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First record of the deadly poisonous *Galerina venenata* (*Hymenogastraceae, Agaricomycotina*) from Türkiye

Ali ÇELİK[1] İbrahim TÜRKEKUL[2] Oğuzhan KAYGUSUZ[3*]
[1]*Aydın Adnan Menderes University, Faculty of Science and Arts, Department of Biology, Aydın, Türkiye*
[2]*Tokat Gaziosmanpasa University, Science and Arts Faculty, Department of Biology, Tokat, Türkiye*
[3]*Isparta University of Applied Sciences, Atabey Vocational School, Department of Plant and Animal Production, Isparta, Türkiye*
**okaygusuz03@gmail.com, [1]acelik@adu.edu.tr, [2]turkoibrahim@yahoo.com*

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Ölümcül zehirli *Galerina venenata* (*Hymenogastraceae, Agaricomycotina*)'nın Türkiye'den ilk kaydı

Abstract: A deadly poisonous fungus, *Galerina venenata* A.H. Sm. collected from the rhizospheric region of *Olea europaea* L., which is distributed in the area with the dominance of Mediterranean climate, is reported for the first time for Türkiye. Molecular phylogenetic analysis based on ITS rDNA sequences from Turkish collections confirmed the position of *G. venenata* in the genus, and being the closest relative of *G. marginata* (Batsch) Kühner. A detailed morphological description of the present species, photographs, and comparison with taxonomically and phylogenetically close species are provided.

Key words: *Agaricales*, macrofungi, phylogeny, ribosomal DNA, taxonomy

Özet: Akdeniz ikliminin hakim olduğu bölgelerde yayılış gösteren *Olea europaea* L.'nın rizosferik bölgesinden toplanan ölümcül zehirli bir mantar olan *Galerina venenata* A.H. Sm., Türkiye için ilk kez rapor edilmektedir. Türkiye koleksiyonlarından elde edilen ITS rDNA dizilerine dayanan moleküler filogenetik analiz, *G. venenata*'nın cins içindeki konumunu ve *G. marginata* (Batsch) Kühner'nın en yakın akrabası olduğunu ortaya koymuştur. Mevcut türün ayrıntılı morfolojik tanımı, fotoğrafları ile taksonomik ve filogenetik olarak yakın türlerle kıyaslanması verilmiştir.

Anahtar Kelimeler: *Agaricales*, makromantar, filogeni, ribozomal DNA, taksonomi

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1. Introduction

The taxonomic classification of *Galerina* Earle (Agaricales) can be traced back to Fries (1821), who initially designated it as the tribe *Galera*, comprising "Mycena-like ocher-brown spored fungi". *Galerina* was traditionally classified in the Hymenogastraceae Vittad., and species of the genus are characterized by small to medium-sized basidiomata with yellow-orange or yellow-brown pileus colour, slender and delicate stipe, the distinctive presence of verrucose basidiospores with rusty brown to brown and typically the presence of cheilocystidia (Ammirati et al., 1985; Gulden, 2012; Landry et al., 2021). Originally, it was assigned to the Cortinariaceae Singer due to the ocher brown and tuberculate nature of its basidiospores (Kirk et al., 2001), but subsequent reevaluation placed it within the Strophariaceae Singer & A.H. Sm. (Kirk et al., 2008). A molecular phylogenetic analysis conducted by Matheny et al. (2006) demonstrated that *Galerina* exhibits a closer evolutionary relationship with genera in the Hymenogastraceae as opposed to those in the Strophariaceae.

Members of *Galerine* grow in moss beds or on moss communities, including *Philonotis*, *Polytrichum*, *Sphagnum* and *Tomenthypnum*. However, certain species can be found colonizing decaying wood, stumps, humus, and soil substrates (Wood, 2001; Gulden 2010, 2012; Grzesiak and Wolski, 2015; Liu and Bau, 2021). Several species belonging to the genera *Amanita* Pers., *Conocybe* Fayod, *Galerina*, and *Lepiota* (Pers.) Gray contains amatoxins in amounts that can seriously poison humans (Ammirati et al., 1985). A recent study reported that *Galerina* contains lethal amatoxins at levels comparable to those in *Amanita phalloides* (Vaill. ex Fr.) Link (Landry et al., 2021). *Galerina badipes* (Pers.) Kühner, *G. castaneipes* A.H. Sm. & Singer, *G. marginata* (Batsch) Kühner, and *G. venenata* A.H. Sm. have been found as poisonous species within the genus (Landry et al., 2021). Amatoxins, α- and β-amanitin, which are toxic peptides found in *Amanita*, were also detected in *G. venenata* (Tyler and Smith, 1963).

Galerina comprises more than 300 species worldwide (Landry et al., 2021). Currently, 493 taxa of *Galerina* have been recorded in the IndexFungorum database (www.indexfungorum.org, accessed 10 January 2024), some of which are illegal names or synonyms. In Türkiye, 15 species have previously been reported and their distribution is limited to a few areas of Türkiye such as *Galerina ampullaceocystis* P.D. Orton, *G. atkinsoniana* A.H. Sm., *G. badipes* (Pers.) Kühner, *G. cinctula* P.D. Orton, *G. clavata* (Velen.) Kühner, *G. clavus* Romagn., *G. graminea* (Velen.) Kühner, *G. marginata* (Batsch) Kühner, *G. moelleri* Bas, *G. mycenoides* (Fr.) Kühner, *G. paludosa* (Fr.) Kühner, *G. pumila* (Pers.) Singer, *G. sideroides* (Bull.) Kühner, *G. sphagnorum* (Pers.) Kühner, and *G. stylifera* (G.F. Atk.) A.H. Sm. & Singer (Sesli et al., 2020; Solak and Türkoğlu, 2022). As part of ongoing investigations to

determine the macrofungal biodiversity in Türkiye, this report presents the first record of *Galerina venenata* in Türkiye based on morphological and molecular data.

2. Materials and Method

2.1. Sampling and morphological study

Galerina specimens were collected during the field trips to the Aydın Province in Western Türkiye in 2020 and 2022. By observing the features of fresh materials and morphological characters were described as follow Vellinga (1988). Dried samples mounted with 3% KOH or Melzer's reagent were observed for microscopic characteristics. A minimum of 30 basidiospores had been measured. In the list of abbreviations provided, "L^m" and "W^m" represent the mean dimensions of basidiospore length and width, respectively. "Q" denotes the length-to-width ratios, while "Q^m" signifies the average ratio value derived from the basidiospores measurements. All of the examined samples are deposited in the fungarium at Isparta University of Applied Sciences (ISUF).

2.2. Molecular genetic analysis

The ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) was used to extract genomic DNA from tiny fragments of dried materials. For the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA), the primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) were utilized. The Polymerase Chain Reaction (PCR) amplification was performed as described by Kaygusuz et al. (2020) and as follows. PCR was conducted in 25 µL reaction volume containing 2 µL DNA template, 8.5 µL ddH2O, 12.5 µL 2 × PCR Master Mix and 1 µL of each primer. For the amplification of the rDNA ITS region, the PCR setup began with an initial denaturation step of 5 minutes at 95°C, followed by 35 cycles that included 1 minute of denaturation at 94°C, annealing for 45 seconds at 54°C, and 1 minute of extension at 72°C. This was concluded with an extension step of 10 minutes at 72°C. PCR products were checked using an ethidium bromide-stained 1.2% agarose electrophoresis gel.

The sequences used for the phylogenetic analysis were selected based on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) results and relevant publications (Landry et al., 2021). DNA sequences of nrITS were aligned using the multiple sequence alignment program MAFFT v7 (Katoh and Standley, 2013) and were manually adjusted in BioEdit v.7.0 (Hall, 2004) when it is necessary.

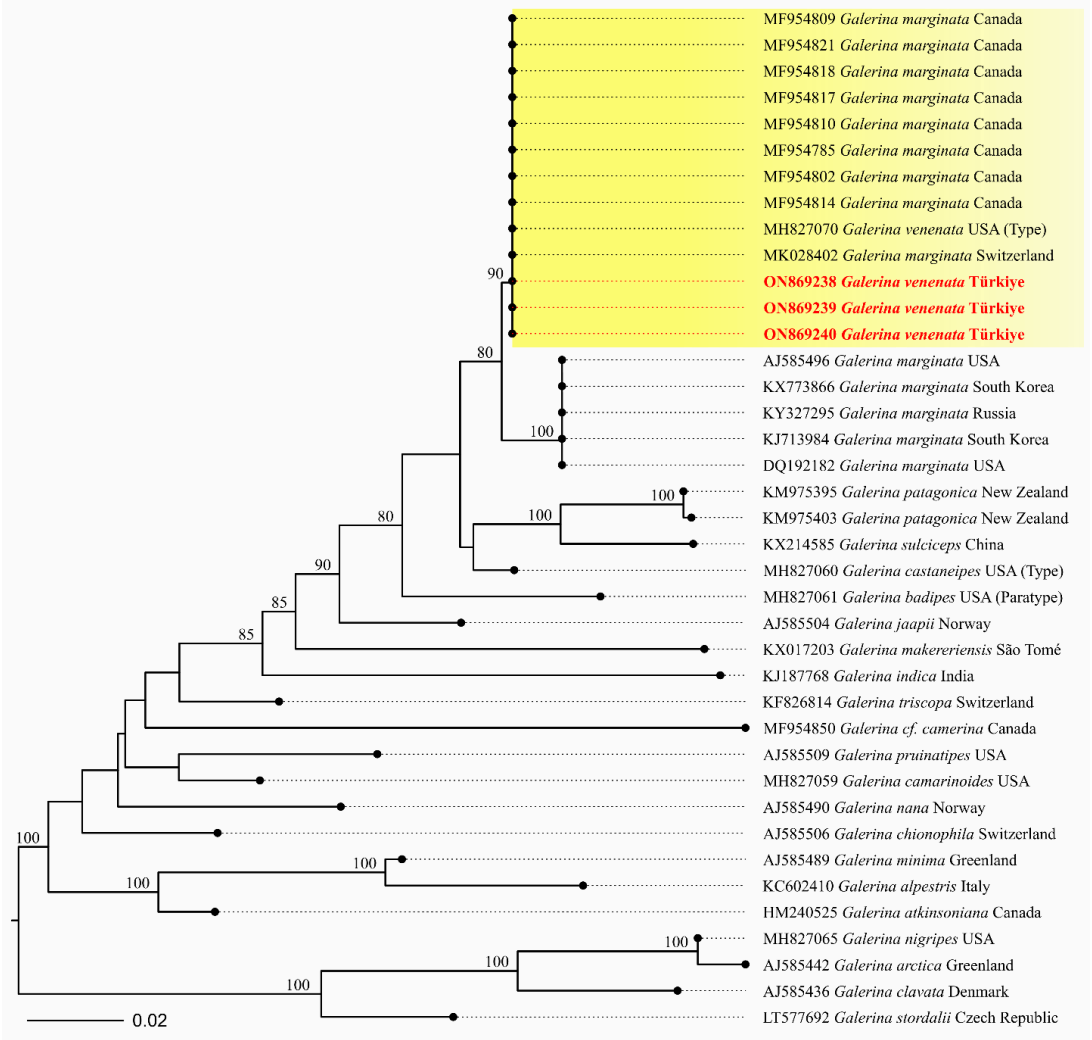

Figure 1. Phylogram generated from Maximum Likelihood (ML) analysis based on nrITS dataset. The tree is rooted with *Galerina arctica* (Singer) Nezdojm., *G. clavata* (Velen.) Kühner, *G. nigripes* A.H. Sm. & Singer and *G. stordalii* A.H. Sm. Newly generated sequences are shown in red

Phylogenetic relationships of taxa were generated through Maximum Likelihood (ML). ML analyses were performed in RAxML v.7.2.6 (Stamatakis et al., 2008) by running 1000 bootstrap (BS) replicates with GTRGAMMA substitution model. Maximum likelihood bootstrap (MLB) values of ≥80% were shown on the branch. The tree was visualized using FigTree v1.4.2 (Rambaut, 2012).

3. Results

3.1. Phylogeny

In this study, three sequences of *Galerina* specimens were generated and deposited in GenBank (ON869238-ON869240). The nrITS data matrix has 39 taxa containing 762 nucleotide sites (including gaps), of which 549 characters were constant and 213 were variable. The sequences from Türkiye were clustered in a separate monophyletic branch with high statistical support with MLB 90% (Fig. 1), with collections including the type sequence of *Galerina venenata* (MH827070).

3.2. Taxonomy

Galerina venenata A.H. Sm., Mycologia 45(6): 922 (1953) (Fig. 2)

Macroscopic and microscopic features:

Pileus 8–20 mm diam., at first convex to broadly convex, finally expanding to applanate, with a broad low umbo or slightly depressed at the centre, surface glabrous, hygrophanous, brightly yellowish white to yellow-brown or cinnamon brown, crenate, and minutely striate margin. Lamellae moderately crowded, subdistant, broadly adnate, yellowish to cinnamon. Stipe 30–65 × 2–6 mm, cylindrical or broadening towards the base, brownish to cinnamon, entirely floccose-fibrillose with white fibrils, without a ring or ring zone. Odour and taste are farinaceous.

Basidiospores (8.0–)9.0–11.8(–12.5) × (4.8–)5.5–6.7(–7.0) µm, $L^m \times W^m = 10.3 \times 6.0$ µm, Q = 1.5–1.9(–2.4), $Q^m = 1.7$, oblong, sometimes subcylindrical, with rugulose-warty in Melzer's reagent, thick-walled. Basidia 20–27 × 7.0–10.0 µm, clavate, 4–spored, thin-walled, hyaline. Pleurocystidia 40–85 × 9.0–15.0 µm, lageniform to fusiform, with a slightly longer neck and acute or subacute apex, thin-walled, hyaline in KOH. Cheilocystidia similar to pleurocystidia 45–80 × 10–18 µm, broadly lageniform to fusiform, with acute or subacute apex, thin-walled, hyaline in KOH. Pileipellis a cutis, terminal hyphae 5.0–18.0 µm wide, intertwined, thin-walled, hyaline in KOH. Caulocystidia 50–70 × 9.0–12.0 µm, lageniform to fusiform, with long tapered apex, mostly at the apex of the stipe, thin-walled, hyaline in KOH. Stipitipellis a cutis, hyphae 5.0–12.0 µm wide, cylindrical, thin-walled, hyaline in KOH. Clamp connections present in all tissues.

Habit and habitat: Gregarious or sometimes in small groups, terrestrial, near rhizosphere of *Olea europaea* L., growing on calcareous sandy soils.

Additional collections examined: Türkiye, Aydın Province, Kuşadası District, around Güzelçamlı, on calcareous soil, near rhizosphere of *Olea europaea*, alt. 10 m, 5 March 2020, leg. O. Kaygusuz, OKA-TR0124, GenBank: ON869238 for nrITS; *ibid.,* on calcareous soil, under *O. europaea*, alt. 5 m, 17 March 2021, leg. O. Kaygusuz, OKA-TR0125, GenBank: ON869239 for nrITS; *ibid.,* on soil, near rhizosphere of *O. europaea*, alt. 8 m, 23 March 2022, leg. O. Kaygusuz, OKA-TR0126, GenBank: ON869240 for nrITS.

4. Discussions

Galerina belongs to the family Hymenogastraceae and is a polyphyletic genus (Gulden et al., 2005). The main colour range of the fruit bodies of members of the genus varies from ochre to reddish brown, and the pileus is hygrophanous, in most species, it is translucently striate (Gulden, 2012). Among them, *Galerina venenata* was originally described by Smith (Smith, 1953) from the USA. Three collections from Türkiye grouped into a monophyletic branch, which includes the holotype sequence of *G. venenata* (Fig. 1). The sequences previously labelled as *G. marginata* from Canada (MF954809, MF954821, MF954818, MF954817, MF954810, MF954785, MF954802, and MF954814) and Switzerland (MK028402) clustered in the same clade as *G. venenata* (Fig. 1). This study is the first report of *Galerina venenata* from Türkiye.

Members of *Galerina* were reported to grow saprotrophic in mosses, on wood or soil, or rarely parasitic (Gulden, 2012). The type specimen of *G. venenata* was recorded from the lawn (Smith, 1953). Türkiye collections of *G. venenata* have been reported from calcareous soil under *Olea europaea*, which is a new possible host or mycorrhizal partner for this species.

Molecular phylogenetic analysis based on ITS rDNA sequences from Türkiye collections revealed the position of *Galerina venenata* within the genus *Galerina*, indicating it as a sister to *G. marginata*. However, *Galerina marginata* differs from *G. venenata* in having a pale to dark ochraceous to yellow pileus colour, and it usually grows in lignicolous habitats (Smith, 1953; Landry et al., 2021). In addition, α-amanitin has been isolated from *G. marginata*, while β-amanitin was detected in *G. venenata* (Landry et al., 2021). Morphologically, *Galerina autumnalis* (Peck) A.H. Sm. & Singer resembles *G. venenata*, but it is distinguished by having a membranous annulus and pleurocystidia that mostly have enlarged apices (Smith, 1953).

For many species of *Galerina*, habitat is a very important characteristic. *G. venenata*, *G. brunneimarginata* (Murrill) A.H. Sm. & Singer, and *G. semilanceata* (Peck) A.H. Sm. & Singer prefers open grassy areas. However, *G. brunneimarginata* and *G. semilanceata* differ from *G. venenata* in the colour and characteristics of the pileus, the small size of the basidiospores, and the absence of pleurocystidia (Smith and Singer, 1964).

Conflict of Interest

The authors have declared no conflict of interest.

Authors' Contribution

The authors contributed equally.

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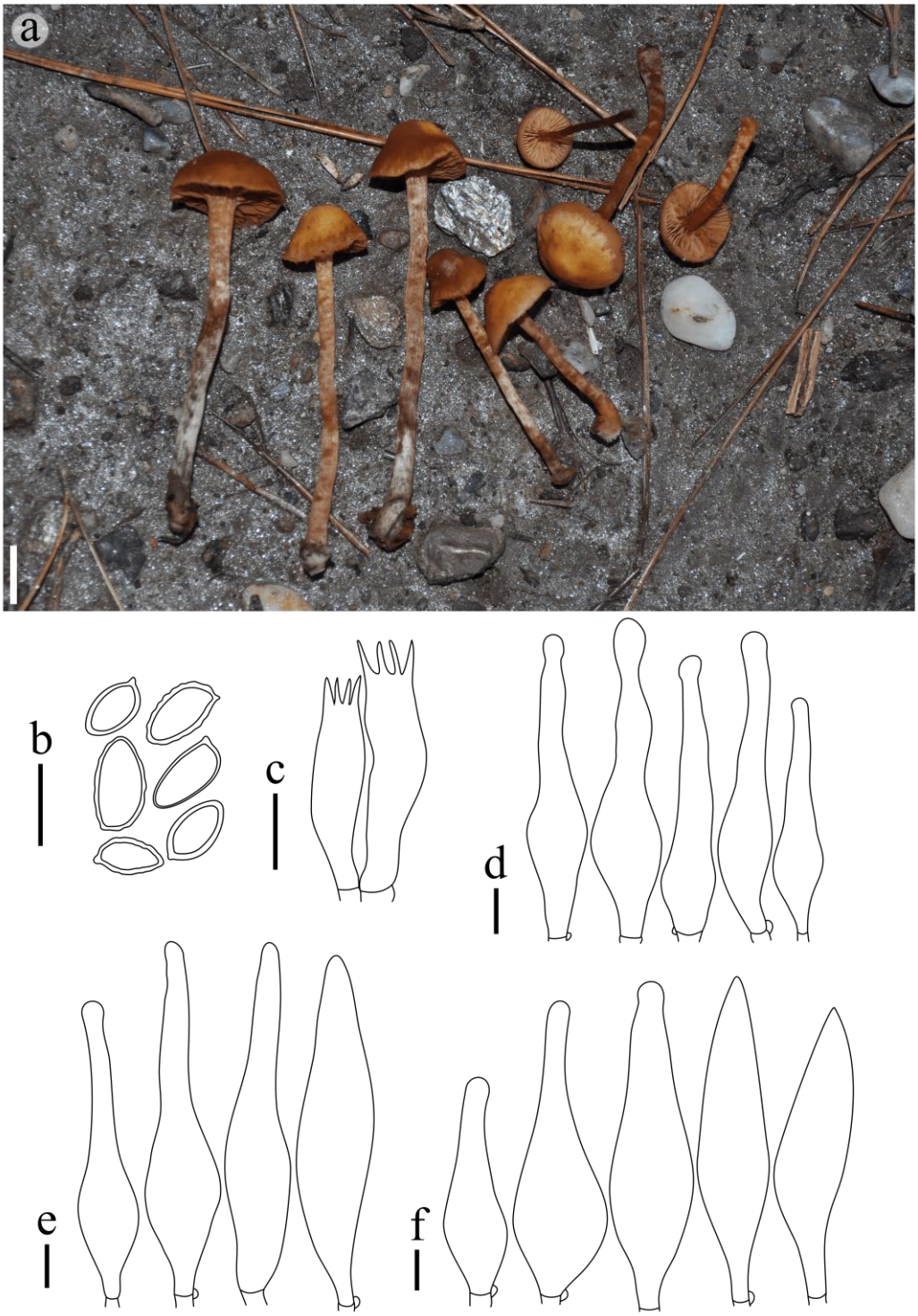

Figure 2. Basidiomata (a), basidiospores (b), basidia (c), caulocystidia (d), pleurocystidia (e) and cheilocystidia (f) of *Galerina venenata* (Scale bars: a- 10 mm, b-f- 10 µm)

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