



First record of *Pluteus tenebromarginatus* (Pluteaceae, Agaricomycetes) from Türkiye, outside its type locality

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Pluteus tenebromarginatus (Pluteaceae, Agaricomycetes)'un tip lokalitesi dışındaki Türkiye'den ilk kaydı

Abstract: The genus *Pluteus* exhibits a cosmopolitan distribution. *Pluteus tenebromarginatus*, which is a member of *Pluteus* section *Celluloderma*, has been recently described from its type locality in France. The present study reports the first record of *P. tenebromarginatus* from Western Eurasia, based on collections from Burdur Province, Türkiye. This finding significantly extends the known distribution of the species beyond its originally described range. The identity of the Turkish specimens was established through an integrative approach, combining detailed macro- and micromorphological analyses with molecular phylogenetic inference based on the nuclear ribosomal internal transcribed spacer (ITS) region. A comprehensive morphological description is provided, supplemented by photographs of the basidiomata and pictures of diagnostic microscopic features. In addition, a phylogenetic tree is presented to confirm the placement of the specimen within the genus.

Key Words: ITS sequences, molecular systematics, new record, *Pluteoid* clade, *Pluteus* section *Celluloderma*

Özet: *Pluteus* cinsi, kozmopolit bir dağılıma sahiptir. *Pluteus* seksiyon *Celluloderma*'nın bir üyesi olan *Pluteus tenebromarginatus*, yakın zamanda tip lokalitesi Fransa'da tanımlanmıştır. Bu çalışma, Burdur İli'nden (Türkiye) toplanan örneklerle, *P. tenebromarginatus*'un Batı Avrasya'daki ilk kaydını rapor etmektedir. Bu bulgu, türün bilinen dağılımını, ilk tanımlandığı alanın ötesinde önemli ölçüde genişletmektedir. Türkiye örneklerinin teşhisi, bütünleştirici bir yaklaşımla, makro- ve mikromorfolojik analizlerin nükleer ribozomal iç transkripsiyon aralayıcı (ITS) bölgesine dayandırılan moleküler filogenetik çıkarımla birleştirilmesi sonucunda doğrulanmıştır. Bazidiyomaların fotoğrafları ve tanılayıcı mikroskobik özelliklerin resimleriyle desteklenen kapsamlı bir morfolojik tanım sunulmuştur. Ayrıca, örneklerin cins içindeki konumunu doğrulamak için bir filogenetik ağaç sunulmaktadır.

Anahtar Kelimeler: ITS dizileri, moleküler sistematik, yeni kayıt, *Pluteoid* klad, *Pluteus* seksiyon *Celluloderma*

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1. Introduction

Pluteus Fr. is a large genus within the family *Pluteaceae* Kotl. & Pouzar, containing more than 525 agaric species with a cosmopolitan distribution across tropical, subtropical, and temperate biomes (Vellinga and Schreurs, 1985; Singer, 1986; Vellinga, 1990; Menolli et al., 2010; Justo et al., 2011a, 2011b; Kaygusuz et al., 2016; Justo et al., 2014; Menolli et al., 2015a; Kaygusuz et al., 2021a, 2021b, 2021c; Ševčíková et al., 2022a, 2022b, 2023; Kaygusuz et al., 2024; Justo et al., 2025; Qi et al., 2025). Members of this genus are identified by a combination of features: a volva is absent, the lamellae are free and become pinkish at maturity, the basidiospores print is pink, the basidiospores are smooth and inamyloid, and the hymenophoral trama is of the inverse type (Lange, 1936; Homola, 1973; Vellinga and Schreurs, 1985; Orton, 1986; Singer, 1986; Vellinga, 1990). Ecologically, they are saprotrophic, typically fruiting on the decaying wood of both angiosperms and gymnosperms (Vellinga and Schreurs, 1985; Orton, 1986; Singer, 1986; Vellinga, 1990). Phylogenetic studies have confirmed the monophyly of *Pluteus* (Moncalvo et al., 2002; Matheny et al., 2006; Menolli et al., 2010; Justo et al., 2011a, 2011b; Justo et al.,

2014) and support its formal division into three sections—*P. sect. Pluteus*, *P. sect. Celluloderma*, and *P. sect. Hispidoderma*—based mainly on the structural features of the pileipellis and pleurocystidia. Specifically, *P. sect. Celluloderma* is characterized by pleurocystidia that lack apical incrustations (non-metuloid) and a pileipellis of either a euhymeniderm or epithelium, the latter consisting of short, clavate or spheropedunculate cells sometimes intermingled with elongated, cystidioid cells (Lange, 1936; Homola, 1973; Vellinga and Schreurs, 1985; Orton, 1986; Singer, 1986; Vellinga, 1990).

In recent years, numerous agaric fungi have been added to the Turkish mycota, supported by both morphological (Aslan et al., 2024; Dalkıran et al., 2024; Keleş et al., 2024; Sesli and Sezgin, 2025; Yorulmazlar et al., 2025) and molecular evidence (Kaygusuz and Şengül Demirak, 2021; Kaygusuz, 2022a, 2022b; Kaygusuz and Şengül Demirak, 2022a, 2022b; Şengül Demirak and Kaygusuz, 2022; Çelik et al., 2024; Kaygusuz, 2024; Kaygusuz and Şengül Demirak, 2025). To date, 45 species of *Pluteus* have been documented from Türkiye (Kaya, 2000, 2009; Kaygusuz et al., 2016, 2019, 2021a, 2021b, 2021c; Sesli et al., 2020; Solak and Türkoğlu, 2022; Kaygusuz et al., 2024). These

include *Pluteus anaticus* Kaygusuz, Knudsen, Menolli & Türkiyeul (Kaygusuz et al., 2021a), *P. canaliculatus* Kaygusuz, Justo & M. Piepenbr. (Kaygusuz et al., 2024), *P. fuscodiscus* Ferisin, E.F. Malysheva, Ševčíková, Kaygusuz, Heilm.-Claus. & I. Saar (Ševčíková et al., 2023), *P. lauracearum* Kaygusuz, Ševčíková & Justo (Kaygusuz et al., 2021b), *P. mediterraneus* Kaygusuz, Knudsen & Menolli (Kaygusuz et al., 2022), and *P. vellingae* Justo,

Ferisin, Ševčíková, Kaygusuz, G. Muñoz, Lebeuf & S.D. Russell (Ševčíková et al., 2022a), all of which were described as new species.

This study reports the first occurrence of *Pluteus tenebromarginatus* Corriol outside its type locality, with the record from Türkiye confirmed through detailed morphological examination and molecular analysis of nrDNA ITS sequences.

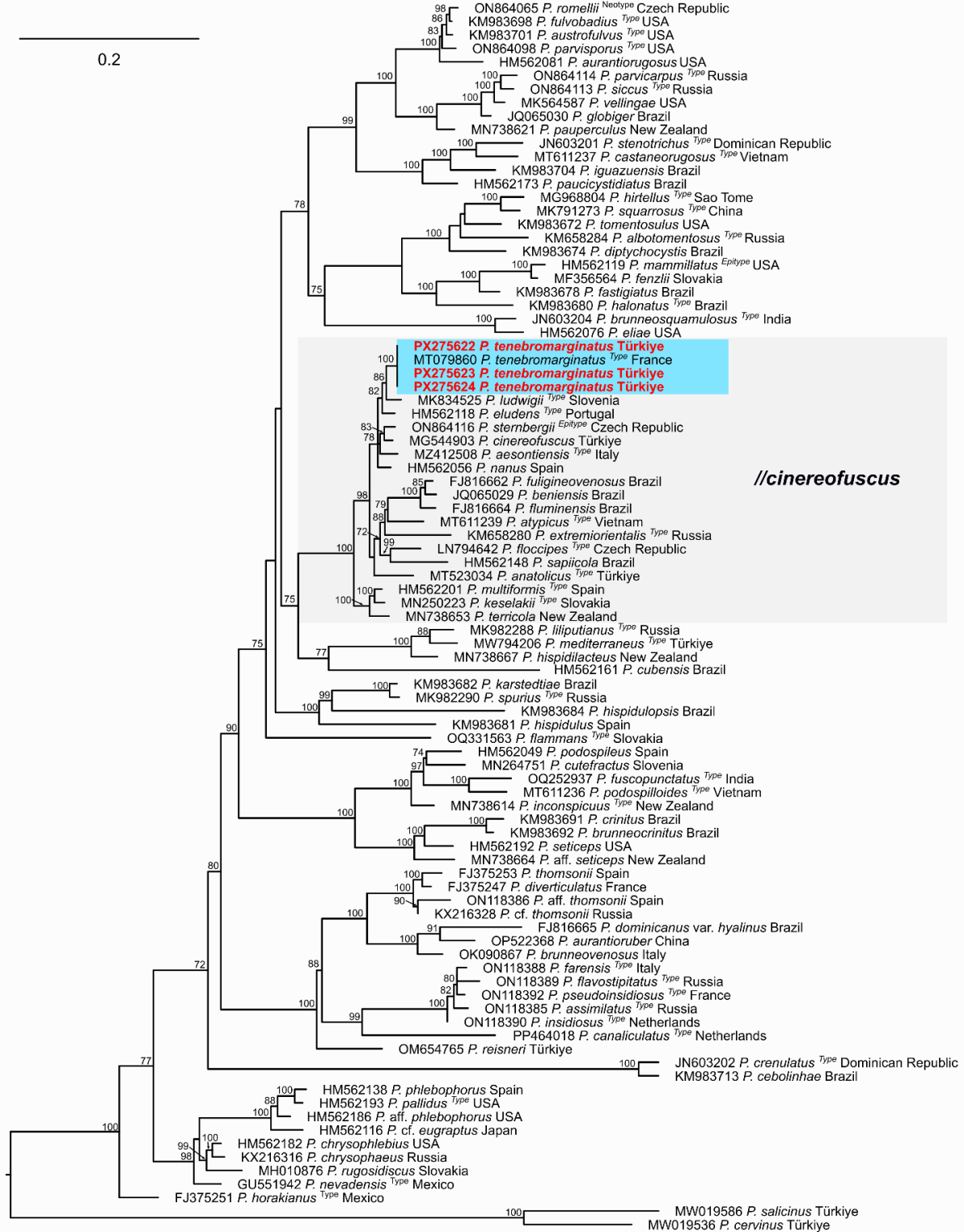


Figure 1. Phylogenetic reconstruction of *Pluteus* sect. *Celluloderma* based on nrDNA ITS sequences, inferred under the Maximum Likelihood criterion. The tree was rooted with *Pluteus cervinus* (Schaeff.) P. Kumm. and *P. salicinus* (Pers.) P. Kumm. Branch supports were assessed using Maximum Likelihood bootstrap analysis, with values $\geq 70\%$ (based on 1,000 replicates) considered significant. The specimens of *Pluteus tenebromarginatus* from Türkiye are shown in bold red to indicate their phylogenetic position.

2. Materials and Method

2.1. Specimen collection and morphological study

The *Pluteus* specimens examined in this study were collected during mycological surveys in Burdur Province, Türkiye, from 2015 to 2022. The basidiomata were dried immediately at a constant temperature of 20–25 °C using an electric dryer to preserve their morphological features. All specimens were preserved in the fungarium of Isparta University of Applied Sciences (ISUF).

Field documentation for all specimens encompassed in situ recording of macro-morphological characters, coordinates, and habitat features, supplemented by digital photography. For microscopic analysis, rehydrated sections of dried tissue were mounted in 3% potassium hydroxide (KOH) and then stained with 1% Congo Red. A minimum of thirty basidiospores per specimen were measured to obtain the mean length (Lm), mean width (Wm), and their mean length-to-width ratio (Qm), alongside individual basidiospore ratios (Q). The descriptive terminology for macromorphological and micromorphological characteristics follows the conventions established by Bas (1969) and Vellinga (1988).

2.2. Molecular analysis

Genomic DNA was extracted from dried basidioma tissue using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, USA), according to the manufacturer's instructions. The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) with the primers ITS1F and ITS4 (White et al., 1990; Gardes and Bruns, 1993). The ITS region was amplified in 25 µl reaction mixtures comprising 2 µl genomic DNA, 1 µl of each forward and reverse primer, 12.5 µl of OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (New England Biolabs, Ipswich, MA, USA), and 8.5 µl ddH₂O. Thermal cycling was carried out with an initial denaturation step of 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 50 s, with a final elongation at 72 °C for 5 min. Amplification success was confirmed by electrophoresis on a 1% agarose gel, and successful products were purified and sequenced. The resulting sequences were assembled and manually edited using BioEdit v. 7.0.5 (Hall, 1999).

The newly obtained sequences were first compared to the NCBI GenBank nucleotide database using the MegaBLAST algorithm. Multiple sequence alignment was then conducted in MAFFT v.7 (Katoh et al., 2019) with the E-INS-I strategy, after which the alignment was manually refined in BioEdit. A Maximum Likelihood (ML) phylogeny was inferred with RAxML v. 8.2.12 (Stamatakis 2014). The analysis employed the GTR+GAMMA+I substitution model and 1000 rapid bootstrap iterations to evaluate branch support. The final phylogenetic tree was visualized using FigTree v.1.4.2 and subsequently edited for publication with Adobe Illustrator CS6.

3. Results

3.1. Molecular phylogeny

The phylogenetic analysis was based on an nrDNA ITS dataset of 91 *Pluteus* sequences, including three newly generated in this study. The ML tree was reconstructed from a 1,837-site alignment that included gap regions. The Turkish specimens (PX275622, PX275623, and

PX275624) clustered with the type sequence of *Pluteus tenebromarginatus* from France (MT079860), forming a strongly supported clade (MLB = 100%, Fig. 1). This lineage was recovered as sister to *P. aesontiensis* Ferisin, Justo & Dovana (MZ412508), *P. cinereofuscus* J.E. Lange (MG544903), *P. eludens* E.F. Malysheva, Minnis & Justo (HM562118), *P. ludwigii* Ferisin, Justo & Dovana (MK834525), *P. nanus* (Pers.) P. Kumm. (HM562056), and *P. sternbergii* Velen. (ON864116). The inclusion of the Turkish collections expands the known range of *P. tenebromarginatus* beyond its French type locality, indicating a wider distribution across Europe and Western Eurasia.

3.2. Taxonomy

Pluteus tenebromarginatus Corriol, in Corriol & Chaduli, Bull. Féd. Assoc. Mycol. Méditerran., N.S. 57: 4 (2020) (Figs. 2, 3)

Macroscopic description: *Pileus* 10–25 mm diam., convex at first, becoming plano-convex and finally nearly applanate, usually with a low, broad umbo; margin straight, smooth, often eroded; surface ochraceous buff, dark brown, chestnut brown to dark sepia brown, with cracked areas contrastingly cream to buff, always darker (dark brown to blackish brown) at the center, slightly reticulately venose around the center, arachnoid-fibrillose when mature, not hygrophanous, margin sometimes translucently striate or not, sometimes slightly cracked. *Lamellae* free, moderately crowded, ventricose, up to 2.0 mm broad, at first pinkish white to pale cream, later pinkish brown, with ochre brown edge. *Stipe* 15–40 × 1.5–2.5 mm, cylindrical, sometimes slightly broadened at the base, whitish-cream, covered with dense, longitudinal fibrillose-striate coating of silvery or pale greyish hue. *Context* white to whitish. *Smell* and *taste* indistinct.

Microscopic description: *Basidiospores* (5.6–)6.0–8.0(–8.5) × (4.5–)4.8–6.4(–6.9) µm, Lm × Wm = 7.0 × 5.7 µm, Q = (1.0–)1.1(–1.3), Qm = 1.2, subglobose to broadly ellipsoid, smooth, guttulate, thick-walled, hyaline in 3% KOH. *Basidia* 25–35 × 8.5–10.5 µm, clavate, tetrasporic, thin-walled, hyaline. *Pleurocystidia* abundant, 35–65 × 12–30 µm, utriform to broadly utriform or fusiform, sometimes with a small pedicel, occasionally ellipsoid or subcylindrical, some with a median constriction, contents with evenly dissolved or sometimes condensed pale brown to brown intracellular pigment in 3% KOH, thin-walled. *Cheilocystidia* numerous, 55–70 × 10–20 µm, clavate, oblong to cylindrical, with evenly dissolved or sometimes condensed pale brown to brown intracellular pigment in 3% KOH, thin-walled. *Pileipellis* an euhymeniderm or epithelium, 30–70 × 15–35 µm, composed of clavate, broadly clavate, spheropedunculate, pyriform to obpyriform elements, containing brown intracellular pigment in 3% KOH, thin-walled. *Stipitipellis* a cutis of cylindrical hyphae, 10–15 µm wide, parallel to the surface, hyaline, thin-walled. *Clamp connections* absent.

Ecology: Basidiomata solitary, on decaying stumps of *Liquidambar orientalis*. Fruiting occurs in early spring (March) and autumn (November).

Materials examined: Türkiye, Burdur Province, Bucak district, close to Karacaören, in the Sweetgum Forest Nature Protection Area, on decaying stumps of *Liquidambar orientalis*, 260 m., 11 November 2015, leg. O.

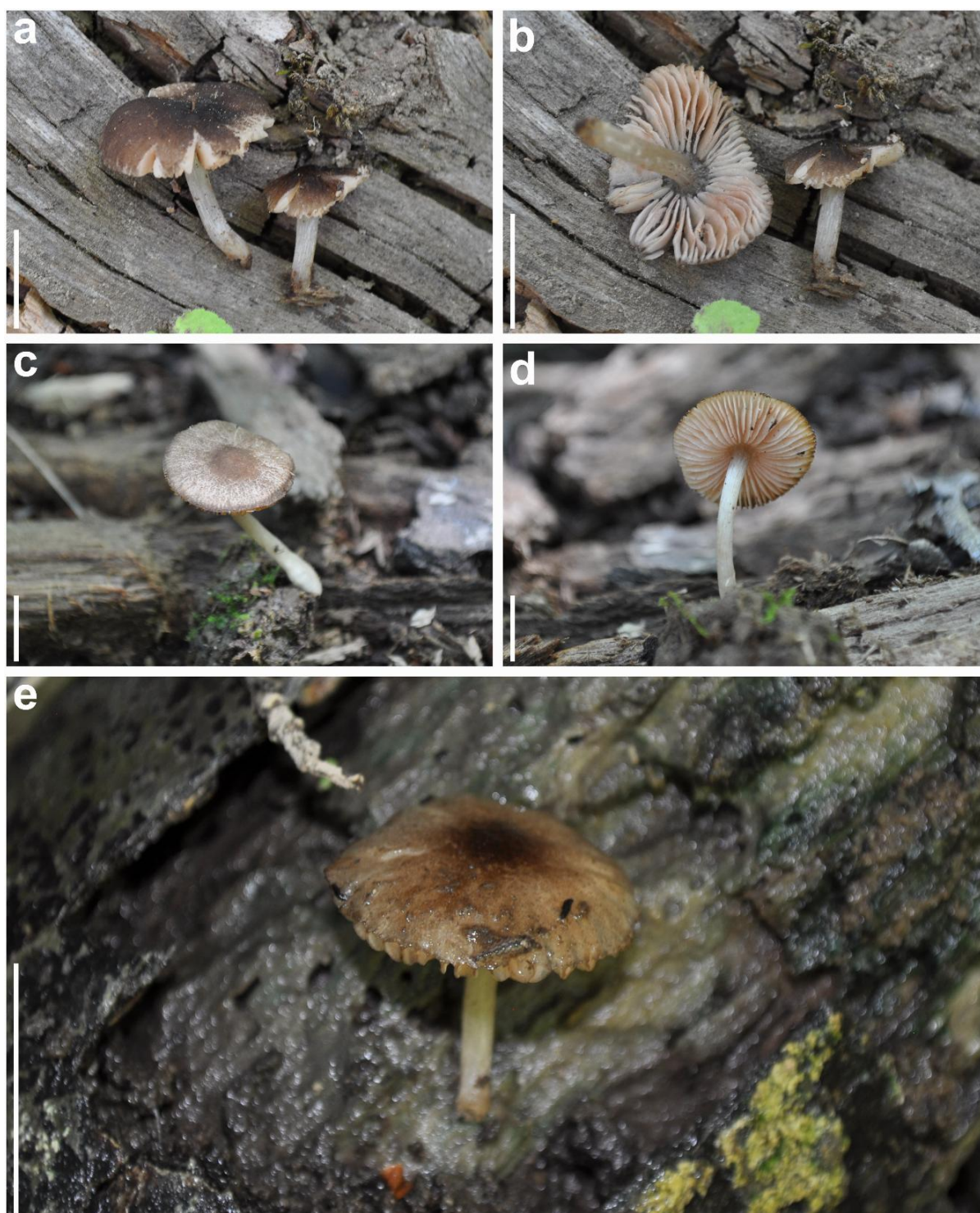


Figure 2. Fresh basidiomata of *Pluteus tenebromarginatus*. (a-b) Collection OKA-TR2003, (c-d) Collection OKA-TR2360, (e) Collection OKA-TR2362. Scale bars = 10 mm.

Kaygusuz (OKA-TR2003, GenBank ITS: PX275622); *ibid.*, on decayed wood of *L. orientalis*, 277 m., 07 March 2017, leg. O. Kaygusuz (OKA-TR2360, GenBank ITS: PX275623); *ibid.*, on logs of *L. orientalis*, 268 m., 16 March 2022, leg. O. Kaygusuz (OKA-TR2362, GenBank ITS: PX275624).

4. Discussions

The Turkish specimens correspond well to the type material of *Pluteus tenebromarginatus* in both morphological features and molecular data (Corriol and Chaduli, 2020)

and are positioned within the *cinereofuscus* clade. Phylogenetic analysis based on nrDNA ITS sequences indicates that *P. tenebromarginatus* is closely related to *P. eludens* (HM562118) and *P. ludwigii* (MK834525). Morphologically, *Pluteus eludens* differs from *P. tenebromarginatus* mainly by having utriform pleurocystidia and cheilocystidia that are narrowly utriform, fusiform, or narrowly lageniform (Justo et al., 2011c), whereas *P. ludwigii* is characterized by smaller basidiospores ($5.3\text{--}6.9 \times 4.9\text{--}6.0 \mu\text{m}$), predominantly fusiform to clavate pleurocystidia (Crous et al., 2019).

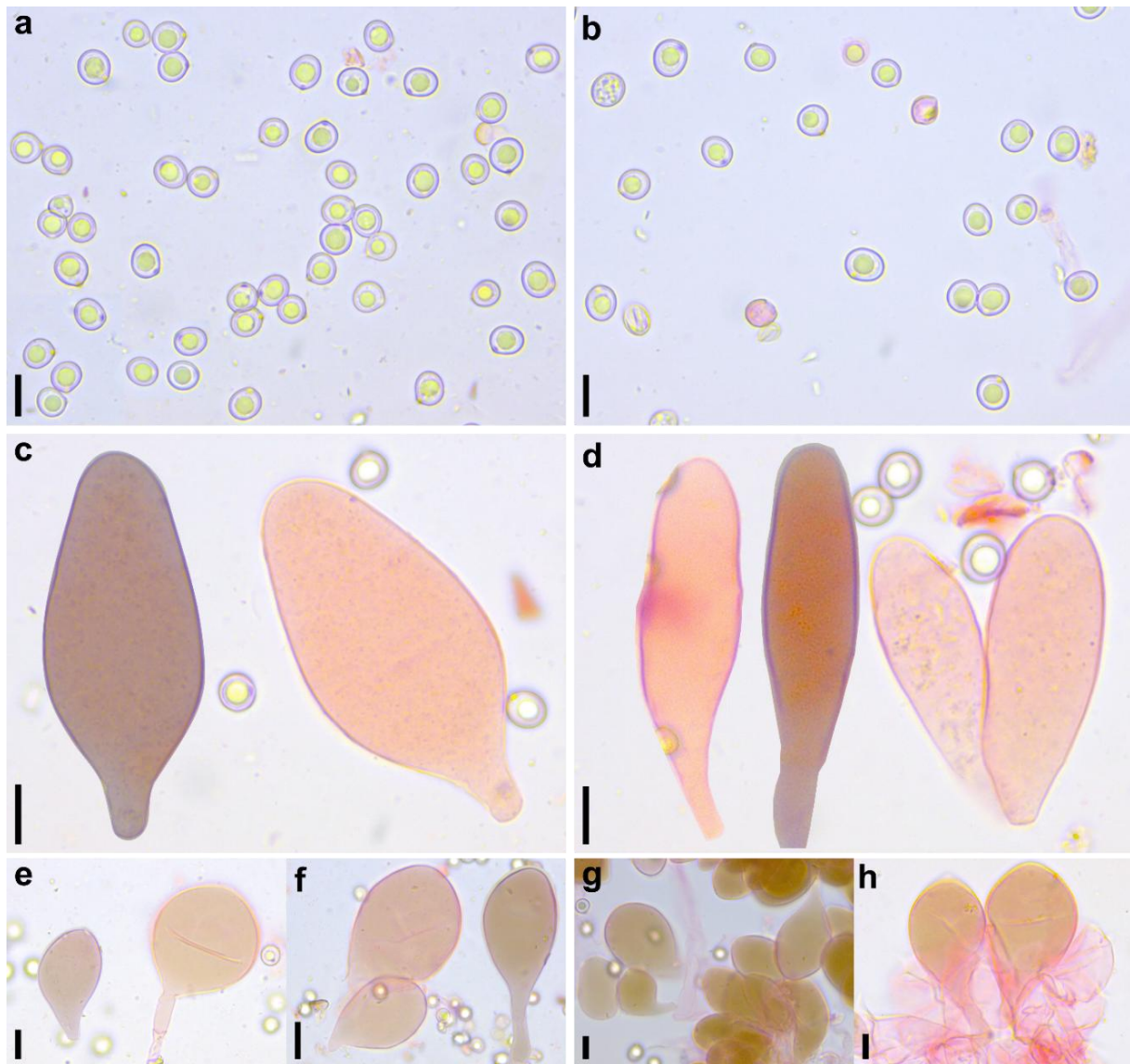


Figure 3. Micromorphological characters of *Pluteus tenebromarginatus*. (a-b) Basidiospores. (c) Pleurocystidia. (d) Cheilocystidia. (e-h) Pileipellis hyphae. Scale bars: 10 µm.

Morphologically, *Pluteus tenebromarginatus* shares similarities with *P. aesontiensis* (MZ412508), *P. cinereofuscus* (MG544903), *P. nanus* (HM562056), and *P. sternbergii* (ON864116), but it can be distinguished from these taxa by several diagnostic characters. *Pluteus aesontiensis* differs from *P. tenebromarginatus* by a reddish brown to deep yellowish brown pileus, non-utriform pleurocystidia, and clavate caulocystidia (Dovana et al., 2022). *Pluteus cinereofuscus* is separated by its greyish brown pileus with an olive tinge at the centre, slightly larger basidiospores ($6.5\text{--}10.5 \times 5.0\text{--}7.5 \mu\text{m}$), and a colourless lamellar edge (Vellinga, 1990).

Pluteus nanus differs in forming a larger pileus (up to 66 mm diam.) often with a slightly greenish or olive tinge, a hyaline lamellar edge, and somewhat larger basidiospores ($6.5\text{--}10 \times 5.5\text{--}7.0 \mu\text{m}$) (Vellinga, 1990). In contrast, *Pluteus sternbergii* is characterized by a rugulose pileus and a smooth, yellow stipe (Ševčíková et al., 2022a).

In conclusion, the present study documents the first occurrence of *Pluteus tenebromarginatus* outside its type locality in France, thereby extending the known distribution

of the species to Western Eurasia. The identity of the Turkish specimens was confirmed through an integrative approach, combining detailed macro- and micromorphological observations with molecular phylogenetic analysis of the ITS region. This finding highlights the importance of continued mycological exploration in Türkiye, a region that has recently yielded numerous taxonomic novelties within *Pluteus*. Expanding the biogeographical knowledge of *P. tenebromarginatus* not only contributes to a more comprehensive understanding of the diversity and distribution patterns of *Pluteus* in Eurasia but also underlines the role of Türkiye as a significant hotspot for fungal diversity. Further systematic surveys and molecular studies will likely reveal additional range extensions and potentially undescribed taxa within this cosmopolitan genus.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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