In Colombia the Eurasian fungus *Amanita muscaria* is expanding its range into native, tropical *Quercus humboldtii* forests

Natalia Vargas, Susana C. Gonçalves, Ana Esperanza Franco-Molano, Silvia Restrepo & Anne Pringle

View supplementary material ☐

Published online: 13 Aug 2019.

Submit your article to this journal ☐

Article views: 119

View related articles ☐

View Crossmark data ☐

In Colombia the Eurasian fungus *Amanita muscaria* is expanding its range into native, tropical *Quercus humboldtii* forests

Natalia Vargas[a], Susana C. Gonçalves [b], Ana Esperanza Franco-Molano[c], Silvia Restrepo[a], and Anne Pringle [d,e]

[a]Laboratory of Mycology and Plant Pathology, Universidad de Los Andes, Bogotá, Colombia; [b]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal; [c]Laboratorio de Taxonomía y Ecología de Hongos, Universidad de Antioquia, Medellín, Colombia; [d]Department of Botany, University of Wisconsin–Madison, Madison, Wisconsin 53706; [e]Department of Bacteriology, University of Wisconsin–Madison, Madison, Wisconsin 53706

ABSTRACT

To meet a global demand for timber, tree plantations were established in South America during the first half of the 20th century. Extensive plantings of non-native species now are found in Brazil, Chile, Argentina, and Uruguay. In Colombia, miscellaneous plantations were established in the 1950s, during a period of intensive local logging, when 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 ectomycorrhizal fungi. We sought to document the origins and spread of the introduced *Amanita muscaria* found in Colombian plantations of the Mexican species *Pinus patula*, North American species *P. taeda*, and Australian species *Acacia melanoxylon* and *Eucalyptus globulus*. In Colombia, *Amanita muscaria* is establishing a novel association with native *Q. humboldtii* and has spread to local *Q. humboldtii* forests. According to a Bayesian phylogeny and haplotype analysis based on the nuclear rDNA internal transcribed spacer region ITS1-5.8-ITS2 (ITS barcode), *A. muscaria* individuals found in four exotic plant species, and those colonizing *Q. humboldtii* roots, have a Eurasian origin and belong to two Eurasian haplotypes. This is the first time the spread of an introduced mutualist fungus into native Colombian *Q. humboldtii* forests is reported. To arrest its spread, we suggest the use of local inocula made up of native fungi, instead of inocula of introduced fungi.

INTRODUCTION

In the Southern Hemisphere, many plantations of non-native trees, including species of *Pinus, Eucalyptus*, and *Acacia*, have been established to make up for local timber shortfalls. Harvested trees are used primarily in industry, for example, for pulping, as well as for timber (Le Maitre 1998; Overbeek et al. 2012). Plantations were first established in the late 17th century in southern Africa and New Zealand (Mirov 1967; Richardson and Higgins 1998). By the 18th century, planted trees had become invasive in Australia and in various 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 plantations of *Eucalyptus* and *Cupressus*, were established in the 1950s–1960s (Cavelier and Tobler 1998; Ramírez et al. 2014) in part to mitigate deforestation. In the early 1900s, intensive harvesting of native forests, including montane

cloud forests, affected *Quercus humboldtii* in particular (Ramírez et al. 2014). This oak is a native species distributed in the Colombian Andes between 750 and 3200 m above sea level (asl) (Avella and Cárdenas 2010). It reaches its greatest extent in the departments of Boyacá and Santander (Fundación Natura 2007; Orwa et al. 2009). An effort to protect native forests focused on establishing forest reserves and creating norms and licenses for use (Ramírez 2009). But at the same time, plantations of non-native trees were developed. An extensive literature documents the problems associated with tree plantations in the Southern Hemisphere; generally, problems are related to conflicts with native ecosystem services (Dickie et al. 2014), including the disruption of abiotic cycles (Nullvalue 1996; Richardson and Higgins 1998; Le Maitre et al. 2000; Céspedes-Payret et al. 2009), biotic interactions (Moran et al. 2000; Simberloff et al. 2010), and social services (Overbeek et al. 2012). In Colombia, there are no formal reports of exotic trees invading into native forests, but we

---

have observed naturally occurring pine trees escaped from original plantations and now growing in mixed pine and oak forests (Vargas, personal observation).

Another unforeseen consequence of tree plantations was the simultaneous introduction of the mycorrhizal fungi typically associated with non-native tree species (Richardson et al. 2000; Keller et al. 2011). The mycorrhizal interaction benefits trees by enhancing access to nutrients, generally increasing survival and growth (Harley and Smith 1983; Read 1998). Commercial tree plantations do not thrive in introduced ranges without compatible mycorrhizal fungi (Nuñez et al. 2009; Nuñez and Dickie 2014). In natural systems, the distributions of mycorrhizal fungi often are shaped by the distributions of their native hosts (Geml et al. 2010). But once a tree and its associated fungi are introduced to a novel habitat, the mycorrhizal fungi not only establish within the plantations but may also disperse to local forests, occasionally establishing novel symbioses with native plant species (Dickie et al. 2010; Pringle et al. 2011; Moeller et al. 2015).

In fact, an analysis of the available data on global ectomycorrhizal (ECM) introductions (Vellinga et al. 2009) suggests that most cases of introduced ECM fungi are recorded from plantations in the Southern Hemisphere. The ECM species *Amanita muscaria* (L.) Lam. (1783) 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 and Totterdell 1988; Sawyer et al. 2001; Dickie and Johnston 2008). In these countries, *A. muscaria* now grows with native *Fuscospora* spp. and *Lophozonia* spp., both formerly in the genus *Nothofagus* (Fuhrer and Robinson 1992; Bougher 1996; Bagley and Orlovich 2004; Orlovich and Cairney 2004; Dickie and Johnston 2008; Robinson 2010). The fungus has also been reported from southern Africa, occurring in pine (Marais and Kotzé 1977; Lundquist 1986; Van der Westhuizen and Eicker 1987; Reid and Eicker 1991) and eucalypt (Ducousso et al. 2012) plantations. In South American countries, where tree plantations were established relatively recently, *A. muscaria* is similarly reported within exotic tree plantations, albeit rarely (Nasi 1977; Pulido 1983; Garrido 1986; Stijve and De Meijer 1993; Malvárez et al. 1997; Franco-Molano et al. 2000; Giachini et al. 2000; De Meijer 2001; Vellinga et al. 2009). One observation was reported with native Nothofagaceae in southern Chile (Nouhra et al. 2019); however, no associations between *A. muscaria* and the roots of native trees in South America have been reported to date.

In Colombia, conserving biodiversity and protecting ecosystems are priority tasks for the government and research institutions. Invasion biology is a developing field, and fauna and flora are already targets, but fungal invasions are generally not studied. During the course of our research, it became clear that *A. muscaria* has established in at least one native forest in the northeastern Andes, near a plantation. We sought 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 the historical events related to the introduction of *A. muscaria* to Colombia and then used root tips to confirm an ectomycorrhizal association between *A. muscaria* and native *Q. humboldtii*. We used phylogenetic approaches to relate Colombian *A. muscaria* to global populations of the fungus. Recently published data suggest that *A. muscaria* is a species complex (Geml et al. 2006, 2008), encompassing multiple, geographically distinct clades; our aim was to understand whether Colombian *A. muscaria* comprise multiple clades and identify which clades are in Colombia. Finally, to place our data in a global context, we collected information from the literature on the numbers of fungal introductions reported for different countries in South America, and we discovered a correlation between fungal introductions and the extent of planted forest in any individual country.

MATERIALS AND METHODS

History: Literature on Colombian tree plantations and reports of A. muscaria.—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. We next searched the database of fungal introductions published by Vellinga et al. (2009) with four search terms: "*Amanita muscaria*," "*Pinus*," "*Eucalyptus*," and "Colombia." To document additional reports of *A. muscaria* made after Vellinga et al. (2009), we searched within the ISI Web of Knowledge using the same search criteria. We also explored the published checklist of Colombian macrofungi (Vasco-Palacios and Franco-Molano 2013) and reports of *A. muscaria* in local newspapers, using online search portals and typing the words "*eucalipto*," "*pino*," "*Amanita*," and/or "*hongo*". Finally, to help establish the earliest dates and initial distribution of *A. muscaria* in Colombia, we use the online specimen data portal *Sistema de Información sobre Biodiversidad* (SIB; www.sibcolombia.net) and explored the fungal collection of two herbaria in Colombia: Herbario

Table 1. Localities for early and recent specimen records of *Amanita muscaria* collected in Colombia.

Department	Locality	Host	Elevation (m asl)
ANT	Estación experimental Piedras Blancas, Corregimiento of Santa Elena, municipality of Medellín	*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	Corregimiento of 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 of 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	*Eucalyptus* sp.	3394
BOY	Via Belén-San José de la Montaña	*Pinus patula*	2911
BOY	Via Arcabuco-Paipa	*Pinus patula*	2958
CUN	Vereda Chiquira, municipality 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	Via Bogotá-Choachí, km 2	*Pinus* sp.	2100
CUN	Via Bogotá-La Calera	*Pinus* sp.	2702
QUI	Via Salento-Armenia	*Pinus* sp.	1987
SAN	Vereda San José de la Montaña, municipality of Belén	*Quercus humboldtii*	3214
SAN	Vereda San José de la Montaña, municipality of Belén	*Pinus patula* and *Acacia melanoxylon*	2905
SAN	Km 9 via municipality of Belén to Vereda San José de la Montaña-municipality of Belén	*Eucalyptus globulus*	3419
TOL	Municipality of Murillo	Mixed forests	2980
VAL	Corregimiento of Dapa, municipality of Yumbo	*Pinus patula*	2000

Note. See SUPPLEMENTARY TABLE 1 for additional information. Departments: Antioquia (ANT), Boyacá (BOY), Cundinamarca (CUN), Quindío (QUI) Santander (SAN), Tolima (TOL), Valle del Cauca (VAL).

Nacional de Colombia (COL) and Herbario de la Universidad de Antioquia (HUA).

A. muscaria collected in this study.—*Amanita muscaria* basidiomes were collected between March 2007 and June 2015 along trails, roads, and forest edges from 24 localities in Colombia (TABLE 1; SUPPLEMENTARY TABLE 1). Dry specimens are stored in the ANDES_F collection in the Museo de Historia Natural (Universidad de Los Andes) and registered in the Specify 6.6.02 software (www.specifysoftware.org; Specify, Lawrence, Kansas, USA). We estimated the sizes of plantations and forests where basidiomes were found, and the distances among target *Q. humboldtii* forests and *P. patula* plantations, by using Google Earth Pro 7.1.5.1557 (Google, Mountain View, California, USA; May 2015) and ArcMap 10.3.1 (ESRI, Redlands, California, USA; May 2015).

Morphological and molecular analyses of fungi and plants of root tips collected from a Q. humboldtii forest.—Soils with root tips were sampled to a depth of 10 cm under the basidiomes of *A. muscaria* growing in a *Q. humboldtii* forest (6°2′33.38″N, 72°59′59.11″W) in the Vereda San José de la Montaña in the department of Santander. Root tips were isolated from soil with a 2 mm sieve and washed with distilled water. Root tips matching the described morphology of *Amanita* root tips (Agerer 2006) were common and were pooled in microcentrifuge tubes with 50% ethanol for root staining or DNA lysis

buffer for DNA extraction. To test whether putative *A. muscaria* form a Hartig net or other features typical of an ectomycorrhizal association, we stained root tips with trypan blue (Brundrett et al. 1996). Stained cross-sections were observed and photographed under a microscope (Axioskop 40; Zeiss, Gottingen, Germany), and anatomical characteristics including mantle type (Agerer 2006) were observed and recorded.

Protocols for DNA extraction, amplification, and sequencing.—To genotype new *A. muscaria* basidiomes and confirm collected root tips as associations between *A. muscaria* and *Q. humboldtii*, we extracted DNA from basidiomes and root tips using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Template DNA for sequencing the fungal nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS barcode) from basidiomes was obtained by polymerase chain reaction (PCR), using primers ITS4 and the ITS5 (White et al. 1990). PCR was performed with a Peltier thermal cycler (Bio-Rad, Hércules, California, USA) 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 10× 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 C for 1 min, followed by 35 cycles of denaturation at 96 C for 2 min, annealing at 55 C for 1 min, and extension at 72 C for 2 min, and a final extension at 72 C for 10 min.

In addition to sequencing basidiomes, we sequenced both plant and fungal markers from root tips. Template DNA for sequencing plant and fungal ITS from root tips was obtained from PCR, using primers ITS 17F/26 (Baraloto et al. 2012) to amplify plant ITS and the same fungal primers as used previously to amplify fungal ITS. PCR was performed with a Peltier thermal cycler (Bio-Rad) in 25-μL reaction mixtures containing double-distilled H$_2$O, 2 μL of DNA template, 1 μL of each 10 μM primer, 2.5 μL of Taq 10× buffer, 0.5 μL of 10 mM dNTP mix, 3 μL of 25 mM MgCl$_2$, 0.5 μL of 10× bovine serum albumin (BSA), and 0.2 μL of 5 U/μL Taq polymerase. Cycling parameters were as follows: initial denaturation at 94 C for 2 min, followed by 35 cycles of denaturation at 94 C for 30 s, annealing at 48 C for 30 s, and extension at 72 C for 2 min, and a final extension at 72 C for 10 min. PCR amplification of *rbcL* was carried out with primers rbcL 1F/724R (Baraloto et al. 2012), using 25-μL reaction mixtures containing double-distilled H$_2$O, 1 μL of DNA template, 1.25 μL of each 10 μM primer, 2.5 μL of Taq 10× buffer, 1 μL of 10 mM dNTP mix, 2.5 μL of 25 mM MgCl$_2$, 0.5 μL of 10× BSA, and 0.2 μL of 5 U/μL Taq polymerase. Cycling parameters were as follows: initial denaturation at 95 C for 4 min, followed by 5 cycles of denaturation at 94 C for 30 s, annealing at 55 C for 1 min, and extension at 72 C for 1 min, 30 cycles of denaturation at 94 C for 30 s, annealing at 54 C for 1 min, and extension at 72 C for 1 min, and a final extension at 72 C 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 ChemiDoc MP Imaging system (Bio-Rad, Hércules, California, USA), and were assembled with Geneious Basic 4.8.5 (Biomatters, Auckland, New Zealand; April 2010).

Phylogenetic analyses.—A total of 141 *A. muscaria* ITS sequences were used to infer a phylogeny and place sequences from Colombia in a global context (SUPPLEMENTARY TABLE 2). The alignment encompassed 24 sequences from Colombian basidiomes and 117 retrieved from GenBank, most of them published previously by Oda et al. (2004) and Geml et al. (2006, 2008). We used *Amanita pantherina* voucher KA12-1393 as an outgroup (Kim et al. 2013).

To confirm the identity of plants from root tips collected in *Q. humboldtii* forests, we compared root tip sequences with previously sequenced ITS amplified from *Q. humboldtii* leaves (collected from Santander forests; Vargas et al., unpublished) and other plant ITS sequences from species in the *Quercus* section *Lobatae*. That analysis included 26 ITS sequences in

section *Lobatae* plus 3 sequences in section *Quercus* as an outgroup (SUPPLEMENTARY TABLE 3).

The *A. muscaria* and *Quercus* ITS data sets were aligned with MUSCLE (Edgar 2004), using default parameters. The alignment files can be accessed on TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23403). Bayesian inference was performed using MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001) in CIPRES Science Gateway 3.3 (Miller et al. 2010). Ten million Metropolis-coupled Markov chain Monte Carlo (MCMCMC) generations were run, using a sample frequency of 1000 and a burn-in of 25%. The selected substitution model for both data sets 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 and Ronquist 2001; Ronquist and Huelsenbeck 2003). Each run was examined using Tracer 1.5 (Rambaut and Drummond 2009) to determine whether the burn-in procedures were correctly assumed and whether 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., Bayesian posterior probability [PP] >0.95) (Dettman et al. 2003). Individual sequences of the clade II *A. muscaria* ITS set, which comprised 97 sequences (each 656 bp), were collapsed into unique haplotypes using Snap Map (Price and Carbone 2005; Monacell and Carbone 2014), recoding indels as unique integers and excluding infinite-sites violation (Geml et al. 2010).

Reports of fungal introductions and the areas of land used for plantations in South America.—We compared Colombian data of numbers of fungal introductions with data of other South American countries (Vellinga et al. 2009). We also searched for published data on the land areas planted with exotic plantations in the different countries (FAO 2010; Overbeek et al. 2012). We tested an apparent correlation between the total area of plantations and fungal introductions with Pearson's product-moment correlation in RStudio 1.1.442 (RStudio 2018).

RESULTS

In Colombia, the trees most often planted for commercial purposes are *P. patula* and *E. grandis*, typically in Andean montane habitats (Sicard and Suarez 1998; Von Christen et al. 1998, Ospina et al. 2011). These species are used for both pulp and timber (Wright et al. 1996; Ramírez 2009; Caro et al. 2012). The extensive literature describing plantations in Colombia contrasts with the scant literature

describing introduced ECM fungi and Colombian *A. muscaria*. However, the history of *A. muscaria* in Colombia is clearly tied to plantation forestry, and the fungus was not recorded in Colombia until plantation forestry was well established (FIG. 1).

In Colombia, *A. muscaria* often associates with *P. patula*, a plant species that occurs naturally in Mexico (Richardson and Rundel 1998). According to Ladrach and Lambeth (1991) and Ospina et al. (2011), seeds of *P. patula* used in Colombia are imported primarily from South Africa, Malawi, Zimbabwe, and Transvaal (South Africa), where advanced genetic breeding programs exist. It is not clear whether soils or fungi were ever imported from these countries. In the literature, ECM spp. used to inoculate *P. patula* seedlings are documented as *Boletus* sp., *Rhizopogon roseolus*, and *Pisolithus tinctorius* (Sicard and Suarez 1998; Rivera et al. 1998). Even though *A. muscaria* is not discussed in this literature, personal communications suggest that *A. muscaria* has also been used: for example, Smurfit Kappa is a global business providing paper-based packaging to world markets, and in Colombia *A. muscaria* basidiomes from their plantations are ground and mixed with soils in nurseries to promote seedling growth (Norman Parra, Smurfit Kappa Cartón de Colombia, pers. comm.).

Amanita muscaria also was observed in *Pinus taeda*, *Eucalyptus globulus*, and *Acacia melanoxylon* plantations (TABLE 1). Loblolly pine (*P. taeda*) comes from the southeast United States (Richardson and Rundel 1998), where it is an important commercial species. It is used widely in South Africa and Zimbabwe, and in South America it is important for pulpwood (Peterson 2001). *Eucalyptus globulus* comes from the southeast and west coast of Tasmania (Hall et al. 1975) and is planted extensively in Southern Hemisphere countries because it grows rapidly (Turnbull and Pryor 1984). *Acacia melanoxylon* also is native to southeast Australia (Cowan and Maslin 2001), and this tree is currently reported as invasive in Colombia (Camelo et al. 2012).

The literature reporting *A. muscaria* in Colombia is scarce but makes clear that the fungus is associated with planted trees. The earliest report of the fungus was made by Nasi (1977) from planted forests near the city of Bogotá and along roads to the cities of Villavicencio and Medellín. Two reports were found in the database of fungal invasions provided by Vellinga et al. (2009): Pulido (1983) described *A. muscaria* associated with *Pinus* plantations located in the departments of Antioquia and Cundinamarca, and Franco-Molano et al. (2000) described the species as commonly associated with introduced pines in the whole country. Vargas et al. (2017) also reported the species as commonly associated with *Pinus* spp. Our additional searches revealed two additional localities: in *P. patula* forests in the department of Antioquia, municipality of Jardín (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* basidiomes were observed in a pine plantation (Shroomery 2015).

Early *A. muscaria* specimen records, deposited in herbaria, dating up to and including 1995 are constrained to the central and eastern cordilleras of the Colombian Andes. The first *A. muscaria* specimen dates to 1968 (Idrobo 6268) (FIG. 1) and was made on the National University campus in the city of Bogotá near a tree of the Californian species *Pinus radiata*. No additional information is provided about the host tree of this first *A. muscaria* specimen record. In the decades following, *A. muscaria* specimen records began to be more frequent (FIG. 1), but none reported outside of plantations.
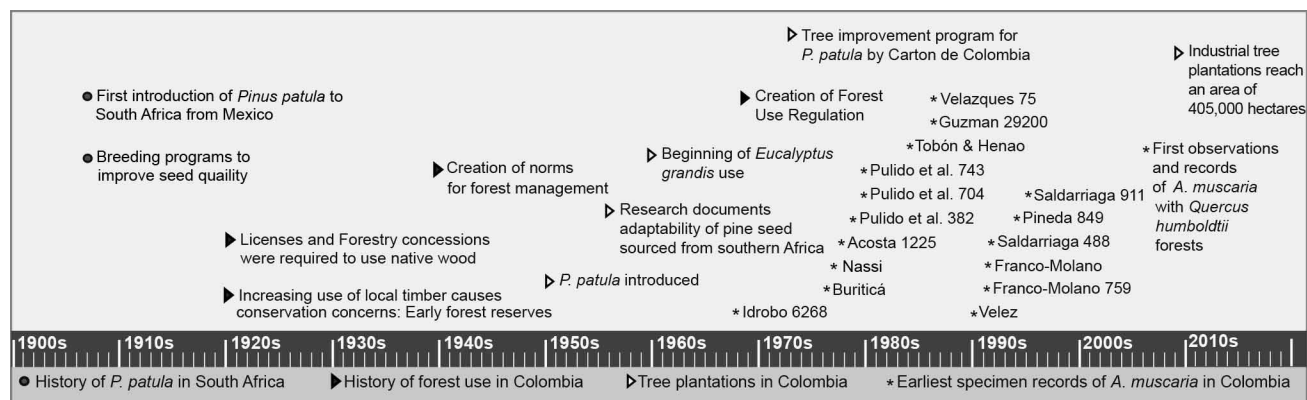

Figure 1. Chronology of events related to Colombian tree plantations and early specimen records of *Amanita muscaria* in Colombia. Events described in Cavelier and Tobler (1998), Le Maitre (1998), Ospina et al. (2011), Poyton (1961), Ramírez (2009), Richardson et al. (1997), Richardson and Rundel (1998), Sicard and Suarez (1998), Wells et al. (1986), and Wormald (1975).

Current distribution of A. muscaria *and its* association with a native oak.—Basidiomes were found fruiting near four exotic tree species: *P. patula, P. taeda, E. globulus,* and *A. melanoxylon,* and near the native *Q. humboldtii.* According to early and recent specimen records, most basidiomes are found or reported in *P. patula* plantations (TABLE 1; SUPPLEMENTARY TABLE 1), 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 (López et al. 2010). Site elevations range from 2100 to 3400 m asl (SUPPLEMENTARY TABLE 1). So far, associations between *A. muscaria* and native *Q. humboldtii* are demonstrated in this study at a single location in the department of Santander (FIG. 2), within three forest patches totaling approximately 3.77 ha (SUPPLEMENTARY TABLE 1). A road divides two of the patches, and the third is approximately 950 m away from the others. The estimated distances between the *Q. humboldtii* forests where *A. muscaria* is found, and local *P. patula* plantations, range from 851 to

2423 m. Recently, additional observations of *A. muscaria* have been made in other oak forests near the municipality of Villa de Leyva, department of Boyacá (Vargas, personal observation).

Morphological and molecular data generated from root tips confirm the association between *A. muscaria* and *Q. humboldtii* (TABLE 2, FIG. 3). The mantle of *A. muscaria–Q. humboldtii* mycorrhizas was characteristic of the genus *Amanita,* with white and bright mycelia (FIG. 3A1–A2), thin-walled, hyaline hyphae (FIG. 3B1–B2), clamp connections, and a lack of cystidia. BLAST results revealed that plant and fungal ITS genes amplified from DNA extracted from single root tips belonged to the genus *Quercus* and the species *A. muscaria,* respectively (TABLE 2). We note that, as far as we are aware, no published molecular phylogeny of *Quercus* includes *Q. humboldtii;* we are the first to sequence the ITS from the species using both leaves and root tip samples. The sequences obtained from root tips cluster together in section *Lobatae* (FIG. 3C), the section thought to encompass *Q. humboldtii* (Rodríguez-Correa et al. 2015).
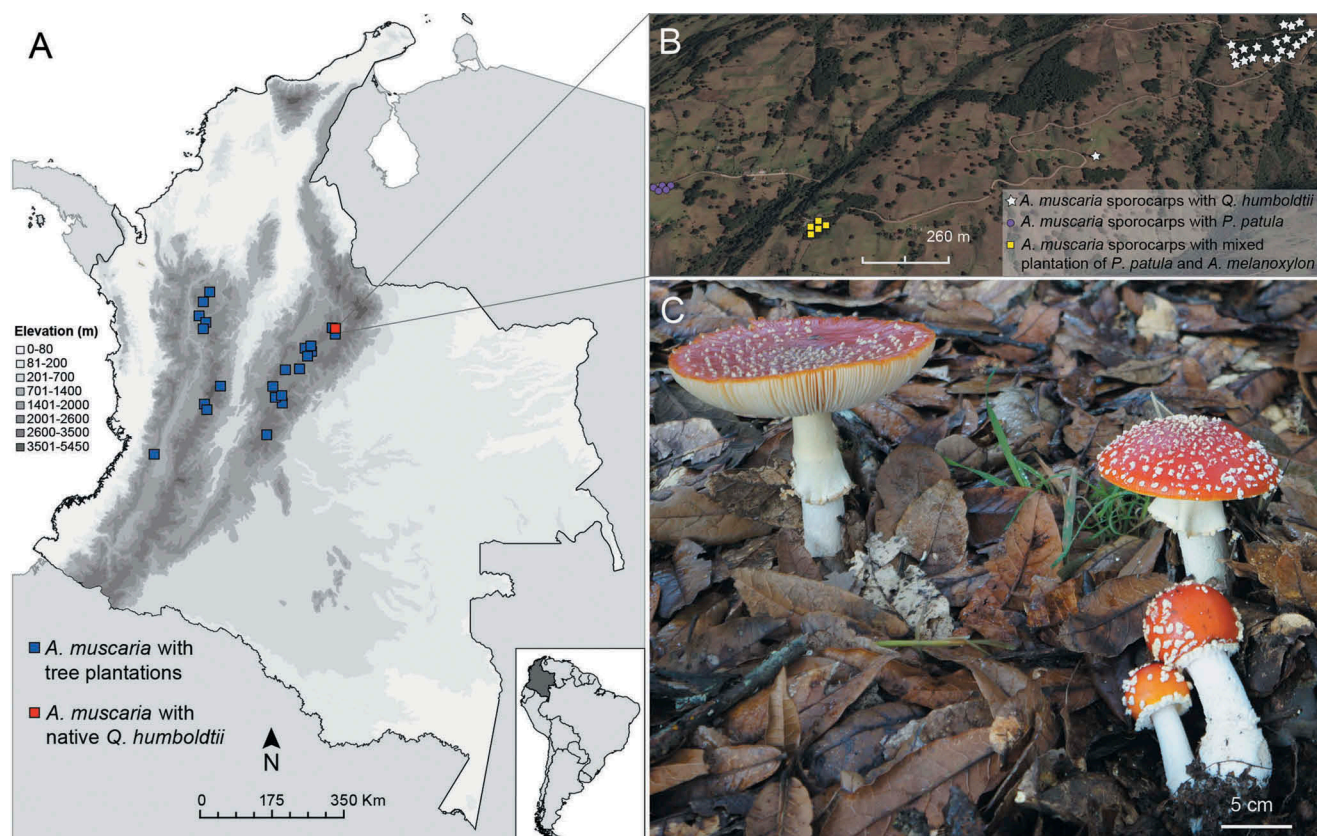

Figure 2. A. Distribution of early and recent specimen records of *A. muscaria* associated to plantations (blue squares) and to *Q. humboldtii* forests (red square). The localities are described in TABLE 1. B. Basidiomes of *A. muscaria* found in *Q. humboldtii* forests (white stars), in *P. patula* plantations (purple circles), and in a mixed plantations of *P. patula* and *A. melanoxylon* (yellow squares). C. Young and mature basidiomes of *A. muscaria* growing near *Q. humboldtii.*

Table 2. BLAST matches and scores for plant and fungal ITS sequences amplified from root tips (NVE_9rt and NVE_11rt) collected under *Amanita muscaria* basidiomes from a *Quercus humboldtii* forest in Colombia.

Root tips sample Primers Accession number	Match sequence Accession number	Max score	Total score	Query cover	E value	Identity percentage
NVE_9rt ITS4-ITS5 MK138671	*Amanita muscaria* isolate ANDES_F401 NVE157, 18S ribosomal RNA gene FJ890026.1	821	821	99%	0.0	91%
NVE_11rt ITS4-ITS5 MK138670	*Amanita muscaria* isolate ANDES_F401 NVE157, 18S ribosomal RNA gene FJ890026.1	217	217	19%	4e-52	99%
NVE_9rt ITS7F-ITS26 MK138673	*Quercus* sp. KK-2014, 18S ribosomal RNA gene KM978077.1	1022	1022	100%	0.0	99%
NVE_11rt ITS7F-ITS26 MK138672	*Quercus* sp. KK-2014, 18S ribosomal RNA gene KM978077.1	974	974	100%	0.0	99%

Eurasian origins of Colombian A. muscaria.—A Bayesian phylogeny reconstructed the clades of *A. muscaria* documented by Geml et al. (2008) with robust statistical support (PP > 0.95) (FIG. 4A). Colombian *A. muscaria* from *P. patula, P. taeda, A. melanoxylon, E. globulus,* and *Q. humboldtii* grouped together within the monophyletic Eurasian clade II sensu Geml et al. (2008) (FIG. 4A; we note that this clade includes samples from the US state of Alaska). Colombian *A. muscaria* are not from the continental United States or Mexico. Instead, Colombian *A. muscaria* group in the same clade as samples from European and Asian countries, including Germany, Scotland, Switzerland, Japan, and Russia. Nevertheless, Colombian samples are genetically heterogeneous and belong to two haplotypes (A and J sensu Geml et al. 2010) (FIG. 4A and C). The two haplotypes 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 Africa, Asia, Europe, and Oceania, but nine Colombian samples are haplotype J, found in England, Germany, and Poland (FIG. 4B and C).

In South American countries, numbers of fungal introductions increase as more land is used for plantation forestry.—Data from 12 countries demonstrate a significant, positive correlation between the area (hectares) of planted exotic trees and records of fungal introductions (FIG. 5), $r(10) = 0.9667, P < 0.001$. However, few reports target *A. muscaria* (FIG. 5): there are five records of *A. muscaria* from Brazil (Stijve and De Meijer 1993; De Meijer 2001; Giachini et al. 2000, 2004; Sobestiansky 2005) and one each from Chile (Garrido 1986), Argentina (Wright and Albertó 2002), and Uruguay (Malvárez et al. 1997).

DISCUSSION

We aimed to decipher the origins of introduced Colombian *A. muscaria* and, in addition to probing the literature, focused on herbarium specimen records, morphological and molecular descriptions of root tips collected from an undisturbed, native forest, sequencing of the ITS region from recently collected Colombian specimens, and subsequent phylogenetic analyses. Evidence suggests that *A. muscaria* first appeared in Colombia in the 1960s (FIG. 1). Nowadays, it is found in tree plantations distributed along three cordilleras (FIG. 2A), and in the northeastern cordillera it is associating with the roots of endemic *Q. humboldtii*. In spite of its conspicuous red-and-white spotted fruiting body, reports of the species in South America remain scarce, and ours is the first report of a shift to a native South American host.

Phylogenetic analyses integrating newly sequenced Colombian specimens with specimens made elsewhere (Oda et al. 2004; Geml et al. 2006, 2008) suggest that (i) introduced Colombian *A. muscaria* have a common Eurasian origin; (ii) *A. muscaria* basidiomes in native forests have the same origin as basidiomes collected from introduced plantations; and (iii) *A. muscaria* collected in Colombia are genetically diverse, made up of at least two Eurasian haplotypes (A and J sensu Geml et al. 2010) (FIG. 4). The association between *A. muscaria* and *P. patula* in Colombia reveals the extent to which novel symbioses can be created by global markets: neither *P. patula* nor *A. muscaria* is native to Colombia, but the plant and fungus now grow together in the country; *P. patula* is Mexican but seeds of *P. patula* in Colombia are typically imported from southern African countries (Ladrach and Lambeth 1991; Ospina et al. 2011), and *A. muscaria* associated with Colombian *P. patula* is Eurasian in origin (FIG. 4).
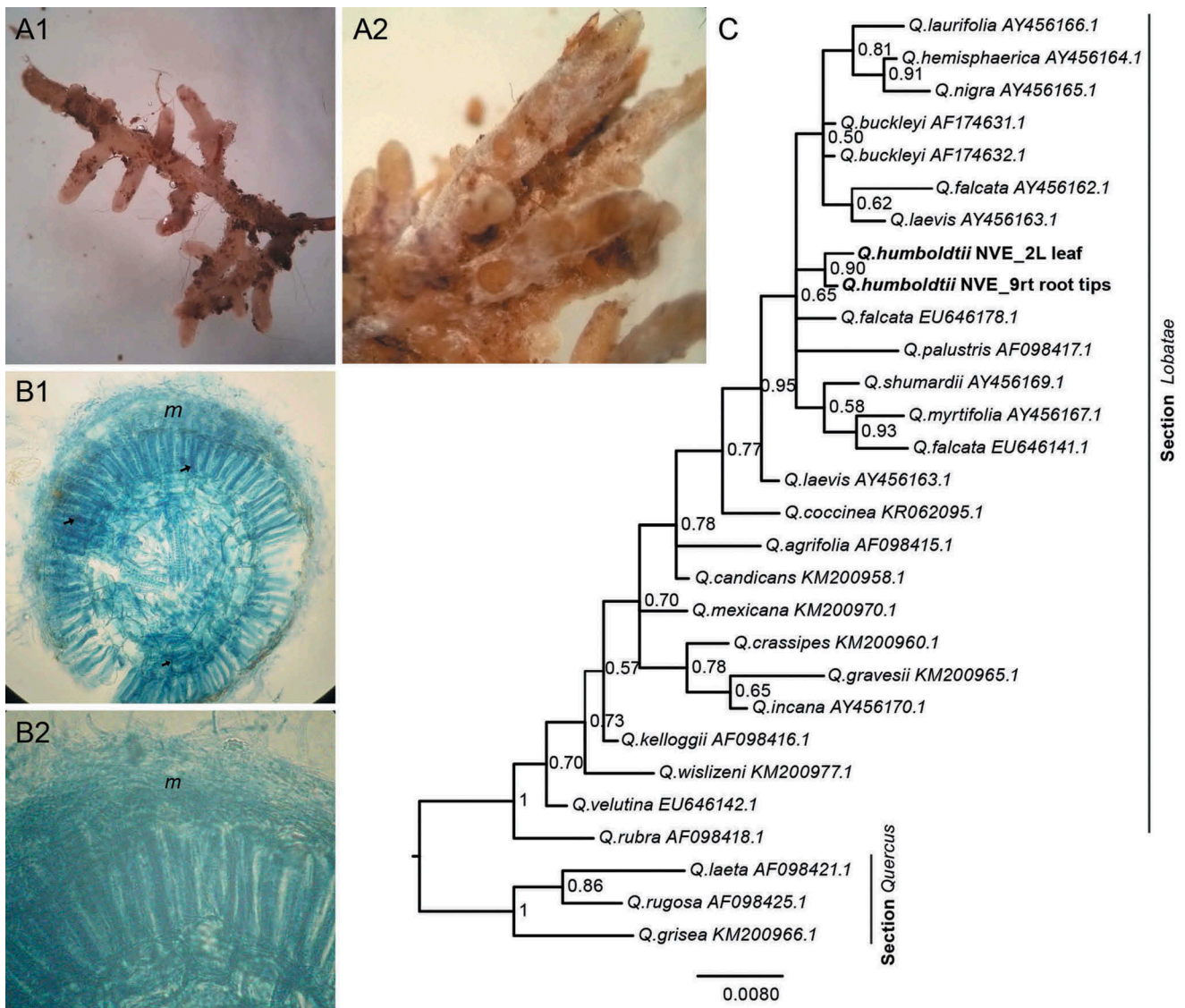
Figure 3. A1–A2. Stereoscope images of root tips isolated from soil collected underneath *A. muscaria* growing near *Q. humboldtii* (sample NVE_9rt). B1–B2. Micrographs of cross-sections treated with trypan blue; m: mantle; black arrows: Hartig net. C. Bayesian phylogenetic reconstruction of sections *Lobatae* and *Quercus* in the genus *Quercus*, based on publicly available ITS sequence data. Posterior probability (PP) values are shown next to the nodes. Plant sequences from our own root tips *Quercus humboldtii* NVE_9rt and leaves *Quercus humboldtii* NVE_2L are shown in bold.

A logical hypothesis for the Eurasian origin of Colombian *A. muscaria* links the early importation of soils from Europe to Africa (Mikola 1970) with the extensive movement of plant material and soils among southern African countries (Deacon 1986; Wells et al. 1986; Richardson et al. 1997, 2014; Read 1998) and subsequent introduction of both soils and seeds to Colombia (FIG. 1). Particularly relevant is the history provided by Mikola (1970). In an attempt to track the movements of soils used to inoculate plants, 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 of African

plantations (Wormald 1975). However, although seeds have clearly moved from southern Africa to Colombia, no literature explicitly records the movement of soils from southern Africa to Colombia.

A. muscaria *is invasive in Colombia*.—Although the term "invasive" is used differently by different authors (Mooney and Drake 1989; Richardson et al. 2000; Rejmánek et al. 2002; Richardson and Rejmánek 2004; Díez 2005; Keller et al. 2011; Simberloff et al. 2012); in general, an invasive species is defined as one that spreads naturally in areas distant from its sites of introduction (even if the spread does not cause any
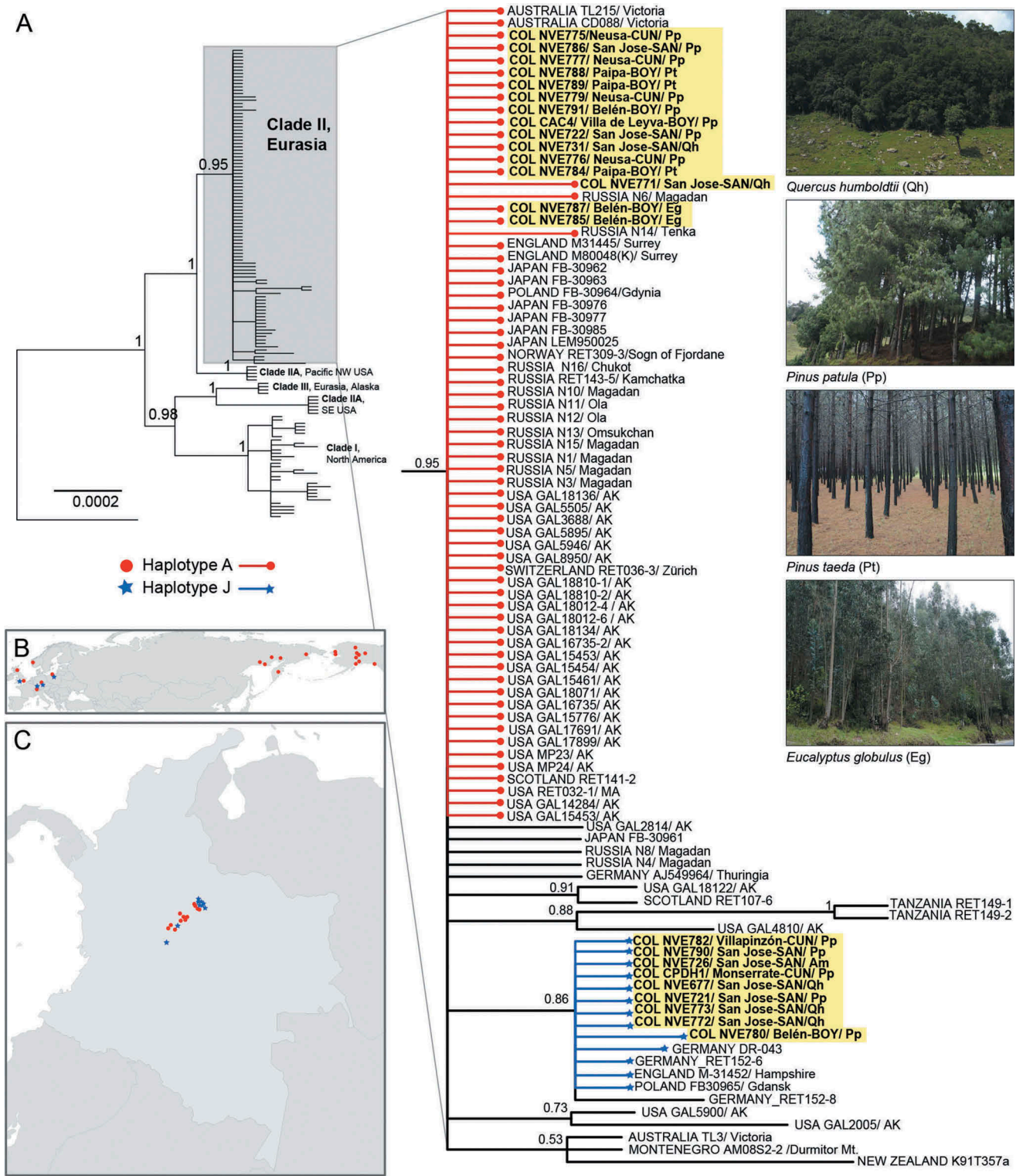
Figure 4. A. Bayesian phylogenetic reconstruction of *A. muscaria* based on publicly available and our own ITS sequence data. Posterior probability (PP) values are shown next to the nodes. Colombian specimen records are highlighted in yellow and named with the following information: COL (Colombia), NVE (collector's name and number)/locality-department: CUN (Cundinamarca), BOY (Boyacá), or SAN (Santander)/abbreviation of the host species (shown in pictures to the right). Other specimen records in the phylogeny were originally collected by and are described in Oda et al. (2004) and Geml et al. (2006, 2008). Haplotypes A and J are shown in red circles and blue stars, respectively, at the tip of branches. B. Distribution of haplotypes A (red circles) and J (blue stars) in the Northern Hemisphere. C. Distribution of haplotypes A (red circles) and J (blue stars) in Colombia.
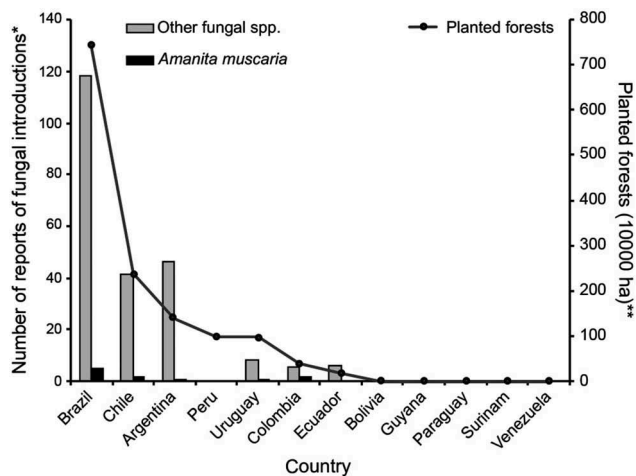
Figure 5. Relationship between the reports of introduced ECM fungi and area (hectares) of industrial tree plantations, for different countries in South America. Sources: *Vellinga et al. (2009); **Overbeek et al. (2012).

economic or environment impact; Richardson et al. 2000; Rejmánek et al. 2002). According to Nuñez and 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.

According to each of these definitions, *A. muscaria* is invasive in Colombia. Although we do not yet know whether *A. muscaria* is causing environmental harm, the fungus is now associating with the roots of a native plant (FIG. 3). The minimum distance between *A. muscaria* associated with that host and *A. muscaria* within a plantation (of *P. patula*) is 851 m, suggesting that the fungus has colonized a novel host at a considerable distance from the parent source (Richardson and Rejmánek 2004). More generally, during the last eight consecutive years, the fungus has persisted, survived, and produced basidiomes in association with *Q. humboldtii* (Vargas, personal observation); the fungus has clearly overcome both geographic and host barriers.

Concluding remarks.—Plantation forestry can have unintended consequences for local biodiversity, although the consequences are not well documented in Colombia. But in Colombia, one consequence of plantation forestry was the introduction of *A. muscaria*. Our data are evidence that two haplotypes of *A. muscaria*, both of Eurasian origin, are present in the Colombian Andes, and that *A. muscaria* has spread from plantations and is now associating with the native Colombian tree *Q. humboldtii*. The costs of eliminating an invasive species can be prohibitive (Keller et al. 2011).

To prevent additional invasions by other fungi, more effective strategies may be the prevention of additional host shifts, or prevention of additional introductions. Exotic tree plantations should be planted apart from native forests (Jairus et al. 2011) to limit potential dispersal to native forests (although exact distances will depend on the dispersal abilities of the associated fungi). It would also be useful to focus on developing local inocula, rather than using inocula of introduced fungi (Schwartz et al. 2006; Dickie et al. 2016). It is quite likely that native fungi can associate with exotic hosts (Bahram et al. 2013), providing the required benefits to planted trees.

The introduction of *A. muscaria* to Colombia was clearly tied to industry and trade, and in fact increases in trade appear to be generally correlated with increases in exotic species introductions (Levine and D'Antonio 2003; Nuñez and Pauchard 2010). In South America, the larger the area used for plantation forestry, the more reports there are of introductions. The expansion of commerce related to plantation forestry seems probable, and unfortunately, more introductions and invasions are likely to occur. Designing policies to reduce the movement and release of non-native species, and to manage those already established (Keller et al. 2011), must emerge as priorities for future programs related to native forest conservation and fungal diversity in Colombia.

ORCID

Susana C. Gonçalves 🆔 http://orcid.org/0000-0001-6308-2662
Anne Pringle 🆔 http://orcid.org/0000-0002-1526-6739

LITERATURE CITED

Agerer R. 2006. Fungal relationships and structural identity of their ectomycorrhizae. Mycological Progress 5:67–107.
Avella A., Cárdenas LM. 2010. Conservación y uso sostenible de los bosques de roble en el corredor de conservación Guantiva-La Rusia-Iguaque, departamentos de Santander y Boyacá, Colombia. Colombia Forestal 13:5–25.

Bagley SJ, Orlovich DA. 2004. Genet size and distribution of *Amanita muscaria* in a suburban park, Dunedin, New Zealand. New Zealand Journal of Botany 42:939–947.

Bahram M, Kõljalg U, Kohout P, Mirshahvaladi S, Tedersoo L. 2013. Ectomycorrhizal fungi of exotic pine plantations in relation to native host trees in Iran: evidence of host range expansion by local symbionts to distantly related host taxa. Mycorrhiza 23:11–19.

Baraloto C, Hardy OJ, Paine CET, Dexter KG, Cruaud C, Dunning LT, Gonzalez MA, Molino JF, Sabatier D, Savolainen V, Chave J. 2012. Using functional traits and phylogenetic trees to examine the assembly of tropical tree communities: assembly of tropical tree communities. Journal of Ecology 100:690–701, doi:10.1111/j.1365-2745.2012.01966.x

Bougher NL. 1996. Diversity of ectomycorrhizal fungi associated with eucalypts in Australia. In: Brundrett M, Dell B, Malajczuk N, Minquin G, eds. Mycorrhizas for plantation forestry in Asia. Canberra, Australia: Australian Centre for International Agricultural Research. p. 8–15.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Canberra, Australia: Australian Centre for International Agricultural Research. 374 p.

Camelo L, Díaz A, Díaz J. 2012. *Acacia melanoxylon*. In: Díaz-Espinosa AM, Díaz-Triana JE, Vargas O, eds. Catálogo de plantas invasoras de los humedales de Bogotá. Bogotá, Colombia: Grupo de restauración ecológica de la Universidad Nacional de Colombia y Secretaría distrital de ambiente. p. 98.

Caro A, Díaz-Espinosa AM, Díaz J. 2012. Pinus patula. In: Díaz-Espinosa AM, Díaz-Triana JE, Vargas O, eds. Catálogo de plantas invasoras de los humedales de Bogotá. Bogotá, Colombia: Grupo de restauración ecológica de la Universidad Nacional de Colombia y Secretaría distrital de ambiente. p. 143–146.

Cavelier C, Tobler A. 1998. The effect of abandoned plantations of *Pinus patula* and *Cupressus lusitanica* on soils and regeneration of a tropical montane rain forest in Colombia. Biodiversity and Conservation 7:335–347.

Céspedes-Payret C, Pineiro G, Achkar M, Gutierrez O, Panario D. 2009. The irruption of new agro-industrial technologies in Uruguay and their environmental impacts on soil, water supply and biodiversity: a review. International Journal of Environment and Health 3:175–197.

Cowan RS, Maslin BR. 2001. *Acacia melanoxylon*. Flora of Australia Online. Australian Biological Resources Study, Canberra, Australia. [cited 2008 Mar 13]. Available from: http://www.environment.gov.au/biodiversity/abrs/online-resources/flora/main/index.html

De Meijer AR. 2001. Mycological work in the Brazilian state of Paraná. Nova Hedwigia 72:105–159.

Deacon J. 1986. Human settlement in South Africa and archaeological evidence from alien plants and animals. In: MacDonalsd IA, Kruger FJ, Ferrar AA, eds. The ecology and management of biological invasions in southern Africa. Cape Town, South Africa: Oxford University Press. p. 4–19.

Dettman JR, Jacobson DJ, Taylor JW. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57:2703–2720.

Dickie IA, Johnston P. 2008. Invasive fungi research priorities, with a focus on *Amanita muscaria*. Landcare Research Contract Report: LC0809/027. Lincoln, New Zealand: Landcare Research. 22 p.

Dickie IA, Bennett BM, Burrows LE, Nuñez MA, Peltzer DA, Porté A, Richardson DM, Rejmánek M, Rundel PW, Van Wilgen BW. 2014. Conflicting values: ecosystem services and invasive tree management. Biological Invasions 16:705–719.

Dickie IA, Bolstridge N, Cooper JA, Peltzer DA. 2010. Co-invasion by *Pinus* and its mycorrhizal fungi. New Phytologist 187:475–484.

Dickie IA, Nuñez MA, Pringle A, Lebel T, Tourtellot SG, Johnston PR. 2016. Towards management of invasive ectomycorrhizal fungi. Biological Invasions 18:3383–3395.

Díez J. 2005. Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian Peninsula. Biological Invasions 7:3–15.

Ducousso M, Duponnois R, Thoen D, Prin Y. 2012. Diversity of Ectomycorrhizal Fungi Associated with *Eucalyptus* in Africa and Madagascar. International Journal of Forestry Research 2012:1–10.

Edgar R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

[FAO] Food and Agriculture Organization. 2010. Global Forest Resources Assessment. FAO Forestry Paper 163. [cited 2015 Jul 22]. Available from: www.fao.org/docrep/013/i1757e/i1757e.pdf

Franco-Molano AE, Aldana-Gómez R, Halling R. 2000. Setas de Colombia (Agaricales, Boletales y otros hongos)—Guía de campo. Medellín, Colombia: Colciencias, Universidad de Antioquia. 156 p.

Fuhrer BA, Robinson RM. 1992. Rainforest Fungi of Tasmania and South-East Australia. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organisation (CSIRO), Melbourne and the Forestry Commission. 95 p.

Fundación Natura. 2007. Elementos conceptuales para la conservación y uso sostenible de los bosques de roble negro (*Colombobalanus excelsa*) y roble común (*Quercus humboldtii*), en jurisdicción de CAS y CORPOBOYACÁ. Bogotá, Colombia: Fundación Natura. 16 p.

Garrido N. 1986. Survey of ectomycorrhizal fungi associated with exotic forest trees in Chile. Nova Hedwigia Kryptogamenkd 43:423–442.

Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL. 2006. Beringian origins and cryptic speciation events in the fly agaric (*Amanita muscaria*): phylogeography of *Amanita muscaria*. Molecular Ecology 15:225–239.

Geml J, Tulloss RE, Laursen GA, Sazanova NA, Taylor DL. 2008. Evidence for strong inter- and intracontinental phylogeographic structure in *Amanita muscaria*, a wind-dispersed ectomycorrhizal basidiomycete. Molecular Phylogenetics and Evolution 48:694–701.

Geml J, Tulloss RE, Laursen GA, Sazanova NA, Taylor DL. 2010. Phylogeographic analyses of a Boreal-Temperate ectomycorrhizal basidiomycete, *Amanita muscaria*, suggest forest refugia in Alaska during the last glacial maximum. In: Habel JC, Assmann T, eds. Relict species:

phylogeography and conservation biology. Berlin and Heidelberg, Germany: Springer-Verlag. p. 173–186.

Giachini AJ, Oliveira VL, Castellano MA, Trappe JM. 2000. Ectomycorrhizal fungi in *Eucalyptus* and *Pinus* plantations in southern Brazil. Mycologia 92:1166–1177.

Giachini AJ, Souza LAB, Oliveira VL. 2004. Species richness and seasonal abundance of ectomycorrhizal fungi in plantations of *Eucalyptus dunnii* and *Pinus taeda* in southern Brazil. Mycorrhiza 14:375–381.

Hall N, Johnston RD, and Chippendale GM. 1975. Forest trees of Australia. Canberra, Australia: Department of Agriculture, Forestry and Timber Bureau. 334 p.

Harley JL, Smith SE. 1983. Mycorrhizal symbiosis. London: Academic Press. 483 p.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Jairus T, Mpumba R, Chinoya S, Tedersoo L. 2011. Invasion potential and host shifts of Australian and African ectomycorrhizal fungi in mixed eucalypt plantations. New Phytologist 192:179–187.

Keller R, Geist J, Jeschke JM, Kühn I. 2011. Invasive species in Europe: ecology, status, and policy. Environmental Sciences Europe 23:23.

Kim CS, Jo JW, Kwag YN, Oh J, Shrestha B, Sung GH, Han SK. 2013. Four Newly Recorded Amanita Species in Korea: Amanita sect. Amanita and sect. Vaginatae. Mycobiology 41:131–138, doi:10.5941/MYCO.2013.41.3.131

Kral R. 1993. *Pinus*. In: Flora of North America Editorial Committee, eds. Flora of North America north of Mexico. Vol. 2. New York: Oxford University Press. p. 352–398.

Ladrach WE, Lambeth C. 1991. Growth and heritability estimates for a seven-year-old open-pollinated *Pinus patula* progeny test in Colombia. Silvae Genética 40:169–173.

Le Maitre DC. 1998. Pines in cultivation: a global view. In: Richardson D, ed. Ecology and biogeography of *Pinus*. Cambridge, UK: Cambridge University Press. p. 407–425.

Le Maitre DC, Versfeld DB, Chapman RA. 2000. Impact of invading alien plants on surface water resources in South Africa: a preliminary assessment. Water South Africa 26:397–408.

Levine JM, D'Antonio CM. 2003. Forecasting biological invasions with increasing international trade. Conservation Biology 17:322–326.

López J, De La Torre F, Cubbage F. 2010. Effect of land prices, transportation costs, and site productivity on timber investment returns for pine plantations in Colombia. New Forests 39:313–328.

Lundquist JE. 1986. Fungi associated with Pinus in South Africa, part I. The Transvaal. South African Forestry Journal 138:1–14.

Malvárez G, Major G, Curbelo V, Frioni L. 1997. Hongos ectomicorrícicos en *Eucalyptus grandis*. Agrociencia, Universidad de la República-Facultad de Agronomía 1:38–43.

Marais LJ, Kotzé JM. 1977. Notes on ectotrophic mycorrhizae of *Pinus patula* in South Africa. South African Forestry Journal 100:61–71.

Mikola P. 1970. Mycorrhizal inoculation in afforestation. In: Romberger H, Mikola P, eds. International review of forest research. New York: Academic Press. p. 123–196.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA. 14 Nov 2010. New Orleans, LA: IEEE. p. 1–8.

Mirov NT. 1967. The genus *Pinus*. New York: The Ronald Press Company. 602 p.

Moeller HV, Dickie IA, Peltzer DA, Fukami T. 2015. Mycorrhizal co-invasion and novel interactions depend on neighborhood context. Ecology 96:2336–2347.

Monacell JT, Carbone I. 2014. Mobyle SNAP Workbench: a Web-based analysis portal for population genetics and evolutionary genomics. Bioinformatics 30:1488–1490.

Montoya F, Arias D, Betancur-Agudelo M. 2005. Contribución al conocimiento de los hongos Macromicetos del resguardo indígena Nuestra Señora de la Candelaria de la Montaña Riosucio, Caldas. Boletín Científico (Centro de Museos) Museo de Historia Natural 9:19–30.

Mooney H, Drake J. 1989. Biological invasions: a SCOPE program overview. In: Drake JA, Mooney HA, di Castri F, Groves RH, Kruger FJ, Rejmánek M, Williamson M, eds. Biological invasions: a global perspective. SCOPE. [cited 2015 Sep 3]. Available from: http://www.scopenvironment.org/downloadpubs/scope37/scope37-ch22.pdf

Moran VC, Hoffmann JH, Donnelly D, Zimmermann HG, Van Wilgen BW, Spencer NR. 2000. Biological control of alien, invasive pine trees (*Pinus* species) in South Africa. In: Spencer NR, eds. Proceedings of the 5th International Symposium on Biological Control of Weeds; Montana State University, Bozeman, Montana; 4–14 July 1999. Bozeman, MT: Montana State University.

Nasi M. 1977. Los hongos superiores de la Sabana de Bogotá y alrededores: descripción botánica, consideraciones ecológicas y bioquímicas, métodos de recolección e identificación, posibilidades de aprovechamiento en Colombia [tesis (Magister en Biología)]. Bogotá, Colombia: Universidad de los Andes.

Nouhra E, Palfner G, Kuhar F, Pastor N, Smith M. 2019. Ectomycorrhizal fungi in South America: their diversity in past, present and future research. In: Pagano M, Lugo M, eds. Mycorrhizal fungi in South America. Fungal Biology. Cham, Switzerland: Springer Nature Switzerland. p. 73–95.

Nullvalue 1996. Los pinos y eucaliptos, un bosque peligroso. Alguna vez ha pensado qué ocurriría si lleva varios camellos del desierto de Sahara a los fríos páramos andinos? El Tiempo. [cited 2015 Jan 20]. Available from: http://www.eltiempo.com/archivo/documento/MAM-299903

Nuñez MA, Dickie IA. 2014. Invasive belowground mutualists of woody plants. Biological Invasions 16:645–661.

Nuñez MA, Horton TR, Simberloff D. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90:2352–2359.

Nuñez MA, Pauchard A. 2010. Biological invasions in developing and developed countries: does one model fit all? Biological Invasions 12: 707–714.

Oda T, Tanaka C, Tsuda M. 2004. Molecular phylogeny and biogeography of the widely-distributed *Amanita* species, *A. muscaria* and *A. pantherina*. Mycological Research 108:885–896.

Orlovich DA, Cairney JW. 2004. Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. New Zealand Journal of Botany 42:721–738.

Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. 2009. Agroforestree Database: a tree reference and selection

guide version 4.0. [cited 2011 May 31]. Available from: http://www.worldagroforestry.org/af/treedb/

Ospina CM, Hernández RJ, Rincon EA, Sánchez FA, Urrego JB, Rodas CA, Ramírez CA, Riaño NM. 2011. El Pino pátula. Guías silviculturales para el manejo de especies forestales con miras a la producción de madera en la zona andina colombiana. Manizales, Colombia: FNC-Cenicafé. 104 p.

Overbeek W, Kröger M, Gerber JF. 2012. An overview of industrial tree plantations in the global south. EJOLT Report No. 03. 100 p. [cited 2015 Nov 10]. Available from: http://www.ejolt.org/2012/06/an-overview-of-industrial-tree-plantations-in-the-global-south-conflicts-trends-and-resistance-struggles/.

Pauchard A, Nuñez MA, Raffaele E, Bustamante RO, Ledgard N, Relva MA, Simberloff D. 2010. Symposium summary: Introduced conifer invasions in South America: an update. Frontiers of Biogeography 2:34–36.

Peterson J. 2001. Pinus taeda L. In: Pines of silvicultural importance. New York: CABI Publishing. p. 470–478.

Posada D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Poyton RJ. 1961. A guide to the characteristics and uses of the trees and shrubs quoted in the price list of the Forest Department. Republic of South Africa: Bulletin of the Department of Forestry No. 39. Pretoria, South Africa: The Government Printer. 50 p.

Price EW, Carbone I. 2005. SNAP: workbench management tool for evolutionary population genetic analysis. Bioinformatics 21:402–404.

Pringle A, Wolfe B, Vellinga E. 2011. Mycorrhiza. In: Simberloff D, Rejmánek M, eds. Encyclopedia of biological invasions. Berkeley, California: University of California Press. p. 468–471.

Pulido M. 1983. Estudios en Agaricales Colombianos: los hongos de Colombia IX. Instituto de Ciencias Naturales, Museo de Historia Natural. Bogotá, Colombia: Universidad Nacional de Colombia. 143 p.

Rambaut A, Drummond A. 2009. Tracer version 1.5 [computer program]. [cited 2015 Sep 2]. Available from: http://beast.bio.ed.ac.uk

Ramírez S. 2009. Reseña histórica de la administración forestal. In: Leguízamo A, ed. Historia y aportes de la ingeniería forestal en Colombia. Bogotá, Colombia: Asociación Colombiana de Ingenieros Forestales. p. 145–170.

Ramírez JA, León-Peláez JD, Craven D, Herrera DA, Zapata CM, González-Hernández MI, Gallardo-Lancho J, Osorio W. 2014. Effects on nutrient cycling of conifer restoration in a degraded tropical montane forest. Plant and Soil 378:215–226.

Read D. 1998. The Mycorrhizal status of Pines. In: Richardson DM, ed. Ecology and biogeography of Pinus. Cambridge, UK: Cambridge University Press. p. 324–337.

Reid DA, Eicker A. 1991. South African fungi: the genus Amanita. Mycological Research 95:80–95.

Rejmánek M, Richardson DM, Babour M. 2002. Biological invasions: politics and the discontinuity of ecological terminology. ESA Bulletin 83: 131–133.

RichardsonDM, Higgins SI. 1998. Pines as invaders in the Southern Hemisphere. In: Richardson DM, ed. Ecology and biogeography of Pinus. Cambridge, UK: Cambridge University Press. p. 450–470.

Richardson DM, Hui C, Nuñez MA, Pauchard A. 2014. Tree invasions: patterns, processes, challenges and opportunities. Biological Invasions 16:473–481.

Richardson DM, Macdonald IA, Hoffmann JH, Henderson L. 1997. Alien plant invasions. In: Cowling RM, Richardson DM, Pierce SM, eds. Vegetation of southern Africa. Cambridge, UK: Cambridge University Press. p. 534–535.

Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ. 2000. Naturalization and invasion of alien plants: concepts and definitions. Diversity and distributions 6:93–107.

Richardson DM, Rejmánek M. 2004. Conifers as invasive aliens: a global survey and predictive framework. Diversity and Distributions 10:321–331.

Richardson DM, Rundel P. 1998. Ecology and biogeography of Pinus: an introduction. In: Richardson DM, ed. Ecology and biogeography of Pinus. Cambridge, UK: Cambridge University Press. p. 3–40.

Richardson DM, Van Wilgen BW, Nuñez MA. 2008. Alien conifer invasions in South America: short fuse burning? Biological Invasions 10: 573–577.

Rivera H, Vega E, Herrera G. 1998. Guia para plantaciones forestales comerciales—Caldas. Serie documental No. 32. Bogotá, Columbia: Corporación Nacional de Investigación y Fomento. 41 p.

Robinson R. 2010. First record of Amanita muscaria in Western Australia. Australasian Mycologist 29:4–6.

Rodríguez-Correa H, Oyama K, MacGregor-Fors I, González-Rodríguez A. 2015. How are oaks distributed in the Neotropics? A perspective from species turnover, areas of endemism, and climatic niches. International Journal of Plant Sciences 176:222–231.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

RStudio. 2018. RStudio: integrated development environment for R (Version 1. 1.442)[computer software]. Boston, Massachusetts. [cited 2018 Feb 2]. Available from: http://www.rstudio.org/

Sawyer J. 1993. Plantation in the tropics: environmental concerns. In: The IUCN Forest Conservation Programme. Gland, Switzerland: UNEP/WWF. 83 p.

Sawyer NA, Chambers SM, Cairney JW. 2001. Distribution and persistence of Amanita muscaria genotypes in Australian P. radiata plantations. Mycological Research 105:966–970.

Shepherd CJ, Totterdell CJ. 1988. Mushrooms and toadstools of Australia. Melbourne, Australia: Inkata Press. 162 p.

Shroomery. 2015. Amanita muscaria in a magic forest in Sumapaz Colombia. [cited 2015 Sep 3]. Available from: http://www.shroomery.org/6675/Amanita-muscaria-in-a-magic-forest-in-Sumapaz

Sicard L, Suarez A. 1998. Efectos de plantaciones forestales sobre suelo y agua. Serie Técnica No. 40. Bogotá, Colombia: Corporación Nacional de Investigación y Fomento, Ministerio del Medio Ambiente.

Simberloff D, Nuñez MA, Ledgard NJ, Pauchard A, Richardson DM, Sarasola M, Van Wilgen BW, Zalba SM, Zenni RD, Bustamante R, Peña E, Ziller SR. 2010. Spread and impact of introduced conifers in South America: lessons from other Southern Hemisphere regions. Austral Ecology 35:489–504.

Simberloff D, Souza L, Nunez MA, Barrios-Garcia MN, Bunn W. 2012. The natives are restless, but not often and mostly when disturbed. Ecology 93:598–607.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecology Letters 9:501–515.

Sobestiansky G. 2005. Contribution to a macromycete survey of the States of Rio Grande do Sul and Santa Catarina in Brazil. Brazilian Archives of Biology and Technology 48:437–457.

Stijve T, de Meijer AA. 1993. Macromycetes from the state of Paraná, Brazil. 4. The psychoactive species. Arquivos de Biologia e Tecnologia 36:313–329.

Turnbull JW, Pryor LD. 1984. Choice of species and seed sources. In: Hillis WE. Brown AG, eds. Eucalyptus for wood production. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organisation (CSIRO), Academic Press. p. 20–22.

Van der Westhuizen GC, Eicker A. 1987. Some Fungal Symbionts of Ectotrophic Mycorrhizae of Pines in South Africa. South African Forestry Journal 143:20–24.

Vargas N, Bernal A, Sarria V, Franco-Molano AE, Restrepo S. 2011. Amatoxin and phallotoxin composition in species of the genus *Amanita* in Colombia: a taxonomic perspective. Toxicon 58:583–590.

Vargas N, Pardo de la Hoz C, Franco-Molano AE, Jimenez P, Restepo S, Grajales A. 2017. Defining the phylogenetic position of *Amanita* species in Colombia. Mycologia 109:261–276.

Vasco-Palacios AM, Franco-Molano AE. 2013. Diversity of Colombian macrofungi (Ascomycota-Basidiomycota). Mycotaxon 121:48.

Vellinga EC, Wolfe BE, Pringle A. 2009. Global patterns of ectomycorrhizal introductions. New Phytologist 181:960–973.

Von Christen H, Ortiz N, de las Salas G. 1998. Los recursos forestales de Colombia y perspectivas para su desarrollo económico. Bogotá, Columbia: Editorial Antares Editores. 136 p.

Wells MJ, Poynton RJ, Balsinhas AA, Musil KJ, Joffe H, Van Hoepen E. 1986. The history of introduction of invasive alien plants to southern Africa. In: MacDonald IA, Kruger FJ, Ferrar AA, eds. The ecology and management of biological invasions in southern Africa. Cape Town, South Africa: Oxford University Press. p. 21–35.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols a guide to methods and applications. New York: Academic Press. p. 315–322.

Wormald TJ. 1975. *P. patula*. Tropical Forestry Papers No. 7. Oxford, UK: Department of Forestry, Commonwealth Forestry Institute, University of Oxford.

Wright JA, Jameel H, Dvorak W. 1996. Laboratory kraft pulping of juvenile tropical pines *P. patula, P. tecunumanii, P. maximinoi* and *P. chiapensis*. Tappi 79:187–199.

Wright JE, Albertó E. 2002. Guía de hongos de la Region Pampeana I. Hongos con lamillas. Buenos Aires, Argentina: LOLA. 279 p.