Chapter 15
Diversity and Importance of Edible Mushrooms in Ectomycorrhizal Communities in Mexican Neotropics

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15.1 Introduction

Fungi carry out multiple ecosystem functions such as decomposing organic material, recycling nutrients, optimizing nutrient availability, and improving soil structure and stability. Due to their essential role in ecosystems, mycorrhizal fungi are one of the most important functional groups. Approximately 95% of terrestrial plants develop mycorrhizae and depend on this relationship to establish, survive, and develop (Finlay and Read 1986; Van der Heijden et al. 2015). Mycorrhizae are basically formed by three major fungi groups: Glomeromycota, which forms arbuscular mycorrhizae; Ascomycota and Basidiomycota, which form ectomycorrhizae, ectendomycorrhizae, arbutoid, and orchidiod mycorrhizae, among others. The ectomycorrhizal association is established between more than 5000 fungal species and plants in the families Betulaceae, Casuarinaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Fagaceae, Myrtaceae, Pinaceae, Salicaceae, etc. Although the number of plant species involved is not high, most of these are dominant shrubs and trees of temperate and boreal ecosystems and therefore of great ecological importance (Brundrett 2009).

Traditionally, the study of ectomycorrhizal fungi (EMF) has been based on their sexual reproductive structures or fruit bodies. However, the knowledge of their diversity is biased by this method because most of these fungi do not have sexual

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reproduction or either, produce hypogeous fruit bodies, or are inconspicuous. Their ecological knowledge is also influenced by this bias, because it is the vegetative state of these fungi that has the most impact in the ecosystem, not their reproductive state. The production of fruit bodies only reflects the energy invested in sexual reproduction and is not an extension of their vegetative state. Therefore, the development of molecular techniques to study mycorrhizae directly from the roots as well as to study the mycelium in the soil has revolutionized our understanding of these fungi. The use of these techniques has shed light on numerous unknown lineages; the fact that many fungi thought to be saprobes are in fact mycorrhizal; and the discrepancy between the diversity and structure of fungi communities when studied by their mycorrhizae than when studied by the fruiting bodies found (Horton and Bruns 2001).

With almost 400 species of edible mushrooms, Mexico possess the second largest edible mushroom biocultural heritage worldwide (Garibay-Orijel and Ruan-Soto 2014), just after China. There, more than 15 million indigenous people and 105 million mestizos have incorporated wild edible mushrooms into their traditions and diet. The genera *Pinus* and *Quercus* have one of their diversification centers in Mexico. The diversity and endemism of both genera in Mexico is, therefore, high (Gernandt and Pérez-de la Rosa 2014; Aguilar-Romero et al. 2016). Both pines and oaks depend on their relationships with EMF for their establishment, growth, and survival. In order to develop a sustainable management of edible ectomycorrhizal mushrooms we must understand their role in ectomycorrhizal communities as these sustain the production of their fruit bodies. The study of EMF as components of mycorrhizal symbiosis provides information not only on their ecology, taxonomy, and systematics but also on their relationship with their hosts and their role in the forest. Therefore, it is now possible to know which species of fungi are associated with certain plant species. This information is essential because it allows us to propose reforestation and restoration plans that are adequate for the plant species and the soil conditions in specific regions. Additionally, this information is useful in forestry plantations, where selecting for the correct fungi would give trees greater growth and higher survival rates. Industrialized countries are already strengthening their forestry sector with the use of this technology. However, in Mexico, the use of this technology is just beginning and it will not continue to develop if the basic information on the fungi diversity and ecology is not produced.

In this chapter we explore the diversity and structure of ectomycorrhizal fungi communities associated with the main forest ecosystems of the Mexican Neotropics, with particular emphasis on edible species.

15.2 Materials and Methods

The convergence of two major ecoregions, the Neartic and the Neotropic, makes Mexico a biodiverse country. Most studies on the diversity and molecular ecology of ectomycorrhizal fungi have taken place in the Holarctic (specifically in USA,

Canada and Europe). Unfortunately, fungi of the temperate forests of the Mexican Neotropics have been poorly studied. Identifying the EMF in the Neotropics is fundamental for our understanding of their biological history, evolution and distribution. This study provides information on the diversity of EMF of the Mexican Neotropics.

We integrated data from fruit body sampling from 2009 to 2015 with data from ectomycorrhizal sampling to evaluate the diversity of EMF. For this purpose, we selected study sites that represented the main vegetation types that host ectomycorrhizal fungi: *Abies religiosa* forests in el Zarco, Estado de Mexico (Argüelles-Moyao et al. 2016); *Alnus* forests in La Malinche, Tlaxcala and in Acatlán, Veracruz (Kennedy et al. 2011); *Pinus* spp. forests in Cuitzeo, Michoacán (Garibay-Orijel 2008); *Quercus* spp. forests in Cuitzeo, Michoacán (García-Guzmán et al. 2017); and tropical dry forests in Chamela, Jalisco (Álvarez-Manjarrez et al. 2018).

15.2.1 Sampling of Fruit Bodies

EMF fruit bodies were collected extensively during the rainy season of the year (June to November) from 2009 to 2015. Most of the sampling took place in Amanalco, "el Zarco," "Nevado de Toluca" and Temascaltepec in "Estado de Mexico," and Cuitzeo in Michoacán. The objective was to collect all EMF species encountered, although emphasis was placed in groups of ectomycorrhizal fungi that had been previously overlooked in mycological studies in Mexico. Therefore, sampling focused on those fungi that produced hypogeous fruit bodies (truffles and false truffles) or resupinate fruit bodies. Collected specimens were described, photographed, and dehydrated according to the techniques suggested by Cifuentes et al. (1986) and Halling (1996). Samples were never dried in temperatures over 60 °C to preserve the DNA, and in some cases small fragments of hymenium (0.5 cm^2) were placed in 96% ethanol. Specimens were identified by observation and measurement of their microscopic characteristics following Largent et al. (1984). Specimens were then deposited in the fungi collection of the "Herbario Nacional" (MEXU) at UNAM. Fungi identification was done using dichotomous keys and specialized monographies. Additionally, we contrasted conventional taxonomic identification with identification with DNA nucleotide similarity sequences based on the ITS region.

15.2.2 Sampling of Mycorrhizae

In each sampling site we made 10 parallel transects where mycorrhizae were sampled. Each transect was 60 m long and separated by 20 m. We took soil cores of 5 cm in diameter by 30 cm long. In each transect we took three soil cores separated

by 20 m. From each sample, we haphazardly obtained 15–20 mycorrhizae. In total, we sampled 500–1000 mycorrhizae per site.

The study of *Abies religiosa* EMF was done at the locality of el Zarco, in "la Sierra de las Cruces, Estado de Mexico" (19° 17′ 36″ N, 99° 21' 18″ W). This locality has a monodominant forest of *Abies religiosa*. Soils in this area are humic Andosols and ocric Andosols, both of medium texture. The area has a mean annual temperature of 10.2 °C with an average rainfall of 1241.7 mm (INEGI 2011). In 2013, we sampled seven plots with similar tree characteristics (diameter at breast height, average distance between trees, data not shown). Details of the study site and sampling design can be found in Argüelles-Moyao et al. (2016).

The study of EMF associated with *Alnus* spp. was done in two sites in Tlaxcala and two sites in Veracruz. The sites in Tlaxcala are located in "La Malinche National Park" (19° 16′ 04″ N, 98° 02′ 07″ W, 3283 m asl and 19° 11′ 17″ N, 97° 58′ 58″ W, 2929 m asl). Soils in these sites are Andosols, climate is cold subtropical (Cw2) with a mean annual temperature that fluctuates between 12 °C and 18 °C, and a mean average rainfall of 620–928 mm. Forests of *Alnus jorullensis* and *Pinus montezumae* are found within these sites. The other two sites are found in the Acatlán Volcano, Naolinco, Veracruz (19° 40′ 29″ N, 96° 51′ 07″ W, 1816 m asl and 19° 40′ 58″ N, 96° 51′ 28″ W, 1880 m asl). These sites have Andosol soils, climate is C(wa) humid subtropical with a mean annual temperature that fluctuates between 15.6 °C and 16.0 °C, and a mean average rainfall of 2400 mm. In these sites the only mycorrhizal host was *A. acuminata.* The four sites were sampled in 2010; details on sampling design and molecular procedures can be found in Kennedy et al. (2011).

The study of EMF associated with oak-pine forests and oak forests were done at Cuitzeo basin. The basin has an area of approximately 4000 km^2 and is located north of Michoacán and south of Guanajuato. Soils in the area are Vertisols, Luvisols, Andosols, and Acrisols. Climate is subtropical highland (Cwb) with summer and winter rains. Mean annual temperature is 15 °C and has a year average of 1000 mm rainfall (Mendoza et al. 2011). Dominant vegetation types are scrubs, *Quercus*, *Pinus-Quercus*, and *Abies* in a fragmented landscape with croplands in mosaic pattern. Sampling of these oak-pine forests was done in 2007. We located eight samplings sites with a dominant vegetation of *Pinus* spp. and with a different proportion of *Quercus* spp. Details on sampling design can be found in Garibay-Orijel (2008).

At Cuitzeo basin four more sampling sites within oak forest were chosen in 2010. In those sites, *Quercus deserticola*, *Q. castanea*, *Q. obtusata*, *Q. magnolifolia*, *Q. rugosa*, and *Q. laeta* are the only hosts for ectomycorrhizal fungi. Sampling details for this site can be found in García-Guzmán et al. (2017).

The study of EMF associated with tropical dry forest was done at Chamela Biological Station, Jalisco (19° 30′ N, 105° 03′ W) during the years of 2012–2014. Soils in this area are heterogeneous: eutric and chromic Cambisol, chromic and haplic Lixisol, haplic Ferrasol, and rendzic, litic Leptosols. Climate is warm subhumid (Aw0) in summer and dry in winter (Bshw). Mean annual temperature is 26.9 °C with an average rainfall of 1394 mm. The dominant plant family in this area is Fabaceae. Sampling details can be found in Alvarez-Manjarrez et al. (2018).

15.2.3 Molecular Techniques

Taxonomic identification of ectomycorrhizae and sporocarps was based on the Internal Transcribed ribosomal Spacer (ITS1 and ITS2) of the nuclear DNA region. This region provides sufficient resolution to differentiate samples at the species level and it is one of the most represented in the genetic databases (Horton and Bruns 2001), mainly because it is used as the barcode of life for fungi (Schoch et al. 2012).

The process of extraction, amplification, and sequencing followed the protocol by Izzo et al. (2005) with modifications. DNA was extracted with the kit XNAP REDExtract-N-Amp (Sigmal-Aldrich, St. Louis, Mo, EUA). The advantage of this method is that it allows the DNA of both the fungus and the plant to be extracted from the mycorrhizae. Therefore, it is possible to identify the fungi that are associated with each tree without ambiguity. Although the DNA of the fungi and the plant are mixed in a solution, it is possible to isolate them by using selective amplification with PCR. For this reason, the fungi ITS (ITS1–5.8S-ITS2) region was amplified with the primers ITS1F, ITS4, or ITS4B (Gardes and Bruns 1993; White et al. 1990). When necessary, the chloroplast *trnL* region was amplified for the plants with the primers proposed by Taberlet et al. (1991). PCR products were cleaned with ExoSAP-IT (USB Corporation, Cleveland, Ohio, EUA) and were sequenced with Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, EUA) under the terms specified by the manufacturers. Finally, DNA sequences were obtained with an ABI 3000 automatic sequencer from the "Laboratorio de Secuenciación de la Biodiversidad y la Salud" of the Biology Institute of the "Universidad Nacional Autónoma de México."

DNA sequences were clustered according to a 97% nucleotide similarity in OTUs (Operational Taxonomic Units). This percentage is accepted as standard in EMF molecular ecology studies (Peay et al. 2008). Sequences of each OTU were later compared with the database in GenBank (NCBI) through BLAST (Altschul et al. 1997) and the assigned taxonomy followed the criteria from García-Guzmán et al. (2017). OTUs were considered species only when they were assigned to a taxon.

Possible degree of endemism was determined following two criteria: (a) distribution in Mexico, if the species had a restricted distribution between study sites; and (b) world distribution, if the species had already been registered in another part of the world. This was determined by the presence of at least one ITS sequence in GenBank. Those species restricted to the Mexican Neotropics and not previously sequenced in another part of the world were considered potentially endemic.

15.3 Results and Discussion

15.3.1 Diversity of Edible Ectomycorrhizal Fungi in the Mexican Neotropics

DNA sequences from the ITS region were obtained for approximately 1300 sporocarps and 2683 ectomycorrhizae, most of these acquired from the "Eje Neovolcánico Transversal" sampling sites. Approximately 4000 samples have been sequenced. These sequences, grouped at 97% of nucleotide similarity, represent 804 fungal species. Out of the 804 species, 693 are ectomycorrhizal. The remaining 111 species are parasitic fungi, saprobes, or endophytes present in sampled mycorrhizae or from fruit bodies of saprobe species. In Mexico, 371 species of wild edible mushrooms are consumed traditionally by indigenous and mestizo people and 229 of these are ectomycorrhizal (Garibay-Orijel and Ruan-Soto 2014). So these 229 edible mushrooms represent 33% of the known ectomycorrhizal fungi from Mexican Neotropics.

The 693 species of the EMF collected belong to 85 genera. The genera with the highest species richness were *Tomentella* (77 species), *Inocybe* (57), *Russula* (54), *Sebacina* (47), *Ramaria* (30), *Amanita* (23), *Thelephora* (21), *Lactarius* (19), and *Clavulina* (16) (Fig. 15.1). These numbers are not estimates, but effectively collected species either as fruit bodies or mycorrhizae. Owing to this sampling effort, one of the most important contributions of this work was the collection of more than 100 fruit bodies of the genus *Tomentella*, out of which only four species were known to Mexico. It is now obvious that this genus is more diverse than what was previously thought. Furthermore, most of these species are new to science (Alvarez-Manjarrez et al. 2016). We were also able to collect more than 50 specimens of the family *Sebacinaceae*, out of which only three species were previously known to Mexico.

Information was generated on the geographic distribution of all species. Species with the widest distribution in the temperate forests were: *Tuber separans* s.l., *Rhizopogon* group *ellenae* sp. 1, *Laccaria vinaceobrunnea*, *Sistotrema confluens*, *Laccaria trichodermophora*, *Amanita rubescens* s.l., *Rhizopogon* group *ellenae* sp. 2, *Suillus pseudobrevipes*, *Helvella* cf. *lacunosa*, and *Laccaria laccata* s.l. Out of these species, *A. rubescens* s.l., *H.* cf. *lacunosa*, *L. laccata* s.l., *L. trichodermophora*, *L. vinaceobrunnea*, and *S. pseudobrevipes* are considered edible fungi and traditionally part of the Mexican cuisine (Garibay-Orijel and Ruan-Soto 2014).

When we include all of the species collected, either by fruit bodies or mycorrhizae, into an accumulation curve with 50 random permutations (Fig. 15.2), it is evident that we have not achieved to reduce the steep slope of species diversity. The diversity of EMF is much greater than what our sampling has managed to capture. Moreover, the richness estimator, Chao 1, determined 2611 potential EMF species for the Mexican Neotropics. Most of our sampling was centered in the temperate zone of the Neotropics; therefore, we do not have sufficient data of the tropical ecosystems in Mexico. Including more sampling sites from the tropics would surely increase Chao 1's estimation. Another reason why the species accumulation curve

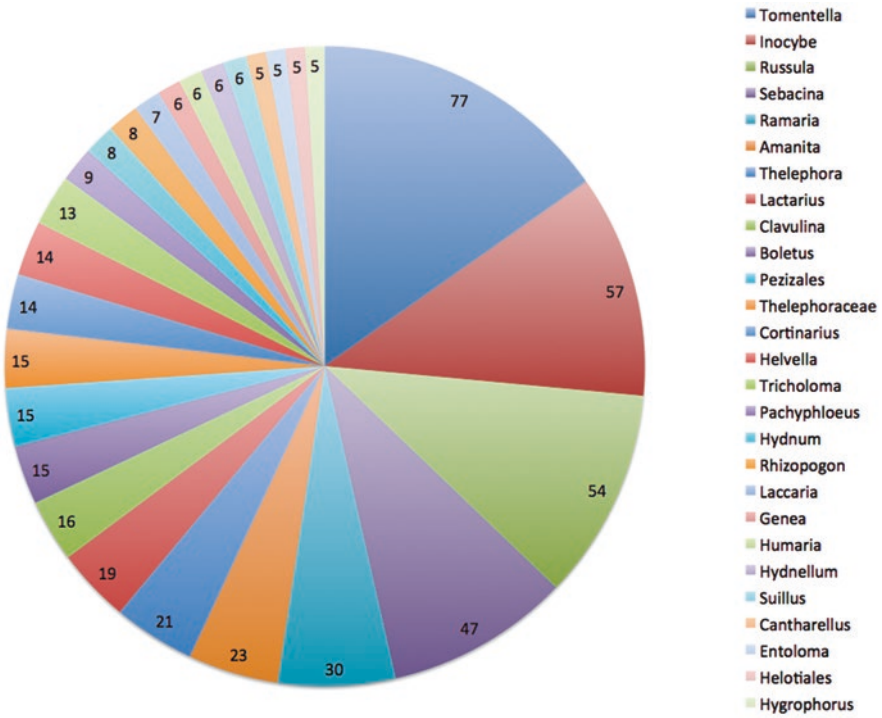

Fig. 15.1 Species richness of ectomycorrhizal fungi by genera. Only genera with the highest richness are shown

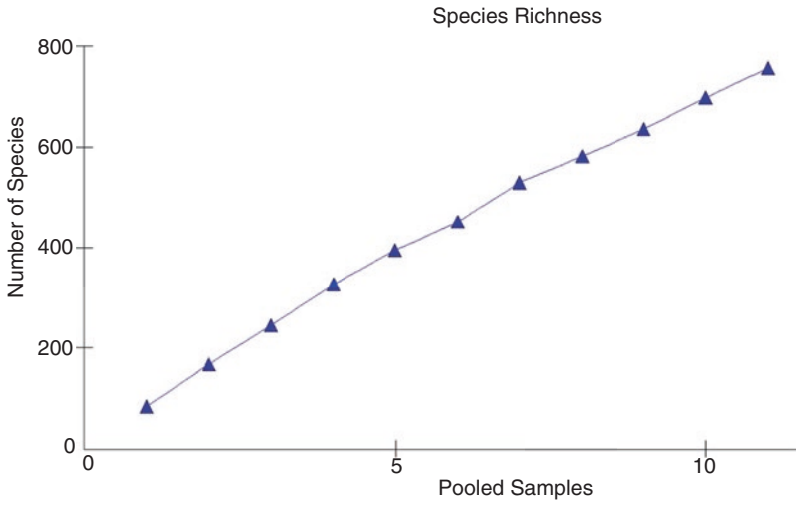

Fig. 15.2 Ectomycorrhizal fungal species accumulation curve of the Mexican Neotropics

has not reached a plateau is that there are very few shared species between study sites: a high level of beta diversity. This is evident in the results of the similarity analysis of the sampling sites. No pair of sites has a species overlap of over 25%. The greatest number of shared species was found in the list of fruit bodies from Temascaltepec and the fruit bodies from Cuitzeo (30 shared species), followed by "Nevado de Toluca" and Amanalco.

15.3.2 Community Structure of EMF in the Main Forest Ecosystems of the Mexican Neotropics

15.3.2.1 *Abies religiosa* Forest

In the *Abies religiosa* forest of el Zarco we collected 856 ectomycorrhizas and we obtained sequences from 591 samples. We had a 69% of sequencing efficiency and the sequences corresponded to 87 species. Out of 87 species, 83 were ectomycorrhizal fungi from which 69 were Basidiomycota and 14 Ascomycota. The ectomycorrhizal species of edible fungi found associated with *A. religiosa* were *Clavulina* cf. *cinerea*, *Clavulina* aff. *rugosa*, *Helvella* cf. *lacunosa*, *Russula americana*, *Russula* cf. *chloroides*, and *Tremelloscypha* sp. 1 (reported as *T. dichroa*). These species have been previously reported as edible in Mexico (Franco-Mass et al. 2012; Burrola et al. 2013; Bandala et al. 2014).

The families with the highest species richness were Inocybaceae (21 species), Thelephoraceae (15 species), Russulaceae, and Sebacinaceae (9 species). The genera with the highest richness were *Inocybe* (21 species), *Tomentella* (10 species), and *Russula* (8 species).

The families with the greatest abundance of mycorrhizae were Clavulinaceae (146 mycorrhizae), Thelephoraceae (115), Inocybaceae (98), and Russulaceae (74). The genera with the greatest abundance of mycorrhizae were *Clavulina* (82 mycorrhizae), *Membranomyces* (62), and *Russula* (22). The species with the highest abundance of mycorrhizae were *Clavulina* cf. *cinerea* (14.39%), *Membranomyces* sp. 1 (10.88%), and *Russula* sp. 1 (3.86%) (Fig. 15.3). Simpson's diversity index for this forest was 0.95 and the Chao 1 richness estimator was 145.3. The 32.2% of species found had values of nucleotide similarity inferior to 97% with their closest sequence in GenBank.

15.3.2.2 *Alnus* spp. Forests

In the *Alnus* forests we collected 562 ectomycorrhizas and obtained 416 sequences, these represent a sequencing efficiency of 74%. We found a total of 23 species in the four sampling sites. Out of the EMF associated with *Alnus*, only the genera *Lactarius* and *Clavulina* have been reported having edible species. Nevertheless, the ITS sequence of *Lactarius* sp. 1 had a 99% similarity to *L. omphaliformis*, *Lactarius* sp.

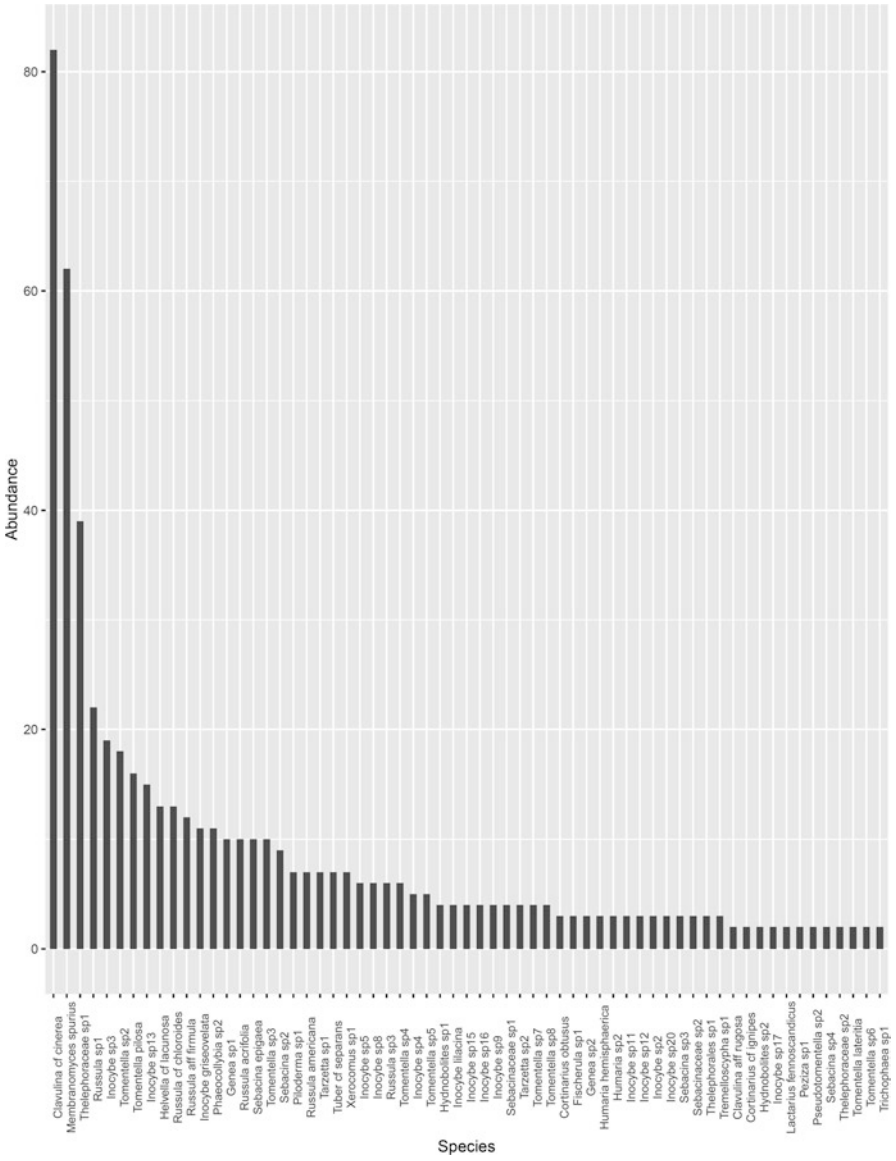

Fig. 15.3 Community structure of ectomycorrhizal fungi associated with *Abies religiosa* in el Zarco, Estado de México

4 had a 99% genetic similarity with *L. cyathuliformis*, both European nonedible species. Montoya et al. (2004) recorded the edible wild mushroom species of La Malinche (including *Alnus* forest). But the species recorded do not match our sampled species. A possibility is that edible mushrooms of this biome are mainly symbionts with pine trees and not *Alnus*, because the forests of La Malinche are mixed

with pine trees. Moreover, the two species of *Lactarius* were only found in the sites of Acatlán volcano and never at La Malinche. Yet, *Clavulina* sp. 1 was dominant in both sites. The species of *Clavulina* are eaten in Europe and Asia (Boa 2005), as well as in tropical America (Henkel et al. 2004). Clavulina sp. 1 presented 99% and 100% genetic similarity with *C. cinerea* and *C. cristata*, respectively. In Mexico, Garibay-Orijel and Ruan-Soto (2014) reported the edibility of *C. cinerea*, *C. coralloides*, and *C. rugosa*. However, *C. cinerea* and *C. cristata* form a taxonomic complex with shared morphological characteristics that has not been resolved, complicating their identification.

Alnus acuminata was associated with EMF of seven families. The families with the greatest richness were Thelephoraceae (7 species) and Sebacinaceae (6 species). Sixteen species of EMF were associated with *A. acuminata.* The most diverse genus was *Tomentella* with seven species. *Alnus jorullensis* had lower richness (9 species) distributed in five families and five genera. The family with the highest richness was Thelephoraceae (3 species) and the most diverse genus was *Tomentella* with three species.

Clavulina sp. 1 was the dominant taxon found in 35% of examined samples. Specifically, this species was the dominant taxon in *A. acuminata*, while *Cortinarius* sp. 1 was the most abundant species found in *A. jorullensis*. Other taxa with high abundances were various species of *Tomentella*, *Alnicola*, and Hymenogastraceae (Fig. 15.4).

15.3.2.3 *Pinus-Quercus* Forests

At the *Pinus-Quercus* forests of the Cuitzeo basin we collected 1600 mycorrhizae and obtained sequences from 978 samples, which represent 61.12% of sequencing efficiency. These samples matched with 206 species of ectomycorrhizal fungi. Out of these, 149 are Basidiomycota and 57 are Ascomycota. In the Cuitzeo basin, 76 species of edible fungi were reported (Reyes-García et al. 2009). The species with the highest biomass production in the basin's forests were *Lyophyllum* aff. *loricatum*, *Lactarius indigo* var. *indigo*, *Laccaria* sp., *Boletus frostii*, and *Amanita arkansana* (Torres-Gómez et al. 2018). Some of these species, like *Lactarius indigo* var. *indigo* and *Laccaria* sp., were found to form mycorrhizas with pine trees but in low frequencies.

The EMF species belonged to 19 families: Thelephoraceae (40 species), Russulaceae and Sebacinaceae (26), and Cortinariaceae (16). The genera with the highest richness were *Tomentella* (26 species), *Russula* (22), *Inocybe* (10), and *Sebacina* (8).

The most abundant species of EMF were Pezizomycetes sp.1, *Phialocephala fortinii*, *Russula* grp. *delica*, *Cenococcum geophilum*, *Rhizoscyphus* sp., *Lactarius* sect. *piperites*, *Russula* aff. *cyanoxantha*, *Wilcoxinia rehmii*, Sebacinaceae sp.1, Ascomycota sp.1, Atheliaceae sp.1, *Russula* grp. *pectinatoides*, *Lactarius* sect. *deliciosi*, Leotiomycetes sp.1, and *Inocybe* sp. Together, these species represent 26.33% of examined samples (Fig. 15.5). However, the most abundant EMF of the area

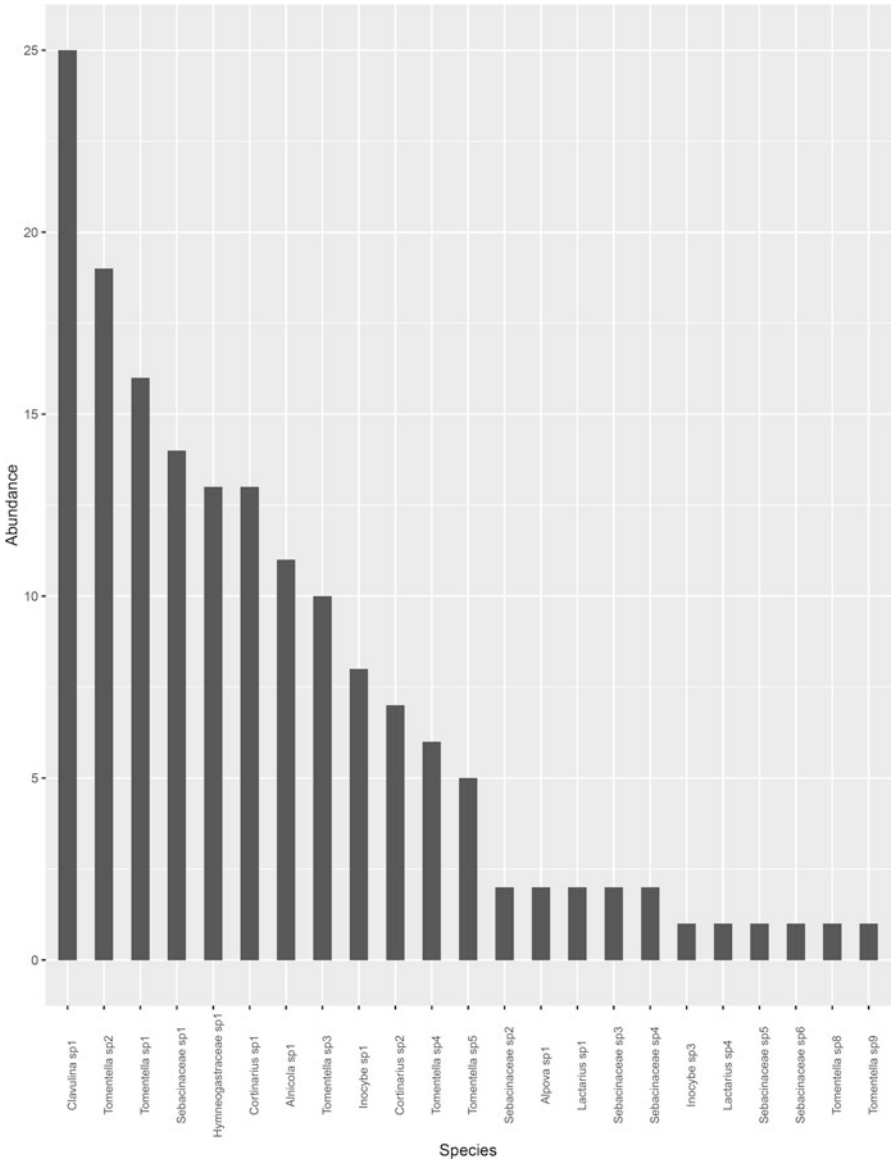

Fig. 15.4 Community structure of ectomycorrhizal fungi associated with *Alnus* forests

(Pezizomycetes sp.1) was only identifiable to the class level. These and many of the other studied species are potentially new taxa for science. The importance of anamorphic (i.e. without evident fruiting bodies) fungal species is clear in these ecosystems; within the most common EMF, anamorphic fungi constituted 60% of the richness and 65.82% of the abundance. We should also highlight the Russulaceae family with 5 species with evident fruiting bodies that in total colonized 27.03% of the roots.

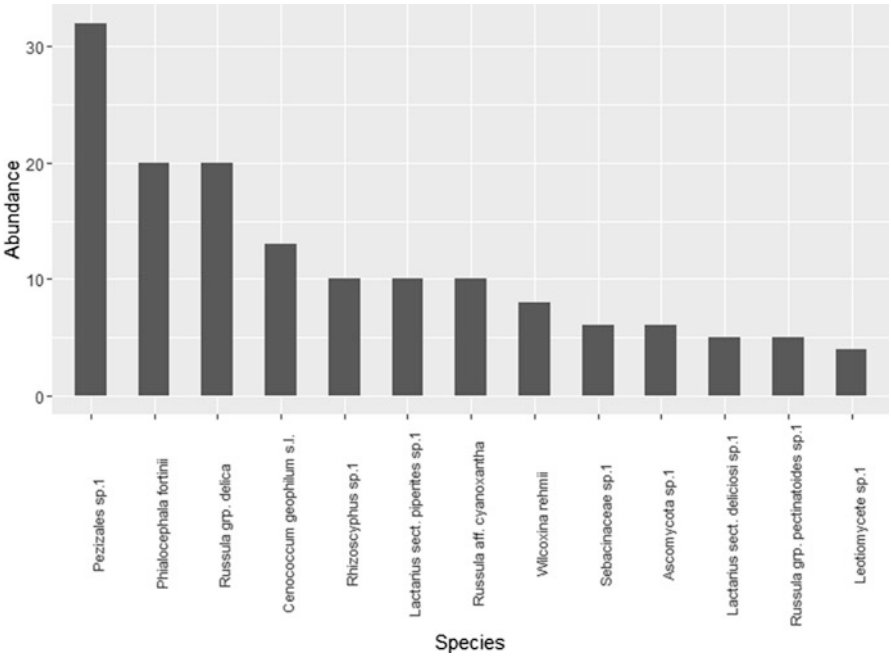

Fig. 15.5 Community structure of ectomycorrhizal fungi associated with *Pinus-Quercus* forests in the Cuitzeo basin, Michoacan. Only the most abundant species are shown

15.3.2.4 *Quercus* spp. Forests

In the oak forests of the Cuitzeo basin we collected 1671 ectomycorrhizas and obtained sequences from 573 samples, which represent a sequencing efficiency of 34.6%. We identified 158 species, of which 140 were ectomycorrhizal fungi, 112 species belonged to Basidiomycota, and 28 species to Ascomycota. The edible ectomycorrhizal fungi found in symbiosis with *Quercus* spp. were *Hydnum repandum*, *Lactarius yazooensis*, *Russula* aff. *brevipes*, *Russula* aff. *cyanoxanta*, and *Russula* aff. *olivacea.*

The community had 22 families; the best represented in terms of richness were Thelephoraceae (31 species), Russulaceae (24), Sebacinaceae (20), and Inocybaceae (13). The species belonged to 34 genera. *Tomentella* (25 species), *Russula* (22), *Sebacina* (19), *Inocybe* (13), and *Pachyphlodes* (5) were the most diverse in this community.

The families with the highest abundant mycorrhizae were Russulaceae (22.79%), Sebacinaceae (25.53%), Thelephoraceae (19.12%), Inocybaceae (6.62%), and Pyronemataceae (5.88%). The genera with higher abundance were *Sebacina* (25.53%), *Russula* (20.77%), *Tomentella* (16.91%), *Inocybe* (6.62%), *Humaria* (3.68%), *Pachyphlodes* (3.31%), and *Clavulina* (2.39%). These seven genera represented 79.21% of the mycorrhizae. The most abundant species in the community

were *Sebacina* sp. 1 with 6.2% of the abundance, followed by *Sebacina* aff. *epigaea* with 4.1% and *Russula* aff. *curtipes* with 3.4% (Fig. 15.6). The site Aguila 1 showed the greatest richness (72 species), the greatest diversity (H′ = 3.99), less dominance (D = 0.019), and greater evenness (1/D = 53.78). In contrast, the site Aguila 2 showed a lower level of richness (38 species), the lowest diversity (H′ = 2.67), the

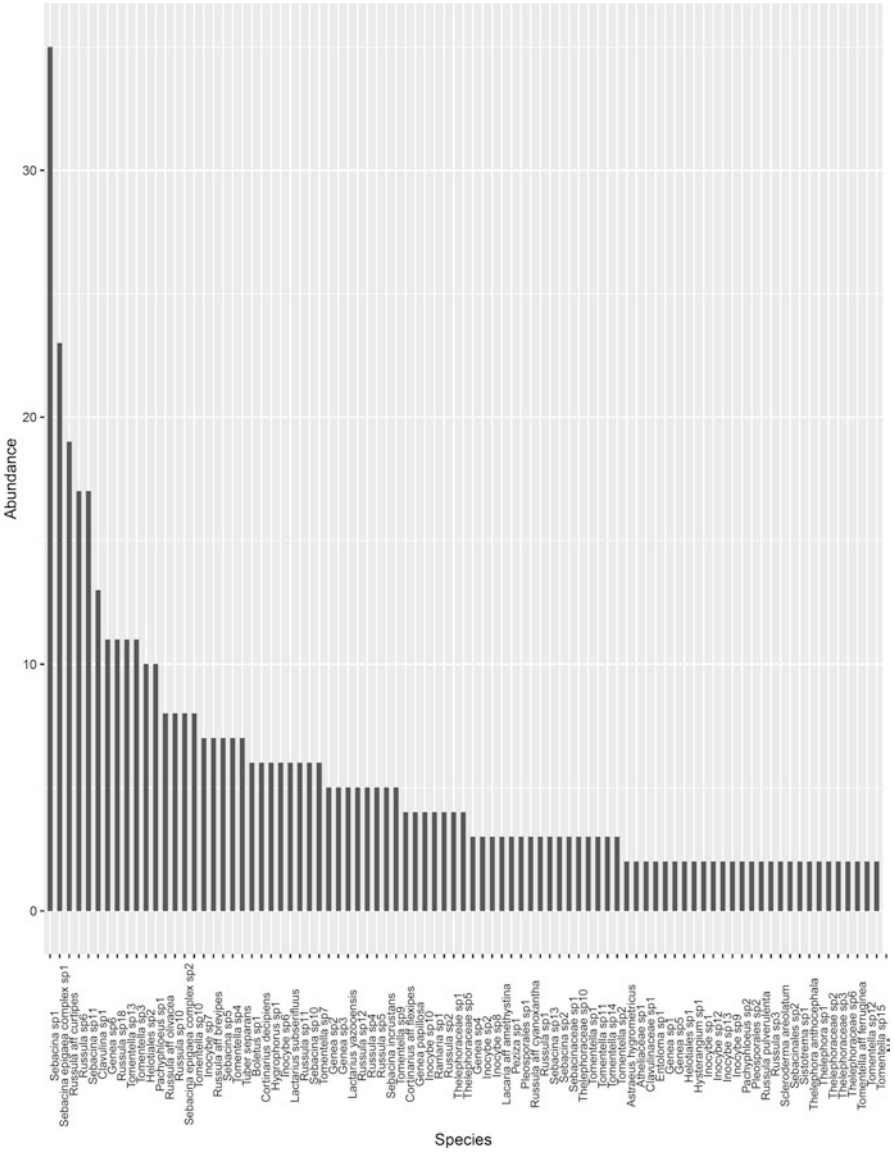

Fig. 15.6 Community structure of ectomycorrhizal fungi associated with *Quercus* forests in the Cuitzeo basin, Michoacán

greatest dominance (D = 0.118), and the lowest evenness (1/D = 8.45). Although these sites are geographically close, they shared few species. This scenario suggests a high species turnover within this forest (García-Guzmán et al. 2017). Nevertheless, both sites are very similar in diversity, dominance and evenness. The Chao 1 estimated a potential richness of 208 species including all sampling sites. The 35.71% of the species had a nucleotide similarity of less than 97% when compared to their best match in GenBank. These results reveal the lack of data and information in the public databases for this geographic region and suggest that these oak forests could sustain important species' endemism.

15.3.2.5 Tropical Dry Forest

At Chamela Biological Station we collected 201 roots that appeared colonized by EMF and obtained sequences from 98 samples, representing a sequencing efficiency of 48.7%. The sequences matched 19 species of ectomycorrhizal fungi: *Clavulina* sp. 1, *Clavulina* sp. 2, *Clavulina* sp. 3, *Clavulina* sp. 4, *Clavulina* sp. 5, *Entoloma* sp., *Inocybe* sp.1, *Inocybe* sp. 2, *Russula* sp. 1, Russulaceae sp., *Sebacina* sp., *Thelephora versatilis*, *Thelephora pseudoversatilis*, *Tomentella brunneoincrustata*, *Tomentella* sp. 1, *Tomentella* sp. 2, *Tomentella* sp. 3, *Tomentella* sp. 4 and *Tremelloscypha dichroa*. Moreover, we collected 101 fruit bodies, out of which, only 28 specimens matched with ectomycorrhizal genera. All the EMF found belonged to Basidiomycota. The families with the highest number of species were Thelephoraceae (8 species) and Clavulinaceae (5 species). The knowledge of edible ectomycorrhizal fungi in this area is null. However, in Chiapas, *Tremelloscypha dichroa* has been reported as edible (Bandala et al. 2014). It seems this is the only edible ectomycorrhizal fungus in the area.

The families with the greatest number of mycorrhizae were Sebacinaceae (41.2%), Thelephoraceae (33.9%), and Clavulinaceae (20%). The genera with the greatest number of mycorrhizae were *Tremelloscypha* (31.1%), *Clavulina* (20.1%), *Tomentella* (19.2%), and *Thelephora* (14.6%). The species with the greatest number of mycorrhizae were *T. dichroa* (26.6%), *Clavulina* sp.1 (14.6%), and *Sebacina* sp. (14.6%) (Fig. 15.7). Simpson's diversity index was 0.85.

The species with the greatest number of fruit bodies were *Thelephora versatilis* (18) and *T. dichroa* (6 specimens). In contrast, we only found one fruit body of *T. brunneoincrustata* and two specimens of *Phaeoclavulina* sp. Unfortunately, for the latter species, we did not find its ectomycorrhizal form. Moreover, for the remaining EMF, we did not find fruit bodies. Interestingly, *T. dichroa* had a high production of fruit bodies, as well as high ectomycorrhizal colonization. None of the mycorrhizae sequences found in this tropical dry forest had nucleotide similarities of 97% or more when compared with the sequences in GenBank. But they were 100% identical to the specimens used recently to describe them as new species (Ramírez-López et al. 2015; Álvarez-Manjarrez et al. 2016).

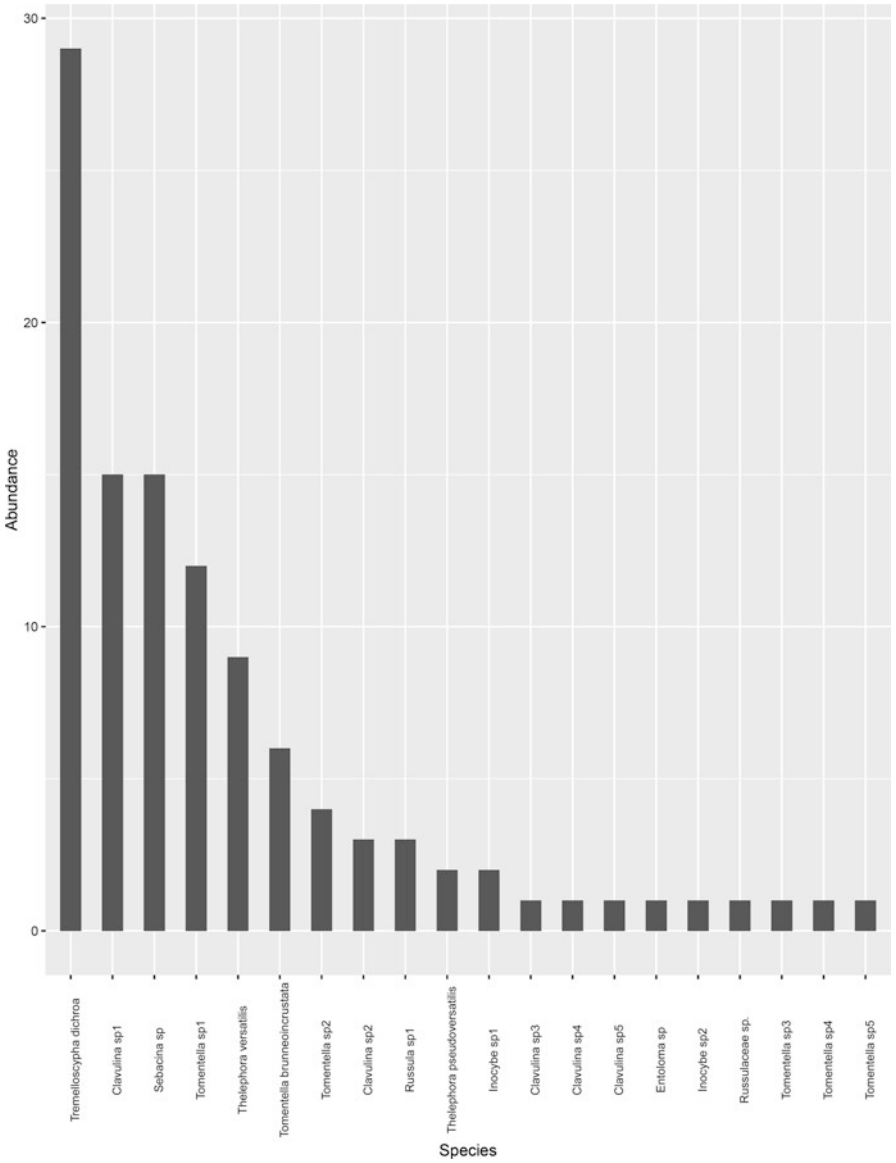

Fig. 15.7 Community structure of ectomycorrhizal fungi associated with the tropical dry forest of Chamela, Jalisco

15.4 Final Considerations

This study highlights the importance of a comprehensive approach when studying EMF. Our study includes EMF found as fruit bodies or as mycorrhizas. If we had only studied fungal diversity by fruit bodies, we would have only found 38% of the species. Likewise, if we had only studied the mycorrhizae, we would have only found 69% of the species.

Overall, edible fungal species are not abundant at the mycorrhizae level, some exceptions include species of Russulaceae like *R. brevipes* s.l., *R. cyanoxantha* s.l., *R. americana*, *R.* cf. *chloroides*, *Lactarius* sect. *deliciosi* sp., and species of *Clavulina*, like *C.* cf. *cinerea* and *C.* aff. *rugosa.* At the fruit body level, species like *A. rubescens* s.l., *H.* cf. *lacunosa*, *L. laccata* s.l., *L. trichodermophora*, *L. vinaceobrunnea*, and *S. pseudobrevipes* proved to be abundant and of a wide distribution.

The ecosystem with the greatest species richness was the pine-oak forest of the Cuitzeo basin with 206 species, followed by the oak forests of Cuitzeo with 153 species and the *Abies* forests of el Zarco with 83 species. *Alnus* forests have little diversity of EMF because this symbiotic relationship is specific, resulting in a limited number of EMF associated with *Alnus* (Kennedy et al. 2011). The tropical dry forest of Jalisco had the lowest number of EMF. Mainly because most of the plants form arbuscular mycorrhizae and the plants forming ectomycorrhizal associations are dispersed within the forest.

The level of endemism is not easy to determine. To be sure a species has a restricted distribution, a wide-ranging sampling effort is necessary. Only then would there be sufficient data to say a species has a limited distribution. We do not have this information for EMF in Mexico because only a few species have been studied to the necessary taxonomic level and sampled intensively. Nevertheless, DNA sequences allow us to compare between them and with public databases, even when their taxonomic identity is uncertain. In the Mexican Neotropics, 421 out of the 690 (71%) species included in the analysis have no GenBank sequences outside Mexico. Therefore, these species are potentially endemic to this area. This level of endemism may seem exaggerated, but it is comparable with the phanerogamic flora of the MegaMexico bioregion, which according to Rzedowski (1991) can hold 72% endemism. Locally, the endemism of EMF ranged between 32.2% in the *A. religiosa* forests up to a 100% in the tropical dry forest.

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