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► **To cite this version:**

Sandra Sleiman, Jean-Michel Bellanger, Franck Richard, Jean Stephan. First molecular-based contribution to the checklist of Lebanon macrofungi. *Mycotaxon*, 2021, 136 (3), pp.687-687. 10.5248/136.687 . hal-04864368

HAL Id: hal-04864368

<https://hal.science/hal-04864368v1>

Submitted on 4 Jan 2025

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First molecular-based contribution to the checklist of Lebanon macrofungi

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ABSTRACT—Across the Mediterranean biodiversity hotspot, our knowledge of fungal diversity is still fragmentary, and the eastern part of the basin remains poorly explored. This is particularly the case in Lebanon, where macrofungal diversity has never been subject to molecular-based investigation. A first checklist was published in 1957 and updated in 1996, based on morphological identification of specimens. In an effort to narrow this gap of knowledge, we designed a field survey in Akkar, north Lebanon in *Abies cilicica*, *Quercus calliprinos* and *Quercus cerris* old-growth stands, carried out between 2018 and 2019. Three sampling plots were monitored in each ecosystem. By combining morphological identification and molecular analysis of 105 collected samples, 79 species were identified, including 62 (i.e., 78.5%) new records for Lebanon, and eight putative undescribed species. Species diversity was the highest under *Quercus calliprinos* and lowest under *Abies cilicica*. Most species were encountered in just one ecosystem type, and only 7 were recorded in two ecosystems. These results highlight the overlooked fungal diversity in this part of the eastern Mediterranean hotspot, and the importance of Lebanese forests for biodiversity conservation. Our results also call for further investigation, in particular under endemic tree hosts, to increment the still incomplete national checklist of macrofungi in Lebanon.

KEY WORDS—basidiomycetes, forest ecosystems, mycology, Mediterranean forests, fungal diversity

Introduction

At least five million fungal species are estimated on Earth, of which ~2% are described (Blackwell 2011; Taylor & al. 2014; Lücking & Hawksworth 2018). A fraction of those are known to produce fruiting bodies large enough to be seen with the naked eyes (Chang & Miles 1992). These macromycetes, primarily produced by *Agaricomycetes* (*Basidiomycota*) and *Ascomycetes* (*Ascomycota*), have been of broad interest to human communities for millennia due to their valuable medicinal and nutritional values (Hall & al. 2003; Peintner & al. 2013). Since the emergence of DNA-based identification methods (Gardes & Bruns 1996), there has been a resurgence of interest by ecologists and conservation biologists who use a combination of field sampling and molecular-based tools to inventory macromycetes in order to 1) provide new insights into fungal diversity in poorly investigated ecosystems (e.g., Loizides & al. 2019); 2) document the distribution of endangered fungal species (Taudière & al. 2017); and 3) investigate biodiversity patterns and fungal-based ecological links in forest ecosystems with high conservation value (Richard & al. 2005; Baptista & al. 2010).

Fungal checklists are far from complete on the global scale and our knowledge of the biogeographical patterns of fungal diversity remains fragmentary (Loizides & al. 2016, 2019; Paz & al. 2017; Taşkın & al. 2016). This is particularly the case in the Eastern part of the Mediterranean, where macromycetes checklists are incomplete, or lacking altogether depending on the considered country. In recent years, there has been a resurgence of initiatives to document fungal diversity in this neglected ecoregion (Kaya 2010; Polemis & al. 2012; Torrejón 2014; Loizides 2016, 2018; Loizides & al. 2018, 2019). However, there is still an urgent need to provide updated checklists and phylogenetic revisions of previous records, to preserve the highly vulnerable biological legacy in this part of the world (Myers & al. 2000; Gogoi & Parkash 2015).

The complex orography and topography that characterize Lebanon contribute to a wide variation in climate and habitats, making the country an integral part of the Mediterranean biodiversity hotspot (Mittermeier & al. 2005).

Because fungal diversity is intimately linked to plant diversity (Hawksworth & al. 2004; Bruns & al. 2002), and because the Mediterranean is a prolific hotspot of diversity for plants, it seems likely that Lebanon may accommodate a high and unique diversity of macromycetes. However, these organisms have received little attention so far in this country. In 1957, a first mycological survey was carried out in Lebanon, describing 207 species of mushrooms across the territory (Lys & Ades 1957). Another survey was carried out 49 years later, mainly targeting habitats within pine forests, and resulted in the addition of 368 species (Modad 2006). From these works, which were only based on field identification, 33 families were represented in Lebanon, out of which 30 are *basidiomycetes* and 3 are *ascomycetes*.

The present study is the first update of the Lebanon macrofungal checklist based on the use of both morphological and molecular methods. We explored three emblematic forest ecosystems of Northern Lebanon in order to provide the first overview of their macrofungal diversity, and detect potential fungal biodiversity stakes in this part of the Mediterranean.

Materials and Methods

Site selection and description

This investigation aims at identifying fungal diversity within three forest ecosystems in Akkar in North Lebanon: Palestine oak (*Quercus calliprinos* Webb.) forest, Turkish oak (*Quercus cerris* L.) forest, and Cilician fir (*Abies cilicica* (Antoine & Kotschy) Carrière) forest. Three public sites were chosen in Tesheh (T), Fnaydeq (F) and Qamouaa (Q) in the mountainous region of Akkar (Figure 1). All study sites were old-growth (more than 100 years old) pure stands dominated by each tree, with limited presence of other putative interacting plants, allowing to confidently assign both mycorrhizal and decaying fungal species to each tree species (Table 1 and 2). In each ecosystem, three plots, 50 m long \times 10 m wide were defined and periodically surveyed. Field surveys were conducted every two weeks from early October to December 2018, until the sites were covered by snow. The long and persisting snow cover did not allow us to conduct further sampling in winter and early spring. Another field survey was performed in early May 2019, but no mushrooms were found.

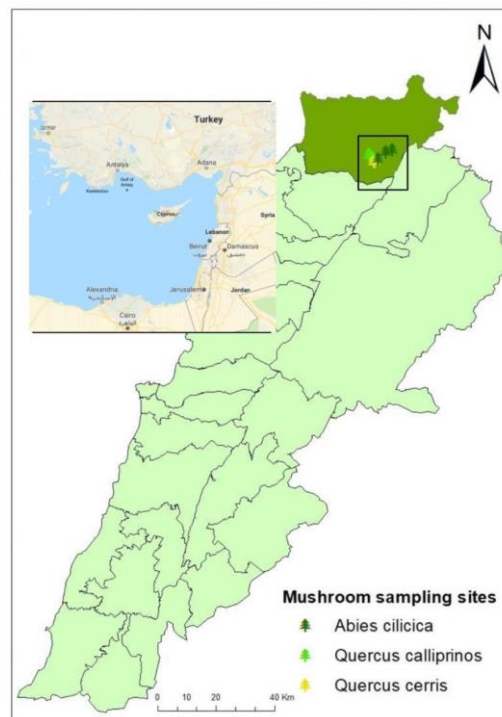


Figure 1: sampling sites location by ecosystem

Sampling and morphological studies:

For mushroom sampling, we followed the methodology of Læssøe & al. (1996). A field note form was adopted from Bremner (2006) for field assessment and mushroom morphological description, based on cap, stem and gills identification traits (Annex I). Photos and mushroom samples backed up with morphological traits described in the field forms were used to identify genus and species, according to Læssøe & al. (1996), Borgarino & Hurtado (2011) and Eyssartier & Roux (2013).

Molecular analysis:

The collected mushrooms were tagged and packed in glassware then dried in an oven at a temperature of 45°C for 72 hours. The dried samples were subsequently shipped to CEFE-CNRS Laboratory (Montpellier, France) for molecular analysis. Dried samples were extracted with the REDEExtract-NAmptm Plant PCR Kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer's instructions. The ITS rDNA locus sequences were generated using primers ITS1F, ITS4B and ITS4 (Gardes & Bruns 1993), following Richard & al. (2015). Sequences were edited and assembled using Codon Code Aligner v. 4.1.1 (CodonCode Corp., Centerville, MA USA). Phylogenetic analyses were conducted online at www.phylogeny.fr (Dereeper & al. 2008). Multiple sequence alignments were performed with MUSCLE v. 3.7 (Edgar 2004), maximum likelihood phylogenetic analyses with PhyML v. 3.0 (Guindon & al. 2010), using the GTR + I + Γ model of evolution and the Shimodaira Hasegawa version of the approximate likelihood-ratio test (SH-aLRT) of branch support (Anisimova & al. 2011). Trees were built using TreeDyn 198.3 (Chevenet & al. 2006) and edited with Inkscape 0.91 (<https://inkscape.org/fr>).

Results and Discussion

Out of the 105 collected samples, a total of 79 taxa were identified and distributed amongst 17 families (Figure 2). Twenty-one samples representing 16 species, belonged to *Cortinarius* which was the best represented genus during the survey, followed by *Hebeloma* (6 species), and *Inocybe*, *Lepiota* and *Tricholoma* (5 species each) (Table 3). Out of the 79 identified samples, 68 were determined at species level and 11 samples at the genus level (Table 3), the latter belonging to *Hebeloma* (3 distinct taxa), *Mycena* (2 distinct taxa), *Coprinellus*, *Inocybe*, *Gymnopus*, *Lyophyllum*, *Lepiota* and *Suillellus* (one taxon each, respectively). The molecular analysis (not shown) did not target all samples; only 67 samples were successfully sequenced while the remaining 38 samples were either very clearly identifiable species or much deteriorated samples with unreadable sequences that should be subject to further investigation.

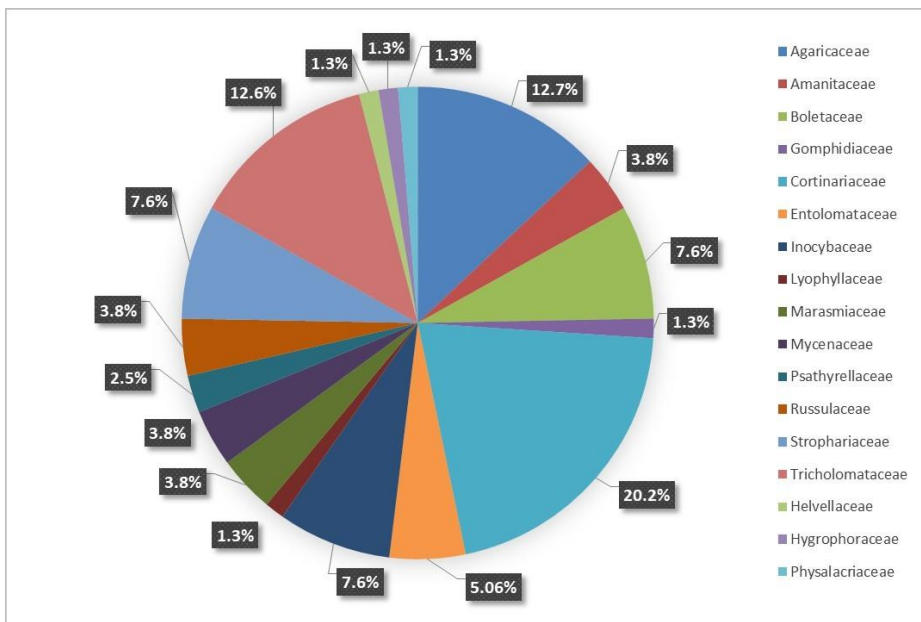


Figure 2: Sampled taxa distribution by family

Out of the analyzed samples, eight species are putatively new to science or not represented in public databases: *Cortinarius* aff. *diffractosuavis*, *C. castaneus* sensu lato, *Inocybe* aff. *hirtella*, *Inocybe* sp., *Lepiota* aff. *echinella*, *Russula* aff. *alutacea* 1, *Russula* aff. *alutacea* 2, and *Suillellus* sp. (Tables 3 and 4). Considering the recent and quite comprehensive survey of *Cortinarius* subgenus *Telamonia* published by Liimatainen & al. (2020) and the unpublished sequences available to us, *Cortinarius* aff. *diffractosuavis* and *C. castaneus* sensu lato, found respectively under *Q. calliprinos* and *A. cilicica*, likely represent new species that deserve further sampling and dedicated studies to be formally described. *Inocybe* aff. *hirtella* and *Inocybe* sp. encountered under *Q. calliprinos* and *A. cilicica* may also represent novel species, but might also be applicable to existing binomials and simply absent from public databases (Ryberg & al. 2008; Vauras & Larsson 2016). Similarly, the two identified lineages close to *Russula alutacea* found under *Q. calliprinos* could be found elsewhere but not yet published, as this genus is currently under revision (Vidal & al. 2019).

Lepiota aff. *echinella* recorded under *Q. calliprinos*, is likely a novel species since this genus is widely covered by literature with new species recently discovered, and this sequence does not match others in public databases (Liang & al. 2018; Hou & Ge 2020). *Suillellus* sp. sampled under *A. cilicica* is also most likely a novelty since the genus is relatively rare in the Eastern Mediterranean (Acar & al. 2019), especially under coniferous trees, and the identified sequence diverges from those belonging to *Boletaceae* recently analyzed in Cyprus (Loizides & al. 2019). Considering that the sister species of the Lebanese collection, *S. comptus*, is present in neighboring Cyprus, the speciation process that led Lebanese populations to diverge has perhaps been driven by host specificity, a hypothesis that needs to be checked by further forays in *Abies cilicica* forests of this tree natural area. The six species in three distinct genera in the *Boletaceae* (*Hortiboletus*, *Rubroboletus*, *Suillellus*) family were distributed in either oak or fir ecosystems, with no overlaps, indicating a highly specific host preferences within this family. Similarly, an inventory conducted on the island of Cyprus has shown a significant diversity of boletoid fungi specifically associated to oaks, especially the species endemic to Cyprus *Q. alnifolia* (Poech) and *Q. calliprinos* (Loizides & al. 2019). Given that the new species might be host-specific to *Abies cilicica*, then boletes in the eastern Mediterranean seem to be highly specific in their host preferences, and not broad-host generalists.

Among the sequenced samples, three identified species are deadly poisonous or at least toxic: *Amanita proxima*, under *Q. calliprinos*, *Omphalotus olearius* under *Q. cerris*, and *Paralepistopsis amoenolens*, under *A. cilicica*. Responsible for erythromelalgia, *P. amoenolens* has been previously reported under *Cedrus* spp., *Cistus ladanifer* L., *Cupressus arizonica* Greene., *Daphne gnidium* L., *Ilex aquifolium* L., *Larix decidua* Mill., *Picea abies* (L.) H.Karst., and *Pinus* spp. (Leonardi & al. 2002; Martínez & al. 2010; Vizzini & Ercole 2012; Çolak & al. 2017). Our finding adds a new ecological niche for this relatively rare and poorly known toxic species, and may call for educational information to caution local collectors harvesting edible mushrooms in *Abies cilicica* forests.

Entoloma plebeioides, the recently described *Rhodocybe matesina*, *Cortinarius semivelatus*, and *Lepiota andegavensis* are four species rarely reported in the literature, either because of rarity, recent description, misidentifications, or a combination of these factors (Mornand 1983; Venturella 2002; Crous & al. 2017). Their occurrence in Lebanese mountains under *Quercus calliprinos* (the first two) and *Q. cerris* (the last two) respectively, represents the eastern-most extension of their known distribution ranges so far.

Overall, only 17 species identified here are present in the current Lebanese checklist, leaving 62 records as new for Lebanon. Particularly striking is the first record for the country of family *Entolomataceae* with the two taxonomically difficult genera *Entoloma* and *Rhodocybe*. These new records are unevenly distributed across the three studied ecosystems; 33 species were found under *Q. calliprinos*, 16 under *Q. cerris*, and 13 under *Abies cilicica* (Table 4). Most species were recorded in a single ecosystem, and only seven were encountered in two ecosystems. No species was documented in

all three studied ecosystems, but *Inocybe bongardii* was found in both *Q. cerris* and *A. cilicica* ecosystems, while *Inocybe* sp., *Tricholoma scalpturatum*, *T. triste* and *T. terreum* were encountered in fir and one oak ecosystems, suggesting that these ectomycorrhizal species are not host-specific mycobionts. *Mycena pura* sensu lato and *Hebeloma sinapizans* were not recorded under fir, but under both oak ecosystems.

Conclusion

This study aimed at updating the macromycete checklist of Lebanon and yielding the first molecular basis for the fungal diversity of the country. Sixty-two species were recorded for the first time in Lebanon, of which eight represent putative novelties for science and four are rarely cited in the literature. In the future, further sampling and dedicated taxonomic analyses should allow the formal description of the species identified as new for science, and also further advance the documentation of fungal communities associated with these poorly known Mediterranean ecosystems. Beyond these interesting findings and perspectives for mycologists and community ecologists, the present work has unveiled the importance of Lebanese mountainous forests as reservoirs of fungal diversity.

The ongoing climate change will particularly impact forest ecosystems in the Eastern Mediterranean during the 21st century (Al-Qaddi & al. 2016; Lopez-Tirado & al. 2021). Here we provide evidence that Lebanon forest soils host organisms of high conservation value that may be part of the monitored biodiversity, and considered as good candidates as flag species for conservation-aimed communication directed towards the scientific community, public institutions and local population. In this respect, *Abies cilicica* and *Quercus calliprinos* forests of Lebanon should be closely followed in the long term, and ecosystems dominated by tree species endemic to Lebanon or the region (i.e., *Cedrus libani* G.Don, *Quercus kotschyana* O.Schwarz, *Q. look* Kotschy, and *Q. ithaburensis* Decne.) should be investigated as well.

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TABLE 1. Detailed site characteristics.

DOMINANT TREE	LOCATION	CODE	PLOT	ELEVATION	LATITUDE	LONGITUDE	UNDERSTORY
<i>Quercus calliprinos</i>	Tesheh	T	T1	1345m	34°49039828	36°19450095	none
<i>Quercus calliprinos</i>	Tesheh	T	T2	1347m	34°49001335	36°19287331	<i>Styrax officinalis</i> + <i>Juniperus oxycedrus</i>
<i>Quercus calliprinos</i>	Tesheh	T	T3	1346m	34°48719103	36°19711446	none
<i>Quercus cerris</i>	Fnaydeq	F	F1	1377m	34°47296148	36°20802682	<i>Juniperus oxycedrus</i>
<i>Quercus cerris</i>	Fnaydek	F	F2	1411m	34°47105669	36°20819483	none
<i>Quercus cerris</i>	Fnaydek	F	F3	1436m	34°47122060	36°20981513	none
<i>Abies cilicica</i>	Qamouaa	Q	Q1	1608m	34°49673301	36°24464733	none
<i>Abies cilicica</i>	Qamouaa	Q	Q2	1600m	34°50119196	36°26362525	none
<i>Abies cilicica</i>	Qamouaa	Q	Q3	1486m	34°47876428	36°21899821	<i>Juniperus oxycedrus</i>

TABLE 2. Soil and dendrometric characteristics of the study sites.

SITE	CODE*	GEOLOGICAL SUBSTRATE	CANOPY COVER	TREES/HA	DBH
T1	QCA	limestone/sandstone	10-40%	<100	50- >70 cm
T2	QCA	limestone/sandstone/ volcanic	40-70%	200-400	30-50cm
T3	QCA	limestone	10-40%	100-200	30-50cm
F1	QCE	limestone	40-70%	200-400	30-50cm
F2	QCE	limestone	40-70%	200-400	30-50cm
F3	QCE	limestone	10-40%	200-400	30-50cm
Q1	AC	limestone	40-70%	<100	>70cm
Q2	AC	limestone	>70%	100-200	>70cm
Q3	AC	limestone	40-70%	100-200	>70cm

*Codes: QCA = *Quercus calliprinos*, QCE = *Quercus cerris* and AC = *Abies cilicica*

Table 3. List of identified species.*

	SPECIES	CHECKLIST STATUS	VOUCHER	ECOSYSTEM	GenBank ITS	TROPHIC MODE
1	<i>Agaricus bohusii</i> Bon	0	T1-6	QCA	MZ088090	saprobic
2	<i>Agaricus cupreobrunneus</i> (F. H. Møller) Pilát	0	F1-17	QCE	NA	saprobic
3	<i>Amanita ovoidea</i> (Bull.) Quéf.	1	T2-1 T3-3	QCA QCA	NA NA	mycorrhizal
4	<i>Amanita proxima</i> Dumeé	0	T3-4	QCA	MZ088129	mycorrhizal
5	<i>Amanita solitaria</i> (Bull.) Fr.	0	Q1-4	AC	MZ088086	mycorrhizal
6	<i>Calvatia excipuliformis</i> (Scop.) Perdeck	1	F1-18 T3-9	QCE QCA	NA NA	saprobic
7	<i>Clitocybe odora</i> (Bull.) P.Kumm.	1	T3-10	QCA	NA	saprobic
8	<i>Collybia butyracea</i> (Bull.) P.Kumm.	0	F1-14 F2-8	QCE QCE	NA NA	saprobic
9	<i>Coprinellus</i> sp.	0	T2-14	QCA	NA	saprobic
10	<i>Coprinopsis atramentaria</i> (Bull. : Fr.) Redhead & Coll.	1	T1-3	QCA	NA	saprobic
11	<i>Cortinarius</i> aff. <i>diffRACTOSUAVIS</i> Chevassut Rob. Henry	0	T3-8	QCA	MZ088093	mycorrhizal
12	<i>Cortinarius caligatus</i> Malençon	0	T2-8	QCA	MZ088122	mycorrhizal
13	<i>Cortinarius castaneus</i> (Bull.) Fr. sensu lato	0	Q1-10 Q1-11 Q1-13	AC AC AC	MZ088103 MZ088104 MZ088106	mycorrhizal
14	<i>Cortinarius cedretorum</i> Maire	1	Q2-3	AC	MZ088108	mycorrhizal
15	<i>Cortinarius epipurrus</i> Chevassut Rob. Henry	0	F3-7	QCE	MZ088098	mycorrhizal
16	<i>Cortinarius luteocingulatus</i> Bidaud & Fillion	0	T2-18	QCA	MZ088128	mycorrhizal
17	<i>Cortinarius olivaceofuscus</i> Kühner	0	Q3-6	AC	MZ088112	mycorrhizal
18	<i>Cortinarius puellaris</i> Brandrud, Bendiksen, & Dima	0	T2-16	QCA	MZ088126	mycorrhizal
19	<i>Cortinarius rapaceoides</i> Bidaud, G.Riousset & Riousset	0	F1-8	QCE	MZ088083	mycorrhizal
20	<i>Cortinarius semivelatus</i> Rob. Henry	0	F1-13	QCE	MZ088094	mycorrhizal
21	<i>Cortinarius splendidior</i> Bidaud	0	T3-15	QCA	MZ088134	mycorrhizal
22	<i>Cortinarius subbulliardoides</i> Rob. Henry	0	T2-9 T3-16 T3-17	QCA QCA QCA	MZ088123 MZ088135 MZ088136	mycorrhizal
23	<i>Cortinarius suillonigrescens</i> Rob.Henry ex Reumaux	0	F2-2 F2-6 T3-7	QCE QCE QCA	MZ088084 MZ088097 MZ088092	mycorrhizal
24	<i>Cortinarius terribilis</i> Reumaux	0	T3-11	QCA	MZ088130	mycorrhizal
25	<i>Cortinarius trivialis</i> J.E.Lange	0	F1-16	QCE	MZ088096	mycorrhizal
26	<i>Cortinarius violaceipes</i> Bidaud & Consiglio	0	T2-10	QCA	MZ088124	mycorrhizal
27	<i>Entoloma inusitatum</i> Noordel., Enderle & H.Lammers	0	T1-11 T1-12 T1-13	QCA QCA QCA	MZ088119 NA NA	saprobic
28	<i>Entoloma lividoalbum</i> (Kühner & Romagn.) Kubička	0	T1-7	QCA	NA	saprobic
29	<i>Entoloma plebeoides</i> (Schulzer) Noordel.	0	T1-8	QCA	MZ088116	saprobic
30	<i>Gomphidius glutinosus</i> (Schaeff. : Fr.) Fr.	1	Q1-1 Q3-4	AC AC	MZ088074 MZ088111	mycorrhizal
31	<i>Gymnopus brassicolens</i> (Romagn.) Antonín & Noordel.	0	T1-9	QCA	MZ088117	saprobic
32	<i>Gymnopus</i> sp.	0	F1-7	QCE	NA	saprobic

	SPECIES	CHECKLIST STATUS	VOUCHER	ECOSYSTEM	GenBank ITS	TROPHIC MODE
33	<i>Hebeloma crustuliniforme</i> (Bull.) Quél.	1	F2-1 F2-3	QCE QCE	NA NA	mycorrhizal
34	<i>Hebeloma mesophaeum</i> (Pers. :Fr.) Quél.	1	Q1-9	AC	MZ088102	mycorrhizal
35	<i>Hebeloma sinapizans</i> (Paulet) Sacc.	0	T3-13 F1-12	QCA QCE	MZ088132 NA	mycorrhizal
36	<i>Hebeloma</i> sp.	0	F1-3 F2-9	QCE QCE	NA NA	mycorrhizal
37	<i>Hebeloma</i> sp. 1	0	T3-6	QCA	NA	mycorrhizal
38	<i>Hebeloma</i> sp. 2	0	F2-5	QCE	NA	mycorrhizal
39	<i>Helvella lacunosa</i> Afzel.	1	Q3-8	AC	NA	mycorrhizal
40	<i>Hortiboletus engelii</i> (Hlaváček) Biketova & Wasser	0	T1-1	QCA	MZ088076	mycorrhizal
41	<i>Hygrophorus roseodiscoideus</i> Bon & Chevassut	0	T2-11	QCA	MZ088125	mycorrhizal
42	<i>Infundibulicybe gibba</i> (Pers.) Harmaja	0	F1-5	QCE	MZ088082	saprobic
43	<i>Infundibulicybe mediterranea</i> Vizzini, Contu & Musumeci	0	F1-6	QCE	NA	saprobic
44	<i>Inocybe</i> aff. <i>Hirtella</i> Bres.	0	Q1-5 Q3-10	AC AC	MZ088087 MZ088113	mycorrhizal
45	<i>Inocybe bongardii</i> (Weinm.) Quél.	0	F1-15 Q3-12	QCE AC	MZ088095 NA	mycorrhizal
46	<i>Inocybe</i> sp.	1	T1-4 Q1-12 Q3-3	QCA AC AC	MZ088115 MZ088088 MZ088105 MZ088110	mycorrhizal
47	<i>Inocybe tarda</i> Kühner		Q1-8	AC	MZ088101	mycorrhizal
48	<i>Inocybe tenebrosa</i> Quél.	0	T2-17	QCA	MZ088127	mycorrhizal
49	<i>Lepiota</i> aff. <i>echinella</i> Quél. & G.E.Bernard	0	T1-14	QCA	MZ088120	saprobic
50	<i>Lepiota andegavensis</i> Mornand	0	T1-2	QCA	MZ088077	saprobic
51	<i>Lepiota clypeolaria</i> (Bull.) Quél.	0	F2-4	QCE	NA	saprobic
52	<i>Lepiota</i> sp.	0	F1-19	QCE	NA	saprobic
53	<i>Lepiota subincarnata</i> J.E.Lange	0	T1-10	QCA	MZ088118	saprobic
54	<i>Lepista nuda</i> (Bull.) Cooke	1	Q3-13	AC	NA	saprobic
55	<i>Lycoperdon perlatum</i> Pers. : Pers.	1	F1-11	QCE	NA	saprobic
56	<i>Lyophyllum</i> sp.	0	T2-12 T2-13	QCA QCA	NA NA	saprobic
57	<i>Macrolepiota procera</i> (Scop.) Singer	1	F1-9	QCE	NA	saprobic
58	<i>Mycena pura</i> (Pers.) P.Kumm. sensu lato	1	F1-10 T2-13	QCE QCA	NA NA	saprobic
59	<i>Mycena</i> sp. 1	0	T2-5	QCA	NA	saprobic
60	<i>Mycena</i> sp. 2	0	Q3-5	AC	NA	saprobic
61	<i>Omphalotus olearius</i> (DC.) Singer	1	F1-1	QCE	MZ088070	saprobic
62	<i>Paralepistopsis amoenolens</i> (Malençon) Vizzini	0	Q3-11	AC	MZ088114	saprobic
63	<i>Pseudosperma rimosum</i> (Bull.) Matheny & Esteve-Rav.	0	Q1-3	AC	NA	mycorrhizal
64	<i>Rhodocybe matesina</i> Picillo & Vizzini	0	F3-2	QCE	MZ088085	saprobic
65	<i>Rubroboletus lupinus</i> (Fr.) Costanzo, Gelardi, Simonini & Vizzini	0	T3-2 F3-1	QCA QCE	MZ088081 MZ088072	mycorrhizal
66	<i>Rubroboletus satanas</i> (Lenz) Kuan Zhao & Zhu L.Yang	1	F1-2	QCE	MZ088071	mycorrhizal
67	<i>Russula</i> aff. <i>alutacea</i> 1 (Fr.) Fr.	0	T2-3	QCA	MZ088079	mycorrhizal

	SPECIES	CHECKLIST STATUS	VOUCHER	ECOSYSTEM	GenBank ITS	TROPHIC MODE
68	<i>Russula</i> aff. <i>alutaceae</i> 2 (Fr.) Fr.	0	T3-1	QCA	MZ088080	mycorrhizal
69	<i>Russula praetervisa</i> Sarnari	0	T1-5	QCA	MZ088089	mycorrhizal
70	<i>Suillellus luridus</i> (Schaeff.) Murrill	0	T2-2	QCA	MZ088078	mycorrhizal
71	<i>Suillellus queletii</i> (Schulzer) Vizzini, Simonini & Gelardi	0	Q1-2	AC	MZ088075	mycorrhizal
72	<i>Suillellus</i> sp.	0	Q-AC	AC	MZ088073	mycorrhizal
73	<i>Tricholoma argyraceum</i> (Bull.) Gillet	0	T2-7	QCA	MZ088121	mycorrhizal
74	<i>Tricholoma orirubens</i> Quél.		Q1-14	AC	MZ088107	mycorrhizal
75	<i>Tricholoma scalpturatum</i> (Fr.) Quél.	1	T2-6 Q3-1	QCA AC	MZ088091 NA	mycorrhizal
76	<i>Tricholoma squarrulosum</i> Bres.	0	T3-12	QCA	MZ088131	mycorrhizal
77	<i>Tricholoma terreum</i> (Schaeff.) Quél.	1	T3-14 Q1-6	QCA AC	MZ088133 MZ088099	mycorrhizal
78	<i>Tricholoma triste</i> (Scop.) Quél.	0	Q1-7 Q3-2	AC AC	MZ088100 MZ088109	mycorrhizal
79	<i>Xerula melanotricha</i> Dörfelt	0	T3-5 Q3-7	QCA AC	NA NA	saprobic

*For checklist status, previously cited species were tagged by (1), and first mention by (0)

TABLE 4. Species richness (S), putative novel species (S_{novelty}) and number of rare species (S_{rare}) in the three studied ecosystems.

Ecosystem type	S	S _{novelty}	Putative novelty species designation	S _{rare}	Rare species names
<i>Abies cilicica</i>	20	4	<i>Cortinarius castaneus</i> sensu lato, <i>Inocybe</i> aff. <i>hirtella</i> , <i>Inocybe</i> sp., <i>Suillellus</i> sp.	1	<i>Paralepistopsis amoenolens</i>
<i>Quercus calliprinos</i>	42	5	<i>Cortinarius</i> aff. <i>diffRACTosuavis</i> , <i>Inocybe</i> sp., <i>Lepiota</i> aff. <i>echinella</i> , <i>Russula</i> aff. <i>alutacea</i> 1, <i>Russula</i> aff. <i>alutacea</i> 2	2	<i>Entoloma plebeioides</i> , <i>Lepiota andegavensis</i>
<i>Quercus cerris</i>	24	0	-	1	<i>Rhodocybe matesina</i>

Annex I: Mushroom collection identification sheet

Date (d/mm/yy):

Collector:

Collection number:

Possible Name:

Location:

Latitude: Longitude:

Habitat (wood, soil, leaf litter, moss, other mushrooms etc. and type of trees nearby):

Growth habit (solitary, gregarious, caespitose)

Pileus

Diameter:

Shape (young/mature): convex, conic, campanulate, depressed, umbonate, papillate, uplifted, parabolic, plane)

Colour:

Colour changes/hygrophanous:

Texture (hairy, scaly, fibrous, smooth, dry, moist, sticky, slimy, other)

Margin (smooth, striate, corrugated, with fringe of tissue)

Stipe Height:

Width:

Shape (tapering at base or apex, flattened, equal, bulbous base, clavate)

Context: solid, stuffed or hollow

Colour (including any changes when cut or bruised)

Texture:

Annulus or Cortina

Present or Absent

Colour:

Texture:

Persistent or disappearing with age

Volva

Present or Absent

Colour:

Texture:

Persistent or disappearing with age

Lamellae, pores or teeth

Form: blade, poroid (circular, angular, elongate, labyrinthiform), toothed

Colour when young:

Colour when mature:

Attachment: free, adnexed, adnate, sinuate, decurrent

Spacing: distant, moderate, close, crowded

Margin: smooth, serrated, wavy, scalloped, eroded; colour

Latex present or absent; if present, colour (including colour changes)

Flesh of Pileus and Stipe

Colour:

Colour changes (if any) when exposed to air:

Thickness:

Odour of whole fruiting body, flesh when cut or crushed

(farinaceous, geranium leaves, fungoid, radish, hot chili, flowers, fruit, almond, fish, garlic, honey, chlorine, rank, liquorice, maple, wintergreen) Other