



Callistosporium luteo-olivaceum (*Callistosporiaceae: Basidiomycota*), an agaric new to Türkiye

Oğuzhan KAYGUSUZ^{1*}, Meryem Şenay ŞENGÜL DEMİRRAK²

¹Isparta University of Applied Sciences, Atabey Vocational School, Department of Plant and Animal Production, Isparta, Türkiye

²Tokat Gaziosmanpaşa University, Faculty of Arts & Sciences, Department of Molecular Biology and Genetics, Tokat, Türkiye

*okaygusuz03@gmail.com, ²msengul@vt.edu

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Callistosporium luteo-olivaceum (*Callistosporiaceae: Basidiomycota*), Türkiye için yeni bir agarik

Abstract: *Callistosporium luteo-olivaceum* collected from Mediterranean region of Türkiye is identified based on morphological characters and combined dataset of nuclear ribosomal ITS and LSU sequences. *Callistosporium luteo-olivaceum* is new to the country's mycobiota and it is the first report of this species from the relict endemic *Liquidambar orientalis* forest. Description of the species based on macro and micromorphological characters, colour photograph from its natural habitat and line-drawings of microscopic features are presented.

Key words: *Basidiomycota*, *Callistosporiaceae*, molecular phylogeny, new record, Türkiye

Özet: Türkiye'nin Akdeniz bölgesinden toplanan *Callistosporium luteo-olivaceum*, morfolojik karakterlere ve nükleer ribozomal ITS ve LSU dizilerinin birleşik veri setine dayalı olarak tanımlanmaktadır. *Callistosporium luteo-olivaceum* ülkenin mikobiyotası için yenidir ve relict endemik *Liquidambar orientalis* ormanından bu türün ilk raporudur. Türün makro ve mikromorfolojik karakterlere dayalı betimlemesi, doğal ortamından renkli fotoğrafı ve mikroskopik özelliklerin çizimleri sunulmaktadır.

Anahtar Kelimeler: *Basidiomycota*, *Callistosporiaceae*, moleküler filogeni, yeni kayıt, Türkiye

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1. Introduction

In previous classification, the genus *Callistosporium* Singer belonged to the family *Tricholomataceae* R. Heim ex Pouzar within *Basidiomycota* R.T. Moore and its members have been regarded in the tricholomatoid clade (Moncalvo et al., 2002). When a relationship with the entolomatoid clade was revealed, the genus was transferred to the *Entolomataceae* Kotl. & Pouzar based on rRNA data (Matheny et al., 2006). Later, a phylogenetic study by Sánchez-García et al. (2016) showed that the genus *Callistosporium* with other genera such as *Catathelasma* Lovejoy, *Guyanagarika* Sánchez-García, T.W. Henkel & Aime, *Macrocybe* Pegler & Lodge, *Pleurocollybia* Singer, and *Pseudolaccaria* (Fr.) Vizzini, Contu & Z.W. Ge have been included within the family *Catathelasmataceae* Wasser. Recently, Raj et al. (2019) added the genus *Anupama* K.N.A. Raj, K.P.D. Latha & Manim from India to this family. Moreover, Vizzini et al. (2020) reorganized the genera *Anupama*, *Callistosporium*, *Guyanagarika*, *Macrocybe*, *Pseudolaccaria* and *Xerophorus* (Bon) Vizzini, Consiglio & M. Marchetti within the family *Callistosporiaceae* Vizzini, Consiglio, M. Marchetti & P. Alvarado.

According to Index Fungorum, the genus *Callistosporium* is represented by about twenty five species (Kirk, 2019) with worldwide distribution from arctic to tropical habitats from North and South America, Europe, and South Asia (Singer, 1970; Hongo, 1981; Redhead, 1982; Horak, 1987; Manimohan and Leelavathy, 1989; Saba and Khalid, 2014;

Sánchez-García et al., 2016; Raj et al., 2019; Vizzini et al., 2020). In Türkiye, only one collection named *Callistosporium olivascens* (Boud.) Bon. was reported as belonging to the genus *Callistosporium* (Akata et al., 2020); however, this species was combined into genus *Xerophorus* (Vizzini et al., 2020). Recently, the first member, *Xerophorus donadinii* (Bon) Vizzini, Consiglio & M. Marchetti, has been presented at genus level from Türkiye (Sesli, 2021).

The genus is characterized by the basidiomata with collybioid habit often fasciculate; pileus with a mixture of yellow, brown and olivaceous colours; the subdecurrent, adnexed or emarginated lamellae; ellipsoid, smooth, inamyloid basidiospores with scarcely or weakly cyanophilic, white deposit; presence or absence of cheilocystidia, pleurocystidia and clamp-connections; a cutis of pileipellis that contains hyphae with encrusting and intracellular pigments; and lilac, violet to olive yellow staining of flesh in KOH reaction (Singer, 1986; Noordeloos, 1995; Arnolds, 2006; Vesterholt and Holec, 2008; Halama and Rutkowski, 2014; Jančovičová et al., 2016; Sánchez-García et al., 2016). *Callistosporium* live as saprotrophic on humus, litter and also other kinds of wood debris, and on the base of palm trees and *Sphagnum*, mostly in forests (Singer, 1986; Noordeloos, 1995; Vesterholt and Holec, 2008; Jančovičová et al., 2016; Sánchez-García et al., 2016).

Callistosporium luteo-olivaceum (Berk. & M.A. Curtis) Singer has been recorded from North America, Canada,

tropical south America, Europe, China, Japan, England, India, Pakistan (Bigelow and Barr, 1969; Singer, 1970; Hongo, 1981; Redhead, 1982; Horak, 1987; Manimohan and Leelavathy, 1989; Roberts, 2009; Saba and Khalid, 2014). It is a decomposer found primarily on dead conifer wood growing alone or gregariously (Kuo, 2016).

In this study, *Callistosporium luteo-olivaceum* is identified both morphologically and molecularly, and presented as the first record from Türkiye. Phylogenetic position of this species is inferred from a combined ITS and LSU rDNA dataset. The results provide taxonomically important features of the Turkish *C. luteo-olivaceum* which enables distinction of its closely related species in the genus *Callistosporium*.

2. Materials and Method

2.1. Morphological analysis

Callistosporium samples were picked up from Muğla province during 2018 season. Pictures are taken in the field. Micro-morphological observations were done using dried samples and using Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany) at magnifications up to 400× and 1000×. Samples were stained with Congo red. The following measurements were taken, which are: L^m and W^m indicating the average length and width of basidiospores, respectively; Q indicating the ratios of length/width and Q^m presenting the average quotient of the measured basidiospores. Specimens were deposited at the fungarium of Isparta University of Applied Sciences, Isparta, Türkiye.

2.2. Molecular analysis

2.2.1. DNA isolation, Polymerase chain reaction (PCR) and Sequencing

The genomic DNA was extracted from dried specimens using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) according to the manufacturer's protocol. Polymerase chain reaction was performed as described by Kaygusuz et al. (2021) using the primer pairs ITS1F/ITS4 (White et al., 1990) and LR0R/LR5 (Vilgalys and Hester, 1990) to amplify nrITS and nLSU gene regions, respectively. Same primer sets were used for sequencing reactions (Source Bioscience, Berlin, Germany). Sequence chromatograms were edited using in BioEdit 7.0.5 (Hall, 1999).

2.2.2. Phylogenetic analyses

Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used for constructing phylogenetic trees. A concatenated dataset was used to perform ML analysis in RAxML v8.2.10 (Stamatakis, 2014) under the GTRGAMMA substitution model with 1.000 bootstrap replicates. The BI analysis was performed using MrBayes 3.2.2 (Ronquist et al., 2012) by Markov chain Monte Carlo (MCMC) method including six simultaneous Markov chains run for 1,000,000 generations where sampling was done for every 100th generation.

3. Results

3.1. Taxonomy

Callistosporiaceae Vizzini, Consiglio, M. Marchetti & P. Alvarado

Callistosporium luteo-olivaceum (Berk. & M.A. Curtis) Singer, Lloydia 89: 117 (1946).

Macroscopic and microscopic features: Habit collybioid. Pileus (Fig. 1a) 15–30 mm diam., convex to plano-convex with centre slightly depressed, margin more or less deflexed or straight, surface smooth and slightly hygrophanous, olivaceous yellow to olivaceous grey brown. Lamellae crowded, regular, ventricose, emarginated, pale yellow or olive yellow. Stipe 35–65 × 3–10 mm, cylindrical with tapering towards at base, sometimes curved, olive brown to yellowish brown or brown, covered with white floccose at the base. Odor and taste indistinct. Spore print white.

Basidiospores (Fig. 1b) (4.5–)5.5–6.5(–7.0) × (3.2–)3.5–4.0(–4.5) μm , $L^m \times W^m = 5.7 \times 3.9 \mu\text{m}$, $Q = 1.3\text{--}1.7$, $Q^m = 1.4$, ellipsoid, with small hilar appendage, guttulate, smooth, thin-walled, hyaline or brownish intracellular pigment, non-dextrinoid, non-amyloid. Basidia (Fig. 1c) (20.0–)24.5–28.5(–32.0) × 5.0–6.5(–7.0) μm , clavate to narrowly clavate, 4-spored or rarely 2-spored, hyaline or violaceous brown and thin-walled. Pleurocystidia absent. Cheilocystidia (Fig. 1d) (25.0–)30.0–45.0(–47.0) × 5.5–10.0 μm , cylindrical to fusiform, hyaline and thin-walled. Hymenial trama made up of clavate, narrowly clavate to cylindrical with obtuse to subcapitate apex, (10.0–)15.0–25.0(–29.0) × 3.5–6.0 μm , hyaline and thin-walled. Pileipellis (Fig. 1e) consisting of cylindrical or clavate, 3.0–10.0 μm wide hyphae, hyaline or some with brown to light brown pigments and thin-walled. Stipitipellis a cutis of cylindrical, 3.5–10.0 μm , hyaline and thin-walled hyphae. Caulocystidia (Fig. 1f) (10.0–)15.5–26.0(–32.0) × 3.5–6.0 μm , narrowly clavate to clavate to cylindrical with obtuse to subcapitate apex, hyaline and thin-walled. Clamp connections absent in all examined tissues.

Ecology: Saprotrophic, in groups on angiosperm wood, present at elev. 5 m, on *Liquidambar orientalis* Mill., growing naturally on rich, deep and moist soils.

Collections examined: TÜRKİYE, Muğla Province, Köyceğiz district, around Döğüşbelen village, on well decayed *Liquidambar orientalis* log, alt. 5 m, 18 January 2018, O. Kaygusuz, OKA-TR1529; GenBank: OK626226 nrITS, OK625531 for nrLSU; *ibid.*, on rotten wood of *L. orientalis*, alt. 5 m, 19 January 2018, O. Kaygusuz, OKA-TR1530; GenBank: OK626227 for nrITS, OK625532 for nrLSU; *ibid.*, on wood of *L. orientalis*, alt. 6 m, 19 January 2018, O. Kaygusuz, OKA-TR1531; GenBank: OK626228 for nrITS, OK625533 for nrLSU.

3.2. Molecular analysis

The combined nrITS+nLSU dataset included sequences from 52 specimens, including 6 newly generated sequences. The ML and BI tree topologies were nearly identical. Therefore, only the ML tree is shown, along with the Maximum-likelihood bootstrap (MLB) and Bayesian posterior probabilities (BPP) values (Figure 2). The phyllogram showed that *Callistosporium luteo-olivaceum* is composed of two considerably distinct clades with sequences from different geographic areas. Sequences of *C. luteo-olivaceum* from Türkiye were clustered in the same branch as sequences produced from Austria, Italy, Japan and Pakistan, with significant statistical support (MLB = 88%, BPP = 0.92, Fig. 2).

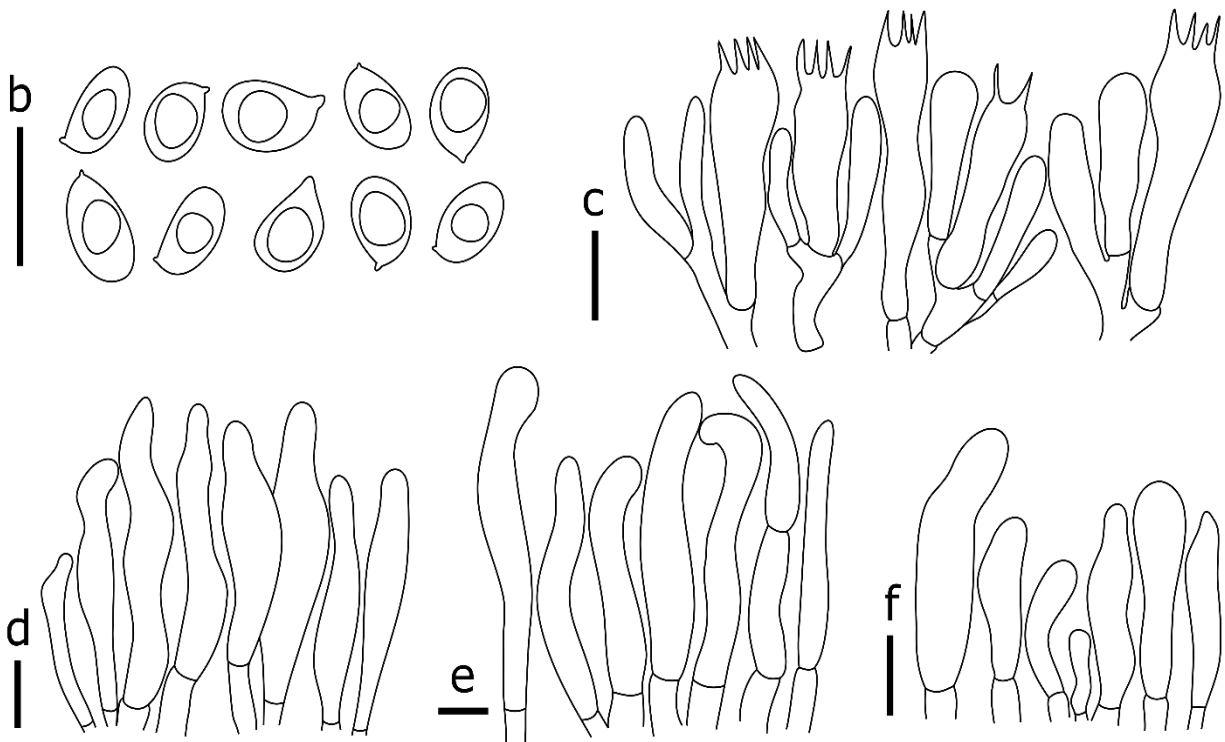


Figure 1. *Callistosporium luteo-olivaceum*: (a) basidiomes growing in its natural habitat, (b) basidiospores, (c) basidia and hymenial elements, (d) cheilocystidia, (e) pileipellis elements, and (f) caulocystidia. Scale bars: a = 10 mm, b-f = 10 μ m.

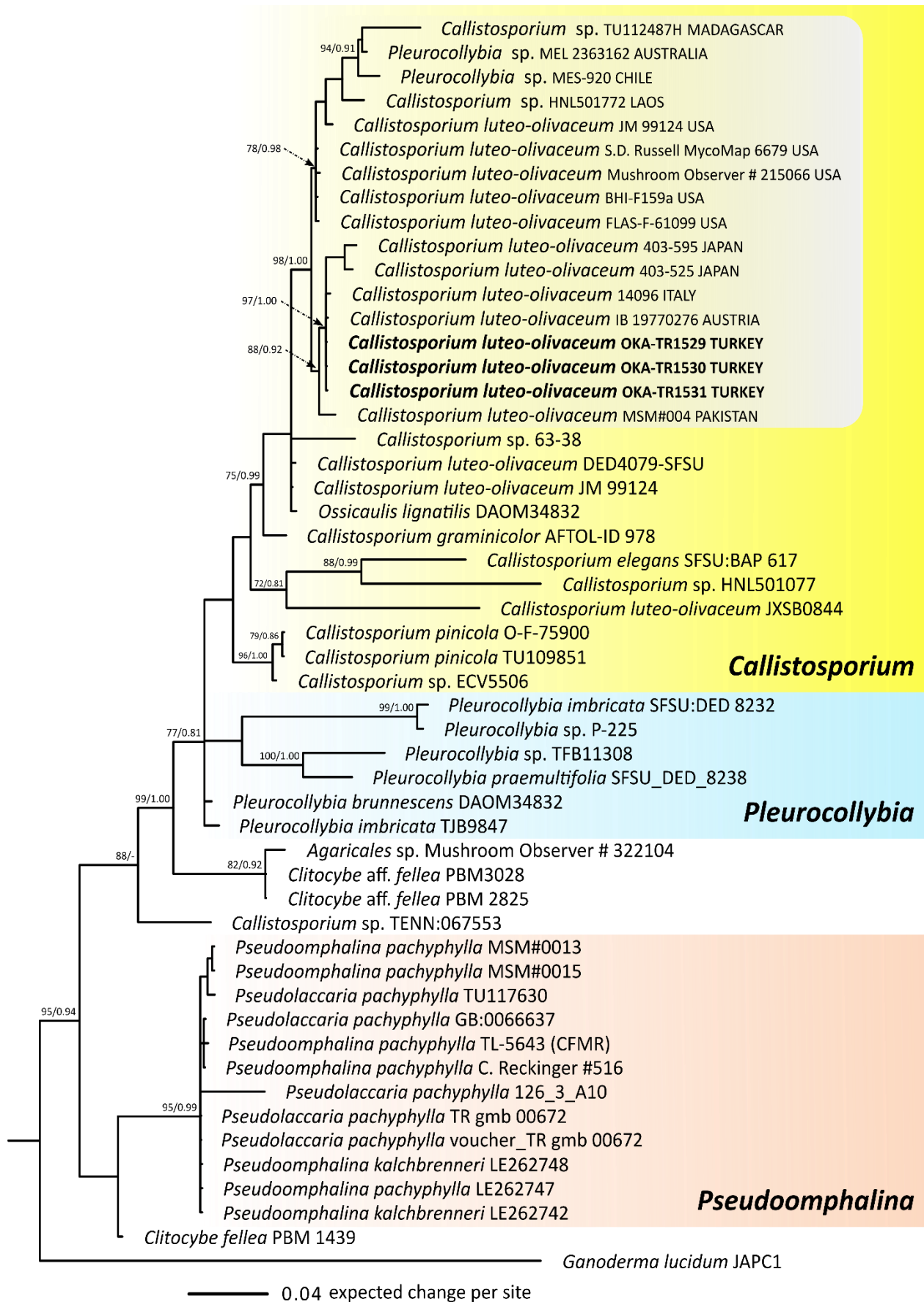


Figure 2. Maximum Likelihood (ML) tree of *Callistosporium* species based on nrITS and nrLSU sequences with the outgroup *Ganoderma lucidum*. Maximum-likelihood bootstrap (MLB) values $\geq 72\%$ and Bayesian posterior probabilities (BPP) ≥ 0.81 are shown on the branches. Collections presented in this study are in bold.

4. Discussions

Callistosporium luteo-olivaceum is regarded as a taxonomically complicated macrofungus due to its variable characters that may arise from its worldwide distribution (Saba and Khalid, 2014). Some researchers have given different names to the species, but critical examination by Redhead (1982) have resulted in elimination of many taxa in this genus, where *C. elaeodes* Bon, *C. favrei* Singer, *C. graminicolor* Lennox, *C. luteofuscum* Singer, *C. luteofuscum* var. *major* Singer, *C. majus* Singer, and *C. xanthophyllum* (Malençon & Bertault) Bon are considered as synonyms of *C. luteo-olivaceum*.

Turkish collection of *C. luteo-olivaceum* is characterized by pileus with yellow olive tinges, frequent pale yellowish and olive yellow lamellae and a white spore print. The features of the Turkish collections of *C. luteo-olivaceum* resembles the species described by Redhead (1982).

Our phylogenetic result confirmed that *C. luteo-olivaceum* is a member of the *Callistosporium* clade within the family *Callistosporiaceae*. Genetically, *Callistosporium luteo-olivaceum* is significantly related to *C. elegans* Desjardin & B.A. Perry, *C. graminicolor* and *C. pinicola* Arnolds. However, they have distinctive morphological characteristics that differentiate each other. *Callistosporium elegans* differs from *C. luteo-olivaceum* mainly by its violet to purple-wine pileus, smaller basidiospores ($4.4\text{-}5.3 \times 3.0\text{-}3.7 \mu\text{m}$) and cylindraceous, subcapitate to bilobed cheilocystidia (Vizzini et al., 2020).

Callistosporium graminicolor is separated from *C. luteo-olivaceum* by having a smaller basidiomata (12-18 mm) and larger basidiospores ($6.0\text{-}8.0 \times 4.0\text{-}5.5 \mu\text{m}$) (Lennox,

1979). It also differs from *C. luteo-olivaceum* by its dark reddish to dark reddish brown color formation with KOH on lamellae and stipe (Vizzini et al., 2020). *Callistosporium pinicola* has somewhat small-sized basidiomes (5-30 mm), usually vividly yellow-brown, orange-brown, rusty-brown to red-brown colored pileus, distinctively smaller basidiospores ($2.5\text{-}4.5 \times 2.0\text{-}3.5 \mu\text{m}$) and prefers decayed wood and conifers (Arnolds, 2006; Halama and Rutkowski, 2014).

Callistosporium elaeodes is morphologically close to *C. luteo-olivaceum*, but it can be distinguished by its smaller basidiomes (5-20 mm), bright olive-green pileus, thicker and green-yellow and olive-brown lamellae, and larger basidiospores ($5.9\text{-}7.6 \times 3.3\text{-}4.3 \mu\text{m}$) and it is mostly associated with broadleaved trees (Vizzini et al., 2020).

With this study, the Turkish *C. luteo-olivaceum* is described, supported with its molecular and morphological features. This species is in the red-list of many European countries and we propose it could be considered as the same for conservation of the Turkish mycobiota.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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