Inocybe mytiliodora: A New Record for Turkey

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Abstract

Aim of the study: The aim of the present study is to identify, characterize and record a new agaric for the first time from Turkey.

Study area: It covers a total area of approximately 20 ha and is located in Ankara within the boundaries of Çankaya district.

Material and method: Basidiomata were collected from Ankara University Tandoğan Campus on September 1, 2018. Macro- and micromorphological properties of basidiomata and nuclear ribosomal large subunit (nrLSU) sequences were used for identification. The voucher specimens were kept at Ankara University Herbarium (ANK).

Main results: Based on the high sequence similarity between the new record (hereafter referred to as 'Ank Akata & Altuntaş 167') and *Inocybe mytiliodora*, the specimen was identified as *I. mytiliodora*. This result is also supported by the morphological data derived from the evaluation of macroscopic and ecological features of the fruiting bodies between 'Ank Akata & Altuntaş 167' and closely related fungi.

Research highlights: As a result, the basidiomata collected from Ankara University Tandoğan Campus represent '*Inocybe mytiliodora*' and this is the first known record for Turkey.

Keywords: *Inocybe mytiliodora*, nrLSU, Ankara, Molecular Phylogeny, New Record

Inocybe mytiliodora: Türkiye İçin Yeni Bir Kayıt

Öz

Çalışmanın amacı: Bu çalışmanın amacı yeni bir şapkalı mantarı Türkiye için ilk kez, tanımlamak, karakterize etmek ve tanıtmaktır.

Çalışma alanı: Çalışma alanı Ankara Çankaya ilçesi sınırları dahilinde bulunmakta ve toplam 195.000 m² lik bir alanı kaplamaktadır.

Materyal ve yöntem: Bazidiyomalar 1 Eylül 2018'de Ankara Üniversitesi Tandoğan Kampüsü'nden toplanmıştır. Teşhis için bazidiyomaların makro ve mikromorfolojik özelliklerinden ve nükleer büyük ribozomal alt ünite (nrLSU) sekanslarından faydalanılmıştır. Kurutulmuş örnekler Ankara Üniversitesi Herbaryumunda (ANK) saklanmaktadır.

Temel sonuçlar: Yeni kayıt (bundan böyle "Ank Akata ve Altuntaş 167" olarak anılacaktır) ve *Inocybe mytiliodora* arasındaki yüksek sekans benzerliklerine dayanarak, ilgili şapkalı mantar *I. mytiliodora* olarak kabul edilmiştir. Bu sonuç aynı zamanda "Ank Akata & Altuntaş 167" ile yakın akraba mantarlar arasındaki morfolojik ve ekolojik benzerliklerin değerlendirilmesi ile de desteklenmiştir.

Araştırma vurguları: Sonuç olarak, Ankara Üniversitesi Tandoğan Kampüsü'nden toplanan bazidiyomalar "*Inocybe mytiliodora*" yı temsil eder ve Türkiye için ilk kayıttır.

Anahtar Kelimeler: *Inocybe mytiliodora*, nrLSU, Ankara, Molecular Filogeni, Yeni Kayıt

Introduction

Inocybe is an ectomycorrhizal genus of the family *Inocybaceae* within the order *Agaricales* (*Basidiomycota*). This genus contains about 500 species, whose distribution range from the tropics to the arctic regions, particularly occurring in coniferous and deciduous woodlands (Akata, 2017; Kirk, Cannon, Minter & Stalpers, 2008).

Inocybe members are characterized by small to medium-sized, collybioid, tricholomatoid or mycenoid fruiting bodies, smooth to fibrillose, squamulose or squarrose, whitish, grey, yellowish, pale brownish or red-brownish pileus, occasionally with red, greenish or purplish tinge, initially conical to campanulate, later convex, plano-convex to applanate, adnate, adnexed or free lamellae, mostly white, greyish, brownish with yellowish, greenish or olivaceous tinge, central and cylindrical stipe, usually spermatic, rarely aromatic or fishy odor, snuff

brown spore print, smooth to tuberculate, angular, nodulose, amygdaloid, or phaseoliform, clavate basidia, mostly thick-walled and metuloid, more rarely thin-walled globose or pyriform cystidia (Knudsen & Vesterholt, 2008; Kuyper, 1986).

According to the current literature on Turkish mycobiota (Akata, 2017; Akata & Uzun, 2017; Akata, Kabaktepe, Sevindik, & Akgül, 2018; Akata, Altuntaş & Kabaktepe, 2019; Doğan & Kurt, 2016; Sesli & Denchev, 2008; Türkekul & Işık, 2016; Uzun and Acar, 2018), more than 75 *Inocybe* species have so far been registered from Turkey but there was not any report of *I. mytiliodora* Stangl & Vauras to date.

The current study aims to contribute to the Turkish *Inocybe*.

Materials and Methods

In this study, both conventional and molecular identifications including evaluation of macro and micromorphologies of the samples and nuclear ribosomal large subunit (nrLSU) sequencing were used to characterize the fungi sample (*Ank Akata & Altuntaş 167*) collected from Ankara University Tandoğan Campus.

Morphological Characterization

Basidiomata were collected from Ankara University Tandoğan Campus (Ankara) on September 2, 2018. During the fieldwork, habitat and macroscopic properties of the Basidiomata were noted. Relevant macroscopic and microscopic data were obtained using standard techniques and identification was performed according to the studies by Hobart and Henrici (2011), Knudsen and Vesterholt (2008) and Stangl and Vauras (1987). The identified samples were kept at Ankara University Herbarium (ANK).

Molecular Characterization
DNA Isolation

The genomic DNAs of the specimens were extracted from the sporophores, according to the method using CTAB extraction buffer (Doyle & Doyle 1987). NanoDrop Lite UV-Vis Spectrophotometer (Thermofisher) was utilized to determine the DNA purity and concentration.

PCR Amplification and Sequencing

The nuclear ribosomal large subunit (nrLSU) region of the rDNA was amplified by PCR using the universal LR5 and LR0R oligonucleotide primers (Stielow et al., 2015). PCR was set up in a reaction volume of 35μl. The final concentrations of the PCR ingredients were designated as follows: 1× Taq DNA polymerase buffer, 2 units of Taq DNA polymerase (Fermentas), 0,4 mM dNTPs, 2 mM MgCl2, and 10 pmol of both LR5 and LR0R primers. PCR was conducted in a Thermal Cycler (ABI MiniAmp Plus) with the below mentioned thermal cycling conditions: first denaturation step of 94°C for 5 minutes, pursued by 35 cycles of 94°C for 25 seconds, 55°C for 25 seconds, and 72°C for 90 seconds, and a last elongation step of 10 minutes at 72°C. The PCR products were electrophoretically analyzed in 1.3% agarose gel including the sybr safe dye. DNA marker (GeneRuler 100 bp Plus DNA Ladder, Thermofisher) was utilized for the sizing of the amplicons. The PCR products were sequenced bidirectionally by using the LR5 and LR0R primers and the standard Sanger dideoxy chain termination method at the laboratory of BM Labosis (Ankara, Turkey).

Sequence Analysis

The amplified fragments were sequenced and the 28S rDNA region sequences from several other fungal species were obtained from GenBank to compare with the *'Ank Akata & Altuntaş 167'* (Supplementary Table 1). The sequences were assembled using Geneious Prime 2019.1.3 software (Biomatters Ltd) and used for the sequence identity analysis with Basic Local Alignment Search Tool (BLAST). The DNA sequences were then aligned using the CLUSTALW. Molecular phylogenetic analyses were conducted by using the maximum likelihood method with the Kimura 2-parameter substitution model via MEGA7 software (Tamura et al., 2011). One thousand bootstrap replicates were applied (Felsenstein, 1985).

Results

Inocybe mytiliodora Stangl & Vauras (Figure 1, 2).

Macroscopic and Microscopic Features

Pileus 10-15 mm across, hemispherical when young, later campanulate or convex to almost plane with a distinct umbo. **Surface** fibrillose to scaly, tawny, yellowish, red to buff. **Margin** strongly incurved. **Cortina** whitish. **Lamellae** greyish when young, then sand-colored to light brown. **Stipe** 25-35 × 5 mm, whitish at the apex, concolorous with pileus. **Flesh** whitish. **Taste** unpleasant. **Odor** distinctly mussel-like (*Mytilus edulis*). **Basidia** 25-30 × 9-10 µm, clavate with 4 sterigmata. **Spores** 6-7 × 9-10 µm, smooth, amygdaliform, thick-walled, yellowish-brown. **Cheilocystidia** 14-21 × 40-55 µm, metuloid, clavate, pyriform or utriform. **Pleurocystidia** were similar to cheilocystidia. **Caulocystidia** not observed. **Pileipellis** 8-12 µm thick, composed of periclinal hyphae.

Ecology: Summer to autumn, under deciduous trees (Knudsen and Vesterholt, 2008).

Specimen examined: TURKEY, Ankara: Ankara University Tandoğan Campus, under pedunculate oak (*Quercus robur* L.), 867 m, 39° 56' N, 32° 50' E, 02.09.2018, ANK Akata & Altuntaş 167. In the current study, the evolutionary history was revealed by using the Maximum Likelihood algorithm with the K2-substitution model for the phylogenetic relationship. The tree with the highest log likelihood (-2171.46) was shown in Figure 3.

Through phylogenetic analysis, we revealed two distinct clades of fungal species as well as an outgroup. The major clade (Clade 1) included all *Inocybe* species together with 'Ank Akata & Altuntaş 167' which was collected from Ankara University Tandoğan Campus (Ankara), while the other clade only included *I. tenebrosa.* On the other hand, *Simocybe sp.* was branched far from other fungi species and generated an outgroup as expected with a bootstrap value of 100% (Figure 3). Just as a side note, more firm conclusions will be possible in future studies when more *Inocybe* species may be included in phylogenetic analyses.
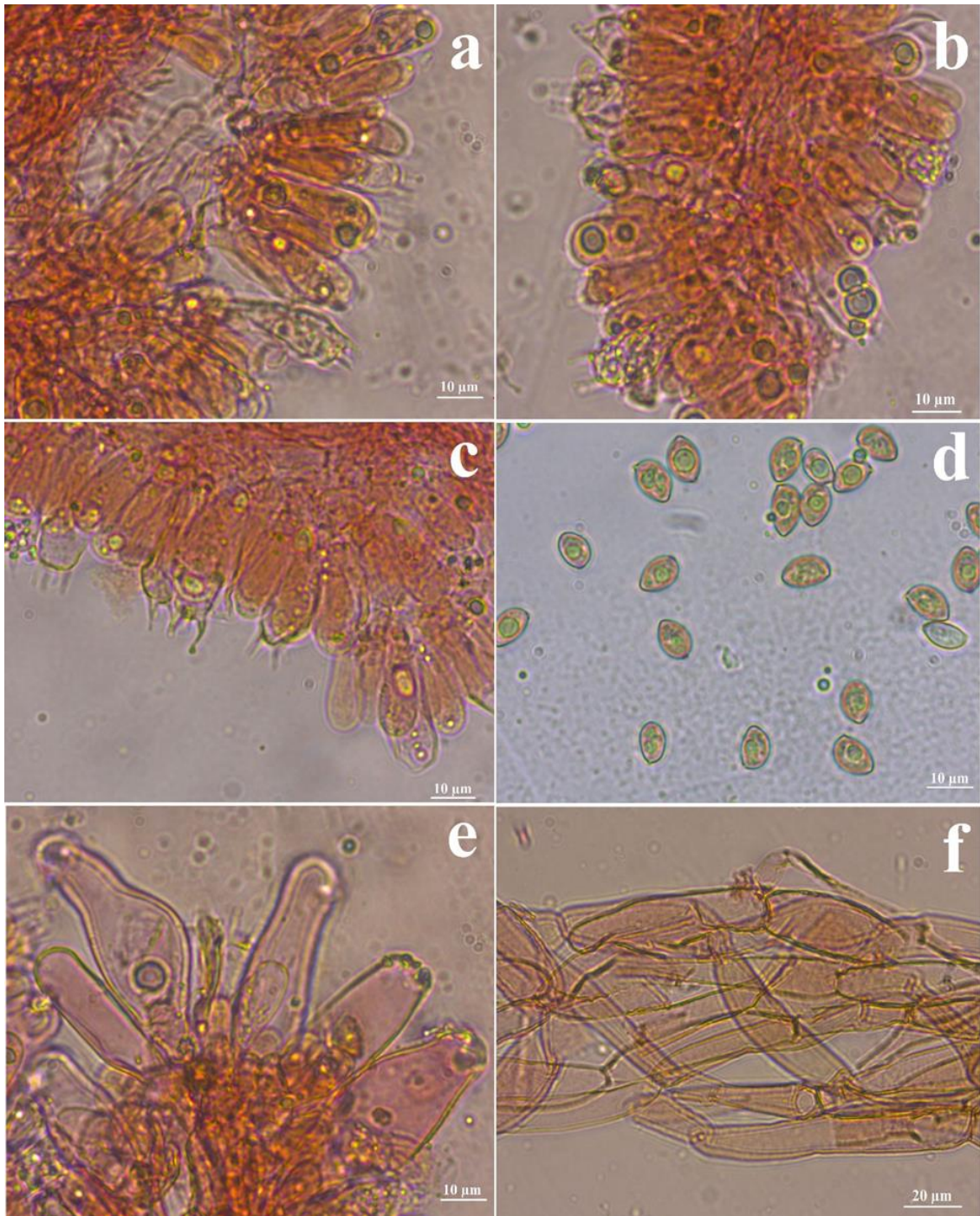

Figure 1. Basidiomata of *Inocybe mytiliodora*

Figure 2. *Inocybe mytiliodora*: a-c. basidia. d. spores. e. cheilocystidia. f. pileipellis.
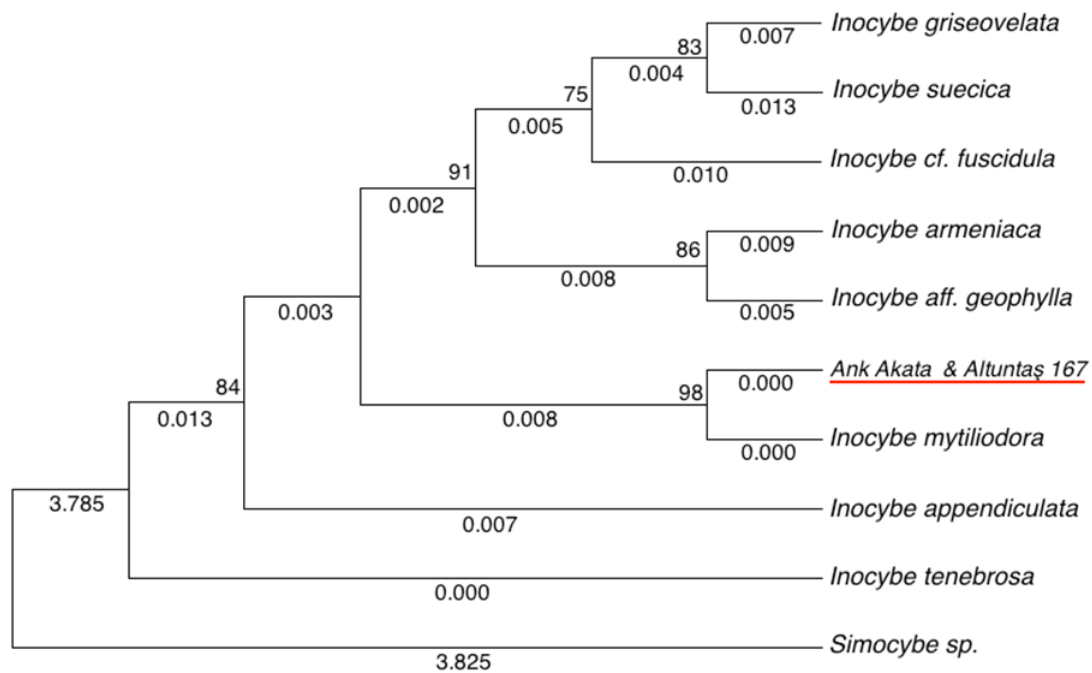
Figure 3. The maximum likelihood topology tree showing the genetic relationships of 10 fungi species with each other based on nrLSU region. Bootstrap values from 1000 bootstrap replicates were shown above the branches. Branch lengths were indicated below the branches.

Moreover, the phylogenetic tree based on nrLSU sequences provided evidence for 100% similarity of newly identified 'Ank Akata & Altuntaş 167' to *Inocybe mytiliodora* with a bootstrap value of 98% (Figure 3).

Taking both the molecular and morphological data into account, we can conclude that 'Ank Akata & Altuntaş 167' is a specimen of *I. mytiliodora* and this study can be considered to be the first study that reveals the presence of *I. mytiliodora* in Turkey.

Discussion

The most important characteristics of *I. mytiliodora* are clavate basidia, smooth and thick-walled spores; metuloid, broad and thick-walled hymenial cystidia, and mussel-like odor. *I. bongardii* (Weinm.) Quél., *I. fulviceps* Murrill, *I. mucidiolens (*Grund & D.E.Stuntz) Matheny, *I. pallidicremea* Grund & D.E.Stuntz, *I. personata* Kühner, *I. geraniodora* J.Favre, and *I. pelargonium* Kühner possess fishy or pelargonium-like odor but they can easily be separated *from I. mytiliodora* by their distinct morphology. *I. pedemontana* may also be confused with *I. mytiliodora* because of their similar

morphology, ecology, and odor but the former species is easily distinguished from the latter by the metuloid caulocystidia at the upper part of the stipe (Knudsen and Vesterholt, 2008; Stangl and Vauras, 1988).

nrLSU (nuclear ribosomal large subunit) is a widely used DNA barcoding marker, which is useful for the identification of fungal samples at the species level (Xiao et al., 2018). As the data of morphological traits is not sufficient alone for precise identification of fungi species, the use of sequence data from conserved DNA regions such as nrITS and nrLSU is considered to be an important tool for researchers with expertise in taxonomy and systematics of fungi since the last three decades (Vellinga, 2004; Chakraborty, Vizzini & Das, 2018; Xiao et al., 2018).

Moreover, the ribosomal RNA region is the most common and abundant DNA barcoding marker, which provides an important source for the researchers to make comparisons of data obtained from their studies with the one found in the GenBank database. For this reason, we used nrLSU region for the molecular identification of this agaric. The new record found 100%

genetically similar to *Inocybe mytiliodora* (GenBank ID: JN974947) and this result is also supported by morphological findings (Figures 1, 2 and 3).

As a result, *I. mytiliodora* is the first record for Turkey and supports the richness of fungal diversity in Ankara University Tandoğan Campus.

Acknowledgments

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