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Agaricus parvitigrinus (*Agaricaceae*): A new record for the *Agaricus* section *Xanthodermatei* from Türkiye

Oğuzhan KAYGUSUZ[1]*, Meryem Şenay ŞENGÜL DEMİRAK[2]
[1]*Isparta Applied Sciences University, Atabey Vocational School, Department of Plant and Animal Production, Isparta, Türkiye*
[2]*Tokat Gaziosmanpaşa University, Science & Arts Faculty, Department of Molecular Biology and Genetics, Tokat, Türkiye*
**oguzhankaygusuz@isparta.edu.tr, [2]msengul@vt.edu*

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Agaricus parvitigrinus (*Agaricaceae*): *Agaricus* seksiyon *Xanthodermatei* için Türkiye'den yeni bir kayıt

Abstract: *Agaricus parvitigrinus*, a member of *Agaricus* section *Xanthodermatei*, was collected under *Pinus brutia* in western Türkiye and is presented and illustrated here as a new record based on its morphological characteristics and molecular analyses of the nuclear rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region. A comprehensive description, color photographs, line drawings of microscopic features, and comparisons with phenotypically similar taxa and phylogenetically related species are provided.

Key words: *Agaricomycetes*, molecular phylogeny, nrDNA ITS, taxonomy, new record

Özet: *Agaricus* seksiyon *Xanthodermatei*'ye ait bir tür olan *Agaricus parvitigrinus*, *Pinus brutia* altında Türkiye'nin batısından toplanmış, morfolojik karakterler ve nükleer rDNA iç transkribe boşluk (ITS1-5.8S-ITS2 = ITS) bölgesini içeren moleküler analizlere dayalı olarak sunulmuş ve tanımlanmıştır. Kapsamlı bir açıklama, renkli fotoğraflar, mikroskobik özelliklerin çizimleri, fenotipik olarak benzer taksonlar ve filogenetik olarak ilişkili türlerle karşılaştırmalar sunulmaktadır.

Anahtar Kelimeler: *Agaricomycetes*, moleküler filogeni, nrDNA ITS, taksonomi, yeni kayıt

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1. Introduction

Agaricus L. is one of the most species-rich genera among agaricoid fungi, comprising more than 500 species (Nauta, 2001; Zhao et al., 2011, 2016; Callac and Chen, 2018; He et al., 2018; Medel-Ortiz et al., 2022; Tarafder et al., 2022; Bashir et al., 2023; Wang and Bau, 2024). It has a cosmopolitan distribution, occurring from sea level to mountainous regions and even in arid environments (Zhao et al., 2011, 2016; Wang and Bau, 2024). The species of this genus are saprotrophic (Zhao et al., 2011; Chen et al., 2017) and include numerous taxa of significant nutritional and medicinal value (Parra, 2013; Kerrigan, 2016; Kaygusuz et al., 2017), such as the widely cultivated and consumed *Agaricus bisporus* (J.E. Lange) Imbach (Zhao et al., 2011; Thongklang et al., 2014). However, members of *Agaricus* section *Xanthodermatei* Singer are known for their toxicity due to the presence of phenol, a toxic compound (Gill and Strauch, 1984; Wood et al., 1998; Parra, 2008).

The genus is distinguished by its pinkish lamellae, which gradually turn brown over time, chocolate-brown spore print, and the presence of a ring-like annulus on the stipe (Parra, 2008; Knudsen and Vesterholt, 2012; Parra, 2013; Kerrigan, 2016). However, species identification within *Agaricus* can be challenging due to limited phenotypic variation, environmental influences, and significant intra-species variability (Zhao et al., 2011). As a result, modern taxonomic studies now emphasize the integration of molecular data alongside traditional morphological methods (He et al., 2018; Cao et al., 2020; Ling et al., 2021; Ortiz-Santana et al., 2021). Based on both morphological and molecular phylogenetic analyses, the genus has been classified into 6 subgenera and 24 sections (Kerrigan, 2016; Chen et al., 2017).

Agaricus sect. *Xanthodermatei* was first described by Singer (1948, 1986), with *A. xanthodermus* Genev. designated as the type species. Molecular phylogenetic analyses have revealed that *A.* sect. *Xanthodermatei*, once regarded as a monophyletic group, is now recognized as polyphyletic (Zhao et al., 2016; Bashir et al., 2021). Members of *A.* sect. *Xanthodermatei* are primarily characterized by distinct chemical reactions, including a negative Schäffer reaction and a bright yellow positive KOH reaction (Parra, 2008). Additionally, many species within this section exhibit key diagnostic traits such as transient yellow discoloration on the pileus surface and stipe base when damaged, a phenolic or iodine-like odor, and the presence of toxic compounds that can induce gastrointestinal distress in humans (Parra, 2008; Parra et al., 2011; Zhao et al., 2016).

According to the checklist of macrofungi in Türkiye and recent studies, a total of 58 species belonging to *Agaricus* have been reported (Sesli et al., 2020; Solak and Türkoğlu, 2022; Aslan et al., 2024; Halıcı and Güllü, 2024). Among these, only four species, namely *A. iodosmus* Heinem., *A. menieri* Bon, *A. placomyces* Peck and *A. xanthodermus*,

have been documented from *A.* sect. *Xanthodermatei*. This study presents the first morphological and molecular description of *Agaricus parvitigrinus* from Türkiye and contributes to the mycobiota of this continent.

2. Materials and Method

2.1. Morphological studies

Three *Agaricus* specimens were collected from Aydın Province, Türkiye. Each specimen was observed in its natural habitat, photographed, and documented with sampling details, including macro-morphological characteristics, geographical location, and habitat type. The specimens were then transported to the laboratory and air-dried using an electric dryer at approximately 20°C. Dried specimens were rehydrated in 3% KOH, stained with Congo Red, and then examined microscopically. Measurements were taken from at least 30 basidiospores. In the provided list of abbreviations, "L^m" and "W^m" denote the mean values of basidiospore length and width, respectively. "Q" represents the length-to-width ratio, while "Q^m" indicates the average Q value calculated from the measured basidiospores. Following morphological and phylogenetic analyses, the specimens were preserved in the fungarium of Isparta University of Applied Sciences (ISUF).

2.2. Molecular procedures

The macrofungus was dried and used for DNA isolation with the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, California), following the manufacturer's protocol. The isolated gDNA was checked on an agorose gel electroporesis for validation. The extracted DNA was subsequently used in polymerase chain reaction (PCR) to amplify the ITS gene region, using ITS1F/ITS4 primers

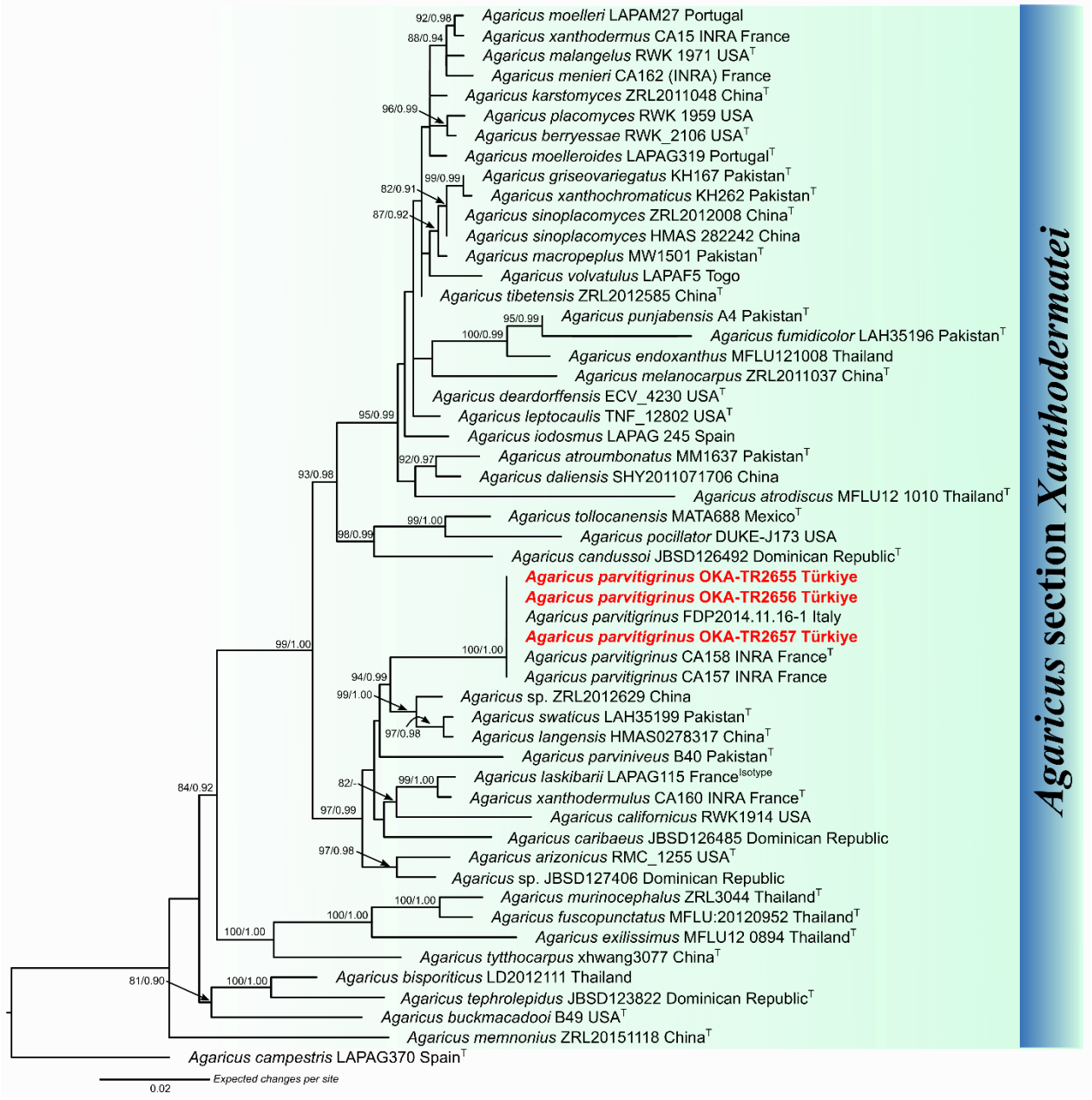

Figure 1. Maximum Likelihood (ML) phylogram of species within *A.* sect. *Xanthodermatei* based on nrDNA ITS sequence data. *Agaricus campestris* L. was used as an outgroup. Maximum Likelihood Bootstrap (MLB) values of ≥ 80% and Bayesian Posterior Probabilities (BPP) values of ≥ 0.90 are shown on the branches. Turkish collections are shown in bold red

Figure 2. Dry basidiomata of *Agaricus parvitigrinus*. Scale bar = 10 mm

(White et al., 1990; Gardes and Bruns, 1993). PCR products were checked on a 1% agarose gel electrophoresis and subsequently sequenced. The sequences were manually edited in BioEdit 7.0.5 (Hall, 1999).

A MegaBLAST search was conducted using the newly obtained sequences to identify closely related taxa in the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/genbank/). Multiple sequence alignment was performed using MAFFT version 7 with the FFT-NS-I strategy (Katoh et al., 2019). The alignment was subsequently reviewed and manually refined in BioEdit. Maximum Likelihood (ML) and Bayesian Inference (BI) methods were applied for phylogenetic analyses, following the methodology outlined by Kaygusuz (2022, 2024). FigTree version 1.4.2 was used to visualize the phylogenetic tree, which was then refined in Adobe Illustrator CS6.

3. Results

3.1. Phylogenetic analyses

Phylogenetic analyses of nrDNA ITS sequences included 53 sequences, three of which were newly generated. Since the BI and ML analyses yielded nearly identical topologies, the ML tree is presented in this study (Fig. 1). The Turkish collections of *Agaricus parvitigrinus* align phylogenetically with European specimens, confirming their taxonomic placement within *A.* sect. *Xanthodermatei*. The Turkish collections of *A. parvitigrinus* (OKA-TR2655, OKA-TR2656, and OKA-TR2657) form a strongly supported clade (MLB = 100%, BPP = 1.00, Fig. 1) alongside specimens from France (CA157, CA158) and Italy (FDP2014.11.16-1). The occurrence of *A. parvitigrinus* in Türkiye, France, and Italy suggests a broader geographical distribution of this species across Europe and Eurasia. Additionally, *Agaricus parvitigrinus* was closely related to *A. swaticus* H. Bashir, S. Jabeen, S. Ullah, Khalid & L.A. Parra (LAH35199) from Pakistan, *A. langensis* M.Q. He & R.L. Zhao (HMAS0278317), and an unidentified *Agaricus* species (ZRL2012629) from China.

3.2. Taxonomy

Agaricus parvitigrinus Guinberteau & Callac, *Mycologia* 97(2): 419 (2005) (Figs. 2, 3)

Macroscopic description: Pileus 25–60 mm diam., hemispherical to convex while young, later plano-convex to expanded, sometimes slightly depressed at center or low to flat umbo, densely covered by dark grayish appressed fibrillose squamules on a whitish background with an entire almost black to blackish grey center. Margin irregular, sometimes incurved in young, white, thick, slightly exceeding the lamellae and fimbriate, sometimes cracked. Lamellae free, unequal, crowded, straight, at first pinkish white, later greyish brown with the edge paler and eroded. Stipe 35–80 × 3–5 mm, smooth, cylindrical, curved at base, slightly bulbous or abruptly bulbous base with rhizomorphs, at first white to dull yellowish white then ferrugineous on handling. Partial veil white to pale gray, thick, membranous, forming a pendant annulus, often with radial squamules on the underside, cogwheel obvious when young. Annulus superous, double, membranous, smooth, white, the lower layer broke in a cogwheel decorating the lower surface. Context white at first, then slightly light yellowish by cutting. Odor phenolic. Taste unpleasant. KOH reaction positive, yellow. Schäffer's reaction negative.

Microscopic description: Basidiospores (4.7–)5.0–6.8(–7.0) × (3.2–)3.3–3.8(–4.2) µm, $L^m \times W^m = 5.6 \times 3.6$ µm, Q = (1.4–)1.8(–1.9), $Q^m = 1.6$, mostly ellipsoid, rarely oblong, smooth, dark brown, granular, guttulate with 1-2 guttules

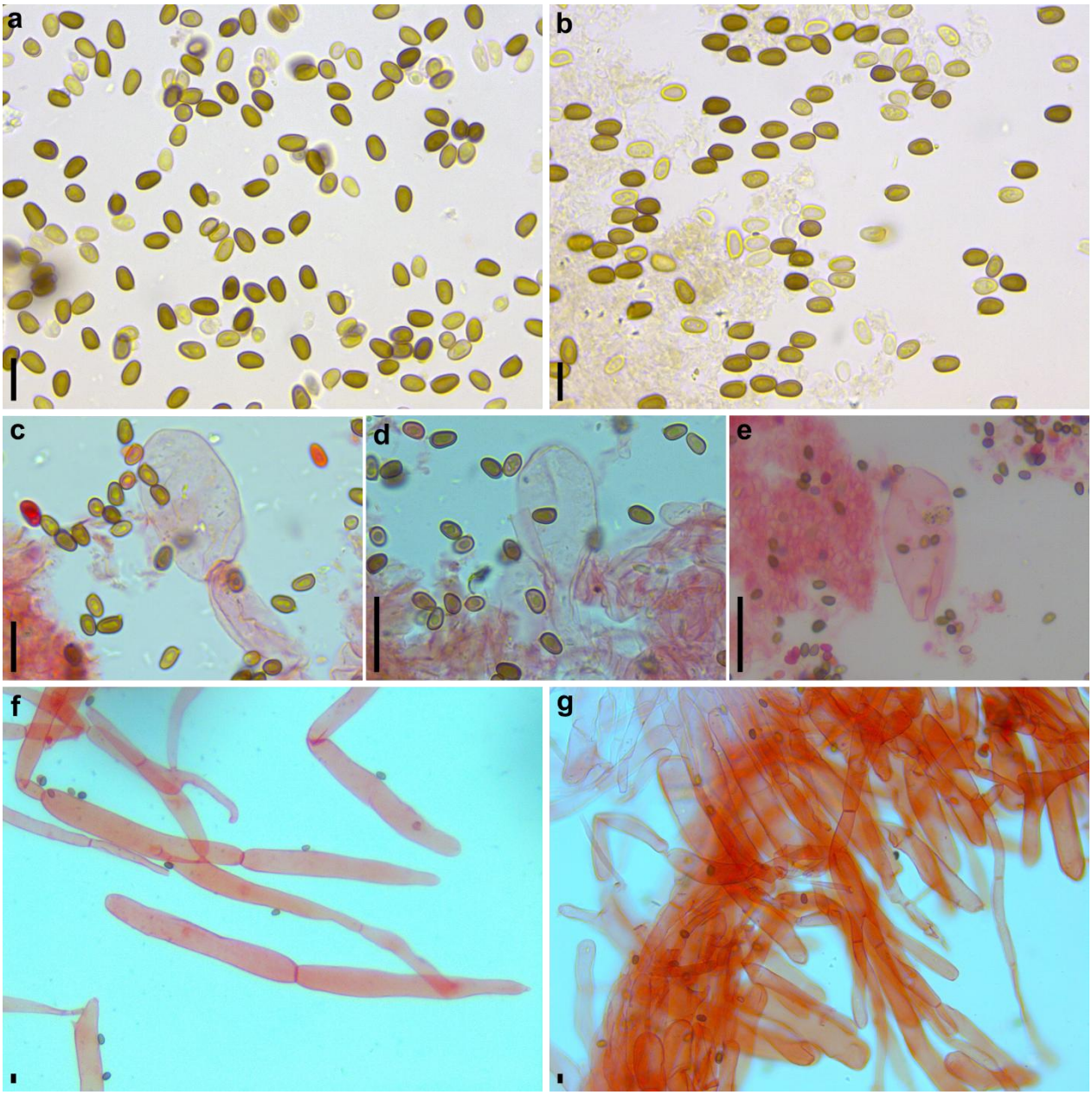

Figure 3. Micromorphological characters of *Agaricus parvitigrinus*. (a-b) Basidiospores. (c-e) Cheilocystidia. (f-g) Pileipellis hyphae. Scale bars: 10 µm

per spore, apiculate. Basidia 17–25 × 5.5–9.5 µm, tetrasporic, clavate, sterigmata up to 2.5 µm long. Cheilocystidia 15–30 × 5–10(–12) µm, clavate to spheropedunculate, rare and difficult to observe, weakly granular or hyaline. Pleurocystidia not observed. Pileipellis a cutis composed of hyphae 10–50 µm in diam., long cylindrical, sometimes curved and branched, containing light brown intracellular pigments, smooth. Clamp connections not observed.

Ecology: Occurring gregariously or in small groups on the forest floor, primarily under *Pinus brutia*, on calcareous sandy soils, predominantly in coastal areas of the Aegean region in western Türkiye.

Specimens examined*:* Türkiye. Aydın Province, Kuşadası district, in Davutlar, on calcareous sandy soil under *Pinus brutia*, alt. 52 m, 10 May 2013, leg. O. Kaygusuz (OKA-TR2655, GenBank accession no.: nrDNA ITS = PV197907); *ibid.*, alt. 45 m, 06 March 2014, leg. O. Kaygusuz (OKA-TR2656, GenBank accession no.: nrDNA ITS = PV197908); *ibid.*, alt. 48 m, 19 March 2014, leg. O. Kaygusuz (OKA-TR2657, GenBank accession no.: nrDNA ITS = PV197909).

4. Discussions

The Turkish specimen both morphologically and molecularly matches the type specimen of *Agaricus parvitigrinus* (Callac and Guinberteau, 2005) and belongs to *A.* sect. *Xanthodermatei*, as confirmed in previous studies (Zhao et al., 2011, 2016; Parra et al., 2018; Phookamsak et al., 2019; Bashir et al., 2021). According to the phylogenetic tree, *A. parvitigrinus* resides in a well-supported clade and is closely related phylogenetically to *A. swaticus* and *A. langensis*. Morphologically, *Agaricus swaticus*, a recently described species from Pakistan, differs from *A. parvitigrinus* by its larger basidiomata (up to 110 mm diam.) with grayish scattered scales, slightly larger basidiospores (on av. 6.1 × 4.0 µm), and its habitat

association with *Cedrus deodara* at high altitudes (Bashir et al., 2021). *Agaricus langensis*, originally described from China, differs from *A. parvitigrinus* by smaller basidiomata (up to 49 mm diam.), larger basidiospores (on av. 7.2 × 4.4 µm), and absence of cheilocystidia (Phookamsak et al., 2019).

Other European species within *A.* sect. *Xanthodermatei*, including *A. menieri*, *A. moelleri*, *A. pseudopratensis* (Bohus) Bohus, and *A. xanthodermus*, may be confused with *A. parvitigrinus*. However, *Agaricus menieri* and *A. moelleri* can be distinguished from *A. parvitigrinus* by larger basidiospores (Heinemann, 1978; Freeman, 1979; Capelli, 1984; Kerrigan, 1986; Nauta, 2001; Parra, 2013). *Agaricus pseudopratensis* exhibits a reddish discoloration when cut (Bohus, 1971; Heinemann, 1978). *Agaricus xanthodermus* differs by its white-toned pileus when young and larger basidiospores (Parra, 2013).

In conclusion, phylogenetic analyses and morphological characteristics confirm that *Agaricus parvitigrinus* is newly recorded from Türkiye and occupies a distinct phylogenetic position within *A.* sect. *Xanthodermatei*. It is considered a rare species, and additional reports from different localities are required to assess potential variations in its characteristics.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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