Research Article GU J Sci 32(4): 1097-1103 (2019) DOI: 10.35378/gujs.523498

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

Calocybe persicolor, A New Record for the Turkish Mycota

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Highlights

- *C. persicolor* was the first time reported from Turkey and hence makes an important contribution to the Turkish mycota.
- Both conventional and molecular methods were used for identification.
- The first ITS sequence data belongs to Calocybe persicolor was uploaded to the GenBank from Turkey.

Article Info

Received: 06/02/2019
Accepted: 23/05/2019

Keywords

Calocybe persicolor
Ankara
Molecular phylogeny
New record

Abstract

The aim of this study is to identify fungal samples from Ankara (Turkey). Identification of the samples was made according to conventional and molecular methods (ITS region of the rDNA). Based on the high sequence similarity of the new record (hereafter will be referred to as '*Ank Akata & Altuntaş 166*') with *Calocybe persicolor* (Fr.) Singer, the relevant agaric is considered to be *C. persicolor*. This result is also supported by the morphological data and proves the presence and distribution of this species in Turkey.

1. INTRODUCTION

Calocybe, a small genus of the family *Lyophyllaceae* (*Agaricales*), includes approximately 40 widely distributed species [1]. Members of the genus are mainly characterized by the tricholomatoid fruiting body, conical to convex or plane pileus, emarginate and crowded lamellae, cylindrical stipe, cylindrical to clavate and siderophilous basidia, whitish to creamy spore print, ellipsoid basidiospores and usually clamped hyphae [2].

Calocybe persicolor (Fr.) Singer appears in late summer and fall growing on soil in grassy meadows, parks, less frequently in woods. Although it is an uncommon species, *C. persicolor* have been reported in Europe, Asia, and America [2].

According to the checklist on Turkish macromycota [3], four *Calocybe* species (*Calocybe carnea* (Bull.) Donk, *C. chrysenteron* (Bull.) Singer, *C. gambosa* (Fr.) Donk and *C. ionides* (Bull.) Donk were collected but, no information has been reported about the presence and distribution of *C. persicolor* in Turkey up to date [3-9]. The present study proves the presence of *C. persicolor* in Turkey and aims to make a contribution to Turkish mycota.

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2. EXPERIMENTAL

2.1. Morphological study

Fungal samples were collected from Ankara University Tandoğan Campus (Ankara) on September 2, 2018. In the research area, relevant macroscopic and ecological features of the fruiting bodies were registered. Routine macroscopic and microscopic investigations were performed with the help of the literature [10,11] in our laboratory. The identified samples were deposited at Ankara University Herbarium (ANK).

2.1. Molecular study

DNA Isolation—The genomic DNA was extracted according to the modified CTAB method [12]. NanoDrop ND-Lite was used to measure DNA concentration and purity of the samples.

Table 1. *GenBank accession numbers of the ITS sequences belonging to the 17 fungi specimens used in this study*

Fungi species	GenBank number (ITS)	Geographical origin	Reference
Leucocybe connata	MG953997	Canada	Unpublished
Leucocybe candicans	KJ681027	Spain	[13]
Tricholomella constricta	JF907769	Italy	[14]
Tephrocybe rancida	EU669250	USA	Unpublished
Tephrocybe ambusta	AF357058	Switzerland	[15]
Tephrocybe anthracophila	KU058523	Australia	[16]
Tephrocybe atrata	AF357053	Switzerland	[15]
Asterophora lycoperdoides	KR673545	South Korea	[17]
AnkAkata&Altuntaş 166 (*Calocybe persicolor*)	SUB5067264	Turkey	Current study
Asterophora parasitica	MG890393	USA	Unpublished
Lyophyllum decastes	HM572548	Northern Fennoscandia	[18]
Lyophyllum semitale	KP192555	France	[19]
Calocybe decolorata	NR156302.1	China	Unpublished
Calocybe obscurissima	AF357031	Switzerland	[15]
Calocybe persicolor	AF357026	Switzerland	[15]
Cortinarius anisatus	NR131788	Sweden	[20]
Amanita muscaria	AB015700	Japan	[21]

PCR Amplification and Sequencing—The ITS region of the rDNA was amplified using ITS1 and ITS4 primers [22,23]. The PCR was performed in 50 µl volumes containing 10× buffer, 1-unit Taq DNA polymerase (Promega, Madison, Wisconsin), 200 µM dNTPs, 2 mM $MgCl_2$, and 10 pmol of both primers, ITS 1 and ITS4. PCR amplification was performed in a Biometra TProfessional Standard thermal cycler (Biometra, GmbH, Germany) with the following thermal cycling conditions: one cycle of 94°C for 2 min, 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and a final extension step of 8 min at 72°C. The amplification products were analyzed by electrophoresis in 1.2 % agarose gel containing ethidium bromide, and the product sizes were determined using a nucleotide size marker (100 bp ladder; Fermentas, Vilnius, Lithuania). The PCR products were sequenced with a BigDye cycle sequencing kit (Applied Biosystems, Foster City, California) using an ABI 3130XL genetic analyzer (Applied Biosystems).

Sequence Analysis—The amplified fragments were sequenced and the ITS gene sequences from several other fungal species were obtained from GenBank to compare with the *'Ank Akata & Altuntaş 166'* (Supplementary Table 1). The DNA sequences were aligned and assembled using Multiple Sequence Comparison by Log-Expectation (MUSCLE) by Geneious R9 [24]. Molecular phylogenetic analyses were

performed using the maximum likelihood method based on the Tamura 3-parameter substitution model via MEGA7 software [25]. One thousand bootstrap replicates were performed [26].

3. RESULTS AND DISCUSSION

The systematics of the new record was in accordance with Kirk et al. [1] and MycoBank (accessed 02.2019) [27]. Short descriptions and the ecology of the species were given with the field photos. Through phylogenetic analysis, we revealed two distinct clades as well as an outgroup. The major clade (Clade 1) included fungi species from the genus *Leucocybe*, *Tricholomella*, *Asterophora*, *Lyophyllum*, and *Tephrocybe*, while the minor clade (Clade 2) contained species only from the genus *Calocybe* together with 'Ank Akata & Altuntaş' with a bootstrap value of 94%. On the other hand, *Cortinarius anisatus* H. Lindstr., Kytöv. & Niskanen and *Amanita muscaria* (L.) Lam. were branched far from the other fungi species and generated an outgroup as expected with a bootstrap value of 100%. Moreover, the phylogenetic tree based on ITS sequences provided evidence for almost 99% similarity of the new record with *C. persicolor*.

3.1. Description of the newly reported species

Lyophyllaceae Jülich
Calocybe persicolor (Fr.) Singer (1962), (Figures 1, 2)
Syn.: *Agaricus persicolor* Fr. (1874) = *Tricholoma persicolor* (Fr.) Sacc. (1887) = *Lyophyllum persicolor* (Fr.) Malençon & Bertault (1975).

Figure 1. *Calocybe persicolor: a-d. basidiomata*

Macroscopic and microscopic characteristics

Pileus 20-25 mm; convex when young, then plane with involute margin. **Surface** cream to pinkish with a light brownish tinge, dry, smooth but pruinose towards to margin. **Lamellae** white, emarginate and crowded. **Stipe** 30-35 × 5 mm, cylindrical, concolorous with pileus somewhat paler, fibrillose. **Flesh** thin and white with a pinkish tinge. **Taste** farinaceous. **Odor** unpleasant. **Basidia** 15.5-17.5 × 4.5-6 µm, clavate, four-spored, with siderophilous granules and basal clamped. **Basidiospores** 4.5-6 × 2-3 µm, smooth, hyaline and ellipsoid. **Cystidia** absent. **Pileipellis** a cutis, consisting of irregular and swollen hyphae, some septa with clamps.
Ecology: Uncommon, summer to autumn, in meadows, parks, garden, on soil, among grasses [11].
Specimen examined: TURKEY—Ankara: Ankara University Tandoğan campus, under Judas-tree (*Cercis siliquastrum* L.), among grasses, 860 m, 39°56'04" N, 32°50'01" E, 02.09.2018, ANK Akata & Altuntaş 166.

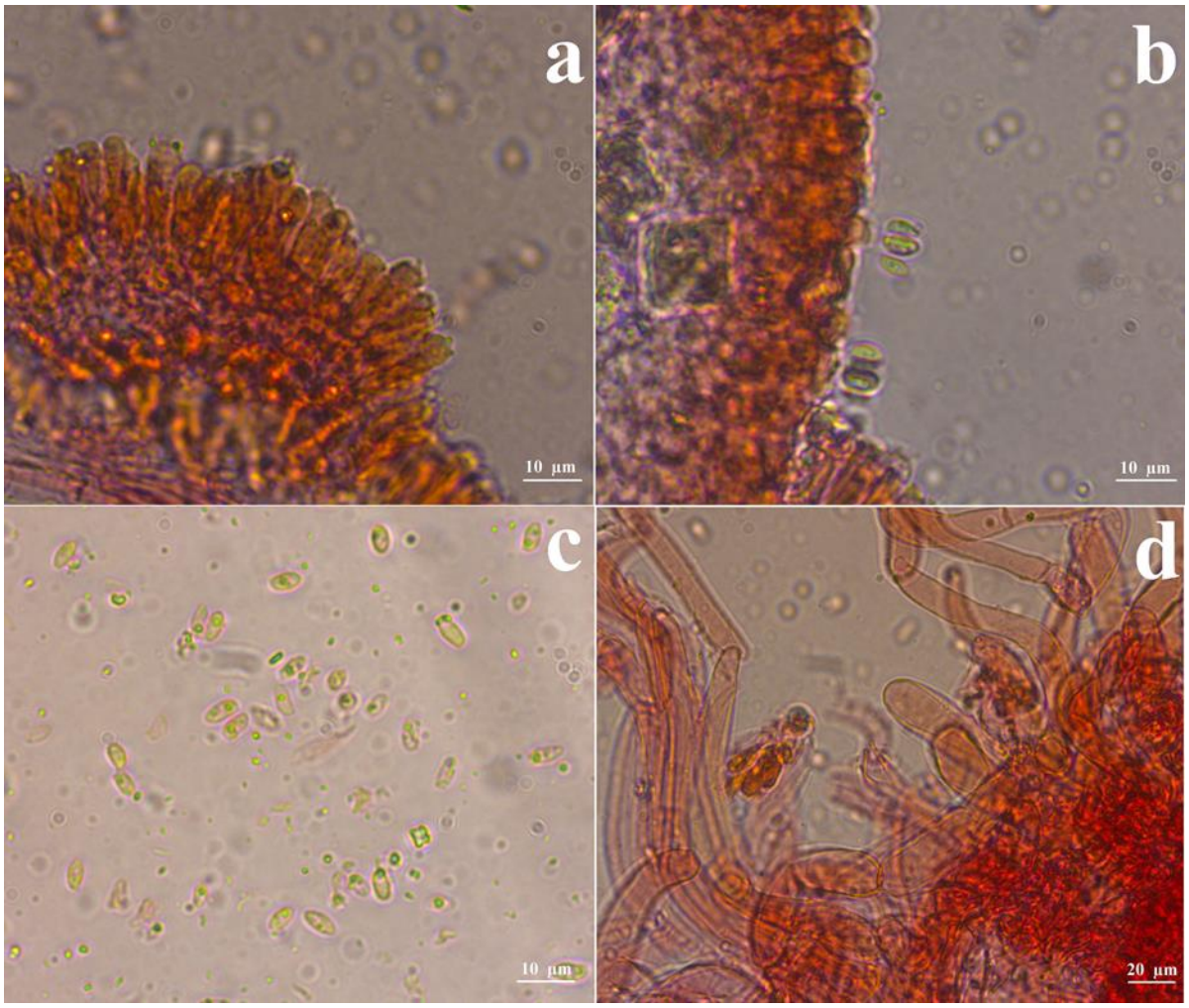

Figure 2. *Calocybe persicolor: a, b. basidia. c. basidiospores d. pileipellis*

The phylogenetic tree which was drawn based on ITS sequences branched into three groups, Clade A and Clade B, and an outgroup (Figure 3). The outgroup included *Cortinarius anisatus* and *Amanita muscaria* as it was expected. The Clade B was seen to be the largest group which included the members of *Asterophora*, *Leucocybe*, *Lyophyllum*, *Tricholomella* and *Tephrocybe* (Figure 3). The Clade A was only comprised of *Calocybe* members and *Ank Akata & Altuntaş 166* as well. In addition, *C. persicolor* showed 99% similarity with *Ank Akata & Altuntaş 166.*

C. persicolor is characterized by its pink to pinkish brown, convex to plane pileus, cylindrical stipe, whitish flesh, four-spored and clavate basidia; elliptic, smooth and hyaline basidiospores. *C. carnea* is a close

species but differs with bright pink to pinkish brown fruiting bodies [10]. As the morphological data is not sufficient for the precise identification of fungi, the use of sequence data from conserved DNA regions such as ITS is considered to be an important tool in taxonomy and systematics since last three decades [28,29]. Moreover, ITS is the most common DNA barcoding marker and thus provides an important source for the researchers to make comparisons of data obtained from their own studies with the one found in the GenBank database. For this reason, we used ITS region for molecular identification and the phylogenetic analysis made based on the ITS regions revealed almost 99% genetic similarity between *Calocybe persicolor* and the new record (GenBank ID: AF357026) (Figure 3).

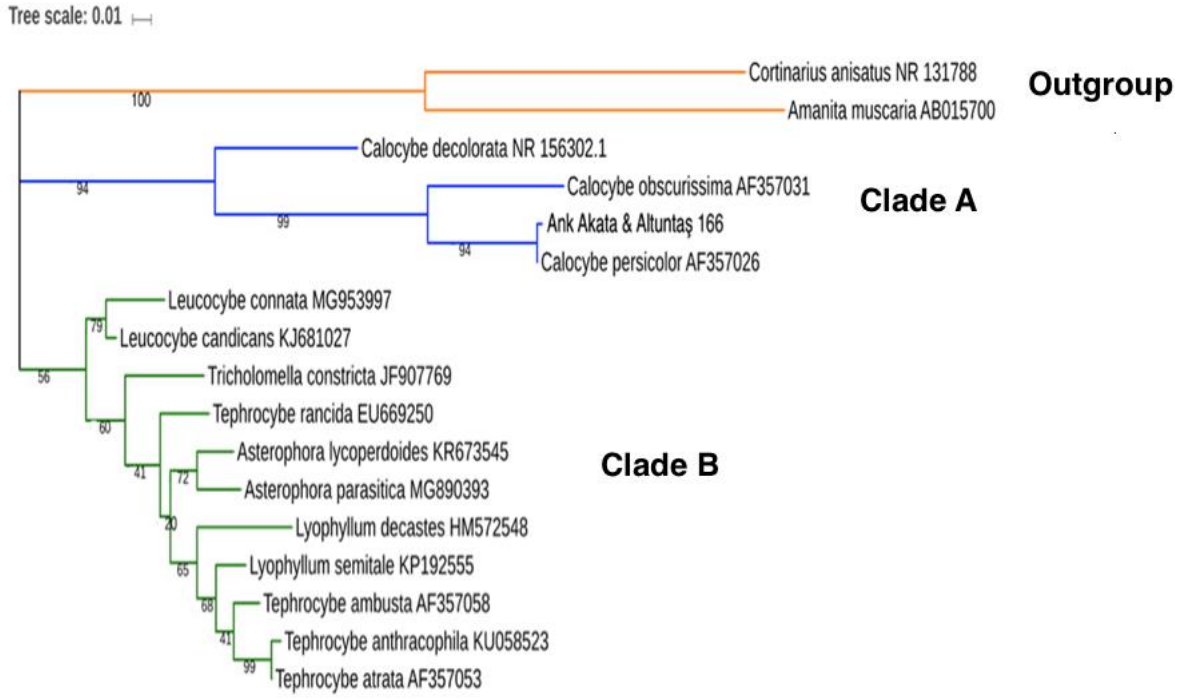

Figure 3. *The maximum likelihood tree showing the genetic relationships between 17 fungi based on the nuclear ITS region. Bootstrap values from 1000 bootstrap replicates were given above the branches. All sequences were retrieved from GenBank except for Ank Akata & Altuntaş 166. Cortinarius anisatus and Amanita muscaria were used as outgroup samples.*

ACKNOWLEDGMENTS

We thank the Ankara University Research Fund (Project no: 18B0430001) for financial support.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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