KSÜ Tarım ve Doğa Derg 27 (Ek Sayı 1), 124-132, 2024
KSU J. Agric Nat 27 (Suppl 1), 124-1320, 2024
DOI:10.18016/ksutarimdoga.vi.1507554

Taxonomic Studies on *Rhodocybe asyae* Specimens Discovered in a New Location

Ilgaz AKATA[1] ✉ , Gülce EDİŞ[2], Eda KUMRU[2], İsmail ACAR[3-4], Ergin ŞAHİN[5-6]

[1] Ankara University, Faculty of Science, Department of Biology, Ankara/TÜRKİYE, [2] Ankara University, Graduate School of Natural and Applied Sciences, Ankara/TÜRKİYE, [3]Van Yüzüncü Yıl University, Başkale Vocational High School, Department of Organic Agriculture, Van/TÜRKİYE, [4]Çanakkale Onsekiz Mart University Çanakkale Vocational School of Health Services, Department of Medical Services and Techniques, Çanakkale/TÜRKİYE, [5]Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir/TÜRKİYE, [6]Dokuz Eylül University, Fauna and Flora Research and Application Center, İzmir/TÜRKİYE

[1]https://orcid.org/0000-0002-1731-1302, [2]https://orcid.org/0000-0001-7038-9865, [3]https://orcid.org/0009-0000-7417-6197
[4]https://orcid.org/0000-0002-6049-4896, [5]https://orcid.org/0000-0003-1711-738X
✉: akata@science.ankara.edu.tr

ABSTRACT

In 2023, fungal specimens were collected from the Tınaztepe Campus of Dokuz Eylül University (İzmir-Türkiye). These samples underwent a comprehensive and meticulous evaluation involving morphological examination and phylogenetic analysis based on nrITS rDNA sequencing. The DEU AKATA & SAHIN 148 samples exhibited microscopic and macroscopic characteristics that closely matched those of *Rhodocybe asyae*, initially described by Sesli & Vizzini. The genetic sequence analysis revealed over 99% similarity with *R. asyae* Sesli & Vizzini. This report represents the second recorded occurrence of *R. asyae* in Türkiye and the third globally. The study documents the collection site, geographical coordinates, habitat observations, the collection date, etc. Furthermore, the research provides macroscopic photographs, microscopic illustrations of the specimens, and detailed discussion, demonstrating the reliability of the present findings.

Mycology

Research Article

Article History
Received: 30.06.2024
Accepted: 17.08.2024

Keywords
Rhodocybe asyae
New locality
Asia Minor
Mycobiota
Türkiye

Yeni Bir Lokasyonu Keşfedilen *Rhodocybe asyae* Örnekleri Üzerinde Taksonomik Çalışmalar

ÖZET

Mantar örnekleri 2023 yılında Dokuz Eylül Üniversitesi Tınaztepe Kampüsü'nden (İzmir-Türkiye) toplanmıştır. Bu örnekler, morfolojik inceleme ve nrITS rDNA dizilemesine dayalı filogenetik analizi içeren kapsamlı ve titiz bir değerlendirmeden geçirilmiştir. DEU AKATA & SAHIN 148 örnekleri, orijinal olarak Sesli & Vizzini tarafından tanımlanan *Rhodocybe asyae* ile yakından eşleşen mikroskobik ve makroskobik özellikler sergilemiştir. Genetik dizi analizi de *R. asyae* Sesli & Vizzini ile %99'un üzerinde benzerlik göstermiştir. Bu rapor, *R. asyae*'nin Türkiye'de kaydedilen ikinci ve Dünya'daki üçüncü kaydını temsil etmektedir. Çalışma, coğrafi koordinatlar, habitat gözlemleri, toplama tarihi ve benzeri bilgileri de içermektedir. Ayrıca, araştırma makroskobik fotoğraflar, örneklerin mikroskobik çizimleri ve bulguların güvenilirliğini gösteren ayrıntılı tartışmalar sunmaktadır.

Mikoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 30.06.2024
Kabul Tarihi : 17.08.2024

Anahtar Kelimeler
Rhodocybe asyae
Yeni lokalite
Küçük Asya
Mikobiyota
Türkiye

Atıf İçin : Akata, I., Ediş, G., Kumru, E., Acar, İ. & Şahin, E. (2024). Taxonomic Studies on *Rhodocybe asyae* Specimens Discovered in a New Location. *KSU J. Agric Nat 27* (Ek Sayı 1), 124-132. DOI: 10.18016/ksutarimdoga.vi.1507554

To Cite: Akata, I., Ediş, G., Kumru, E., Acar, İ. & Şahin, E. (2024). Yeni Bir Lokasyonu Keşfedilen *Rhodocybe asyae* Örnekleri Üzerinde Taksonomik Çalışmalar. *KSÜ Tarım ve Doğa Derg 27* (Suppl 1), 124-132. DOI: 10.18016/ksutarimdoga.vi.1507554

INTRODUCTION

The genus *Rhodocybe*, established by Maire (1926) as a part of the *Entolomataceae*, is distinguished by its variable-shaped, often dull-coloured basidiomata, which typically vary from conical to funnel-shaped with a central depression. The colours of the pileus range from pinkish to vinaceous cinnamon. The lamellae are attached in ways that range from adnate to decurrent, occasionally exhibiting slight notches (Bas et al., 1988; Sun & Bau, 2023). The genus produces a spore print varying from salmon to brownish pink, and its basidiospores vary from

globose to ellipsoid or tear-shaped, characterized by a subtly nodulose or gently angular surface with pustulate details. In the polar view, the basidiospores are angular with 6–12 facets. Importantly, the genus lacks clamp connections. Hymenial cystidia may be absent or present as pseudocystidia with brightly coloured contents or as hyaline leptocystidia appearing as cheilocystidia and sometimes as pleurocystidia (Baroni, 1981; Vizzini et al., 2018). The genus members are primarily saprotrophic, thriving in soil among debris and occasionally on decaying wood. These species are found extensively across temperate and tropical regions in the northern and southern hemispheres. (Vizzini et al., 2016; Sesli & Vizzini, 2017).

Originally categorized in 1981 by Baroni, the genus *Rhodocybe* was organized into seven distinct sections, including *Claudopodes*, *Crepidotoides*, *Decurrentes*, *Rhodocybe*, *Rhodophana*, *Rufobrunnea*, and *Tomentosi*. The *Rufobrunnea* section is distinguished by its unique colouration, featuring shades of reddish-beige, salmon pink, pinkish-brown, or ochre on the pileus. Additionally, this section is noted for its adnate to decurrent gills and the notable absence of pseudocystidia (Vizzini et al., 2018). Further research, including molecular studies, has validated that this section is monophyletic, confirming its natural classification within the genus (Kluting et al., 2014; Sesli & Vizzini, 2017; Sun & Bau, 2023).

Around 50 species of the genus are recognized globally (Sun & Bau, 2023), but only six have been reported in Türkiye (Sesli, 2021; Sesli & Vizzini, 2017; Sesli et al., 2020; Vizzini et al., 2018). Within these, *R. asanii* Sesli & Vizzini and *R. asyae* Sesli & Vizzini, belong to *Rhodocybe* sect. *Rufobrunnea* (Sesli & Vizzini, 2017; Vizzini et al., 2018).

Rhodocybe asyae features distinctive clitocyboid basidiomata with exceptionally thin flesh. The species is noted for its smooth, dish-shaped pileus that exhibits a subtle salmon-pink hue. The lamellae are decurrent, transitioning in colour from whitish to light ivory, and may also have a hint of reddish beige. The stipe is pruinose and cylindrical. It possesses uniquely shaped basidiospores, which are ellipsoid to broadly ellipsoid, slightly angular, and have a warty texture. This species typically has two to four spored basidia and shows diversity in the form of its cheilocystidia (Sesli & Vizzini, 2017).

This study aims to enrich the knowledge of the rare species *R. asyae* by reporting a newly discovered location in Türkiye, thus contributing to the species' global distribution records.

MATERIALS and METHODS

The research employed a comprehensive approach, combining morphological assessments with molecular methods to examine and categorize specimens collected from İzmir province, Türkiye. This study involved a thorough macroscopic and microscopic examination of the samples, which was enhanced by analyzing ribosomal DNA (rDNA) sequences using Internal Transcribed Spacer (ITS) sequencing techniques.

Morphological Characterization

The collection of the specimens was followed by an initial assessment of their macroscopic features and environmental conditions at the collection site. The samples were examined in detail using a binocular light microscope (Euromex Oxion). To ensure the reliability of the findings, each microscopic characteristic was measured using 30 different samples. 5% potassium hydroxide (KOH) and Congo red were used to prepare the material for examination.

For scanning electron microscopy (SEM), small fragments of the fungal samples were mounted on stubs using double-sided tape and coated with gold. These samples were then analyzed using an EVO 40XVP SEM from LEO Ltd., based in Cambridge, UK, operating at a 20 kV accelerating voltage.

The method for morphological identification of the samples followed the detailed protocols outlined in studies by Sesli & Vizzini (2017) and Aplin et al. (2022). These research papers were crucial in guiding the identification process and ensuring accuracy and adherence to established scientific standards. After accurately identifying the samples, they were carefully preserved in the Fungarium at Ankara University, Faculty of Science, Department of Biology.

Molecular Characterization

Genomic DNA Isolation From Fungal Specimens

In the genomic DNA isolation method, 50 mg of dry sporophore samples were mechanically powdered with a mill grinder, placed in 1.5 mL microcentrifuge tubes, and mixed with 700 µL of CTAB lysis buffer (pH 8.0) containing 3% w v^{-1} cetyl trimethyl ammonium bromide (CTAB), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris, 3% w v^{-1} polyvinylpyrrolidone (PVP), and 0.2% v v^{-1} β-mercaptoethanol. The samples were vortexed for 1 minute and incubated at 65 °C for 30 minutes. Following lysis, the samples were centrifuged at 13.000 rpm for 10 minutes, and 500 µL of the supernatant was transferred to new tubes. An equal volume of chloroform-isoamyl alcohol (24:1) solution was added, vortexed briefly, and centrifuged again at 13.000 rpm for 5 minutes. The resulting supernatants were carefully transferred to new tubes, mixed with an equal volume of cold isopropanol, and stored at -20 °C for 30 minutes.

After this incubation, the samples were centrifuged at 13.000 rpm for 10 minutes to precipitate the genomic DNA as pellets. The supernatants were discarded, and the DNA pellets were washed twice with 70% ethanol. Residual ethanol was removed by incubating the DNA pellets at 60 °C. The partially dried DNA pellets were dissolved in an appropriate volume of nuclease-free distilled water. The concentration and purity of the isolated genomic DNA were determined spectrophotometrically using the Nanodrop Lite (Thermo Scientific) device. The integrity of the DNA was verified by agarose gel electrophoresis using a TAE buffer (40 mM Tris-acetate 1 mM EDTA; pH 8.3) with 0.8% agarose. Electrophoresis was performed at a standard voltage of 5 volts per centimetre. Imaging was done using a safe blue light transilluminator and safe green dye. A 1 kb Plus DNA Ladder was the DNA marker in the agarose gel electrophoresis.

PCR Amplification of the Internal Transcribed Spacer (ITS) rDNA Region and Determination of Nucleotide Sequences for Molecular Phylogeny of the Specimens

Genomic DNA samples isolated from macrofungal sporophores using the CTAB method were used as templates to amplify the ribosomal DNA region (ITS) for fungal molecular phylogeny studies. To prevent the formation of undesirable primer dimers during PCR, a hot start DNA polymerase enzyme was used. The PCR reaction was performed in 200 µL polypropylene tubes with a total volume of 50 µL. The reaction mixture included 5 µL of 10X DNA polymerase buffer (containing 25 mM $MgCl_2$), 1 µL of a deoxynucleoside triphosphate (dNTP) mixture (10 mM of each nucleotide), 300-400 ng of genomic DNA template, 1 µL of each 10 µL sequence-specific oligonucleotide primer, five units of DNA polymerase enzyme, and enough nuclease-free dH_2O to bring the total volume to 50 µL. The sequences and melting temperatures (Tm) of the oligonucleotide primers used in PCR are provided in Table 1. PCR thermocycling conditions were optimized based on the Tm of each primer pair, the lengths of the amplified gene regions, and their copy number in the genome. The "Touchdown" approach was employed to minimize undesirable primer dimer formation.

The general PCR conditions were 30 seconds at 95 °C, 15 seconds at 65-50 °C (using the touchdown approach), and 15-30 seconds at 72 °C for 35 cycles, following an initial 2-minute denaturation step. The extension time at 72 °C was adjusted according to the length of the amplicon and the DNA polymerase enzyme used. The reaction concluded with a final extension step of 7 minutes at 72 °C. Thermal cycling was conducted using the MiniAmp Plus Thermal Cycler (Applied Biosystems). The resulting amplicons were analyzed by electrophoresis in a 1% agarose gel to assess the success and quality of the amplification, indicated by the presence of a single band in the gel and the absence of non-specific amplifications. Agarose gel electrophoresis was performed as described earlier. A GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific) was used as a size marker for the DNA amplicons.

Amplicons of confirmed quality were then cleaned and purified using the GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific) according to the manufacturer's instructions. The concentration and purity of the purified amplicons were measured spectrophotometrically using the Nanodrop Lite device. DNA sequencing was performed using the Sanger dideoxy chain termination method. The same oligonucleotide primers used for PCR were used to sequence the amplicons. DNA sequence analysis of the amplicons was outsourced to an external facility.

Table 1. Sequences and Tm temperatures of Oligonucleotide Primers Used in the PCR
Çizelge 1. PCR'da Kullanılan Oligonükleotid Primerlerin Dizileri ve Tm sıcaklıkları

LOCUS	Primer	Oligonucleotide Sequence (5'-3')	Tm Temperature (°C)
ITS	ITS1	TCCGTAGGTGAACCTGCGG	62
	ITS4	TCCTCCGCTTATTGATATGC	53

Molecular Phylogenetic Analyses of the Fungal Specimen

Molecular phylogenetic analyses of the specimens were conducted using MEGA-X software (https: www.megasoftware.net) based on their nucleotide sequences. The amplicon sequences were initially analyzed with NCBI's Nucleotide BLAST (Basic Local Alignment Search Tool) to identify similar sequences. The sequences in the GenBank DNA database that showed the highest similarity to the analyzed amplicon sequences were selected as the ingroup for phylogenetic analyses. Additionally, sequences from distantly related macrofungi that did not show similarity to the analyzed amplicon sequences were chosen as the outgroup for the analyses.

The sequences were first aligned with the ingroup and outgroup sequences using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithms to construct a phylogenetic tree. The most appropriate nucleotide substitution model was then determined, and phylogenetic trees were constructed using the Neighbor-Joining algorithm. To assess the reliability of the tree branches, 1000 bootstrap replicates were applied.

RESULTS

The specimens collected from Dokuz Eylül University Tınaztepe Campus were identified as *Rhodocybe asyae*. This species was briefly described, encompassing macroscopic and microscopic characteristics observed in the samples. The documentation includes the collection date, the precise location of the samples, habitat descriptions, geographic coordinates, and unique collection identifiers. Macroscopic photos, illustrations of microscopic structures, and Scanning Electron Microscope (SEM) images of the basidiospores were also provided.

Taxonomic overview

Basidiomycota R.T. Moore
Agaricales Underw.
Entolomataceae Kotl. & Pouzar
Rhodocybe asyae Sesli & Vizzini (2017), (Figure 1-3).
Sesli & Vizzini (2017) thoroughly characterized the type specimens.

Figure 1. *Rhodocybe asyae*: a-d basidiomata (scale bars: 10 mm)
Şekil 1. Rhodocybe asyae: a-d basidiomata (ölçekler: 10 mm)

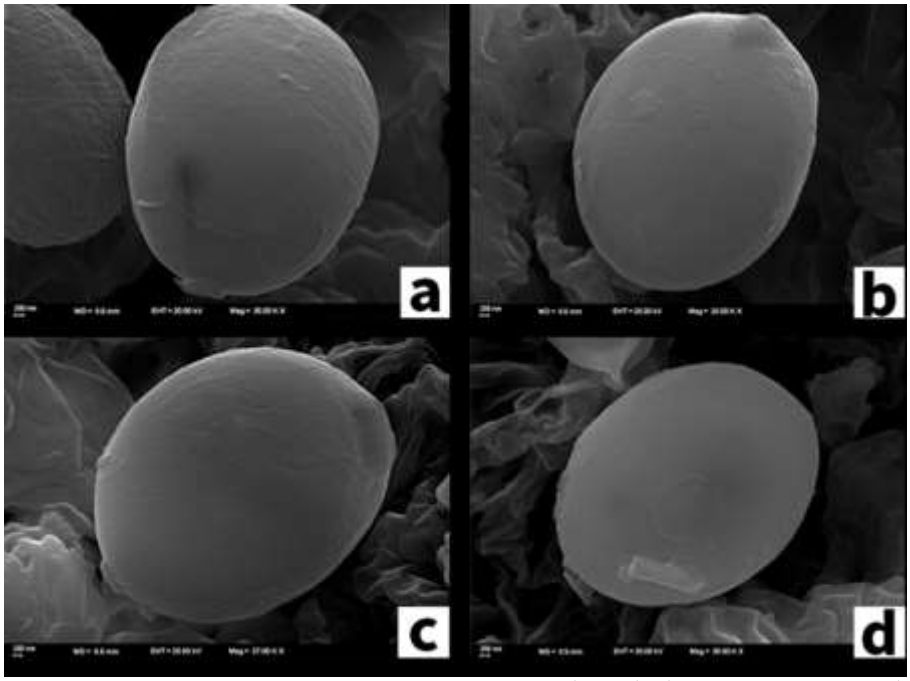

Figure 2. *Rhodocybe asyae* a-d. basidiospores (SEM), (scale bars: 200 nm)
Şekil 2. Rhodocybe asyae: a-d. sporlar (SEM), (ölçekler: 200 nm)

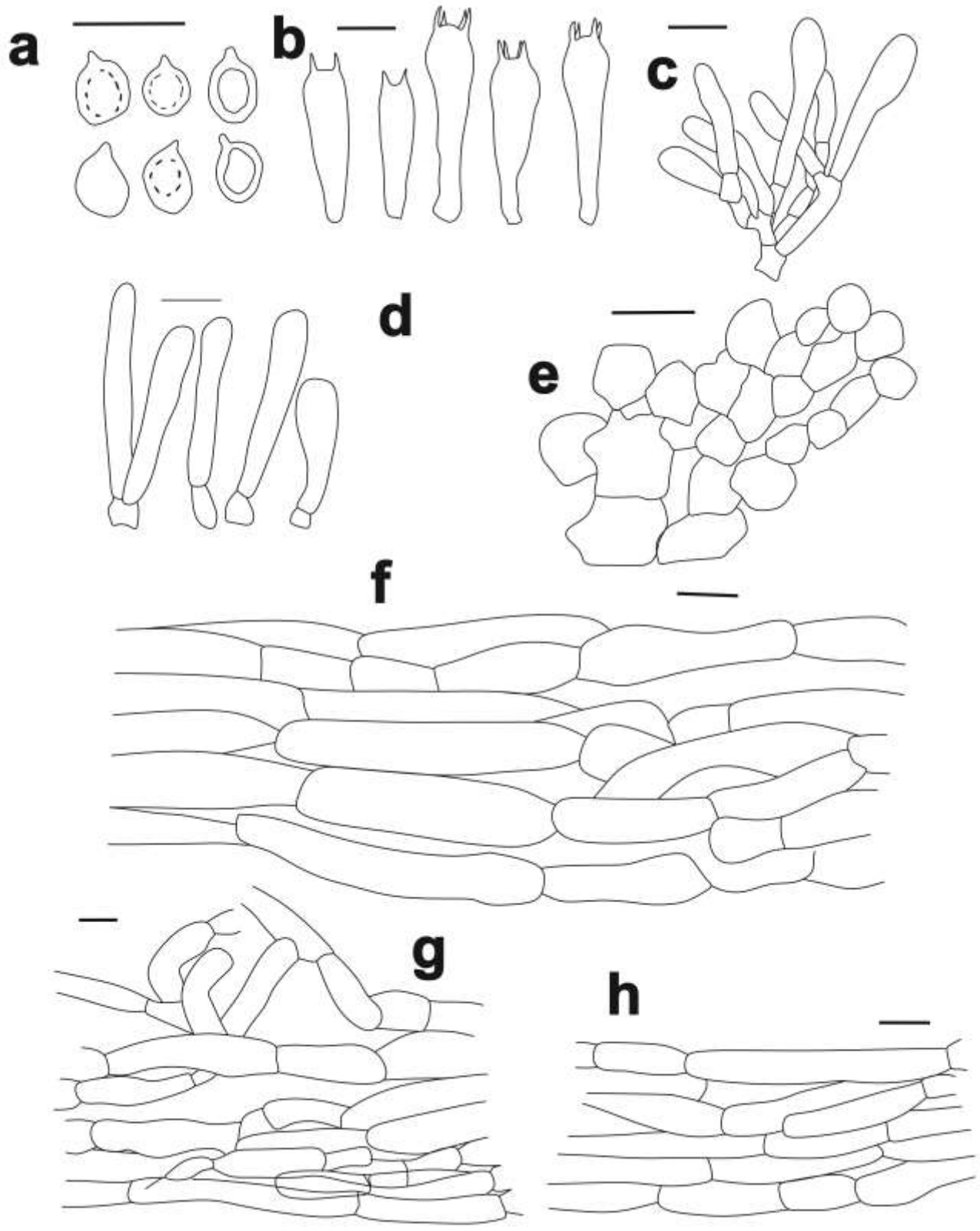

Figure 3. *Rhodocybe asyae*: a. basidiospores, b. basidia, c. basidioles, d. cheilocystidia, e. subhymenium, f. hyphae of the hymenophoral trama, g. pileipellis, h. stipitipellis (scale bars: 10 µm).

Şekil 4. Rhodocybe asyae: a. basidiosporlar, b. bazidiyumlar, c. bazidiyoller, d. keylosistidyumlar, e. subhimeniyum, f. himenoforal trama hifleri, g.pileipellis, h. stipitipellis (ölçekler:10 µm).

Macroscopic and microscopic features

Habit tricholomatoid. **Pileus** 15–30 mm broad, convex at first, soon convex to the plane, slightly depressed in the centre, salmon pink with ivory to beige hue, paler pinkish towards the margin, reddish brown in the centre, dry, smooth, slightly hygrophanous. Margin slightly inrolled when young, soon plane, ivory to beige. **Lamellae are** adnate to subdecurrent, whitish, light ivory to beige orange, fragile and regular. **Stipe** 25–30 × 5 mm, cylindrical, hollow, intensely pruinose at apex, typically tapering towards base, light pinkish to light orange-brown, and with a white-tomentose bulb. **Odor** and **Taste** not distinctive. **Flesh** thin, whitish to ivory or grayish-beige. **Basidiospores** (4–)4.5–7.5 (–8) × (3–)3.2–5.3(–5.6) µm including apiculus [(n = 30), Q = 1.18–1.45 Qav = 1.39], ellipsoid to broadly ellipsoid and subangular in profile view, containing tiny droplets or granules, hyaline, and inamyloid. **Basidia** 24–32 × 6.5–9 µm, 2–4 spored, slenderly clavate, thin-walled, and has 3–5 µm long sterigmata. Basal clamp not observed. **Basidioles** similar to basidia and18–25 × 4.5–6.5 µm. **Subhymenium** ranges from lobed and rounded to nearly spherical, broadly elliptical, and occasionally even angular, 4.5- 7 × 5.5-9 µm. **Hymenophoral trama** regular, consisting of cylindrical and septate hyphae, measuring 6.5–12.5 µm broad. **Pleurocystidia** not observed. **Cheilocystidia** rarely observed, 18–24 × 4–6 µm, slenderly cylindrical to slightly subclavate and thin-walled. **Pileipellis** is a cutis comprising two distinct layers: A thin upper layer of narrow, 3–4.5 µm cylindrical hyphae and a thicker lower layer consisting of multicellular structures of 6.5–10 µm broad. **Stipitipellis** is a cutis comprising cylindrical, septate, and thin-walled, 4–8 µm broad hyphae. **Clamp connections** not observed.

Ecology and distribution: The species was described from Türkiye (Trabzon) and recorded in the United Kingdom (East Sussex). It usually grows singly or in small groups within coniferous forests, including pine, spruce, and fir trees. It is often found amid fallen pine needles and grass tufts, predominantly emerging during autumn (Sesli & Vizzini, 2017; Aplin et al., 2022).

Material examined: TÜRKİYE— İzmir, Dokuz Eylül University Tınaztepe Campus, under Turkish pine (*Pinus brutia* Ten), 210 m, 38° 22' 13" N, 27° 12' 43" E, 01.12.2023, DEU AKATA & SAHİN 148 (nrITS rDNA sequence GenBank accession number: PP944722.1).

Evolutionary History of DEU AKATA & SAHIN 148

The evolutionary lineage of specimen DEU AKATA & SAHIN 148 was examined based on its nrITS rDNA sequence, which was obtained using standard molecular techniques and archived in the NCBI GenBank under accession number PP944722.1. To explore its evolutionary relationships, nrITS rDNA sequences from various members of the *Rhodocybe* genus were selected for comparison, with *Tuber melanorufum*'s nrITS rDNA sequence serving as an outgroup. Molecular phylogenetic analysis identified ten distinct clades, including Clade 1, which contained different isolates of *Rhodocybe asyae* and DEU AKATA & SAHIN 148. The remaining clades (Clade 2-10) included other *Rhodocybe* species. *T. melanorufum* formed a separate branch, confirming its role as the outgroup. BLAST analyses showed over 99% similarity between the nrITS rDNA sequences of DEU AKATA & SAHIN 148 and various isolates of *R. asyae*. Phylogenetic analyses confirmed the close relationship between DEU AKATA & SAHIN 148 and *R. asyae*, with bootstrap branch support validating the reliability of their grouping.

DISCUSSION and CONCLUSION

The genetic diversity of fungal species exceeds their morphological diversity, prompting the integration of genetic information with traditional morphological methods for more precise species identification. Various genetic markers, such as rRNA gene regions like nrITS, nrSSU, and nrLSU, as well as protein-coding gene sequences, have been utilized in molecular systematic studies for decades (Raja et al., 2017; Akata et al., 2023; 2024a; 2024b; Altuntaş et al., 2021). The ITS region stands out in fungal molecular taxonomy, offering valuable insights. High-throughput sequencing technologies and bioinformatics tools have advanced whole genome comparisons and phylogenomic analyses among fungal taxa, potentially replacing molecular phylogenetic analyses based on a few marker genes (Marian et al., 2024). In the present study, nuclear ITS rDNA sequences were employed for the molecular identification of DEU AKATA & SAHIN 148, revealing a similarity of over 99% with the specimen (GenBank ID: PP944722.1) and *R. asyae* (Figure 4).

Rhodocybe asyae, a species within Section *Rufobrunnea*, shares several morphological and ecological traits with its close relatives. Notably, it exhibits similarities to species such as *R. asanii* E. Sesli & Vizzini, *R. fusipes* Silva-Filho, D.L. Komura & Wartchow, *R. fumanellii* R.J. Ferrari, Vizzini & Fellin and *R. subasyae* T. Bau & Y.L. Sun.

Rhodocybe asanii shares similar habitats (pine, spruce, and fir) and several characteristics with *R. asyae*, including a small pileus size (20–45 mm) and similar size of basidia (20–30 × 7–8 µm) and basidiospores (5.4–6.8 × 3.9–4.9 µm). Both species exhibit a change in the surface color of the pileus upon aging or when damaged. Despite these similarities, *R. asanii* is distinct in its tricholomatoid morphology. The pileus of *R. asanii*, described as light ivory to beige-red, displays a range of shapes from

convex to plane or even irregular and is notably fragile. The lamellae are adnexed to sinuate and transition from whitish to reddish beige, with a tendency to become more reddish when injured. The stipe of *R. asanii* is pruinose, contributing to its unique textural qualities and lack of cheilocystidia (Sesli & Vizzini, 2017).

R. fumanellii is recognized by its solid, tricholomatoid basidiomata, vibrant reddish-brown tinges, and closely spaced adnate lamellae. Microscopically, it stands out due to its long cheilocystidia (35–95 × 3–6.5 mm) and its ellipsoid basidiospores. Caulocystidia are also present. *R. fumanellii* has larger basidiomata (pileus: 35–100 mm and stipe: 40–70 × 5–15 mm). Additionally, this species has rhizomorphs at the stipe base and 4-spored basidia and usually grows among the leaf litter under deciduous trees (Vizzini et al., 2018).

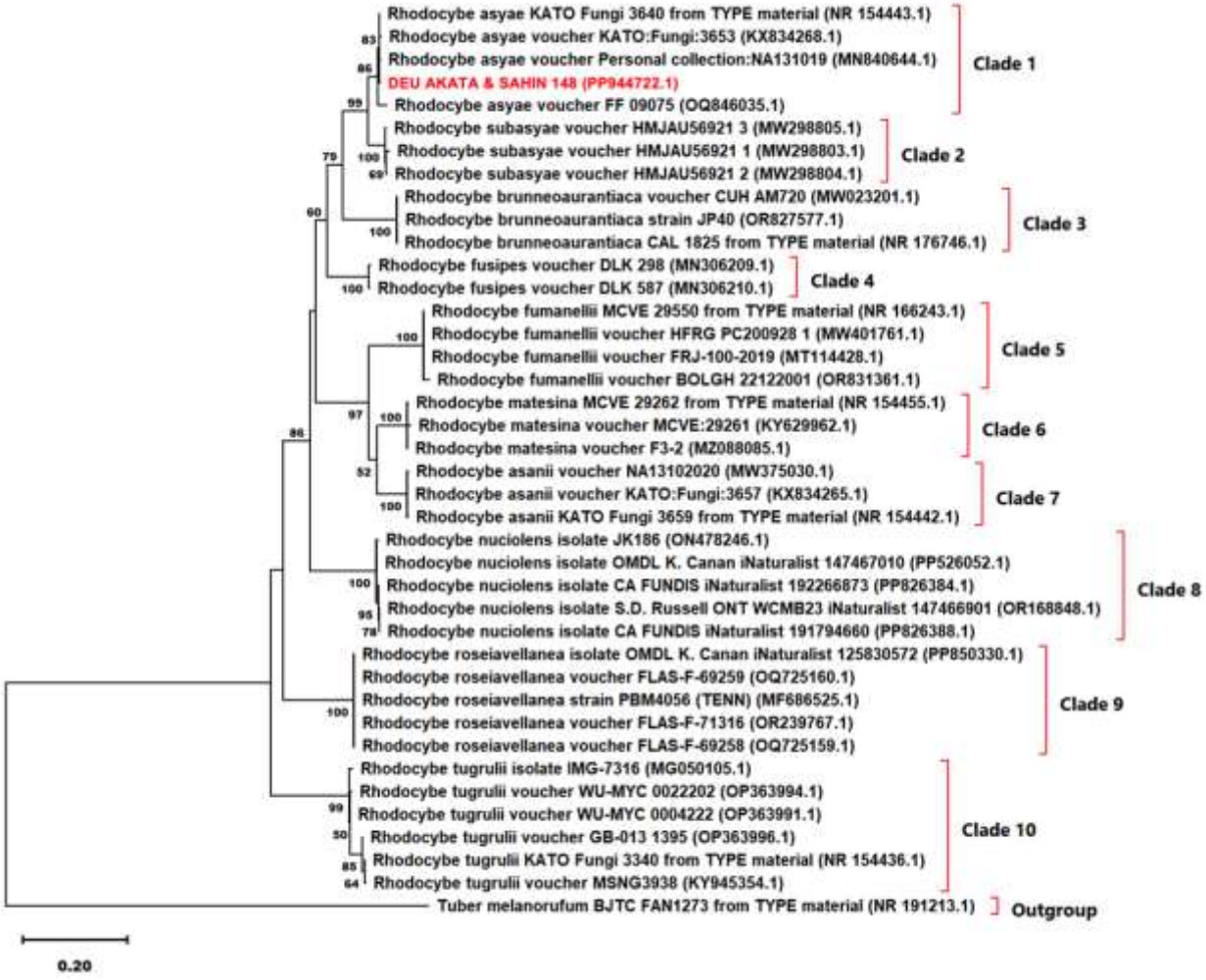

Figure 4. The phylogenetic tree, constructed using the nrITS rDNA region and the Neighbour Joining algorithm with the T92+G nucleotide substitution model, reveals the evolutionary relationships among 40 fungal specimens. Confidence levels were indicated by assigning bootstrap rates (≥50) to each branch. The sequences used in constructing the tree were obtained from the NCBI GenBank, except DEU AKATA & SAHIN 148. Additionally, *Tuber melanorufum* was included in the phylogenetic tree as the outgroup representative. A GenBank accession number accompanies each sequence, and a scale bar in the lower left corner represents a genetic distance of 0.20.

Şekil 4. nrITS rDNA bölgesi ve T92+G nükleotit yerini alma modeli ile Komşu Birleştirme algoritması kullanılarak oluşturulan filogenetik ağaç, 40 mantar örneği arasındaki evrimsel ilişkileri ortaya koyuyor. Güven seviyeleri, her şubeye önyükleme oranları (≥50) atanarak belirtildi. Ağacın oluşturulmasında kullanılan diziler, DEU AKATA & SAHIN *148 hariç, NCBI GenBank'tan elde edildi. Ek olarak Tuber melanorufum, dış grup temsilcisi olarak filogenetik ağaca dahil edildi. Ağaçtaki her diziye ait GenBank erişim numarası belirtilmiş ve sol alt köşedeki ölçek çubuğu 0.20'lik genetik mesafeyi temsil etmektedir.*

Rhodocybe fusipes are distinguished by their orange to red colored pileus and prominently expansive umbonate center. Its lamellae are white, adnate to slightly decurrent, close or crowded, and sometimes forked. The stipe transitions from white to a subtle sordid orange and is characterized by a tapering that culminates in a distinctively fusiform base. It produces subglobose to broadly ellipsoid basidiospores and differs with the absence of cheilocystidia. Pileipellis is similar to a cutis and additionally, *R. fusipes* lacks hymenial pseudocystidia and clamp connections. The central stipe of this species displays a reddish hue. It is found in the biodiverse Amazonian forests, which are rich in families such as *Lecythidaceae*, *Sapotaceae*, *Burseraceae*, and *Fabaceae* members but do not include coniferous trees. *R. fusipes* can be distinguished from *R. asyae* with the color of pileus, larger stem, unique spore morphology, and the absence of cheilocystidia (Silva-Filho et al., 2020).

R. subasyae differs from orange-white to beige-red and 19–25 mm pileus. It grows under the canopy of mixed forests composed of pine, larch, and oak trees. It is distinguished with sinuate to adnate lamellae, ellipsoid to broadly ellipsoid, and 5.4–6.8 × 3.9–4.9 µm basidiospores. Its lamellae range from adnexed to sinuate. The stipe is 22–37 × 5–7 mm, cylindrical, fibrillose, and orange-white and with white rhizomorphs. Cheilocystidia are 22.4–28.2 × 3.9–6.8 µm, slenderly clavate, and occasionally branched (Sesli & Vizzini, 2017; Sun & Bau, 2023).

In the study conducted by Sesli and Vizzini (2017), the morphological traits of *Rhodocybe asyae* samples are thoroughly documented, detailing specific features of various macroscopic structures, the properties, and dimensions of basidiospores, basidia, cheilocystidia, and other microscopic structures in the hymenium, pileus, and stipe. Similarly, Aplin et al. (2022) described some macroscopic structures and the properties and dimensions of basidiospores and basidia. Table 2. compares the specimens of *R. asyae* analyzed in the present study with those documented by Sesli and Vizzini (2017) and Aplin et al. (2022). This table thoroughly details the dimensions of various macroscopic and microscopic structures observed in the presented samples, providing a comprehensive overview of their measurements. The detailed comparison highlights the similarities and differences in measurements and characteristics between the present study's samples and those of the type specimens and the specimens analyzed by Aplin et al. (2022).

The comparative analysis of the macroscopic and microscopic features of *R. asyae* specimens with those documented by Sesli & Vizzini (2017) and Aplin et al. (2022) highlights a significant degree of congruence, particularly regarding dimensional attributes. This consistency reinforces the reliability of these characteristics as critical identifiers for the species. However, the present findings also reveal some notable discrepancies. Specifically, the habit of the present specimens diverges from the previously reported descriptions. While Sesli & Vizzini (2017) characterized the type specimen as having a different habit (clitocyboid), the present specimens exhibit a distinct tricholomatoid habit. This variation in habit could suggest potential intraspecific diversity or environmental influences affecting morphological expression. Additionally, the analysis of microscopic features aligns with previous studies, further validating the species identification.

Table 2. Comparison of various measurements of the morphological structures of *Rhodocybe asyae*
Çizelge 2. Rhodocybe asyae'nin morfolojik yapılarının çeşitli ölçümlerinin karşılaştırılması

Dimensions	Sesli and Vizzini (2017)	Aplin et al. (2022)	Current study
Habit	clitocyboid	not provided	tricholomatoid
Pileus	10–30 mm broad	up to 35 × 5 mm	15–30 mm broad
Stipe	25–30 × 2–5 mm	up to 35 mm	25–30 × 5–7 mm
Basidiospores (average)	(4.8–)5–7(–8) × 4–5(–5.5) µm	5.2–6.8 x 3.5–4.6 µm	(4–)4.5–7.5 (–8) × (3–)3.2–5.3(–5.6) µm
Basidiospores (Q-values)	Q = 1.1–1.4, Qav = 1.3	not provided	Q = 1.18–1.45 Qav = 1.39
Basidia	20–30 × 6.5–8.5 µm	not provided	24–32 × 6.5–9 µm
Sterigmata	3–8 µm long	not provided	3–5 µm long
Cheilocystidia	20–30 × 4–6 µm	not observed	18–24 × 4–6 µm
hymenophoral trama (hyphae)	6–15 µm broad	not provided	6.5–12.5 µm broad
Pileipellis (upper layer)	3–5 µm broad	not provided	3–4.5 µm broad
Pileipellis (lower layer)	6–12 µm broad	not provided	6.5–10 µm
Stipitipellis	7–16 µm broad	not provided	4–8 µm broad

Contribution Of The Authors As Summary

The authors declare that their contributions are equal.

Statement Of Conflict Of Interest

The authors have declared no conflict of interest.

REFERENCES

Akata, I., & Erdoğdu, M. (2020). First report of *Rutstroemia elatina* (Ascomycota) from Turkey. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *23*(2), 391-395.

Akata, I., Kumru, E., Ediş, G., Özbey, B.G. & Şahin. E. (2023). Three New Records For Turkish *Agaricales* Inhabiting Ankara University Beşevler 10th Year Campus Area. *Kastamonu University Journal of Forestry Faculty*, *23*(3), 250-263.

Akata, I., Kumru, E., Sahin, E., Acar, İ. & Kaya, E. (2024a). *Amanita vidua*: A new record for Turkish *Amanita* Section *Phalloideae* based on morphological and molecular data. *Trakya University Journal of Natural Sciences*, *25*(1), 97-110.

Akata, I., Ediş, G., Kumru, E., Acar, İ. & Şahin, E. (2024b). Initial Report of Inocybe costinitii in Türkiye with Morphological and Molecular Data. KSU J. Agric Nat *27* (6), 1304-1312.

Altuntaş, D., Sahin, E., Kabaktepe, Ş., Alli, H., & Akata, I. (2021). *Albatrellopsis flettii*, A New Genus for Turkish Albatrellaceae. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *24*(4), 815-819.

Aplin, N., Cullington, P., Douglas, B., & Janke, E. (2022). DNA barcoding reveals three *Rhodocybe* species new to Britain. *Field Mycology*, *23*(2), 41-47.

Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (1988). *Flora Agaricina Neerlandica*. Vol. 1. Rotterdam, the Netherlands: Balkema.

Baroni, T.J. (1981). A revision of the genus *Rhodocybe* Maire (Agaricales). *Beihefte Zur Nova hedwigia* 67, 1-198.

Kluting KL, Baroni TJ & Bergemann SE. (2014). Toward a stable classification of genera within the Entolomataceae: a phylogenetic re-evaluation of the Rhodocybe-Clitopilus clade. *Mycologia 106*(6), 1127-1142.

Marian, I.M., Valdes, I.D., Hayes, R.D., La Butti, K., Duffy, K., Chovatia, M., Johnson J., Ng, V., Lugones L.G., Wösten, H.A.B., Grigoriev, I.V. & Ohm, R.A. (2024). High phenotypic and genotypic plasticity among strains of the mushroom-forming fungus *Schizophyllum commune*. *BioRxiv,* 2024-02.

Raja, H.A., Miller, A.N., Pearce, C.J. & Oberlies, N.H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. Journal of Natural Products, *80*(3), 756-770.

Sesli, E. (2021). *Rhodocybe cistetorum* (Basidiomycota, Entolomataceae), is a new species from the Colchic ecoregion of Turkey. *Nordic Journal of Botany*, *39*(4),1-9.

Sesli, E., & Vizzini, A. (2017). Two new *Rhodocybe* species (sect. *Rufobrunnea*, Entolomataceae) from the east black sea coast of Turkey. *Turkish Journal of Botany*, *41*(2), 200-210.

Sesli, E., Asan, A., Selçuk, F., Arabacı Günyar, Ö., Akata, I., Akgül, H., Aktaş, S., Alkan, S., Allı, H., Aydoğdu, H., Berikten, D., Demirel, K., Demirel, R., Doğan, H.H., Erdoğdu, M., Ergül, C.C., Eroğlu, G., Giray, G.., Halik Uztan, A., Kabaktepe, Ş., Kadaifçiler, D., Kalyoncu, F., Karaltı, İ., Kaşık, G., Kaya, A., Keleş, A., Kırbağ, S., Kıvanç, M., Ocak, İ., Ökten, S., Özkale, E., Öztürk, C., Sevindik, M., Şen, B., Şen, İ., Türkekul, İ., Ulukapı, M., Uzun, Ya., Uzun, Yu. & Yoltaş, A. (2020). *Türkiye Mantarları Listesi*. Ali Nihat Gökyiğit Vakfı Yayını, İstanbul, 1177 pp.

Silva-Filho A.G.S., Baroni T.J., Komura D.L., Moncalvo J. M., Baseia I.G. & Wartchow F. (2020). *Rhodocybe fusipes* (Entolomataceae) is a new species from Amazonian 'terra-firme' Forest of Brazil. *Sydowia* 72,163-170.

Sun, Y. L., & Bau, T. (2023). *Rhodocybe subasyae*, a new species of *Rhodocybe* sect. Rufobrunnea (Entolomataceae, Agaricales) from northeast China. *Mycoscience*, *64*(3), 96-100.

Vizzini A, Baroni TJ, Sesli E, Antonín V. & Saar I (2016). *Rhodocybe tugrulii* (Agaricales, Entolomataceae), a new species from Turkey and Estonia based on morphological and molecular data, and a new combination in *Clitocella* (Entolomataceae). *Phytotaxa,* 267, 1-15.

Vizzini, A., Ferrari, R. J., Ercole, E. & Fellin, A. (2018). A new species of *Rhodocybe* sect. *Rufobrunnea* (Entolomataceae, Agaricales) from Italy. *MycoKeys* 36, 21-33.