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Contribution to the mycobiota of Türkiye: New macrofungal records from Koramaz Valley revealed by molecular methods

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Abstract

Purpose: This study aimed to document and identify the macrofungal diversity of Koramaz Valley (Kayseri, Türkiye) by combining classical morphological examinations—including critical microstructures such as basidia and basidiospores,—with molecular DNA barcoding of the ITS region. Türkiye still harbors a largely underexplored mycobiota. Since the scope and title of this work emphasize "new macrofungal records," generalizations in the study are based solely on the number of newly recorded taxa. Koramaz Valley, a unique ecosystem listed on the UNESCO World Heritage Tentative List, had not previously been investigated using such an integrative molecular and morphological framework for macrofungi.
Method: Fieldwork involved the systematic collection and photographic documentation of macrofungal specimens, with detailed recording of ecological and morphological features. DNA was extracted from dried samples, the ITS region amplified and sequenced, and the resulting data compared to reference sequences using BLAST and phylogenetic analyses (Maximum Likelihood in MEGA). Morphological and molecular data were integrated to achieve reliable species identification.
Findings: As a result, four macrofungal species—***Coprinellus saccharinus*** (Romagn.) P. Roux, Guy García & Dumas, ***Fomes inzengae*** (Ces. & De Not.) Cooke and ***Lepista ricekii*** Bon—were recorded for the first time in Türkiye based on molecular evidence. Phylogenetic trees constructed for each genus revealed strong bootstrap support for the identification and placement of the studied specimens, with some samples demonstrating close but distinct relationships to reference taxa. These findings highlight the effectiveness of the ITS region in resolving species-level relationships and underscore the need for molecular approaches in fungal taxonomy.
Conclusion: In conclusion, the study significantly expands the known macrofungal diversity of Koramaz Valley and Türkiye. It demonstrates the indispensable role of integrating molecular and morphological methods in the discovery, documentation, and conservation of regional fungal biodiversity, and indicates the potential for novel species in the area.
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Keywords: Koramaz Valley, macrofungi, DNA barcoding, ITS gene region, biodiversity

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Türkiye mikobiyotasına katkı: Koramaz Vadisi'nden moleküler yöntemlerle yeni makromantar kayıtları

Özet

Amaç: Bu çalışma, Koramaz Vadisi'nin (Kayseri, Türkiye) makrofungal çeşitliliğini ortaya koymak ve tanımlamak amacıyla yürütülmüş olup, temel mikro yapılar (basidyum ve basidiyospor) dâhil klasik morfolojik incelemeler ITS bölgesine yönelik moleküler DNA barkodlama ile birleştirilmiştir. Türkiye mikotası hâlen büyük ölçüde yeterince araştırılmamıştır. Çalışmanın kapsamı ve başlığı "yeni makrofungal kayıtlar" üzerine yoğunlaştığından, yapılan genellemeler yalnızca yeni kaydedilen taksonların sayısına dayandırılmıştır. UNESCO Dünya Mirası Geçici Listesi'nde

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yer alan ve kendine özgü ekosistemlere sahip Koramaz Vadisi, bu çalışmadan önce makrofunguslar açısından bütüncül bir moleküler ve morfolojik yaklaşımla incelenmemiştir.
Metod: Arazi çalışmalarında makromantar örnekleri sistematik olarak toplanmış, ekolojik ve morfolojik özellikleri ayrıntılı biçimde kaydedilmiştir. Kurutulmuş örneklerden DNA izole edilmiş, ITS bölgesi çoğaltılıp dizilenmiş ve elde edilen veriler BLAST ile referans dizilerle karşılaştırılarak filogenetik analizler (MEGA'da Maksimum Olasılık yöntemiyle) gerçekleştirilmiştir. Tür teşhisinde morfolojik ve moleküler veriler birlikte değerlendirilmiştir.
Bulgular: Sonuç olarak, dört makromantar türü ***Coprinellus saccharinus*** (Romagn.) P. Roux, Guy García & Dumas, ***Fomes inzengae*** (Ces. & De Not.) Cooke ve ***Lepista ricekii*** Bon, moleküler kanıta dayalı olarak Türkiye'den ilk kez kaydedilmiştir. Her bir cins için oluşturulan filogenetik ağaçlarda örneklerin teşhisi ve konumlandırılması için yüksek bootstrap desteği elde edilmiş, bazı örneklerin referans taksonlara yakın ancak farklı ilişkiler gösterdiği belirlenmiştir. Bulgular, ITS bölgesinin tür düzeyinde ayırt edici gücünü ve mantar taksonomisinde moleküler yaklaşımların gerekliliğini ortaya koymaktadır.
Sonuç: Sonuç olarak bu çalışma, Koramaz Vadisi ve Türkiye'nin bilinen makromantar çeşitliliğini önemli ölçüde genişletmiş; keşif, dokümantasyon ve koruma için moleküler ve morfolojik yöntemlerin entegrasyonunun vazgeçilmezliğini göstermiştir. Ayrıca, bölgede yeni türlerin bulunabileceğine işaret etmektedir.

Anahtar kelimeler: Koramaz Vadisi, makromantar, DNA barkodlama, ITS gen bölgesi, biyolojik çeşitlilik

1. Introduction

Numerous systematic investigations have focused on macrofungi in Türkiye, and while considerable advancements have been made recently, the mycota of Türkiye remains incompletely documented [1–4]. A significant number of studies addressing macrofungal diversity have been conducted, and many are still ongoing both within Türkiye and globally. These efforts have notably enriched the known macrofungal diversity of Türkiye. In 2020, a comprehensive checklist of Turkish fungi was published, compiled through extensive collaboration among Turkish mycologists [5]. According to this checklist, 5865 fungal taxa have been recorded in Türkiye to date, including 2782 Basidiomycota, 2728 Ascomycota, 282 Myxomycota, 2 Chytridiomycota, 33 Oomycota, and 38 Zygomycota species. In addition, there are about 300 wild edible mushroom species in Türkiye [6]. Once the biodiversity of macrofungi in Türkiye is fully cataloged, it will be possible to clearly determine the number of macrofungal taxa present in the country, their geographic distributions, which species are edible or toxic, and to what extent these mushrooms are recognized and consumed by both local and national populations.

Fungi are eukaryotic, chlorophyll-lacking, heterotrophic, spore-producing organisms that typically possess a cell wall containing chitin and exhibit saprophytic, parasitic, or symbiotic lifestyles. These organisms play a crucial role in ecosystems as decomposers, forming an essential link in nutrient cycles. Some fungi break down dead organic matter and return it to the soil, while others establish symbiotic relationships with plant roots as mycorrhizae; in the form of lichens, they live in mutual association with algae or cyanobacteria. Throughout human history, fungi have been used for nutritional, medicinal, and cultural purposes. Today, the pharmaceutical, food, biotechnological, and industrial applications of fungi are steadily increasing. Although systematic studies of fungi date back some 250 years, advancements in molecular biology—particularly DNA sequence analyses based on the ITS gene region—have become highly significant in taxonomic identification.

Koramaz Valley, with its rich biodiversity and unique ecosystems, holds significant scientific and conservation value and is included on the UNESCO World Heritage Tentative List. Located on the Anatolian plateau of Türkiye, this valley has been the subject of only a few studies regarding its macrofungal diversity—these being mostly limited to morphological observations, with no molecular research to date. This gap has formed the basis for the present study.

The first study on the macrofungi of Koramaz Valley was conducted by Allı (2021). In this research, 64 macrofungal specimens were collected from various localities in Koramaz Valley (Melikgazi, Kayseri, Türkiye) and its surroundings during 2019–2020. As a result of field and laboratory investigations on these specimens, a total of 36 taxa belonging to 17 families and two divisions (Ascomycota and Basidiomycota) were identified, and their morphology, habitat, ecology, and distribution data were presented. In this study, Entoloma alpicola was also reported as a new record for Türkiye, and detailed information was provided regarding its morphological description, locality, collection date, and distribution [7].

In this article, three macrofungal species distributed in Koramaz Valley were examined comprehensively using both morphological characteristics and DNA barcoding techniques targeting the ITS gene region. Fieldwork and laboratory analyses revealed that molecular identification was particularly valuable for distinguishing species that are difficult to differentiate morphologically or appear similar to one another. Based on the findings, the species ***Coprinellus saccharinus*** (Romagn.) P. Roux, Guy García & Dumas, ***Fomes inzengae*** (Ces. & De Not.) Cooke, and ***Lepista ricekii*** Bon were definitively recorded from Türkiye for the first time through molecular methods.

Ultimately, this research provides up-to-date and significant insights into the macrofungal biodiversity of Koramaz Valley, contributing both new records to the Turkish mycobiota and presenting a notable example with the potential to represent a novel species. Furthermore, the study demonstrates once again the effectiveness of integrating

DNA barcoding and phylogenetic analyses with traditional morphological approaches for revealing the true fungal diversity of Türkiye.

2. Materials and methods

2.1. Collection of Mushroom Samples and Documentation of Morphological Features

Prior to collection, the mushroom specimens were photographed, as certain morphological characteristics (such as coloration and size) may alter after drying. Additionally, information about the habitat—including vegetation type, GPS (Global Positioning System) coordinates, altitude, and other relevant data necessary for morphological assessment—was recorded at the collection site. The samples were assigned numbers and then air-dried. Subsequently, each specimen was placed in ziplock plastic bags to be preserved as fungarium material.

During morphological investigation, various features were noted, including the dimensions, color, and shape of the basidiocarps (cap), whether the surface was moist or dry, the coloration of the flesh, the size and color of the stem (as well as any color changes upon cutting), its shape, the presence or absence of a swollen base, and whether the stipe was straight or curved.

2.2. Molecular Characterization

2.2.1. DNA Extraction, PCR, and Sequencing

For molecular analysis, dried macrofungal material was utilized. DNA was extracted following the protocol of the Nucleogene Plant Kit. In this investigation, the non-coding ITS rDNA region was targeted for sequence analysis. The ITS1F and ITS4 primer pairs were employed for both the PCR amplification and subsequent sequencing steps of the extracted DNA [8,9]. PCR products were separated on a 1.5% agarose gel via electrophoresis, stained with ethidium bromide, and visualized using a UV transilluminator. Sequencing was carried out at Nucleogene company.

2.2.2. Sequence alignment and phylogenetic analysis

The resulting DNA sequences were compared to those in GenBank (NCBI) using the BLAST tool to verify identity. Raw sequence data were aligned and edited with BioEdit version 7.2.5 (Biological Sequence Alignment Editor) software [10]. Sequence alignment was conducted using the Clustal W algorithm within BioEdit. The ITS gene region sequences acquired from our samples were compared with those of *Agaricus* L. species listed in GenBank. To explore phylogenetic relationships and construct evolutionary trees, the Maximum Likelihood method was applied in the MEGA 7.0 (Molecular Evolutionary Genetics Analysis) software, using 1000 bootstrap replications to assess support for branches [11].

3. Results

3.1. List of Newly Recorded Species, Their Descriptions, Ecologies, and Molecular Assessments

3.1.1. *Coprinellus saccharinus* (Romagn.) P. Roux, Guy García & Dumas

Coprinellus saccharinus (Romagn.) P. Roux, Guy García & Dumas is a small, short-lived macrofungal species belonging to the family Psathyrellaceae and displays a saprotrophic lifestyle [12]. Initially placed within the genus *Coprinus*, the species was later reassigned to the genus *Coprinellus* following molecular phylogenetic studies [13]. The species epithet "saccharinus" refers to the presence of sparkling, sugar crystal-like granules found on the cap surface in young specimens, which serve as a key morphological identifier, especially when fresh [12].

Description:

The cap measures 1–3 cm in diameter and starts out elliptical or ovoid, maturing into a bell-shaped or slightly convex form (Fig. 1). Its coloration ranges from grey to beige tones. Fresh individuals exhibit shiny, sugar-like granules on the surface of the cap. The cap is striate and tends to fade with age; in wet conditions, it may become slightly sticky. Initially white, the gills shift to grey and eventually black as they mature. Autodigestion (deliquescence), in which the gills liquefy as they age, is a defining feature of the genus *Coprinellus* P. Karst. The stipe is slender, hollow, and fragile, measuring 3–7 cm in length, with a whitish coloration and sometimes a powdery surface. The basidiospores of *Coprinellus saccharinus* are ellipsoid, smooth, and brown in color, measuring 8–10 × 4.5–6 µm, with a distinct germ pore (Fig. 2). However, since the basidiospores themselves do not show any special discriminating features, additional micromorphological parameters such as basidia and cystidia are also considered in the description. No annulus is present.

The mushroom has a faint, unremarkable odor. Taste is generally not assessed, and due to its unknown edibility, sampling for taste is not recommended.

Coprinellus saccharinus is a saprotroph commonly found growing singly or in small clusters on decaying tree bark, woody debris, or humus-rich soils [12]. It appears in temperate regions, typically during spring and autumn, and prefers shaded, moist environments such as parks, gardens, and forest clearings. This study presents the first confirmed report of the species from Türkiye.

There is no reliable information on the edibility of this species. Its small size, rapid deterioration, and the risk of confusion with similar species make it unsuitable for consumption. Caution is also advised due to the potential for adverse reactions with alcohol, a trait documented in some related *Coprinellus* and *Coprinopsis* species [14].

Specimen examined: Türkiye, Kayseri, Melikgazi, Koramaz Valley, Bağpınar site, 38° 41' 51" N, 35° 35' 15" E, alt.1330m, 21.10.2024, leg. M. Güllü and M.G. Halıcı (M87).

Comparison with Similar Species:

Coprinellus micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson, which also displays granules on the cap surface, can be confused with *C. saccharinus*, though the granules in *C. micaceus* are typically larger and more golden in color. *Coprinellus disseminatus* (Pers.) J.E. Lange is much smaller, forms dense clusters, and lacks shiny cap granules. *Coprinopsis lagopus* (Fr.) Redhead, Vilgalys & Moncalvo is characterized by a larger size, darker coloration, and a generally hairy stipe. Careful observation of cap granulation, deliquescent gills, and habitat is necessary to accurately distinguish these species [12,14,15].

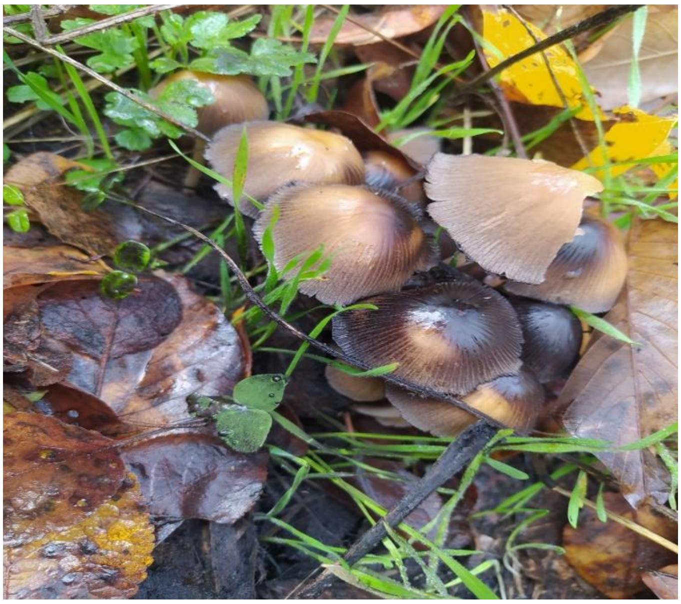

Figure 1. General appearance of *Coprinellus saccharinus* from Koramaz Valley

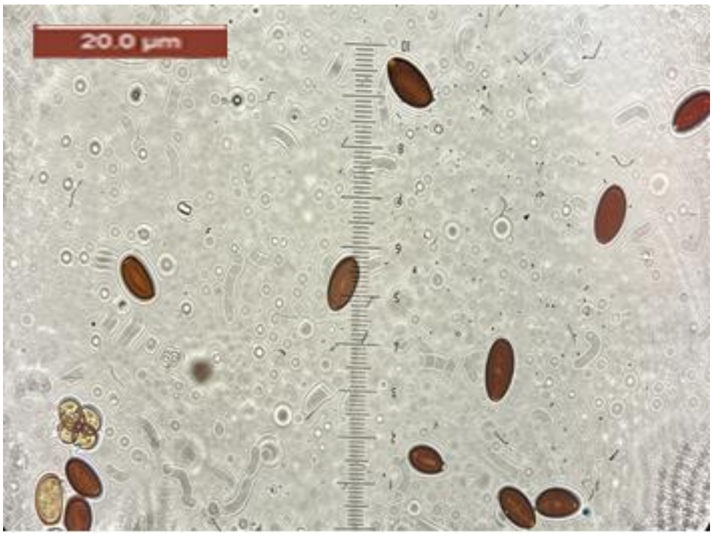

Figure 2. *Coprinellus saccharinus.* Basidiospores.

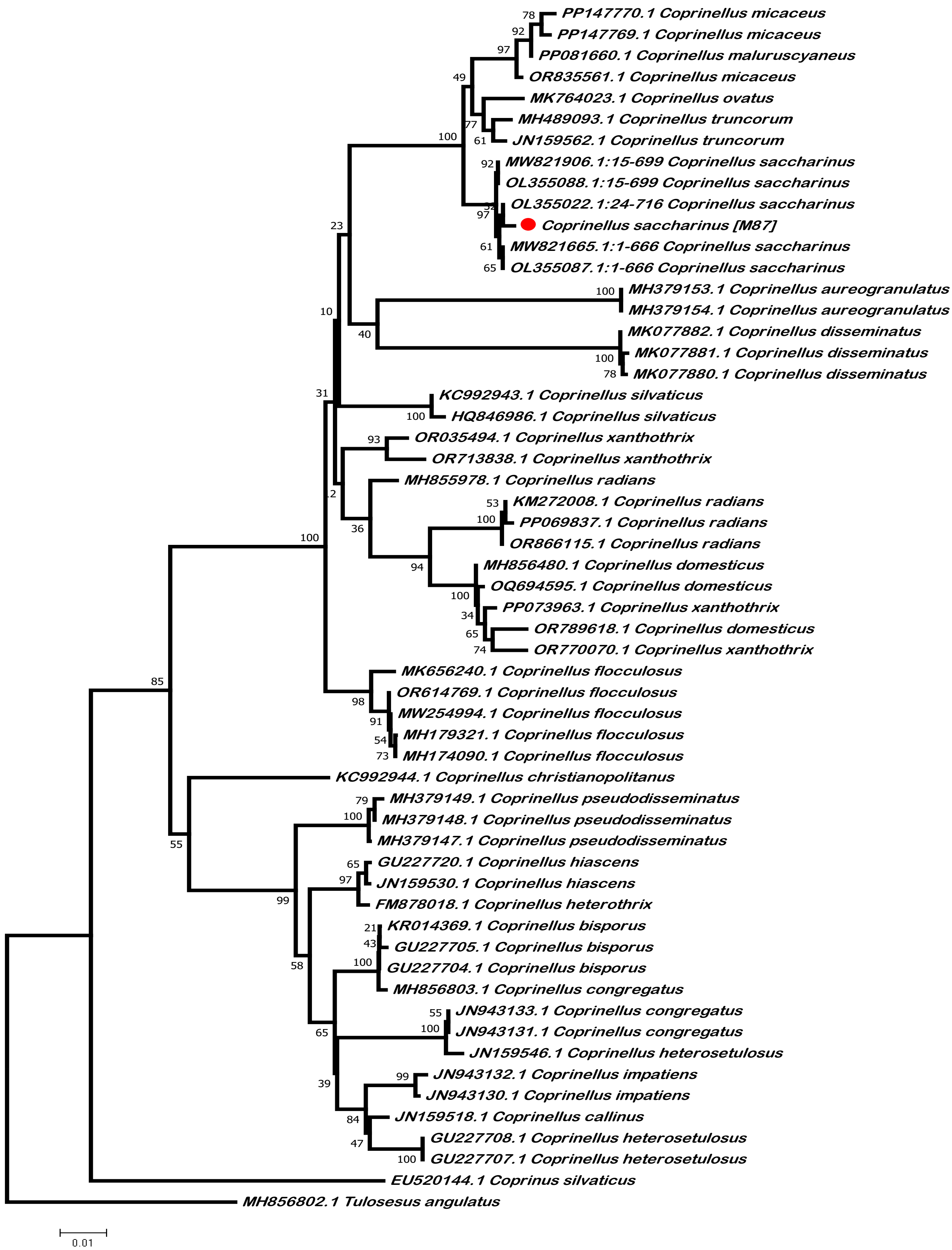

Figure 3. Maximum Likelihood dendrogram based on ITS gene region sequences of species belonging to the genus *Coprinellus*. Outgroup: *Tulosesus angulatus*. The studied specimen is indicated by the code M87.

The phylogenetic tree in Figure 3 was constructed based on ITS gene region sequences of species belonging to the genus *Coprinellus* and strongly reveals the phylogenetic relationships among the species. The specimen labeled M87 perfectly matches *Coprinellus saccharinus* sequences and is positioned in a phylogenetic location that overlaps with this species with almost 100% support; this indicates that the molecular identification of the specimen is highly reliable. The clear distinctions observed throughout the tree demonstrate that the ITS region is effective for species-level identification within the genus *Coprinellus*, particularly by showing that the M87 specimen exhibits high genetic similarity with its respective species and can be reliably identified.

3.1.2. Fomes inzengae **(Ces. & De Not.) Cooke**

Fomes inzengae (Ces. & De Not.) Cooke is a wood-decaying fungus in the family Polyporaceae. Originally described as *Polyporus inzengae*, it was reassigned to the genus *Fomes* by Cooke in 1885. Until recently, this species was frequently confused with *Fomes fomentarius* (L.) Fr., but molecular analyses (using ITS, LSU, RPB1, RPB2, and TEF1 gene regions) have established that, although both belong to the same species complex, they are distinct taxa [17].

Description

The fruiting body is perennial and hoof-shaped, sometimes reaching up to 60 cm in width. It is thick, tough, and exhibits noticeable zoning (Fig. 4). Upper surface: generally smooth, with light brown to gray zones, and may develop cracks with age. The pore layer is creamy to gray, containing 13–32 pores per centimeter; the pore density in F. inzengae tends to be slightly higher compared to F. fomentarius. The dimensions of the spores and the diameter of the skeletal hyphae are highly similar to those of related species; therefore, additional micromorphological parameters such as basidia and cystidia, together with molecular markers, are essential for accurate identification (Fig. 5).

Fomes inzengae is predominantly distributed in Southern Europe and regions with a Mediterranean climate. It primarily colonizes hardwood hosts such as oak, beech, chestnut, and plane trees. While it can occur alongside *F. fomentarius* in similar habitats, *F. inzengae* is more commonly found at lower elevations and in warmer environments. The first records of this species come from countries such as Italy, Austria, the Czech Republic (Moravia), Spain, Slovakia, and Belgium. Both *F. inzengae* and *F. fomentarius* are perennial, woody, polypore Basidiomycetes that grow on the bark of living or dead trees. Given their morphological similarities, *F. inzengae* was often misidentified as *F. fomentarius* in the past.

This species is considered inedible. It causes "white rot" as it decays wood and should not be consumed either raw or processed. Nonetheless, species within the genus *Fomes* have been traditionally utilized for purposes such as tinder, in biotechnology, and as sources of various bioactive compounds.

Specimen examined: Türkiye, Kayseri, Melikgazi, Koramaz Valley, Southwest of Keşlik District, 38° 41' 31" N, 35° 33' 23" E, alt.1285m, 09.09.2024, leg. M. Güllü and M.G. Halıcı (M12, M25, M28 and M30).

Comparison with Similar Species

Fomes fomentarius and *Fomes inzengae* are morphologically and ecologically close species that have often been confused in the past. Both taxa produce perennial, hoof-shaped basidiocarps that may reach large sizes and display a tough, woody consistency. However, subtle but consistent differences allow them to be distinguished. The upper surface of *F. fomentarius* typically shows a gray to dark gray coloration with concentric zoning, while *F. inzengae* usually exhibits lighter brown to grayish zones and may show a slightly smoother texture [18].

Pore density is another discriminating trait. *F. fomentarius* generally has 2–5 pores per mm, whereas *F. inzengae* tends to have a higher pore density, with 3–6 pores per mm. Microscopically, their basidiospores are highly similar in size and shape, rendering molecular data essential for reliable identification. Recent phylogenetic studies based on ITS sequences have confirmed that these two species form distinct but closely related clades, highlighting the necessity of integrating molecular approaches with traditional morphology [18].

Figure 4. General appearance of *Fomes inzengae* from Koramaz Valley.

Figure 5. *Fomes inzengae.* Basidiospore.

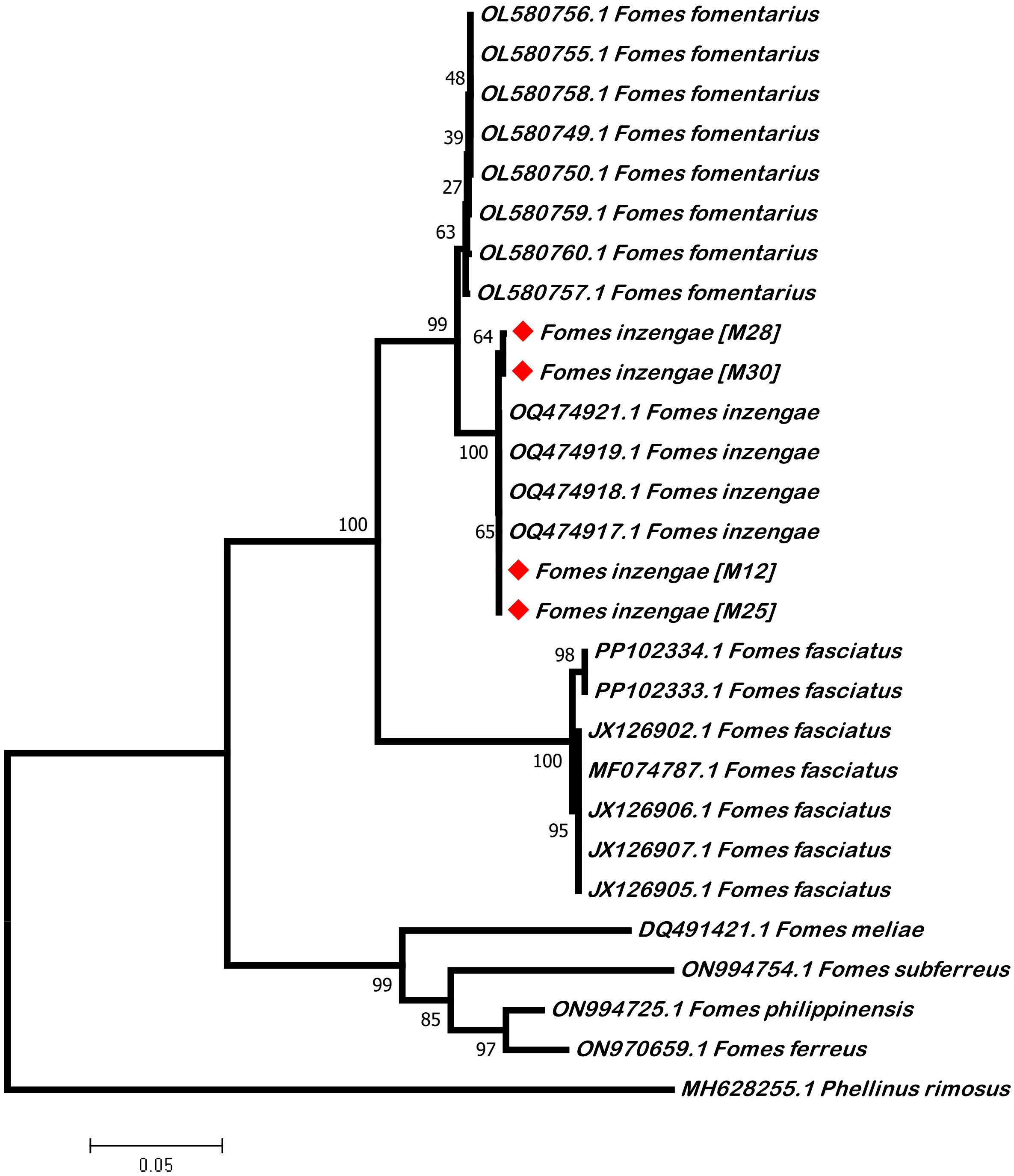

Figure 6. Maximum Likelihood dendrogram based on ITS gene region sequences of species belonging to the genus *Fomes*. Outgroup: *Phellinus rimosus*. The studied specimens are indicated by the codes M12, M25, M28, and M30

The phylogenetic tree in Figure 6 was constructed based on ITS gene region sequences of species belonging to the genus Fomes and reliably reveals the evolutionary relationships among the specimens. In particular, the samples coded M12, M25, M28, and M30 were remarkably matched with *Fomes inzengae* when compared to reference species.

M12, M25, M28, and M30 are located within a strongly supported clade with 100% bootstrap support, directly grouped with the *Fomes inzengae* reference sequences (OQ474918.1, OQ474919.1, OQ474920.1, OQ474921.1). This high support value clearly indicates that these four specimens undoubtedly belong to *Fomes inzengae* at the molecular level. The studied specimens are grouped closest to *F. fomentarius* at the molecular level. This finding reveals that, although the samples may resemble *F. fomentarius* morphologically, they are definitively *F. inzengae* at the molecular level.

Across the tree, other species such as *Fomes fasciatus* (Sw.) Cooke, *F. meliae* (Underw.) Murrill, *F. subferreus* Murrill, *F. philippinensis* Murrill, *and F. ferreus* Sacc. are identified as distinct, highly supported clades. This structure demonstrates that the ITS gene region is a highly reliable molecular marker for distinguishing Fomes species. Phellinus rimosus was used as the outgroup to root the tree.

In conclusion, all specimens (M12, M25, M28, M30) matched *Fomes inzengae* with high bootstrap values of around 100% in the ITS-based analyses, demonstrating that their species identification is strongly supported at the molecular level.

3.1.4. Lepista ricekii Bon

Lepista ricekii Bon is a small to medium-sized macrofungal species exhibiting a saprotrophic lifestyle. It was first described scientifically by Bon in 1983, with the species epithet referencing its distinctly bitter or peppery taste. The species had previously been referred to invalidly as *Lepista piperata* (Bon, 1983). It is most commonly encountered in moist meadows and forest clearings throughout Europe. To date, there have been no confirmed records of this species in the mycobiota of Türkiye.

Description:

Measuring 4–10 cm in diameter, the cap is convex when young and becomes slightly depressed at the center with maturity (Fig.7). Its surface is slightly rough or scaly, with coloration varying from pale brown and grey-beige to yellowish tones. The cap surface may be sticky or shiny when wet, turning matte upon drying. The gills are crowded and free from the stipe, initially whitish and gradually shifting to a grey-brown hue as the mushroom ages. Stipe length is 3–6 cm, cylindrical, with a fibrous surface, and coloration similar to or paler than the cap. The stem is often eccentrically positioned rather than centrally attached. The spore print is pale grey-pink. Spores are elliptical, thin-walled, and minutely roughened, measuring 5–6 × 3–4 µm (Fig.8). There is no notable odor, but the taste is distinctly bitter or peppery—an important diagnostic feature distinguishing it from other *Lepista* species.

Lepista ricekii functions as a saprotroph, typically occurring in humus-rich, moist grasslands, forest clearings, and among herbaceous plant debris. It can be observed from late spring to late autumn. The species is particularly reported from Central and Western Europe. This study documents the first confirmed record of *L. ricekii* from Türkiye.

This species is generally regarded as inedible. Its flesh is insubstantial, and the strong bitter or peppery taste discourages consumption. Misidentification with other *Lepista* species is possible and caution is advised. While some sources note that it may be conditionally edible, its culinary value is considered negligible [11,17].

Specimen examined: Türkiye, Kayseri, Melikgazi, Koramaz Valley, Southwest of Keşlik District, 38° 41' 31" N, 35° 33' 23" E, alt.1285m, 09.09.2024, leg. M. Güllü and M.G. Halıcı (M47 and M58).

Comparison with Similar Species:
According to the literature, the studied species has been compared with morphologically similar taxa as outlined below:

- *Lepista nuda* (Bull.) Cooke: Characterized by a purple hue, pleasant fragrance, and edibility; in contrast, *L. ricekii* is paler and bitter.
- *Lepista saeva* (Fr.) P.D. Orton: Has a thicker flesh, denser gills, and a more greyish-beige color.
- *Collybia sordida* (Schumach.) Z.M. He & Zhu L. Yang: Smaller and paler, with even smaller spores.
- *Lepista ricekii* can be recognized by its peppery taste, and pale pink spore print.

According to the phylogenetic analysis, the newly recorded *Lepista ricekii* clustered most closely with *Lepista irina* (Fr.) H.E. Bigelow. Morphologically, however, *L. ricekii* can be distinguished by its paler coloration with pinkish tones, peppery taste, and pale pink spore print, whereas *L. irina* typically has a whitish to cream cap with a farinaceous odor, mild taste, and slightly larger spores.

The phylogenetic tree presented in Figure 9 was constructed based on ITS gene region sequences from species within the genus *Lepista*, providing a reliable depiction of evolutionary relationships among the taxa. Samples M47 and M58 are positioned within a strongly supported clade, receiving 100% bootstrap support, and are clearly separated from species closely related to *Lepista nuda*. These samples are clustered with reference sequences such as KJ681016.1, DQ659047.1, and OR863453.1, all forming a group under *Lepista ricekii*. This high level of support unequivocally demonstrates that M47 and M58 can be definitively assigned to *Lepista ricekii* at the molecular level.

Throughout the tree, several species—such as *Lepista nuda* (Bull.) Cooke, *Lepista glaucocana* (Bres.) Singer, *Lepista saeva (*Fr.) P.D. Orton, *Lepista sordida* (Schumach.) Singer, *Lepista tarda* (Peck) Murrill, *Lepista irina* (Fr.) H.E. Bigelow, *Lepista densifolia* (J. Favre) Singer & Clémençon, and *Lepista subconnexa* (Murrill) Harmaja —form well-supported, distinct clades. This highlights the strong discriminatory power of the ITS gene region as a molecular marker for species identification within the genus *Lepista*. The use of *Clitocybe metachroa* (ON323015.1) as an outgroup facilitated the rooting of the tree, thereby contributing to the accurate inference of evolutionary relationships.

In conclusion, M47 and M58 display a perfect match with *Lepista ricekii*, supported by 100% bootstrap value. The placement of these samples within the same phylogenetic branch as reference sequences provides robust scientific validation for their molecular identification. The overall topology of the phylogenetic tree supports consistent species delimitation and demonstrates that reliable classification can be achieved based on ITS sequences.

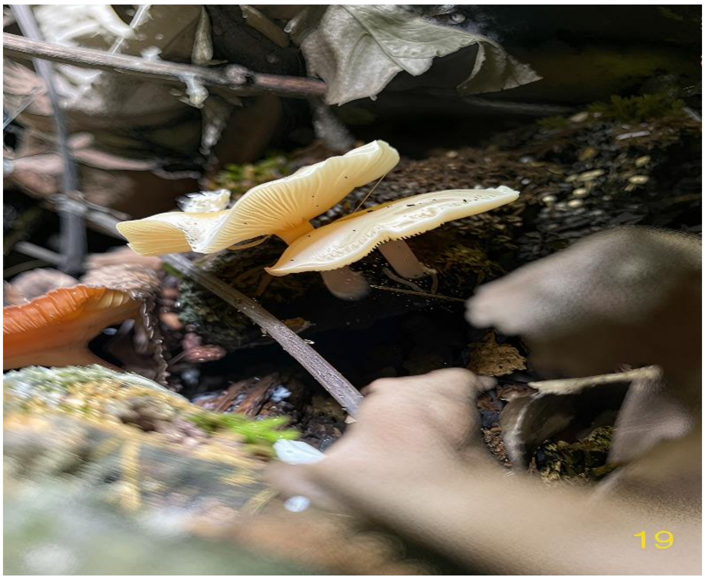

Figure 7. General appearance of *Lepista ricekii* from Koramaz Valley

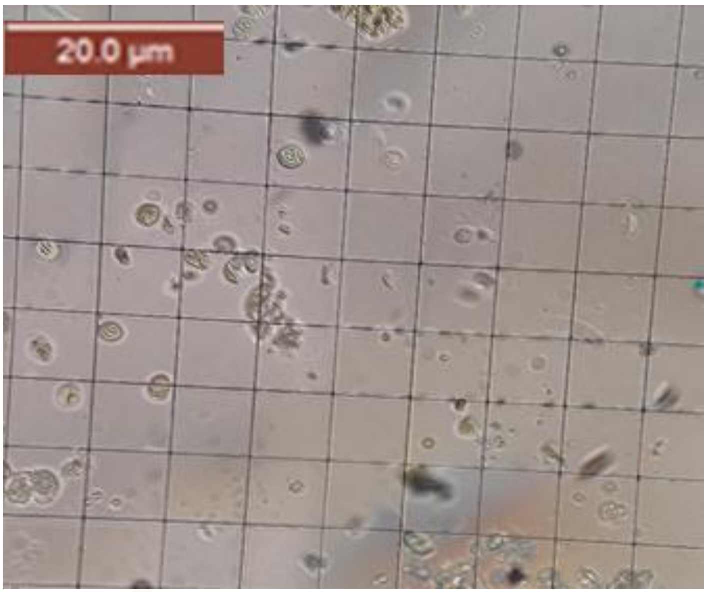

Figure 8. *Lepista ricekii.* Basidiospores

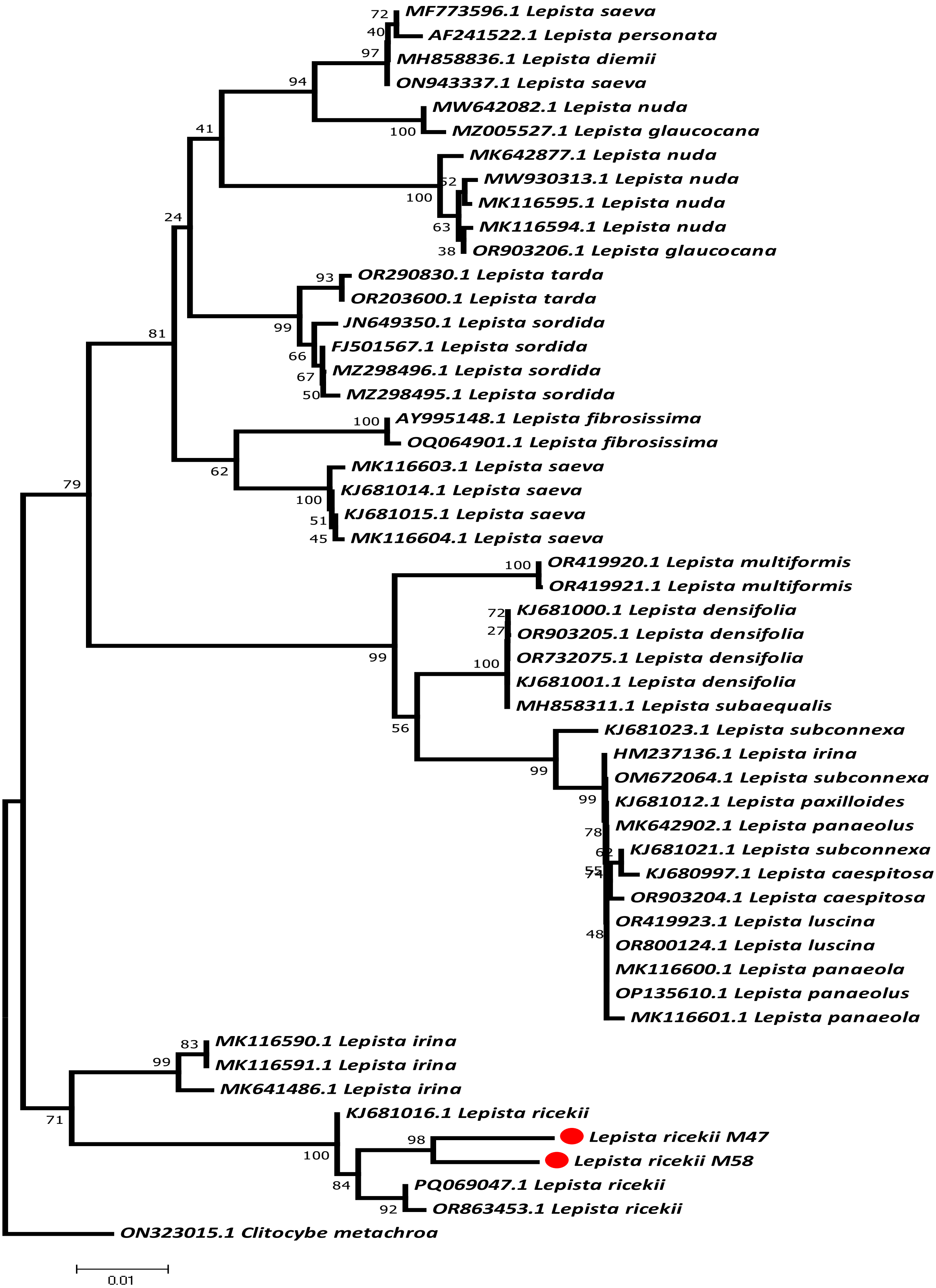

Figure 9. Maximum Likelihood dendrogram based on ITS gene region sequences of *Lepista ricekii* and related species. Outgroup: *Clitocybe metachroa*. The studied specimens are indicated by the codes M47 and M58

4. Conclusions and discussion

This study aimed to identify some macrofungi samples collected in Koramaz Valley (Kayseri) using both morphological examination and DNA barcoding (ITS gene region), thereby confirming the current distribution of known species as well as documenting taxa potentially new to the Turkish mycobiota. The findings reaffirm that when molecular identification is combined with traditional morphological methods, it is crucial for achieving precise species delimitation.

For species that are morphologically difficult to distinguish or display divergent characteristics, molecular comparison and the elucidation of phylogenetic relationships are essential. Research of this kind contributes not only to the discovery but also to the conservation of Türkiye's rich genetic resources. Comprehensive knowledge of regional diversity is the first critical step in compiling the mycot of any country or area. Subsequent phases involve detailed taxonomic revision of the collected taxa, followed by the synthesis of floristic works informed by all available data. For Turkish macrofungi, substantial progress has been made at the initial stage, and it is now possible to advance to revisionary studies while continuing floristic documentation [19].

Additionally, Koramaz Valley is listed on the UNESCO World Heritage Tentative List, and the assessment of its macrofungal biodiversity will provide valuable insights. Identifying edible and toxic fungi by integrating morphological and anatomical approaches with molecular techniques will lead to more accurate and reliable results.

The first study on the macrofungi of Koramaz Valley was conducted by Allı (2021), who examined 64 macrofungal specimens collected from the valley and its surroundings during 2019–2020. Based solely on morphological and anatomical analyses, Allı identified 36 taxa belonging to 17 families and reported *Entoloma alpicola* as a new record for Türkiye [7].

In contrast, the present study examined three distinct macrofungal species distributed in Koramaz Valley using an integrative approach that combined morphological, anatomical, and molecular techniques (ITS DNA barcoding). Three of these species—***Coprinellus saccharinus*** (Romagn.) P. Roux, Guy García & Dumas, ***Fomes inzengae*** (Ces. & De Not.) Cooke, and ***Lepista ricekii*** Bon—are reported from Türkiye for the first time based on molecular confirmation.

Thus, while Allı (2021) contributed valuable morphological data on the valley's fungal diversity, this study advances the knowledge further by applying molecular tools to confirm species identities and by recording several taxa as new to the Turkish mycobiota. Moreover, the phylogenetic analyses revealed that some specimens exhibit substantial divergence from currently known species, suggesting the potential for new taxa. Further detailed investigations of these unique samples may ultimately lead to the description of novel species in the scientific literature. Overall, these findings highlight the indispensable role of DNA barcoding and phylogenetic approaches in uncovering the true diversity of the Turkish mycobiota.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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