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The First Identification of a Rare Fungus *Calonarius fulvocitrinus* (Cortinariaceae, Agaricales) from *Quercus* forests of the Black Sea Region of Türkiye

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ABSTRACT
Calonarius fulvocitrinus is reported for the first time from Türkiye based on morphological description and molecular evidence. It is characterised by a yellow to greenish-yellow pileus with tiny scales in the center, yellowish lamellae, and is associated with Fagaceae. Based on phylogenetic analysis of the nuclear internal transcribed spacer (nrITS) sequences, the Turkish collection is placed in a well-supported clade along with its European representatives within the / *Calochroi* clade. Our results demonstrate the first distribution of this taxon outside the European continent and new to Türkiye.

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Türkiye'de Karadeniz Bölgesi Meşe Ormanlarından Nadir Bir Mantar olan *Calonarius fulvocitrinus*'un (Cortinariaceae, Agaricales) ilk defa Belirlenmesi

ÖZET
Calonarius fulvocitrinus, morfolojik tanımlama ve moleküler kanıtlara dayanarak Türkiye'den ilk kez rapor edilmiştir. Ortada küçük pullu, sarı ila yeşilimsi sarı pileus ile sarı lameller ile karakterize edilmekte ve Fagaceae familyası ile ilişkilidir. Nükleer ribosomal iç transkribe ara parçası (nrITS) dizilerinin filogenetik analizine dayanarak, Türkiye'deki koleksiyonun, / *Calochroi* kladındaki Avrupa temsilcileriyle birlikte iyi desteklenen bir kladda yer almaktadır. Sonuçlarımız, bu taksonun Avrupa kıtası dışındaki ilk dağılımını ve Türkiye için yeni olduğunu göstermektedir.

Mikoloji

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INTRODUCTION
Calonarius Niskanen & Liimat is a genus of Basidiomycetes in the family Cortinariaceae, represented by about 200 species worldwide (Liu et al., 2024; Index Fungorum, 2025). Until 2022, the family Cortinariaceae has been represented by one genus, *Cortinarius* (Pers.) Gray, including more than 5000 species worldwide. Nomenclature of the species in this genus has been problematic due to renaming existing species as new and the interpretation of its species based solely on morphological characters. With the recent genomic analyses by Liimatainen et al. (2022), revision of the family Cortinariaceae at the genus level is accomplished. Accordingly, the family Cortinariaceae is divided into ten new genera, which are *Cortinarius, Aureonarius, Austrocortinarius, Calonarius, Cystinarius, Hygronarius, Mystinarius, Phlegmacium, Thaxterogaster,* and *Volvanarius.*

The genus *Calonarius* is divided into three subgenera, *C.* subgen. *Calonarius*, *C.* subgen. *Calochroi,* and *C.* subgen. *Fulvi*. Most species are rare and have limited ecological preferences, endemic to the Northern Hemisphere (Frøslev et al., 2005; Garnica et al., 2009; Liimatainen et al., 2022). *Calonarius* species are ectomycorrhizal in association mainly with Fagaceae, Pinaceae, Betulaceae, Cistaceae, Malvaceae, Caesalpiniaceae, Dipterocarpaceae, Malvaceae, Myrtaceae, Nothofagaceae, Rhamnaceae, Rosaceae, and Salicaceae (Frøslev et al., 2007; Garnica et al., 2011; Liu et al., 2024). The genus contains species with medium to large-sized, pileocarpic, agaricoid (phlegmacioid) or sometimes sequestrate, often brightly coloured basidiomata with a usually distinctly marginated bulb at the base of the stipe (Frøslev et al., 2007; Liimatainen et al., 2022). The structure of the pileipellis is simple, with a more or less developed hypoderm, and basidiospores are amygdaliform to citriform, coarsely verrucose.

Calonarius subgen. *Calochroi* comprises members of the sections *Calochroi*, *Nymphicolores*, *Platypodes*, and *Sodagniti*. Most species are non-anthraquinonoid with a calochroid appearance, violaceous color on the lamella, ochraceous to yellow or brown pileus with bluish, purplish, greenish or olivaceous tints, a broad marginate bulb, a simplex pileipellis, amygdaloid to citriform basidiospores (Frøslev et al., 2007; Liimatainen et al., 2022). Species show ectomycorrhizal associations mainly with the species of Fagaceae and Betulaceae, rarely with Pinaceae, Cistaceae, and Malvaceae. *Calonarius* subgen. *Calochroi* species differ from *C.* subgen. *Fulvi* in such that the latter has yellow color on the lamella and/or stipe, and some have anthraquinonoid pigments. *Calonarius* subgen. *Calochroi* has larger spores and pileus, lamella rarely with a purplish tint, and show no red color formation with KOH (Liimatainen et al., 2022). Phylogeny also supports them as separate lineages (Frøslev et al., 2005, 2007; Liimatainen et al., 2022).

Cortinarius fulvocitrinus is currently transferred to *Calonarius*, with the new name *Calonarius fulvocitrinus* (Brandrud) Niskanen & Liimat. (Basionym: *Cortinarius fulvocitrinus* Brandrud), which belongs to *Calonarius* subgen. *Calochroi*. *Calonarius fulvocitrinus* is the only taxon within the /*Calochroid* subclade with anthraquinoid pigments, uncommon for the other species, a feature useful for the separation of the closely related species in sect. *Calochroi* (Frøslev et al., 2005). It is described only from a few European countries, such as Italy, France, Denmark, Spain, and Germany (Garnica et al., 2003; Peintner et al., 2004; Frøslev et al., 2005, 2007; Bellanger, 2015; Mahiques et al., 2018). Since the new classification of the genus *Cortinarius*, the studied species has not been reported from a non-European continent, including Türkiye (Sesli et al., 2020; Solak & Türkoğlu, 2022; Sesli, 2025). In this study, we provide a new distribution of this species and a new record of *Calonarius* outside Europe, supported by macro- and micro-morphological characters and phylogenetic analysis.

MATERIAL and METHOD

Collection and morphological analysis: During field trips in 2020, fresh basidiomata specimens were collected from Erbaa, Tokat Province. Macroscopic features, such as color and habitat, are noted in their natural environment. Specimens were dried out, spore prints were taken from the mature samples, and micromorphological analyses were conducted using dried specimens. The specimens were kept at the Fungarium of the Department of Biology, Tokat Gaziosmanpasa University, Turkey (GOPUF).

Molecular and phylogenetic analysis: The genomic DNA was isolated from dried specimens using GeneMATRIX Plant & Fungi DNA purification kit (EURx, Poland) following the manufacturer's protocol. For amplification of the internal transcribed spacer (ITS) region, primer pair ITS4-ITS5 was used (White et al., 1990). Polymerase Chain Reaction (PCR) was performed in a total of 30 µl volume, which included 15 µl 2X Dream Taq DNA master mix (Thermo Scientific), 3 µl of each primer pair, 8 µl gDNA, and 1 µl sterile double-distilled H2O. PCR protocol was performed as described by Şengül Demirak et al. (2020). The PCR product was visualized using 1 % agarose gel electrophoresis. The purified DNAs were sequenced in both directions by Sanger sequencing (Macrogen Europe).

Forward and reverse sequences were assembled and manually checked for sequencing errors. The newly generated ITS sequence was examined for similar sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) of the National Center of Biotechnology Information (NCBI, 2025) using BLAST (Basic Local Alignment Search Tool). Selected sequences were used for alignment (ClustalW), and a phylogenetic tree was constructed using MEGA7.0 (Kumar et al., 2016). Maximum Likelihood (ML) phylogenetic inference was based on heuristic searches obtained by Neighbor-Join and BioNJ algorithms and using the Tamura-Nei model (Tamura & Nei, 1993), while Maximum Parsimony (MP) analysis was obtained using the Tree-Bisection-Regrafting algorithm, both included 1000 repeats for bootstrapping. *Cortinarius violaceus* (AY669579) was used as the outgroup taxon following Garnica et al. (2009). The bootstrap support values (≥ 50%) were shown on the tree branches.

RESULTS

Taxonomy

Fungi
Basidiomycota R. T. Moore
Cortinariaceae R. Heim ex Pouzar
Calonarius fulvocitrinus (Brandrud) Niskanen & Liimat., Fungal Diversity 112: 131 (2022)
MB#554421 (Figures 1 & 2)

Figure 1. *Calonarius fulvocitrinus* (Collection HIS-48): a basidiomata, b- basidia and basidiol (Scale bars: a = 20 mm; b = 20 µm).

Şekil 1. Calonarius fulvocitrinus (Koleksiyon HIS-48): a- bazidiyokarp, b- bazidyumlar ve bazidiyol (Ölçek çubuğu: a = 20 mm; b = 20 µm).

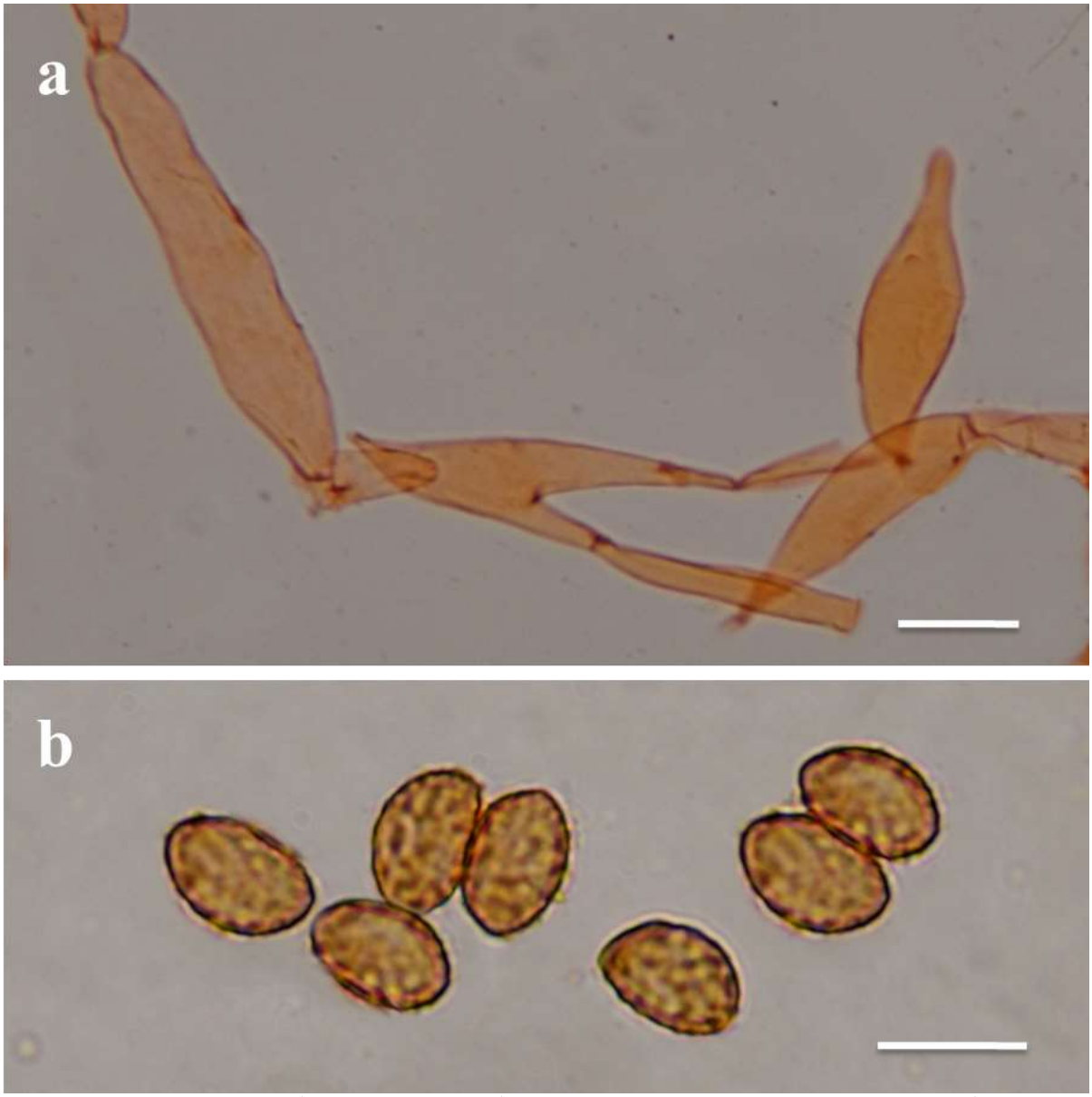

Figure 2. *Calonarius fulvocitrinus* (Collection HIS-48): a- pileipellis elements, b- basidiospores (Scale bars: a = 30 µm, b = 10 µm).

Şekil 2. Calonarius fulvocitrinus (Koleksiyon HIS-48): a- pileipellis elementleri, b- bazidiosporlar (Ölçek çubuğu: a = 30 µm, b = 10 µm).

Pileus 40–70 (90) mm; center yellow, yellowish green to olive, pale colour when young with a chrome-yellow to citrinous margin. Flesh intensely yellow to greenish yellow, paler in cap. Odor is trivial or nil. Lamellae are mostly crowded, yellowish. Stipe 60-70(90) × 0,8-11(13) mm, with a marginate bulb; yellow to citrinous, later yellow. Veil brilliantly chrome-yellow, later darkening to brown or purple-brown. Basidiospores 8.5–10 × 5.5–7.0 µm, obtusely amygdaloid to citriform, rather coarsely verrucose, yellow-brown. Basidiol 35-40(50) × 10-15(18) µm, clavate, with 4 sterigmata and basal clamp. Pileipellis filamentous, cylindrical, and tubular hyphae, septate with clamps. Cortina white to pale citrinous colour.

Habitat: This species is found in calcareous, deciduous forests, associated with *Quercus sp.* and *Fagus sylvatica* L., in groups of 3-5 specimens (Garnica et al., 2003; Frøslev et al., 2007; Mahiques et al., 2018).

Specimen examined: Türkiye. Tokat Province, Erbaa County, Benli village, 912 m., 40°35'33"N, 36°32'14"E, 12 November 2020, HIS-48; in *Quercus* sp. forest. GenBank PV197263.

Phylogenetic Results

The amplified ITS sequence was blasted in the non-redundant nucleotide database (NCBI), and the best matches were selected for phylogenetic analysis. Twenty-five sequences (including the outgroup species) were retrieved from the GenBank database and aligned for phylogeny. Six nrITS gene sequences of *C. fulvocitrinus* (including type material) from European collections were used, which showed 100% identity with that of the newly identified Turkish collection. The phylogenetic relationships inferred with ML and MP methods were in agreement, and only ML tree results are presented (Fig. 3). The ML tree supports the monophyly of the clade, which comprises taxa including *C. fulvocitrinus,* in which the studied specimen was grouped in with a high bootstrap support (98%). Our specimen also matched perfectly with the type material from France (DQ663309). Two members of the *Calonarius* section Nymphicolores Niskanen & Liimat., *C. molochinus* and *C. nymphicolor*, form a separate clade, sister to *C. fulvocitrinus*. In outer clades, distantly related taxa were also supported by high bootstrap supports.

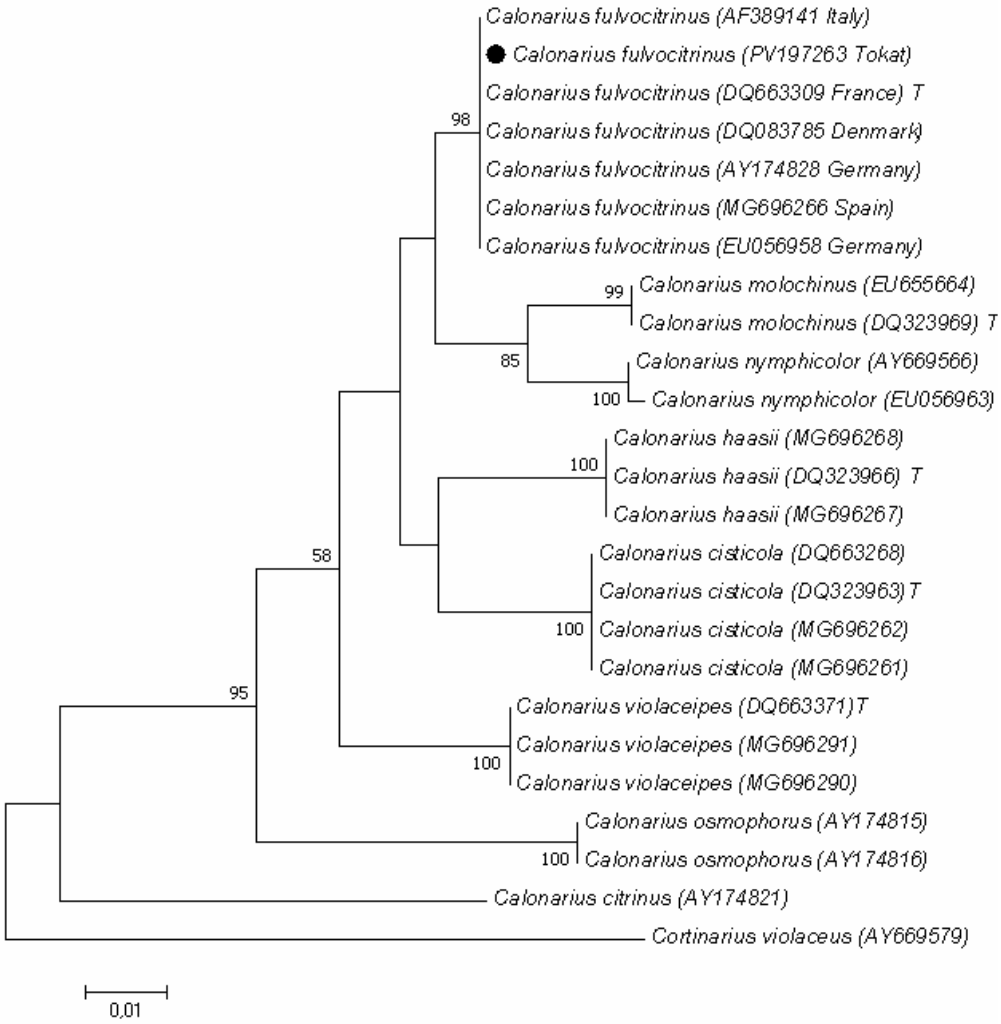

Figure 3. Maximum Likelihood (ML) analysis of nrITS sequences of the *Calonarius* species for phylogenetic inference. The Turkish *Calonarius fulvocitrinus* is indicated with a black circle. Bootstrap support values ≥ 50% from ML analysis are shown on the branches. *Cortinarius violaceus* (AY669579) is the outgroup species. Bar indicates 0.01 expected change per site per branch. T: type material.

Şekil 3. Filogenetik çıkarım için Calonarius türlerine ait nrITS sekanslarına dayalı Maximum Likelihood (ML) analiz. Türkiye'ye ait Calonarius fulvocitrinus siyah yuvarlak ile gösterilmiştir. ML analizlerinde, ≥ %50 bootstrap destek değerleri dalların üstünde gösterilmiştir. 0.01 çizgisi, her daldaki her noktaya ait tahmini değişimi ifade etmektedir. T: tip materyal.

DISCUSSION

Within the family Cortinariaceae, the genus *Cortinarius* is the largest genus, including thousands of species exhibiting morphological diversity, broad habitat preferences, and a wide host range. Previous studies were mainly conducted for the delimitation of the infrageneric groupings within *Cortinarius*. However, as proposed by

Liimatainen et al. (2022), they have not been able to resolve the higher-level classification of the groups, and thus, forced the attempts for a genus-wide revision with the help of genome-based phylogenetic inference. Accordingly, a revised classification of the genus *Cortinarius* has been performed by Liimatainen et al. (2022), and seven genera, ten subgenera, and four sections were newly introduced within the family Cortinariaceae. A disagreement with the new classification of the genus *Cortinarius* has been proposed in the recent publication by Gallone et al. (2024). They re-analyzed Liimatainen et al. (2022) dataset and concluded that the genus *Cortinarius* should not yet be split due to the irreproducibility of their phylogenetic analyses, which also had poor bootstrap support for most of the branches in the phylogeny. They further addressed that phylogenetic uncertainty would undoubtedly cause taxonomic uncertainty with important consequences. Despite this controversy, nomenclature now follows Liimatainen et al. (2022) and is presented accordingly in Index Fungarum (2025). Based on this new classification, some *Cortinarius* species were moved to the genus *Calonarius* and renamed accordingly. *Cortinarius fulvocitrinus* is currently named as *Calonarius fulvocitrinus,* and we follow this nomenclature.

The Turkish collection's morphological features were consistent with the European collections, such that it has a greenish-yellow pileus surface, a strongly marginate bulb, yellowish lamellae, yellow-green flesh, amygdaliform to slightly citriform basidiospore shape and size, and similarities in the habitat preferences (Garnica et al., 2005; Frøslev et al., 2007; Bellanger, 2015; Mahiques et al., 2018). Molecular evidence also supports the species delimitation in this section, and morphological separation can be correlated with the genetic differentiation. According to the phylogenetic analysis, the Turkish *Calonarius fulvocitrinus* formed a separate clade with the previously described European counterparts, supported with high bootstrap support values (Fig. 3). Phylogenetically, *C. fulvocitrinus* formed a separate clade (including the type material) and separated from the species of the *Calonarius* section Nymphicolores, including *C. molochinus* (Bidaud & Ramm) Niskanen & Liimat. and *C. nymphicolor* (Reumaux) Niskanen & Liimat. These species typically have purplish pileus and stipe and often lamellae, veil initially white or yellow, which differs from *C. fulvocitrinus*. Based on molecular data, *C. cisticola* (Frøslev & T.S. Jeppesen) Niskanen & Liimat. and *C. haasii* (M.M. Moser) Niskanen & Liimat. are also distantly related to *C. fulvocitrinus*. Morphologically, *C. cisticola* and *C. fulvocitrinus* both have yellowish pileus, but *C. fulvocitrinus* has greenish yellow colours on pileus and bulb context, slightly smaller spores, yellowish lamellae and stipe, and *C. cisticola* has yellow pileus with whitish to brownish veil patches, pale greyish violet to violaceous lamellae and a shorter, white stipe (Frøslev et al., 2006). *Calonarius haasii* also differs from *C. fulvocitrinus* with a smaller orange to greyish orange pileus, lilac or lilac violet lamellae, and larger spores (Ortega et al., 2008). Phylogenetically, *C. citrinus* (P.D. Orton) Niskanen & Liimat., belonging to section Fulvi, is also genetically isolated from *C. fulvocitrinus* and in congruence with the findings of Garnica et al. (2003). Overall, the phylogenetic separation reflects the species separation based on morphology, where morphologically different taxa are distantly related to *C. fulvocitrinus*, such as *C. osmophorus* (P.D. Orton) Niskanen & Liimat. and *C. citrinus*.

To date, only six *Cortinarius* species have been reported from Tokat Province with the support of molecular and morphological data, which include *C. rapaceoides* Bidaud, G. Riousset & Riousset (Şengül Demirak & Işık, 2020), *C. rufoolivaceus* (Pers.) Fr. (Şengül Demirak et al., 2020), *C. lilacinovelatus* Reumaux & Ramm (Şengül Demirak & Türkekul, 2021a), *C. bulliardi* (Pers.) Fr. (Şengül Demirak & Türkekul, 2021b), *C. eucaeruleus* Rob. Henry (Şengül Demirak et al., 2022) and *C. strenuipes* Rob. Henry (Şengül Demirak et al., 2024). All these species share similar habitat preferences as they are found in *Quercus sp.* forests. Only *C. lilacinovelatus* and *C. fulvocitrinus* (this study) belong to section *Calochroi,* but they differ morphologically; the former has ochre white to pale ochre brown pileus, light ochraceous to clay brown lamella, whitish to pale yellowish stipe, and slightly smaller spores. Both species share similar habitats, such that *C. lilacinovelatus* was found in association with *Quercus*, *Carpinus* L., and *Fagus*, while *C. fulvocitrinus* has only been reported from *Quercus sp.* and *Fagus* habitats and stated that it is more selective to its host plant (Bidaud et al., 2001; Frøslev et al., 2006, 2007; Mahiques et al., 2018; Şengül Demirak & Türkekul, 2021a). With the support of morphological and molecular data, we provide here, for the first time, *C. fulvocitrinus* from Tokat province as a new record for the Turkish mycobiota.

CONCLUSION

Here, we provide the first molecular and morphological identification of *Calonarius fulvocitrinus*, recently transferred to the genus *Calonarius*, from Türkiye. As proposed by Frøslev et al. (2006), fungal species identification and delimitation can be achieved by the inclusion of the molecular data from type material along with a large set of molecular data. This can be accomplished where more collections from different regions of the world are included in a thorough phylogenetic analysis to eliminate biased sampling issues. Morphology-based species discovery and delimitation have been problematic in *Calonarius* subgen. *Calochroi* and molecular evidence should be used additionally for a reliable taxonomic conclusion. It is important to act cautiously in the revision of taxonomic rankings and nomenclature. More extensive sampling, including various locations, is urgently needed to resolve the taxonomic and nomenclatural problems associated with Cortinariaceae.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

Authors have declared no conflict of interest.

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