ANDES_F2281	
Colombia/Cundinamarca/Embalse del	NVE775	KU693309
Neusa	ANDES_F2285	
Colombia/Cundinamarca/Embalse del	NVE776	KU693310
Neusa	ANDES_F2286	
Colombia/Cundinamarca/Embalse del	NVE777	KU693311
Neusa	ANDES_F2287	
Colombia/Cundinamarca/Embalse del	NVE779	KU693312
Neusa	ANDES_F2289	
	NVE782	KU693313
Colombia/Cundinamarca/Villapinzón	ANDES_F2292	
Colombia/Santander/San Jose de la	NVE677	KU693328
Montaña	ANDES_F2180	

Colombia/Santander/San Jose de la Montaña	NVE721 ANDES_F2224	KU693321
Colombia/Santander/San Jose de la Montaña	NVE722 ANDES_F2225	KU693322
Colombia/Santander/San Jose de la Montaña	NVE726 ANDES_F2229	KU693323
Colombia/Santander/San Jose de la Montaña	NVE727 ANDES_F2230	KU693324
Colombia/Santander/San Jose de la Montaña	NVE731 ANDES_F2234	KU693329
Colombia/Santander/San Jose de la Montaña	NVE771 ANDES_F2282	KU693332
Colombia/Santander/San Jose de la Montaña	NVE772 ANDES_F2283	KU693331
Colombia/Santander/San Jose de la Montaña	NVE773 ANDES_F2288	KU693330
Colombia/Santander/San Jose de la Montaña	NVE786 ANDES_F2296	KU693320
Colombia/Santander/San Jose de la Montaña	NVE790 ANDES_F2300	KU693319
England/Hampshire	31452	AB080777.1
England/Surrey	31445	AB080778.1
England/Surrey	80048(K)	AB080779.1
Germany/	DR 043	EU346871.1
Germany/Baden-Württemberg	RET152-6	EU071897.1
Germany/Bavaria	RET152-8	EU071920.1

Germany/Thuringia/Uranium mining site near Ronneburg	Taxon: 41956	AJ549964.1
Japan/	LEM950025	AB015700.1
Japan/Aomori/Aomori-shi	FB-30961	AB080980.1
Japan/Gifu/Ohno-gun	FB-30977	AB081295.1
Japan/Gifu/Ohno-gun	FB-30985	AB096048.1
Japan/Nagano/Kiso-gun	FB-30976	AB081294.1
Japan/Yamanashi/Kitakoma-gun	FB-30962	AB080981.1
Japan/Yamanashi/Kitakoma-gun	FB-30963	AB080982.1
Korea/	KA12-1393	KF017944.1
Mexico/Tlaxcala	RET144-10	EU071913.1
Mexico/Tlaxcala	RET145-1	EU071921.1
Mexico/Tlaxcala	RET145-2	EU071903.1
Mexico/Veracruz/Parque Nacional Cofre De Perote	AM08S2-2	JX122508.1
Montenegro/Durmitor Mt.	AMD-X-08	JQ685713.1
New Zealand/	K91T357a	GQ267468.1
Norway/Fjordane/Sogn og Fjordane	RET309-3	EU071914.1
Poland/Gdynia	FB-30964	AB080983.1
Poland/Gdynia	FB-30965	AB080984.1
Russia/Autonomous/Anadyr district, Chukot	NS16	EU071933.1
Russia/Kamchatka	RET143-5	EU071915.1
Russia/Magadan	NS8	EU071932.1
Russia/Magadan	NS10	EU071905.1
Russia/Magadan	NS5	EU071924.1
Russia/Magadan	NS6	EU071927.1

Russia/Ola	NS1	EU071934.1
Russia/Ola	NS11	EU071935.1
Russia/Ola	NS12	EU071923.1
Russia/Ola	NS3	EU071922.1
Russia/Ola	NS4	EU071904.1
Russia/Omsukchan	NS13	EU071925.1
Russia/Tenka	NS14	EU071928.1
Russia/Tenka	NS15	EU071930.1
Scotland/Highlands & Islands Reg	RET107-6	EU071909.1
Scotland/Highlands & Islands Reg	RET141-2	EU071918.1
Switzerland/Zürich	RET036-3	EU071912.1
Tanzania/Southern Highlands Prov	RET149-1	EU071895.1
Tanzania/Southern Highlands Prov	RET149-2	EU071894.1
U.S.A/Alabama/Shelby Co.	RET151-6	EU071891.1
U.S.A/Alabama/Talladega Co.	RET151-4	EU071892.1
U.S.A/Alaska/Bird Creek Campground, Anchorage	MP23	EU071929.1
U.S.A/Alaska/Bird Creek Campground, Anchorage	MP24	EU071931.1
U.S.A/Alaska/Bonanza Creek LTER site	GAL16775	DQ060892.1
U.S.A/Alaska/Bonanza Creek LTER site	GAL18122	EU071944.1
U.S.A/Alaska/Bonanza Creek LTER site	GAL18134	EU071946.1
U.S.A/Alaska/Bonanza Creek LTER site	GAL18136	EU071949.1
U.S.A/Alaska/Bonanza Creek LTER site	GAL2005	EU071947.1
U.S.A/Alaska/Dalton Hwy, mile 122	GAL2810	DQ060904.1
U.S.A/Alaska/Dalton Hwy, mile 123	GAL2814	DQ060897.1

U.S.A/Alaska/Denali National Park	GAL14284	DQ060895.1
U.S.A/Alaska/Denali National Park	GAL4810	EU071937.1
U.S.A/Alaska/Denali National Park	GAL5505	DQ060908.1
U.S.A/Alaska/Denali National Park	GAL8950	DQ060901.1
U.S.A/Alaska/Eagle Summit	GAL3169	DQ060905.1
U.S.A/Alaska/Fairbanks	GAL15330	DQ060871.1
U.S.A/Alaska/Fairbanks	GAL15335	EU071945.1
U.S.A/Alaska/Fairbanks	GAL15336	EU071906.1
U.S.A/Alaska/Fairbanks	GAL15776	DQ060893.1
U.S.A/Alaska/Fairbanks	GAL16654	DQ060907.1
U.S.A/Alaska/Fairbanks	GAL16735	DQ060896.1
U.S.A/Alaska/Fairbanks	GAL16735-2	EU071998.1
U.S.A/Alaska/Fairbanks	GAL16735-3	EU071941.1
U.S.A/Alaska/Fairbanks	GAL17647	EU071907.1
U.S.A/Alaska/Fairbanks	GAL17691	EU071956.1
U.S.A/Alaska/Fairbanks	GAL17899	EU071950.1
U.S.A/Alaska/Fairbanks	GAL17982	EU071938.1
U.S.A/Alaska/Fairbanks	GAL17984	EU071908.1
U.S.A/Alaska/Fairbanks	GAL18012-2	GAL18012-2
U.S.A/Alaska/Fairbanks	GAL18012-2	EU071952.1
U.S.A/Alaska/Fairbanks	GAL18012-4	EU071953.1
U.S.A/Alaska/Fairbanks	GAL18012-6	EU071951.1
U.S.A/Alaska/Fairbanks	GAL18071	EU071940.1
U.S.A/Alaska/Fairbanks	GAL18076	EU071942.1
U.S.A/Alaska/Glacier Hwy, mile 30, Juneau	GAL3643	EU071948.1
U.S.A/Alaska/Glacier Hwy, mile 30, Juneau	GAL3688	DQ060906.1

U.S.A/Alaska/Glacier Hwy, mile 30, Juneau	GAL4247	DQ060894.1
U.S.A/Alaska/Glacier Hwy, mile 30, Juneau	GAL4302	DQ060910.1
U.S.A/Alaska/Homer	GAL18810-1	EU071955.1
U.S.A/Alaska/Homer	GAL18810-2	EU071954.1
U.S.A/Alaska/Kougarok Rd., mile 49, Nome	GAL6027	DQ060909.1
U.S.A/Alaska/North Pole	GAL15453	DQ060899.1
U.S.A/Alaska/North Pole	GAL15454	EU071943.1
U.S.A/Alaska/North Pole	GAL15461	DQ060900.1
U.S.A/Alaska/Quartz Creek, E. of Nome	GAL5946	DQ060903.1
U.S.A/Alaska/Serpentine Hot Springs, N. of Nome	GAL5895	DQ060898.1
U.S.A/Alaska/Serpentine Hot Springs, N. of Nome	GAL5900	DQ060902.1
U.S.A/Arizona/Cochise Co.	CMP0648	EU071910.1
U.S.A/Arizona/Cochise Co.	CMP1345	EU071902.1
U.S.A/Arizona/Cochise Co.	CMP3143	EU071889.1
U.S.A/California	45863(NY)	AB080787.1
U.S.A/California/Santa Cruz Island	LG382	EU071957.1
U.S.A/California/Santa Cruz Island	LG882	EU071960.1
U.S.A/California/Santa Cruz Island	LG1045	EU071961
U.S.A/California/Santa Cruz Island	LG862	EU071958.1
U.S.A/California/Santa Cruz Island	LG864	EU071959.1
U.S.A/California/Sonoma Co.	7086	GQ250402.1
U.S.A/Idaho/Fremont Co.	RET320-1	EU071911.1
U.S.A/Massachusetts	RET124-2	EU071896.1

U.S.A/Massachusetts/Essex Co	RET032-1	EU071890.1
U.S.A/Massachusetts/Hampshire	45785	AB080789.1
U.S.A/Massachusetts/Hampshire	45843	AB080788.1
U.S.A/Massachusetts/Lawrence	45840	AB080791.1
U.S.A/Mississippi/Harrison Co.	RET024-3	EU071886.1
U.S.A/Mississippi/Harrison Co.	RET112-5	EU071887.1
U.S.A/Mississippi/Harrison Co.	RET112-6	EU071888.1
U.S.A/New Jersey/Burlington Co.	RET158-7	EU071916.1
U.S.A/New Jersey/Cape May Co.	RET289-3	EU071901.1
U.S.A/New Jersey/Monmouth Co.	RET303-4	EU071917.1
U.S.A/New Jersey/Somerset Co.	RET271-3	EU071919.1
U.S.A/New Jersey/Sussex Co.	RET271-2	EU071899.1
U.S.A/New Jersey/Sussex Co.	RET328-2	–
U.S.A/New Jersey/Sussex Co.	RET328-2	EU071926.1
U.S.A/New York/Bronx	45820	AB080790.1
U.S.A/Washington/Clallam Co.	RET338-9	EU071900.1
U.S.A/Washington/Skamania Co.	RET264-7	EU071898.1
U.S.A/Washington/Whatcom Co.	RET136-2	EU071936.1

IV. Population genetics of the invasive species *Amanita muscaria* in the native oak forest (*Quercus humboldtii)* in Colombia

Natalia Vargas-Estupiñán, Laura Natalia Gonzalez, Silvia Restrepo

Laboratory of Mycology and Plant Pathology, Universidad de Los Andes, Bogotá, Colombia

Anne Pringle

Departments of Botany and Bacteriology, University of Wisconsin-Madison, Madison, WI, U.S.A

Introduction

ECto**M**ycorrhizal **F**ungi (ECM) establish symbiotic ecological relationships, throughout a mutually beneficial ecological interaction between plant roots and fungal mycelium. This ecological interaction plays an essential role in the dynamics of forest ecosystems. Lists of the ECM species occurring naturally in oak ecosystems have been reported in several studies, but no evolutionary studies in foreign invasive fungal species colonizing native forest *Quercus humboldtii* in Colombia have been conducted yet. This project will focus on the elucidation of the population genetic factors characteristic of the invasive species *Amanita muscaria,* whose natural host is *Pinus patula,* and is currently colonizing oak forest in the department of Santander. The use of a next generation sequencing technology such as Genotyping by Sequencing (GBS), will provide us robust data to understand the differences between the populations adapted to two distinct forests types: pine and oak forest ecosystems.

Oak ecosystems are distributed in the three cordilleras of Colombia (Cárdenas & Salinas, 2006) and occupy small discontinuous relicts in the Colombian departments of Antioquia, Boyacá, Cundinamarca, Huila, Nariño, Santander and Tolima. According to the Fundación Natura (2007) nearly 10,500 hectares of natural ecosystems have been replaced by

miscellaneous forests and to a lesser extent by areas for agricultural activities. This fragmentation constitutes one of the major threatens to the biodiversity associated oak forest, and renders oak a vulnerable (VU) species since it is considered a source for charcoal production and tanning processes (Barrios *et al*. 2006, Cardenas and Salinas 2006). However one underestimated problem facing the diversity associated to oak forest, is the colonization of invasive fungal species such us *Amanita muscaria,* occurring naturally in introduced *Pinus* spp. plantations in Colombia and now have appeared colonizing the native species *Quercus humboldtii* forests.

The native fungal diversity associated to a particular ecosystem can be threatened by invasive species in several ways. Ectomycorrhizal fungi compete for root (plant host, source of carbon) and soil resources (Kennedy *et al*. 2009). Particularly, *A. muscaria* produces a high density of sporocarps, so it might be hypothesized that this species is reducing the availability of resources to other native species (Dickie and Johnston 2008). This research will be one of the first efforts to test the evolutionary aspects based on the genetics of the adaptation of *A. muscaria* to oak forest. Other ectomycorrhizal fungi may be or may become invasive producing inconspicuous sporocarps, however its highly visible sporocarps makes *A. muscaria* an appropriate species for the population analysis in this case study.

Invasive ectomycorrhizae

Plantations of forestry tree species such as *Pinus* and *Eucalyptus* have introduced several other non-native species of ectomycorrhizal fungi to Colombia, which with time have evolved to adapt and colonize the native oak species habitat. One of the most common ECM showing a notable spreading onto native host is *Amanita muscaria*, which is one of the most dominant fungi in *Pinus patula* plantations in Colombia.

Pringle *et al.* (2011) described some factors related to biological invasions. The novel invasion of habitat can be established by mycorrhizal species associated with introduced plants, or after association with native plants. Second, mychorrizal invasions can promote the invasion of introduced plants. Third, native mycorrhizal fungi will respond to invasions by associating with introduced plants, or by declining after the introduction of other biotic sources that could attack native host plants.

Genotyping by Sequencing and population genetics

The molecular data used in the majority of the studies have been obtained from few regions of the genome, covering a limited subset of particular regions. Recently, next generation sequencing and computational platforms have been designed for the discovery of a large quantity of polymorphisms, allowing the researcher to screen the genome in a more densely way and to observe specific genetic patterns across the genomes of populations (Narum *et al*. 2013)

Genotyping by Sequencing (GBS) is a strategy that uses the Illumina platform to generate hundreds of thousands or millions of sequenced reads (Elshire *et al*. 2011). In contrast to the common use of neutral markers that appropriately allow solving a wide range of evolutionary questions related to gene flow, effective population sizes, genetic drift, among others, GBS greatly enables the discovery of specific molecular regions that have experienced natural selection. The manipulation of this amount of data requires the use of pipelines that in general terms, first filter out poor-quality reads, then classify reads based on sequence barcodes, and finally identify loci *de novo* or align reads to a reference genome to discover Single Nucleotide Polymorphisms (SNPs).

Population genetics is considered one of the core tools of analyses in molecular

ecology and conservation biology, providing a framework for understanding the distribution of genetic variability among populations from molecular data (Pool *et al*. 2010). In order to assess the population genetics, calculation of summary statistics such as F_{ST}, nucleotide diversity and linkage disequilibrium analyses are being carried out to solve specific biological questions.

Implicit is the view that a population is a single entity where processes such as mating and movement of individuals are uniform throughout, a condition often called panmixia (Hamilton 2009). Several processes and features in actual populations make this perspective of population uniformity unlikely to hold true for many populations. One of the reasons to make that panmixia can not be hold in natural populations is the phenomenon of isolation by distance, which explains that increasing distance separating individuals consequently will decrease the chances of mating (Hamilton 2009). One effect of isolation by distance is the change in the chance that two individuals mate because of their location within the population: this refers to population structure. Population structuring can lead to changes in allele frequency in the population, with local regions approaching fixation or loss of alleles (Hamilton 2009).

This research aims to study the population genetics of foreign populations of the ectomycorrhizal *A. muscaria* expanding its range to *Q. humboldtii* ecosystems, determining if population structure exists between populations of *A. muscaria* associated to *Q. humboldtii* forests and to *P. patula.* This will be the first study using a high-throughput strategy to elucidate the molecular population genetics of an ectomycorrhizal invasive species switching to a native Colombian oak host.

Materials and Methods

Sampling and DNA extraction–. Fruiting bodies of ectomycorrhizal *A. muscaria* were collected in three forests: 1) *Pinus patula* forest in Boyacá (called hereafter Pine_B): Vereda Capilla 05°42.982" N 73°28.199" W (Villa de Leyva, Boyacá), 2) *P. patula* forest in the department of Santander (called hereafter Pine_S): Vereda San Jose de la Montaña 06°02.54" N 72°59.99" W, and 3) *Quercus humboldtii* forest (called hereafter Oak_S) in the department of Santander, separated 2216 m from Pine_S (Fig. 1). In the Santander locality we searched for all the pine forest near the oak were *A. muscaria* is observed: Figure 1 show all pines (with or without *A. muscaria* fruiting bodies) near oak forests within the locality. The sampling consists in collecting conspicuous *A. muscaria* sporocarps, separated each other > 8 m according to previously reported genet size of *A. muscaria*. Piece of the stipe context were collected and immersed in CTAB solution for DNA extraction and molecular analyses. The fruiting bodies were dried and packaged in plastic bags, stored at the ANDES Herbarium (Universidad de los Andes) and registered in the SPECIFY database. DNA extraction was perfomed with DNeasy Plant Minikit (Qiagen, USA). Extracted genomic DNA was normalized to a concentration of 30 ng/lL in 96-well plates and digested with the restriction enzyme *Ape*K1 (New England Biolabs, UK), samples were processed in the Biotechnology Center of the University of Wisconsin-Madison.

DNA sequencing–.DNA was sequenced in 1 lane on an Illumina HiSeq2000, single-end 100 bp according to manufacturer's instructions. An analysis of the quality of the reads was made by using Fastqc version 0.10.1. A perl script run_demultiplex.sub process_radtags was used to demultiplex and clean the raw data. Raw sequence reads were demultiplexed using Stacks pipeline (Catchen *et al*. 2011). Only those reads of sufficiently

high sequencing quality, and having the correct barcode, were retained (Table 1). A *de novo* reassembling and SNP calling was performed by using *ustacks*, the catalog of loci were created by using *cstacks* and the *sstacks* was used to match loci to the catalog (Catchen *et al*. 2011) (supplementary Figure 1). SNPs were determined and genotypes called using a maximum-likelihood statistical model implemented in Stacks (Hohenlohe *et al*. 2010, Catchen *et al*. 2011).

Population genetics analysis–.To include a locus in the analysis, we set the conditions for the loci to be present in the three populations and genotyped in at least 75% of the samples. Summary statistics including major allele frequency, percent polymorphic loci, nucleotide diversity (p) and Wright's F statistics F_{IS} and F_{ST}) were calculated for every SNP using the POPULATIONS program in Stacks.

To analyse the organization of the populations, we used the POPULATIONS program in Stacks to output multilocus genotypic data into a STRUCTURE-format file (Pritchard *et al*. 2000; Falush *et al*. 2007; Hubisz *et al*. 2009). For all analyses, 100,000 burnin steps and 100,000 replicates were used, with 20 replicates for values of 1-10 K (where K is number of genotypic combinations). The optimal K for each analysis was chosen using the online program STRUCTURE HARVESTER (Earle *et al*. 2012) which implement the deltaK method of Evanno *et al*. (2005).

Results

Sequence data quality and processing.–The total number of reads were 16x107 bp, with a sequence read lenght of 101 observed in the sequence length distribution, and a 53% of GC. The quality control analysis of the set of reads, reach a failure in the base pair 101, since the

lower quartile for the base 101 was less than 5 (Supplementary Fig. 2). According to the per sequence quality score there was no indication of a general loss of quality within a run, the most frequently observed mean quality was not below 27. The per sequence GC content showed a normal distribution where the reference distribution corresponds to the overall GC content of the genome. No proportions of Ns were called at any position, which indicate a no loss of quality. Between 10 and 500 sequences have duplication events (Supplementary Fig. 3), which can be explained by the fact that some wells were technically duplicated. There were no overrepresented sequences, meaning that, there was no library contamination. The percentage of adaptor at the end of the sequences was high starting at the 55th position in the read over 10% up to 45% at the end of the sequences, which was trimmed during the cleaning process before proceeding with the STACKS analysis. One lane of sequencing produced a total of more than 16 million reads derived from 72 individuals, A total of 6539 loci were included in the population analysis after requiring loci to be present in the three populations and in 75% of individuals.

Population structure analysis.–For all loci that were polymorphic, the average major allele frequency (P) ranged from 0.728 to 0.738 and the average observed heterozygosity ranged from 0.3588 to 0.4205 (Table 1). When considering variant and fixed positions, the values for P increase to 0.999 and the observed average heterozygosity decreases to 0.0001. The three populations demonstrated equal levels of genetic diversity that was evident when comparing the percentage of polymorphic loci and the nucleotide diversity (π) (Table 1)

The allele frequency spectra of major alleles across loci were similar among the three populations (Fig. 2A), showing different percentage of loci with variable values of alleles frequencies between 0.4 and 1. Whereas, the pine populations (Boyacá and Santander)

exhibited a distribution of major allele frequencies towards 0.5, the oak population has a high percentage of loci with frequencies towards 1.0 (Fig. 2A). The measure of F_{ST} was estimated to assess the genetic relatedness of *A. muscaria* populations belonging to the three forests, we found that although all values are statistically significant (with an alpha = 0.05), the values are very close to zero and therefore are indicative of no structure among populations, however the Santander forests showed less differentiation to one another in contrast to the population of *A. muscaria* associated to the pine forest in Boyacá (Table 2). Overall F_{IS} estimates showed that the majority of the loci in the three populations have zero or negative values (Table 1, Fig. 2B) which are indicative of no cryptic structure or assortative mating commonly ocurring in the populations.

When analyzing the K number of genotypic combinations, in the three forests Oak_S, Pine_B and Pine_S, a value of K =4 [L(K)=-5590.2, var [LnP(D) 364.3] (Fig. 3, yellow, blue, red and green) was found when applying the Evanno *et al* (2005) method (Supplementary Fig. 4). The four genotypic combinations were found in all the three forests being yellow the dominant genoptypic combination in Oak_S and Pine_B forests (Table 3, Fig. 3), and in Pine_S the more frequent was the red genotypic combination (Table 3). The populations of *Q. humboldtii* and *Pinus patula* showed very low structure. Although there is no evident structure according to the F_{ST} analysis, STRUCTURE analysis, nor an independen clustering of forest populations (Fig. 4), the populations showed scattered distribution among them (Fig. 4).

Discussion

The populations of *A.muscaria* associated to *P. patula* and *Q.humboldtii* forests, are genetically diverse. Evidence of an equilibrium of the allele frequencies showed that the populations are stable with high genetic diversity, a characteristic that has been considered necessary for invasive populations to adapt to new environments (Stapley *et al*. 2015) and that are recently colonizing a new host. According to the history of introduction of *A. muscaria* shown previously (chapter 2, Vargas-Estupiñán et al. unpub.) the populations were introduced recently during the 1950s.

All the three forests showed close genetic relatedness to one another. The three forests show no population structure (F_{ST} values from 0.020-0.025) despite the Pine_B forest in Boyacá is separated approximately 60 Km. Similarly, the STRUCTURE analysis of the three populations provided evidence of a lack of structure among forests which may be a sign of gene flow ocurring among forests, as well as of a recent introduction of the species in Colombia, and a consequent recent colonization into the new host (*Q. humboldtii* forests). Furthermore the oak forest individuals represent a range of mixtures of genotypic combinations present in pine forest populations.

On the other hand, the statistic F_{IS} provides evidence of a recent population expansion, since variation in Fis values is seen across the three populations (Fig. 2B). Furthermore, the majority of these values were negative representing an excess of heterozygosity compare to the expected by Hardy-Weinberg. Patterns of high variance in F_{IS} with significant negative values, can exist in non-equilibrium populations due to founder effects and rapid expansion with high gene flow (Catchen *et al*. 2013).

A high percentage of loci with allele frequencies P=1.0 was observed in the oak population showing evidence that this population is facing a more recent colonization in the

Q. humboldtii forest. This might be indicative of a probable small number of founder individuals that are new colonizers into oak forest, and in order to reach an evolutionary equilibrium new mutants or migrant must be adquired (Hart & Clark 2007). Moreover estimates of the inbreeding coefficient (F_{IS}) which measures reductions in observed heterozygosity with respect to that expected heterozygosity (Slatkin 1991, Charlesworth 1998), showed no indication of cryptic assortative mating or hidden population structure within the three populations, this characteristic is related to the characteristics of invader populations entering a new geographic range and a new host.

The present study shows some of the genetic characteristics in the population of *A. muscaria* colonizing a new host as a recent introduction by humans in Colombia. A future perspective of study in this recently explored field of biological invasión in ectomycorrhizal fungi in Colombia, will be to analyze the genomic regions compared to a reference genome of the species and to know which regions have lead to the local adaptation into a new oak host, regions that probable are under selective pressures.

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Tables

Table 1. Summary genetic statistics for the three populations calculated for all nucleotide positions across all restriction-site associated DNA (GBS) sites: variant (left) or variant and fixed (right). These statistics include the the number of polymorphic sites (*P sites*) across the data set (*Sites*), percentage of polymorphic loci *(% P Loci),* the average frequency of the major allele (*P*), the average observed heterozygosity and homozygosity per locus, the average nucleotide diversity (π), and the average Wright's inbreeding coefficient (F_{IS})

Variant

	Num. Ind.	*P*	*Obs Het*	*Obs Hom*	F_{IS}	π
Pine_B	9	0.738	0.3852	0.6148	-0.0405	0.3567
Pine_S	19	0.7282	0.4205	0.5795	-0.1167	0.3638
Oak_S	35	0.735	0.3588	0.6412	-0.0292	0.3417

Variant and Fixed

	P	*Obs Het*	*Obs Hom*	*Fis*	π	*Sites*	*V Sites*	*P Sites*	*% P Loci*
Pine_B	0.9999	0.0001	0.9999	0	0.0001	388924	143	128	0.0329
Pine_S	0.9999	0.0001	0.9999	0	0.0001	285207	98	92	0.0323
Oak_S	0.9999	0.0001	0.9999	0	0.0001	294141	101	101	0.0343

Table 2. Summary Fst

	Pine_B	**Pine_S**	**Oak_S**
Pine_B	-	0.0211762	0.0252657
Pine_S	-	-	0.0204641

Table 3. Number of individuals with the dominant genotypic combination in each forest population

	Genotypic combination			
Forest	Yellow	Blue	Red	Green
Pine_B	4	0	3	2
Pine_S	6	2	7	4
Oak_S	22	11	1	1

Figures

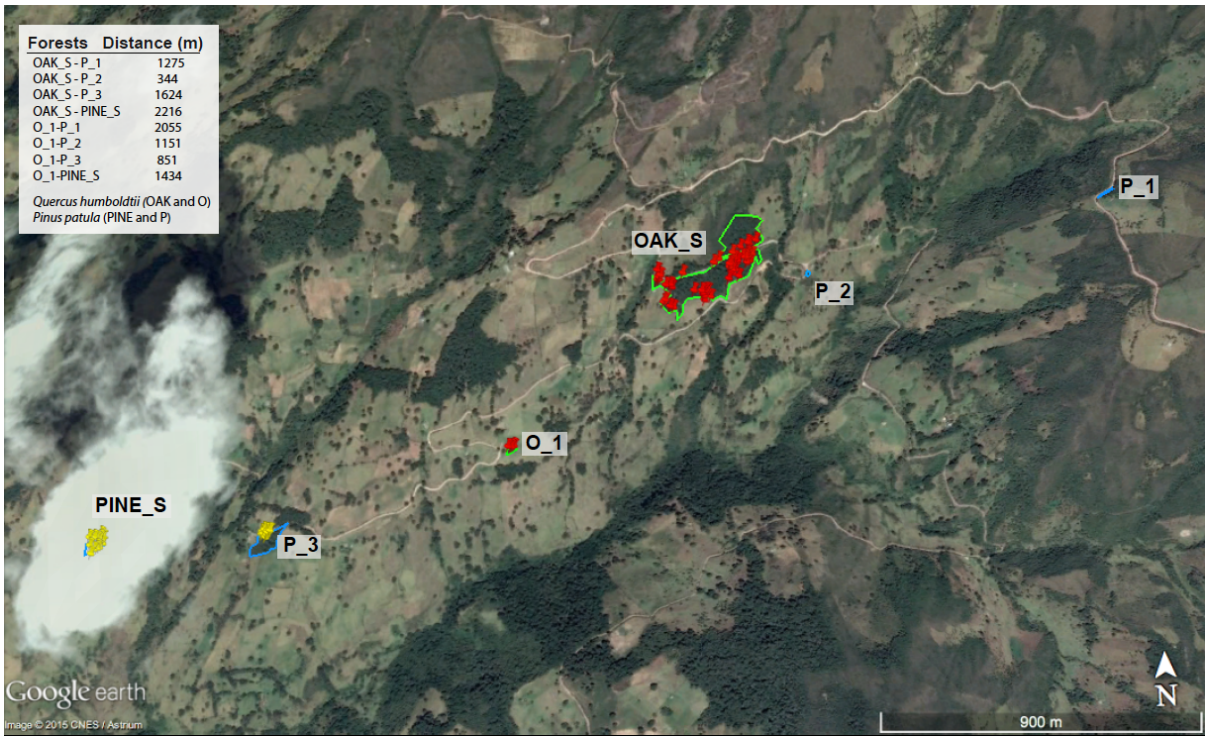


Figure 1. Forests sampled in Santander. Red placemarks represent individuals in *Q. humboldtii* forest; yellow placemarks indicate individuals of *A. muscaria* in *Pinus patula* forests. Individuals included in the GBS analysis were collected in OAK_S and PINE_S.

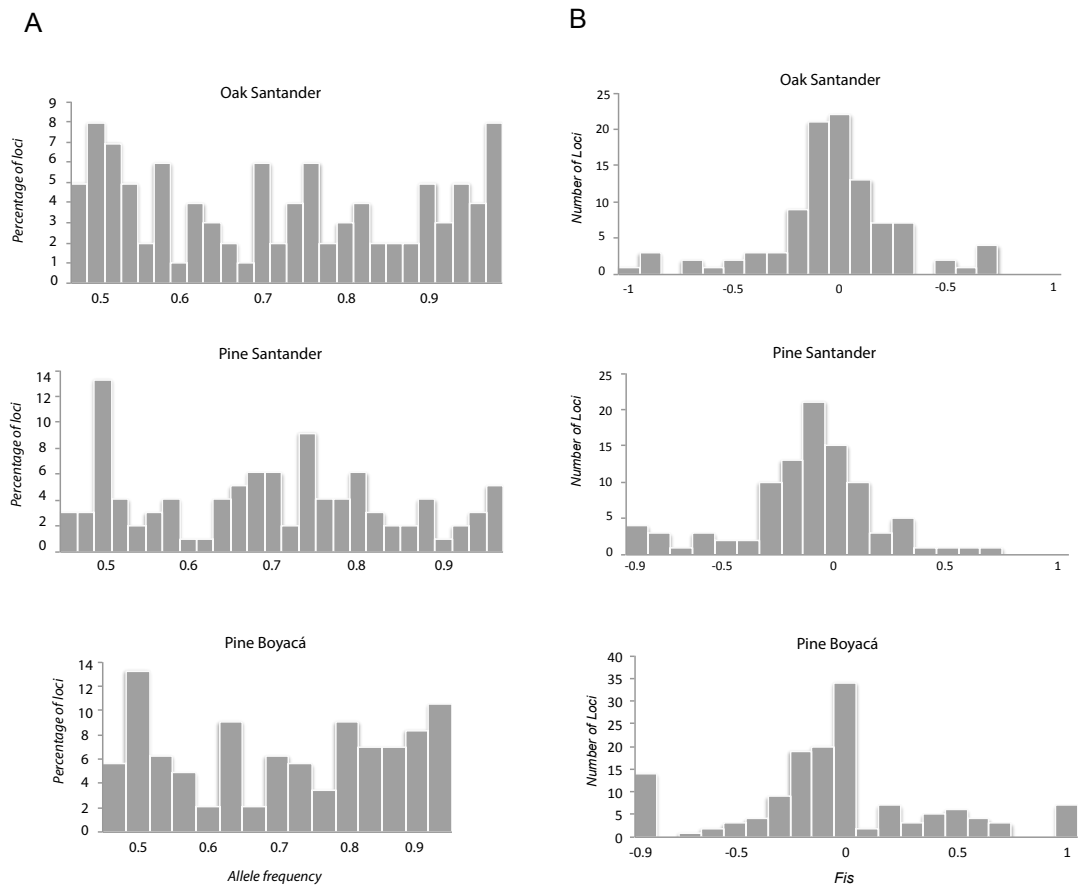


Figure 2. Population statistics obtained with POPULATIONS program (Stacks).

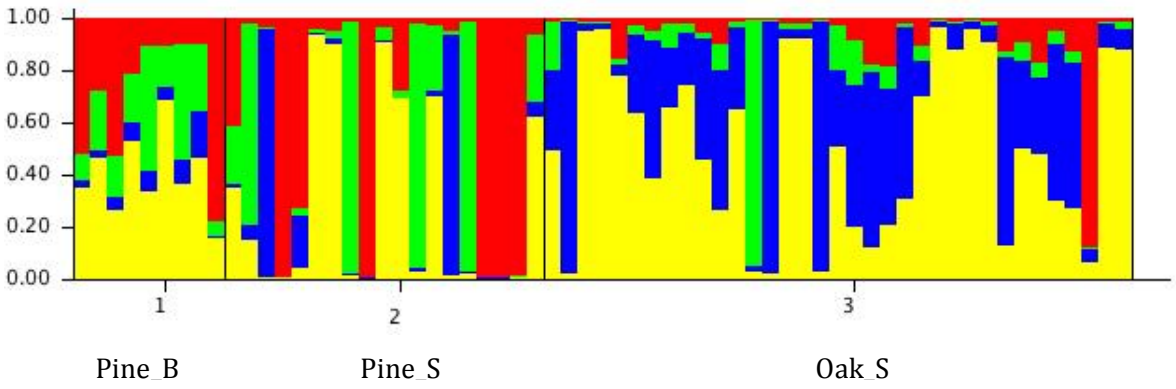


Figure 3. Results of a structure analysis showing plots of posterior probabilities of group assignment of individuals into 4 genotypic combinations. The x axis show the population of origin for each individual. The color proportion in each vertical bar represents the posterior

probability of assignment of an individual to one of the 4 genotypic combinations.

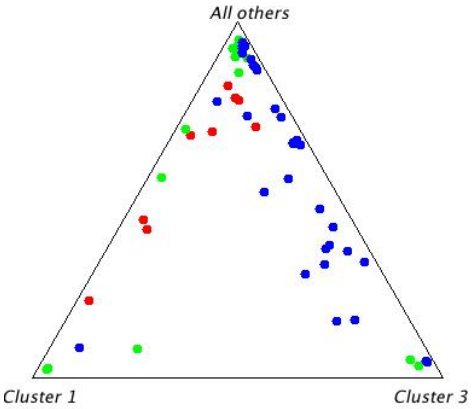
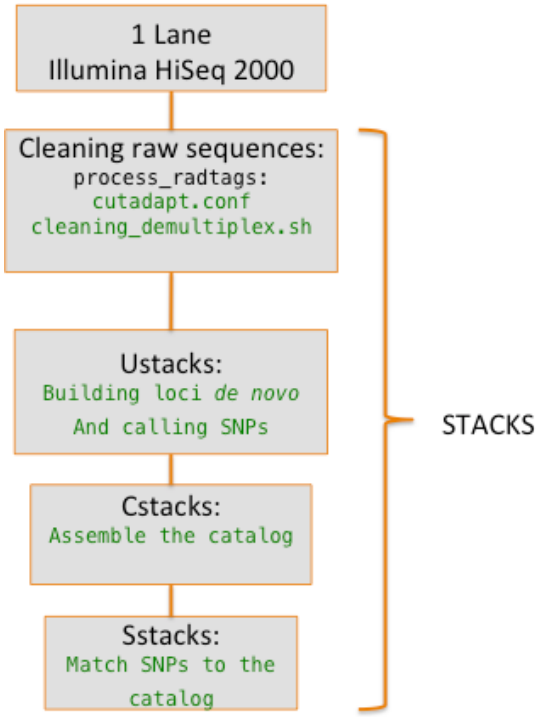
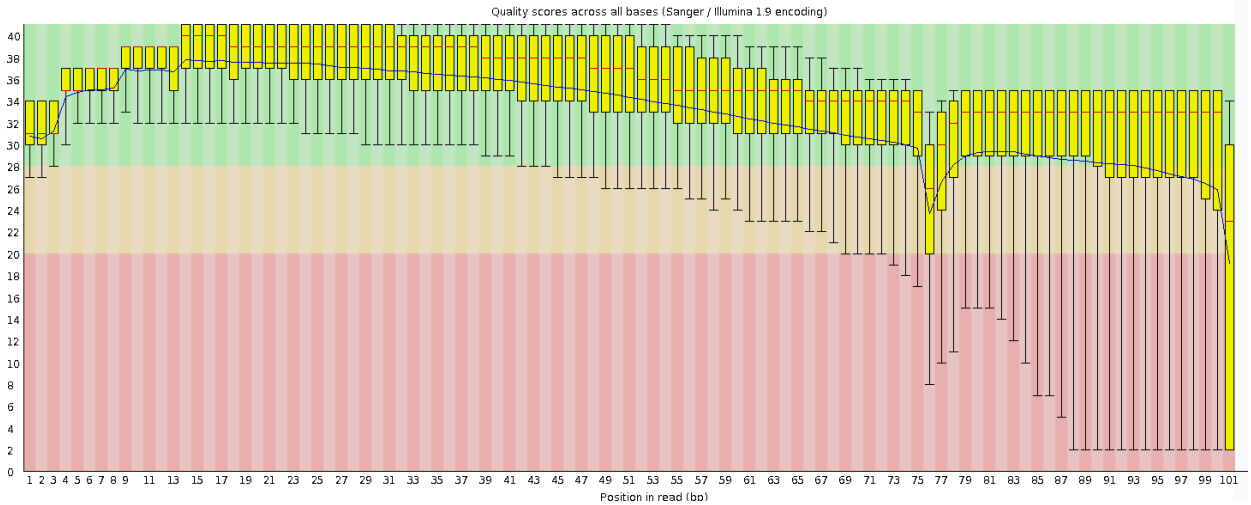


Figure 4. A cluster analysis of forest populations performed by STRUCTURE. Pine_S (red), Pine_B (green) and Oak_S (blue).

Supplementary Figures

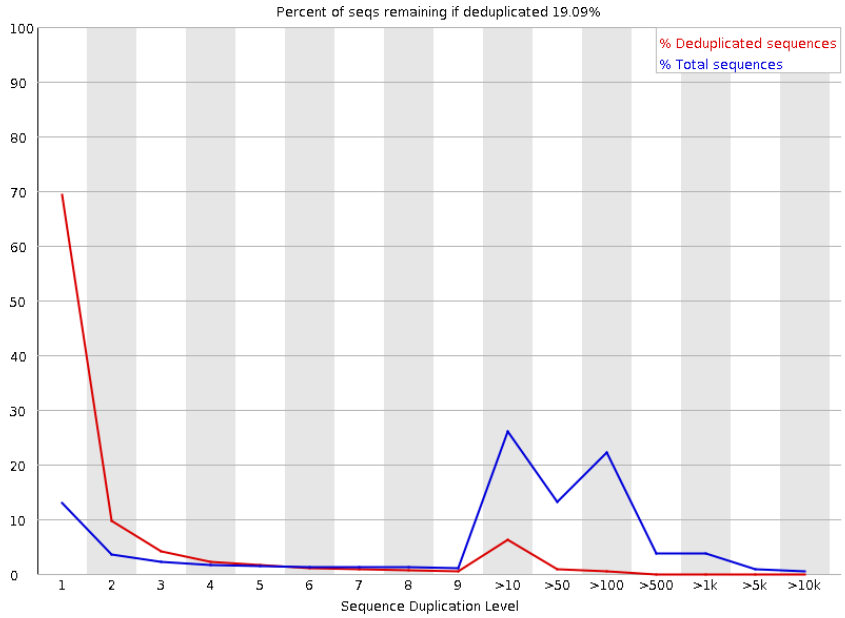


Supplementary Figure 1. Diagram of the analysis using Stacks

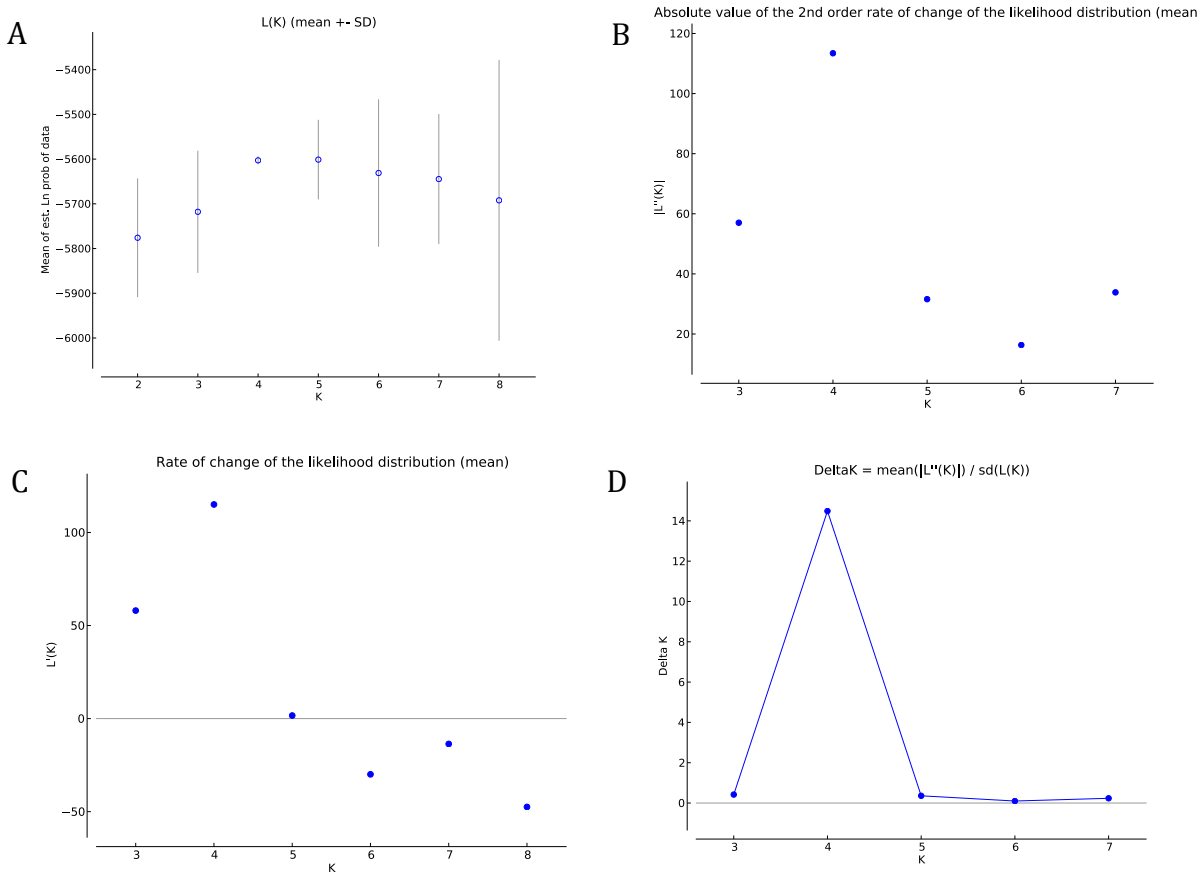


Supplementary Figure 2. Overview of the range of quality values across all bases at each

position in the reads of GBS data. The y-axis shows the quality scores. The higher the score the better the base call. The background of the graph divides the y axis into very good quality calls (green), calls of reasonable quality (orange), and calls of poor quality (red). The yellow box represents the inter-quartile range (25-75%), and the upper and lower whiskers represent the 10% and 90% points. The blue line and the central red line represent the mean quality and the median value, respectively.



Supplementary Figure 3**.** Relative number of GBS sequences with different degrees of duplication



Supplementary Figure 4. Parameters used to obtain the most probable K by using Structure Harvester

CHAPTER 3

SOCIAL ASPECTS IN THE CONSERVATION OF MACROFUNGI

1) To investigate the current state-of-the-art of ECM fungi associated to *Quercus humboldtii* in Colombia.
2) To elaborate an illustrated booklet with recommendations for sustainable collection and management of invasive species.
3) To include information of macrofungi in Colombia, in the Barcode of Life Database system.
4) To integrate the cultivation of *Pleurotus ostreatus* as an economic and nutritive income alternative for rural communities in Villapinzón, a locality in Cundinamarca, Colombia.

INTRODUCTION

The initiative to conserve fungi began in Oslo in 1995, with the consolidation of the *European Council for Conservation of Fungi.* Afterwards, the first international Congress on Fungal Conservation (Cordoba, Spain-2007) brought onto the world stage the idea to protect fungi. During the 3rd International Congress in fungal conservation I decided to devote a chapter of this thesis that seeks to promote macrofungi conservation from a social perspective. In this chapter I further describe a series of activities that I organized to create public awareness and knowledge on macrofungi.

The global cases

The current president of the British Mycological Society, Dr. David Minter, described some facts about the global infant movement in fungal conservation; highlighting the urgent need to protect them and to generate national activities to promote a general public awareness of this still unfamiliar kingdom (Minter 2011). Among the baseline activities that he proposes mycologists should implement at a local level are:

- He suggests to include fungi as part of biodiversity; the terms "Mycota" or "Fungi" are some terms that should be used. Indeed, scientists frequently use phrases including "Fauna and Flora, "Plants and Animals" "botany and zoology" as encompassing the whole diversity in biodiversity projects.
- He strongly suggests using precise terminology when referring to these organisms and not to ignore its misuse: an example is to use a correct term for the fungal storage place, such us "Fungarium", as has been adopted by the Royal Botanical Gardens Kew.
- Also, mycologists should be convoked when Biodiversity inventories are proposed, particularly when national projects are planned.
- The skills of mycologists should be considered to enhance the conservational value of a particular ecosystem or place to protect. Taking into account the value of a taxonomist recognizing species, the ecology of the specimens, etc. Indeed, trained personnel in mycology, particularly in traditional taxonomy, will assure that a more comprehensive knowledge on the diversity will be maintained, ensuring that the fungal diversity is not overlooked.

A common pattern when reviewing the efforts for conservation of fungi in countries where these organisms are starting to be part of planning conservation activities, is to

enhance and promote the public awareness highlighting the importance of fungi (Grgurinovic & Simpson 2001, Minter Allen & Lendemer 2015). Similarly, the lack of mycologists contributing to increase the knowledge on fungal diversity is a common factor:

- In the USA the conservation of fungi is starting to promote the inclusion of these organisms as essential elements in nature. Particularly it is proposed that increasing the basic research on fungal diversity for people outside the mycological community is a key step for the incorporation of fungi as elements of conservation planning. In a similar way, the number of trained professions in the field should be increased, and promoting laws related to fungal conservation should be promoted (Allen & Lendemer 2015).
- In Australia a list of species following the International Union for the Conservation of Nature (IUCN) Red List criteria were revised, including their distribution and conservation status for Southern Australia (Grgurinovic & Simpson 2001).
- In India, 10 % of the fungal diversity in the country is known. The lack in fungal identification training and the absence of reference books are among the constrains that hider research in this area. Furthermore, there is a difficulty in convincing politicians of the importance in conserving these organisms (Sankaran 2013).
- In Malaysia vast areas are unexplored, many mycologists and financial aid are needed to initiate appropriate strategies for protection of fungi (Ngadin and Razali 2013).
- Russia reports that 80 administrative regions out of 83 have Red Data Books (RDB). However few active mycologist are working in the conservation of the fungal diversity in this country. The gathering of knowledge on species with very little information available is among their efforts to protect fungi (Svetasheva 2013).

- The case for North African countries stands for the same issues as in many countries, and some edible species such as the desert truffles clearly need conservation efforts since they are widely collected without regulation (Abdel-Azeem and Salem 2013).
- In New Zealand, conservation status for some specific groups have been assessed but the majority of species are data deficient making difficult to be catalogued according to threat categories (Buchanan and Cooper 2013).
- Serbia represents a good example of a country that is devoting its attention to fungal conservation. This is a consequence of an early assertion of mycologists, who started conserving some species that were threatened due to habitat deterioration. The Law on Environmental Protection states that fungi alone, are a single reason to protect a certain area (Ivancevic 2013).

Currently, the Micheli Guide to Fungal Conservation [http://www.fungal-conservation.org/micheli.htm] states five criteria to evaluate countries according to their activities and plans related to fungal diversity and conservation. This are based on the adequacy of local Biodiversity reports:

The five criteria for adequacy are:

- Fungi mentioned in a conservation context.
- Fungi explicitly recognized as different from animals, microorganisms, and plants and lichens recognized as fungi.
- Strategic consideration explicitly given to fungal conservation (example indicators: separate texts devoted to fungal conservation; lists of important fungal areas / fungal biodiversity hotspots; deficiencies in legal protection for fungi

identified and plans presented to rectify those deficiencies; threats to fungi identified; fungal red lists mentioned).

- Principal fungal habitats and roles taken into account (decomposers, dung fungi, endobionts, freshwater fungi, fungi on man-made products, fungi on naturally occurring inanimate substrata, lichen-forming fungi, marine fungi, mycorrhizal fungi, parasitic fungi).
- Gaps related to what is known about fungal diversity and plans to address those gaps.

According to these criteria, in the 4th National Report of the Convention of Biological Diversity in Colombia, fungi were mentioned once in the context of biological control for *Hypothenemus hampei* (the coffee berry borer), categorizing Colombia as totally deficient in reports on fungal conservation initiatives.

Increasing the baseline of fungal knowledge for the public outside from the mycological community is a crucial step towards incorporating fungi as integral components of conservation planning. The following reports presented in this chapter can contribute with the knowledge on ECM fungi particularly for policy-makers, institutions working in biodiversity, regional corporations, and public in general. It is important to highlight that although within the National Protected areas in Colombia fungal diversity is assumed to be encompassed, as long as we continue to assume that by regarding plant and animal biodiversity as representatives of the "whole diversity" still hurdle the need to devote specific attention to groups like fungi.

On the other hand, in order to encourage the public awareness of fungi and its biology, in this chapter we develop a social activity with low-income rural women in the

Municipality of Villapinzón, including the oyster mushrooms cultivation as an alternative to help reduce the poverty in this community.

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V. Ectomycorrhizal species associated with *Quercus humboldtii* in Colombia

Natalia Vargas-Estupiñán[1] and Silvia Restrepo[2]

Laboratorio de Micologia y Fitopatología, Universidad de Los Andes, Bogotá, Colombia

[1]n.vargas20@uniandes.edu.co

[2]srestrep@uniandes.edu.co

INTRODUCTION

The native *Quercus humboldtii* is a dominant species in the Andean montane system, expanding from the Darien in Panama (8º N) to the southern montane Cordilleras in the Colombian Andes (1º N) (Pulido *et al*. 2006; Cárdenas & Salinas 2006, Orwa *et al.,* 2009), having a wide altitudinal range from 1,100 to 3,200 m.a.s.l. These ecosystems occupy small continuous and discontinuous relicts in the Colombian departments of Antioquia, Boyacá, Caldas, Cundinamarca, Huila, Nariño, Santander, Valle del Cauca and Tolima.

Oak forests establish symbiotic ecological relationships with **EC**to**M**ycorrhizal (ECM) fungi. This type of association is established by a beneficial ecological interaction between plant roots and fungal mycelium, playing an essential role in the dynamics of forest ecosystems: it allows the exchange of nutrients especially phosphorus from the plant to fungi, the exchange of carbohydrates from the host to the fungi and constitutes an overall communication system between several trees by translocating nutrients (Harley & Smith 1983, Read 1998).

Most studies that have been carried out related to macrofungi in Colombia have focused on oak forests (*Quercus humboldtii*), because it is estimated that fungal diversity in

these ecosystems is high (Franco *et al*. 2000). Moreover nearly 99% of the fungal diversity has been reported in the Andean cordilleras including saprotroph, pathogens, entomopathogenic and ectomycorrhizal fungi (Vasco-Palacio & Franco-Molano 2013). A contrast to the high diversity expected in the Andean montane ecosystems, the oak forests have been the focus of anthropocentric activities and thousands of hectares of natural ecosystems have been replaced by miscellaneous forests and plantations and to a lesser extent by areas for agricultural activities (Fundación Natura 2007). This fact renders oak a vulnerable (VU) species (Barrios *et al*. 2006; Cárdenas & Salinas, 2006), and consequently the fungal ECM partner can be threatened by habitat loss or loss of symbiotic hosts.

In this report we seek to compile the current ECM diversity knowledge collected under *Q. humboldtii* in Colombia and confirming the status of ectomycorrhizal of the reported genera. In addition, this study provide new reports in the departments of Boyacá and Santander where the diversity has barely been reported, and three new reports for the country, this way encouraging the knowledge of the Colombian mycota and highlighting the importance of initiate research in ECM macrofungi.

Methods

Sampling.–Fruiting bodies of ectomycorrhizal fungi associated with *Quercus humboldtii* were collected in forests in the Departments of Boyacá, Santander and Huila, during the fruiting seasons in April-May 2014 and 2015: Boyacá, Municipio de Villa de Leyva, Vereda Capilla 05°39’ 26.78’’ N, 73°30’ 46.41’’ O; Boyacá, Municipio de Arcabuco, Vereda Piedras Blancas, 05°48.546” N, 73°28.751” O; Boyacá Municipio de Arcabuco, 5°45’ 35.38’’ N, 73°26’ 47.10’’ O; Santander, Vereda San Jose de La Montaña (Dept. Santander), 06°02’ 29.82’’ N, 73°00’ 02.8’’ O; Huila, Parque Natural Los Guacharos 01°38.280” N, 76°06.109”

W. They were documented for macroscopic and microscopic features. After documentation, the fruiting bodies were dried and packaged in plastic bags, stored at the ANDES Herbarium (Universidad de los Andes, Bogotá, Colombia) and registered in the SPECIFY database. Taxonomic keys and guides were used to the identification: Arora (1986), Franco-Molano (1999), Phillips (2005), Tulloss (2000, 2002, 2005), Franco-Molano *et al.* (2000), Mata *et al.* (2003), Halling (1989), Halling & Mueller (2005).

Search for ECM spp. in Colombia and the trophic status of macrofungi considered ECM.–We made an extensive literature search on fungal diversity lists for the country, including references that confirmed the collection under *Q. humboldtii*. We searched for ecology reports in the ISI web of Knowledge for ectomycorhizal trophic status of each genus documented for ECM species (no information on ectomycorrhizal status at species level was included). The family and author of each species were confirmed in Index Fungorum (http://www.indexfungorum.org/names/names.asp).

Results and discussion

A total of 128 species followed the search criteria (Table 1): collected under *Q. humboldtii* and belonging to a genus that has been reported with an ectomycorrhizal trophic status. Figure 1 shows the departments where ECM fungi have been reported under *Q. humboldtii*. This distribution nearly encompasses the Andean regions constrained to the three cordilleras where the host species occurs. 12 departments are delimited in the mountain Andean system (Fig. 1) and for 11 departments reports on ECM species exist but no reports for Risaralda was found. The families with the major number of ECM species are the Boletaceae (in 7

departments), Amanitaceae (in 2), Russullaceae (in 1) and Cantharellaceae (in 1).

The analysis by department reflects the undersampled areas and lack of reports in departments including Caldas, Quindio and Tolima, showing the lowest number of reports. This probably reflects the lack of research centers or institutions working with these specific organisms. For the particular case of Santander there was no previously ECM reports and we documented in the present study the firsts reports. The department of Tolima particularly the town of Murillo located at *ca*. 2950 masl (http://www.murillo-tolima.gov.co/informacion_general.shtml) where several field trips and collections exist in *Q. humboldtii* forests, still needs reports of the diversity present in this department. The departments with the highest reports are Antioquia, followed by Boyacá and Cundinamarca, probably reflecting the frequent research conducted in these regions.

A total of 24 species are proposed as new reports for Colombia: 3 national and 21 reports (Table 2) for the departments of Boyacá, Santander and Huila. Information on the ECM trophic status for the genera included in this study was searched in literature where anatomical, chemical and/or molecular analyses confirmed the trophic ectomycorrhizal status (Table 3). Fruiting bodies of collections included as new reports for departments and for the country are shown in Figure 2 and 3, respectively.

Taxonomic description of three national reports

Inocybe tahquamenonensis D.E. Stuntz 1954. Material studied NVE 302 Fig. 3A – Colombia, Boyacá, Municipio de Arcabuco, Vereda Peñas Blancas, 20 May. 2012, under *Q. humboldtii.* This species occur in north temperate regions in eastern North America (Phillips 2005; Matheny and Moreau 2009). *Pileus*: 1.5–4cm cm wide, convex to plano convex to

decurved when mature, dark purplish brown to reddish- or blackish-brown, with pronounced scales concolorus with the pileus surface; margin even with scales. *Context* reddish-purple. *Lamellae* adnexed to adnated, concolorous with the pileus surface, close to slightly distant. Spore print brown. *Stipe* 3–6 x 0.4–0.7 cm, concolorus with the pileus surface, cylindrical, with abrupt scales. *Basidiospores*: 6-9 x 5-7.5 µm, cruciform. *Basidia*: 24–31 x 13–16 µm. *Hymenophoral trama* interwoven.

Russula foetens Pers. 1796. Material studied NVE 302 Fig. 3B – Colombia, Boyacá, Municipio de Villa de Leyva, vereda Capilla. 20 May. 2012, under *Q. humboldtii.* This species occur in north temperate regions, found in Europe and North America (Phillips 2005). *Pileus*: 5–8cm wide, globose at first, then convex, finally flattening, with a central depression, pale yellowish to dull brownish ochre towards the center and when mature towards the margin, thick-fleshed, rigid, slimy or glutinous; margin furrowed with strong striations. *Context* white. *Lamellae* adnexed, cream color, often with brown spots, thick and distant. Stipe 6-10 x 1.5-4 cm, whitish to buff, cylindrical or swollen in the middle, hard and rigid, easily breaking. Spore print pale to medium cream. Spores: 8–10 x 7–9 µm, globose with abrupt projections, up to 2 µm long, with a line joining them. Basidia: 45–70 x 13–20 µm. Hymenophoral trama with ovoid to globose cells up to 55 µm long, regularly arranged. Pleurocystidia cylindrical, tapering or spindle-shaped.

Russula sardonia Fr. 1838. Material studied NVE 633 Fig. 3C – Colombia, Boyacá, Municipio de Arcabuco, Km 5 via Arcabuco-Gachantiva. 15 Dec. 2013, under *Q. humboldtii.* This species occur in north temperate regions, found in Europe and western North America

(Phillips 2005).

Pileus: 4–6.5 cm wide, convex, to flat in mature specimens and with a depression, violet-, purplish- or brownish-red, greenish or ochre to yellowish, hard, glabrous. *Context* white, 1-2 cm wide. *Lamellae* adnexed to slightly decurrent, at first cream to pale golden yellow, narrow. *Stipe* 3.0–8.0 cm long x 1.0–1.5 cm wide, whitish to very pale lilac upper half, to greyish dark lilac in the lower half, uniform; surface fibrillose to slightly pruinose. *Spore print* cream. *Basidiospores*: 7–9 x 6–8 µm, ovoid with warts up to 0.5 µm high, joined into ridges forming fine irregular lines or rugose ornamentation. *Basidia*: 50–60 x 10–14 µm. *Hymenophoral trama* with ovoid cells up to 30 µm long, regularly arranged. *Pleurocystidia* spindle-shaped or cylindrical, without septa.

Notes on the ECM taxa

Basidiomycota

Amanitaceae*:* All the species in Colombia, with the exception of *A. savannae* (which was described in wet savanna by Tulloss & Franco-Molano (2008), belong to a clade of symbiotic species (Subgenera *Lepidella* and *Amanita*) (Wolfe *et al* (2012). We did not include the species *A. ceciliaes* previously reported by Vasco-Palacio & Franco-Molano (2013), whose presence in Colombia does not coincide with Pulido (1983). The species *A. muscaria* has been widely reported with exotic plantations (Pulido 1983, Franco-Molano *et al*. 2000, Franco-Molano & Uribe-Calle 2000, Montoya *et al.* 2005), but Vargas *et al*. unpubl. data b reported it in association with *Q. humboldtii* in the department of Santander.

Cortinariaceae: Despite the fact that the genus *Cortinarius* is one of the most speciosus genera containing over 2000 spp. (Kirk *et al*. 2008), in Colombia a large number of these

species occur without determination and are among the families with the lowest number of reported species.

Entolomataceae: Although the genus is considered to have ectomycorrhizal species (Rinaldi *et al*. 2008) the species *E. ferrugineogranulatum* reported by Soto-Medina & Bolaños-Rojas (2013) was collected in open lands (potreros) and by Horak (1977) on rotten wood in rain forest so we did not included it, the same for the species *E. lyophylliforme* reported by Horak (1977) in a tropical rain Forest near Buenaventura at 180 m.a.s.l.

Tricholomataceae: Whilst the genus *Leucopaxilus* has been reported as ECM (Lu *et al*. 1998, Matheny *et al* 2006, Rinaldi *et al*. 2008), the species *L. gracillimus* is not reported as mycorrhizal (Franco-Molano *et al*., 2000).

Hydnangiaceae: The species *Laccaria ohiensis* has been collected in forests dominated by the oak species *Colombobalanus excelsa* in the department Valle del Cauca (Soto-Medina and Bolaños-Rojas 2013).

Boletaceae: the species *Boletus orquidianus* has been reported in no association with Fagaceae in Colombia (Halling 1989), however it has been collected under oak forest (Franco-Molano *et al*. 2000). The species *Boletus pavonius* and *B. purpurascens* are reported for Santander (Hooker & Kunth 1822; Vasco-Palacio & Franco-Molano 2013) on the banks of the Magdalena River growing on decomposed wood (Hooker & Kunth 1822), and was not included in this study. The species *B. reticulatus* was collected in temperate region between Popayan and Almaguer (Hooker & Kunth 1822), which probable show distribution of oak

forests, however no vegetation information for this specimen was reported.

We did not include species in the genus *Phlebopus*, since the ecology of the genus is ambiguous showing some species cultivated as saprotrophs (Thoen & Ducousso 1990, Wilson *et al* 2012).

Strobilomyces confosus was reported first in parque Nacional Puracé (Dept. Huila) (Halling 1989); here we report it in Parque Natural Los Guacharos (Dept. Huila) under *Q. humboldtii* (Fig. 2) (Table 2).

Tylopilus obscurus was collected in exotic plantations along with stands of *Q. humboldtii* forests and is likely considered to be associated with *Q. humboldtii* as well (Halling 1989); we reported here the species under *Q. humboldtii* (Fig. 2-D2).

We included *Suillus luteus* collected in *Q. humboldtii* (Fig. 2-D4). This constitutes a first report of this exotic species expanding its range into native oak forest (Fig. 2-D4); previous studies reported this species in Colombia associated to conifers (Franco-Molano *et al*. 2000) and introduced with *Pinus* spp. (Guzman & Varela 1978) in the Departments Antioquia, Caldas and Cundinamarca.

Cantharellaceae*:* we did not include the species *Cantharellus cinereus* previously reported by Vasco-Palacio & Franco-Molano (2013), whose presence in Colombia was not reported by Guzman & Varela (1978).

Gomphales

Most of the species in the genus *Ramaria* show ectomycorrhizal ecology, however species such as *Ramaria stricta* and the genus *Lentaria* demonstrate to be basal in phylogenetic

analysis and have been considered likely saprotrophs (Hosaka *et al*. 2006, Rinaldi *et al*. 2008)

Ascomycota

Pezizales.– Within this order several species are expected to form ectomycorrhizal symbiosis Indlufing species in the genera *Humaria, Genea, Trichophaeta, Geopora, Helvella, Hygnodtria, Peziza* and *Sarcosphaera* (Tedersoo *et al*. 2006). Although no specific analysis testing the mycorrhizal ecology of the species *Peziza patena,* we considered as putative ECM.

Notes on other orders

Geastrales.–We did not listed *Geastrum* as ECM according to the analyses made by Rinaldi *et al*. (2008) and the phylogenetic analysis by Hosaka *et al*. (2006).

Trechisporales.–Dunham *et al* (2007) observed some traits that characterizes ECM formation in species from the genus *Trechispora*. In Colombia 8 species are reported, but no one was included here; further analysis must be made for this particular case within the corticioid members.

Endemic ECM species and some ECM used as edible in the country

According to the list proposed by Halling and Mueller (2005) of putatively endemic species associated to *Quercus*-dominated forests, some species reported here are: *Amanita arocheae, A. burnneoclocularis, A. flavoconia, A. fuligineodisca, Boletus neoregius, Craterellus boyasensis, Laccaria gomezii, Leccinum andinum, L. talamancae, Phylloporus centramericanus. P. phaeoxanthus, Rozites colombiana, Tylopilus bulbosus* and *T. obscurus.*

On the other hand, reports on the knowledge about the traditional use of edible ECM species by local farmers, living nearby oak forests, have been reported for the department of Boyacá: *Ramaria secunda* and *Lactarius indigo* reported by Ruíz & Henao-M (2006), *Tylopilus indecisus, Russula* sp.1, *Ramaria flava, R. cyaneigranosa, R. botrytis*, and 5 more species of *Ramaria* reported by Peña-Cañon & Enao-Mejía (2014), *Lactarius deceptivus* (pers. comm. Yeina Niño Fernandez), *Russula cyanoxantha* (pers. comm. Angelica Ruiz),

Concluding remarks

The characterization of the Andean biodiversity with special emphasis in the fungal diversity associated to *Q. humboldtii*, a species in the vulnerable category under the IUCN, is a priority objective to start including fungi in National Biodiversity reports. The training in fungal taxonomy is the basis to achieve accurate determinations to generate inventories and local knowledge on ectomycorrhizal diversity. This has to be simultaneous performed by encourgaing academic centers and institutions in the national Departments, particularly those that has less known or unexplored diversity, to initiate research in this field, knowing that a high diversity of fungi is expected in native oak ecosystems (Franco *et al*. 2000), moreover increasing the knowledge in infrequent or rare ECM species makes the oak forests in Colombia priorities ecosystems to conserve (Soto-Medina and Bolaños-Rojas 2013).

Finally it is important to reiterate that this report constitutes a baseline study to advance in the knowledge of macrofungi and public awareness of ECM fungi in the country as a viable strategy to coordinate initiatives in the protection of macrofungal diversity and oak ecosystems. Following the evaluation criteria made by the *Micheli Guide To Fungal Conservation* (http://www.fungal-conservation.org/micheli.htm) this report constitues a tool

to be taking into account for future National Biodiversity reports, since we accomplished the following citeria: ECM fungi were mentioned in a conservation context, listing departments in the Colombian Andes where ECM fungal species are potentially associated to native *Q. humboldtii* host, and showing deficiencies in exploration of fungal diversity in differents departments in the country.

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Tables

Table 1. Distribution of ectomycorrhizal species collected under *Q. humboldtii*

Species	Department	Collected under *Q. humboldtii* reference(s)	References where the species is cited
BASIDIOMYCOTA			
Agaricales			
Amanitaceae			
Amanita			
A. advena Tulloss, Ovrebo & Halling 1992	ANT	Tulloss, Ovrebo & Halling (1992)	Tuloss *et al* (1992), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
A. arocheae Tulloss, Ovrebo & Halling 1992	ANT, **BOY**	Tulloss, Ovrebo & Halling (1992), Halling & Mueller (2005), Vargas *et al*. Unpublished data a	Tuloss *et al* (1992), Franco-Molano & Uribe-Calle (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013), Vargas *et al*. Unpublished data a
A. aureomonile Tulloss & Franco-Mol. 1992	VAL	Tulloss, Ovrebo & Halling (1992)	Tuloss *et al* (1992), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
A. brunneolocularis Tulloss, Ovrebo & Halling 1992	ANT, BOY, VAL	Tulloss, Ovrebo & Halling (1992), Franco-Molano *et al* (2000)	Saldarriaga *et al* (1988), Tullos *et al* (1992), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013), Soto-Medina and Bolaños-Rojas (2013)
A. capillensis Vargas, Franco-Molano & Restrepo	**BOY**	Vargas *et al*. Unpublished data a	Vargas *et al*. Unpublished data a
A. citrina Pers. 1797	**BOY**	Vargas *et al*. Unpublished data a	Vargas *et al*. Unpublished data a

A. colombiana Tulloss, Ovrebo & Halling 1992	ANT, **BOY**	Tulloss, Ovrebo & Halling (1992), Franco-Molano *et al* (2000), Vargas *et al*. Unpublished data a	Tuloss *et al* (1992), Franco-Molano *et al* (2000), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
A. flavoconia G.F. Atk. 1902	ANT, CUN, BOY, **SAN**	Tulloss, Ovrebo & Halling (1992), Halling & Mueller (2005), Franco-Molano *et al* (2000), Vargas *et al*. Unpublished data a	Saldarriaga *et al* (1988), Tullos *et al* (1992), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Halling & Mueller (2005), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013), Soto-Medina and Bolaños-Rojas (2013)
A. fuligineodisca Tulloss, Ovrebo & Halling 1992	ANT, BOY, NAR, **SAN**	Tulloss, Ovrebo & Halling (1992), Halling & Mueller (2005), Franco-Molano *et al* (2000), Vargas *et al*. Unpublished data a	Saldarriaga *et al* (1988), Tullos *et al* (1992), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013), Soto-Medina and Bolaños-Rojas (2013)
A. gemmata (Fr.) Bertill. 1866	CUN	Guzman & Varela (1978)	Nasi (1977), Guzman & Varela (1978), Tulloss *et al* (1992), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
A. humboldtii Singer 1963	CUN, NAR	Singer (1963), Guzman & Varela (1978)	Singer (1963), Denis (1970), Guzman & Varela (1978), Pulido (1983), Tulloss *et al* (1992), Wu *et al* (1997), Vasco-Palacio and Franco-Molano (2013)
A. inaurata Secr. 1833	BOY	Singer (1963)	Singer (1963), Denis (1970), Pulido (1983), Tullos *et al* (1992), Vasco-Palacio and Franco-Molano (2013)
A. muscaria (L.) Lam. 1783	**SAN**	Vargas *et al*. Unpublished data b	Vargas *et al*. Unpublished data b
A. picea Tulloss, Ovrebo & Halling 1992	BOY	Tulloss, Ovrebo & Halling (1992)	Tulloss *et al* (1992), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)

A. sororcula Tulloss, Ovrebo & Halling 1992	ANT, BOY	Tulloss, Ovrebo & Halling (1992), Franco-Molano *et al* (2000), Vargas *et al*. Unpublished data a	Tuloss *et al* (1992), Franco-Molano *et al* (2000), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013), Vargas *et al*. Unpublished data a
A. xylinivolva Tulloss, Ovrebo & Halling 1992	ANT, CAU, CUN, NAR, BOY, **SAN**	Tulloss, Ovrebo & Halling (1992), Franco-Molano *et al* (2000), Vargas *et al*. Unpublished data a	Saldarriaga *et al* (1988), Tullos *et al* (1992), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Vasco-Palacio and Franco-Molano (2013), Vargas *et al*. Unpublished data a
Cortinariaceae			
Cortinarius			
C. boyasensis Singer	BOY	Singer (1963)	Denis (1970), Singer (1963), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
C. iodes Berk. & M.A. Curtis 1853	ANT, NAR, BOY, **SAN**	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010), López-Quintero *et al* (2007), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
C. violaceus (L.) Gray 1821	ANT, **BOY**	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010), Vasco-Palacio and Franco-Molano (2013)
Rozites colombiana Halling & Ovrebo	ANT	Franco-Molano *et al* (2000)	Halling & Obrevo (1987), Saldarriaga *et al* (1988), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
Entolomataceae			
Entoloma			

E. venezuelanum (Dennis) E. Horak 1978	VAL	Forests dominated by *Colombobalanus excelsa* (Soto-Medina and Bolaños-Rojas 2013)	Soto-Medina and Bolaños-Rojas (2013)
Hydnangiaceae			
Laccaria			
L. amethystina Cooke	ANT, CUN, **SAN**	Guzman & Varela (1978), Franco-Molano *et al* (2000)	Guzman & Varela (1978), Mueller (1996), Franco-Molano *et al* (2000); Halling & Mueller (2005);Vasco-Palacio and Franco-Molano (2013)
L. gomezzi Singer and Mueller 1988	ANT, HUI	Mueller & Singer (1988)	Mueller & Singer (1988), Mueller (1996), Franco-Molano & Uribe-Calle (2000), Franco-Molano *et al* (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
L. laccata (Scop.) Cooke 1884	ANT, BOY, CAL, CUN, QUI, **SAN**	Pulido (1983), Franco-Molano *et al* (2000), Halling and Mueller (2005)	Pulido (1983), Saldarriaga *et al* (1988), Mueller (1996), Nieves-Rivera *et al*. (1997), Franco-Molano and Uribe-Calle (2000), Franco-Molano *et al* (2000), Montoya *et al*. (2005), Betancur *et al* (2007), López-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
L. ohiensis (Mont.) Singer 1947	VAL	Mueller (1996)	Mueller (1996), Soto-Medina and Bolaños-Rojas (2013), Vasco-Palacio and Franco-Molano (2013)
L. proxima (Boud.) Pat. 1887		Mueller (1996)	Mueller (1996), Vasco-Palacio and Franco-Molano (2013)
Hygrophoraceae			
Hygrophorus			
H. hondurensis (Murrill) Murrill 1912	PNN	Boekout & Pulido (1989)	Boekout & Pulido (1989), Franco-Molano *et al* (2010), Vasco-Palacio and Franco-Molano (2013)

H. obconicus Peck 1909	PNN	Boekout & Pulido (1989)	Boekout & Pulido (1989), Franco-Molano *et al* (2010), Vasco-Palacio and Franco-Molano (2013)
H. quercuum Singer 1973	BOY	Singer (1973)	Singer (1973), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000)
Inocybaceae			
Inocybe			
I. calamistrata (Fr.) Gillet 1876	ANT	Franco-Molano *et al*. (2010)	Franco-Molano *et al*. (2010)
I. fastigiata (Schaeff.) Quél. 1872	CUN	Guzman & Varela (1978)	Guzman and Varela (1978)
I. hystrix (Fr.) P. Karst. 1879	ANT	López-Quintero *et al* (2007)	López-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
I. jalapensis (Murrill) Singer 1958	CUN	Singer (1963), Guzman & Varela (1978)	Singer (1963), Denis (1970), Guzman & Varela (1978), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
I. rimosa (Bull.) P. Kumm. 1871	CUN		Guzman & Varela (1978), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
I. tequendamae Singer 1963	CUN	Singer (1963)	Singer (1963), Denis (1970), Guzman & Varela (1978), Wu *et al* (1997), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
I. tahquamenonensis D.E. Stuntz 1954	**BOY**	This study	This study
Tricholomataceae			
Leucopaxilus gracillimus Singer and A.H Sm	ANT	Franco-Molano *et al*. (2010)	Franco-Molano *et al* (2000, 2010)
Tricholoma			

T. cystidiosum A.H. Sm. 1941	ANT	Saldarriaga *et al* (1988)	Saldarriaga *et al* (1988), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
T. caligatum (Viv.) Ricken 1914	**BOY**	This study	Cepero de García *et al* (2012)
Boletales			
Boletaceae			
Aureoboletus auriporus (Peck) Pouzar 1957	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Austroboletus subvirens (Hongo) Wolfe 1980	ANT, HUI	Halling (1989), Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Boletellus			
B. ananas (M.A. Curtis) Murrill 1909	ANT, VAL	Franco-Molano *et al* (2000)	Halling (1989, 1996), Singer (1970), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. russellii (Frost) Gilbert	CAU	Halling (1989)	Halling (1989), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Boletus			
B. atkinsonianus (Murrill) Sacc. & Trotter 1912	ANT	Halling (1989)	Halling (1989, 1996), Vasco-Palacio and Franco-Molano (2013)
B. fuligineotomentosus Singer 1973	VAL	Singer (1973)	Singer (1973), Halling (1989), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. neoregius Halling & G.M. Muell. 1999	ANT, CUN, **BOY**	Franco-Molano *et al* (2000), Halling & Mueller (2005)	Franco-Molano *et al* (2000, 2010); Halling & Mueller (2005);Vasco-Palacio and Franco-Molano (2013)

B. orquidianus Halling 1989	ANT	Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. pseudorubinellus A.H. Sm. & Thiers 1971	ANT, CAU	Halling (1989), Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. pulverulentus Opat. 1836	CUN	Halling (1989), Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. pyrrhosceles Halling 1992	ANT, NAR	Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. reticulatus Schaeff. 1763	CAU	NA	Hooker & Kunth (1822)
B. subtomentosus L. 1753	ANT, BOY	Halling (1989)	Halling (1989), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
B. truncatus (Singer, Snell & E.A. Dick) Pouzar 1966	ANT, BOY	Halling (1989)	Halling (1989), Franco-Molano and Uribe-Calle (2000), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
Chalciporus caribaeus Pegler 1983	ANT	López-Quintero *et al* (2007)	López-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
Chalciporus piperatus (Bull.) Bataille 1908	ANT, TOL	Franco-Molano *et al*. (2010)	Franco-Molano *et al*. (2010)
Xerocomellus chrysenteron (Bull.) Sutara 2008	ANT, NAR, **BOY**	This study	Saldarriaga *et al* (1988), Franco-Molano *et al* (2010), Vasco-Palacio and Franco-Molano (2013)
Leccinum			

L. andinum Halling	ANT	Halling (1989), Franco-Molano *et al* (2000)	Halling (1989), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000), Halling & Mueller (2005), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
L. rugosiceps (Peck) Singer	ANT, CAU, TOL	Franco-Molano *et al* (2000)	Halling (1996), Franco-Molano *et al* (2000, 2010), halling and Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
L. talamancae Halling, L.D. Gómez and Lannoy	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010), Halling & Mueller (2005), López-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
Phylloporus			
P. phaeoxanthus Singer and L.D Gómez	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010),Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
P. fibulatus Singer, Ovrebo & Halling	ANT, NAR	Singer *et al* (1990), Franco-Molano *et al* (2000)	Singer *et al* (1990), Halling *et al* (1999), Wu *et al* (1997), Franco-Molano *et al* (2000), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
P. centroamericanus Singer & L.D. Gómez 1984	ANT, **BOY**	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
P. purpurellus Singer	CAU	Singer (1973)	Singer (1973), Wu *et al* (1997), Halling *et al* (1999), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Pulveroboletus ravenelii (Berk. & M.A. Curtis) Murrill 1909	ANT, VAL	Franco-Molano *et al* (2000)	Boekout & Pulido (1989), Franco-Molano *et al* (2000, 2010), Soto-Medina and Bolaños-Rojas (2013)

Strobilomyces confusus Singer 1945	HUI	Halling (1989)	Halling (1989), Franco-Molano and Uribe-Calle (2000), Halling and Mueller (2005), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
Suillus luteus (L.) Roussel 1796	**BOY**	This study	This study
Tylopilus			
T. bulbosus Halling & G.M. Muell. 2001	ANT	Sierra *et al* (2011)	Sierra *et al*. (2011)
T. indecisus (Peck) Murrill 1909	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
T. obscurus Halling 1989	ANT, **HUI**	Halling (1989), Halling & Mueller (2005), Franco-Molano *et al* (2000), This study	Halling (1989), Franco-Molano *et al* (2000, 2010) Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
T. umbrosus (G.F. Atk) A.H. Sm. & Thiers	NAR	Franco-Molano *et al*. (2010)	Franco-Molano *et al*. (2010)
Calostoma cinnabarinum Desv. 1809	ANT, HUI, CAL, **BOY**	López-Quintero *et al* (2007), This study	Saldarriaga *et al* (1988), Dumont & Umaña (1978), López-Quintero *et al* (2007), Betancur *et al* (2007), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
Xanthoconium separans (Peck) halling and Both.	ANT, NAR	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010)
Gyroporaceae			
Gyroporus castaneus (Bull.) Quél. 1886	CAU	Halling & Mueller (2005)	Halling & Mueller (2005), Franco-Molano *et al* (2010)

Sclerodermataceae			
Scleroderma			
S. albidum Pat. & Trab.	CUN	Guzman and Varela (1978)	Guzman and Varela (1978)
S. citrinum Pers. 1801	QUI	Franco-Molano (2002)	Franco-Molano (2002)
Cantharellales			
Cantharellaceae			
Cantharellus			
C. cibarius Fr. 1821	ANT, CUN, **BOY**	Guzman & Varela (1978), López-Quintero *et al* (2007)	Guzman and Varela (1978), Franco-Molano & Uribe-Calle (2000), López-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
C. cinnabarinus (Schwein.) Schwein. 1832	CUN	Guzman & Varela (1978)	Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
C. lateritius (Berk.) Singer 1951	NAR	Petersen and Mueller (1992)	Petersen and Mueller (1992), Franco-Molano and Uribe-Calle (2000), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
Craterellus			
C. boyacensis Singer 1963	ANT, BOY, HUI	Singer (1963)	Singer (1963), Denis (1970), Wu *et al* (1997), Franco-Molano & Uribe-Calle (2000), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
C. fallax A.H. Sm. 1968	CUN, **BOY**	This study	Wu & Mueller (1995), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Pseudocraterellus sinuosus (Fr.) Corner 1958	ANT	Wu & Mueller (1995)	Wu & Mueller (1995), Franco-Molano & Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
Hydnaceae			
Hydnum repandum L. 1753	ANT	Lopez-Quintero *et al* (2007)	Henao (1989), AMVA (200), Lopez-Quintero *et al* (2007), Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)

Gomphales			
Gomphaceae			
Ramaria			
R. botrytis (Pers.) Ricken 1918	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
R. cyaneigranosa Marr & D.E. Stuntz 1974	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
R. flava (Schaeff.) Quél. 1888	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
Ramaria sp.1	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
Ramaria sp.2	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
Ramaria sp. 3	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
Ramaria sp.4	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
Ramaria sp.5	BOY	Peña-Cañón and Enao-Mejía (2014)	Peña-Cañón and Enao-Mejía (2014)
R. formosa (Pers.) Quél. 1888	CAL	Montane Forest-Betancur *et al*. (2007)	Betancur *et al*. (2007), Vasco-Palacio and Franco-Molano (2013)
R. secunda (Berk.) Corner,	BOY	Ruiz & Henao-M (2006)	Ruiz & Henao-M (2006)
R. stricta (Pers.) Quél. 1888	CAL	Montane Forest-Betancur *et al*. (2007)	Betancur *et al*. (2007), Vasco-Palacio and Franco-Molano (2013)
Hymenochaetales			
Hymenochaetaceae			
Coltricia			

C. cinnamomea (Jacq.) Murrill 1904	ANT	Henao (1989)	Henao (1989), Vasco-Palacio and Franco-Molano (2013)
C. focicola (Berk. & M.A. Curtis) Murrill 1908	CUN	Guzman & Varela (1978)	Guzman & Varela (1978), Vasco-Palacio and Franco-Molano (2013)
C. perennis (L.) Murrill 1903	ANT	Henao (1989)	Henao (1989), Vasco-Palacio and Franco-Molano (2013)
Russulales			
Russulaceae			
Lactarius			
L. atroviridis Peck	ANT, BOY **SAN**	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010); Halling & Mueller (2005);Vasco-Palacio and Franco-Molano (2013)
L. caucae Singer	CAU	Singer (1973)	Singer (1973), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
L. chrysorrheus Fr. 1838	ANT, CUN, **SAN**	Guzman & Varela (1978), Franco-Molano *et al* (2000)	Guzman & Varela (1978), Franco-Molano *et al* (2000), Franco-Molano and Uribe-Calle (2000),Vasco-Palacio and Franco-Molano (2013)
L. costaricensis Singer	NAR	Halling & Mueller (2005)	Franco-Molano *et al* (2010); Halling & Mueller (2005);Vasco-Palacio and Franco-Molano (2013)
L. deceptivus Peck	ANT, BOY, **SAN**	Halling & Mueller (2005), This study	Franco-Molano *et al* (2000, 2010), Halling & Mueller (2005), Vasco-Palacio and Franco-Molano (2013)
L. fragilis (Burl.) Hersler and A.H Sm.	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010), Vasco-Palacio and Franco-Molano (2013)
L. gerardii Peck	ANT, **BOY**	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000), Vasco-Palacio and Franco-Molano (2013)

L. indigo (Schwein)	ANT, BOY, CUN, NAR	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010); Halling & Mueller (2005); Cepero de García *et al* (2012), Vasco-Palacio and Franco-Molano (2013)
L. quercuum Singer	BOY	Singer (1963)	Singer (1963), Denis (1970), Wu *et al* (1997), Vasco-Palacio and Franco-Molano (2013)
L. rimosellus Peck 1906	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010)
Russula			
R. boyacensis Singer	BOY	Singer (1963)	Singer (1963), Denis (1970), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
R. brevipes Peck	CAL, CUN	Guzman & Varela (1978)	Guzman & Varela (1978), Montoya *et al*. (2005), Vasco-Palacio and Franco-Molano (2013)
R. caucaensis Singer 1989	CAU	Singer (1989)	Singer (1989), Franco-Molano and Uribe-Calle (2000), Mueller and Wu (1997), Vasco-Palacio and Franco-Molano (2013)
R. columbiana Singer	CUN	Singer (1963)	Singer (1963), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
R. compacta Frost	ANT	Halling & Mueller (2005)	Franco-Molano *et al* (2010); Halling & Mueller (2005);Vasco-Palacio and Franco-Molano (2013)
R. cyanoxantha (Schaeff) Fr.	CUN, **BOY**	Guzman & Varela (1978)	Guzman and varela (1978), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
R. emetica (Schaeff) Fr.	ANT, BOY	Singer (1963)	Singer (1963), Saldarriaga *et al* (1988), Franco-Molano and Uribe-Calle (2000), Sierra *et al* (2011), Vasco-Palacio and Franco-Molano (2013)
R. foetens Pers. 1796	**BOY**	This study	This study
R. humboldtii Singer	CUN	Singer (1963)	Singer (1963), Denis (1970), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)

R. idroboi Singer	CUN	Singer (1963)	Singer (1963), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000), Vasco-Palacio and Franco-Molano (2013)
R. peckii Singer	ANT	Franco-Molano *et al* (2010)	Franco-Molano *et al* (2010), Vasco-Palacio and Franco-Molano (2013)
R. puiggarii (Speg.) Sing.	ANT	López-Quintero *et al* (2007)	Lopez-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
R. sardonia Fr. 1838	**BOY**	This study	This study
R. semililacea Singer	CUN	Singer (1989)	Singer (1989), Wu *et al* (1997), Franco-Molano and Uribe-Calle (2000),Vasco-Palacio and Franco-Molano (2013)
R. silvestris (Singer) Reumaux	ANT	López-Quintero *et al* (2007)	Lopez-Quintero *et al* (2007), Vasco-Palacio and Franco-Molano (2013)
R. virescens (Schaeff) Fr.	ANT	Franco-Molano *et al* (2000)	Franco-Molano *et al* (2000, 2010); Vasco-Palacio and Franco-Molano (2013)
Thelephorales			
Thelephoraceae			
Thelephora			
T. cervicornis Corner 1968	QUI	Franco-Molano (2002)	Franco-Molano (2002), Vasco-Palacio and Franco-Molano (2013)
T. palmata (Scop.) Fr. 1821	ANT	Henao (1989)	Henao (1989), Vasco-Palacio and Franco-Molano (2013)
ASCOMYCOTA			
Eurotiales			
Elaphomycetaceae			
Elaphomyces muricatus Fr. 1829	CUN	Guzman and Varela (1978)	Guzman and Varela (1978), Vasco-Palacio and Franco-Molano (2013)
Pezizales			
Helvellaceae			
Helvella			
H. lacunosa Afzel. 1783	ANT, **BOY**	Tobon (1991), This study	Tobon (1991), Vasco-Palacio and Franco-Molano (2013)

H. macropus (Pers.)P. Karst	ANT	Tobon (1991)	Tobon (1991), Vasco-Palacio and Franco-Molano (2013)

*Abbreviations for the Departments: Antioquia (ANT), Boyacá (BOY), Caldas (CAL), Cauca (CAU), Cundinamarca (CUN), Huila (HUI), Nariño (NAR), Quindio (QUI), Santander (SAN), Tolima (TOL), Valle del Cauca (VAL). The new reports in the department of Boyacá and Santander are in bold (**BOY** and **SAN**).

NA: No information on the host available

Table 2. Localities of new distributions from ECM collected under *Q. humboldtii*

Species	Collection(s) *	Department	Locality
Amanita xylinivolva	NVE 490, 491, 504, 511, 535, 670, 671, 672, 735, 744, 747	BOY, SAN	Km 0.8 Via Arcabuco-Gachantiva, Municipio de Arcabuco
Amanita colombiana	NVE 410	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Amanita citrina	NVE 600, 616	BOY	Km 0.8 Via Arcabuco-Gachantiva, Municipio de Arcabuco
Amanita muscaria	NVE 662-665, 728-732	SAN	Vereda San jose de la Montaña
Boletus neoregius	NVE 474	BOY	vereda Capilla, Municipio de Villa de Leyva
Calostoma cinnabarinum	NVE 315, 462, 697	BOY	Km 0.8 Via Arcabuco-Gachantiva, Municipio de Arcabuco; vereda Capilla, Municipio de Villa de Leyva
Cantharellus cibarius	NVE 312, 414	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Cortinarius iodes	BOY: NVE 233-235, SAN: NVE 482, 483, 489, 733, 734	BOY, SAN	Vereda Peñas Blancas, Municipio de Arcabuco; Vereda San jose de la Montaña
Cortinarius violaceus	NVE 405	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Craterellus falax	NVE 307	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Inocybe tahquamenonensis	NVE 303	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Laccaria laccata	NVE 291	SAN	Vereda San jose de la Montaña

Lactarius atroviridis	BOY: NVE 317, SAN: NVE 765	BOY, SAN	Vereda Peñas Blancas, Municipio de Arcabuco
Lactarius gerardii	NVE 337, 617	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Lactarius chrysorrheus	NVE 696	SAN	Vereda San jose de la Montaña
Lactarius deceptivus	NVE 682, 736	SAN	Vereda San jose de la Montaña
Phylloporus centroamericanus	NVE 429	BOY	Vereda Capilla, Municipio de Villa de Leyva
Russula cyanoxantha	NVE 244, 460	BOY	Vereda Peñas Blancas, Municipio de Arcabuco
Russula foetens	NVE 302	BOY	vereda Capilla, Municipio de Villa de Leyva
Russula sardonia	NVE 633	BOY	Km 5 via Arcabuco-Gachantiva, Municipio de Arcabuco
Strobilomyces confusus	Observation	HUI	Parque Natural Los Guacharos, Municipio de Palestina
Tricholoma caligatum	Observation	BOY	Vereda Capilla, Municipio de Villa de Leyva
Tylopilus obscurus	NVE 380	HUI	Parque Natural Los Guacharos, Municipio de Palestina
Xerocomellus chrysenteron	NVE 449	BOY	Km 2 Via Gachantiva-Arcabuco

***** Collections made by Natalia Vargas Estupiñán (NVE)

Table 3. Host associations references to ECM genera included in this study

Species	References
Amanita	Cripps & Miller (1995), Högberg *et al* (1999), Hobbie *et al* (2001 2002), Mleczko (2004), Rinaldi *et al* (2008), Wolfe *et al* (2012),
Aureoboletus	Binder & Hibbett (2006), Rinaldi *et al* (2008)
Austroboletus	Binder & Hibbett (2006), Rinaldi *et al* (2008)
Boletellus	Trappe (1962), Binder & Hibbett (2006), Rinaldi *et al* (2008)
Boletus	Hahn (2001), Högberg *et al* (1999), Hobbie *et al* (2001), Rinaldi *et al* (2008)
Calostoma	Wilson *et al* (2007)
Cantharellus	Trappe (1962), Mleczko (2004), Countess and Goodman (2000,) Danell (1994), Högberg *et al* (1999), Hobbie *et al*. (2001, 2002), Moncalvo *et al*. (2006), Rinaldi *et al* (2008)
Chalciporus	Binder & Hibbett (2006), Rinaldi *et al* (2008)
Coltricia	Agerer (2006), Thoen and Ba (1989), Tedersoo *et al* (2007), Danielson (1984), Larsson *et al* (2006), Rinaldi *et al* (2008)
Cortinarius	Godbout & Fortin (1983), Högberg *et al* (1999,) Kuss *et al* (2004, Rinaldi *et al* (2008)
Craterellus	Trappe (1962), Fransson (2004), Högberg *et al* (1999), Moncalvo *et al* (2006), Rinaldi *et al* (2008)
Elaphomyces	Trappe (1969), Agerer (1999), Tedersoo *et al* (2003), Miller and Miller (1984), Rinaldi *et al* (2008)
Entoloma	Agerer (1997), Högberg *et al* (1999), Montecchio *et al* (2006), Rinaldi *et al* (2008)
Gyroporus	Agerer (2009), Rinaldi *et al* (2008)

Helvella	Trappe (1969), Tedersoo *et al* (2006), Kjøller *et al* (2006), Högberg *et al* (1999), Hobbie *et al* (2001), Hansen and Pfister (2006), Rinaldi *et al* (2008)
Hydnum	Trappe (1962), Agerer *et al* (1996), Lu *et al*. (1998), Högberg *et al*. (1999), Moncalvo *et al*. 2006), Rinaldi *et al* (2008)
Hygrophorus	Högberg *et al* (1999), Cullings *et al* (2000), Rinaldi *et al* (2008)
Inocybe	Cripps & Miller (1995, Magyar *et al* (1999), Högberg *et al* (1999), Hobbie *et al* (2001), Rinaldi *et al* (2008)
Laccaria	Högberg *et al* (1999), Hobbie *et al* (2001), Buée *et al* (2005),Rinaldi *et al* (2008)
Lactarius	Trappe (1962), Eberhartd *et al* (2000), Nuytinck *et al* (2004), Flores *et al* (2005), Högberg *et al* (1999), Hobbie *et al* (2001, Miller *et al* (2006), Rinaldi *et al* (2008)
Leccinum	Molina & Trappe (1982), den Bakker *et al* (2004), Rinaldi *et al* (2008)
Leucopaxilus	Lu *et al* (1998), Rinaldi *et al* (2008)
Peziza	Molina *et al* (1992), Valentine *et al* (2004), Tedersoo *et al* (2006), Warcup (1990), Hobbie *et al* (2002), Hansen *et al* (2005), Rinaldi *et al* (2008)
Phylloporus	Binder & Hibbett (2006), Rinaldi *et al* (2008)
Pulveroboletus	Binder & Hibbett (2006), Rinaldi *et al* (2008)
Ramaria	Trappe (1962), Nourha *et al* (2005), Hobbie *et al* (2001, 2002), Hosaka *et al* (2006), Rinaldi *et al* (2008)
Russula	Trappe (1962), Benken (2001, 2001), Taylor and Alexander (1989), Högberg *et al* (1999), Hobbie *et al* (2001), Miller *et al* (2006), Rinaldi *et al* (2008)
Scleroderma	Trappe (1962), Ingleby (1999), Valentine *et al* (2004,) Mohan *et al* (1993), Sims *et al* (1999), Binder and Hibbett (2006), Hosaka *et al* (2006)
Strobilomyces	Sato *et al*. (2007), Rinaldi *et al* (2008)
Suillus	Trappe (1962), Horton *et al* (2005), Samson and Fortin (1988), Högberg et a (1999), Binder and Hibbett (2006), Rinaldi *et al* (2008)
Thelephora	Trappe (1962), Agerer and Weiss (1989), Mahmood *et al* (1999), Mohan *et al* (1993), Rinaldi *et al* (2008)

Tricholoma	Brunner *et al* (1992), Högberg *et al* (1999), Hobbie *et al* (2001), Rinaldi *et al* (2008)
Tylopilus	Raidl and Hahn (2006), Jonsson *et al* (1999), Burke *et al* (2005, 2006),Rinaldi *et al* (2008)
Xanthoconium	Brundrett *et al*. (1996), Binder and Hibbett (2006), Rinaldi *et al* (2008)
Xerocomellus	Hahn (2001), Högberg *et al* (1999), Hobbie *et al* (2001), Rinaldi *et al* (2008)

Figures

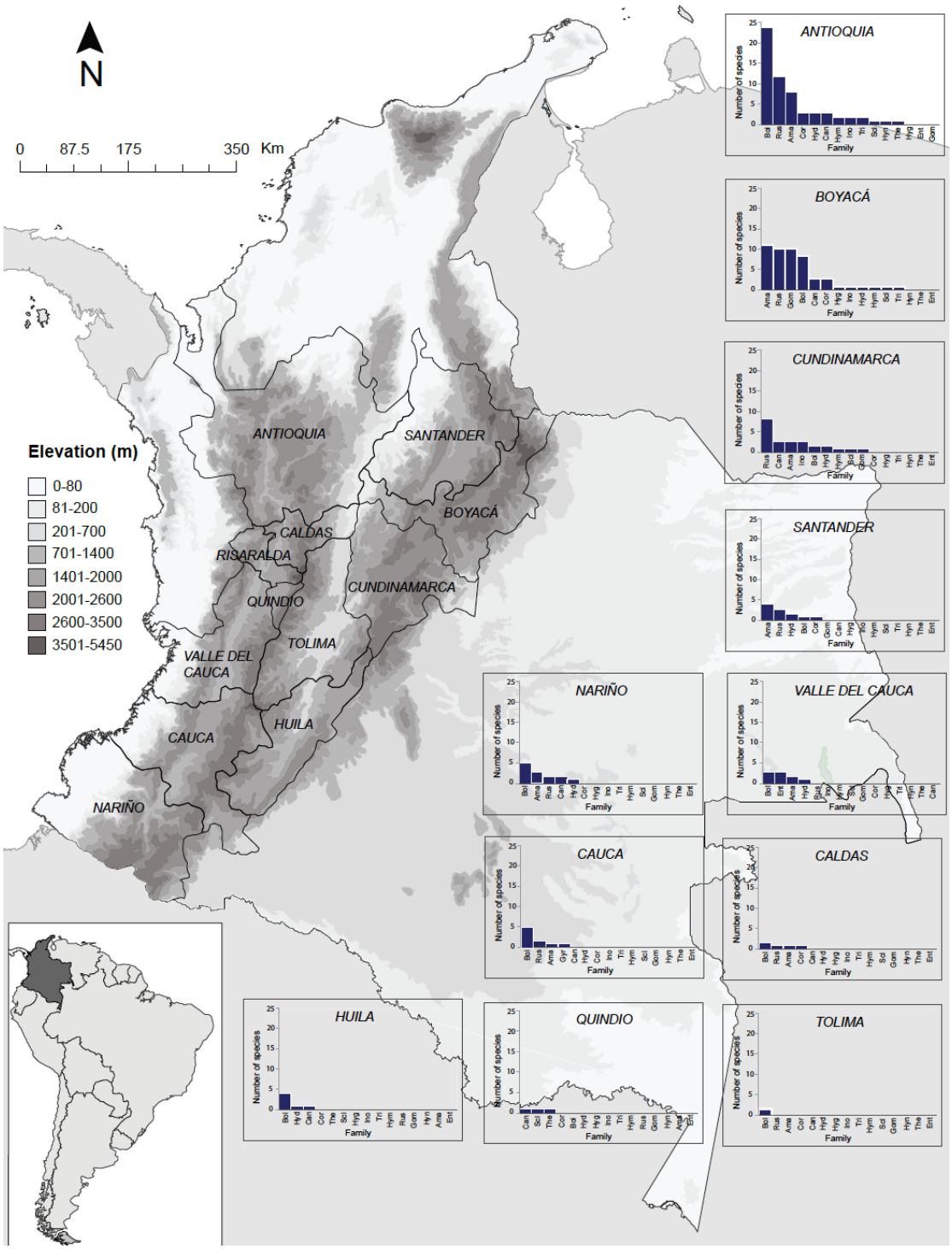


Figure 1. Map of Colombia with departments where ECM species occur with oak *Quercus humboldtii*. Abbreviation for each family inside the graphics: Amanitaceae (Ama), Boletaceae (Bol), Cantharellaceae (Can), Cortinareaceae (Cor), Inocybaceae (Ino), Tricholomataceae (Tri), Hymenochaetaceae (Hym), Sclerodermataceae (Scl), Hydnaceae

(Hyn), Thelephoraceae (The), Entolomataceae (Ento), Gomphaceae (Gom)

Figure 2. Fruiting bodies of new reports collected under *Q. humboldtii* for the departments of Boyacá and Santander. A1) *Amanita fuligineodisca,* A2) *A. citrina,* A3) *A. colombiana,* A4) *A. flavoconia,* A5) *A. arocheae var. alba,* A6) *Amanita sp NVE 562.* B1) *Cortinarius iodes,* B2) *Rozites colombiana,* B3) *C. violaceus,* B4) *Cortinarius sp. NVE.* C1) *Tricholoma caligatum,* D1) *Strobilomyces confuses,* D2) *Tylopylus obscurus,* D3) *Boletus neoregius,* D4) *Suillus luteus,* D5) *Xerocomellus chrysenteron,* D6) *Phylloporus centroamericanus,* D7) *Calostoma cinnabarinum.* E1) *Craterellus boyacensis,* E2) *Cantharellus cibarius,* E3) *Craterellus fallax*. F1) *Lactarius gerardii,* F2) *L. chrysorrheus,* F3) *L. atroviridis,* G1) *Helvella lacunosa.* White scale bars correspond to 1 cm.

Figure 2. Continued

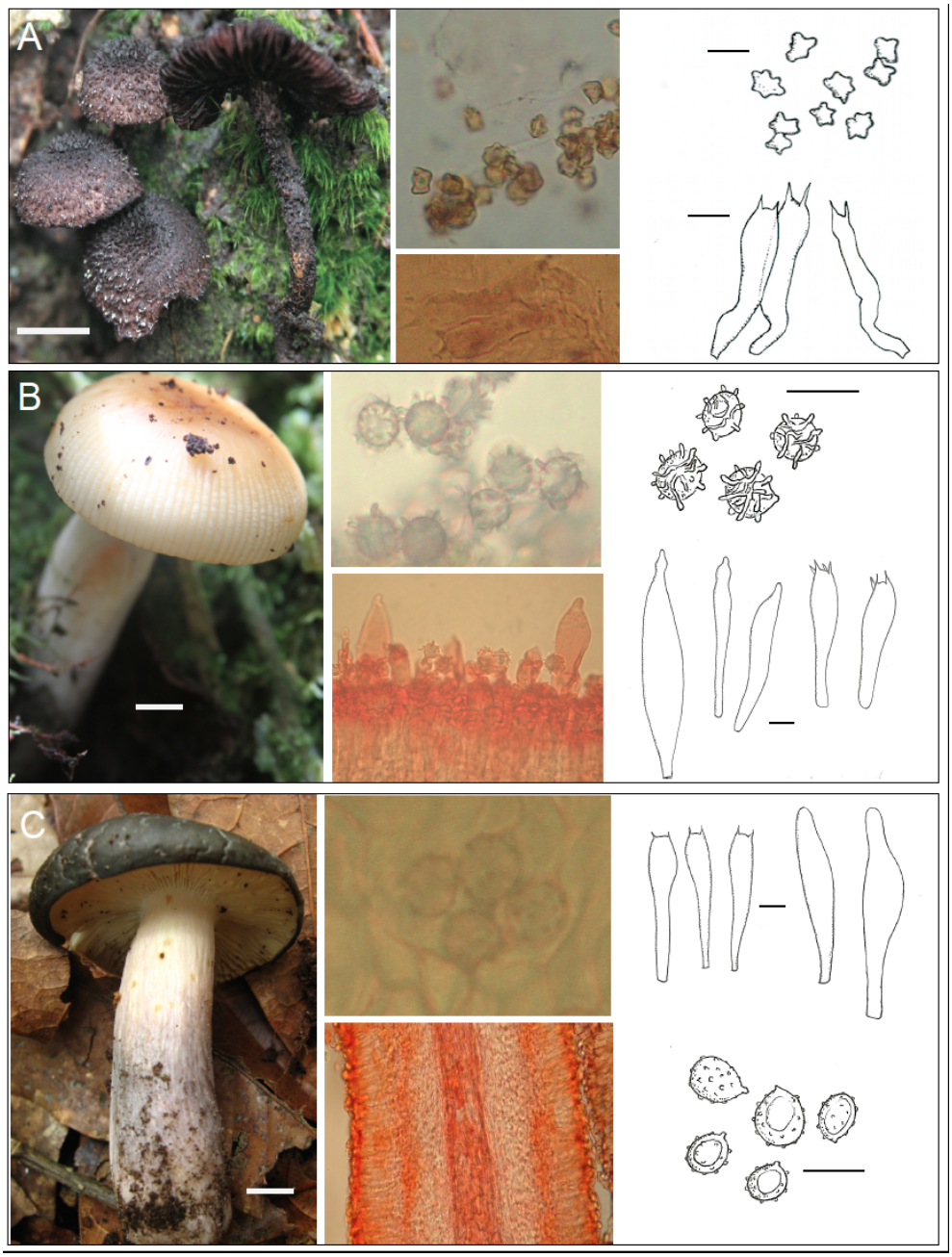


Figure 3. Macroscopic and microscopic characteristics of new national reports. A) *I. tahquamenonensis*: basidiocarp, spores and basidia. B) *R. foetens*: basidiocarp, spores, basidia and pleurocystidia. C) *R. sardonia*: basidiocarp, hymenophoral trama, spores, basidia and pleurocystidia. White scale bars correspond to 1 cm. Black scale bars correspond to 10 µm

An approach to conserve macrofungi in oak forests: socializing conservation aspects

The booklet constructed in this section of the chapter 3 (Figure 1) is intended for people living nearby oak forests as starting point to encourage public awareness of macrofungal biology and how to manage them in a sustainable way, in localities where the edibility is known through traditional knowledge. Given that the diversity gives environmental goods and services its maintainance and protection is vital (Chaves *et al*. 2007). In countries such as United States, and some countries in Europe, conservation activities have been proposed base on the fact that population declining of native edible fungi is observed throughout years of harvesting by local people. The recommendations provide in this booklet will inform people helping with information to gradually create a consciousness of the important function of the macrofungi in the ecosystems and how to perform a sustainable harvest. Moreover recommendations to reduce the spread of *A. muscaria* are explained in this booklet for people developing their activities near these ecosystems.

¿CÓMO AYUDAR A LA CONSERVACIÓN DE MACROHONGOS EN BOSQUES DE ROBLE?

¿Qué son los hongos?

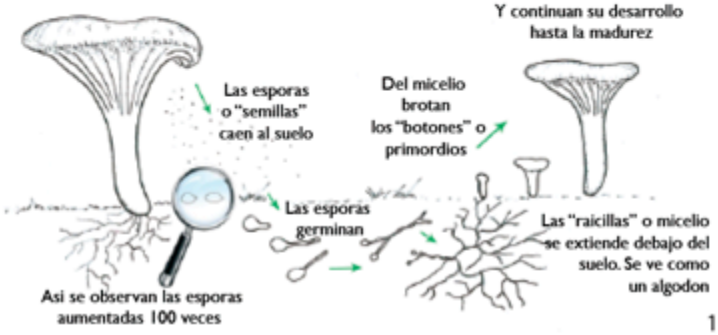
Son seres vivos que estan agrupados en el reino Hongos diferente al reino Plantas y Animales. Los macrohongos son faciles de ver y ubicar por su tamaño y cumplen diversas funciones en los ecosistemas:

Algunos interactuan con las raíces de las plantas para intercambio de nutrientes (por parte del hongo), azucares (por parte de la planta). Se les llama micorrizas

Varios macrohongos crecen sobre madera en descomposición, ayudando al "reciclaje" de nutrientes

Varios animales los usan como comida, muchos de estos animales sirven de alimento a otros. Así contribuyen a mantenerlos ciclos de intercambiode nutrientes, denominados"redes tróficas".

¿Cómo se reproducen?



1

¿CÓMO AYUDAR A UNA COLECTA SOSTENIBLE?

En países donde el consumo de hongos comestibles se ha hecho durante decadas, se ha demostrado que luego de años de cosechas no sostenibles, existe una disminución en la producción de hongos.

Para asegurar:

- la producción luego de varios años,
- que no disminuyan sus fructificaciones
- una protección del bosque nativo
- el buen estado de una cosecha

a continuación se muestran algunas recomendaciones que ayudan al mantenimiento de los macrohongos:

1) Antes de retirar el hongo del suelo o tronco, dele unos golpecitos para ayudar a que más esporas se queden en su habitat

2) Cuando los colecte, girelos y tirelos levemente a la vez, para sacarlos completos. Con el cuchillo, corte la base del tallo o "estipite" que tiene micelio unido, y dejelo caer en el suelo. Esto permite que el micelio pueda continuar su desarrollo

3) Evite colectar hongos jovenes, ellos deben crecer hasta madurar y tambien dejar caer esporas en el bosque

4) Utilice una canasta que tenga huecos, esto ayuda a que a medida que transporta los hongos, sus esporas vayan cayendo al suelo.

2

¿EXISTEN HONGOS QUE NO SEAN NATIVOS DE BOSQUES DE ROBLE COLOMBIANOS?

Amanita muscaria, "hongo hamburguesa" como lo llaman en algunas zonas de Santander, no es nativo de bosques de roble de Colombia. Es un hongo micorriza tóxico que comunmente interactua con los pinos que habitan regiones no tropicales.
Y asi, como las plantaciones de pino han sido traidas a Colombia por humanos, de esta manera este hongo ha sido introducido en el país con las plantaciones de pino, como resultado esta colonizando los bosque de roble nativo

Ayude a que este hongo no se disperse a los bosques de roble. ¿Cómo hacerlo?

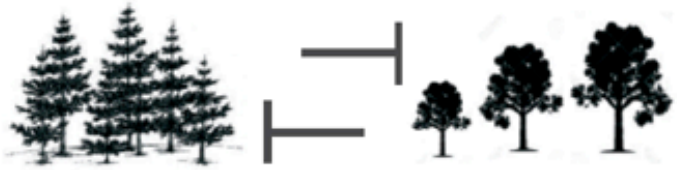
Además del viento y el agua, muchos animales actuan como propagadores y ayudan a la dispersión de esporas de los hongos.

1) Usted puede ayudar a evitar que el ganado que ha pastado en potreros cercanos a plantaciones de pino, no se trasladen inmediatamente a potreros cercanos a bosques de roble

3

2) Si por alguna razón manipula el hongo *Amanita muscaria* trate de no cruzar a bosques nativos, o procure limpiar sus manos, ropa y botas. Ésto evita que se dispersen las esporas de el hongo micorriza

3) Evite las sembradios de plantaciones de pino y eucalipto cerca de bosque nativo. Ésto ayuda a prevenir que especies invasoras colonicen bosque nativo. Recuerde que al estar cerca dos bosques los seres vivos se trasladan entre bosques.



ES MUY IMPORTANTE QUE CONTRIBUIR A QUE LOS BOSQUES ESTEN LIMPIOS

Evite botar basuras en los bosques para que esten libres de residuos químicos.

Fotografía de basura en un bosque de roble que fue tomada cerca al municipio de Arcabuco (Boyacá)

4

Este instructivo no es una guia para la identificación de hongos comestibles. Los autores de esta guia no se hacen responsables de ningun uso o identificación erronea de los hongos. Su uso como comestible debe ser guiada por un conocedor local.
Elaborado por Natalia Vargas Estupiñán y Silvia Restrepo, en Junio del 2016.
Laboratorio de Micología y Fitopatología- Universidad de Los Andes
Fotografias de Natalia Vargas Estupiñán.
Diseño de graficos por Orlando Vargas Díaz

LAMFU

Figure 1. Four pages of the Booklet.

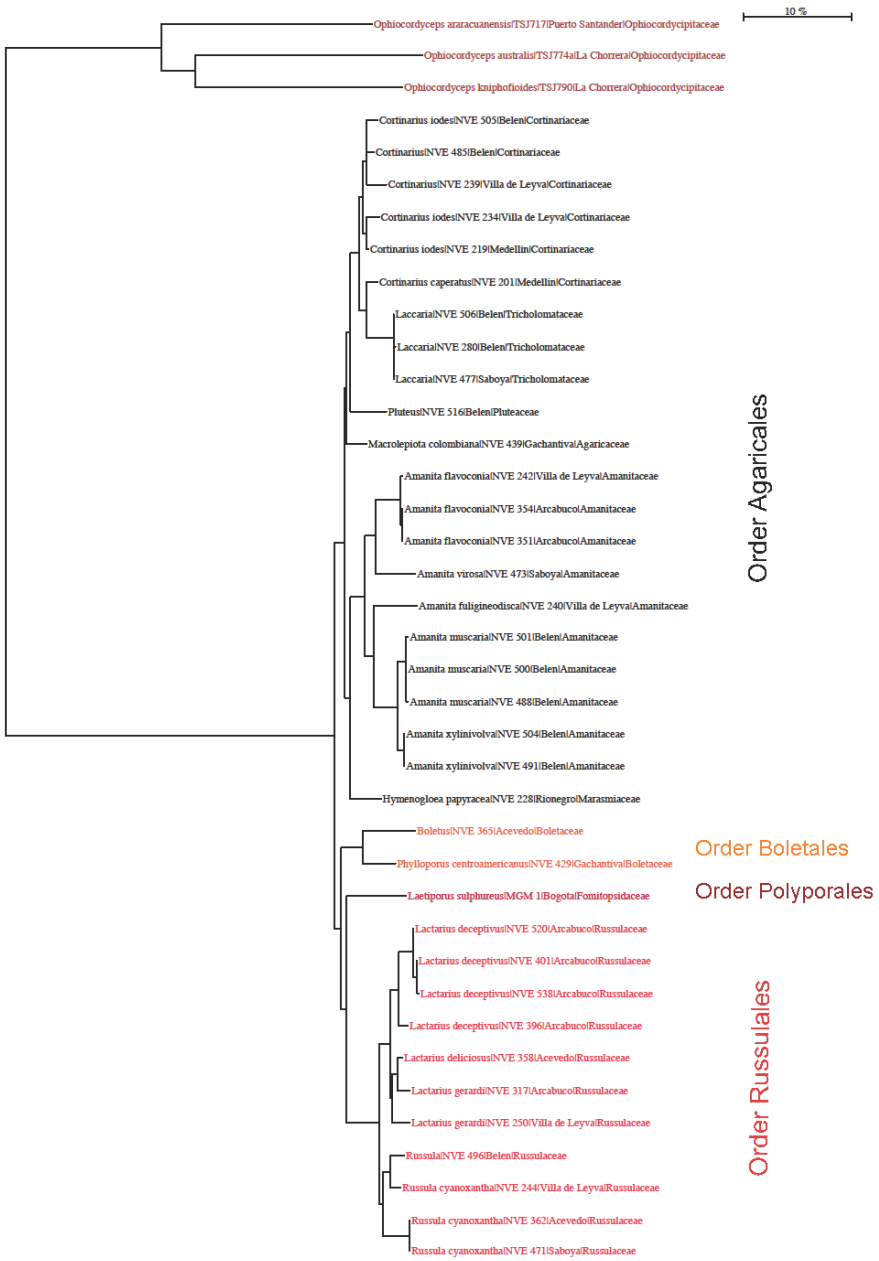
International barcode of life database

In order to contribute with information of the biological diversity in Colombia we conducted a systematization of information into a worldwide database, compiling data, of ectomycorrhizal and saprotroph species, and upload the barcoding regions ITS and nLSU.

Results

The chromatograms, barcode sequences, collection data and photographs of 32 ECM and 4 saprotroph species were uploaded in the database of the Barcode of Life Data Base (BOLD) under the project name FUNCO (http://www.boldsystems.org/index.php/MAS_Management_OpenProject?code=FUNCO). A taxon ID tree constructed with the distance model Jukes Cantor, the alignment for nLSU will be performed using MUSCLE, is shown in figure 1.

Figure 1. A taxon ID tree generated by the BOLD system by using Neighbor Joining as clustering method. The outgroup belongs to the genus *Ophiocordyceps* in the phylum Ascomycota. This is one of the tools available in the BOLD platform



VI. Oyster mushroom cultivation as an economic and nutritive alternative for rural low-income women in Villapinzón (Colombia)

Natalia Vargas-Estupiñán, Silvia Restrepo

Laboratory of Mycology and Phytopathology, Department of Biological Sciences, Universidad de los Andes, Bogotá Colombia

Laura Mesa, María-Alejandra Díaz, and Nubia Velasco

Industrial Engineering Department, Universidad de los Andes, Bogotá Colombia

Carmen Gutiérrez

School of Government Alberto Lleras Camargo, Universidad de los Andes

Abstract

Eradicating extreme poverty and hunger, *promoting gender equality, empowering women,* and *ensuring environmental sustainability* are among the eight Millennium Development Goals (MDGs). According to FAO, most of the world's poor people live in rural areas where hunger and food insecurity are the main expressions of rural poverty. In an attempt to reduce poverty, several countries throughout the world promote the cultivation of edible fungi as a means of providing nutritive alternatives and earning opportunities for people living in marginal rural areas. However, in Colombia the edibility of fungi is not as yet well known. The present project takes an interdisciplinary approach to implement the cultivation of *Pleurotus ostreatus,* develop a business plan, and establish a local company, *Orellanas de la Villa*. It is presented as an alternative method of reducing hunger and rural poverty in a

community with low-income rural women living in the municipality of Villapinzón–Cundinamarca. Over several visits, the interdisciplinary group established the technological settings and found the available resources to facilitate mushroom production. The purpose of each visit was to teach the women a new technique, to promote the community's familiarization with the fungal product, and demonstrate the nutritive properties and different ways of consuming the mushrooms. In collaboration with the community, different production problems were identified, and relevant solutions proposed. Considering a selling price that varies between US$ 3.91 to US$ 7.84/ kg depending on the product presentation, the business plan, showed that each woman has to produce 60 bags/household in order to earn a quarter of the Colombian minimum wage. This study aims to contribute an interdisciplinary approach to the mushroom cultivation process and to create an accessible, nutritive, and economic alternative to diversify some of the local agricultural resources (i.e., the potato).

Keywords: Mycelia, Fruiting body, Oyster mushroom production, Business plan, Empowerment of women.

1. Introduction

Eradicating extreme poverty and hunger, *promoting gender equality, empowering women,* and *ensuring environmental sustainability* are among the eight Millennium Development Goals (MDGs) to be achieved by 2015. A meaningful path out of poverty requires a strong economy that provides full and productive employment with good wages. In view of this, expanding women's opportunities in different sectors, and providing them with a stable source of income, can accelerate economic growth and turn into a vital path to poverty eradication. Sustainable growth must be environmentally sound and target the development of management practices aimed at biodiversity conservation, while, at the same time, meeting the necessary human production needs (Pilz & Molina, 1996).

The project described in this study is the continuation of a mixed analysis that previously diagnosed poverty conditions in Villapinzón, Cundinamarca, (Bautista & Torres, 2012). The authors identified priority areas for intervention and proposed alternatives for overcoming poverty in the municipality. Its results, according to a multidimensional poverty indicator, established that 38% of the population in Villapinzón is poor, and that housing (overcrowding), education (low level of education, educational lag and illiteracy), and work (very high rate of informal employment) are priority areas for intervention (Bautista & Torres, 2012).

The local government of Villapinzón and Universidad de los Andes have worked together to propose interventions for the priority areas identified. In December 2013, the Mayor of Villapinzón, the Villapinzón Women's Association (VWA), the School of Government, the School of Management, and the Department of Biological Sciences at Universidad de los Andes, all agreed to begin a pilot program for the production of *Pleurotus ostreatus*

(commonly known as the oyster mushroom). The initiative focuses on the cultivation and marketing of these fungi as a stable and sustainable source of income for the inhabitants of Villapinzón, specifically for the women of the VWA. The process is participatory and implemented by the community and municipal government, once they defined their own priorities.

Until recently, the main economic activities in Villapinzón were in the primary and secondary sectors, potato crop cultivation being one of the most important. The vast majority of farmers are smallholders, specifically, 48% own less than one hectare and 85% own less than three hectares (DANE, 2001). The producers' economy is strongly undermined by the unstable and uncertain potato price cycle (FEDEPAPA, 2013), and not all farmers are landowners; about 52% of the cultivated area is leased, leading to job instability. Lastly, according to DANE (2001), approximately 89% of the producers did not receive technical assistance in the previous year, leading to low levels of production and deficient marketing (DANE, 2001).

The edible fungi industry has proven to be a viable alternative in many countries, as a means of providing incentives for biodiversity use, the diversification of products, and the improvement of income opportunities in marginal rural areas (Ortega-Martínez & Martínez-Peña, 2008; Cai *et al*., 2011). According to the newsletter published by the "Corporación Internacional de Colombia" (2004), worldwide edible fungi consumption is approximately 3 million tons per year, consisting of thirty different fungal species. Latin American countries, such as Mexico, Guatemala, Chile, Peru, and Argentina are aware of the importance of fungal use (Boa, 2004), and they have traditionally introduced several species into their diets. According to a FAO study (Boa, 2004), 2166 species of wild edible mushrooms are known

worldwide, proving their relevance as a food source.

Along these lines, a strategy that explores crop diversification, establishing an alternative source of nutrition, and a stable source of income for farmers in the region, could contribute to the eradication of extreme poverty in Villapinzón. In this study, we used the *Pleurotus ostreatus* species (commonly known as the oyster mushroom) because i) it is considered to be a very nutritive biological food source with a high content of amino acids, crude protein, vitamins, fiber, and unsaturated fatty acids (Cheung, 2010; Michael et al. 2011; Rathee *et al*. 2012; Kalač, 2013), ii) it has antioxidant and anti-inflammatory properties (Puttarajo *et al*. 2006; Rathee *et al*. 2012), and iii) it is an accessible, low-investment production option, which can be grown in a great variety of substrates.

Poverty conditions in towns like Villapinzón have become an additional obstacle for small producers to gain access to markets and subsidy programs. Partnerships and collaborations like the one between Universidad de los Andes and the VWA can help projects overcome these barriers, meaning that they have a greater chance of succeeding. The Department of Biological Sciences at Universidad de los Andes provided the women with the required technical information and management practices to grow oyster mushrooms, ensuring a good end product. The School of Government monitored the group of women providing them with leadership workshops that targeted the development and management of emotional and self-awareness skills, as well as entrepreneurial tools to increase their chances of success. Finally, the School of Management helped design the business plan and develops the production process and supply chain management. These partnerships put strategies in place to empower women with the appropriate knowledge, thus enabling them to sustainably use, reproduce, and exploit the oyster mushroom cultivation. In this sense, the relevance of

this project responds to the patterns of land ownership and the need for crop diversification and technical assistance in the municipality. More specifically, the initiative focuses on generating a stable and sustainable source of income over time for the members of the Villapinzón Women's Association.

2. Materials and Methods

In order to develop the project presented in this study, we proposed a methodological approach based on three main steps: i) the characterization of the municipality and the VWA; ii) teaching all interested women how to produce the mushrooms, and iii) the business plan design. At the end of this process, a survey was applied to the women in order to identify the main difficulties that arose during the cultivation process, and the results were used to adjust the project as required.

2.1 Study site and the Villapinzón Women's Association (VWA)

The municipality of Villapinzón has about 18,764 inhabitants, of which, according to the multi-dimensionally indicator, 67% reside in rural areas; 86% are classified as part of SISBEN[2] levels 1-3, and 38% are considered to be poor. Also, over 77% of the labor market in Villapinzón is informal, which is a higher rate than the 53% national average (DANE, 2005) reflecting a high rate of job instability. Studies have shown that high rates of informal work and a higher rate of unemployment, mostly among women, leads to income instability (Journard & Londoño, 2013).

The Villapinzón Women's Association (VWA) has been working informally with the Mayor's office since 2012, and only legally constituted in 2014. The purpose of the Women´s Association is to provide employment alternatives to single-parent households, and its legal

[2] SISBEN is an instrument that obtains socioeconomic information on specific groups in the country. It is the main instrument for targeting social programs to poor and vulnerable groups.

documents include details on 35 women who live mostly in the rural areas of the municipality with limited and unstable sources of income. The Association has worked for three consecutive years with the Mayor´s office to determine possible employment alternatives for them and to train them with specific technical farming skills.

2.2 Teaching the cultivation process

The applied teaching techniques were very important for our methodological approach. We exercised a degree of caution throughout the steps detailed and during the cultivation process, ensuring that the new technical language shared with the VWA was clear and understandable. Rural women participated during the cultivation teaching process, carried out in six rural homes

2.2.1 *Preparing the grain spawn.* Over the course of the first visits, we taught the women how to undertake the complete process under sterilized conditions. The methodology described was that used in the six pilots, in which we used special instructions—given in the appropriate language for the women's level of understanding—using the equipment and materials available in each house. For grain spawn preparation, pearl barley, wheat, and rice were used to determine which of these was more efficient for mycelial growth. For the sterilization process, we suggested that they use a pressure cooker. We constructed a handmade mesh with four screws, to be put at the bottom of the pot (Supplementary Fig. 1A-B), so the jars were not touching the bottom of the pot, and a maximum temperature was used to heat the pot for one hour. This process was repeated once more for an additional hour, replacing the water used before. After two hours the bottles were allowed to cool at room temperature.

2.2.2 *Inoculation and pilot incubation room.* The production process should be carried out in an aseptic area that is free of contaminants. On a prewashed table with two lighters surrounding the inoculation area (Fig. 1A), the women, using the tip of a scalpel or knife (previously briefly held in the fire), placed 1 cm x 1 cm square portions of mycelium growing in the *petri* dish on the surface of each of the flasks containing the wet and sterilized grain. They then closed the bottles with foil and rubber bands and left them in the designed area for the incubation periods.

2.2.3 *Use of the available resources: sterilization of the substrates.* The women used the substrates available in their houses. They added 300 g of calcium carbonate (5%– substrate weighed), and 15 % (dry weight of substrate) of supplement (molasses and wheat bran) to 6 kg of dry substrate, placing these in a 70 L pot (all packed in a clean potato bag).

2.2.4 *Bags setting incubation, fruiting bodies collection, and packaging.* On a disinfected table with two lighters surrounding the area of inoculation, the women placed a substrate layer inside 31 x 45 cm transparent bags using the tip of a scalpel or knife. They then placed portions of the "seed" on the substrate layer, repeating this process until making each layer homogeneous (Fig. 1B-C). They pressed each bag down slightly and used a piece of PVC pipe with a gauze, tied with a rubber band, to allow gas exchange in the top of the bag (Fig. 1D). The cultivation process consisted of two phases: a) a darkness phase for bag incubation, and b) a light phase for the production of fruiting bodies. The VWA women designed a zone covered with black bags for the darkness phase area, and another area in the room for the light phase.

Dark phase, *after preparing the bags.* The women injected 10 mL of boiled water into one of the holes in the gauze every day in order to keep the bags damp, and, as instructed, they

recorded the days when the white mycelium started to spread and to cover the whole substrate.

Light phase. Once the white mycelium had covered the whole substrate, the women opened 2 cm-holes randomly distributed over the bag surface. They observed the substrate on a daily basis, while keeping the bags damp as explained above, until they started to produce primordia and then mature fruiting bodies (Fig. 2 A, B, respectively).

Collection. Once the fungus was of a good size (pileus: 5-8cm) and before becoming dry, the women harvested it by cutting with a clean knife or razor, and then storing it in a container in the freezer. The women measured the following parameters during the incubation and production phases: time for the mycelium to cover the grain and substrate, number of production days, and fresh weight (gr) of fruiting bodies/bag (Albertó, 2008; Sher, 2011). The statistical summary parameters of the grain coverage, substrate coverage and biological efficiency were obtained by using R (R Development Core Team, http://www.r-project.org/). The mushroom yield was calculated according to Albertó (2008) as a percentage: e.g., a 3Kg/bag of fresh mushrooms from 10Kg of wet substrate = 30% yield.

Packaging. Once individual 250 gr quantities were collected, the women packed the fungal product according to client requirements.

2.2.5 *Microbiological and statistical analysis.* Experts at the Laboratory of Food Microbiology (LEMA) at *Universidad de los Andes* carried out routine analyses of the fresh and dehydrated fruiting bodies, and water from two localities in the municipality, as well as direct microscopic examinations of green appearance colonies growing in the cultivated bags in order to describe and classify the fungal genera.

2.3 Business plan — Industry and sector analyses

We proposed the development of a business plan in order to identify whether oyster mushroom production is a good opportunity for the VWA women. The different tools we used in the methodology allowed us to diagnose the appropriate conditions for a feasible business idea. According to Ehmke & Akridge (2005), a business plan has six components: business description, market analysis, competitor assessment, marketing plan, operating plan and, financial plan.

The business description includes a mission statement, the company objectives, the legal structure and the owner(s), information about the nature of the business, how the company will start, a general description of the products/services, and the target market. The market analysis section describes the market characteristics and customer profiles. We carried out a competitor assessment to find the competitors' profiles and their strengths and weaknesses. The marketing plan describes the products and services, identifies their features and benefits, and discusses the needs and problems they addressed, and describes and specifies the pricing. Finally, we present the logistic management plan, focusing on the distribution channels and client approaches.

The operating plan section includes two main components: ownership and management, and resources and production. The former describes the key people in the organization and the external advisors. The resources and production section presents the production process, equipment, and facilities.

Finally, the financial plan describes the current financial status of the project, and presents forecasts of future financial statements as well as the potential return on investment.

This section shows how, or under which conditions, the proposal is feasible.

2.4 Perception of the cultivation process

In order to identify the women's perception of different parts of the cultivation process as well as their acceptance of a new product in their daily nutrition, we informally surveyed the six groups of VWA women involved in the process during the first visits.

3. Results

3.1 Cultivation process

Rural women participated in the cultivation pilot programs carried out in six rural households, where small rooms adapted for bag incubation and, small greenhouses were built and adapted with the women's consent when necessary. During the first visits, in an attempt to promote sustainable production, we found that the available resources in the region that could be used as substrates were hay, rice husk, and sawdust.

3.1.1 Grain coverage and substrate coverage. For grain assembly, the women were instructed not to fill the entire jar with grain, as this would not have left the necessary space for the fungus to grow. The average time for *P. ostreatus* to cover the grains was 21.92 days (*SD*=3.47), from 12 jars (two in each of the six houses). The substrate that showed the highest biological efficiency was rice husk supplemented with molasses (24%, 1.2 Kg fresh mushroom/ 5 Kg fresh substrate, during the first cycle of production) (Fig. 3A), and the mycelia proved to be highly efficient, covering the substrate in an average of 30.25 days (*SD*=2.78) (48 bags, eight bags in each of the six houses).

3.1.2 Common problems associated to the cultivation process. The women came up with many ideas to solve problems such as contamination, dehydration (above 40°C), and homogenization of the substrate (Fig. 3B). The most common problem was dehydration of the fruiting bodies once the bags were opened and the women attributed this to the warm days, which caused a slower production of fruiting bodies during the production period. To solve this, the women increased the frequency with which they sprayed the substrate. Some women also noticed green colonies inside some of the bags, indicating a source of contamination (Table 1). As such, we instructed the women to check the efficacy of the sterilization process in order to decrease contamination by other fungal species, identified as *Penicillium* and *Trichoderma* spp.

Microbiological analysis: The results of the water and mushroom analysis showed the presence of *Listeria* sp. and a high fecal coliform loading. Hence, following *Medicins Sans Frontieres* (MSF) recommendations, we suggested the women to treat each liter of water, used during incubation and spraying steps, with a drop of hypochlorite

3.1.3 The women's perception of the cultivation process and the inclusion of a new fungal product. In every meeting with the group of women, we explained the nutritional properties of the oyster mushrooms as an alternative to supplement the absence of meat-based protein. Some common perceptions and comments about the cultivation process are summarized in Table 1. The general perception regarding the production process was positive and the women agreed that it was a promising option for their families' daily nutrition. Progressively, they began to consume the mushrooms as creamy soups, grilled with butter and garlic, with chicken breasts, and cooked in soups and spaghetti. The women perceived that the mushrooms taste like chicken, and that they are harder to chew than normal

champignons.

3.2 Business plan

This section, outlines the components of the business plan: First, we present a general description of the company, followed by the market study. We then detail the technical and organizational study as well as the economic analysis shown in Section 3.2.4 Finally, we propose an implementation plan in section 3.2.5.

3.2.1. *Business description:* the VWA women chose the name *"Orellanas de la Villa"* for their company, which seeks to satisfy the demand of gourmet restaurants, organic markets in Bogotá, local customers, and ultimately improve the economic conditions of the Villapinzón population.

We applied an in-depth SWOT analysis to the company in order to study its Strengths, Weaknesses, Opportunities and Threats (SWOT) (Fig. 4). The tool assessed internal and external aspects and identified the positive characteristics that could help develop the business, as well as the negative issues that could be harmful to the company. Figure 4 summarizes this analysis.

Orellanas de la Villa's mission is to capture and retain customers, providing them with a quality product that is 100% organic and contributes to a healthy diet. It should do this while, at the same time, maintaining high quality standards that are reflected in an outstanding business performance, adequately rewarding all those who invest ideas and work in the company. By 2019, *Orellanas de la Villa* will be one of the leading brands in the organic mushroom market, serving major markets and restaurants in Colombia. This will be represented by a gradual increase in sales, and a reduction of poverty in the municipality of

Villapinzón, as mushroom sales become a stable source of income for the workers and stakeholders. The main strategy is to reduce the number of intermediaries by using the municipality's geographical positioning as an advantage to quickly access markets, and develop its own distribution channel from the producer directly to the client. The company's production system guarantees an organic product that will soon be certified.

3.2.1 *Market Study.* According to Macro Setas Colombia (Macro Setas, 2012), the orellana market in the country is growing at a rate of 0,0015 tons a year, meaning that the projected demand for the coming years is estimated at 728 tons a year. This market is satisfied by national production that currently has a capacity to harvest 11 tons a month (Perilla *et al*. 2005), or 132 tons per year, representing less than 20% of the market. Other companies could potentially cover the remaining 80% gap in the market. More specifically, in Bogotá alone, and only considering gourmet restaurants and organic markets—with more than 4000 restaurants located near 12 organic markets (Acodres, 2006) and more than one million inhabitants in the high-income bracket—the demand is estimated at 18 tons a year. This potential market of 18 tons a year is seven tons more than the existing national production.

Once we identified the target market, we contacted some restaurants and markets in Bogotá (Supplementary Table 1), finding that *Orellanas de la Villa* has the opportunity to start producing and commercializing approximately 765 kg a month, representing 50% of the demand in Bogotá. The company's ten-year goal is to be supplying both national and international markets.

Nationally, the competitors' analysis shows that there are four potential competitors: 1) *ASOFUNGICOL*: a farmers' association in the department of Huila that produces and commercializes oyster mushrooms; 2) *CasOrellana*: a group of companies located in the

Valle del Cauca region that produces oyster mushrooms on local and regional levels; 3) *Setas de Boyacá*: a producers' network that collects from more than 100 farming families in the Boyacá region; 4) *AMUSEF*: a women's association that produces a diversity of edible mushrooms in Usme - Bogotá.

In order to evaluate the competitive positioning of *Orellanas de la Villa,* we developed a curve value chart (Supplementary Fig. 2), which shows seven features associated with the production, commercialization and distribution of the fungal product: price, organic certification, social responsibility, market access, distribution channels, product presentation, and technical support. We can see that, for these seven attributes, *Orellanas de la Villa* has a better competitive position than other producers. Given the that the company's proposal is to a) reduce intermediaries, use b) geographical positioning to quickly access markets, and c) to take control of distribution activities, the price will be favorable for both farmers and clients.

The marketing strategy will be developed based on organic certification and the fact that the growers themselves harvest and commercialize the product. Also, given that oyster mushroom consumption is not common in Colombia, *Orellanas de la Villa* will add an oyster mushroom recipe on their labels for the product (Supplementary Fig. 3).

3.2.2 *Technical Study: Production process.* We structured the production process into eight stages—from grain preparation to distribution—based on the cultivation process undertaken in six households. Table 2 specifies the raw materials, the resources required, and the processing time for each stage. The production process is currently being undertaken at the women's houses, and stages 1 to 4 are executed in their kitchens using the equipment with which they usually cook. Before each stage, they must sanitize all the equipment and clean the kitchen to guarantee the conditions required. During the dark and light phases, the bags

must be irrigated at least twice a day. For these stages, each woman has adapted a space of approximately 10m^2, where they can simultaneously assemble ten plastic bags.

Raw materials, suppliers and production equipment. Universidad de los Andes and the local government provided technical support to identify who in the community could supply the most important raw materials required. *Universidad de los Andes* provided the fungal strain; the plastic bags to be used in steps 4 to 7 were sometimes recycled from the women's homes (i.e., from empty bags of sugar or rice); and the remaining materials were provided by local markets (see a summary of materials, suppliers, and prices in supplementary Table 2). The equipment required to produce the oyster mushrooms is summarized in supplementary Table 3, which also outlines additional information regarding costs.

For a fixed production level of 765 kg a month (see §3.2.1), the following materials are required: 765 plastic bags, 1913 kg of substrate, 38 kg of supplement, 3.8 m of PVC piping, 6.1 m of chiffon, 38 kg of grain, and 3825 m^3 of water. If the production process were to be concentrated in one specific area, it would require 142 m^2. This distribution is presented in supplementary Figure 4.

3.2.3 *Organizational Study.* As part of the technical support, an organizational analysis was carried out to identify the relationships within the community, and to determine the appropriate organizational structure for the development of company activities.

Organizational structure. Orellanas de la Villa is a company created with and for the VWA, with the technical support of *Universidad de los Andes*. Its purpose is to promote the production, marketing, and consumption of organic oyster mushrooms, using the available

raw substrates in rural Villapinzón. At the same time, the company was designed to provide an economic alternative to the women in the community, initially guaranteeing a monthly income of a quarter of the Colombian minimum wage.

The proposed organizational structure for *Orellanas de la Villa* is based on the VWA's current established organization. High ranked positions are to be filled by external staff, as they require more specific knowledge in the management and operational processes. It is important to highlight that some of these management positions could be filled with people from the community or the association if they have the necessary expertise; thus, giving them the opportunity to climb the organizational structure.

The salaries associated to each job are presented in supplementary Table 4. The General Manager and Operations Manager are currently from the *Universidad de los Andes* team, but, in the near future, it should be the women who assume this responsibility. As the production system is not yet working, a financial advisor is not yet necessary. The VWA also has a legal advisor who works with the local government and usually supports the women's activities.

In order to legally establish *Orellanas de la Villa*, and according to national policies, a set of activities need to be carried out such as commercial registration, the payment of commercial fees, and adherence to the required regulations that guarantee adequate food handling and processing. For this process, the company must seek advice from a legal consultant or the Bogotá Chamber of Commerce (CCB).

3.2.4 *Financial Study.* To formally establish *Orellanas de la Villa,* an estimated initial investment of 10 million COP is required. The financial analysis was developed on the basis that: 1) according to the market study, *Orellanas de la Villa* will sell 765 kg a month, or 51%

of the current market; 2) *Orellanas de la Villa's* target market will consist of 80% restaurants (the product will be provided in 1 kg bags) and 20% organic markets (the product will be provided in a 250 gr tray); 3) Oyster mushroom consumption will present a growth of 13% (Macro Setas Colombia, 2012); 4) the safety stock will be 1% of supplies required; 5) the clients will pay on delivery; 6) The annual increase in expenses will be of 3% IPC; and 7) *Orellanas de la Villa* will pay suppliers when the raw materials are acquired.

Considering only the variable costs, the production cost of 1 kg of oyster mushroom is US$ 0.94, as shown in Table 3. The equipment required for the production process and investment in additional equipment are given in supplementary Table 3 and 5, respectively. Including the packing costs, presented in supplementary Table 6, we can estimate that the total production cost of a 1 kg-bag is US$ 1.07 and US$ 0.38 for a 250-gr tray. This final investment considers all administrative requirements to establish the company and to obtain an organic certificate. Taking into consideration the production, packing, and equipment costs, the analysis estimates an approximate selling price of US$ 3.91 for a 1 kg-bag of oyster mushroom, and US$ 1.96 for each tray. Given these prices, and assuming that 80% of the product is stored in 1 kg-bags, the equilibrium point for the two types of packaged products will be to produce and sell 7294 1kg-bags and 5608 250gr-trays a year, corresponding to a yearly production of 8696 kg.

Finally, we carried out a financial simulation to assess the financial sustainability of *Orellanas de la Villa,* by assessing three scenarios, summarized in Table 5: (i) A pessimistic scenario that considers a 5% market growth rate, and in which there is no company expansion; (ii) a neutral scenario, which considers a normal annual growth rate of 13%; and (iii) an optimistic scenario, which considers a 16% annual market growth rate.

In these scenarios, and contemplating the Net Present Value (NPV) for a period of up to ten years, the results show that even in the most pessimistic scenario the company will generate profit (Table 4). According to the business plan and merchandising analyses, each woman has to cultivate 60 bags per month in order to earn a quarter of a Colombian minimum wage (Table 5).

3.2.5 *Implementation plan.* We propose a plan of action to develop the company over a ten-year period. In the short term, the company will have a monthly income of US$ 119, considering the economic constraints that oyster mushrooms will be under at the beginning of the project. During this phase, the company will develop all microbiological tests of the fungal product, obtain the organic certification, develop an intensive marketing strategy, and increase its production. In the mid-term, after the fifth year, the company will increase workers' salaries, guaranteeing a minimum wage for each technician, and it will be able to build four new greenhouses, allowing for an increase in production to 940 kg a month. In the long-term, at the end of tenth year, the company will be well-established in the national market, and will have developed a plan to export to the USA, Canada, the United Kingdom, and Germany.

4. Discussion

We are convinced that in order to meet the millennium development goals (MDG), rural communities must be aware of the importance of diversifying their products, and of expanding the possibilities to take greater advantage of nutritive biological sources. In this project, we have proposed a sustainable food-based approach for low-income rural women in Villapinzón (VP) by establishing the conditions for oyster mushroom production with local

resources. To do this, we analyzed the viability of the product's commercialization in the proposed business plan, as well as in local stakeholder meetings in rural communities in VP. Despite mushrooms not being regularly consumed in the area, the new information we offered to rural women dealt with the technical aspects of cultivation, fungus biology, and their health-promoting properties. These have been essential steps in establishing sustainable management of a daily dietary source and income.

4.1 How rural women became involved in the cultivation process

Teaching the women how to cultivate the mushrooms was successfully implemented using the substrates available in the rural communities. This is due, in part, to the high adaptability of *P. ostreatus* to a wide variety of substrates, making it possible to take advantage of a sub-utilized substrate. The women are aiming to reach a biological efficiency of 25%, which will make supplying the product economically viable.

Low-income rural women from the VWA intend to replicate the process by teaching other women and by overcoming the difficulties that occur during the cultivation process. Although production is not labor-intensive, the women had to acquire new skills that are very different from those required for potato cultivation. These include sterilization with pressure and heat, disinfecting surfaces, the use of tools that are always boiled in water, the use of gloves and surgical masks, the maintenance and replication of the fungal source (mycelium), and the recognition of every stage in the mushroom production, among others.

The learning of new alternatives to the common potato crop for product diversification involved providing information about oyster mushrooms' nutritional facts as well as therapeutic benefits, and post-harvest uses. *P. ostreatus* has a high content of protein, vitamins and polyunsaturated fatty acids, high antioxidant and anti-inflammatory properties

(Rathee *et al*. 2012), accessible production and cultivation processes, post-harvesting activities are also beneficial in that they provide opportunities to promote the sustainability of small farming systems, given that residues can be used as a growing substrate and then returned to the land as fertilizer (Marshal & Nail, 2009), compost, or animal feed (i.e., for cattle).

Threats to the cultivation process always demanded VWA action. The proactive initiative of women when facing problems such as contamination and dehydration helped to solve these issues; for example, they learned how to eliminate contaminants (commonly associated to species of mold such as *Trichoderma* spp.) when they appeared in the bags. A number of workshops that are currently being run with the women have highlighted the need for incubating rooms, in which the humidity and temperature can be controlled, allowing the mushrooms to withstand high dry-season temperatures and dehydration. This will lead to an optimal production of *P. ostreatus,* and ensure the demand for personal nutrition and business activities. The women recently visited the *Universidad de Los Andes* campus in what proved to be a very enriching experience, as they were able to compare their homemade process with a laboratory one.

4.2 Advantages of cultivating oyster mushrooms

There are a few advantages of growing this fungal species over other fungal species. The cultivation process is easy when compared to the infrastructure required for other edible fungi such as traditional champignon mushrooms, it does not require a large initial investment, and the oyster mushroom is a fairly complete nutritional source. Moreover, according to financial and marketing studies, there is a high level of unmet demand in the Colombian oyster mushroom market, and more specifically, demand was found to exist in the organic market.

In Colombia, oyster mushrooms producers recorded a monthly production of around 11 tons, or 132 tons per year; however, in 2014, the same studies established a potential national market of 728 tons per year, showing an opportunity for growth (Mesa, 2014). Whereas continents such as Europe and Asia are traditionally known for their high levels of production and consumption, in Colombia, mushroom cultivation is still an emerging activity that is considered a good and viable alternative to traditional cultivation, given the sustainability of substrates and the null environmental impact. Small farmers are the main producers; however, the low quantities produced and the high demand of gourmet restaurants still make imports an important option when it comes to accessing the product. As such, oyster mushroom production is a highly viable business opportunity for the municipality of Villapinzón.

4.2 Future perspectives: A potential new project based on the previous case experience

Several countries have attempted to promote the edible fungi industry as a means of providing incentives for forest conservation and improving the earning opportunities of people living in marginal rural areas (Ortega-Martínez & Martínez-Peña, 2008; Cai *et al*., 2011). For many decades, interest in the commercial harvesting of wild edible fungi has increased considerably in many regions. For instance, in the USA, recreational and subsistence mushroom harvesters have found an abundance of mushrooms in nearby forests, which has led unemployed timber workers to sell mushrooms as an alternative source of income (Pilz *et al*., 2003).

Following this perspective, we argue that proposing strategies to characterize and recognize the species of saprotrophic edible fungi that can be cultivated will enhance local

knowledge about the use of fungal diversity, and the understanding of its importance as part of the ecosystem. Considering the above, rural farmers have been flexible in incorporating new alternatives, such as the oyster mushroom, to compensate for the lack of nutritive food and to potentially improve their economic situations with the establishment of *Orellanas de la Villa* company.

Additionally, the Colombian State is in the process of building the Plan for Food Security and Nutrition, and, as such, the search for alternatives to contribute to the management of the community's complementary nutritional needs as a relevant issue for the country's development. This project will seek to further explore the potential of saprotrophic fungal species as a Non Wood Forest Product (NWFP). As stated by FAO (2001): [it is…] "an interesting product to be used by human society, regarding its nutrition values."

5. Concluding remarks

The interdisciplinary project that received conjunct contribution from three research areas, proved to be a strategic approach that promoted the knowledge appropriation of producing a new product, and its subsequent development. The case study shown here was proposed as a promising option to help mitigate the effects of poverty, hunger, inequality, and consequently, as a basis for further studies related to the emergent research field in conservation and the sustainable use of fungi in Colombia. Moreover, the community involved in the project was very interested in learning about an unfamiliar agricultural activity, and so they were attentive, ready to solve cultivation issues, and creative in the way they integrated oyster mushroom consumption.

According to the municipality development plan (2012-2016), entitled "Villapinzón, the

path to progress," the government is aiming to produce favorable employment conditions for companies to increase the productive sector (Development Plan, 2012). Drawing on this precept, this study uses a strategy that relies on the promotion of economic and nutritional improvement by turning a cultivation activity into a company, *Orellanas de la Villa*. The successful results obtained from the experience and the organic product itself are being shown to potential consumers, who have agreed to taste and to classify a high quality product.

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Tables

Table 1. Women's perception of oyster cultivation

Category	Women´s answers appear in *italics*
Which were the main difficulties you found associated to the first cultivation pilot?	*"The routine of the process is sometimes exhausting"* "*The control of mosquitoes is hard to handle*" "*Sometimes I forgot to boil the water and use the syringe"* *"It is not as simple as it is to cultivate potatoes, we have to be more careful about contamination"* Contamination was a common problem "*This green colour in the bag is growing"* *"I take the green part from the bag with a cleaned knife"* *"All the bags filled with hay were contaminated"*
Could you teach the whole process to another person?	Every woman said that she was prepared to replicate the whole process by herself and to teach it to other farmers: " *The first time I did not understand very much, but you learn as you do it*" *"It is not a complex process, and rice husk is easier to handle"* *"I will teach the whole process since it is easy"* *"Yes I will teach the process with what I have learned so far"* *"Yes, it is like raising chickens or plants, is not complicated"*
Which is your first perception of the oyster mushrooms harvesting process?	" *I thought that the culture was damaged since a brown colour appeared in the cap*" *"In the morning, I found like an old white dust on the tables"*– We explained that this was the colour of fungal spores, and that spores from different fungi are different colours, additionally we explained that if we were growing common champignons, the *dust* would be brown *"They are like little umbrellas, they are beauty"* *"They are like grey ears, I can't wait to eat them"* *"For me they are like cup-shaped trees"* One woman named the "seed": *"el cocido"*
Did anyone help you in the process or did you carry out the complete procedure?	*"I did everything by my self"* *"A friend helped me with the irrigation of the bags"* *"Sometimes my children helped to accommodate the bags"*
What do you think about the oyster mushroom product	"*They taste like chicken"*, "*it is delicious*", "*they are more hard to chew than common champignons*" "*My mom loves this…*" "*…we like this protein source because it is healthy",* *"My godfather stopped by and said, I am interested in this fungus",* *"on occasions the oyster mushrooms replace meat", "my daughter likes the taste, and it is good for her because she suffers from hypoglycaemia".*

How many bags are you willing to prepare?	"*Maybe 20*" *"I will make 10 bags"* "*I will culture all the bags that fit on the shelves"*

Table 2. Oyster mushroom production process requirements

Stage	Raw material	Resource	Time
1) Grain preparation and sterilization (section 2.1.2)	Wheat Grain (rice) Hot water	Pot Glass bottle Marmite	150 min
2) Inoculation (section 2.1.3)	Mycelium Grain preparation	Sterilized storage area Burner	24 d
3) Substrate sterilization (section 2.1.3)	Substrate (sawdust, rice husk, hay) Supplements (molasses, coffee grounds waste)	Marmite (70 l) Fabric sac	10,5 h
4) Bags setting (section 2.1.4)	Micelle Sterilized substrate Plastic bags Tube Chiffon	Burner	15 min
5) Dark phase (section 2.1.4)	Sterilized water	Syringes Black plastic Greenhouse	35 min several times a day until
6) Light phase (section 2.1.4)	Sterilized water	Syringes Greenhouse	35 min several times a day until…
7) Collection (section 2.1.4)	Collected oyster mushroom	Cutter Freezer	10 min a bag
8) Package (section 2.1.4)	Bags, Tray, Labels	Balance	20 min

Table 3. Total cost production of 1 kg of oyster mushrooms

Production cost (1 kg)			**Observation**
Material	*Quantity*	*Cost (US$)*	
Plastic bag	1	0,20	Polypropylene bags
Substrate	2,5 kg	0,16	55 kg rice hulls US3,2$
Supplement	50 gr	0,01	8 kg Molasses US$ 2,4
PVC pipe	5 cm	0,02	2 m of PVC pipe of 6 cm of diameter – US$ 0,80
Chiffon	8 cm	0,04	Box of 4 m US$ 1,6
Rubber	1	0,03	Box of 100 units US$ 3,0
Grain	50 gr	0,08	2 kg US$ 1,60
Water	5 m^3	0,40	1 l US$ 0,08
Total production cost		**0,94**	

Table 4. NPV for three scenarios analysed

Scenario (NPV 10 years)		Value
Pessimistic: considers a market growth rate of 5%. There is no company expansion	$	5.962.180
Neutral: considers the normal annual growth rate of 13%	$	110.826.615
Optimistic: considers that the market growth rate is of 16% a year	$	242.588.452

Table 5. Bags produced related to the Colombian salary

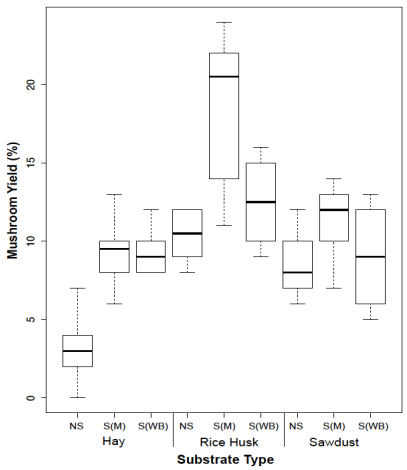
	Half a salary– 344,000 (COP)	**Quarter of a salary– 172,000 (COP)**
Workers	9	9
Bags per woman	60	45
Total Kg per month	765	405

Figures

Figure 1. A) Clean table for seed preparation. B)-C) Women of the AWV during the bags assemblage; and D) Finished bags

Figure 2. A)- E). Fruiting bodies production

A.



B.

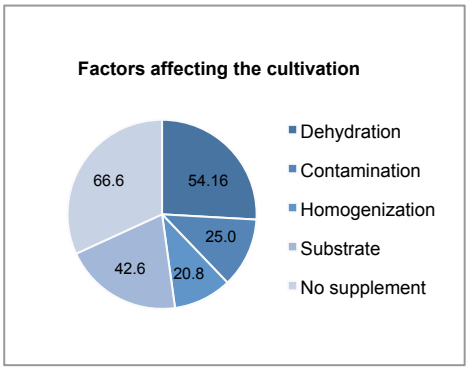


Figure 3. Production process. A) Percentage of mushroom production by treatment for each of the 6 houses. Dark horizontal lines represent the mean, with the box representing the 25th and 75th percentiles, and whiskers the minimum and maximum values. NS: No supplement, SM: Supplemented with molasses, SWB: Supplemented with Wheat Bran. B) Percentage of bags affected by common problems: dehydration (26 out of 48 bags); contamination (12 out of 48 bags); homogenization (10 out of 48 bags); substrate (23 bags showing less than 10 % of production, out of 54 bags; no supplement (12 bags showing less than 10 % of production out of 18 Bags).

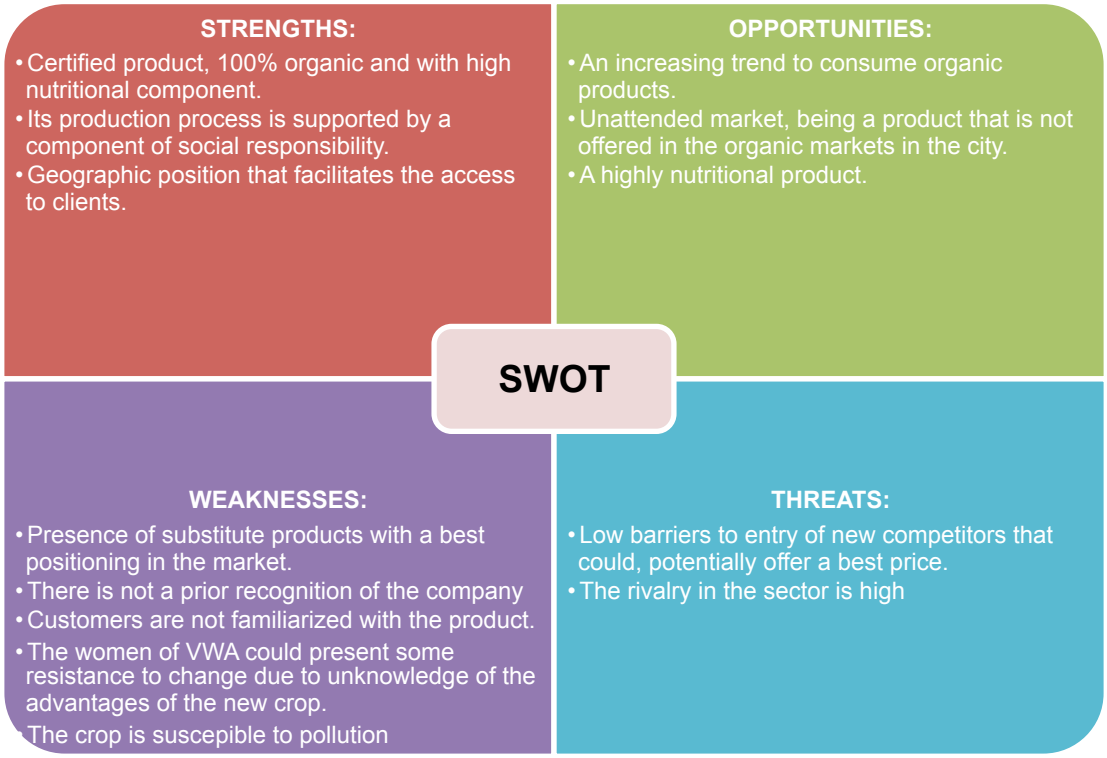


Figure 4. SWOT Matrix

Supplementary information

Supplementary Table 1. Potential clients and situation of negotiation

Establishment	Activity	State
Restaurants		
Wok	The procurement manager is interested in acquiring Orellana from *Orellanas de la Villa*. She has been constantly in contact	It is necessary to produce a sample
Balzac (Harry Sasson)	The Main Chef is interested in acquiring oyster mushrooms from *Orellanas de la Villa.*	It is necessary to produce a sample
Café Renault	They already know the product and the company	Continuing to contact them
Teriyaki	They already know the product and the company.	Continuing to contact them
La Monferrina	The procurement manager is interested in buying the product.	It is necessary to produce a sample and guarantee procurement
Organic markets		
Escarola	The client was visited	It is important to generate an agreement. It is important to define the time and volume to procure each week and define when production will start
Bioplaza	The client was visited	It is necessary to generate a proposal with the price and the lead-time defined.
Vivir Bonito	The client was visited	It is necessary to generate a proposal with the price and the lead-time defined.
Clorofila	The client was visited	They demanded a proposal and a sample
Supermarkets		
Jumbo	The client was contacted	They demanded a proposal following company criteria

Supplementary Table 2. Raw materials and their suppliers

Raw material	Supplier	Price
Grain (rice)	Local market	2 US$/kg
Hot water	Local drinking water distribution system	0,1 US$/ L
Mycelium	The Laboratory of Mycology and Phytopathology of Universidad de los Andes taught the women to produce the seed from the one used in previous assays.	10 units – 5 US$
Substrate (sawdust, rice-husk, hay)	The rural community	55kg - 4 US$
Supplements (molasses, Coffee grounds)	The rural community	8 kg – 3 US$
Plastic bags	Local market Home recycling bags	0,25 US$ / Unit
Chiffon	Local market	
Tube	Local market – it could be reused	2 m – 1 US$
Sterilized water	Local drinking water distribution system	0,1 US$/ l
Bags to pack	Local market	5000 units - 4,5 US$
Tray to pack	Local market	500 units – 12,4 US$
Labels to pack	Printers from Bogota	1000 units – 150 US$

Supplementary Table 3. Equipment required for the production process

Equipment	Quantity	Cost (US$)*	Total
Steel tables	2	100,0	200,0
Fridge	1	416,0	416,0
Cooker	2	32,0	64,0
Industrial hob	2	107,0	214,0
Plastic containers	20	4,0	80,0
Balance	2	16,0	32,0
Buckets	4	2,5	10,0
Packing machine	1	218,0	218,0
Burners	4	1,5	6,0
Thermometer	1	4,5	4,5
Thermos PS	5	8,5	42,5
Total investment in equipment			1287,0

*Costs have been estimated in Colombian pesos (COP), this table shows the prices in USD using a TRM of 1 USD = 2522 $COL

Supplementary Table 4. Organizational team and salaries at *Orellanas de la Villa*

Position	Number of employees	Salary * (US$)	Observation
General Manager	1	460,0	
Production Manager	1	131,5	
Financial advisor	1	198,2	Advisors are hourly paid
Juridical advisor	1	198,2	Advisors are hourly paid
Technicians	8	920,5	*Orellanas de la Villa* starts its production with eight women that belong to the association

*Salaries include all benefits according to Colombian labour laws

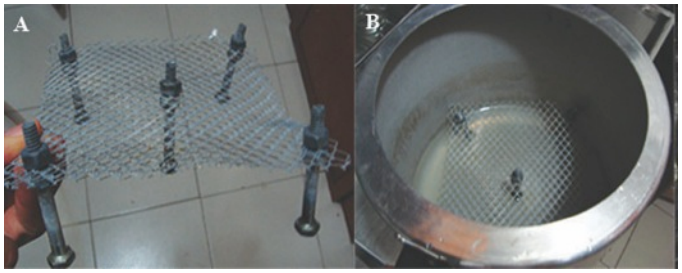
Supplementary Table 5. Other initial investment

Item	Cost (US$)
Computer	294,0
Software	63,5
Company establishment	307,0
Working capital	79,5
Initial inventory	630,0
Greenhouse adjustments	397,5
Organic certification	516,5
Organoleptic tests	274,0
Total	2562,0

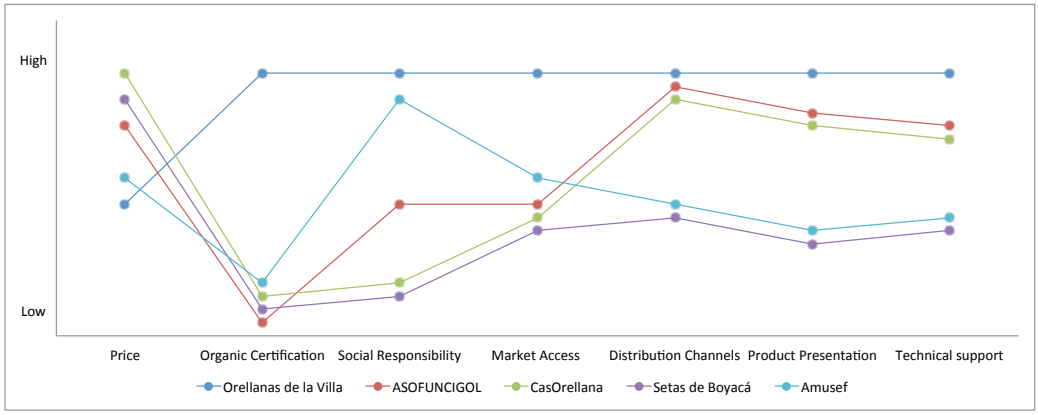
Supplementary Table 6. Packing cost

Packing material	Presentation	Cost
Polystyrene tray	500 units	10,0
Cling film	1500 m	18,5
Plastic Bag 1 kg	100 units	3,5
Label	1000	119,0

Supplementary Figures

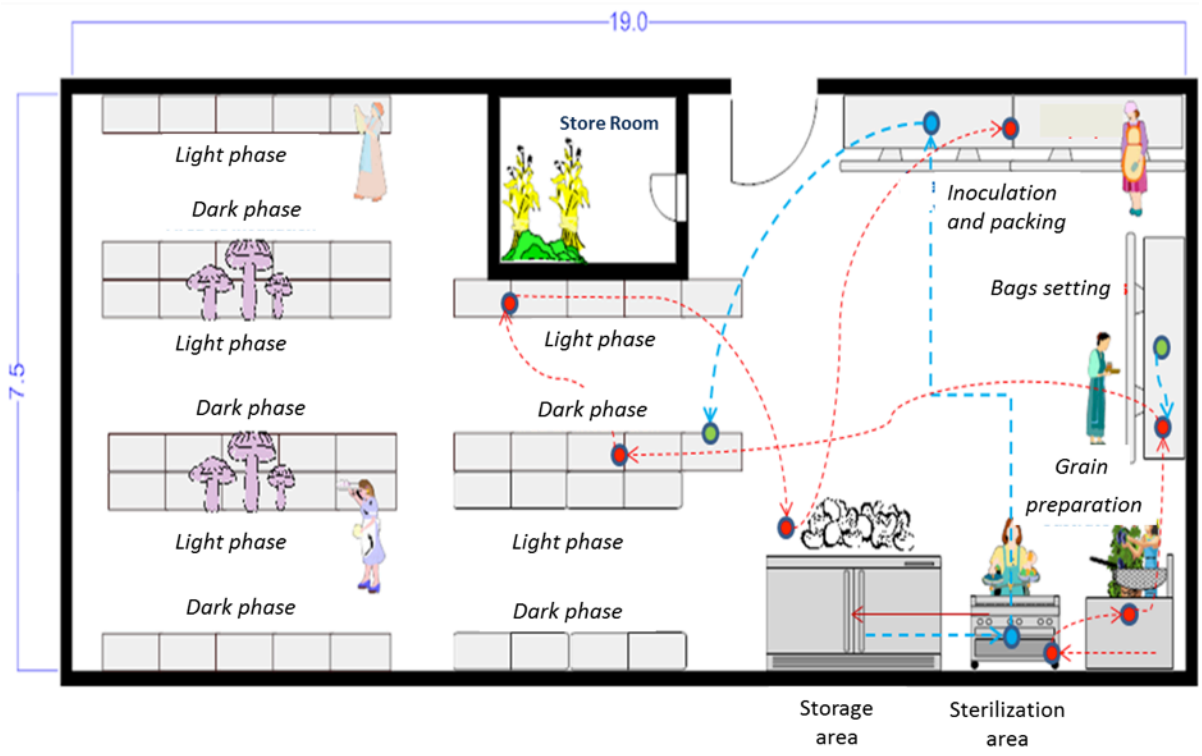


Supplementary Figure 1. Handmade mesh.



Supplementary Figure 2. A value curve chart for *Orellanas de la Villa*'s product

Supplementary Figure 3. Product label



Supplementary Figure 4 Facility layout

Concluding remarks

This thesis gathered robust scientific data to contribute to the knowledge on ECM macrofungi in Colombia, and as a tool for National Biodiversity reports. Colombia has adhered to the Strategic Plan for Biological Diversity (*Plan Estratégico de Diversidad Biológica* (PEDB 2011-2020) that defines the lines to apply coherently and efficiently the three objectives of the Convention on Biological Diversity (CBD). Among the targets identified by the CBD (https://www.cbd.int/sp/targets/) we highlight the advance in the scientifically sound knowledge of biological diversity and the incorporation of its ecological aspects in all governmental and social contexts. The inclusion of fungal diversity into these biodiversity reports should be a priority in Colombia.

This thesis proposes a comprehensive study of macrofungi in Colombia:

1) The basic research conducted on species within the genus *Amanita* gave us clues about the origin and phylogeography of this important genus. In Chapter 1, the origin of this neotropical ectomycorrhizal genus distributed in the Andean mountains was assessed. In Chapter 2 I investigate the historical events that lead to the introduction of *A. muscaria,* an exotic ECM species, and its population genetics structure. This is the first report of an ectomycorrhizal fungus that after its introduction expands its range into native oak forests. This provides a strong justification to initiate strategies to control, prevent, and/or limit the spread of *A. muscaria.* Our results constitute the basis for designing decision-making tools in regions, particularly located in the Andes cordilleras where a great activity of exotic tree plantations is being carried out. Additionally, this study is a starting point to initiate a research line in fungal biological invasions in the country.

2) The list of ECM fungi presented in Chapter 3 provides valuable information on the distribution and diversity of ECM fungi in Colombia. This study also constitutes a first attempt to show which are the regions where research on ECM fungal diversity is lacking and should be addressed. For decades plant and animals have been the focus of National Biodiversity reports and the basis in the formulation of projects towards the conservation and sustainable use of ecosystems in the country (Chaves *et al*. (2007). The scientific data provided here should be used to encourage the public awareness of fungal diversity. Additionally, the booklet intended for people living near oak forests is a strategy to encourage public awareness of macrofungal conservation and how to sustainably manage these fungi in localities where their edibility is traditionally known. Given that the biological diversity of a species gives environmental good and services, its maintenance and protection is vital (Chaves *et al*. 2007).
3) Finally, we aimed to have a social impact developing this project. Therefore, as an alternative for the reduction of poverty which is one of the Millennium Goals we taught the process of oyster mushrooms cultivation to low-income rural women.

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Chaves, M.E., M. Santamaría & E. Sánchez. 2007. Alterativas para la conservación y uso sostenible de la biodiversidad en los Andes Colombianos. Resultados 2001-2007. Instituto de Investigaciones de Recursos Biológicos Alexander van Humbodlt. Bogotá, Colombia.

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