KSÜ Tarım ve Doğa Derg 28 (3), 843-854, 2025
KSU J. Agric Nat 28 (3), 843-854, 2025
DOI:10.18016/ksutarimdoga.vi.1622161

Morphological and Molecular Characterization of *Terfezia claveryi* and its Distribution in Türkiye

Ilgaz AKATA[1✉], Eda KUMRU[2], Gülce EDİŞ[3], Beste Gizem Özbey[4], İsmail ACAR[5], Ergin ŞAHİN[6,7]

[1,4]Ankara University, Faculty of Science, Department of Biology, Ankara, Türkiye, [2,3]Ankara University, Graduate School of Natural and Applied Sciences, Ankara, Türkiye, [5]Van Yüzüncü Yıl University, Başkale High School, Department of Organic Agriculture, Van, Türkiye, [6]Dokuz Eylül University, Department of Biology, İzmir, Türkiye, [7]Dokuz Eylül University, Fauna and Flora Research and Application Center, İzmir, Türkiye

[1]https://orcid.org/0000-0002-1731-1302, [2]https://orcid.org/0009-0000-7417-6197, [3]https://orcid.org/0000-0001-7038-9865
[4]https://orcid.org/0000-0002-9777-6598, [5]https://orcid.org/0000-0002-6049-4896, [6]https://orcid.org/0000-0003-1711-738X
✉: akata@science.ankara.edu.tr

ABSTRACT

The samples were collected from Ankara, Gaziantep, Konya, Niğde, and Şanlıurfa (Türkiye), between 2020 and 2023. Morphological analyses and nrITS rDNA sequence-based phylogenetic methods were used to examine the samples. Twenty-one specimens displayed morphological features consistent with *Terfezia claveryi* Chatin at both macro and micro levels. Genetic analysis revealed over 99% sequence similarity with this species. Detailed documentation included habitat descriptions, geographical coordinates, collection dates, and photographic records of macroscopic and microscopic structures. These findings contribute valuable insights into the distribution and taxonomy of *T. claveryi* in Türkiye.

Mycology

Research Article

Article History
Received : 17.01.2025
Accepted : 06.03.2025

Keywords
Terfezia claveryi
Mycobiota
Biodiversity
Türkiye

Terfezia claveryi'nin Morfolojik ve Moleküler Karakterizasyonu ve Türkiye'deki Dağılımı

ÖZET

Örnekler, Türkiye'nin Ankara, Gaziantep, Konya, Niğde ve Şanlıurfa illerinden 2020 ile 2023 yılları arasında toplanmıştır. Örneklerin incelenmesinde morfolojik analizler ve nrITS rDNA dizisine dayalı filogenetik yöntemler kullanılmıştır. Yirmi bir örnek, makro ve mikro seviyede *Terfezia claveryi* Chatin ile uyumlu morfolojik özellikler sergilemiştir. Genetik analiz, bu türle %99'dan fazla dizi benzerliği olduğunu ortaya koymuştur. Detaylı dokümantasyon kapsamında habitat tanımları, coğrafi koordinatlar, toplama tarihleri ve makroskobik ile mikroskobik yapıların fotoğrafik kayıtları yer almıştır. Bu bulgular, Türkiye'deki *T. claveryi*'nin dağılımı ve taksonomisi hakkında değerli bilgiler sunmaktadır.

Mikoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 17.01.2025
Kabul Tarihi : 06.03.2025

Anahtar kelimeler
Terfezia claveryi
Mikobiyota
Biyoçeşitlilik
Türkiye

Atıf İçin: Akata, I., Kumru, E., Ediş, G., Özbey, B. G., Acar, İ., & Şahin, E (2025). Morphological and Molecular Characterization of *Terfezia claveryi* and its Distribution in Türkiye. *KSÜ Tarım ve Doğa Derg 28* (3), 843-854-000. DOI: 10.18016/ksutarimdoga.vi.1622161.

To Cite: Akata, I., Kumru, E., Ediş, G., Özbey, B. G., Acar, İ., & Şahin, E (2025). Morphological and Molecular Characterization of *Terfezia claveryi* and its Distribution in Türkiye. *KSU J. Agric Nat 28* (3), 843-854. DOI: 10.18016/ksutarimdoga.vi.1622161.

INTRODUCTION

Commonly known as the "desert truffle" the genus *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul. belongs to the order *Pezizales* J. Schröt. and the family *Pezizaceae* Dumort., and typically appears during the rainy season between March and May (Alsheikh, 1994). Species within this genus are notable for producing edible subterranean fruiting bodies and are primarily associated with arid and semi-arid regions (Zambonelli et al., 2014). Despite their preference for such climates, *Terfezia* species display remarkable ecological adaptability, thriving in diverse environments, including deciduous and coniferous forests, prairies, and heathlands. These fruiting bodies are highly valued for culinary and medicinal purposes, particularly in regions such as the Middle East, North Africa, and the Mediterranean. The genus is also known for forming symbiotic mycorrhizal associations with specific plants, with a marked affinity for members of the family *Cistaceae* Juss., particularly the species of *Helianthemum* Mill. However, some species are also collected in habitats dominated by oaks and pines. The genus demonstrates significant versatility in its mycorrhizal associations, capable of forming various types such as sheeting

ectomycorrhizae, endomycorrhizae, and ectomycorrhizae (Bordallo et al., 2015; Zitouni-Haouar et al., 2018).

Terfezia members have long been valued for their dual role in culinary and medicinal applications, owing to their high nutritional content and the presence of bioactive compounds (Veeraraghavan et al., 2021). Historically, desert truffles were primarily consumed as a dietary staple in specific regions. However, their significance has only recently garnered broader attention. Today, they are highly regarded for their economic and nutritional contributions and emerging scientific research potential (Bordallo et al., 2015).

According to the Mycobank (https://www.mycobank.org/), *Terfezia* comprises approximately 55 currently recognized species. Seven species have been documented in Türkiye: *T. albida* Ant. Rodr., Muñ.-Moh. & Bordallo, *T. arenaria* (Moris) Trappe, *T. boudieri* Chatin, *T. cistophila* Ant. Rodr., Bordallo, Kaounas & Morte, *T. claveryi* Chatin, *T. leptoderma* (Tul. & C. Tul.) Tul. & C. Tul., and *T. olbiensis* (Tul. & C. Tul.) Sacc. (Akata et al., 2022). These findings highlight the diversity of *Terfezia* species in the region, contributing to understanding truffle biodiversity in Türkiye.

T. claveryi Chatin is extensively distributed across North African and Arabian Peninsula countries, including Morocco, Egypt, Syria, Iraq, Kuwait, and Iran. Its range also spans Mediterranean countries such as Spain, Portugal, Italy, France, Hungary, and Türkiye. These regions are characterized by arid and semi-arid ecosystems where *T. claveryi* primarily associates with *Helianthemum* species in alkaline soils (Marasas & Trappe, 1973; Jamali & Banihashemi, 2012; Abdelaziz, 2018; Zitouni-Haouaret al., 2018). Morphologically, this species is distinctive, producing hypogeous ascomata with a subglobose to turbinate form, weighing between 20 and 350 grams. The mature peridium varies in colour from reddish-brown to blackish-brown, while the gleba is firm and fleshy. The asci are hyaline, generally containing 7-8 spores, which may be globose, ellipsoidal, or subglobose in shape. The ascospores are globose, light brown, and exhibit a reticulate surface texture, occasionally adorned with truncated warts (Jamali & Banihashemi, 2012).

T. claveryi, a desert truffle of significant commercial value, has been traditionally utilised in Middle Eastern folk medicine to address conditions affecting the eyes and skin (Mandeel & Al-Laith, 2007). This species is renowned for its rich nutritional profile, which includes high concentrations of proteins, carbohydrates, and dietary fibre, alongside an array of bioactive compounds such as ascorbic acid, anthocyanins, phenolics, flavonoids, and carotenoids (Veeraraghavan et al., 2021). These attributes, combined with its diverse biological properties, position *T. claveryi* as a medicinally valuable species. Its biological activities encompass anticancer, antimicrobial, antidiabetic, hepatoprotective, antimutagenic, and anti-inflammatory effects, emphasising its potential as a therapeutic agent (Janakat & Nassar, 2010; Akyüz et al., 2015a; Dahham et al., 2018; Malik et al., 2018; AlAhmed & Khalil, 2019).

Economically, *T. claveryi* is highly prized for its culinary and medicinal applications, commanding significant market value. In Europe, its price ranges from 20 to 60 Euros per kilogram, while in Arab countries such as the United Arab Emirates, Qatar, Kuwait, and Saudi Arabia, prices can soar to 220 Euros per kilogram, reflecting its high demand and cultural importance (Abdelaziz, 2018). Furthermore, *T. claveryi* serves as a host for diverse mycoviruses, with recent studies identifying five distinct mycoviruses in its isolates, thereby opening avenues for exploring its role in fungal virology (Sahin et al., 2023; Akata et al., 2024a).

Akata et al. (2022) documented the presence of *T. claveryi* in 15 locations across various provinces of Türkiye, including Adana, Aksaray, Elazığ, Denizli, Diyarbakır, Karaman, Konya, Malatya, Şanlıurfa, and Yozgat, as part of the Checklist of Turkish Truffles. These reports primarily relied on morphological traits for species identification. In contrast, the present study adopts an integrated methodology that combines morphological observations with molecular techniques, offering a more precise and robust framework for identifying *T. claveryi*.

Beyond the fundamental objective of identifying this species, the study seeks to deepen scientific insights into its geographical range, contributing valuable data to expand the understanding of *T. claveryi* distribution across Türkiye.

MATERIAL and METHOD

The study employed an integrative approach that combined conventional morphological methods with advanced molecular techniques to identify and classify specimens collected from Gaziantep, Niğde, Şanlıurfa, Ankara, and Konya in Türkiye (Figure 1). Detailed analyses of macroscopic and microscopic features were conducted to characterize the samples comprehensively. Additionally, the study incorporated ribosomal DNA (rDNA) analysis, focusing on the Internal Transcribed Spacer (ITS) region, to enhance the accuracy of species identification through molecular sequencing. This multi-faceted methodology ensured a robust and reliable classification of the collected specimens.

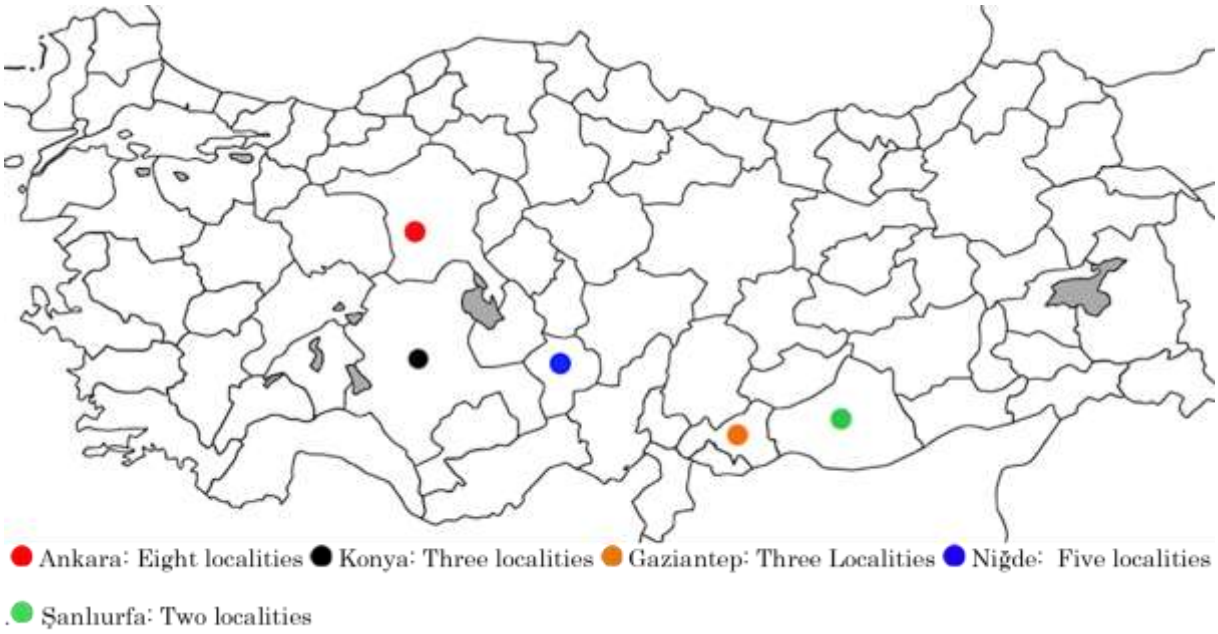

Figure 1. Distribution of *Terfezia claveryi* specimens collected during the present study.
Şekil 1. Bu çalışma sırasında toplanan Terfezia claveryi örneklerinin dağılımı.

Morphological Study

Specimens of *Terfezia claveryi* ascomata were collected during field studies conducted in 2020 and 2023, with detailed documentation of their macroscopic characteristics and ecological surroundings performed on-site. Microscopic examinations were conducted using a Euromex Oxion Trinocular light microscope (LM) and a ZEISS EVO 40XVP scanning electron microscope (SEM). The LM analyses were conducted at 100x magnification, with approximately 30 measurements taken for each feature, subsequently subjected to statistical evaluation. Chemical reagents, including Melzer's reagent, 5% KOH, and Congo red, were used during the analytical processes. For SEM imaging, sections of the gleba were mounted on stubs using double-sided adhesive tape and coated with a thin layer of gold particles to improve conductivity. Imaging was performed under a 20 kV accelerating voltage using the ZEISS EVO 40XVP SEM. The specimens were preserved and catalogued in the Fungarium of the Faculty of Science at Ankara University upon identification.

Molecular Characterization

Determination of the ITS rDNA sequences

Genomic DNA extraction was successfully performed on 21 specimens of the genus *Terfezia*. Afterwards, the nuclear ribosomal internal transcribed spacer (nrITS) rDNA regions were amplified through polymerase chain reaction (PCR). The genomic DNA isolation and ITS rDNA amplification processes adhered strictly to the methodologies outlined by Rogers and Bendich (1994) and White et. al (1990) as well as those described in subsequent studies (Akata & Erdoğdu, 2020; Akata et al., 2024b, 2024c, 2024d), ensuring both the accuracy of the procedure and the reproducibility of the findings.

Molecular Phylogeny Study

A comprehensive phylogenetic analysis of fungal samples was conducted using MEGA-X software, with nucleotide sequences derived directly from the collected specimens (Kumar et al., 2018). Comparative sequences were carefully selected from GenBank, with closely related fungal taxa forming the ingroup and more distantly related sequences designated as the outgroup, identified via NCBI BLAST searches. Sequence alignment was executed using the MUSCLE algorithm, enabling the determination of the most appropriate nucleotide substitution model (Kimura, 1980). Phylogenetic trees were subsequently constructed using the Neighbor-Joining approach, and the reliability of branching patterns was assessed through 1000 bootstrap replicates by Felsenstein (1985). The methodology for this analytical framework aligns closely with the protocols detailed by Akata et al. (2024c; 2024d).

RESULTS

Terfezia claveryi Chatin, (1892), (Figure 2-4).

Macroscopic and microscopic features

Ascomata 40-90 mm diam., initially grows underground but gradually emerges as it matures, semi-globular or pear-shaped, and sometimes lobed, or irregular, **Surface** smooth, commonly developing grooves or becoming wrinkled with age, pale yellow combined with orange-brown tones or ochre-salmon at first, reddish-brown to brownish-black at maturity, **Gleba** solid, fleshy, and succulent texture, the initial colour range of whitish to creamy or pale pinkish, transitioning to a yellowish to pinkish-salmon shade upon maturation, fertile sections are interspersed with sterile veins of a whitish-pink, occasionally spotted with patches of yellow to yellow-brownish discolorations. **Peridium** 500–600 µm thick, whitish to pale yellow, sometimes with a pinkish hue, with a slender brown region present at the surface, the outermost layer made up of hyaline and thin hyphae, innermost layer composed of thick hyphae with yellowish walls. **Asci** 60–90 x 55–65 µm, spherical to pear-shaped, non-amyloid, and 7-8 spored. **Ascospores** 16–18 (19) µm, spherical, excluding ornamentation, initially smooth and hyaline, yellowish to pale brown at maturity, with irregular and coarse reticulum and embellished with rounded warts, occasionally truncated.

Material examined: TÜRKİYE— **Gaziantep**, ***Dülük***, near *Helianthemum salicifolium,* 25 Apr. 2020, 927 m, 37° 08' N, 37° 21' E, ANK AKATA & SAHIN 008, (GenBank accession number: MZ089983.1); ***Küllü***, near *H. ledifolium*, 25 Apr. 2020, 763 m, 37° 03' N, 37° 34' E, ANK AKATA & SAHIN 009, (GenBank Accession number: MZ089984.1); 782 m, 37° 03' N, 37° 35' E, ANK AKATA & SAHIN 010, (GenBank Accession number: MZ089985.1); **Niğde**, ***Gümüşköy***, near *H. canum*, 26 Apr. 2020, 1290 m, 37° 29' N, 34° 36' E, ANK AKATA & SAHIN 011, (GenBank Accession number: MZ089986.1); ***Ulukışla***, near *H. canum,* 1 May 2020, 1380 m, 37° 32' N, 34° 30' E, ANK AKATA & SAHIN 014, (GenBank Accession number: MZ089989.1); ***Yeniköy***, near *H. canum,* 1 May 2020, 1046 m, 37° 43' N, 34° 18' E, ANK AKATA & SAHIN 015, (GenBank Accession number: MZ089990.1); ***Zengen***, near *H. canum,* 2 May 2020, 1042 m, 37° 45' N, 34° 15' E, ANK AKATA & SAHIN 016, (GenBank Accession number: MZ089991.1); ***Altay***, near *H. canum,* 2 May 2020, 1228 m, 37° 38' N, 34° 27' E, ANK AKATA & SAHIN 017, (GenBank Accession number: MZ089992.1); **Şanlıurfa**, ***Yılmaz***, near *H. ledifolium*, 28 Apr. 2020, 650 m, 37° 04' N, 37° 59' E, ANK AKATA & SAHIN 012, (GenBank Accession number: MZ089987.1); ***Uğurcuk***, near *H. ledifolium*, 28 Apr. 2020, 370 m, 37° 03' N, 37° 59' E, ANK AKATA & SAHIN 013, (GenBank Accession number: MZ089988.1); **Ankara, Polatlı, *Üçpınar***, near *H. ledifolium*, 18 Apr. 2023, 1054 m, 39° 36' N, 32° 06' E, ANK AKATA 8764, (GenBank Accession number: OR398211.1); ***Kuşçu,*** near *H. canum,* 18 Apr. 2023, 1008 m, 39° 37' N, 32° 14' E, ANK AKATA 8768,(GenBank Accession number: PP494016. 1);1028 m, 39° 35' N, 32° 15' E, 8772,(GenBank Accession number: PP494016.1); ***Beyceğiz***, near *H. ledifolium*, 18 Apr. 2023, 1000 m, 39° 39' N, 32° 08' E, ANK AKATA 8770,(GenBank Accession number: OR398223.1); ***Kargalı***, *H. canum,* 18 Apr. 2023, 1065 m, 39° 36' N, 32° 15' E, ANK AKATA 8773, (GenBank Accession number: PP494023.1); ***Hacıtuğrul***, near *H. ledifolium*, 30 Apr. 2023, 970 m, 39° 44' N, 32° 13' E, ANK AKATA 8779, (GenBank Accession number: OR394962.1); ***Macun***, near *H. ledifolium,* 30 Apr. 2023, 980 m, 39° 39' N, 32° 16' E, ANK AKATA 8780,(GenBank Accession number: PP494025.1); ***Eskipolatlı***, near *H. ledifolium,* 30 Apr. 2023, 930 m, 39° 31' N, 32° 12' E, ANK AKATA 8784,(GenBank Accession number: PP494026.1); **Konya**, ***Hadim***, near *H. ledifolium*, 11 May 2023, 1382 m, 37° 04' N, 32° 27' E, ANK AKATA 8785,(GenBank Accession number: PP494038.1); ***Sarayköy***, near *H. canum,* 11 May 2023, 1303 m, 37° 53' N, 32° 22' E, ANK AKATA 8789,(GenBank Accession number: PP494039.1); ***Karadiğin***, near *H. ledifolium*, 11 May 2023, 1179 m, 37° 45' N, 32° 22' E, ANK AKATA 8790,(GenBank Accession number: OR394967.1).

Evolutionary History of *T. claveryi* Specimens

The evolutionary relationships of this *T. claveryi* samples were assessed using nuclear ribosomal ITS (nrITS) rDNA sequences, which were generated through standard molecular protocols and archived in the NCBI GenBank database. Comprehensive details on the collection sites and corresponding GenBank accession numbers are presented in Table 1. To elucidate phylogenetic relationships, nrITS rDNA sequences from multiple species within the genus *Terfezia* were analyzed, with the nrITS rDNA sequence of *Mattirolomyces terfezioides* (Mattir.) E. Fisch. designated as the outgroup (Figure 4). Molecular phylogenetic reconstruction revealed the presence of seven distinct clades. Clade 1 exclusively included isolates of *T. claveryi*, while Clades 2 to 7 represented other *Terfezia* species. Placing *M. terfezioides* on a separate branch confirmed its role as the outgroup. BLAST analysis demonstrated over 99% similarity between the nrITS rDNA sequences of Turkish *T. claveryi* specimens and various isolates of *T. claveryi*. Phylogenetic results further substantiated the strong genetic affinity of Turkish *T. claveryi* specimens to other isolates of the same species, with high bootstrap support values underscoring the robustness of their clustering.

Figure 2. Ascomata of *Terfezia claveryi* .
Şekil 2. Terfezia claveryi 'nin askokarpları.

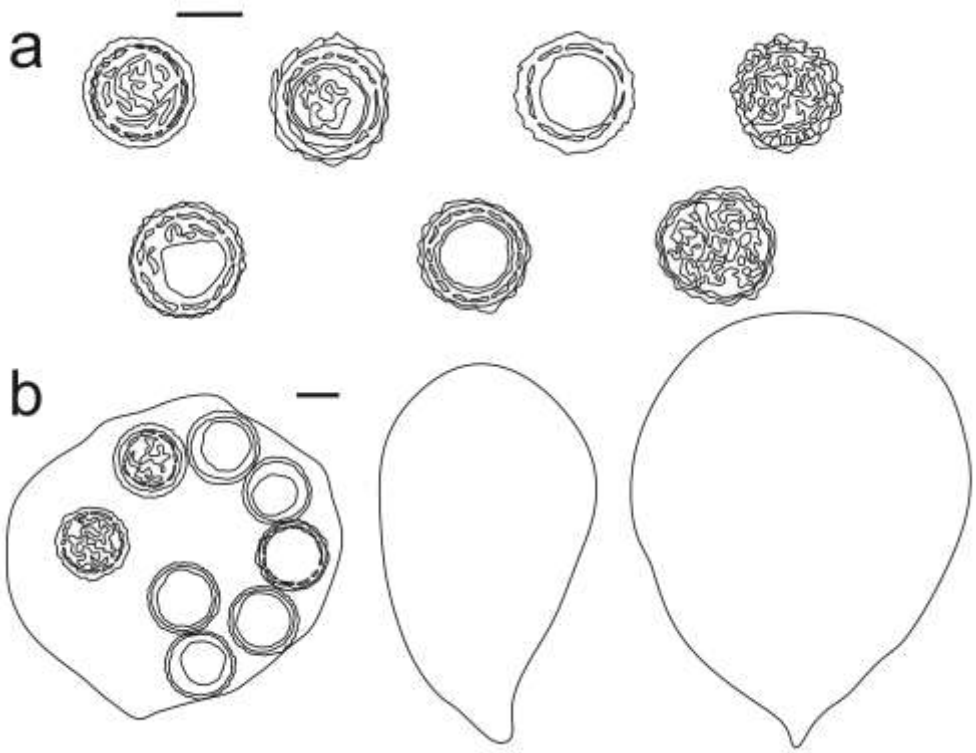

Figure 3. *Terfezia claveryi* : a. spores, b. asci (scale bars: 10 µm).
Şekil 3. Terfezia claveryi: a. sporlar, b. ascuslar (ölçek: 10 µm).

Figure 4. A single spore of *Terfezia claveryi* (SEM)
Şekil 4. Terfezia claveryi'nin sporu (SEM)

Table 1. GenBank accession numbers and localities of the *Terfezia* specimens analyzed in this study
Çizelge 1. Bu çalışmada analiz edilen Terfezia örneklerinin GenBank erişim numaraları ve lokaliteleri

Species	Specimen Voucher/Isolate/Strain	nrITS GenBank Accession Number	Geographical origin	Reference
Terfezia claveryi	ANK AKATA 8764	OR398211.1	Türkiye: Ankara	Current study
	ANK AKATA 8768	PP494016.1	Türkiye: Ankara	Current study
	ANK AKATA 8770	OR398223.1	Türkiye: Ankara	Current study
	ANK AKATA 8772	PP494020.1	Türkiye: Ankara	Current study
	ANK AKATA 8773	PP494023.1	Türkiye: Ankara	Current study
	ANK AKATA 8779	OR394962.1	Türkiye: Ankara	Current study
	ANK AKATA 8780	PP494025.1	Türkiye: Ankara	Current study
	ANK AKATA 8784	PP494026.1	Türkiye: Ankara	Current study
	ANK AKATA 8785	PP494038.1	Türkiye: Konya	Current study
	ANK AKATA 8789	PP494039.1	Türkiye: Konya	Current study
	ANK AKATA 8790	OR394967.1	Türkiye: Konya	Current study
	ANK AKATA & SAHIN 008	MZ089983.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 009	MZ089984.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 010	MZ089985.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 011	MZ089986.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 012	MZ089987.1	Türkiye: Sanliurfa	Current study
	ANK AKATA & SAHIN 013	MZ089988.1	Türkiye: Sanliurfa	Current study
	ANK AKATA & SAHIN 014	MZ089989.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 015	MZ089990.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 016	MZ089991.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 017	MZ089992.1	Türkiye: Nigde	Current study
	J029	MK910035.1	Iraq: Kahlar	Aish et al. 2020
	MZR12	MK967454.1	Iraq	-
	RA80	MH810271.1	Iraq	-
	LBMB 8	MF940187.1	Algeria: Saida	Zitouni-Haouar et al. 2018
	RUF-TC3	MK610443.1	Iran	-
	Fars Province, Iran	MN583175.1	Iran	Behzadi et al. 2021
	Fa1	GQ888693.1	Iran: Fars	Mostowfizadeh-Ghalamfarsa et al. 2010
Terfezia arenaria	MA:FU 59216	HQ698069.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 40130	HQ698066.1	Spain: Badajos	Kovacs et al. 2011

	NE_Algeria_1	KP217812.1	Algeria	Dafri & Beddiar 2017
	NE_Algeria_3	KP217814.1	Algeria	Dafri & Beddiar 2017
	NE_Algeria_5	KP217816.1	Algeria	Dafri & Beddiar 2017
	2004244	MW508705.1	Portugal: Setúbal	Santos-Silva et al. 2021
	j466	OP458227.1	Portugal: Lavre	-
	F	LT718230.1	Tunisia	Radhouani et al. 2019
	K1	LT718227.1	Tunisia	Radhouani et al. 2019
Terfezia boudieri	type 1	AF092096.1	Israel	Ferdman et al. 2005
	j371	OP458234.1	United Arab Emirates	-
	bou04	AF276672.1	Kuwait	Díez et al. 2002
	LBMB 19	MF940178.1	Algeria: Oued Daoura	Zitouni-Haouar et al. 2018
	105ET	AF387656.1	-	-
	99TC	AF387657.1	-	-
Terfezia olbiensis	AH 46143	MF940204.1	Spain: Tocon de Quentar, Granada	Zitouni-Haouar et al. 2018
	j588	OP458229.1	Greece: Attica, Artemis	-
	MA:FU 5408	HQ698102.1	Spain: Madrid, Toledo	Kovacs et al. 2011
Terfezia aff. olbiensis	MA:FU 54676	HQ698147.1	Spain: Valladolid, Castromonte, La Espina	Kovacs et al. 2011
	tO16	HM056221.1	Spain: Albacete	Bordallo et al. 2013
Terfezia albida	MUB:Fung-0029 (Type Material)	NR_137053.1	Spain: Albacete	Bordallo et al. 2013
	j574	OP458226.1	Spain: Albacete, Lezuza	-
	j113	KP728824.1	Spain: Badajoz	Bordallo et al. 2015
	j384	KP728826.1	Greece: Rafina Attica	Bordallo et al. 2015
	j479	KP728829.1	Greece: Zagora Magnesia	Bordallo et al. 2015
Terfezia cistophila	MUB Fung-j477 (Type Material)	NR_160445.1	Greece: Nea Makri Attica	Bordallo et al. 2015
	2004865	MW508653.1	Portugal: Portalegre	Santos-Silva et al. 2021
	MA:FU 65481	HQ698097.1	Spain: Toledo	Kovacs et al. 2011
	MA:FU 24971	HQ698090.1	Spain: Segovia	Kovacs et al. 2011
	MA:FU 57171	HQ698093.1	Spain: Caceres	Kovacs et al. 2011
Terfezia leptoderma	MA:FU 59232	HQ698096.1	Spain: Badajos	Kovacs et al. 2011
	MA:FU 26757	HQ698087.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 28367	HQ698088.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 41323	HQ698092.1	Spain: Madrid	Kovacs et al. 2011
Mattirolomyces terfezioides	943	MT890667.1	-	Lu et al. 2022

DISCUSSION and CONCLUSION

T. claveryi stands out as a distinctive species, characterized by its complex, reticulate spores, a peridium with shades ranging from reddish-brown to brownish-black, and gleba that exhibits reddish hues (Díez et al., 2002). While this species shares some morphological and ecological similarities with other *Terfezia* species reported in Türkiye (Díez et al., 2002; Kovács et al., 2011; Akata et al., 2022), it can be differentiated from them. For instance, *T. arenaria* and *T. boudieri* differ from *T. claveryi* based on distinct morphological characteristics. However, differentiating *T. claveryi* from species like *T. albida*, *T. cistophila*, *T. leptoderma*, and *T. olbiensis* is more challenging, as the differences in their physical features are not as pronounced (Kovács et al., 2011; Bordallo et al., 2013; Türkoğlu & Castellano, 2014; Türkoğlu et al., 2015). This complexity in distinguishing *T. claveryi* from certain other *Terfezia* species highlights the need to carefully examine and compare their specific traits.

T. boudieri typically thrives in basic soils, which are either rich in calcareous or contain gypsiferous marl, and associate with *Helianthemum* species. This species is notably characterized by its sizeable ascomata and distinct spores adorned with warts on a reticular structure (Moreno et al., 2002; Sbissi et al., 2011; Akyüz et al., 2012). Similarly, *T. claveryi* is found in comparable soil types and bears a macroscopic and mycorrhizal association resemblance to *T. boudieri*, making it challenging to distinguish between the two species based solely on their appearance (Díez et al., 2002; Moreno et al., 2002). Pay attention to the distinctive ornamentation patterns on their spores for precise identification and distinction between *T. claveryi* and *T. boudieri* (Zitouni-Haouar et al., 2018). The key distinguishing feature lies in their spore structures: Spores of *T. claveryi* are reticulate but lack warts, presenting a smoother appearance under microscopic observation. In contrast, the spores of *T. boudieri* are characterised by the presence of warts superimposed on a reticulate background (Moreno et al., 2002; Kovács et al., 2011). This subtle yet significant difference in spore ornamentation is crucial in correctly identifying and differentiating these species.

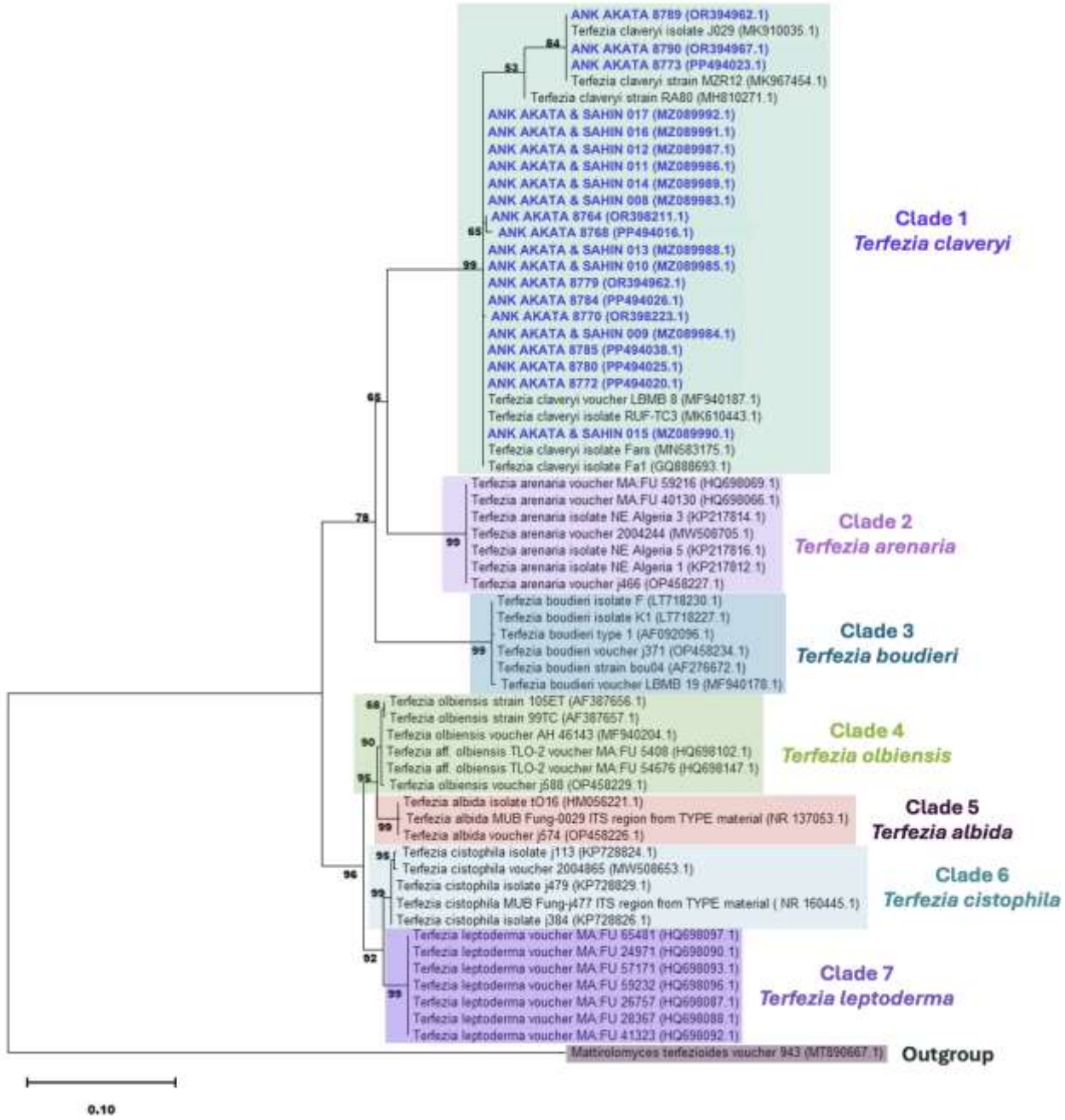

Figure 5. The evolutionary connections among 63 fungal specimens are illustrated in a phylogenetic tree created using the nrITS rDNA region and the maximum likelihood (ML) method. Bootstrap rates (≥50) are allocated to each branch to denote confidence levels. Sequences utilized for tree construction were obtained from the NCBI GenBank database. Additionally, *Mattirolomyces terfezioides* was included in the phylogenetic tree as the representative outgroup. Each sequence is accompanied by its respective GenBank accession number, and a scale bar in the lower left corner indicates a genetic distance of 0.10.

Şekil 5. nrITS rDNA bölgesi ve maksimum olabilirlik (ML) yöntemi kullanılarak oluşturulan bir filogenetik ağaçta 63 mantar örneği arasındaki evrimsel bağlantılar gösterilmektedir. Güven seviyeleri, her şubeye önyükleme oranları (≥50) atanarak belirtildi. Ağacın oluşturulması için kullanılan diziler NCBI GenBank veri tabanından elde edilmiştir. Ek olarak, Mattirolomyces terfezioides temsili dış grup olarak filogenetik ağaca dahil edilmiştir. Her diziye ilgili GenBank erişim numarası belirtilmiş ve sol alt köşedeki ölçek çubuğu 0.10'luk bir genetik mesafeyi temsil etmektedir.

T. arenaria is known for its unique peridium, which initially appears whitish with delicate pink undertones and black speckles. As this species matures, its peridium transforms, adopting a more brownish hue. A standout feature of *T. arenaria* is its robustly warty spores, which distinctly sets it apart from closely related species like *T. claveryi*. The latter is recognized for its reticulate spores, distinguishing between these species (Díez et al., 2002; Kovács et

al., 2011).

T. cistophila, known for its spiny-spored structure, is notably characterized by a peridium that turns intensely black. The gleba presents a light ochre colour, distinctively different from the outer peridium. A remarkable feature of this species is its spermatic odour, and it thrives in acidic soils and is typically found in areas where *Cistus* species are prevalent (Bordallo et al., 2015). On the other hand, *T. claveryi* is distinguishable from *T. cistophila* in several aspects. One of the most notable differences is its spore structure; *T. claveryi* produces reticulate spores devoid of spines, contrasting sharply with the spiny spores of *T. cistophila*. The peridium of *T. claveryi* ranges in colour from reddish-brown to brownish-black, and a pinkish-salmon hue characterises its gleba. It does not emit a spermatic odour (Díez et al., 2002). Furthermore, its habitat preferences are quite distinct; *T. claveryi i*s primarily found in arid and semi-arid regions, favouring calcareous, clayey, or sandy alkaline soils (Díez et al., 2002; Akyüz et al., 2015b; Türkoğlu et al., 2015). This species tends to associate with *Helianthemum* species, indicating a specific ecological relationship akin to that observed in *T. cistophila* with *Cistus* species (Díez et al., 2002; Bordallo et al., 2015).

T. albida is another species that shares its habitat preferences with *T. claveryi*, predominantly thriving in arid and semi-arid regions. This species has a particular affinity for calcareous, alkaline soils, similar to *T. claveryi*. However, a key aspect of the environmental association of *T. albida* is its tendency to grow in conjunction with *Helianthemum* species, a characteristic it shares with *T. claveryi* (Bordallo et al., 2013). Despite these similarities in habitat, *T. albida* is distinctly set apart from *T. claveryi* in several notable ways. The most striking difference lies in the colouration of its peridium, which is white, contrasting sharply with the reddish-brown to brownish-black peridium of *T. claveryi*. Additionally, the gleba of *T. albida*, presents a grayish-green coloration, offering a unique visual differentiation from the pinkish-salmon gleba of *T. claveryi* (Díez et al., 2002; Bordallo et al., 2013; Türkoğlu et al., 2015). Another distinguishing feature of *T. albida* is its spore structure. Like *T. cistophila, T. albida* produces spiny spores, a trait that diverges from the reticulate spores of *T. claveryi*. Furthermore, *T. albida* emits a spermatic odour, an attribute it shares with *T. cistophila* but not with *T. claveryi* (Díez et al., 2002; Bordallo et al., 2013; 2015)

T. olbiensis is characterized by its brown peridium and greenish-grey gleba, with its spiny spores being a notable attribute. This species often becomes a target for larvae and rabbits, likely because it emerges early in the year during humid, low-light conditions. It emits a unique scent and has a milder flavour than other *Terfezia* species (Bordallo et al., 2013). *T. olbiensis* is known to favour environments with limestone and clayey soils, commonly reported to be associated with pine and oak trees. According to research by Bordallo et al. in 2013, this species is often found in areas where the soil composition includes limestone and clay, conducive to the growth of pine and oak forests. Conversely, studies conducted by Akyüz et al., 2015b; Türkoğlu & Castellano, 2014, have documented the occurrence of this species in Türkiye, explicitly noting its association with *Helianthemum* species. The distinctions in morphology, including differences in peridium and gleba colourations and spore ornamentation, combined with their unique habitat and soil preferences and mycorrhizal partnerships, provide valuable criteria for differentiating *T. claveryi* from *T. olbiensis*.

The genetic diversity among fungal species far exceeds their morphological variation, highlighting the importance of integrating genetic data with conventional morphological techniques to improve the accuracy of species identification. Over the years, several genetic markers, including ribosomal RNA gene regions such as nrITS, nrSSU, and nrLSU, along with protein-coding gene sequences, have played a pivotal role in molecular systematics (Raja et al., 2017). The internal transcribed spacer (ITS) region is particularly valued for its high resolution in fungal molecular taxonomy. Recent advancements in high-throughput sequencing technologies and bioinformatics have further facilitated detailed genome-wide comparisons and phylogenomic analyses, which may soon surpass traditional molecular phylogenetic approaches that rely on a limited number of genetic markers (Marian et al., 2024). This study employed nuclear ITS rDNA sequences to characterize T. claveryi specimens collected from regions across Türkiye molecularly. The analysis revealed a sequence similarity exceeding 99% between these specimens and other *T. claveryi* isolates with sequence data available in the GenBank database, underscoring the efficacy of ITS-based approaches in identifying and confirming fungal taxa.

This study enhances the understanding of *Terfezia claveryi* distribution in Turkey, identifying 21 new localities through combined morphological and molecular methods (ITS rDNA). The findings improve taxonomic accuracy, provide insights into the species' ecology, and support conservation, sustainable harvesting, and commercial potential. This study underscores the value of integrating traditional and molecular approaches for studying desert truffles in arid ecosystems.

ACKNOWLEDGMENTS

The Ankara University Research Funding Unit supported the study with Project number FBA-2022–2383.

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.

KAYNAKLAR

Abdelaziz, M. (2018). *Discovering the environmental factors affecting the distribution of Terfezia claveryi Chatin in the Northwest of the Region of Murcia*. [Master thesis, Lleida, University of Lleida, p 31].

Aish, A. A., Abdulmalek, S. T., Kareem, T. A., Yasir, L. B., & Matny, O. (2020). Molecular identification and genetic diversity study of the Iraqi truffles. *Journal of Phytology*, *12,* 121-126. https://doi.org/10.25081/jp.2020.v12.6599

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. https://doi.org/10.18016/ ksutarimdoga.vi. 626466

Akata, I., Şen, İ., Sevindik, M. & Kabaktepe, Ş. (2022). Truffle checklist of Turkey II with a new record. *Turkish Journal of Agriculture-Food Science and Technology*, *10*(10), 1913-1920. https://doi.org/10.24925/ turjaf.v10i10.1913-1920.5482

Akata, I., Edis, G., Ozbey, B. G., Keskin, E., & Sahin, E. (2024a). Complete Genome Analyses of a Novel Flexivirus with Unique Genome Organization and Three Endornaviruses Hosted by the Mycorrhizal Fungus *Terfezia claveryi*. *Current Microbiology*, *81*(7), 1-11. https://doi.org/10.1007/s00284-024-03745-2

Akata, I., Kumru, E., Sahin, E., Acar, I., & 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 costinitii* in Türkiye with Morphological and Molecular Data. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 27*(6), 1304-1312. https://doi.org/10.18016/ksutarimdoga.vi.1467628

Akata, I., Ediş, G., Kumru, E., Acar, İ., & Sahin, E. (2024d). Taxonomic Studies on *Rhodocybe asyae* Specimens Discovered in a New Location. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 27*(Suppl 1), 124-132. https://doi.org/10.18016/ksutarimdoga.vi.1507554

AlAhmed, A., & Khalil, H. E. (2019). Antidiabetic Activity of *Terfezia Claveryi*: An In Vitro and In Vivo Study. *Biomedical and Pharmacology Journal*, *12*(2), 603-608. https://dx.doi.org/10.13005/bpj/1680

Akyüz, M., Kirbağ, S., & Kurşat, M. (2012). Ecological aspects of the arid and semi-arid truffle in Turkey: evaluation of soil characteristics, morphology, distribution, and mycorrhizal relationships. *Turkish Journal of Botany*, *36*(4), 386-391. https://doi.org/10.3906/bot-1103-4

Akyüz, M., Kırbağ, S., & Bircan, B. (2015a). Medical characteristics of arid-semi arid Truffle (*Terfezia* and *Picoa*) in the Elazığ-Malatya region of Turkey. *Hacettepe Journal of Biology and Chemistry*, 43(4), 301-308. https://doi.org/10.15671/HJBC.20154315989

Akyüz, M., Kirbag, S., Bircan, B., & Gurhan, Y. (2015b). Diversity and distribution of arid-semi arid truffle (*Terfezia* and *Picoa*) in Elazig-Malatya region of Turkey. *Mycosphere Journal of Fungal Biology*, *6*(6): 766–783. https://doi.org/10.5943/mycosphere/6/6/11

Alsheikh, A. M. (1994). *Taxomony and mycorrhizal ecology of the desert truffles in the genus Terfezia.* [Doktoral Thesis, Corvallis, Oregon State University, p 239].

Behzadi, A. A., Zareie, M., Abbasi, A., Masoumi, B., Ashrafi-Dehkordi, E., & Morte, A. (2021). Physicochemical properties, nutritional composition, and phylogenic analysis of black truffles grown in Fars Province, Iran. *International Journal of Nutrition Sciences*, *6*(1), 45-51. https://doi.org/10.30476/IJNS.2021.90183.1128

Bordallo, J. J., Rodríguez, A., Kounas, V., Camello, F., Honrubia, M., & Morte, A. (2015). Two new *Terfezia* species from southern Europe. *Phytotaxa*, *230*(3), 239-249. https://doi.org/10.11646/phytotaxa.230.3.2

Bordallo, J. J., Rodríguez, A., Muñoz-Mohedano, J. M., Suz, L. M., Honrubia, M., & Morte, A. (2013). Five new Terfezia species from the Iberian Peninsula. *Mycotaxon*, *124*(1), 189-208. https://doi.org/10.5248/124.189

Dafri, A., & Beddiar, A. (2017). Desert truffles from northeastern Algerian coasal dunes: Ecology, identification and symbiosis. *Journal of Fundamental and Applied Sciences*, *9*(1), 154-169. http://dx.doi.org/10.4314/jfas.v9i1.11

Dahham, S. S., Al-Rawi, S. S., Ibrahim, A. H., Majid, A. S. A., & Majid, A. M. S. A. (2018). Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle *Terfezia claveryi*. *Saudi Journal of Biological Sciences*, *25*(8), 1524-1534. https://doi.org/10.1016/j.sjbs.2016.01.031

Díez, J., Manjón, J. L., & Martin, F. (2002). Molecular phylogeny of the mycorrhizal desert truffles (*Terfezia* and *Tirmania*), host specificity and edaphic tolerance. *Mycologia*, *94*(2), 247-259.

https://doi.org/10.1080/15572536.2003.11833230

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*(4), 783-791. https://doi.org/10.2307/2408678

Ferdman, Y., Aviram, S., Nurit, R. B., Trappe, J. M., & Kagan-Zur, V. (2005). Phylogenetic studies of *Terfezia pfeilii* and *Choiromyces echinulatus* (Pezizales) support new genera for southern African truffles: *Kalaharituber* and *Eremiomyces*. *Mycological Research*, *109*(2), 237-245. https://doi.org/10.1017/S0953756204001789

Jamali, S., & Banihashemi, Z. (2012). Hosts and distribution of desert truffles in Iran, based on morphological and molecular criteria. *Journal of Agricultural Science & Technology*, *14*(6), 1379-1396.

Janakat, S., & Nassar, M. (2010). Hepatoprotective activity of desert truffle (*Terfezia claveryi*) in comparison with the effect of *Nigella sativa* in the rat. *Pakistan Journal of Nutrition*, *9*(1), 52-56. https://doi.org/10.3923/pjn.2010.52.56

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16,* 111-120. https://doi.org/10.1007/BF01731581

Kovács, G. M., Balazs, T. K., Calonge, F. D., & Martin, M. P. (2011). The diversity of *Terfezia* desert truffles: new species and a highly variable species complex with intrasporocarpic nrDNA ITS heterogeneity. *Mycologia*, *103*(4), 841-853. https://doi.org/10.3852/10-312

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

Lu, B., Zhang, F. M., Yu, F. Q., & Rinaldi, A. C. (2022). Ethnobiological notes and volatile profiles of two rare Chinese desert truffles. *Mycology*, *13*(3), 177-184. https://doi.org/10.1080/21501203.2022.2035005

Malik, H. M., Munazza Gull, M. G., Ulfat Omar, U. O., Kumosani, T. A., Al-Hejin, A. M., Marzouki, H. Z., & Iyer, A. P. (2018). Evaluation of the antibacterial potential of desert truffles (*Terfezia* spp) extracts against Methicillin Resistant *Staphylococcus aureus* (MRSA). *Journal of Experimental Biology and Agricultural Sciences*, *6,* 652–660. https://doi.org/10.18006/2018.6(4).652.660

Mandeel, Q. A., & Al-Laith, A. A. A. (2007). Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. *Journal of Ethnopharmacology*, *110*(1), 118-129. https://doi.org/10.1016/j.jep.2006.09.014

Marian, I. M., Valdes, I. D., Hayes, R. D., LaButti, 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*. *Fungal Genetics and Biology, 173,* 103913. https://doi.org/10.1016/j.fgb.2024.103913

Marasas, W. F. O. & Trappe, J.M. (1973). Notes on Southern African *Tuberales*. *Bothalia 11,* 139-141. https://doi.org/10.4102/abc.v11i1/2.1994

Moreno, G., Diez, J., & Manjon, J. L. (2002). *Terfezia boudieri*, first records from Europe of a rare vernal hypogeous mycorrhizal fungus. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, *17*(4), 637-641.

Mostowfizadeh-Ghalamfarsa, R., Jamali, S., & Banihashemi, Z. (2010). Molecular phylogeny of three desert truffles from Iran based on ribosomal genome. *Rostaniha*, *11*(2), 151-162.

Radhouani, F., Zampieri, E., Guasmi, F., Ferchichi, A., & Mello, A. (2019). Assessment of ß-tubulin gene as a complementary molecular marker for desert truffle identification: A study case on Tunisian samples. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *153*(1), 19-24. https://doi.org/10.1080/11263504.2018.1435571

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

Rogers, S.O., Bendich, A.J. (1994). Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin, S.B., Schilperoort, R.A. (eds) Plant *Molecular Biology Manual*. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-0511-8_12

Sahin, E., Ozbey Saridogan, B. G., Keskin, E., & Akata, I. (2023). Identification and complete genome sequencing of a novel betapartitivirus naturally infecting the mycorrhizal desert truffle *Terfezia claveryi*. *Virus Genes*, *59*(2), 254-259. https://doi.org/10.1007/s11262-023-01972-6

Santos-Silva, C., Louro, R., Natário, B., & Nobre, T. (2021). Lack of knowledge on ecological determinants and cryptic lifestyles hinder our understanding of *Terfezia* diversity. *MycoKeys*, *84,* 1-14. https://doi.org/10.3897/mycokeys.84.71372

Sbissi, I., Ghodhbane-Gtari, F., Neffati, M., Ouzari, H., Boudabous, A., & Gtari, M. (2011). Diversity of the desert truffle *Terfezia boudieri* Chatin. in southern Tunisia. *Canadian Journal of Microbiology*, *57*(7), 599-605. https://doi.org/10.1139/w11-040

Türkoğlu A., Castellano M. A., Trappe J. M., & Güngör M. Y. (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

Türkoğlu A., & Castellano M. A. (2014). New Records of Some Ascomycete Truffle Fungi from Turkey. *Turkish Journal of Botany*, *38,* 406-416. https://doi.org/10.3906/bot-1303-24

Veeraraghavan, V. P., Hussain, S., Papayya Balakrishna, J., Dhawale, L., Kullappan, M., Mallavarapu Ambrose, J., & Krishna Mohan, S. (2021). A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia claveryi*, *Terfezia boudieri,* and *Tirmania nivea*. *Food Reviews International*, *38*(sup1), 846–865. https://doi.org/10.1080/87559129.2021.1889581

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.

Zambonelli, A., Donnini, D., Rana, G. L., Fascetti, S., Benucci, G. M. N., Iotti, M., Khabar L,, Bawadekji, A, Piattoni, F., Campagno, R., Venturella, G. (2014). Hypogeous fungi in Mediterranean maquis, arid and semi-arid forests. *Plant Biosystems-An International Journal Dealing With all Aspects of Plant Biology*, *148*(2), 392-401. https://doi.org/10.1080/11263504.2013.877537

Zitouni-Haouar, F. E. H., Carlavilla, J. R., Moreno, G., Manjon, J. L., & Fortas, Z. (2018). Genetic diversity of the genus *Terfezia* (*Pezizaceae, Pezizales*): New species and new record from North Africa. *Phytotaxa*, *334*(2), 183-194. https://doi.org/10.11646/phytotaxa.334.2.7