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Research Article

Callistosporium SINGER, A NEW GENUS RECORD FOR TURKISH MYCOBIOTA

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Abstract: This study aims to describe and introduce a new record for the Turkish mycobiota. Based on the similar macro- and micromorphology, and high nuclear ribosomal large subunit sequence similarity, the mushroom was identified as *Callistosporium olivascens* (Boud.) Bon. According to the literature research, we found out that this finding is the first record of the genus Callistosporium in Turkey.

Özet: Bu çalışmanın amacı Türkiye mikobiyotası için yeni bir kaydı tanıtmaktır. Benzer makro- ve mikromorfoloji ve yüksek çekirdek ribozomal büyük alt ünite dizi benzerliğine bakılarak bu mantar *Callistosporium olivascens* (Boud.) Bon. olarak teşhis edildi. Literatür araştırmalarına göre bu bulgu cinsin Türkiye'deki ilk kaydıdır.

Introduction

Callistosporium Singer is a small genus of the family *Tricholomataceae* within the order *Agaricales* (*Basidiomycota*). According to Index fungorum, sixteen confirmed species (*C. amazonicum* Singer, *C. chrysophorum* Singer, *C. elegans* Desjardin & B.A. Perry, *C. olivascens* (Boud.) Bon, *C. luteo-olivaceum* (Berk. & M.A. Curtis) Singer, *C. foetens* E. Ludw., *C. galerinoides* Singer, *C. heimii* (Singer) Singer, *C. krambrukum* Grgur., *C. marginatum* (Peck) H.E. Bigelow, *C. palmarum* (Murrill) Singer, *C. pinicola* Arnolds, *C. purpureomarginatum* Fatto & Bessette, *C. terrigenum* Singer, *C. vinosobrunneum* Desjardin & Hemmes, and *C. xerampelinum* Pegler) currently exist in the genus. Its members are characterized by collybioid or mycenoid basidiomata, convex to plane, umbilicate or umbonate, thin and firm, hygrophanous, yellow, olivaceous, brown, sometimes dark vinaceous brown pileus, frequently farinaceous odor; thick and subdistant, adnexed to adnate, dark vinaceous brown to yellow and waxy lamellae, dark vinaceous brown, yellowish to greenish, central and tough stipe, white spore print, hyaline, four- spored, sometimes pigmented basidia, subglobose to ellipsoid, hyaline or intracellular pigmented basidiospores, generally lacking of cystidia and clamp connections (Singer 1944, Kühner & Romagnesi 1954, Lennox 1979, Bon 1984, Moser 1986, Contu 1993, Bas *et al*. 1996, Jančovičová *et al*. 2016).

According to literature on Turkish mycobiota (Sesli & Denchev 2008, Uzun *et al*. 2014, Akata & Doğan 2015, Sesli *et al*. 2016, Acar *et al*. 2017, Öztürk *et al*. 2017, Akata *et al*. 2018, 2019, Altuntaş *et al*. 2019, Acar *et al*. 2020), approximately 2500 macrofungi species have thus far been registered from Turkey but there exists no report related to the genus *Callistosporium* Singer in the country. This study aims to introduce this genus in Turkey and to make a new contribution to Turkish mycobiota.

Materials and Methods

Basidiomata of the study were collected from Turkey, Ankara University Beşevler 10.Yıl campus (date: 20.09.2018). Color, odor, surface structure and mycorrhizal relationships of fruiting bodies were noted in the field. Freehand sections were obtained from pileus, stipe, and lamellae to examine the microscopic structures. Sections were mounted in both distilled water and concentrated ammonia. They were then stained with Congo red and examined using the Euromex Oxion Trinocular microscope. 100X magnification rates were used for microscopic structures and at least 20 measurements were performed. Identification was made using morphological and molecular methods (Singer 1944, 1946, Kühner & Romagnesi 1954, Lennox 1979, Bon 1984, 1991, Moser 1986, Contu 1993, *Bas et al.* 1996, Jančovičová *et al.* 2016, Pancorbo *et al.*2016,

Conca *et al.* 2017). The identified samples are deposited in Ankara University herbarium (ANK).

Molecular characterization

DNA Isolation

The genomic DNA of the specimens ANK Akata & Altuntas was isolated from the fruit bodies according to the CTAB method (Doyle & Doyle 1987). NanoDrop One© Microvolume UV-Vis Spectrophotometer (Thermofisher) was used to measure DNA concentration and purity.

PCR Amplification and Sequencing

The nuclear ribosomal large subunit (nrLSU) region of the rDNA was PCR amplified using the universal LR0R and LR5 oligonucleotide primers (Stielow *et al*. 2015). PCR was conducted in a reaction volume of 25µmL. The final concentrations of the PCR ingredients were adjusted as follows: 1 × Taq DNA polymerase buffer, 1 unit of Taq DNA polymerase (Fermentas), 0.4 mM dNTPs, 2.5 mM MgCl2, and 10 pmol of both LR0R and LR5 primers. PCR was carried out in a Thermal Cycler (Applied Biosystems MiniAmp Plus) with the following thermal cycling conditions: initial denaturation step of 95°C for 5 min, followed by 35 cycles of 95°C for 30s, 55°C for 30 s, and 72°C for 90 s, and a final extension step of 7 min at 72°C. The PCR amplicon was electrophoretically separated in 1% agarose gel containing the intercalating dye ethidium bromide, and the amplicon size was determined using a DNA marker (GeneRuler 100 bp Plus DNA Ladder, Thermofisher). The amplicon was sequenced bidirectionally using the LR0R and LR5 primers and the standard Sanger dideoxy chain termination method at the laboratory of Macrogen Europe (Amsterdam, The Netherlands).

Sequence Analysis

The nrLSU gene sequences of some relevant fungal species were obtained from GenBank and used for the phylogenetic analysis of the specimen Ank Akata & Altuntas 172. While the nrLSU sequences of the genera *Callistosporium* Singer, *Singerocybe* Harmaja, *Tricholoma* (Fr.) Staude and *Lepista* (Fr.) W.G. Sm. as some of the well-known genera of *Tricholomataceae* R. Heim ex Pouzar were selected as in-group sequences, the nrLSU sequences of *Agaricus campestris* L. and *Marasmius oreades* (Bolton) Fr. were selected as the out-group sequences. 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 analysis was conducted using the neighbor-joining method based on the K2 + G substitution model. MEGAX software was used for constructing the phylogenetic tree by applying one thousand bootstrap replicates (Kumar *et al*. 2018, Felsenstein 1985).

Results

Family *Tricholomataceae* R. Heim ex Pouzar

Genus *Callistosporium* Singer

Callistosporium olivascens (Boud.) Bon, 1976. (Figs 1, 2).

Syn.: *Collybia aerina* Quél. 1884, Assoc. Franç. Avancem. Sci., Congr. Rouen 12: 498 (1884).

= *Tricholoma olivascens* Boud., Bull. Soc. Mycol. Fr. 33(1): 7 (1917).

= *C. olivascens* var. *aerinum* (Quél.) Bon, Docums Mycol. 6(23): 286 (1976).

Macroscopic and microscopic features

Basidiomata clustered to solitary. **Pileus** 20-50 mm, hemispherical when young, later campanulate, convex to almost plane or funnel-shaped depending on weather conditions, with a wide umbo. Margin straight to wavy, entire, translucently striate; surface smooth, velutinous to apparently glabrous; mixture of brown, yellow and green pigments and hygrophanous. **Lamellae** sparse, L = 25-35, l = 1-3, emarginate, pale yellowish to beige when young, then rusty yellow or olive-yellow**. Stipe** 20-50 × 2-4 mm, generally central, cylindric, fused into a cluster, mostly curved, longitudinally compressed, fistulose, brown, yellowish-brown and olive-brown, sometimes minutely floccose or finely longitudinally fibrillose. **Context** 1.5-2 mm thick, olive-brown or yellowish. **Taste** mild, smell like beeswax. **Basidiospores** 7.5-9.5 × 5-6.5 µm, ellipsoid with small but distinct hilar appendage, hyaline, smooth, thin-walled with various content. **Basidia** 35-40 × 6-7 µm, clavate to cylindrical, 4-spored, clavate, thin-walled, hyaline with globose droplets. **Hymenial cells** are clavate, narrowly utriform and cylindrical with obtuse apex**. Cheilocystidia** and **pleurocystidia** not seen. **Pileipellis** a cutis, about 30-70 mm deep, made up of cylindrical, smooth or incrusted, thin- to thick-walled, 3-8 mm wide hyphae; Terminal cells cylindrical, narrowly cylindrical, clavate, narrowly clavate or narrowly lageniform. **Stipitipellis** a cutis of cylindrical, smooth or slightly incrusted, thin- to slightly thick-walled, up to 9 mm wide hyphae. **Clamp-connections** absent in all tissues.

Specimen examined: TURKEY-Ankara: Ankara University Beşevler 10.Yıl campus, under deodar cedar (*Cedrus deodara* (Roxb. ex D.Don) G.Don), 867 m, 39°56'04" N, 32°50'00" E, 20.09.2018, ANK Akata & Altuntas 172.

Molecular Phylogeny of the Specimen

As a result of the phylogenetic analysis, four distinct clades were revealed along with an out-group. The clade 1 contained *Callistosporium* species and the specimen Ank Akata & Altuntas 172. The Clades 2, 3 and 4 included species from the genera *Singerocybe*,

Fig. 1. a-c. Basidiomata of *Callistosporium olivascens* (photographed by Ilgaz Akata).

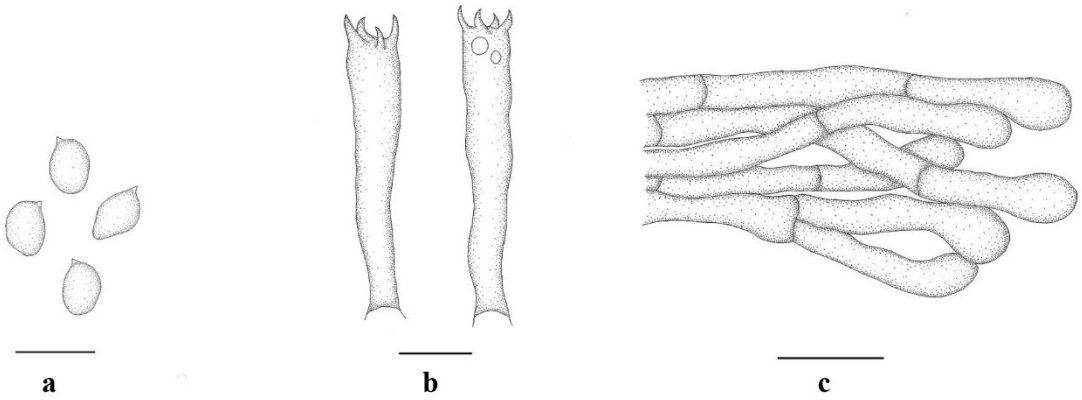

Fig. 2. *Callistosporium olivascens* (illustrated by Deniz Altuntaş) a. basidiospores, b. basidia, c. pileipellis (bar: 10 µm).

Tricholoma and *Lepista*, respectively. On the other hand, *A. campestris* and *M. oreades* were branched far from the rest of the fungi species and formed an out-group as anticipated. The BLAST analysis carried out with the nrLSU sequences of Ank Akata & Altuntas 172 provided evidence for the 99.89% similarity of this new record with the two separately collected *C. olivascens* specimens. The phylogenetic analyses conducted based on the nrLSU sequences of this specimen further supported the close identity relationship of the specimen with *C. olivascens* with a percent bootstrap value of 98 (Fig. 3).

Discussion

Although the genus *Callistosporium* includes sixteen confirmed species, the most known European members are *C. olivascens* (Boud.) Bon, *C. luteo-olivaceum* (Berk. & M. A. Curtis) Singer and *C. pinicola* Arnolds (Jančovičová *et al.* 2016). These species may be confused in the field due to their collybioid habit but *C. olivascens* can be distinguished from the latter species by its different morphology and ecology. *Callistosporium olivascens* was described in this study with 20-50 mm, hemispherical, campanulate, convex to almost plane or funnel-shaped pileus; emarginate, pale yellowish to beige, rusty yellow or olive-yellow lamellae, 7.5-9.5 × 5-6 µm and ellipsoid basidiospores. *Callistosporium luteo-olivaceum* and *C. pinicola* have narrower pileus and spores. While *C.* luteo-*olivaceum* has up to 35 mm broad pileus and (4.2-)4.7-5.6(-6) × (3-)3.3-4(-4.2) µm, basidiospores, *C. pinicola* up to 32 mm broad pileus and (2.5-)3-4(-4.5) × 2-3(-3.5) µm spores (Antonín *et al.* 2009, Jančovičová *et al.* 2016).

Since the morphological data is not always adequate for the precise identification of fungal species, the sequence data from the conserved DNA regions such as ITS, nrSSU and nrLSU has been employed as a convenient tool in taxonomic studies in the last three decades (Raja *et al.* 2017). Furthermore, nrLSU is one of the most common DNA barcoding markers and thus confers important information for molecular phylogenetic studies. Therefore, we used nrLSU region for the molecular identification of the specimens Ank Akata & Altuntas 172. The phylogenetic analysis conducted based on the nrLSU regions revealed almost 100% genetic similarity between the *C. olivascens* (GenBank ID: MK277665) and the new record (GenBank ID: MN486509 for Ank Akata & Altuntas 172) (Fig. 3).

The molecular phylogeny of *C. olivascens* demonstrated herein, points out its significant distinction from the other species of the genus *Callistosporium* as *C. olivascens* clusters in a separate branch within the clade 1 (Fig. 3). Based on this finding, it is plausible to state that the taxonomic revision of this species is likely to be addressed in the future.

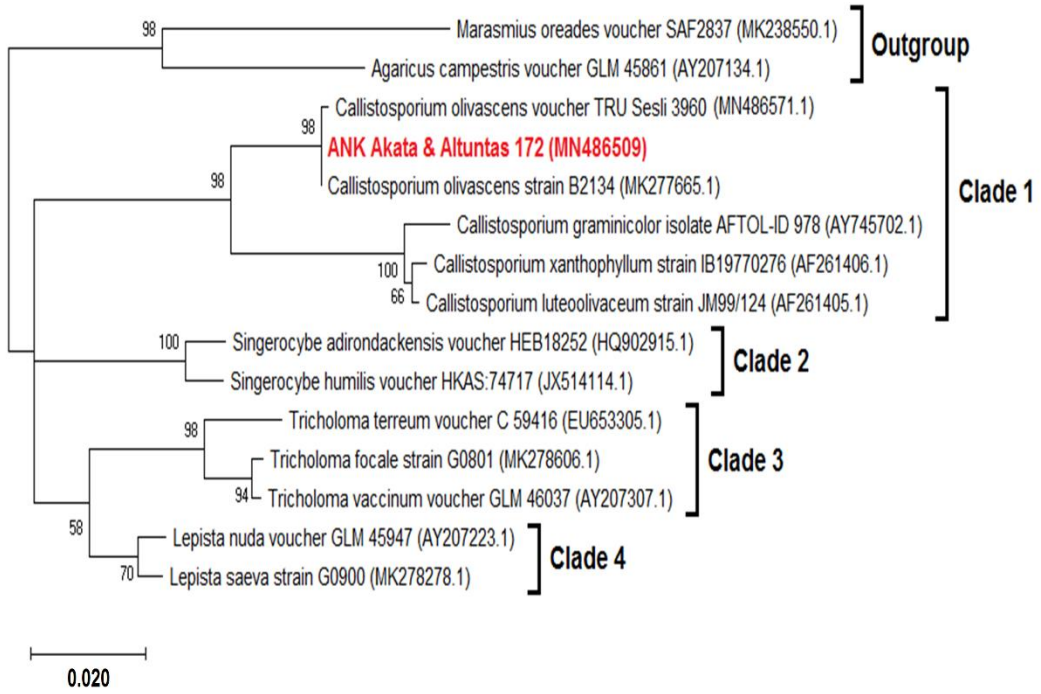

Fig. 3. The neighbor-joining tree demonstrating the phylogenetic relationships of 15 fungi inferred from the nrLSU region. Percentage bootstrap values obtained from 1000 replicates were given next to the branches. All the sequences used in the phylogenetic analysis were obtained from GenBank except for Ank Akata & Altunta 172. *Agaricus campestris* and *Marasmius oreades* were used as the outgroup samples. Accession numbers are given in parentheses. The scale bar at the lower left represents a genetic distance of 0.02

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