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First Report of *Rhodocybe fumanellii* (*Entolomataceae*) from Türkiye with Morphological and Molecular Characterisation

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ABSTRACT

This study presents a comprehensive taxonomic assessment of fungal specimens collected from Çanakkale Province in northwestern Türkiye. An integrative approach was employed, combining detailed macro and micromorphological analyses with molecular phylogenetic methods based on the internal transcribed spacer (nrITS, rDNA) region of nuclear ribosomal DNA. The results confirmed the identity of the specimens as *Rhodocybe fumanellii* R.J. Ferrari, Vizzini & Fellin, representing the first record of this species in Türkiye. As a newly documented member of section *Rufobrunnea*, this finding broadens the known geographical distribution of the species and contributes to understanding the genus *Rhodocybe* in the region. The study includes thorough morphological descriptions, ecological data, precise locality information with geographic coordinates, collection details, and photographic documentation, thereby enriching the knowledge of fungal biodiversity in Türkiye.

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Rhodocybe fumanellii (*Entolomataceae*)'nin Türkiye'deki İlk Kaydı ve Morfolojik-Moleküler Analizi

ÖZET

Çalışma, Türkiye'nin kuzeybatısındaki Çanakkale ilinden toplanan mantar örneklerinin kapsamlı bir taksonomik değerlendirmesini sunmaktadır. Ayrıntılı makro ve mikromorfolojik analizler ile nükleer ribozomal DNA'nın iç transkripsiyonlu spacer (nrITS, rDNA) bölgesine dayanan moleküler filogenetik yöntemleri birleştiren bütünleştirici bir yaklaşım kullanılmıştır. Sonuçlar, örneklerin Rhodocybe fumanellii R.J. Ferrari, Vizzini & Fellin olarak kimliğini doğrulamış ve bu türün Türkiye'deki ilk kaydını temsil etmiştir. *Rufobrunnea* bölümünün yeni belgelenmiş bir üyesi olarak bu bulgu, türün bilinen coğrafi dağılımını genişletmekte ve bölgedeki Rhodocybe cinsinin anlaşılmasına katkıda bulunmaktadır. Çalışma, kapsamlı morfolojik tanımları, ekolojik verileri, coğrafi koordinatlarla birlikte kesin lokalite bilgilerini, toplama detaylarını ve fotoğrafik dokümantasyonu içermekte ve böylece Türkiye'deki mantar biyoçeşitliliği bilgisini zenginleştirmektedir.

Mikoloji

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INTRODUCTION

Rhodocybe, belonging to the *Entolomataceae* family, comprises saprotrophic fungi recognized for their muted-colour basidiomata, commonly conical to funnel-shaped and often exhibit a central depression (Vizzini et al., 2016; Sun & Bau, 2023). The pileus surface typically ranges from pale pink to deep vinaceous cinnamon. The lamellae

display variable attachment patterns, including adnate and decurrent types, and may occasionally display shallow notches at the attachment point (Dutta et al., 2021; Akata et al., 2024a). Spores are usually globose to tear-shaped and bear angular or pustulate ornamentation, resulting in a polygonal structure with 6 to 12 facets when observed under polar view (Sesli & Vizzini, 2017; Vizzini et al., 2016, 2018). The spore print is generally salmon to brownish pink in hue. One key taxonomic feature of this genus is the absence of clamp connections. When present, hymenial cystidia may appear either as vividly pigmented pseudocystidia or as transparent leptocystidia (Akata et al., 2024a; Kluting et al., 2014; Sun & Bau, 2023). Species within the genus are broadly distributed across temperate and tropical ecosystems, typically occurring in nutrient-rich forest soils or on decomposing wood substrates. The genus is categorized into seven distinct sections: *Claudopodes, Crepidotoides, Decurrentes, Rhodocybe, Rhodophana, Rufobrunnea*, and *Tomentosi* (Akata et al., 2024a).

Rhodocybe sect. *Rufobrunnea* Singer ex T.J. Baroni comprises cryptic species exhibiting minimal macroscopic differences, including basidioma habit, cap colour, lamellae attachment, odor, and rhizomorph presence, while the influence of environmental factors on these traits remains unclear (Sesli & Vizzini, 2017; Akata et al., 2024a). Microscopic identification relies on limited features such as cheilocystidia, spore morphology, and pileipellis structure, challenging species delimitation (Sesli & Vizzini, 2017; Sun & Bau, 2023). However, ribosomal sequence analysis provides a more precise taxonomic resolution (Sesli & Vizzini, 2017; Akata et al., 2024a).

Rhodocybe fumanellii, classified in the *Rufobrunnea* section, is characterized by its robust basidiomata, which display a tricholomatoid shape and have a distinctive reddish-brown colour. The species features adnate lamellae that are not strongly decurrent, and a noticeably swollen stipe base adorned with elongated and well-developed rhizomorphs. At the microscopic level, it is distinguished by slender, elongated cheilocystidia and ellipsoid basidiospores. These microscopic features and the cheilocystidia are crucial in its taxonomic classification within the section (Vizzini et al., 2018).

Existing literature indicates that only two species, *Rhodocybe asanii* Sesli & Vizzini and *R. asyae* Sesli & Vizzini, within the *Rufobrunnea* section, have so far been documented in Türkiye (Sesli & Vizzini, 2017; Vizzini et al., 2018; Akata et al., 2024a). This limited representation suggests that the diversity of this section in the region remains underexplored, thereby warranting further taxonomic and ecological investigations to assess its true extent.

This study enhances understanding of *R. fumanellii* by documenting its first occurrence in Türkiye. This record extends the species' known distribution and provides essential insights into the biogeography of the *Rhodocybe* sect. *Rufobrunnea*, contributing to a more comprehensive perspective on its range.

MATERIAL and METHOD

This study employed a comprehensive approach, integrating morphological assessments with molecular analyses to investigate and classify specimens from the province of Çanakkale in Türkiye. Detailed examinations of macroscopic and microscopic features were conducted alongside ITS sequencing of ribosomal DNA (rDNA) to enhance taxonomic resolution.

Morphological Study

Field surveys were carried out to document macroscopic traits and environmental conditions. In the laboratory, microscopic analyses were performed using a Euromex Oxion Trinocular light microscope at 100x magnification to observe fine structural details. Each microscopic feature underwent approximately 30 measurements to ensure accuracy, followed by statistical analysis. Once identified, the specimens were deposited in the Fungarium of Ankara University, located within the Department of Biology at the Faculty of Science.

Molecular Characterization

Determination of the ITS rDNA sequences

Genomic DNA was isolated from the fungal specimen ANK ACAR 1549, and the nuclear ribosomal internal transcribed spacer (nrITS) region of the rDNA was subsequently amplified using polymerase chain reaction (PCR). The process adhered to established protocols from previous studies (Akata & Erdoğdu, 2020; Akata et al., 2024a, 2024b, 2024c, 2024d, 2025), ensuring methodological consistency and reproducible results.

Molecular Phylogeny Study

Phylogenetic relationships among the fungal specimens were reconstructed through comparative analysis of nucleotide sequences obtained from the sampled isolates, utilizing MEGA 11 software (Tamura et al., 2021) for computational phylogenetic inference. Specifically, for specimen ANK ACAR 1549, the nuclear ribosomal ITS region was amplified and sequenced via Sanger sequencing employing the ITS1/ITS4 primer set (White et al.,

1990). The resultant electropherograms were base-called, quality-filtered, and assembled into consensus sequences before subsequent phylogenetic interrogation within the MEGA 11 bioinformatics platform. Reference sequences retrieved from GenBank were carefully curated through a stringent screening process, in which closely related taxa were designated as the ingroup. In contrast, phylogenetically distant sequences, identified via BLASTn homology searches, served as the outgroup. Multiple sequence alignment was executed using the ClustalW algorithm to ensure optimal accuracy and alignment consistency. Phylogenetic reconstruction was performed under the Maximum Likelihood statistical method, employing the Tamura 3-parameter (T92) substitution model with gamma-distributed rate heterogeneity (Tamura, 1992). Branch support was evaluated through 1,000 bootstrap replicates to assess the robustness of the inferred topology.

RESULTS

The classification of the newly documented species was based on the taxonomic framework outlined in the Index Fungorum (accessed 25 March 2025). A comprehensive description of the species was provided, including essential details such as collection dates, precise geographical coordinates, habitat characteristics, and unique collection identifiers. Additionally, macroscopic and microscopic morphological traits were thoroughly examined to ensure accurate taxonomic identification.

Taxonomic overview

Rhodocybe fumanellii R.J. Ferrari, Vizzini & Fellin (2018). (Fig. 1, 2)
Vizzini et al. (2018) offer an in-depth account of the type specimens found within the original collections.

Figure 1. Basidiomata of *Rhodocybe fumanellii*
Şekil 1. Rhodocybe fumanellii'nin basidiomatası

Macroscopic and microscopic features

Basidiomata presenting a tricholomatoid habit. **Pileus** 50–60 mm across; initially convex with a prominent central umbo; expansion leading to a flattened configuration with irregular or undulate margins; surface smooth and dry under standard conditions; greasy when moist; hygrophanous changes negligible or absent; pigmentation ranging from reddish-brown in immature basidiomata to brick-reddish or pale orange in maturity. **Lamellae** narrow, adnate, and closely spaced; colouration shifts from whitish cream in early developmental stages to pinkish tones in aged specimens; edges are irregularly eroded and match in colour. **Stipe** centrally positioned and solid; shape varying from cylindrical to clavate; surface pinkish with a dense white flocculent-pruinose covering, mainly concentrated near the apex; basal region enveloped in thick white tomentum with copious whitish rhizomorphs.

Flesh whitish, displaying pinkish marbling; thickness most significant at the center of the pileus and diminishing toward the margin. **Odor** and **taste are** indistinct. **Spore print** pinkish. **Basidia** 26–32 × 7–8 µm, clavate, thin-walled, four-spored. **Basidioles** 23–34 × 5–7 µm, clavate. **Basidiospores** (5.6–)5.9–7.1(–7.4) × (3.8–)4.1–4.9(–5.2) µm [(n = 30), Q = (1.25–)1.31–1.68(–1.76), Qav = 1.42], ellipsoid, hyaline, surface ornamentation finely verrucose to pustulate; inamyloid, walls showing cyanophilic reaction. **Cheilocystidia** 55–85 × 3.5–6 µm, scattered, slender, and flexuose-cylindrical in shape; occasional apical protuberances are septate and thin-walled. **Pleurocystidia** not observed. **Hymenophoral trama** subregular, composed of parallel, cylindrical hyphae intermixed with short, inflated elements, with a width of up to 13 µm. **Caulocystidia** 35–45 × 3–4 µm, slender with an irregular, cylindrical outline and thin walls. **Pileipellis** forming a xerocutis, composed of subparallel, thin-walled hyphae, 5.5–13 µm wide, pigmentation yellowish to orange, brown. **Clamp connections** were not observed.

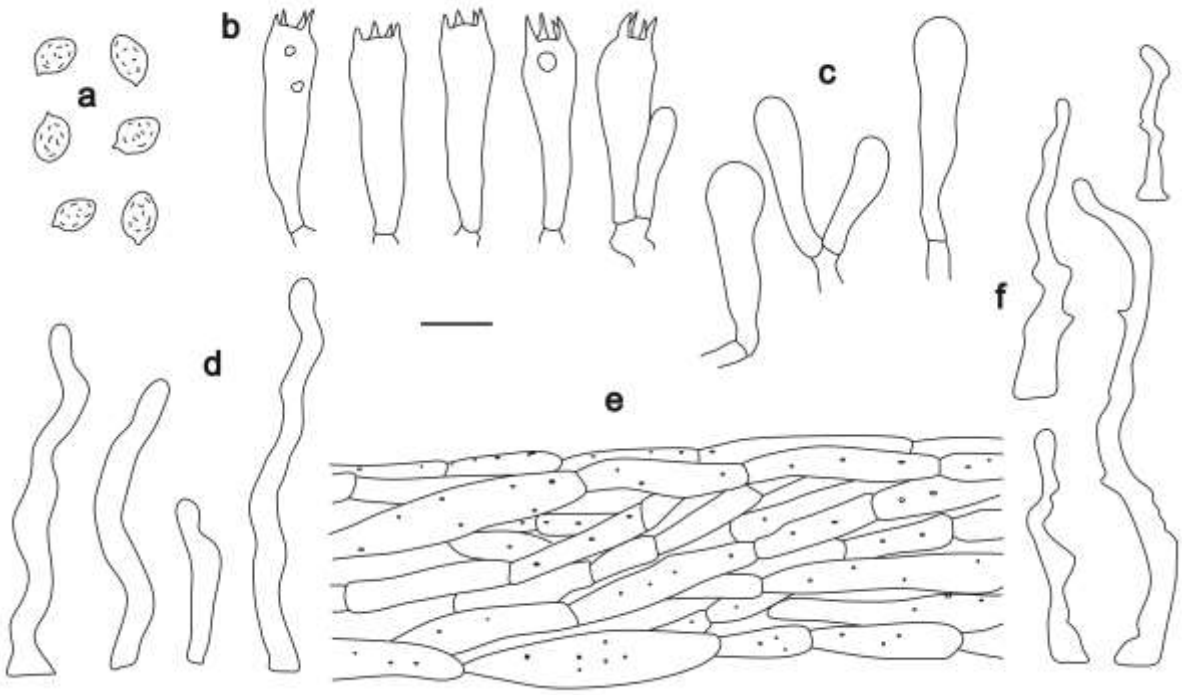

Figure 2. *Rhodocybe fumanellii* : a. spores, b. basidia, c. basidiols, d. cheilocystidia, e. pileipellis, f. caulocystidia (scale bars: 10 µm).

Şekil 2. Rhodocybe fumanellii: a. sporlar, b. bazidiyumlar, c. bazidioller, d. keylosistidyumlar, e. pileipellis, f. kaulosistidyumlar (ölçek: 10 µm).

Material examined: TÜRKİYE—Çanakkale, Çanakkale Onsekiz Mart University, Terzioğlu campus area, under Turkish pine (*Pinus brutia* Ten), 40° 06' 45"N, 26° 25' 56"E, 220 m, 20 Dec. 2023, ANK ACAR 1549 (nrITS rDNA sequence GenBank accession number: PV363011).

Evolutionary History

The evolutionary lineages of the specimens, including ANK ACAR 1549, were investigated through molecular phylogenetic analysis based on their nuclear ribosomal internal transcribed spacer (nrITS) rDNA sequences. These sequences were acquired using advanced molecular techniques and deposited in NCBI GenBank, with corresponding accession numbers in Table 1. To enlighten phylogenetic relationships, nrITS rDNA sequences from multiple representatives of the genus *Rhodocybe* were selected for comparative analysis, while sequences from *Entoloma flavoconicum* and *Entoloma subaraneosum* were designated as outgroups to root the phylogenetic tree.

The molecular phylogenetic reconstruction delineated nine well-supported clades (Fig. 3). Clade 3 exclusively comprised specimens of *R. fumanellii*, including ANK ACAR 1549, whereas the remaining clades encompassed other *Rhodocybe* species. As anticipated, *E. flavoconicum* and *E. subaraneosum* formed a distinct, early diverging branch, corroborating their utility as outgroups. BLAST analyses revealed exceptionally high sequence similarity (>99%) between the examined specimens and their respective *Rhodocybe* counterparts within established clades. Furthermore, robust bootstrap support values confirmed the phylogenetic affinities between the specimens and were interpreted to assess clade reliability, especially highlighting Clade 3, where *R. fumanellii* clustered and their closely related *Rhodocybe* species, thereby reinforcing the reliability of the inferred taxonomic groupings.

Table 1. List of taxa, specimens, locations, and GenBank accession numbers of sequences used in this study.
Çizelge 1. *Bu çalışmada kullanılan taksonların, örneklerin, lokasyonların ve dizilerin GenBank erişim numaralarının listesi.*

Species	Specimen Voucher /Isolate/Strain	nrITS GenBank Accession Number	Geographical origin	Reference
	ANK ACAR 1549	**PV363011**	**Türkiye, Çanakkale**	**Current study**
	FRJ-100-2019	MT114428	-	Unpublished
	BOLGH_22122001	OR831361	Italy: Tuscany, Bolgheri	Unpublished
Rhodocybe fumanellii	iNaturalist.org/254213473	PQ810552	-	Unpublished
	MCVE:29550	NR_166243	Italy: Veneto	Vizzini et al. 2018
	HFRG_PC200928_1	MW401761	United Kingdom: Buckinghamshire	Aplin et al. 2022
	CAL 1825	NR_176746	India: West Bengal	Dutta et al. 2021
Rhodocybe brunneoaurantiaca	CUH AM720	MW023201	India: West Bengal	Dutta et al. 2021
	JP40	OR827577	-	Unpublished
Rhodocybe fusipes	DLK 587	MN306210	Brazil	Silva-Filho et al. 2020
	DLK 298	MN306209	Brazil	Silva-Filho et al. 2020
	KATO Fungi 3640	NR_154443	Türkiye: Trabzon	Sesli & Vizzini 2017
Rhodocybe asyae	KATO Fungi 3653	KX834268	Türkiye: Trabzon	Sesli & Vizzini 2017
	DEU AKATA & SAHIN 148	PP944722	Türkiye: İzmir	Akata et al. 2024a
	Personal collection:NA131019	MN840644	United Kingdom: Sussex	Aplin et al. 2022
	KATO Fungi 3659	NR_154442	Türkiye: Trabzon	Sesli & Vizzini 2017
Rhodocybe asanii	KATO Fungi 3657	KX834265	Türkiye: Trabzon	Sesli & Vizzini 2017
	NA13102020	MW375030	United Kingdom: Sussex	Aplin et al. 2022
	OKA-TR2383	PQ619404	Türkiye: Isparta	Kaygusuz 2024
	MCVE 29261	KY629962	Italy: Campania	Crous et al. 2017
Rhodocybe matesina	MCVE 29262	NR_154455	Italy: Campania	Crous et al. 2017
	F3-2	MZ088085	Lebanon: Fnaydek	Sleiman et al. 2021
Rhodocybe cistetorum	KATO Fungi 4260	NR_176724	Türkiye: Trabzon	Sesli 2021
	HAY-F-002060	PP357297	USA: California	-
	KATO Fungi 3340	NR_154436	Türkiye: Trabzon	Vizzini et al. 2016
Rhodocybe tugrulii	WU-MYC 0022202	OP363994	Austria: Niederoesterreich	Vizzini et al. 2023
	WU-MYC 0003753	OP363993	Austria: Niederoesterreich	Vizzini et al. 2023
	WU-MYC 0010084	OP363995	Austria: Burgenland	Vizzini et al. 2023
	MSNG3938	KY945354	Italy	Unpublished
	FLAS-F-69259-Rhodocybe_roseiavellanea	OQ725160	USA: University of Florida Deluca Reserve	Unpublished
	FLAS-F-69286-Rhodocybe_roseiavellanea	OQ725177	USA: University of Florida Deluca Reserve	Unpublished
Rhodocybe roseiavellanea	FLAS-F-71316_ITS1F	OR239767	USA: Florida	Unpublished
	PBM4056 (TENN)	MF686525	USA: Tennessee, north Knoxville residential area	Unpublished
	OMDL iNat # 125830572	PP850330	USA: Florida, Alachua County, Gainesville	Unpublished
	iNAT 55427666	MT939496	USA: New York	Unpublished
Entoloma flavoconicum	UCH 9226	NR_177616	Panama: Chiriquí	Reschke et al. 2022
Entoloma subaraneosum	GDGM 28823	NR_120052.1	China: Jilin	Schoch et al. 2014

DISCUSSION and CONCLUSION

Within the section *Rufobrunnea*, several closely related taxa have been described, each exhibiting diagnostic features that allow for their distinction from *Rhodocybe fumanellii*. Among Turkish representatives, *R. asyae* Sesli & Vizzini is the earliest reported species, easily recognized by its small-sized basidiomata, slender stipes, decurrent gills, and absence of rhizomorphs (Sesli & Vizzini, 2017; Akata et al., 2024a). Microscopically, it displays 2–4 spored basidia and lacks caulocystidia (Vizzini et al., 2018). *R. asanii* Sesli & Vizzini shares certain morphological affinities with *R. fumanellii*, but can be differentiated by its paler, more fragile pileus, adnexed to sinuate lamellae, and smaller, weakly angular spores (Sesli & Vizzini, 2017; Vizzini et al., 2018). Likewise, *R. subasyae* T. Bau & Y.L. Sun exhibits a comparable basidiomata architecture and gill attachment to *R. fumanellii*, yet it differs in its lighter pileus pigmentation, occasionally branched cheilocystidia, and less pronounced spore morphology (Vizzini et al., 2018; Sun & Bau, 2023). Additionally, *R. brunneoaurantiaca* A.K. Dutta, G.M. Gates & K. Acharya, while displaying similar warm-toned basidiomata and a centrally placed stipe, is distinct in having a smaller pileus, markedly decurrent lamellae, and the absence of both clamp connections and hymenial pseudocystidia (Dutta et al., 2021; Vizzini et al., 2018). Its cheilocystidia are narrower and exhibit considerable morphological variability. Finally, *R. roseiavellanea* (Murrill) Singer, despite its superficially similar robust pileus and stout stipe, can be separated based on its shortly decurrent gills, absence of basal rhizomorphs, shorter cheilocystidia, and larger, almond-shaped spores (Vizzini et al., 2018).

Genetic diversity among fungal species is significantly greater than morphological diversity, necessitating the integration of genetic data with traditional morphological methods for accurate species identification (Lücking et al., 2021). Several genetic markers are commonly utilized in molecular systematics, including rRNA gene regions (nrITS, nrSSU, nrLSU) and protein-coding genes such as TEF1a and TUB2 (Raja et al., 2017). The ITS region has

been identified as the universal primary fungal barcode marker region for fungi (Schoch et al., 2012). This study employed nuclear ITS rDNA sequences to identify fungal specimen ANK ACAR 1549. The molecular analysis indicated a genetic similarity of over 99% between reference sequences of *R. fumanellii* and their closely related taxa, including members of the genus *Rhodocybe* (GenBank accession number: PV363011).

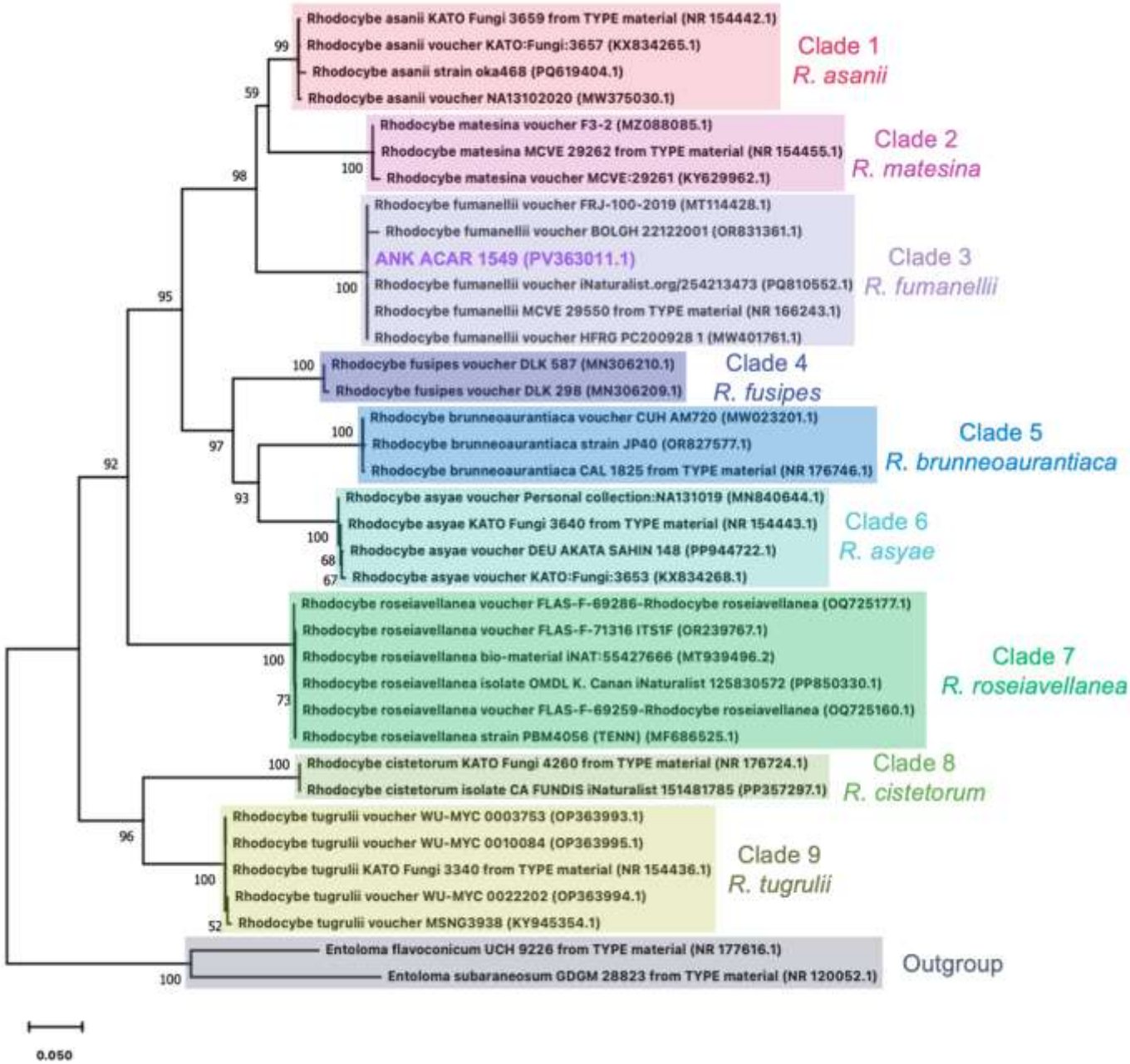

Figure 3. The phylogenetic tree illustrates the evolutionary relationships among 37 fungal specimens, reconstructed using the nrITS rDNA region and the maximum likelihood (ML) method. Branch support is indicated by bootstrap values (≥50), and all sequences were sourced from the NCBI GenBank database. *Entoloma flavoconicum* and *Entoloma subaraneosum* were included as the outgroup. Each sequence is labeled with its GenBank accession number, and a scale bar (0.050 genetic distance) is provided for reference.

Şekil 3. Filogenetik ağaç, nrITS rDNA bölgesi ve maksimum olabilirlik (ML) yöntemi kullanılarak yeniden yapılandırılan 37 mantar örneği arasındaki evrimsel ilişkileri göstermektedir. Dal desteği bootstrap değerleri (≥50) ile gösterilmiştir ve tüm diziler NCBI GenBank veritabanından alınmıştır. Entoloma flavoconicum ve Entoloma subaraneosum dış grup olarak dahil edilmiştir. Her sekans GenBank erişim numarası ile etiketlenmiştir ve referans için ölçek çubuğu (0.050 genetik mesafe) verilmiştir.

The presence of *R. fumanellii* has been recently documented for the first time in Türkiye, thereby enhancing the region's fungal diversity. This identification was based on a thorough morphological analysis of specimens (ANK ACAR 1549), which displayed key characteristics specific to the species. Additionally, molecular phylogenetic analysis using ITS rDNA further confirmed the morphological findings. This new addition brings the number of recognized species within the *Rhodocybe* sect. *Rufobrunnea* in Türkiye to three.

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors have declared no conflict of interest.

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