



Melanoleuca tristis (Basidiomycota, Agaricales), a new record from western Mediterranean region of Türkiye

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Türkiye'nin Batı Akdeniz bölgesinden yeni bir kayıt, *Melanoleuca tristis* (Basidiomycota, Agaricales)

Abstract: A rare species, *Melanoleuca tristis* is reported for the first time from Türkiye on sandy soil under *Pinus nigra* subsp. *pallasiana* forest, a characteristic habitat of the Mediterranean region. Phylogenetic analysis based on a dataset of the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences is performed to support the identification and to compare with similar taxa. The description, a color photograph of the basidiomata and microscopic drawings are given.

Key words: Basidiomycota, *Melanoleuca*, new record, phylogenetic analysis, taxonomy, Türkiye

Özet: Nadir bir tür olan *Melanoleuca tristis*, ilk defa Türkiye'de, Akdeniz bölgesinin karakteristik bir habitatu olan *Pinus nigra* subsp. *pallasiana* ormanındaki kumlu toprak üzerinden rapor edilmiştir. Nükleer ribozomal DNA (rDNA) internal transcribed spacer (ITS) sekanslarının bir veri setine dayanan filogenetik analiz, türün belirlenmesini desteklemek ve benzer taksonlarla karşılaştırmak için gerçekleştirilmiştir. Bazidiyomatının betimlemesi, renkli fotoğrafı ve mikroskopik çizimler verilmiştir.

Anahtar Kelimeler: Bazidiyomikota, *Melanoleuca*, yeni kayıt, filogenetik analiz, taksonomi, Türkiye

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1. Introduction

Melanoleuca Pat. (Yılanmantarı) is an agaric genus in *Tricholomataceae* R. Heim ex Pouzar (Singer, 1986; Sesli et al., 2020). According to the Index Fungorum, it is represented by more than 400 species, while only 60 species are accepted worldwide (He et al., 2019).

Melanoleuca species exhibit very similar macroscopic features that make morphological identification difficult or misleading. Furthermore, its species may have variable characters influenced by environmental conditions, which makes species delimitation more complicated (Bon, 1991; Boekhout, 1999). With the advent of molecular studies, it is now accepted that morphology-based classification of its species is insufficient. In addition to some features, such as basidioma colour, odour, stipe ornamentation, presence/absence of cheilocystidia, shape of cystidia, spore size and ornamentation, and pileipellis structure, phylogenetic methods should also be considered for a reliable taxonomic identification and infrageneric classification in this genus (Vizzini et al., 2011).

Melanoleuca species are often characterised by a collybioid to tricholomatoid basidioma, convex to slightly depressed pileus, emarginated to adnate to shortly decurrent lamellae, warty and strongly amyloid spores, white to pale-yellowish spore print, cutis to trichoderm pileipellis, mostly hymenial cystidia and lack of clamp connections (Singer 1986; Boekhout 1988, 1999; Bon 1991; Vesterholt 2008; Vizzini et al., 2011).

In the last few decades, molecular studies have revealed important information about the infrageneric classification and species identification in the genus *Melanoleuca* and have indicated that *Melanoleuca* species are closely related to those of *Pluteaceae* Kotl. & Pouzar and *Amanitaceae* E.-J. Gilbert (Moncalvo et al. 2002; Matheny et al. 2006; Garnica et al. 2007; Justo et al. 2011; Vizzini et al. 2011; Sánchez-García et al. 2014; Antonín et al., 2014, 2017, 2021; Binder et al. 2014; Yu et al. 2014; Kalmer et al., 2018; Xu et al., 2019; Pei et al., 2021). Vizzini et al. (2011) revealed that *Melanoleuca* is monophyletic, and divided into two subgenera, subg. *Urticocystis* and subg. *Melanoleuca*, in which the former includes taxa mainly with urticocystidia but also with macrocystidia and brightly coloured pilei, while the latter possesses basidiomata with non-septate macrocystidia, or rarely without cystidia.

In Turkey, only 28 *Melanoleuca* species have been reported so far, most of which lack molecular support (Sesli et al., 2020; Solak and Türkoğlu, 2022). In this study, molecular and morphological investigations of collections from the Mediterranean region of Turkey are provided. Our results indicate that the specimen is *Melanoleuca tristis* M.M. Moser, a new record from a non-European continent and for Turkish mycobiota.

2. Material and Method

2.1. Morphological studies

Specimens were collected from Isparta Province during the autumn season in 2012. Pictures were taken in the field and

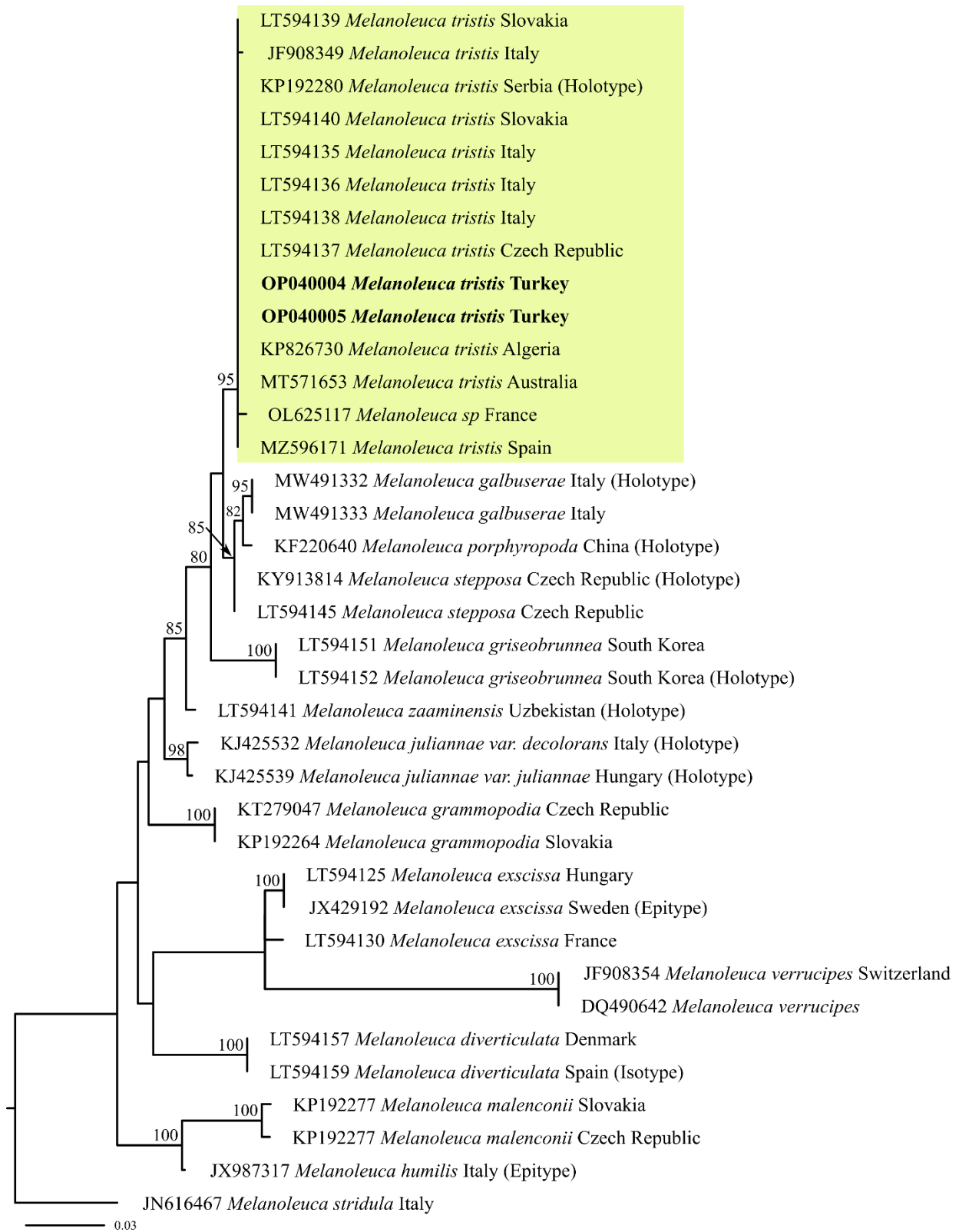


Figure 1. Maximum likelihood tree illustrating the phylogeny of *Melanoleuca tristis* and related taxa based on nrITS dataset (ML \geq 80% is indicated). The tree is rooted with *Melanoleuca stridula* (Fr.) Singer (JN616467). Newly generated sequences are shown in bold.

macroscopic characters were recorded using fresh materials. Micro-morphological observations were made using dried samples and a Leica DM750 light microscope (Leica Microsystems, Wetzlar, Germany) at magnifications of up to 400 \times and 1000 \times . Specimens were deposited at the fungarium of Isparta University of Applied Sciences, Turkey.

2.2. Phylogenetic studies

2.2.1. DNA isolation, Polymerase chain reaction and Sequencing

Using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, Irvine, California) in accordance with the manufacturer's instructions, the total genomic DNA was

extracted from specimens. To amplify the nrITS region, the primer pairs ITS1F/ITS4 (Gardes & Bruns 1993; White et al. 1990) were chosen. Polymerase chain reaction was performed as described by Kaygusuz et al. (2021). Using the same primer sets, sequencing was carried out (Source Bioscience, Berlin, Germany). Editing of sequence chromatograms was done with BioEdit 7.0.5. (Hall, 1999).

2.2.2. Phylogenetic analysis

The sequences for phylogenetic analysis were chosen based on Antonín et al. (2017). The Maximum Likelihood (ML) approach was used to build the phylogenetic tree. A dataset was used for the ML analysis in RAxML v8.2.10 (Stamatakis, 2014), with 1.000 bootstrap replicates and the GTRGAMMA substitution model.

3. Results

3.1. Molecular analysis

The phylogenetic tree inferred from nrITS gene sequences was obtained from 37 fungal samples representing 779 characters. The phylogenetic analysis based on the nrITS sequences resulted in the well-supported separation of the species belonging to subg. *Urticocystis* and subg. *Melanoleuca* and supported the monophyly of the *Melanoleuca* species. Additionally, Turkish collections presented as *Melanoleuca tristis* were grouped with the holotype from Serbia (KP192280) with high statistical support (MLB = 95%, Fig. 1).

3.2. Taxonomy

Melanoleuca tristis M.M. Moser, Boletus 15: 66 (1991)

(Fig. 2)

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Macroscopic and microscopic features:

Pileus 35–50 mm diam, at first convex, then applanate with undulating margin, depressed or umbilicate at the center, non-striate at the edge, not hygrophanous, non-pruinose, smooth, glabrous, brown to dark brown (Fig. 2a). Lamellae crowded, emarginate, sinuate, grayish to gray, with concolorous edge. Stipe 20–30 × 3–6 mm, cylindrical or slightly widened under pileus, longitudinally coarsely fibrillose, dark grey-brown to black-brown. Flesh dark grey-brown. Smell and taste indistinct.

Basidiospores (7.3–)7.8–9.7(–10.0) × (5.3–)5.8–6.2(–6.8) µm, ellipsoid to broadly ellipsoid, verruculose, irregular warts, thin walled, amyloid (Fig. 2b). Basidia 30–45 × 8–12 µm, clavate, hyaline, 4-spored (Fig. 2c). Cheilocystidia frequent, 30–50 × 6–10 µm, clustered, fusiform to subulate to subcylindrical, hyaline, thin-walled (Fig. 2d). Pleurocystidia absent or very scattered. Pileipellis ixocutis, composed of cylindrical, 4–10 µm wide hyphae, hyaline, thin-walled.

Habit and habitat: Solitary or in small groups, on sandy soil, under *Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe.

Materials examined: Turkey, Isparta Province, Sav district, on soil under *Pinus nigra* subsp. *pallasiana*, alt. 1000 m, 01 November 2012, O. Kaygusuz, OKA-TR1220; GenBank: nrITS OP040004; idib, under *P. nigra* subsp.

pallasiana, alt. 1050 m, 02 November 2012, O. Kaygusuz, OKA-TR1221; GenBank: nrITS OP040005.

4. Discussions

Melanoleuca tristis is a rare species which is distributed on the European continent. Its distinctive features include large basidiomata, a dark brown pileus, a stipe that varies in color from dark gray-brown to black-brown, grayish to gray lamellae, somewhat narrow basidiospores, a well-developed caulohymenium and large cheilocystidia (Moser, 1991; Antonín et al., 2017, 2018). The Turkish specimens also fit well with the characters of the originally described *Melanoleuca tristis* (Moser, 1991; Antonín et al., 2017, 2018).

Based on the molecular data, species of the genus *Melanoleuca* formed a monophyletic group, and our results are congruent with those of Vizzini et al. (2011). However, both molecular and morphological characters should be considered for a reliable identification. According to the ITS region phylogeny, *Melanoleuca tristis* is phylogenetically closely related to *Melanoleuca galbuserae* Antonín, Ševčíková, Para & Tomšovský, *M. porphyropoda* X.D. Yu and *M. stepposa* Vacek. However, morphologically, *Melanoleuca galbuserae* differs from *M. tristis* by smaller basidiomata (up to 40 mm broad), a light beige to dirty ochre-brown pileus, dirty whitish lamellae, ochre to pale brown or gray-brown stipe, and a different habitat (Antonín et al., 2021). *Melanoleuca porphyropoda*, originally described from China, differs from *M. tristis* by having an orange-cinnamon pileus, a purplish stipe, larger basidiospores (8.0–12.0 × 4.5–8.0 µm) and lacking cystidia (Yu et al., 2014). *Melanoleuca stepposa* has a dirty grey-yellow or yellow-brown pileus and white or pale cream lamellae (Antonín et al., 2017). *Melanoleuca tristis* also differs from *M. galbuserae* and *M. griseobrunnea* by a well-developed caulohymenium (Antonín et al., 2017).

Macroscopically *Melanoleuca tristis* is similar to *M. malenconii* Bon, *M. humilis* (Pers.) Pat., *M. grammopodia* (Bull.) Murrill and *M. excissa* (Fr.) Singer. However, *Melanoleuca malenconii* differs from *M. tristis* in having a gray, ochraceous to dirty yellow pileus, a pale to grayish yellow lamellae and a more distinctly floccose stipe (Antonín et al., 2018). *Melanoleuca humilis* has a paler gray-brown pileus and stipe, which is paler in color (Antonín et al., 2018). *Melanoleuca grammopodia* differs in having grayish, whitish or brownish pileus, larger basidiomata (up to 125 mm broad) and gray-brown stipe (Antonín et al., 2018). *Melanoleuca excissa* has a gray-brown or beige-gray pileus, whitish, grayish or pale ochraceous stipe, cream-coloured lamellae, and longer basidiospore size (7.5–11.0 × 5.0–7.0 µm) (Antonín et al., 2018).

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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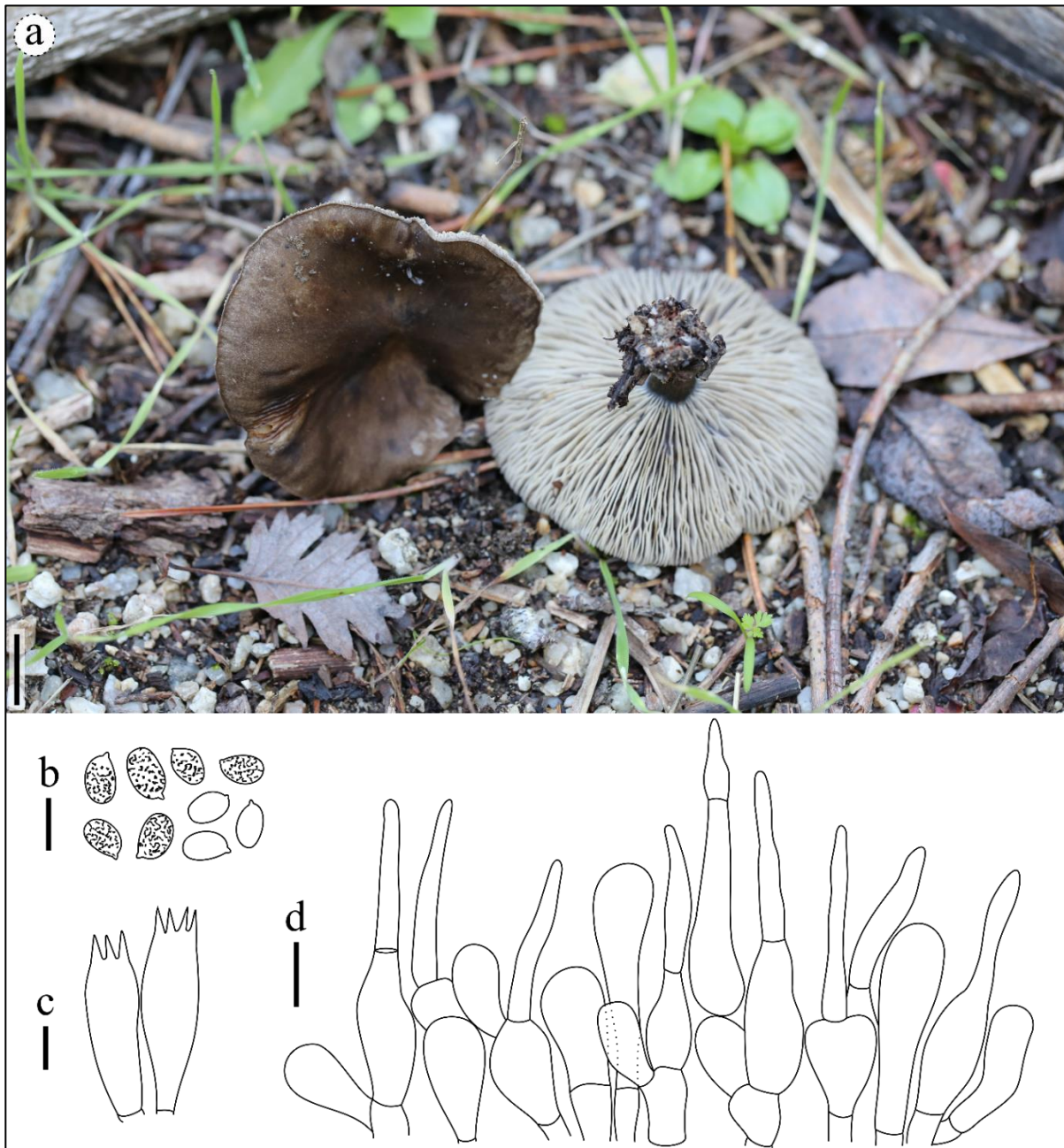


Figure 2. *Melanoleuca tristis*: a. Basidiomata, b. Basidiospores, c. Basidia, d. Cheilocystidia Scale bars: a = 10 mm, b-d = 10 µm.

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