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Morphology and phylogeny of *Cortinarius strenuipes* (*Basidiomycota, Agaricales*) reported for the first time from Türkiye

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Türkiye'den ilk kez kaydedilen *Cortinarius strenuipes* (*Basidiomycota, Agaricales*)'in morfolojisi ve filogenisi

Abstract: *Cortinarius strenuipes* Rob. Henry is reported for the first time from Türkiye based on morphological features and molecular analysis. It is found in mixed forest and distinguished by a gray or reddish-brown pileus with blackish spots, dark brownish ochre to chocolate-brown lamellae, brown or brownish-gray, cylindrical stipe slightly bulbous at base. Internal transcribed spacer region (ITS) and the large subunit (LSU) of nuclear ribosomal RNA region sequences of the specimen are determined and compared with similar taxa.

Key words: *Basidiomycota*, *Cortinariaceae*, new record, phylogenetic analysis, Türkiye

Özet: *Cortinarius strenuipes* Rob. Henry morfolojik özellikleri ve moleküler analizlere dayalı olarak Türkiye'den ilk kez rapor edilmiştir. Karışık ormanlarda bulunur ve siyahımsı benekli, kahverengimsi veya kırmızımsı-kahverengi şapka, koyu okra-kahverengi ile çikolata-kahverengi lamelleri ve önce beyazımsı, sonra kahverengi veya kahverengimsi-gri, tabanda hafif şişkin silindirik sap ile ayırt edilir. Örneğin, ribozomal RNA bölgesine ait transkribe edilen aralayıcı bölge (ITS) ve büyük altbirim (LSU) sekansları belirlenip benzer taksonlarla karşılaştırılmıştır.

Anahtar Kelimeler: *Basidiyomycota, Cortinariaceae,* yeni kayıt, filogenetik analiz, Türkiye

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1. Introduction

Cortinarius (Pers.) Gray is the largest genus of Agaricales with an estimated number of more than 5,000 scientific names according to the IndexFungorum (Kirk et al., 2008; Liimatainen et al., 2014) and about 150 species in Turkey (Sesli et al., 2020; Şengül Demirak et al., 2022; Sesli, 2023). Members of the genus distributed in temperate and subtropical forests and they form mycorrhizal associations with a wide range of tree and plant families including, Caesalpiniaceae, Cistaceae, Dipterocarpaceae, Fagaceae, Malvaceae, Myrtaceae, Nothofagaceae, Pinaceae, Rhamnaceae, Rosaceae and Salicaceae (Frøslev et al., 2006; Garnica et al., 2011; Liimatainen et al., 2014; Soop et al., 2019).

Classification of species within the genus *Cortinarius* is very problematic due to convergence of macroscopic and microscopic features which causes description of the same species under different names. These problems are partly solved by the use of molecular techniques where the nuclear ribosomal internal transcribed spacer (ITS) is widely used as a universal barcode marker for fungal barcoding (Schoch et al., 2012). More specifically, it is suggested that ITS is suitable for species delimitation in *Cortinarius* (Frøslev et al., 2005, 2007; Garnica et al., 2011; Liimatainen et al., 2014). Thus, molecular studies are necessary for an accurate identification of a *Cortinarius* species.

The present study identifies a *Cortinarius* species, *Cortinarius strenuipes* Rob. Henry, a new record for the Turkish mycota, based on molecular and morphological analyses.

2. Materials and Method

2.1. Morphological studies

Fresh specimens of *Cortinarius* were collected from Avlunlar village (Tokat) on November 2018. Color photographs were taken in the field and macromorphological and ecological features were noted. The samples were transported to the laboratory wrapped in aluminum foil, then dried using a fan heater and placed in zip lock bags for further studies. The dried samples are kept in the Fungarium of the Biology Department, Tokat Gaziosmanpasa University (GOPUF). The measurements of micromorphological structures were determined using dried samples and chemicals such as 5% KOH, Melzer reagent, 1% Congo Red under a Nikon Research Microscope (100x). Micromorphological features were examined and descriptions were made following Bidaud (1992), Muñoz (2018), Gane (2016) and Maletti (2021).

2.2. Molecular studies

Genomic DNA was extracted from lamella using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) as described by the manufacturer's protocol. The ITS1-5.8S-ITS2 region of the rDNA gene was amplified using the primer pair ITS4-ITS5 (White et al., 1990) and the 28S LSU gene region was amplified using the primer pair LROR-LR5 (Vilgalys and Hester, 1990).

Polymerase chain reactions (PCR) were prepared in a 30 µl final volume mixture containing 3 µl 10X buffer, 3 µl dNTP mix, 3 µl primer pair (final concentration of 1 µM each), 0.3 µl Dream Taq DNA polymerase (Thermo), 10 µl gDNA and 7.7 µl sterile double distilled H_2O. PCR amplifications for ITS and LSU gene regions included 5 min initial denaturation at 95ºC followed by 40 cycles of denaturation at 95ºC for 30 sec, annealing at 53ºC for ITS and 48ºC for LSU for 30 sec, and extension at 72ºC for 1 min and a final extension for 10 min. PCR products were verified by using 1 % agarose gel electrophoresis and sent for sequencing (Aquatayf Biotechnology Laboratories, Istanbul, Türkiye).

For both ITS and LSU genes, sequences were generated from both ends and then assembled to produce a final gene sequence. These sequences were BLASTed for homology based searches using Basic Local Alignment Search Tool (BLAST) program. Best matches were retrieved from GenBank for phylogenetic analysis. Sequence alignment is performed using ClustalW and phylogenetic tree is constructed using MEGA 6.0 (Tamura et al., 2013). Phylogenetic trees were constructed using the maximum likelihood (ML) method where Tamura-Nei model (Tamura and Nei, 1993) was used to construct the ML tree with bootstrap support of 1000 replicates and default settings. The bootstrap support values ≥ 50% were marked on the branches of the phylogenetic tree.

3. Results

3.1. Taxonomy

Cortinarius strenuipes Rob. Henry, Bulletin de la Société Mycologique de France 71 (3): 230 (1956) (Fig. 1,2)

Mycobank MB# 295972

Macroscopic and microscopic features: Pileus 60-100(130) mm across, firstly convex, later plane-convex, finally flat-convex and even slightly depressed; brown-gray, brown-cream or reddish-brown; margin regular, smooth, firstly inrolled and then incurved; surface dirty in appearance, dry, hygrophanous, initially covered with a grayish veil, with abundant whitish fibrils cuticle and dark blackish spots. Lamellae dark ocher-brown to chocolate-brown; not very tight, thick, broad, several lengths, crowded, convoluted indentation, slightly decurrent. Stipe 60-70(100) x 15-20(25) mm, dry, solid, fleshy, hard, rusty brown fibrillose, cylindrical, slightly bulbous at base;

Figure 1. *Cortinarius strenuipes* (Collection HIS-47): a- basidiomata in situ, b- basidiospores, (Scale bars: a = 40 mm; b = 10 µm).

whitish, then slightly brown, covered with a grayish silky veil; cortina short-lived, whitish, rarely leaves a slight ring-shaped scar on the upper. Flesh thick, whitish grayish on stem, pale reddish brown on pileus; unpleasant weak odor. Basidiospores 8.5–11.5(13.0) x 6.0–7.0(8.0) µm, ellipsoidal to amygdaliform, fine to medium-sized warts. Basidia 33-35(45) x 10-12(14) µm, clavate, 4-spored. Marginal cells 5-7 µm in diameter, clavate. Pileipellis filamentous, cylindrical hyphae, septate with clamps. Clamp connections present. Potassium hydroxide (KOH) on the cuticle black, on the flesh dark gray.

Ecology and distribution: Rare, in hygrophilous (moisture-loving) deciduous forests in summer and autumn, under trees (beech, hornbeam and oak), on calcareous soils, in groups of 6-8 specimens. Reported from Mediterranean regions.

Specimen examined: Türkiye. Tokat province, Avlunlar village, in a mixed forest on calcareous soil. 24.11.2018, 40°32ˈ35"N, 36°45ˈ41"E, 1146 m, HIS-47.

3.2. Phylogeny

Approximately a 580 bp ITS and 960 bp LSU gene sequences for *C. strenuipes* are generated in this study with accession numbers PP425965 and PP425973, respectively. The nrITS data set included 865 sites, of which 582 were conserved, 105 were variable, and 61 were parsimony informative. According to ITS sequence analyses, the Turkish specimen was identified as belonging to *C. strenuipes*, where each species formed a monophyletic clade with high bootstrap support (Fig. 3). Within the clade, sequence analyses between our collection and the remaining species indicated an intraspecific genetic

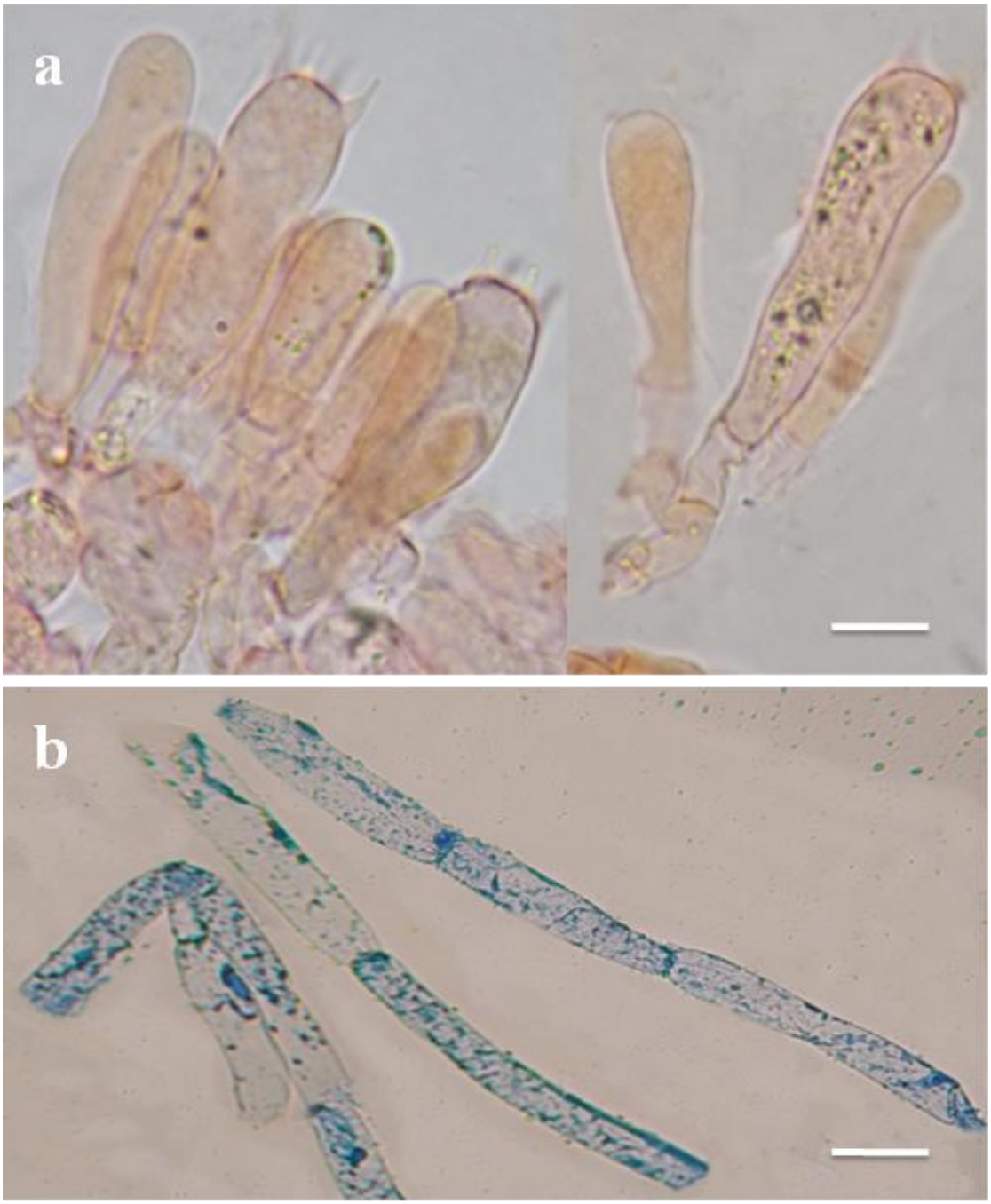

Figure 2. *Cortinarius strenuipes* (Collection HIS-47): a- basidia and basidiole, b- pileipellis (Scale bars: a = 10 µm, b = 30 µm).

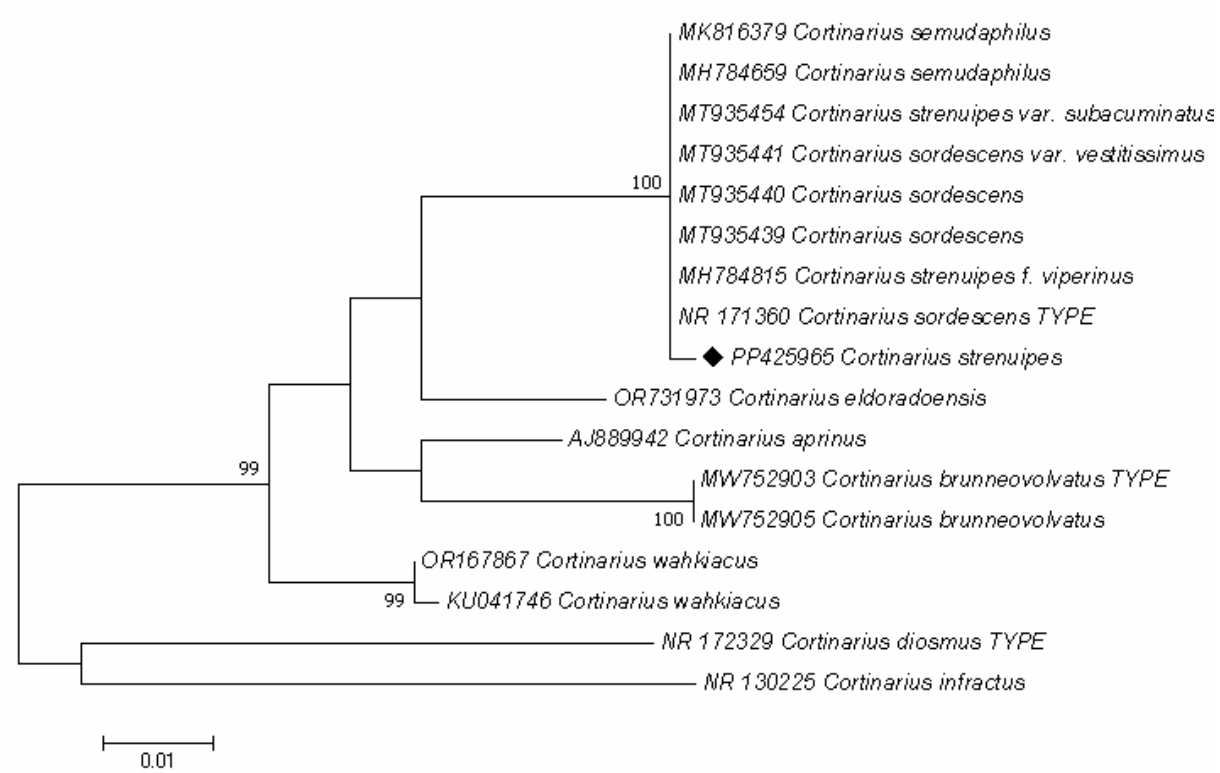

Figure 3. Maximum likelihood tree generated from nrITS sequences belonging to Turkish collection (labelled in diamond) in this study and closely related taxa. Bootstrap support values ≥ 50% from ML analysis were shown on the branches. Bar indicates 0.01 expected change per site per branch.

variation of 3 to 6 bp. Analysis with LSU sequence did not show any significant results since no sequence from this gene region existed for the studied specimen and closely related species for an accurate comparison. LSU based tree is uninformative, thus is not presented here.

4. Discussions

This study reports *Cortinarius strenuipes* Rob. Henry for the first time from Türkiye with molecular data and morphological description. This species belongs to C. subg. Dermocybe (Fr.) Trog, C. sect. Sericeocybe (P.D. Orton) Melot., and C. subsect. Strenuipedes (Moser and Horak, 1975). Its characteristic features include a gray or reddish-brown, hygrophanous, fibrous pileus; cylindrical, slightly bulbous at the base, brownish fibrillose stipe; loosely packed, thick, ocher brown to chocolate brown lamella. It is a rare species reported only from Mediterranean regions including Spain and France in *Quercus ilex* L. forests on calcareous soil (Henry, 1956; Mahiques, 2010; Muñoz, 2018).

According to IndexFungorum (2024), this taxon appears in two forms and two varieties, which are *Cortinarius strenuipes* f. *strenuipes*, *Cortinarius strenuipes* f. *viperinus* Reumaux, *Cortinarius strenuipes* var. *strenuipes* and *Cortinarius strenuipes* var. *subacuminatus* Rob. Henry ex Reumaux. The latter species has shorter pileus and stipe, cream-colored and rusty brown lamella, slightly smaller basidiospores when compared to *C. strenuipes* Rob. Henry, and differs from *C. strenuipes* var. *strenuipes* based on negative reaction to phenol anilin and positive for guaiac tincture (Bidaud et al., 2002; Mahiques et al. 2013). More recently, Liimatainen et al. (2020) used the name of *C. sordescens* Rob. Henry as the current name of *C. strenuipes* var. *subacuminatus* based on molecular analyses.

In the literature, there is no molecular sequence data exists for *C. strenuipes* Rob. Henry. This study provides the first molecular data from this species. While *C. strenuipes* and its varieties macroscopically differ from each other, sequence analysis revealed high sequence identity among them. Our phylogenetic analysis showed that *C. strenuipes* is closely related to *C. strenuipes* var. *strenuipes*, *C. strenuipes* f. *viperinus*, and *C. sordescens* with high support and clustered them together. The observation of little intraspecific genetic variation among them could be due to no enough time to for ITS sequence divergence. It is clear that multiple collections of this taxon from different geographical locations should be analzyed for a better explanation of morphological and genetic variations.

Cortinarius is an extremely difficult genus for species identification due to overlapping characters among its species. In the last decade, studies including DNA sequence analyses have helped delimitation problems. It is evident that both molecular and morphological investgation should be conducted for accurate nomenclature of *Cortinarius* species.

Conflict of nterest

Authors have declared no conflict of interest.

Authors' Contribution

Dr. Şengül Demirak conducted the molecular studies, wrote original draft, revised and edited the manuscript. Drs. Türkekul and Işık conducted morphological studies.

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