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Madre Monte Natural Conservation Area in the Colombian Andes as Model for Preservation of Fungi in *Quercus humboldtii* Forests

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HIGHLIGHTS

- Small preservation areas are very important to preserve macrofungi biodiversity.
- The Madre Monte Preservation reserve in the Colombian Andes has contributed to preserve at last 3 rare species, 19 new citations to the Andes and 2 new to the country.
- Change the strategy creating small preservation areas instead of large ones could be an important step in preserving macrofungi.

Abstract: Few conservation studies have been done with Agaricales fungi (mushrooms) of the Andes and conservation areas created in many countries have no priorities in protecting fungi. Small areas can be also very important to preserve rare and unknown fungi species, but evaluations are still needed. Applying the technique of the transects in the natural reserve of Madre Monte in Arcabuco, Boyacá Province, in the Colombian Andes (a characteristic oak forest – *Quercus humboldtii* - Fagaceae), the diversity and ecological data were collected and compared to the fungi already known in the country and their conservation status, to understand how fungi are protected indirectly with the creation of preservation areas. The samples were identified at the Laboratorio del Grupo de Investigación Biología para la Conservación de la Universidad Pedagogica y Tecnológica of Colombia. A total of 331 specimens and 46 species of Agaricales were collected and identified. The presence of rare species, like *Lactifluus gerardii*, *Cortinarius violaceus* and *Cortinarius boyacensis*, reinforces the importance of creating more small areas in the preservation of mushroom species. Only 43 species of Agaricales were reported to the province, being 19 new citations to the area, what is an indication that the diversity in the Colombian Andes is higher than suspected and that even small conservation areas have of profound impact in fungi conservation. Some of the mushrooms found are also

first references to Colombia, like *Panaeolus rickenii* and *Protostropharia dorsipora*, but these were found growing in horse manure, being introduced. The impact of domestic animals and exotic trees in the mycobiota of preservation areas needs to be better evaluated.

Keywords: mycobiota; mushrooms; oak; Agaricales; andine.

INTRODUCTION

Agaricales fungi from Colombia were initially studied in the first half of the last century thanks to Chardon's studies [1]. The most recent list counts for at least 537 species of Agaricales, many of them found only in this country and some exclusive from the Andes region [2]. The studies done so far are essentially taxonomic, and rarely ecological/conservational or involving relationships between the fungi and the forest.

The ectomycorrhizal fungi complex of Central America is also found in Colombia, as *Quercus* forests penetrate this country as well [3], but only one species is known from Colombia: *Quercus humboldtii* Bonpl. – Fagaceae [4]. Studies with this forest fungi are scarce and often restricted to mycorrhizal fungi, and their mycosociology is poorly known [5]. There are also citations of fungi introduced into this forest, including *Amanita muscaria* (L.) Lam., usually associated with pine plantations and found associated with this oak species [8]. The impact of these exotic species to this exclusive forest needs to be better studied.

Among the Andine species, only 43 Agaricales fungi were reported to Boyacá Province, but many more are supposed to occur [2]. The creation of new preservation areas in the Andes region can help in the preservation of rare and undescribed species also of mushrooms, but this needs to be better evaluated and accordingly valued. Few studies have been dedicated to surveys of Agaricales in Colombia and even less in their relationship with conservation areas

The Madre Monte Reserve is located in the Arcabuco municipality and has 17,71 hectares of Andine forests, composed mainly by *Quercus humboldtii* trees, a native oak of the Andes and the unique species of this genus in Colombia. The area is used by the locals as beekeeping being the final products a result of the native nectar and pollen availability. *Eucalyptus* spp. (originally from Australia) are being introduced for this reason too. Seven species of Agaricales were preliminary reported to this preservation area, with 4 new occurrences to Colombia [9].

Even though small reserves are extremely common around the world, empirical studies on their conservational value are disproportionally uncommon in scientific works, which usually focus on defining their value relative to one large reserve, to one or two taxa or ecosystem types [10, 15].

In order to describe species and their interactions, list species, assess conservation status of Agaricales s. l. in a small reserve with a forest of *Quercus humboldtii* (unique oak in Colombia) we proposed the present work, dealing with the taxonomy and mycosociology of this order in the natural reserve of Madre Monte – Arcabuco – Colombia.

MATERIAL AND METHODS

In order to verify the occurrence of Agaricales fungi in the period of early October rainfall (2018) in an area with predominance of *Quercus humboldtii*, a survey on Agaricales mushrooms was carried out in the natural reserve of Madre Monte, in the Arcabuco Municipality, Boyacá Department, in Colombia (Figure 1). The area is located at 5°46'59,32" N and 73°25' 26,85" W, at an elevation of ca. 2500 m. The climate is considered to be Cfb (Köppen-Geiger) with average annual temperature of 14 °C (warm and temperate) and rainfall averages of 1564 mm.

The occurrence of Agaricales mushrooms in four forest areas and in one open field was studied applying transects to compare the results to the list of Agaricales already known from the area. Five transects of 30 meters long and with 40 people searching for fungi up to 4 meters on each side of the line were studied, being 1.200 m^2 the total area of the site examined.

The following areas were sampled (Figure 2):

Area 1- Forest border: some young oaks associated to other trees;
Area 2- Forest border: some young oaks associated to other trees;
Area 3- Open field: open field vegetation, sometimes occupied by horses.
Area 4- Forest interior: old oak trees associated to other trees;
Area 5- Forest interior: old oak trees associated to other trees.

The macrofungi were sought on soil, trunks of dead and living trees, litter, manure and associated to other natural substrates.

All the mushrooms found were collected and taken to the Laboratorio del Grupo de Investigación Biología para la Conservación de la Universidad Pedagogica y Tecnológica de Colombia in Boyacá Department for identification. All samples were photographed and the identification was made with the use of microscopes and usual morphological-anatomical techniques, as well as the specific bibliography available for the area.

The existing list of the Agaricales found in Boyacá Department was compared with the results of this survey and to discuss the importance of preservation areas to preserve Agaricales fungi species [2].

The species were identified using the usual taxonomic works available to Colombia and South America [2, 3, 5, 9, 11, 13, 16].

Figure 1. Map of the studied area in Colombia (left – scale = 100 km) and in Arcabuco municipality (right – scale = 5 km).

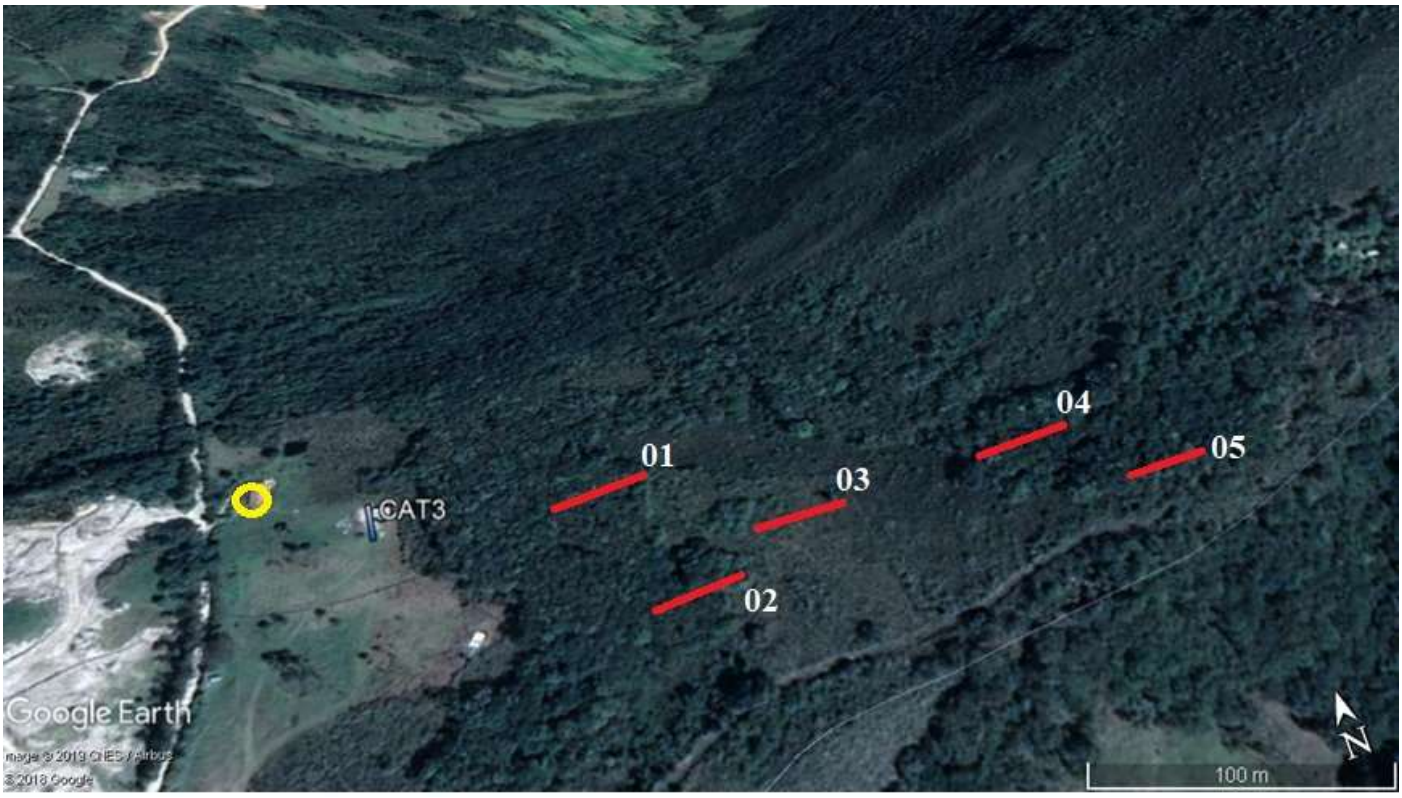

Figure 2. Image of the Madre Monte Reserve and the exact points of each transect studied. (red lines). Circle = reception; CAT3 = beginning of the forested area and trail.

RESULTS AND DISCUSSION

A total of forty-six species of Agaricales fungi (331 specimens) were found in the studied area (Table 1) what represents 8,6% of the Colombian Agaricales list and 19 are new references to the Boyacá Province (Table 1). Only 43 species of Agaricales (excluding *Lycoperdum perlatum* and *Boletales* fungi) were reported to this province (see list below) showing that this survey is an important addition to the agaricobiota of this area [2]. The Marasmiaceae was the most abundant in number of species (14) and in specimens collected, followed by Mycenaceae (10), Cortinariaceae (5), Strophariaceae (3) and Pleurotaceae (2) (Figures 2–3). These numbers per family are in accordance with the literature for similar areas.

It is important to mention that some very large mushrooms have been also found and represent edible species, showing the potential of these in local cuisine if the management plan of the preservation area will allow this. Among these species it can be listed: *Agrocybe praecox, Laccaria fraterna, Lactifluus gerardii*, *Lentinula boryana* and *Pleurotus ostreatoroseus*, all of them showing many basidiomata during the collecting period.

The following species were collected in each sampling area (Table 1):

Table 1. List of species found on each transect (line) with the number of basidiomata collected.

	LINE 01	LINE 02	LINE 03	LINE 04	LINE 05
Agrocybe praecox (Pers.) Fayod (S) – new reference to B - (1)			4		
Campanella aeruginosa Singer (M) – new reference to B - (2)	3				
Campanella elongatispora Singer (M) – new reference to B - (3)	2				
Cheimonophyllum candidissimum (Berk. and Curt.) Singer (Cy) – new reference to B - (4)	26				
Collybia fusipes (Bull.) Quél. (= *Gymnopus*) (M) – new reference to B - (5)					7
Cortinarius boyacensis Singer (C) - (6)					1
Cortinarius violaceus (L.) Gray (C) – new reference to B - (7)					1
Cortinarius sp. 1 (young) (C) - (8)				1	2
Cortinarius sp. 2 (young) (C) - (9)				1	1
Cortinarius sp. 3 (young) (C) - (10)				1	2
Craterellus fallax A. H. Sm. (C1) – new reference to B - (11)					1
Crepidotus croceitinctus Peck (C2) – new reference to B - (12)					19
Favolaschia dealbata Singer (My) – new reference to B - (13)	5				
Galerina sp. (C) - (14)					1
Hydropus sp. (M) - (15)					2
Hypholoma subviride (Berk. & Curt.) Dennis (S) – new reference to B - (16)				3	
Laccaria fraterna (Cooke & Mass. ex Sacc.) Pegler (H) – new reference to B - (17)					5
Lactifluus gerardii (Peck) Kuntze (R) – new reference to B - (18)	2	2			
Lentinula boryana (Berk. Mont.) Pegler (M) - (19)	16	8		4	3
Marasmiellus bolivarianus Singer (M) – new reference to B - (20)		8			
Marasmiellus sp. 1 (M) - (21)	3	8		4	
Marasmiellus sp. 2 (M) - (22)	2			2	
Marasmius sp. 1 (M) - (23)	2	2			
Marasmius sp. 2 (M) - (24)	1	2			
Marasmius sp. 3 (M) - (25)	2	2			
Marasmius sp. 4 (M) - (26)				97	2
Marasmius sp. 5 (M) - (27)				1	3
Marasmius sp. 6 (M) - (28)				2	3
Mycena sp. 1 (My) - (29)	2	2			
Mycena sp. 2 (My) - (30)	3	2			
Mycena sp. 3 (My) - (31)		1			
Mycena sp. 4 (My) - (32)	1	1		1	6
Mycena sp. 5 (My) - (33)	2			3	4
Mycena sp. 6 (My) - (34)	2				
Mycena sp. 7 (My) - (35)		2			
Mycena sp 8 (My) - (36)		1			
Omphalina sp. 4 (T) - (37)	1				
Panaeolus rickenii Hora (C3) – new reference to B- (38)			1		
Pleurotus sp. (P) - (39)				5	
Pleurotus ostreatoroseus Singer (P) – new reference to B - (40)					3
Pluteus chrysophlebius (Berk. & Curt.) Sacc. (P1) – new reference to B - (41)	3	2		4	2
Psilocybe coprophila (Bull.) Kumm. (= *Deconica*) (S) - (42)			1		
Protostropharia dorsipora (Esteve-Rav. & Barrasa) Redhead (S) – new reference to B - (43)			1		
Protostropharia semiglobata (Fr.) Redhead (S) – new reference to B - (44)			4		
Xerocomus sp. (B) - (45)			1		
Xeromphalina campanella (Batsch) Maire (My)–new reference to B - (46)					1
Total number of basidiomata	78	43	12	129	69

Legend: B- Boyacá Province; (C) – Cortinariaceae; (C1) - Craterellaceae; (C2) - Crepidotaceae; (C3) - Coprinaceae; (Cy) – Cyphellaceae; (H) - Hydnangiaceae; (M) - Marasmiaceae; (My) – Mycenaceae; (P) – Pleurotaceae; (P1) – Pluteaceae; (R) - Russulaceae; (S) - Strophariaceae; (T) – Tricholomataceae; (X) - Xerocomaceae. Numers in parenthesis are the species shown in Figure 3 and 4.

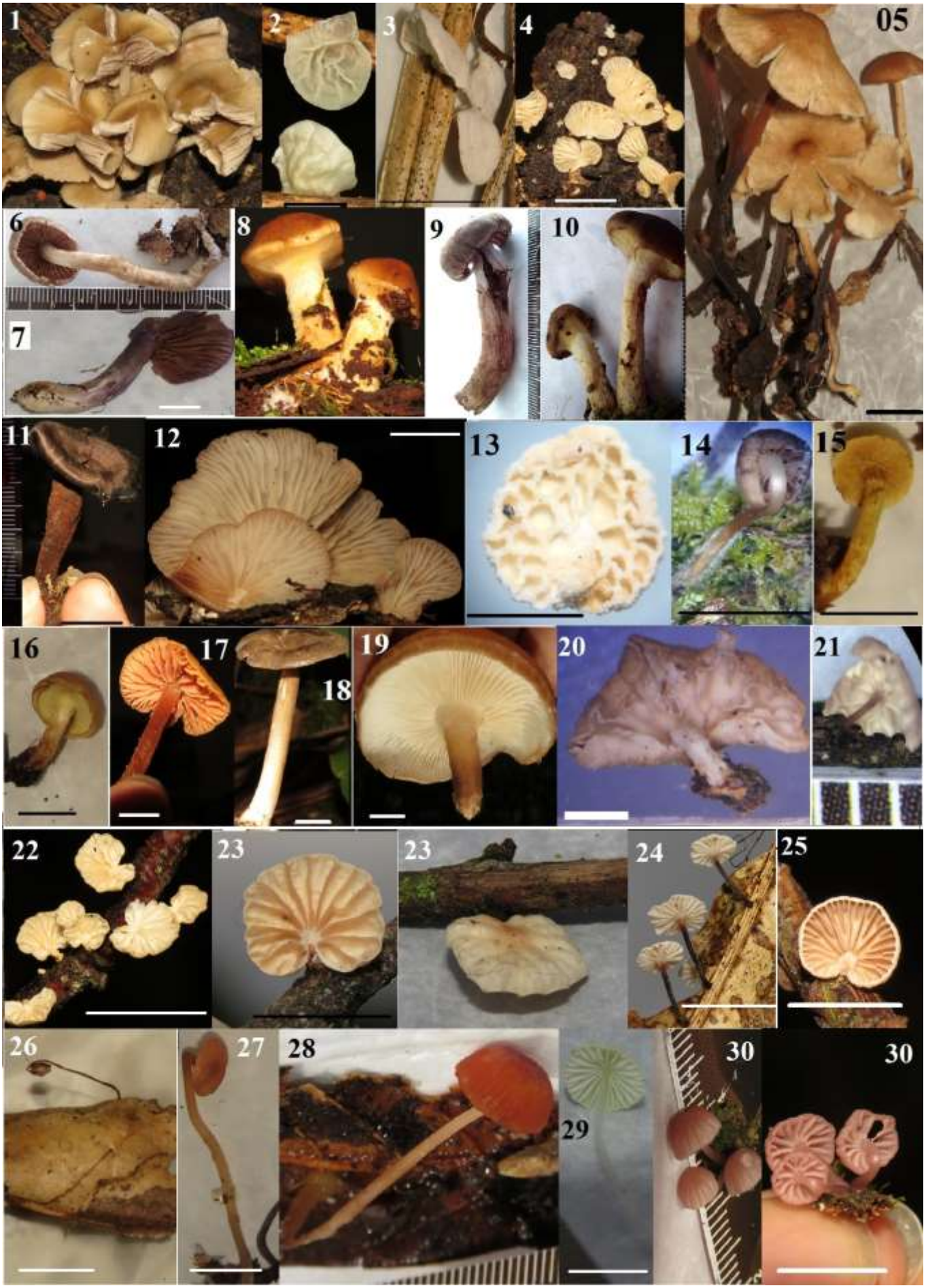

Figure 3. Species of Agaricales found with numbers according to Table 1.

Figure 4. - Species of Agaricales found with numbers according to Table 1.

Line 1 – Forest border:

In this area many small basidiomata were found, especially *Campanella* spp. (two species), *Mycena* (5), *Marasmiellus* (2) and *Marasmius* (3). In addition, *Cheimonophyllum candidissimum*, *Omphalina* sp. and *Favolaschia dealbata* were also found. This is due to the fact that a thick layer of foliage and small twigs are present in the studied environment and, as these genera prefer this organic source, they were found associated. Many of these fungi occur on fallen wooden stick not exactly on the ground but suspended among other trees, so that the collector needs to look upwards sometimes to find them. As for example *Campanella*

aeruginea and *C. elongatispora* were found at up to 2 m from the soil level. As forest litter is maintained inside conservation areas, where fires are controlled, this group of important fungi is also preserved.

Lactifluus gerardii a typical mycorrhizal fungus was also found, reported before only from Antioquia department in Colombia [11]. The species is found also in North America and Asia but probably they were not conspecific. It is used as food in Asia [12].

Line 2 – Forest border:

In this area, large-sized mycorrhizal mushrooms of the genus *Cortinarius* were absent like in Line 01. *Mycena* (six species), *Marasmiellus* (two species) and *Marasmius* (three species) were also found on the leaflet. *Marasmiellus bolivarianus* was collected and seems to be restricted also to the Andean environment in South America (Venezuela and Colombia), despite a citation as *aff.* to Indonesia [13]. Here again *Lactifluus gerardii* was also found.

Line 3 – Open Field:

Mushrooms associated with manure of horses grazing in the sector were found, besides *Agrocybe praecox*, a wood decomposer. Occurring on horse manure, there were found:

- *Stropharia semiglobata*
- *Stropharia dorsipora*
- *Panaeolus rickii*
- *Psilocybe coprophila*

Panaeolus rickenii and *Protostropharia dorsipora* are new to the Colombian mycobiota. It is very important to study the interference of domestic animals in the Andes ecosystem, not only in phanerogams composition but also on biodiversity changes in fungi composition and try to register the fungi introduced up to now in this ecosystem and the implications of that.

Xerocomus sp. (Boletaceae – Boletales) found 20 m from the forest border is probably mycorrhizal and associated to trees found nearby. This shows that mycorrhizal fungi of forest are sometimes found forming basidiomata outside the oak forest. This was the only fungi found of this order in all transects studied.

Line 4 – Inside forest:

In this line there were found at least 3 species of *Cortinarius*, which were probably developing due to the intense rain that occurred in this period, but have not yet reached maturity, on contrary of Line 5, where *Cortinarius* were well developed, but of different species. Even in the humid chamber it was impossible to develop these primordia to maturity, indicating a very slow growth or dependence on the forest tree associated (mycorrhizal).

Also, in this transect the first lignicolous fungi of larger diameter were found, because many arboreal individuals are dead inside this sector. *Pleurotus* sp. is prominent in this area, occurring in many basidiomata. *Mycena* spp., *Marasmiellus* (two species) and *Hypholoma* sp. were also found. *Marasmius* sp. 4 (97) had the highest number of basidiomata among all collections, typical of this group when growing on small twigs.

Line 5 – Inside forest:

Again, as in the previous transect (Line 4), young individuals of *Cortinarius* spp. but some already more developed of *Cortinarius violaceus* and *Cortinarius boyacensis* were found. Also common in larger trunks were individuals of *Crepidotus croceitinctus*, *Hydropus* sp., *Mycena* spp. and *Galerina* sp. *Collybia fusipes* and *Mycena* spp. were found on plant debris. *Laccaria fraterna* and *Craterellus fallax* also appeared in this transect, both growing as mycorrhizae and new citations to this province, but the first species probably associated to *Eucalyptus* sp. trees planted near the native forest as source of nectar for honey production. This introduction is changing the microflora as different mycorrhiza fungi are also introduced.

Common to all transects studied inside forests and in the border were the specimens of *Lentinula boryana* (that is reported for high mountain forests of *Quercus*) and *Pluteus chrysophlebius* (deep yellow basidiomata), both growing on wood, besides one *Mycena* sp. It is surprising that all other species were found common to only two transects or exclusive to only one. This probably indicates that the diversity is very high in the *Quercus humboldtii* forest and that more transects are needed to complete the species list for this season and region.

Lentinula boryana is important locally since it forms large basidiomata and is edible, being commonly found from 1800 to 2900 m of elevation [2]. This is the case also of the *Pleurotus* spp., all of them edible and also cultivated in Colombia [14].

The number of mature basidiomata formed in *Marasmius* sp. 4 (97 – line 3), *Cheimonophyllum candidissimum* (26 – line 1) and *Crepidotus croceitinctus* (19 – line 5) where the biggest and reflects the small size of these species, usually growing aggregate on dead trunks. The largest number of basidiomata was found in the Line 4 (Inside forest) followed by forest border (Line 1), inside forest (line 5), border (Line 2) and open field (Line 3). These numbers are affected by the availability of organic matter, humidity and other factors, including predation. It is also important to mention that the mycorrhizal fungi found can survive with the help of the tree associated, especially the native oak, not depending so much on these abiotic factors exposed above.

More than half of the samples (24 collections) were identified only at genus level, many of them probably new species to science and further studies are needed to confirm this. Of the remaining samples, almost all (19 of 22 collections) were new references to Boyacá Province (elevating the list of species from 46 to 62), showing that more taxonomic studies are needed in the oak forest to complete the list.

List of agaricoid species formerly reported to Boyacá Province [2] (* = new references to Boyacá Province from this work):

Agaricus bisporus (J. E. Lange) Pilát
**Agrocybe praecox* (pers.) Fayod
Amanita brunneolocularis Tulloss, Ovrebo & Halling
Amanita ceciliae (Berk. & Broome) Bas. (sin. *Amanita inaurata* Secr.)
Amanita fuligineodisca Tulloss, Ovrebo & Halling
Amanita muscaria (L.) Lam.
Amanita picea Tulloss, Ovrebo & Halling
Amanita sororcula Tulloss, Ovrebo & Halling
* *Campanella aeruginosa* Singer
Campanella castaneipes Singer
* *Campanella elongatispora* Singer
Chaetocalathus liliputianus (Mont.) Singer
**Cheimonophyllum candidissimum* (Berk. & Curt.) Singer
* *Collybia fusipes* (Bull.) Quél.
Cortinarius boyacensis Singer
**Cortinarius violaceus* (L.) Gray
Craterellus boyacensis Sing.
**Craterellus fallax* A. H. Sm.
**Crepidotus croceitinctus* Peck
**Favolaschia dealbata* Singer
Filoboletus gracilis (Klotzsh ex Berk.) Singer
Fistulina hepatica (Schaeff.) With.
Galerina columbiana Singer
Galerina oligocalyptrata Singer
Hydropus marasmioides Singer
Hygrocybe rosea Murrill
Hygrophorus cossus (Sowerby) Fr.
Hygrophorus quercuum Singer
**Hypholoma subviride* (Berk. & Curt.) Dennis
* *Laccaria fraterna* (Cooke & Mass. ex Sacc.) Pegler
Laccaria laccata (Scop.) Cooke
**Lactifluus gerardii* (Peck) Kuntze
Lentinula boryana (Berk. & Mont.) Pegler
Macrolepiota colombiana Franco-Mol.
**Marasmiellus bolivarianus* Singer
Marasmius berteroi var. *major* Singer
Marasmius haematocephalus var. *pseudotageticolor* Singer
Marasmius nebularum Singer
Marasmius rotalis Berk.

Melanomphalia columbiana Singer
Panaeolus antillarum (Fr.) Dennis
Panaeolus cyanescens (Berk. & Broome) Sac.
* *Panaeolus rickenii* Hora
Panellus nubigenus Singer
Pholiota privigna (Speg.) Singer
* *Pleurotus ostreatoroseus* Singer
Pluteus cervinus P. Kumm
**Pluteus chrysophlebius* (Berk. & Curt.) Sacc.
* *Protostropharia dorsipora* (Esteve-Rav. & Barrasa) Redhead
* *Protostropharia semiglobata* (Fr.) Redhead
Pseudomphalina arsitophylli Singer
Psilocybe argentina (Speg.) Singer
Psilocybe bullacea (Bull.) P. Kumm.
Psilocybe coprophylla (Bull.) P. Kumm.
Psilocybe cubensis (Earle) Singer
Psilocybe phyllogena (Peck) Peck
Psilocybe subcubensis Guzmán
Rickenella fibula (Bull.) Raith.
Schizophyllum commune Fr.
* *Xeromphalina campanella* (Batsch) Maire
Xeromphalina helbergeri Singer
Xeromphalina tenuipes (Schwein.) A. H. Sm.

BOLETALES
Boletus subtomentosus L.

Gasteromycete
Lycoperdon perlatum Pers.

This survey is an indication that even small protected areas like Madre Monte Natural Conservation Area, with only 17,71 ha, can house many species of Agaricales among those already described for the Andes, being an excellent way to preserve them [6, 7].

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