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A macrofungus taxon that is commonly eaten by the folk in Central Anatolia but never reported from Türkiye: *Agaricus pequinii* (Boud.) Konrad & Maubl.

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Abstract

As in the world, macrofungi are one of the important food sources in terms of closing the food deficit in our country. *Agaricus pequinii* (Boud.) Konrad & Maubl.is a rare and edible mushroom with a mild taste that grows mainly on forest soils and on meadows. Although this taxon is very common in the meadows of Kayseri, located in central Anatolia (Türkiye) and frequently collected and eaten by the folk between October and the end of December, it was never reported in the checklists of Türkiye. In this study, *Agaricus pequinii* was studied in terms of morphological, anatomical and molecular aspects and added to the fungal checklist of Türkiye.

Key words: *Agaricaceae*, biodiversity, edible mushroom, ITS gene region.

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Orta Anadolu'da halk tarafından yaygın olarak yenilebilen ancak Türkiye'den hiç bildirilmemiş bir makrofungus taksonu: *Agaricus pequinii* (Boud.) Konrad & Maubl.

Özet

Makrofunguslar dünyada olduğu gibi ülkemizde de besin açığının kapatılması açısından önemli besin kaynaklarından biridir. *Agaricus pequinii* (Boud.) Konrad & Maubl., ağırlıklı olarak orman topraklarında ve çayırlarda yetişen, hafif bir tada sahip, nadir ve yenilebilir bir mantardır. Bu takson, İç Anadolu'da (Türkiye) yer alan Kayseri'nin çayırlarında çok yaygın olmasına ve Ekim ile Aralık sonu arasında halk tarafından sıklıkla toplanıp yenmesine rağmen, Türkiye kontrol listelerinde hiç bildirilmemiştir. Bu çalışmada, *Agaricus pequinii* morfolojik, anatomik ve moleküler yönden incelenmiş ve Türkiye mantar kontrol listesine eklenmiştir.

Anahtar kelimeler: *Agaricaceae*, biyoçeşitlilik, yenilebilir mantar, ITS gen bölgesi.

1. Introduction

Although there are around 144,000 identified fungal species in the world, it is estimated that the total number of fungal species may be between 2.2 and 3.8 million, which is more than 6 times the estimated number of plants [1]. These revised estimates are based on the analysis of environmental sequence data, which has grown rapidly as a result of particularly reliable statistical and phylogenetic approaches. Many systematic studies have been carried out on macrofungi in Türkiye and although significant progress has been made in recent years, Türkiye's mycota has not been completed yet [2-4]. Many studies on macrofungal diversity were carried out and yet many are still ongoing both in Turkey and in world. As a result of these studies, significant contributions have been made to the macrofungal diversity of Türkiye. A checklist of the fungi of Türkiye was published in 2020 with broad cooperation of Turkish mycologists [5]. According to this checklist, a total of 5865 fungal taxa, including 2782 Basidiomycota, 2728 Ascomycota 282 Myxomycota, 2 Chytridiomycota, 33 Oomycota and 38 Zygomycota species identified in Turkey have been listed so far. There are approximately 300 edible nature mushrooms in Türkiye [6]. With the completion of macrofungi biodiversity in Türkiye,

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the number of macrofungi taxa distributed in Türkiye, the distribution areas of these taxa, which ones are edible or poisonous, and how much the local and general local population witness and consume these mushrooms will be understood.

In this article, *Agaricus pequinii*, an edible macrofungi species that is consumed by the folk in Kayseri, has been studied in terms of morphological, anatomical and molecular aspects. As a result of the molecular studies, it was concluded that this species is a new record for Türkiye. This result was clearly determined by the phylogenetic tree created with the species belonging to the genus *Agaricus* taken from the Genbank (Figure 1).

2. Materials and methods

2.1. Collection of Mushroom Sample and Recording of Morphological Characteristics

Photographs were taken of the mushroom sample before it was collected (due to the fact that some morphological characters (color, dimensions, etc.) change when the mushrooms are dried). In addition, vegetation (vegetation) characteristics of the area where the fungus was found, GPS (Global Positioning System) coordinates, altitude above sea level and some other necessary information for morphological diagnosis were recorded. The numbered sample was dried. Then, the collected mushroom was kept in ziplock plastic bags as a fungarium sample.

For example, when performing morphological characterization, the size, color, shape, slippery or dryness of the basidiocarps (cap), flesh color, stem dimensions, color (changes in color when cut or not), shape, whether the base of the stem is swollen or not, whether the stem is straight or completely or not.

2.2. Molecular Characterization

2.2.1. DNA Isolation, PCR and Sequencing

For the molecular characterization of the sample, a dried macrofungi sample was used. DNA isolation from the macrofungi sample was performed using the protocol included in the DNeasy Plant Mini Kit (Qiagen, Catalog No: 69104). In the study, the ITS rDNA gene region, which cannot be transcribed, was used for DNA sequence analysis. ITS1F and ITS4 primers were used for both PCR amplification and DNA sequencing of isolated DNAs [7,8]. At the end of PCR applications, the amplified gene regions were run on agarose gel (1.5%) with the help of electrophoresis, and then stained with ethidium bromide and visualized with the help of UV imaging system. Sequencing was done at the BM Labosis laboratory.

2.2.2. Sequence alignment and phylogenetic analysis

Sequences obtained from the analyzed samples were compared with the samples registered in the GenBank (NCBI) using the BLAST program and their suitability was determined. Alignment and editing of the raw data obtained from the DNA sequence analysis system was performed using the BioEdit 7.2.5 (Biological Sequence Aligment Editor) software program [9]. Alignment was performed using the Clustal W module in the BioEdit 7.2.5 software program. The sequence data we obtained from the fungus species used in our study were compared with the sequences of the ITS gene regions of the species belonging to the genus *Agaricus* in the GenBank. In order to obtain the phylogenetic relationship and evolutionary trees between the samples, the Maximum Likelihood method was used by selecting the 1000 repetitive Bootstrap value from the MEGA 7.0 (Molecular Evolutionary Genetics Analysis) modules and the results were evaluated [10].

3. Results

3.1. Phylogenetics analyses

The dataset comprised 25 sequences. The final alignment comprised a total of 718 characters, of which 378 were conversed sites, 323 variable sites and 136 parsimony-informative. The phylogenetic trees generated by ML. Collections ERC M 0.001 clustered in the *Agaricus gennadii*, *A.nevoi* and *Agaricus pequinii* clade with strong support (1/98) (Figure 1).

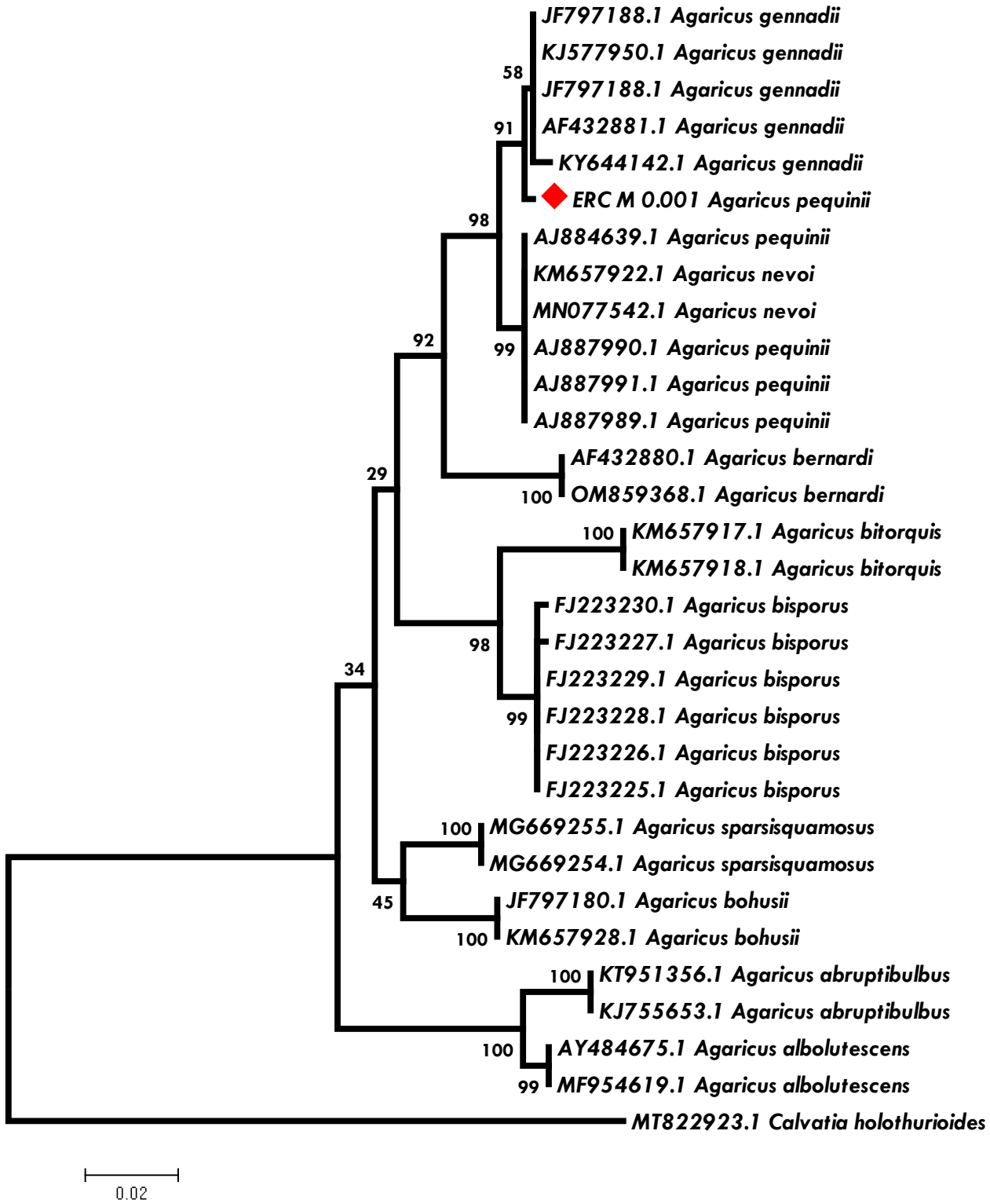

Figure 1. Maximum likelihood analysis inferred from ITS gene region sequences of *Agaricus pequinii* and related species

3.2. Taxonomy

3.2.1. Agaricus pequinii (Boud.) Konrad & Maubl.

Pileus 3-7 cm in diam., thick-fleshed, at first spherical or hemispherical, then convex to depressed, margin with brown membranous remains, sometimes dirty ochraceous in the centre (Figure 2 and Figure 3). Gills free, thin, crowded,

with an even sterile margin, at first pink, later dark brown, chocolate-brown. Basidia 4 (sometimes 1-3) spored, 23-28 x 7-10 µm, clavate. Spore print dark brown. Spores 5-7.5 x 4.5-6 µm, pale brown, broadly ovoid, with refractive droplets (Figure 4). Flesh white, unchanging on exposure, or becoming slightly pinkish. Odor fugacious. Taste acidulous. Cross reaction with Schaeffer's reagent negative [11].

Specimen examined: Türkiye, Kayseri, Kocasinan, Buğdaylı, 38° 48' 05" N, 35° 32' 17" E, alt.1072 m, 15.10.2022.

Figure 2. *Agaricus pequinii* in natural habitat

Figure 3. *Agaricus pequinii.* Cleaned and ready for cooking

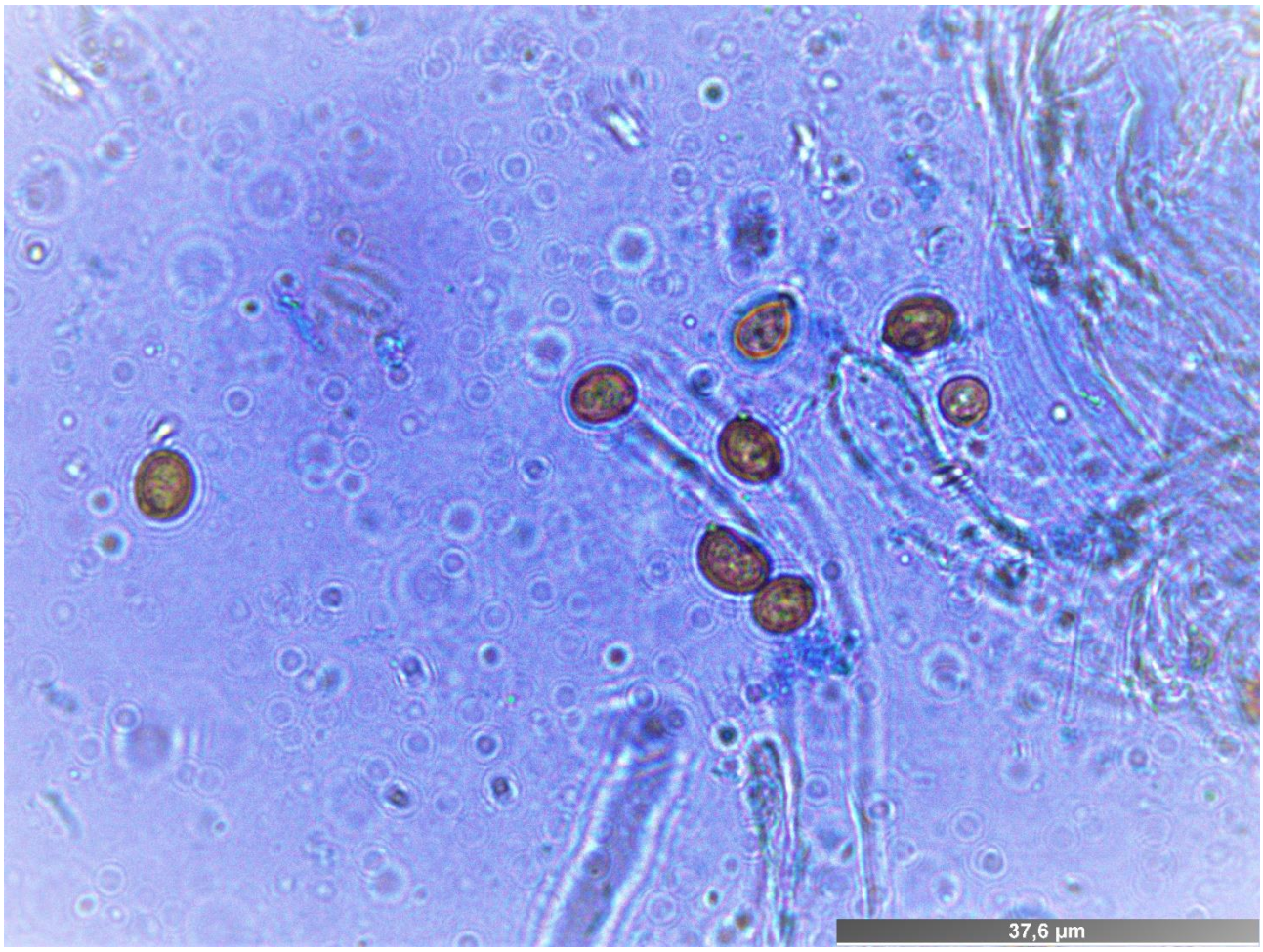

Figure 4. *Agaricus pequinii.* Basidium and basidiospores

4. Conclusions and discussion

According to the phylogenetic tree (Figure 1), *Agaricus pequinii* (Boud.) Konrad & Maubl.is phylogenetically close to *A. nevoi* Wasser and *A. gennadii* (Chatin & Boud.) P.D. Orton. It differs from *A. nevoi* in the character of the remnants of its general veil and spore size [12]. *Agaricus pequinii* differs from *A. gennadii* in having spores of a smaller size, and in the presence of numerous white scales on the stipe and surface of the volva-like remnants of the general veil. This species has a distribution in Europe (Italy, Prance, Hungary, Ukraine) and Asia [13].

Morphological and microscopic identification methods are no longer sufficient for species identification. Morphological features can sometimes be easily affected by environmental factors. As a result of this effect, changes in properties such as color, mushroom size and shape may occur, which may lead to misdiagnosis. Microscopic analyzes, on the other hand, may not be distinctive because sometimes very close results are obtained (for example, very close results are obtained in spore measurements). For this reason, molecular methods based on DNA sequence analysis have been started to be used in taxonomic studies on fungi. However, although molecular methods provide important clues, they must be evaluated by combining them with microscopic and morphological studies. The first national study to include the molecular taxonomy of a fungal genus in Türkiye was carried out by Taşkın et al. (2010, 2012, 2016) for the genus *Morchella* [14-16]. Therefore, it is necessary to increase such studies in our country.

References

[1] Hawksworth, D. L., & Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum*, *5*(4), 5-4. doi: 10.1128/microbiolspec.FUNK-0052-2016

[2] Solak, M. H., Işıloğlu, M., Kalmış, E., Allı, H. (2007). *Macrofungi of Turkey Checklist*. Üniversiteliler ofset, İzmir, 254 s.

[3] Sesli, E., Denchev, C. M. (2010). Checklists of the myxomycetes, larger ascomycetes and larger basidiomycetes in Turkey. *Mycotaxon* 106, 65–67.

[4] Allı H, Çöl, B, Şen İ (2017). Macrofungi biodiversity of Kütahya (Turkey) province. *Biological Diversity and Conservation* 10/1: 133-143.

[5] Sesli, E., Asan, A., Selçuk, F. (eds), Abacı Günyar, Ö., Akata, I., Akgül, H., "…" Yoltaş, A. (2020). Türkiye Mantarları Listesi. Ali Nihat Gökyiğit Vakfı Yayını. İstanbul.

[6] Kıvrık, M., Süfer, Ö., & Bozok, F. (2022). A Research on Quality Evaluation of Eight Wild Edible Macrofungi Collected from East Mediterranean Region of Turkey. *Chemistry & Biodiversity*, 19(3), e202100967. https://doi.org/10.1002/cbdv.202100967

[7] Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular ecology*, 2(2), 113-118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

[8] White, T., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18(1), 315-322.

[9] Hall, TA. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. *Acids Symposium Series*, 41: 95-98.

[10] Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33(7), 1870-1874. doi: 10.1093/molbev/msw054

[11] Wasser, S. P. (2000). A contribution to the taxonomy and species diversity ofthe Agariceae tribe (Higher Basidiomycetes) of Israel mycobiota. *Flora Mediterr*, 10, 191-221.

[12] Wasser, S. P. (1995). New and noteworthy species of the genus Agaricus L.: Fr. emend. Karst. from Israel. *Documents Mycologiques*, 25, 98-100.

[13] Wasser, S. P. (1996). New, remarkable and rare higher Basidiomycetes from Israel. *Czech Mycol*, 48(4), 237-250.

[14] Taşkın, H., Büyükalaca, S., Doğan, H. H., Rehner, S. A., & O'Donnell, K. (2010). A multigene molecular phylogenetic assessment of true morels (*Morchella*) in Turkey. *Fungal Genetics and Biology*, 47(8), 672-682. doi: 10.1016/j.fgb.2010.05.004

[15] Taşkın, H., Büyükalaca, S., Hansen, K., & O'Donnell, K. (2012). Multilocus phylogenetic analysis of true morels (Morchella) reveals high levels of endemics in Turkey relative to other regions of Europe. *Mycologia*, 104(2), 446-461. doi: 10.3852/11-180

[16] Taşkın, H., Doğan, H. H., Büyükalaca, S., Clowez, P., Moreau, P. A., & O'Donnell, K. (2016). Four new morel (Morchella) species in the elata subclade (M. sect. Distantes) from Turkey. *Mycotaxon*, 131(2), 467-482. doi: 10.5248/131.467