



Newly Reported *Elaphomyces* Species from Kırklareli, Türkiye: A Morphological and Molecular Approach

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ABSTRACT

This study aims to enhance our understanding of *Elaphomyces* diversity in Türkiye by combining morphological and molecular techniques to document newly discovered species. Fungal samples were collected from Kırklareli Province between October and December 2022. Their morphological characteristics were examined using light microscopy and scanning electron microscopy, while molecular phylogenetic analyses of nrITS (rDNA) sequences provided complementary evidence for species identification. The research identified three new Turkish collections: *Elaphomyces aculeatus*, *E. americanus*, and *E. virgatosporus*, whose ITS sequences showed over 99% similarity to corresponding entries in the GenBank database. Additionally, this study provides detailed morphological descriptions, habitat information, and phylogenetic analyses.

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Kırklareli, Türkiye'den Yeni Bildirilen *Elaphomyces* Türleri: Morfolojik ve Moleküler Bir Yaklaşım

ÖZET

Bu çalışma, yeni rapor edilen türleri belgelemek amacıyla morfolojik ve moleküler teknikleri bir araya getirerek Türkiye'deki *Elaphomyces* çeşitliliğine dair bilgilerimizi geliştirmeyi hedeflemektedir. Mantar örnekleri, Ekim-Aralık 2022 tarihleri arasında Kırklareli ilinden toplanmıştır. Morfolojik özellikleri ışık mikroskobu ve taramalı elektron mikroskobu kullanılarak incelenmiş, nrITS (rDNA) dizilerine dayalı moleküler filogenetik analizler ise türlerin teşhisinde tamamlayıcı kanıtlar sağlamıştır. Araştırma sonucunda, ITS dizileri GenBank veritabanındaki karşılık gelen kayıtlarla %99'un üzerinde benzerlik gösteren, Türkiye'den üç yeni kayıt belirlenmiştir: *Elaphomyces aculeatus*, *E. americanus* ve *E. virgatosporus*. Ayrıca, bu çalışma ayrıntılı morfolojik tanımlar, habitat bilgileri ve filogenetik analizler sunmaktadır.

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INTRODUCTION

Elaphomyces T. Nees, commonly referred to as "deer truffles," is a genus of significant ecological and biological importance within the family *Elaphomycetaceae* Tul. ex Paol., order *Eurotiales* G.W. Martin ex Benny & Kimbr., and division *Ascomycota* (Castellano et al., 2019; Cabero et al., 2019). This subterranean ectomycorrhizal (ECM) fungus plays a crucial role in the functionality and sustainability of temperate and subarctic forest ecosystems. By forming mutualistic symbiotic relationships with various hardwood and coniferous trees, *Elaphomyces* enhances

nutrient absorption, promotes tree health, and supports the structural integrity and resilience of forest environments (Castellano & Stephens, 2017; Paz et al., 2017; Castellano et al., 2018). In addition to its ecological importance, this genus is essential for supporting complex forest food webs, as it serves as a key dietary component for mycophagous animals, thereby contributing to the maintenance of biodiversity (Castellano et al., 2012a; Paz et al., 2017; Elliott et al., 2022).

Elaphomyces species are primarily found at greater soil depths than other hypogeous fungi. Nevertheless, their distinctive aroma enables trained dogs to detect them easily, facilitating efficient foraging. A notable characteristic that sets this genus apart from many truffle species is its year-round detectability; it is not limited by seasonal changes, making it a consistent and reliable resource for truffle hunters (Molia et al., 2020). Geographically, *Elaphomyces* exhibits remarkable adaptability, with a distribution that spans several continents, including Europe, North America, South America, Oceania, Africa, and Asia, underscoring its widespread presence (Shirakawa & Tanaka, 2020).

Initially classified by Persoon as *Scleroderma cervina* and later verified by Fries, this genus comprises notable species, including *Elaphomyces granulatus* and *E. muricatus* (Molia et al., 2020). Young *Elaphomyces* specimens are characterized by a large central cavity filled with cottony hyphae, which eventually transform into a powdery mass of ascospores (Castellano et al., 2021). These spores, which vary in colour from yellow to black, are enclosed within a thick peridium, a distinctive feature of the genus (Castellano & Stephens, 2017). The genus currently comprises approximately 100 recognised species worldwide, with Europe hosting more than 35 described taxa, reflecting its diversity in the region (Castellano et al., 2011, 2012a, 2012b, 2016, 2018, 2021; Buyck et al., 2016; Paz et al., 2017; Sukarno et al., 2018; Cabero et al., 2019; Crous et al., 2020, 2021; Shirakawa & Tanaka, 2020; Tan et al., 2022; de la Fuente et al., 2023a, 2023b).

Despite significant efforts in mycological research within Türkiye, the classification of *Elaphomyces* species has predominantly relied on traditional morphological approaches. These conventional methods, focused on macroscopic and microscopic characteristics, provide only a preliminary understanding of species diversity and fail to meet the rigorous standards of contemporary taxonomy, which increasingly prioritises molecular data for accurate identification. Türkiye's current *Elaphomyces* records include eight species: *E. anthracinus* Vittad., *E. citrinus* Vittad., *E. cyanosporus* Tul. & C. Tul., *E. decipiens* Vittad., *E. granulatus* Fr., *E. leucocarpus* Vittad., *E. muricatus* Fr., and *E. septatus* Vittad. (Türkoğlu et al., 2015; Uzun and Kaya, 2019, 2020, 2021; Uzun, 2021). However, these identifications are predominantly based on basic morphological observations, often neglecting detailed structural features and molecular evidence. Such a limited approach not only obscures the accurate delimitation of species but also poses challenges in distinguishing morphologically similar taxa. Moreover, the absence of molecular validation raises concerns about the reliability of these classifications, leaving the identification of cryptic species, the assessment of intraspecific genetic diversity, and phylogenetic relationships largely unexplored. Consequently, the current understanding of *Elaphomyces* diversity in Türkiye remains fragmented, underscoring the critical need to integrate molecular techniques to establish a more accurate and comprehensive taxonomic framework.

This study combines morphological and molecular techniques to create a more accurate taxonomic framework for *Elaphomyces* in Türkiye. By utilising DNA sequencing alongside traditional assessments, it aims to uncover unrecognised species, resolve taxonomic uncertainties, and establish a dependable classification of *Elaphomyces*. Molecular techniques, particularly phylogenetic analyses, will enhance our understanding of species boundaries and evolutionary relationships, providing a robust evaluation of *Elaphomyces* diversity. This method addresses the deficiencies of previous studies and lays a precise groundwork for future mycological research in Türkiye.

MATERIAL and METHOD

This study employed a comprehensive approach combining traditional and molecular methods. The goal is to identify and classify *Elaphomyces* specimens collected from the European region of Türkiye. The extensive analysis included detailed morphological assessments, sequence comparisons, and phylogenetic analyses focused on the nrITS region of rDNA.

Study Area

Kırklareli, situated in the Thracian region of northwestern Türkiye, spans an area of 6,650 km², characterised by diverse geographical features shaped by the Yıldız Mountains and the Ergene Plain. It is positioned between latitudes 41° 13' 34" N and 42° 05' 03" N, and longitudes 26° 54' 14" E and 28° 06' 15" E. Bordered by Bulgaria to the north, the Black Sea to the northeast, Tekirdağ to the south and southeast, and Edirne to the west, Kırklareli is an essential ecological transition zone. The region boasts lush forests predominantly composed of hornbeam, oak, and beech, alongside distinctive species such as alder, ash, and willow, providing habitats for various species and enhancing ecological resilience (Kültür, 2007; Morgül, 2014). Kırklareli exhibits notable climatic variability;

the northern slopes of the Yıldız Mountains have a humid Black Sea climate, in contrast to the continental climate prevalent in the inland Ergene Basin, characterised by substantial seasonal variations in temperature and precipitation (Gençer & Yüksek, 2022). December records the highest average rainfall at 68.81 mm, while August has the least at 21.54 mm. The temperature ranges from an average high of 36.14 °C in July to a low of -8.16 °C in January. Annual precipitation increases with elevation on the northern slopes yet decreases towards the south (Yılmaz, 2023). These climate variations, along with diverse microhabitats and soil types, create optimal conditions for fungal development, particularly for *Elaphomyces* species.

Figure 1 displays the map of the study area, clearly indicating the locations where *Elaphomyces* specimens were collected. Similarly, Table 1 offers detailed information for each sampling site, including site names, elevation, and geographic coordinates, providing important context for understanding the distribution of the collected material.



Figure 1. Map of study area.

Şekil 1. Araştırma alanının haritası.

Table 1. Localities, elevations, and coordinates of collected *Elaphomyces* specimens.

Çizelge 1. Toplanan *Elaphomyces* örneklerine ait lokaliteler, yükseltiler ve koordinatlar.

Localities	Coordinates	Altitude (m)
Locality 1 (Centre)	41°56' N, 27°20' E	524
Locality 2 (Demirköy)	41°48' N, 27°48' E	212
Locality 3 (Demirköy)	41°54' N, 27°56' E	16
Locality 4 (Vize)	41°31' N, 27°54' E	21
		200

Field study

Elaphomyces samples were gathered with the assistance of truffle dogs (Lagotto Romagnolo) (Figure 2), renowned for their remarkable truffle detection abilities (Rusbridge & Wilkins, 2002). Initially bred as water retrievers, these dogs have an exceptional sense of smell, making them particularly skilled at finding truffles (Olivier et al., 2018; Čejka et al., 2022). Their notable olfactory skills allow them to detect the unique scent of truffles that are buried underground, facilitating effective and precise harvesting. As a result, Lagotto Romagnolo dogs have emerged as the favoured companions for truffle hunters globally, providing a reliable and sustainable approach to unearthing these valuable fungi (Allen & Bennett, 2021; Bach et al., 2021). The sampling primarily took place in oak and beech forests (Figure 3), which serve as excellent habitats for *Elaphomyces*. These forests provide essential ecological conditions, including suitable soil composition, adequate moisture, and a symbiotic relationship with host trees that promotes the growth of *Elaphomyces*. Careful observations were made during the collection process to ensure the comprehensive documentation of each specimen's ecological context. Throughout the sampling process, thorough records were kept, capturing critical details such as collection dates, precise GPS coordinates, in-depth habitat descriptions, and specific information about the sampling sites.



Figure 2. Field studies with truffle dogs (Lagotto Romagnolo).

Şekil 2. Trüf köpekleri (Lagotto Romagnolo) eşliğinde arazi çalışmaları.

Molecular Characterization

The specimens were collected and examined for their macroscopic traits and environmental conditions. Thorough examinations were performed with a binocular light microscope (LM) for precise observation. To enhance structural visualisation, samples were treated with a 5% potassium hydroxide (KOH) solution and other reagents before analysis. For scanning electron microscopy (SEM), fungal samples were placed on stubs with double-sided tape and coated with a thin layer of gold for conductivity. High-resolution imaging and surface assessments were conducted with an EVO 40XVP SEM (LEO Ltd., Cambridge, UK) at 20 kv. After identification, the specimens were preserved in the Fungarium at Ankara University's Faculty of Science, Department of Biology, for long-term storage and research availability.

Extraction of Genomic DNA from Fungal Samples

To extract genomic DNA, 50 mg of dried sporophore samples were ground to a fine powder using a mechanical mill grinder and placed in 1.5 mL microcentrifuge tubes. The powdered material was mixed with 700 µL of CTAB lysis buffer (pH 8.0), containing 3% cetyltrimethylammonium bromide, 1.4 M sodium chloride (NaCl), 20 mM ethylenediaminetetraacetic acid (EDTA), 100 mM Tris-HCl, 3% polyvinylpyrrolidone (PVP), and 0.2% β-mercaptoethanol. This mixture was vortexed for one minute and incubated at 65 °C for 30 minutes to facilitate lysis. After centrifuging at 13,000 rpm for 10 minutes, 500 µL of the supernatant was transferred to new tubes. To remove impurities, an equal volume of chloroform-isoamyl alcohol (24:1) was added, followed by brief vortexing and centrifugation at 13,000 rpm for 5 minutes. The upper aqueous layer was carefully collected and mixed with an equal volume of chilled isopropanol. The mixture was then stored at -20 °C for 30 minutes to precipitate the DNA. The samples were centrifuged again at 13,000 rpm for an additional 10 minutes to collect the DNA pellets, which were then washed twice with 70% ethanol to remove contaminants. The residual ethanol was evaporated by drying the pellets at 60 °C, after which they were dissolved in nuclease-free distilled water. DNA concentration and purity were measured using a Nanodrop Lite spectrophotometer (Thermo Scientific). The integrity of the genomic DNA was confirmed via agarose gel electrophoresis on a 0.8% agarose gel with TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.3), performed at 5 V/cm and visualised with a blue-light transilluminator using a safe green dye. A 1 kb Plus DNA Ladder served as the size marker for the electrophoresis analysis (Akata et al., 2024a, 2024b, 2024c, 2024d, 2025a).

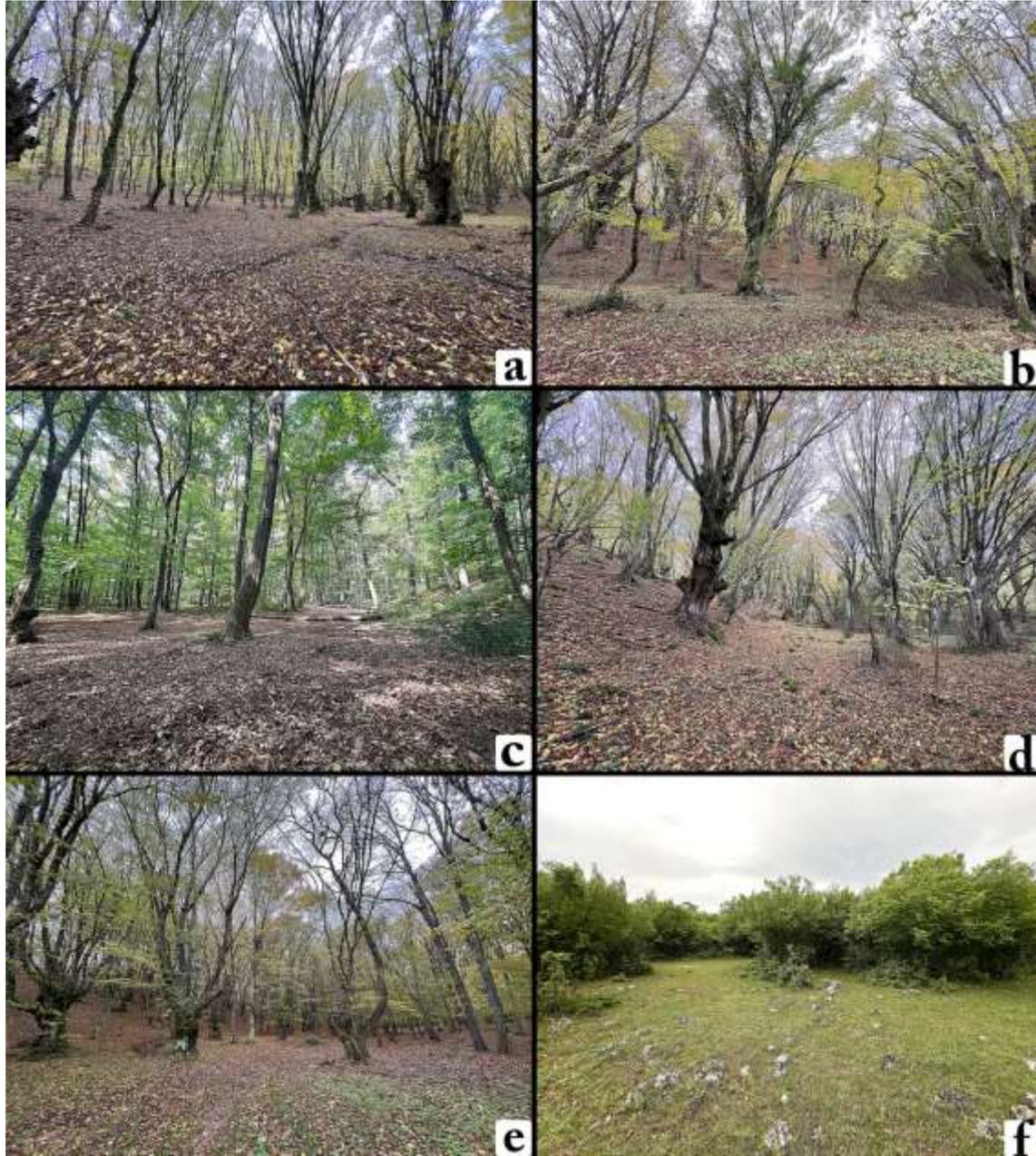


Figure 3. Collection areas: a-e. oriental beech forests, f. oak forest.

Şekil 3. Örnekleme alanları: a-e. doğu kayını ormanları, f. meşe ormanı.

PCR Amplification of ITS rDNA and Nucleotide Sequencing for Molecular Phylogenetic Analysis of Specimens

Genomic DNA was extracted from *Elaphomyces* ascomata utilising the CTAB method to amplify the internal transcribed spacer (ITS) region, which is essential for molecular phylogenetic analysis of fungi. A hot-start DNA polymerase was employed to minimise non-specific primer dimers during PCR. PCR reactions were conducted in 0.2-mL tubes, each containing a total volume of 50 μ L. This mixture comprised 5 μ L of 10X DNA polymerase buffer (25 mM $MgCl_2$), 1 μ L of a 10 mM dNTP mix, between 300-400 ng of genomic DNA, 1 μ L of forward primer ITS1 (10 μ M) and 1 μ L of reverse primer ITS4 (10 μ M), yielding a final concentration of 0.2 μ M for each primer in a 50 μ L reaction five units of DNA polymerase, and nuclease-free distilled water to reach the final volume. PCR conditions were optimised for each primer pair in accordance with their T_m and the lengths of the target genes. To minimise off-target amplification and primer dimer formation, a "Touchdown" PCR protocol was utilised (Akata et al., 2025b; Ediş et al., 2025; Kumru et al., 2025).

The refined thermocycling process commenced with an initial denaturation step at 95 °C for 2 minutes, followed by 35 cycles consisting of denaturation (30 seconds at 95 °C), annealing (15 seconds with a gradual temperature decrease from 65 °C to 50 °C via the touchdown method), and extension (15-30 seconds at 72 °C, adjusted based on

amplicon length and the DNA polymerase utilised). The protocol concluded with a 7-minute extension at 72 °c. All reactions were performed using the MiniAmp Plus Thermal Cycler (Applied Biosystems). Successful amplification was verified by running the PCR products on a 1% agarose gel prepared with 1× TBE buffer and electrophoresed at 140 V for 40 min, where a single, distinct band indicated successful amplification, free from non-specific products, assessed with a GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific) as the molecular size marker (Akata et al., 2024a, 2024b).

PCR products were purified using the GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific) according to the manufacturer's guidelines, by applying the PCR cleanup protocol without gel extraction. The concentration and purity of the purified amplicons were assessed with a Nanodrop Lite spectrophotometer. DNA sequencing was performed using the Sanger method with the same oligonucleotide primers used in PCR amplification. Sequencing and analysis of the amplicons were outsourced to an external laboratory to ensure high-quality sequence data for phylogenetic studies (Akata et al., 2024a, 2024b, 2024c, 2025a, 2025b; Ediş et al., 2025; Kumru et al., 2025).

Molecular Phylogenetic Analyses

A molecular phylogenetic analysis of fungal samples was conducted using MEGA-X software (<https://www.megasoftware.net>) with nucleotide sequences. The amplicon sequences were queried in NCBI's Nucleotide BLAST to identify homologous sequences. Sequences from the GenBank DNA database that matched the analyzed amplicon sequences were chosen as the ingroup for further examination. Conversely, distantly related macrofungi sequences without significant similarity were designated as the outgroup. Sequences were aligned with MUSCLE as implemented in MEGA X under default parameters (Kumar et al., 2018). The final alignment comprised 635 bp, including 118 variable and 87 parsimony-informative sites, under pairwise deletion. The best-fit nucleotide substitution model, Tamura–Nei (TN93) with gamma-distributed rate variation (+G), was selected using MEGA X's "Find Best DNA/Protein Models" tool according to the Bayesian Information Criterion (BIC) (Tamura & Nei, 1993). Phylogenetic trees were inferred using the Neighbor-Joining method under TN93+G distances. Node support was assessed using standard nonparametric bootstrap resampling with 1,000 pseudoreplicates (random seed initialised by the software); bootstrap values ≥50% are shown at corresponding nodes. Phylogenetic trees were generated using the Neighbour-Joining algorithm, a well-recognised method. To evaluate the robustness of the tree's branching patterns, a bootstrap analysis with 1,000 replicates was conducted, providing statistical validation for the inferred relationships (Akata et al., 2025a, 2025b; Ediş et al., 2025; Kumru et al., 2025).

RESULTS

This study examined both the macroscopic and microscopic characteristics of our samples and analysed their ribosomal DNA (rDNA) sequences using Internal Transcribed Spacer (ITS) sequencing.

Taxonomic overview

1. *Elaphomyces aculeatus* Vittad. (1831), (Figure 4a,b, Figure 5).

Macroscopic and microscopic features

Ascomata 15-20 mm broad, globose to subglobose, featuring a purplish-brown to reddish-brown and black peridial surface, often with a reddish hue, and covered with pyramidal warts, each tipped with black. **Gleba** solid, with a texture ranging from powdery to cottony, exhibiting a blackish-brown color interspersed with white streaks. **Peridium** overall up to 1 mm thick, composed of two distinct layers. **Outer layer** composed of reddish-brown hyphae measuring up to 4 µm in width, thick-walled. **Inner layer** pseudoparenchymatous, made up of hyaline, angular cells measuring up to 6 µm in width, often organised in intermingled hyphae up to 6 µm in width, hyaline, and septate. **Spores** 15.5–19 µm (mean: 17.4 µm), globose, brown to blackish-brown, and thick-walled, densely ornamented with fine spines, forming an irregularly asperulate surface.

Ecology and distribution: This rare European species primarily forms ectomycorrhizal associations with common hazel (*Corylus avellana*), beech (*Fagus sylvatica*), lime (*Tilia* spp.), and oak (*Quercus* spp.), typically on calcareous soils; it has also occasionally been recorded with chestnut (*Castanea sativa*) and hornbeam (*Carpinus betulus*). (Bratek et al., 2013; Paz et al., 2017; Chachula et al., 2020; Molia et al., 2020).

Material examined: Locality 1, under beech, 6 Oct. 2022, ANK AKATA TT 153 (nrITS rDNA sequence GenBank accession number: PV069102).

2. *Elaphomyces americanus* Castellano (2017), (Figure 4c,d, Figure 6).

Macroscopic and microscopic features

Ascomata 10-15 mm broad, almost globose, forming an irregular husk with soil, ectomycorrhizal roots, and debris, yellowish-brown peridial surface covered mainly with acutely pointed warts, along with some blunt ones. **Gleba**

packed with dark brown, powdery spores entwined with fragile, cobweb-like hyphae. **Peridium** overall up to 1.4 mm thick, composed of two distinct layers. **Outer layer** composed of densely packed yellow-brown hyphae measuring up to 4 µm in width, tightly agglutinated, compact, curly, and contorted, with walls beneath the wart-like projections, along with a secondary layer of septate, hyaline hyphae measuring up to 7 µm broad, forming a parallel network. **Inner layer** composed of hyphae up to 5 µm broad, densely packed with pigment granules, producing a uniform dark coloration, masking the boundary between white and dark veins, ensuring a smooth transition with a macroscopically consistent texture. **Spores** 24–28 µm (mean: 25.5 µm), globose, brown, thick-walled, and adorned with tufted, coalescent rod-like projections on the surface.

Distribution and Ecology: This species has been recorded in North America, mainly found in sandy or clay-rich soils and often forming associations with conifers like balsam fir, Jack pine, white pine, and Canadian hemlock. It typically fruits from June to October, with a rare instance noted in December. (Castellano & Stephens, 2017).

Material examined: Locality 2, under beech, 8 Oct. 2022, ANK AKATA TT 160 (nrITS rDNA sequence GenBank accession number: PV069103).

3. *Elaphomyces virgatosporus* Hollós (1908), (Figure 4e,f, Figure 7).



Figure 4. Peridium: a,b. *Elaphomyces aculeatus*, c,d. *E. americanus*, e,f. *E. virgatosporus*.
Şekil 4. Peridyum: a,b. *Elaphomyces aculeatus*, c,d. *E. americanus*, e,f. *E. virgatosporus*.

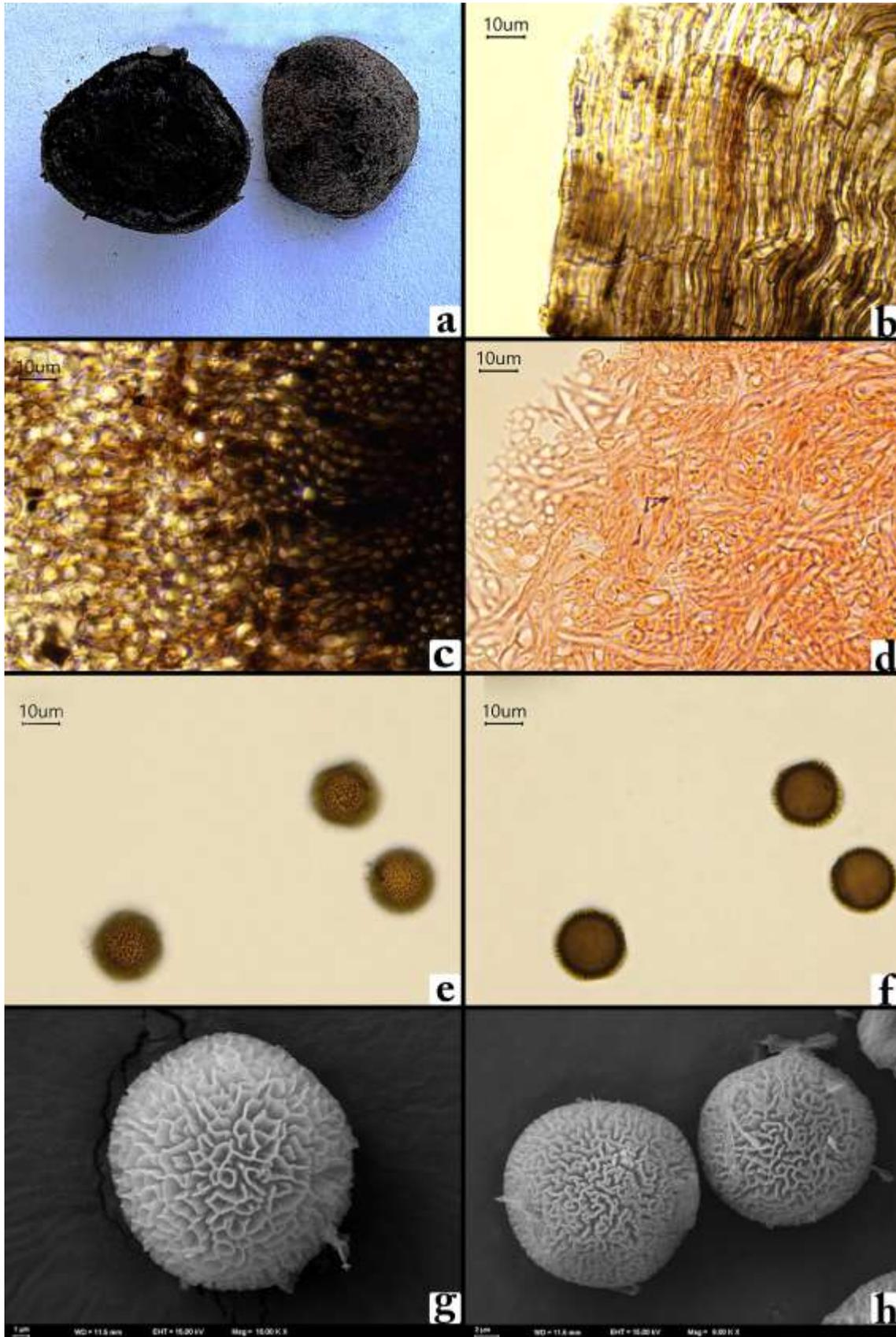


Figure 5. *Elaphomyces aculeatus* : a. ascomata, b-d. details of the peridium, e,f. spores (LM), g,h. spores (SEM),
Şekil 5. *Elaphomyces aculeatus* : a. askomata, b-d. peridyumun ayrıntıları, e,f. sporlar (LM), g,h. sporlar (SEM).

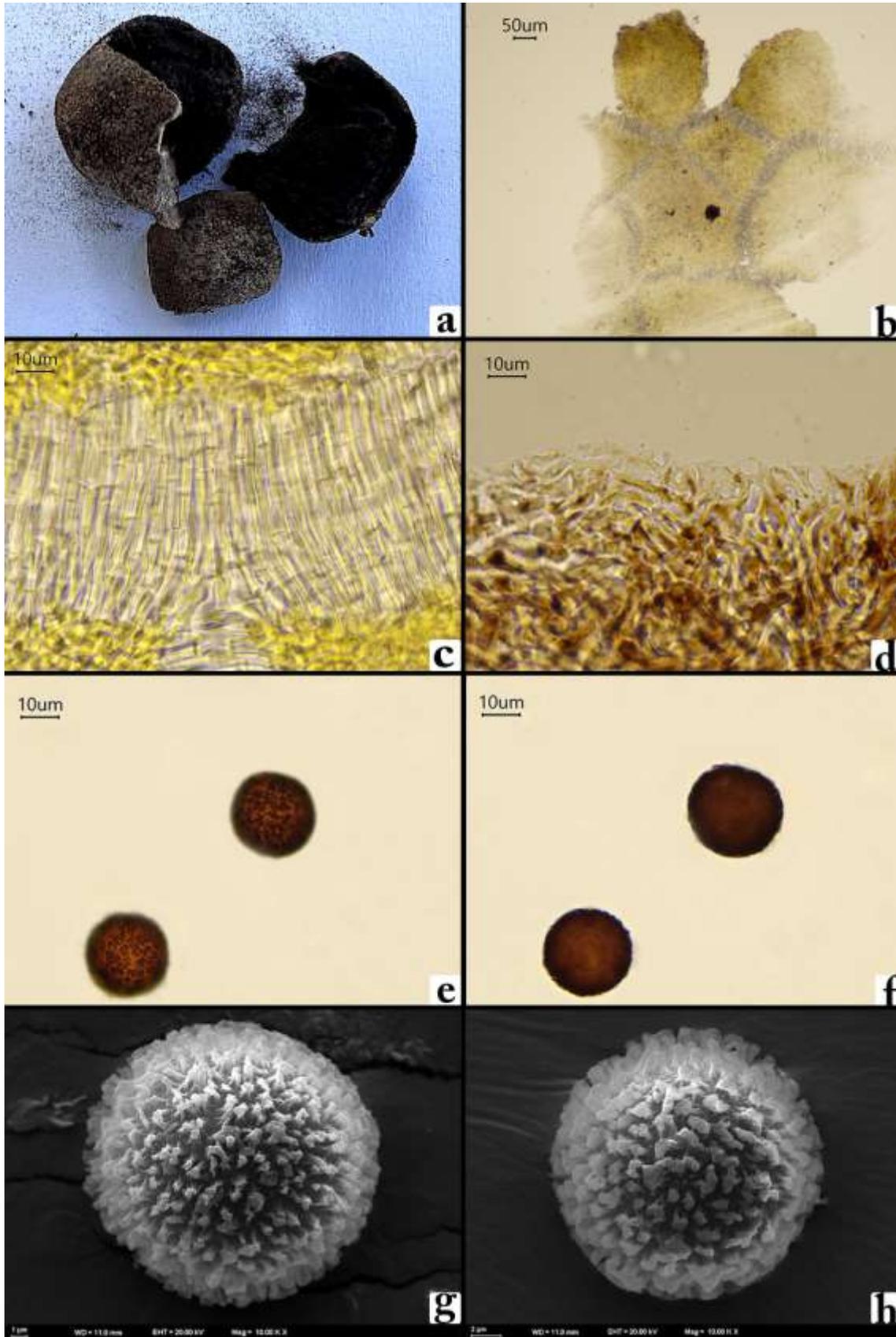


Figure 6. *Elaphomyces americanus*: a. ascomata, b-d. details of the peridium, e,f. spores (LM), g,h. spores (SEM),
Şekil 6. *Elaphomyces americanus* : a. askomata, b-d. peridyumun ayrıntıları, e,f. sporlar (LM), g,h. sporlar (SEM).

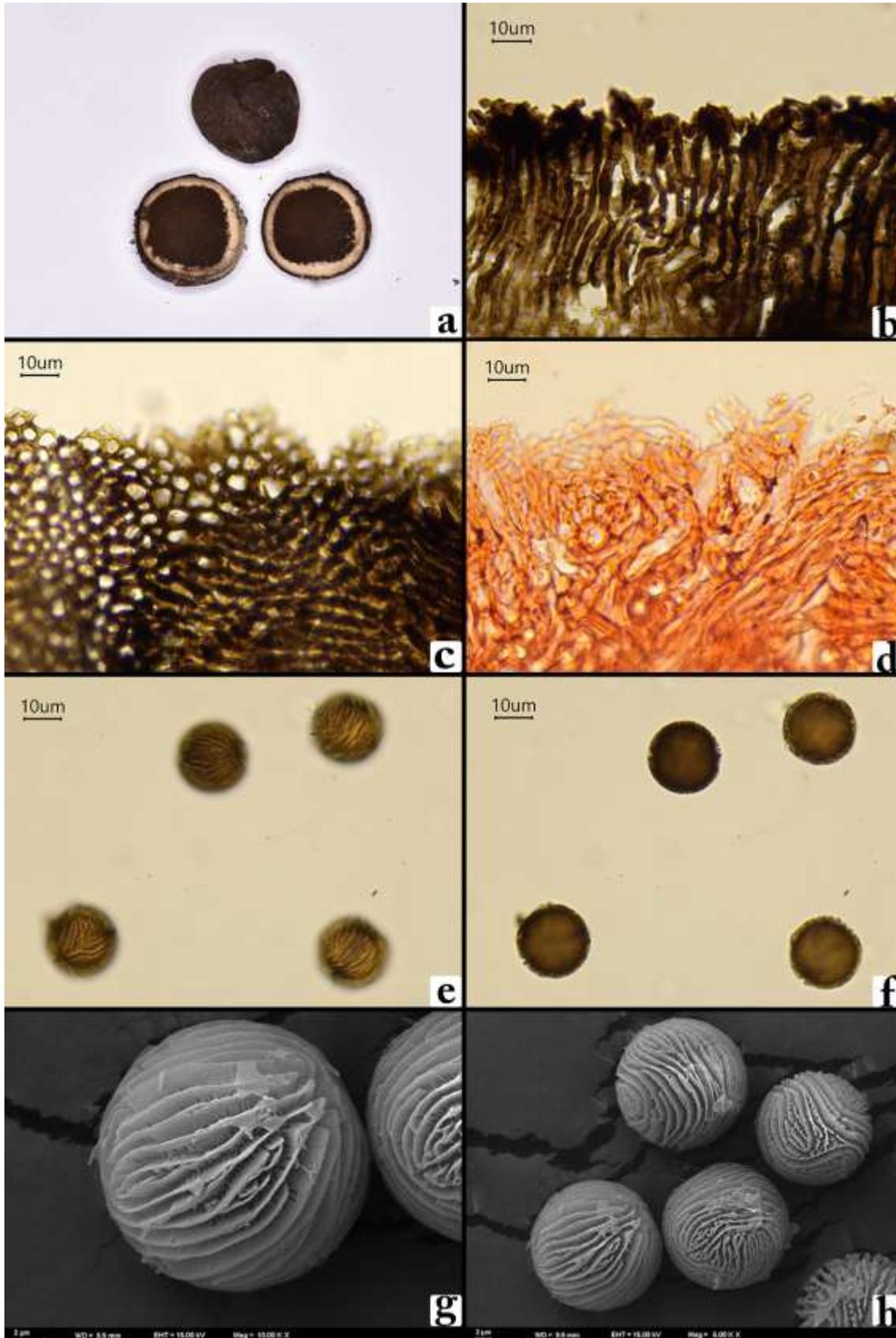


Figure 7. *Elaphomyces virgatosporus*: a. ascomata, b-d. details of the peridium, e,f. spores (LM), g,h. spores (SEM),
Şekil 7. *Elaphomyces virgatosporus*: a. askomata, b-d. peridyumun ayrıntıları, e,f. sporlar (LM), g,h. sporlar (SEM).

Macroscopic and microscopic features

Ascomata 20-25 mm broad, globose and firmly encrusted with a thick layer of soil, accompanied by some reddish-

brown mycelial cords. dark brown to black, covered with tiny, prominent, sharp, somewhat irregular verrucae warts. **Gleba** initially hollow and white, somewhat dull, but later matures to a dark reddish-brown. **Peridium** overall up to 1.2 mm thick, composed of two distinct layers. **Outer layer** formed by hyphae composed of short cells up to 7 µm broad, very intertwined, with thick walls and pigmented dark brown, almost black, alongside other globular and irregular hyphae. **Inner layer** composed of tightly twisted, creamy-white hyphae measuring 4–7 µm broad, and globular, irregular hyphae up to 8 µm broad, enveloping the gleba like a membrane. **Spores** 16–22.5 µm (mean: 20.7 µm), globose, and reddish-brown with an intricate ornamentation of more or less parallel ridges creating a striped impression.

Distribution and Ecology: This rare species, native to Europe, forms ectomycorrhizal relationships with deciduous trees such as hazel, oak, beech, and hornbeam, preferring calcareous, nutrient-rich soils (Siller et al., 2005; Paz et al., 2017; Molia et al., 2020) outside Europe, it has only been found in Mississippi, USA, where it is associated with Southern live oak (Kers, 1997; Læssøe et al., 2009).

Material examined: Locality 3, under beech, 19 Nov. 2022, ANK AKATA TT 229 (nrITS rDNA sequence GenBank accession number: PV069104); *ibid.*, ANK AKATA TT 237 (nrITS rDNA sequence GenBank accession number: PV069105); *ibid.*, ANK AKATA TT 259 (nrITS rDNA sequence GenBank accession number: PV069106); Locality 4, under oak, 8 Dec. 2022, ANK AKATA TT 358 (nrITS rDNA sequence GenBank accession number: PV069108).

Evolutionary History

The phylogenetic positions of specimens ANK AKATA TT 153, 160, 229, 237, 259, and 358 were assessed through analysis of their nuclear ribosomal internal transcribed spacer (nrITS) rDNA sequences. These sequences were generated via standard molecular protocols and are available in the NCBI GenBank, with accession numbers listed in Table 2.

Table 2. GenBank accession numbers and collection sites of *Elaphomyces* specimens examined.
 Çizelge 2. İncelenen *Elaphomyces* örneklerinin GenBank erişim numaraları ve toplama yerleri.

Species	Specimen Voucher/Isolate/Strain	nrITS GenBank Accession Number	Geographical origin	Reference
	AKATA TT 229	PV069104.1	Türkiye: Kırklareli, Demirkoy	Current study
	AKATA TT 237	PV069105.1	Türkiye: Kırklareli, Demirkoy	Current study
	AKATA TT 259	PV069106.1	Türkiye: Kırklareli, Demirkoy	Current study
	AKATA TT 358	PV069108.1	Türkiye: Kırklareli, Vize	Current study
<i>Elaphomyces virgatosporus</i>	personal collection:A. Paz IC26051213	KX238811.1	Spain: Asturias, San Esteban de Cunaba	Paz et al., 2017
	personal collection:A. Paz IC26031101	KX238809.1	Spain: Asturias, San Esteban de Cunaba	Paz et al., 2017
	personal collection:A. Paz IC06031103	KX238802.1	Spain: Asturias, San Esteban de Cunaba	Paz et al., 2017
	OF22179	KR029778.1	-	Direct submitted
<i>Elaphomyces aculeatus</i>	AKATA TT 153	PV069102.1	Türkiye: Kırklareli, Center	Current study
	JNitare820730	KR029777.1	-	Direct submitted
<i>Elaphomyces anthracinus</i>	OF22177	KR029774.1	-	Direct submitted
	OF22176	KR029773.1	-	Direct submitted
	LIP:0001145	NR_158403.1	Spain: Asturias, San Esteban de Cunaba	Paz et al., 2017
<i>Elaphomyces anthracinus f. talosporus</i>	personal collection:A. Paz IC06070803	KX238800.1	Spain: Cantabria, Saja	Paz et al., 2017
	AM35-14	KR029753.1	Sweden, Småland	Direct submitted
<i>Elaphomyces asperulus</i>	OF21354	KR029754.1	-	Direct submitted
	OF22178	KR029755.1	-	Direct submitted
<i>Elaphomyces americanus</i>	AKATA TT 160	PV069103.1	Türkiye: Kırklareli, Demirkoy	Current study
	OSC:81113	NR_198386.1	USA: West Virginia	Castellano & Stephens, 2017

<i>Elaphomyces decipiens</i>	personal collection:A. Paz IC27111118	KX238842.1	Spain: Asturias, San Esteban de Cunaba	Paz et al., 2017
	LIP-0001134	KX238832.1	Spain: Cantabria, Saja	Paz et al., 2017
	EL268-19	MT872011.1	-	Direct submitted
<i>Elaphomyces citrinus</i>	16955	JF907986.1	Spain	Direct submitted
	LIP-0001141	KX238822.1	Spain: Caceres, Jarandilla de la Vera	Paz et al., 2017
<i>Elaphomyces roseolus</i>	MUB:Fung-925	PQ346457.1	Spain: Albacete, Masegoso	Direct submitted
<i>Tuber castellanoi</i>	JMV20141916-1	KX238860.1	Spain: Girona	Paz et al., 2017
	OSC:131470	NR_121355.1	USA	Bonito et al., 2010

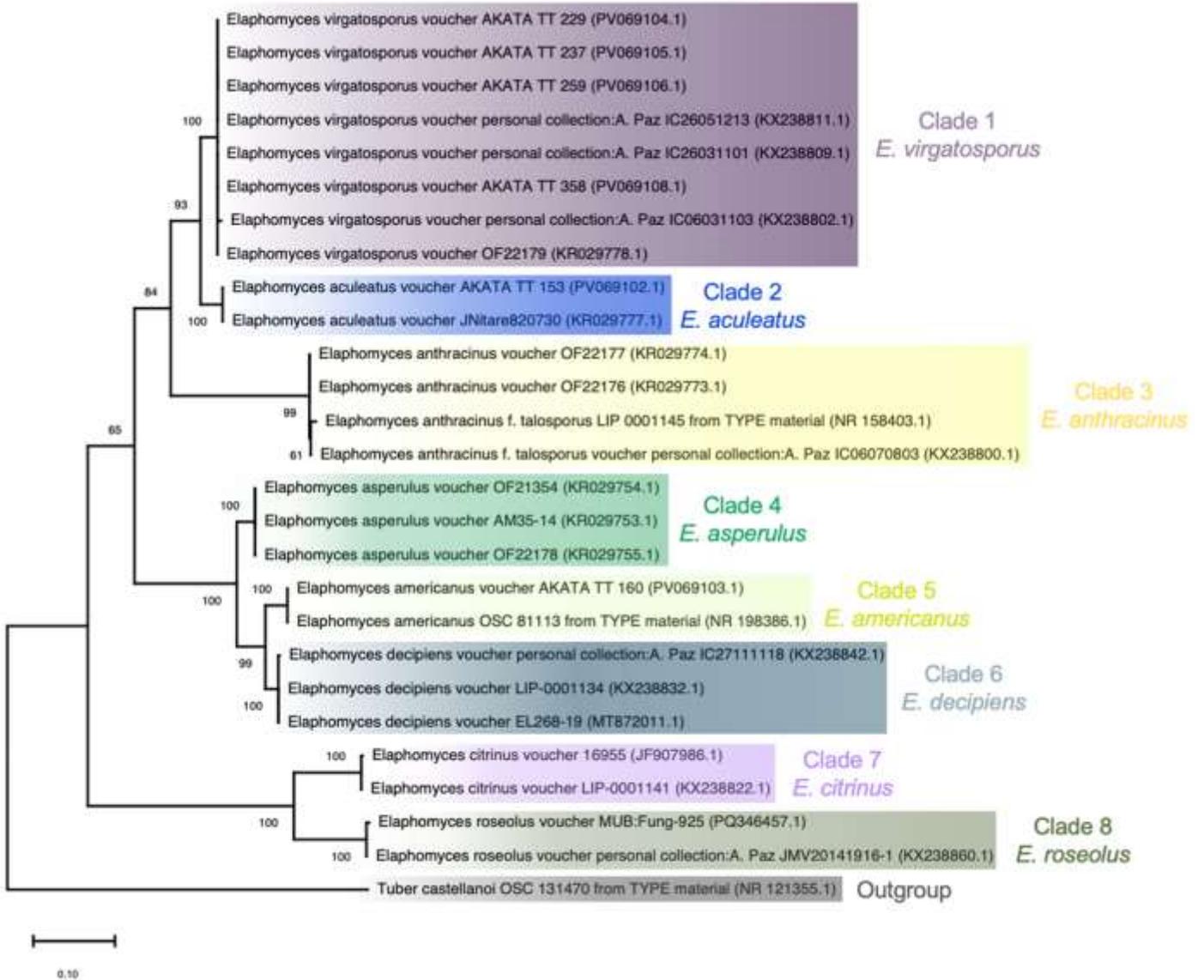


Figure 8. Maximum likelihood (ML) phylogram constructed from nrITS rDNA sequences of 26 *Elaphomyces* specimen, with *Tuber castellanoi* designated as the outgroup to root the tree. Bootstrap values are shown for nodes with support equal to or greater than 50%. GenBank accession numbers are provided alongside each corresponding sequence. The scale bar located at the bottom left represents a genetic divergence of 0.1 substitutions per site.

Şekil 8. *Elaphomyces* cinsine ait 26 örneğin nrITS rDNA dizilimlerine dayanarak oluşturulan maksimum olasılık (ML) filogramı; ağaç, dış grup olarak belirlenen *Tuber castellanoi* ile köklendirilmiştir. %50 ve üzeri destek değerine sahip dallarda ön yükleme değerleri gösterilmiştir. Her bir dizinin yanında ilgili GenBank erişim numarası verilmiştir. Sol alt köşede yer alan ölçek çubuğu, sitede 0.1 düzeyinde genetik farklılığı temsil etmektedir.

To elucidate their evolutionary affiliations, comparative analyses were conducted using nrITS rDNA sequences from diverse *Elaphomyces* taxa, with *Tuber castellanoidi* serving as the outgroup. The resulting phylogenetic tree resolved eight distinct clades (Fig. 8). Clade 1 comprised multiple isolates of *E. virgatosporus* along with specimens ANK AKATA TT 229, 237, 259, and 358. Clade 2 included both an *E. aculeatus* isolate and specimen ANK AKATA 153. In Clade 5, specimen ANK AKATA 160 grouped with a single isolate of *E. americanus*. The other clades (3, 4, 6, 7, and 8) encompassed additional species within *Elaphomyces*. The phylogenetic analysis demonstrated strong bootstrap support for the placement of the studied specimens within their respective clades, confirming their close genetic affiliation with the corresponding *Elaphomyces* taxa.

DISCUSSION and CONCLUSION

Elaphomyces aculeatus, *E. virgatosporus*, and *E. anthracinus* Vittad. exhibit considerable morphological resemblance, making taxonomic differentiation particularly challenging (Paz et al., 2017). A defining characteristic that distinguishes these taxa from other *Elaphomyces* species is their smooth peridial surface, which lacks the mycelial patches commonly found in related species (Castellano & Stephens, 2017; Molia et al., 2020).

Elaphomyces aculeatus is characterized by globose ascomata (10–50 mm) usually encrusted with soil, a thick whitish peridium covered by a purple to purplish-red surface covered with prominent black-tipped warts, and spores measuring 13–20 µm that have thin, rod-like warts converging at the apex to form distinct folds (Paz et al., 2017). In contrast, *E. virgatosporus* has ascomata measuring 10–35 mm, typically embedded in a compact substrate interwoven with reddish-brown mycelial strands, a dark brown to nearly black peridial surface densely covered in small warts contrasting with the cream-white peridium, and larger spores (16–22 µm) ornamented with sharp ridges and wavy striate crests (Læssøe et al., 2009; Paz et al., 2017; Molia et al., 2020). Conversely, *E. anthracinus* is characterized by dark brown to black, globose to pyriform ascomata that often grow in gregarious clusters, with a slightly granulose, charcoal-like peridial surface, a thick yellowish-white peridium, and an almost black gleba (Paz et al., 2017).

Elaphomyces americanus was first identified in North America and is distinguished from its regional counterparts by a uniquely marbled inner peridium. Although it shares morphological and ecological traits with *Elaphomyces muricatus*, it differs notably in spore morphology, as *E. muricatus* has spores that are slightly smaller (16–23 µm) (Paz et al., 2017). *Elaphomyces americanus* exhibits more complex ornamentation, with a higher density of linear ridges rather than rod-like shapes. Additionally, its ornamentation is more prominent, with increased height and coarseness, setting it apart from *E. muricatus* (Castellano & Stephens, 2017).

The genetic diversity within fungal species often greatly exceeds what can be observed through morphological traits alone, highlighting the importance of combining molecular data with traditional morphology-based identification methods. For many decades, molecular systematics has used genetic markers, especially regions of ribosomal RNA genes such as nrITS, nrSSU, and nrLSU, alongside various protein-coding genes, to clarify fungal taxonomy (Raja et al., 2017). Among these markers, the internal transcribed spacer (ITS) region has become the most commonly used in fungal molecular classification, providing vital taxonomic information (White et al., 1990). Additionally, recent advances in high-throughput sequencing and computational tools have enabled genome-wide comparisons and phylogenomic studies, which may soon surpass traditional phylogenetic methods based on a limited set of genetic markers (Qu et al., 2025).

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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.

REFERENCES

- Akata, I., Ediş, G., Kumru, E., Acar, İ., & Şahin, E. (2024a). Taxonomic Studies on *Rhodocybe asyae* Specimens Discovered in a New Location. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature* 27 (Suppl 1), 124–132. <https://doi.org/10.18016/ksutarimdog.vi.1507554>
- Akata, I., Kumru, E., Sahin, E., Acar, İ., & Kaya, E. (2024b). *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. <https://doi.org/10.23902/trkjnat.1446327>

- Akata, I., Ediş, G., Kumru, E., Acar, İ., & Şahin, E. (2024c). Initial Report of *Inocybe costinittii* in Türkiye with Morphological and Molecular Data. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature* 27(6), 1304–1312. <https://doi.org/10.18016/ksutarimdog.vi.1467628>
- Akata, I., Kumru, E., Ediş, G., Acar, İ., & Şahin, E. (2024d). Two Newly Reported *Agaricales* Species from Türkiye with Morphological and Molecular Data. *Kastamonu University Journal of Forestry Faculty*, 24(3), 260–280. <https://doi.org/10.17475/kastorman.1599952>
- Akata, I., Kumru, E., Ediş, G., Özbey, B. G., Acar, İ., & Şahin, E. (2025a). Morphological and Molecular Characterization of *Terfezia clavervyi* and its Distribution in Türkiye. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature* 28(3), 843–854. <https://doi.org/10.18016/ksutarimdog.vi.1622161>
- Akata, I., Ediş, G., Kumru, E., Keskin, E., & Şahin, E. (2025b). *Stasesia pompholyx*: A Newly Reported Species for Turkish Hysterangiales. *Kastamonu University Journal of Forestry Faculty*, 25(1), 1–8. <https://doi.org/10.17475/kastorman.1660474>
- Allen, K. & Bennett, J. W. (2021). Tour of truffles: aromas, aphrodisiacs, adaptogens, and more. *Mycobiology*, 49(3), 201–212. <https://doi.org/10.1080/12298093.2021.1936766>
- Bach C., Beacco P., Cammaletti P., Babel-Chen, Z., Levesque, E., Todesco, F., Cotton, C., Robin, B., & Murat, C. (2021). First production of Italian white truffle (*Tuber magnatum* Pico) ascocarps in an orchard outside its natural range distribution in France. *Mycorrhiza*, 31(3), 383–388. <https://doi.org/10.1007/s00572-020-01013-2>
- Bonito, G., Trappe, J. M., Rawlinson, P., & Vilgalys, R. (2010). Improved resolution of major clades within *Tuber* and taxonomy of species within the *Tuber gibbosum* complex. *Mycologia*, 102(5), 1042–1057. <https://doi.org/10.3852/09-213>
- Bratek, Z., Merényi, Z., & Varga, T. (2013). Changes of hypogeous fungi in the Carpathian Pannonian region in the past centuries. *Acta Mycologica*, 48(1), 33–39. <https://doi.org/10.5586/am.2013.005>
- Buyck, B., Hosaka, K., Masi, S., & Hofstetter, V. (2016). Molecular analyses of first collections of *Elaphomyces* Nees (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from Africa and Madagascar indicate that the current concept of *Elaphomyces* is polyphyletic. *Cryptogamie, Mycologie*, 37(1), 3–14. <https://doi.org/10.7872/crym/v37.iss1.2016.3>
- Cabero, A., Alvarado, P., Alonson, J., Salcedo, I., & Moreno-Mateos, D. (2019). *Elaphomices lilacinus* sp. nov. (*Ascomycota*, *Eurotiomycetes*), a new hypogeous species from Navarre (Spain). *Ascomycete.org*, 11:229–232. <https://doi.org/10.25664/art-0279>
- Castellano, M. A., Trappe, J. M., & Vernes, Karl. (2011). Australian species of *Elaphomyces* (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*). *Australian Systematic Botany*, 24, 32–57. <https://doi.org/10.1071/SB10012>
- Castellano, M. A., Beever, R. E., & Trappe, J. M. (2012a). Sequestrate fungi of New Zealand: *Elaphomyces* (*Ascomycota*, *Eurotiales*, *Elaphomycetaceae*). *New Zealand Journal of Botany*, 50(4), 423–433. <http://doi.org/10.1080/0028825X.2012.725057>
- Castellano, M. A., Henkel, T. W., Miller, S. L., Smith, M. E., & Aime, M. C. (2012b). New *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from Guyana. *Mycologia*, 104(5), 1244–1249. <https://doi.org/10.3852/12-061>
- Castellano, M. A., Dentinger, B. T. M., Séné, O., Elliott, T. F., Truong, C., & Henkel, T. W. (2016). New species of *Elaphomyces* (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from tropical rainforests of Cameroon and Guyana. *IMA Fungus* 7, 59–73. <https://doi.org/10.5598/imafungus.2016.07.01.05>
- Castellano, M. A. & Stephens, R. B. (2017). *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*) from bartlett experimental forest, New Hampshire, USA. *IMA fungus*, 8(1), 49–63. <https://doi.org/10.5598/imafungus.2017.08.01.04>
- Castellano, M. A., Elliott, T.F., & Trappe, J.M. (2018). Three new black *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from eastern North America with notes on selected European species. *Fungal Systematics and Evolution*, 1, 1–12. <https://doi.org/10.3114/fuse.2018.01.01>
- Castellano, M. A., Crabtree, C. D., Mitchell, D., & Healy, R. A. (2021). Eight new *Elaphomyces* species (*Elaphomycetaceae*, *Eurotiales*, *Ascomycota*) from eastern North America. *Fungal Systematics and Evolution*, 7(1), 113–131. <https://doi.org/10.3114/fuse.2021.07.06>
- Čejka, T., Thomas, P. W., Oliach, D., Stobbe, U., Egli, S., Tegel, W., Centenaro, G., Sproll, L., Bagi, I., Trnka, M., & Büntgen, U. (2022). Understanding the performance of Truffle Dogs. *Journal of Veterinary Behavior*, 52–53, 8–13. <https://doi.org/10.1016/j.jveb.2022.04.002>
- Chachula, P., Fiedor, M., Rutkowski, R., & Dorda, A. (2020). New record of macrofungi for the mycobiota of the Cieszyn municipality (Polish Western Carpathians) including new species to Poland. *Acta Mycologica*, 55(1), 5511. <https://doi.org/10.5586/am.5511>
- Crous, P. W., Wingfield, M. J., Chooi, Y. H., Gilchrist, C. L., Lacey, E., Pitt, J. I., Roets, F., Swart, W. J., Cano-Lira, J. F., Valenzuela-Lopez, N., Hubka, V., Shivas, R. G., Stchigel, A. M., Holdom, D. G., Jurjević, Ž., Kachalkin, A. V., Lebel, T., Lock, C., Martín, M. P., Tan, Y. P., Tomashevskaya, M. A., Vitelli, J. S., Baseia, I. G., Bhatt, V. K.,

- Brandrud, T. E., De Souza, J. T., Dima, B., Lacey, H. J., Lombard, L., Johnston, P. R., Morte, A., Papp, V., Rodríguez, A., Rodríguez-Andrade, E., Semwal, K. C., Tegart, L., Abad, Z. G., Akulov, A., Alvarado, P., Alves, A., Andrade, J. P., Arenas, F., Asenjo, C., Ballarà, J., Barrett, M. D., Berná, L. M., Berraf-Tebbal, A., Bianchinotti, M. V., Bransgrove, K., Burgess, T. I., Carmo, F. S., Chávez, R., Čmoková, A., Dearnaley, J. D. W., de A Santiago, A. L. C. M., Freitas-Neto, J. F., Denman, S., Douglas, B., Dovana, F., Eichmeier, A., Esteve-Raventós, F., Farid, A., Fedosova, A. G., Ferisin, G., Ferreira, R. J., Ferrer, A., Figueiredo, C. N., Figueiredo, Y. F., Reinoso-Fuentealba, C. G., Garrido-Benavent, I., Cañete-Gibas, C. F., Gil-Durán, C., Glushakova, A. M., Gonçalves, M. F. M., González, M., Gorczak, M., Gorton, C., Guard, F. E., Guarnizo, A. L., Guarro, J., Gutiérrez, M., Hamal, P., Hien, L. T., Hocking, A. D., Houbraken, J., Hunter, G. C., Inácio, C. A., Jourdan, M., Kapitonov, V. I., Kelly, L., Khanh, T. N., Kislo, K., Kiss, L., Kiyashko, A., Kolařík, M., Kruse, J., Kubátová, A., Kučera, V., Kučerová, I., Kušan, I., Lee, H. B., Levicán, G., Lewis, A., Liem, N. V., Liimatainen, K., Lim, H. J., Lyons, M. N., Maciá-Vicente, J. G., Magaña-Dueñas, V., Mahiques, R., Malysheva, E. F., Marbach, P. A. S., Marinho, P., Matočec, N., McTaggart, A. R., Mešić, A., Morin, L., Muñoz-Mohedano, J. M., Navarro-Ródenas, A., Nicolli, C. P., Oliveira, R. L., Otsing, E., Ovrebo, C. L., Pankratov, T. A., Paños, A., Paz-Conde, A., Pérez-Sierra, A., Phosri, C., Pintos, A., Pošta, A., Prencipe, S., Rubio, E., Saitta, A., Sales, L. S., Sanhueza, L., Shuttleworth, L. A., Smith, J., Smith, M. E., Spadaro, D., Spetik, M., Sochor, M., Sochorová, Z., Sousa, J. O., Suwannasai, N., Tedersoo, L., Thanh, H. M., Thao, L. D., Tkalčec, Z., Vaghefi, N., Venzhik, A. S., Verbeken, A., Vizzini, A., Voyron, S., Wainhouse, M., Whalley, A. J. S., Wrzosek, M., Zapata, M., Zeil-Rolfe, I., & Groenewald, J. Z. (2020). Fungal Planet description sheets: 1042–1111. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 44, 301. <https://doi.org/10.3767/persoonia.2020.44.11>
- Crous, P. W., Cowan, D. A., Maggs-Kölling, G., Yilmaz, N., Thangavel, R., Wingfield, M. J., Noordeloos, M. E., Dima, B., Brandrud, T. E., Jansen, G. M., Morozova, O. V., Vila, J., Shivas, R. G., Tan, Y. P., Bishop-Hurley, S., Lacey, E., Marney, T. S., Larsson, E., Le Floch, G., Lombard, L., Nodet, P., Hubka, V., Alvarado, P., Berraf-Tebbal, A., Reyes, J. D., Delgado, G., Eichmeier, A., Jordal, J. B., Kachalkin, A. V., Kubátová, A., Maciá-Vicente, J. G., Malysheva, E. F., Papp, V., Rajeshkumar, K. C., Sharma, A., Spetik, M., Szabóová, D., Tomashevskaya, M. A., Abad, J. A., Abad, Z. G., Alexandrova, A. V., Anand, G., Arenas, F., Ashtekar, N., Balashov, S., Bañares, Á., Baroncelli, R., Bera, I., Biketova, A. Y., Blomquist, C. L., Boekhout, T., Boertmann, D., Bulyonkova, T. M., Burgess, T. I., Carnegie, A. J., Cobo-Díaz, J. F., Corriol, G., Cunnington, J. H., da Cruz, M. O., Damm, U., Davoodian, N., de A Santiago, A. L. C. M., Dearnaley, J., de Freitas, L. W. S., Dhileepan, K., Dimitrov, R., Di Piazza, S., Fatima, S., Fuljer, F., Galera, H., Ghosh, A., Giraldo, A., Glushakova, A. M., Gorczak, M., Gouliamova, D. E., Gramaje, D., Groenewald, M., Gansch, C. K., Gutiérrez, A., Holdom, D., Houbraken, J., Ismailov, A. B., Istel, Ł., Iturriaga, T., Jeppson, M., Jurjević, Ž., Kalinina, L. B., Kapitonov, V. I., Kautmanová, I., Khalid, A. N., Kiran, M., Kiss, L., Kovács, Á., Kurose, D., Kušan, I., Lad, S., Læssøe, T., Lee, H. B., Luangsa-Ard, J. J., Lynch, M., Mahamedi, A. E., Malysheva, V. F., Mateos, A., Matočec, N., Mešić, A., Miller, A. N., Mongkolsamrit, S., Moreno, G., Morte, A., Mostowfizadeh-Ghalamfarsa, R., Naseer, A., Navarro-Ródenas, A., Nguyen, T. T. T., Noisripoom, W., Ntandu, J. E., Nuytinck, J., Ostrý, V., Pankratov, T. A., Pawłowska, J., Pecenka, J., Pham, T. H. G., Polhorský, A., Pošta, A., Raudabaugh, D. B., Reschke, K., Rodríguez, A., Romero, M., Rooney-Latham, S., Roux, J., Sandoval-Denis, M., Smith, M. T., Steinrücken, T. V., Svetasheva, T. Y., Tkalčec, Z., van der Linde, E. J., V D Vegte, M., Vauras, J., Verbeken, A., Visagie, C. M., Vitelli, J. S., Volobuev, S. V., Weill, A., Wrzosek, M., Zmitrovich, I. V., Zvyagina, E. A., & Groenewald, J. Z. (2021). Fungal Planet description sheets: 1182–1283. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 46, 313. <https://doi.org/10.3767/persoonia.2021.46.11>
- de la Fuente, J. I., García-Jiménez, J., Raymundo, T., Sánchez-Flores, M., Valenzuela, R., Guevara-Guerrero, G., Pérez-Ovando, E. C., & Martínez-González, C. R. (2023a). *Elaphomyces castilloi* (Elaphomycetaceae, Ascomycota) and *Entoloma secotioides* (Entolomataceae, Basidiomycota), two new sequestrate fungi from tropical montane cloud forest from south Mexico. *MycKeys* 96, 127–142. <https://doi.org/10.3897/mycokeys.96.98320>
- de la Fuente, J. I., Pérez-Moreno, J., Martínez-Reyes, M., Ayala-Vásquez, O., Martínez-González, C. R., & Aguirre-Acosta, C. E. (2023b). *Elaphomyces readii* (Elaphomycetaceae, Eurotiomycetes), a new medicinal species of hypogeous fungus with biocultural importance from Mexico. *Phytotaxa*, 594(4), 241–250. <https://doi.org/10.11646/phytotaxa.594.4.1>
- Ediş, G., Kumru, E., Acar, İ., Karabıylık, H., & Akata, I. First Report of *Rhodocybe fumanellii* (Entolomataceae) from Türkiye with Morphological and Molecular Characterisation. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, 28(5), 1197–1205. <https://doi.org/10.18016/ksutarimdoga.vi.1703181>
- Elliott, T. F., Truong, C., Jackson, S. M., Zúñiga, C. L., Trappe, J. M., & Vernes, K. (2022). Mammalian mycophagy: a global review of ecosystem interactions between mammals and fungi. *Fungal Systematics and Evolution*, 9(1), 99–159. <https://doi.org/10.3114/fuse.2022.09.07>
- Gençer, F. & Yüksek, İ. (2022). Examination of the Diversity in Rural Architecture in Kırklareli Through

- Factors. *International Journal of Architecture and Planning*, 10(1), 299–324. <https://doi.org/10.15320/ICONARP.2022.204>
- Kers, L. E. (1997). *Elaphomyces virgatosporus* funnen i Sverige. *Svensk botanisk tidskrift*, 91, 25–36.
- Kültür, Ş. (2007). Medicinal plants used in Kırklareli province (Turkey). *Journal of Ethnopharmacology*, 111(2), 341–364. <https://doi.org/10.1016/j.jep.2006.11.035>
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Kumru, E., Ediş, G., Sahin, E., Keskin, E., & Akata, I. (2025). Expanding the diversity of *Genea* Vittad. (*Ascomycota, Pezizales*) in Türkiye: Morphological and molecular insights into newly recorded species. *Trakya University Journal of Natural Sciences*, (Online First). <https://doi.org/10.23902/trkjnat.1640957>
- Læssøe, T., Jordal, J. B., Nielsen, J. G. B., Holtan, D., & Larsen, P. G. (2009). *Elaphomyces virgatosporus* in NW Norway—the northernmost records of a rare truffle. *Agarica*, 28, 43–49.
- Molia, A., Larsson, E., Jeppson, M., Læssøe, T., & Larsson, K. H. (2020). *Elaphomyces* section *Elaphomyces* (Eurotiales, Ascomycota) taxonomy and phylogeny of North European taxa, with the introduction of three new species. *Fungal Systematics and Evolution*, 5(1), 283–300. <https://doi.org/10.3114/fuse.2020.05.14>
- Morgül, Ş. (2014). Kırklareli İlinde Eko Turizm Olanakları. *Electronic Journal of Vocational Colleges*, 4(4), 27–38. <https://doi.org/10.17339/ejovoc.75153>
- Olivier, J.-M., Savignac, J.-C., Sourzat, P., 2018. Truffe Et Trufficulture. Fanlac, France, p. 352.
- Paz, A., Bellanger, J. M., Lavoise, C., Molia, A., Ławrynowicz, M., Larsson, E., Iburguren, I. O., Jeppson, M., Læssøe, T., Sauve, M., Richard, F., & Moreau, P. A. (2017). The genus *Elaphomyces* (*Ascomycota, Eurotiales*), A ribosomal DNA-based phylogeny and revised systematics of European ‘deer truffles’. *Persoonia*, 38(1), 197–239. <https://doi.org/10.3767/003158517X697309>
- Qu, H., Cai, Q., Redhead, S. A., Chen, X., Ge, Z. W., & Yang, Z. L. (2025). Exploring criteria for constructing suprageneric classifications of fungi in the genomic era: a case study of suborders Agaricineae, Pluteineae, and Tricholomatineae (Agaricales). *Fungal Diversity*, 1–24. <https://doi.org/10.1007/s13225-025-00557-y>
- 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. <https://doi.org/10.1021/acs.jnatprod.6b01085>
- Rusbridge, C. & Wilkins, P. (2002). Neuronal heterotopia in a Lagotto Romagnolo dog. *Neuropathology and Applied Neurobiology*, 28(2), 162–162. https://doi.org/10.1046/j.1365-2990.2002.39286_39.x
- Shirakawa, M. & Tanaka, M. (2020). Two new deer truffle species, *Elaphomyces marmoratus* and *Elaphomyces fuscus* spp. nov., from a secondary forest in Japan. *Mycoscience*, 61(6), 315–322. <https://doi.org/10.1016/j.myc.2020.07.002> 1340-3540
- Siller, I., Vasas, G., Pál-Fám, F., Bratek, Z., Zagyva, I., & Fodor, L. (2005). Hungarian distribution of the legally protected macrofungi species. *Studia botanica hungarica*, 36, 131–163.
- Sukarno, N., Listiyowati, S., Rahayu, N., & Nara, K. (2018). *Elaphomyces tropicalis* sp. nov.: A new ectomycorrhizal fungus associated with dipterocarps from tropical Indonesia. *Mycoscience*, 60(2), 83–88. <https://doi.org/10.1016/j.myc.2018.12.004>
- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512–526. <https://doi.org/10.1093/oxfordjournals.molbev.a040023>
- Tan, Y. P., Bishop-Hurley, S. L., Shivas, R. G., Cowan, D. A., Maggs-Kölling, G., Maharachchikumbura, S. S., Pinruan, U., Bransgrove, K. L., De la Peña-Lastra, S., Larsson, E., Lebel, T., Mahadevakumar, S., Mateos, A., Osieck, E. R., Rigueiro-Rodríguez, A., Sommai, S., Ajithkumar, K., Akulov, A., Anderson, F. E., Arenas, F., Balashov, S., Bañares, Á., Berger, D. K., Bianchinotti, M. V., Bien, S., Bilański, P., Boxshall, A. G., Bradshaw, M., Broadbridge, J., Calaça, F. J. S., Campos-Quiroz, C., Carrasco-Fernández, J., Castro, J. F., Chaimongkol, S., Chandranayaka, S., Chen, Y., Comben, D., Dearnaley, J. D. W., Ferreira-Sá, A. S., Dhileepan, K., Díaz, M. L., Divakar, P. K., Xavier-Santos, S., Fernández-Bravo, A., Gené, J., Guard, F. E., Guerra, M., Gunaseelan, S., Houbraken, J., Janik-Superson, K., Jankowiak, R., Jeppson, M., Jurjević, Ž., Kaliyaperumal, M., Kelly, L. A., Kezo, K., Khalid, A. N., Khamsuntorn, P., Kidanemariam, D., Kiran, M., Lacey, E., Langer, G. J., López-Llorca, L. V., Luangsa-Ard, J. J., Lueangjaroenkit, P., Lumbsch, H. T., Maciá-Vicente, J. G., Mamatha Bhanu, L. S., Marney, T. S., Marqués-Gálvez, J. E., Morte, A., Naseer, A., Navarro-Ródenas, A., Oyedele, O., Peters, S., Piskorski, S., Quijada, L., Ramírez, G. H., Raja, K., Razzaq, A., Rico, V. J., Rodríguez, A., Ruszkiewicz-Michalska, M., Sánchez, R. M., Santelices, C., Savitha, A. S., Serrano, M., Leonardo-Silva, L., Solheim, H., Somrithipol, S., Sreenivasa, M. Y., Stępniewska, H., Strapagiel, D., Taylor, T., Torres-Garcia, D., Vauras, J., Villarreal, M., Visagie, C. M., Wołkowycki, M., Yingkunchao, W., Zapora, E., Groenewald, J. Z., Crous, P. W., & Marqués-Gálvez, J. E. (2022). Fungal Planet description sheets: 1436–1477. *Persoonia-Molecular Phylogeny*

- and Evolution of Fungi*, 49(1), 261–350. <https://doi.org/10.3767/persoonia.2022.49.08>
- Türkoğlu, A., Castellano, M. A., Trappe, J. M., & Yaratanakul GÜngör M. 2015. Turkish truffles I: 18 new records for Turkey. *Turkish Journal of Botany*, 39(2), 359–376. <https://doi.org/10.3906/bot-1406-42>
- Uzun, Y. (2021). *Elaphomyces anthracinus*, a new hypogeous ascomycete record for Turkish mycota. *Anatolian Journal of Botany*, 5 (1), 29–31. <https://doi.org/10.30616/ajb.857752>
- Uzun, Y. & Kaya, A. (2019). *Elaphomyces granulatus*, A New Hypogeous Ascomycete Record for Turkey. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, 22(1), 85–88. <https://doi.org/10.18016/ksutarimdog.vi.466008>
- Uzun, Y. & Kaya, A. (2020). *Elaphomyces citrinus* and *E. cyanosporus*, new for Turkey. *Mycotaxon*, 135, 339–344. <https://doi.org/10.5248/135.339>
- Uzun, Y. & Kaya A. (2021). First Record of *Elaphomyces decipiens* for the Mycobiota of Turkey. *Mantar Dergisi*, 12(2), 134–137. <https://doi.org/10.30708.mantar955067>
- White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315–322. In: Innis, M.A. & Gelfand, D.H. (eds). PCR Protocols: A Guide To Methods And Applications. Academic Press, London, 482 pp.
- Yılmaz, M. U. (2023). Keşif kuraklık indeksi ve standartlaştırılmış yağış indeksi kullanılarak kırklareli ilinde kuraklığın eğilimi ve zamansal değişkenliği. *Doğal Afetler ve Çevre Dergisi*, 9(2), 341–364. <https://doi.org/10.21324/dacd.1296428>