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Verified Occurrence of *Terfezia albida* in Türkiye: An Integrative Approach Combining Morphological and Molecular Evidence

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ABSTRACT

This study undertakes a taxonomic examination of hypogeous fungi collected in Konya Province, Türkiye, on May 11, 2023. An integrative method merges macroscopic and microscopic morphological evaluations with molecular phylogenetic assessments of the nuclear ribosomal internal transcribed spacer (nrITS) region. The phylogenetic analysis indicates more than 99% sequence similarity to *Terfezia albida* Ant. Rodr., Mohedano & Bordallo, thus confirming the specimens' taxonomic classification. Although *Terfezia albida* was previously reported in Türkiye based on morphological features, this research marks the first molecularly verified occurrence outside the Iberian Peninsula, supported by comprehensive morphological and ecological data. The study also includes documentation of habitat specifics, geographic coordinates, collection dates, and photographic evidence. These results expand the range of *Terfezia albida* and highlight the importance of integrative taxonomic methods in deepening our understanding of hypogeous fungal diversity.

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Türkiye'de *Terfezia albida*'nın Doğrulanmış Kaydı: Morfolojik ve Moleküler Kanıtların Birleştirilmesiyle Bütüncül Bir Yaklaşım

ÖZET

Bu çalışma, 11 Mayıs 2023 tarihinde Türkiye'nin Konya ilinden toplanan hipogeik mantar örneklerinin taksonomik açıdan değerlendirilmesini amaçlamaktadır. Araştırmada, makroskobik ve mikroskobik morfolojik özelliklerin incelenmesi, nrITS bölgesine ait moleküler filogenetik analizlerle birleştirilerek bütüncül bir yöntem benimsenmiştir. Filogenetik analizler, örneklerin *Terfezia albida* Ant. Rodr., Mohedano & Bordallo türüyle %99'un üzerinde benzerlik gösterdiğini ortaya koyarak taksonomik teşhislerini doğrulamaktadır. *Terfezia albida*'nın Türkiye'de daha önce yalnızca morfolojik özelliklere dayanılarak bildirildiği göz önüne alındığında, bu çalışma türün İber Yarımadası dışındaki ilk moleküler doğrulamalı kaydını sunmakta; morfolojik ve ekolojik verilerle desteklenmiş önemli bir katkı sağlamaktadır. Çalışma kapsamında ayrıca örnekleme alanlarına ait habitat bilgileri, coğrafi koordinatlar, toplanma tarihleri ve fotoğraflı belgeler de sunulmuştur. Elde edilen bulgular, *Terfezia albida*'nın bilinen yayılış alanını genişletmekte ve hipogeik mantar çeşitliliğinin anlaşılmasında bütüncül taksonomik yaklaşımların önemini vurgulamaktadır.

Mikoloji

Araştırma Makalesi

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Anahtar kelimeler
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INTRODUCTION

The genus *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul., in the *Pezizaceae* family and the *Pezizales* order, includes edible underground fungi known as "desert truffles". These fungi primarily grow in arid and semi-arid environments, especially in the Mediterranean region (Bordallo et al., 2015). While *Terfezia* species are characterized by underground fruiting bodies and a preference for dry climates, they also exhibit ecological adaptability, occurring in diverse habitats, including deciduous and coniferous forests, grasslands, and heathlands (Akata et al., 2025a). They are a significant component of the Mediterranean mycobiota, forming important mycorrhizal relationships with many plants, particularly within the family *Cistaceae* Juss., especially the genera *Helianthemum* Mill., *Cistus* L., and *Tuberaria* (Dunal) Spach (Louro et al., 2020). However, species are also frequently reported under *Quercus* L. and *Pinus* L. (Zitouni-Haouar et al., 2014).

The growing importance of biotechnology and plant nutrition has spurred increased research into fungal diversity and molecular phylogeny, with desert truffles emerging as a key focus. Notably, *Terfezia* species have attracted significant interest because of the rising demand for their ascocarps, leading to greater appreciation of their value in Mediterranean cuisine (Santos-Silva et al., 2021).

Initial taxonomic efforts for *Terfezia* relied mainly on morphological traits, including spore shape, peridial features, and gleba color (Bordallo et al., 2013, 2015). However, these characteristics proved insufficient due to extensive morphological convergence and homoplasy associated with their hypogeous adaptations, leading to the description of many redundant or poorly defined species (Louro et al., 2020; Santos-Silva et al., 2021). The advent of molecular techniques, especially ITS sequencing, has enabled a global reassessment of *Terfezia* collections, refining taxonomic boundaries and revealing previously misidentified specimens, although ongoing challenges remain (Bordallo et al., 2013; Zitouni-Haouar et al., 2014; Louro et al., 2020; Akata et al., 2025a).

Terfezia albida features subglobose to turbinate ascomata that are hypogeous to partially emergent, with a smooth white to cream surface, a pseudoparenchymatous peridium up to 500 µm thick, and a grayish-green gleba that matures and is separated by whitish or pinkish veins. It emits a strong spermatic odor, produces globose, spiny ascospores (18–23 µm), and associates with members of *Helianthemum* Mill. in calcareous, alkaline soils, fruiting from late April to mid-May (Bordallo et al., 2013).

Terfezia albida was first recorded in the southeastern Iberian Peninsula, and GenBank data indicate that all 18 documented specimens are from Spain, establishing Spain as its main habitat. Previous research mentioning *T. albida* in Türkiye (Uzun & Kaya, 2016) relied on morphological observations, providing limited macroscopic and microscopic descriptions without a full ecological context. This narrow perspective has left significant gaps in understanding the species' distribution, habitat preferences, and ecological relationships outside its native range.

This study aims to confirm the identification of *Terfezia albida* in Türkiye using an integrative approach that combines morphological and molecular methods. This approach ensures accurate verification and provides a framework for distinguishing *Terfezia albida* from similar species. Additionally, the research improves understanding of the species' distribution in Türkiye, offering insights into its ecological preferences, geographic range, and interactions with host plants. This investigation broadens our knowledge of *Terfezia albida* and records its presence in the country.

MATERIAL and METHOD

This research used a comprehensive methodology that combines traditional morphological analysis with modern molecular techniques to accurately identify and classify *Terfezia* specimens collected in Konya, Türkiye. Detailed evaluations of macroscopic and microscopic features were conducted, resulting in a thorough characterization of the samples. Additionally, molecular sequencing improved species identification accuracy by using ribosomal DNA (rDNA) analysis, specifically targeting the ITS region. This approach yielded a reliable and detailed classification of the collected specimens.

Morphological Study

Terfezia samples were carefully collected from the study area, with their observable features and environmental conditions thoroughly documented on-site. Following field observations, the samples were transported to a laboratory for detailed microscopic analysis. This involved using a Euromex Oxion Trinocular light microscope at 100x magnification, enabling detailed examination of structural characteristics. Around 30 observations were noted for each microscopic feature to ensure accurate measurements. To improve visibility, various chemical

reagents, including 5% potassium hydroxide (KOH), Melzer's reagent, and Congo red, were used (Figure 1). For ultrastructural analysis, small specimen pieces were mounted on stubs using double-sided adhesive tape and coated with a thin layer of gold in preparation for scanning electron microscopy (SEM). SEM analysis was conducted using an EVO 40XVP SEM (LEO Ltd., Cambridge, UK) at an acceleration voltage of 20 kV, providing high-resolution images of surface structures (Figure 2).

Molecular Characterization

Determination of the ITS rDNA sequences

Genomic DNA was extracted from two *Terfezia* samples. Subsequently, the nrITS regions of nuclear ribosomal DNA were amplified by polymerase chain reaction (PCR) using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). DNA isolation and ITS amplification followed standard protocols described by Rogers and Bendich (1994) and White et al. (1990), and were supplemented with optimized, reproducible protocols from Akata et al. (2024a, 2024b, 2024c, 2024d; 2025a, 2025b), Ediş et al. (2025), and Kumru et al. (2025). This approach aimed to ensure the reliability of the molecular data and the methodological integrity of the study.

Molecular Phylogeny Study

Detailed phylogenetic analyses of the fungal samples were conducted using nucleotide sequences obtained by Sanger sequencing after PCR amplification of DNA directly from the collected materials with ITS primers. These analyses were performed in MEGA11, and all phylogenetic evaluations were conducted on this platform (Tamura et al., 2021). Sequences showing morphological similarity to *Terfezia albida* were retrieved from the GenBank database. Closely related species served as the ingroup, while species with more distant phylogenetic positions, as determined by NCBI BLAST searches, served as the outgroup. Sequence alignment was performed with the MUSCLE algorithm to ensure high accuracy and consistency. Based on the appropriate nucleotide substitution model, the Tamura-3-parameter model with a gamma distribution (T92 + G) was selected as the best model (Tamura & Kumar, 2002). A phylogenetic tree was constructed using the Maximum Likelihood method to reveal the evolutionary relationships among taxa. The statistical reliability of the tree was assessed using 1000 bootstrap replications, in accordance with the method proposed by Felsenstein (1985). This entire analysis process was conducted in accordance with the standard protocols presented by Akata et al. (2024c, 2024d, 2025a, 2025b), ensuring methodological integrity and reliability.

RESULTS

Terfezia albida Ant. Rodr., Mohedano & Bordallo (Figure 1, 2).

Macroscopic and microscopic features

Ascomata 30–40 mm across, hypogeous or occasionally partially emergent at full maturity, generally exhibiting rounded to irregular shapes, such as nearly spherical, top-like, or cushion-shaped forms. **Surface** smooth, initially vivid, bright white, gradually fading to pale cream with maturity, and often develops blackish or brownish patches in areas exposed to sunlight or frequent handling, greenish with age on injured areas. **Gleba** dense, displaying a white colouration in immature stages and gradually shifting to greyish-green with age, fertile tissues separated by a network of veins retaining a whitish appearance throughout development, sometimes exhibiting faint pinkish discolourations. **Odour** spermatic, **taste** mild without notable characteristics. **Peridium** 300–450 µm thick, merging gradually and without clear separation from the underlying gleba, presenting a uniform white appearance in cross-section, composed of a pseudoparenchymatous tissue formed by irregularly arranged, subglobose cells, innermost layers made up of hyaline and thin-walled, whereas those in the outermost layers become yellowish and develop thicker walls. **Asci** 75–90 x 65–80 µm, spherical to broadly pear-shaped, sessile or with short stalk, thick-walled, 6-8 spored and inamyloid. **Ascospores** (18.5)19–20(–21) µm, including ornamentation, and (15)16–17.5(–18) µm, without ornamentation, initially hyaline, smooth, and containing a prominent single guttule, gradually transition to a yellowish-ochre hue at maturity, the surface becoming ornamented with distinct conical to blunt spines, predominantly straight, sometimes presenting cylindrical, curved, or truncated form at their apex, reaching lengths of up to 3 µm.

Material examined: TÜRKİYE— Konya, Karacadağ, on calcareous, alkaline soils, associated with *Helianthemum* sp., 450 m, 39°06'44.9"N 32°55'19.5"E, 11.05.2023, ANK Akata 8786, (GenBank accession number: OR398194); Hasköy, on calcareous, alkaline soils, associated with *Helianthemum* sp., 938 m, 38°53'50.5"N 32°56'13.8"E, 11.05.2023, ANK Akata 8787, (GenBank accession number: OR398208).

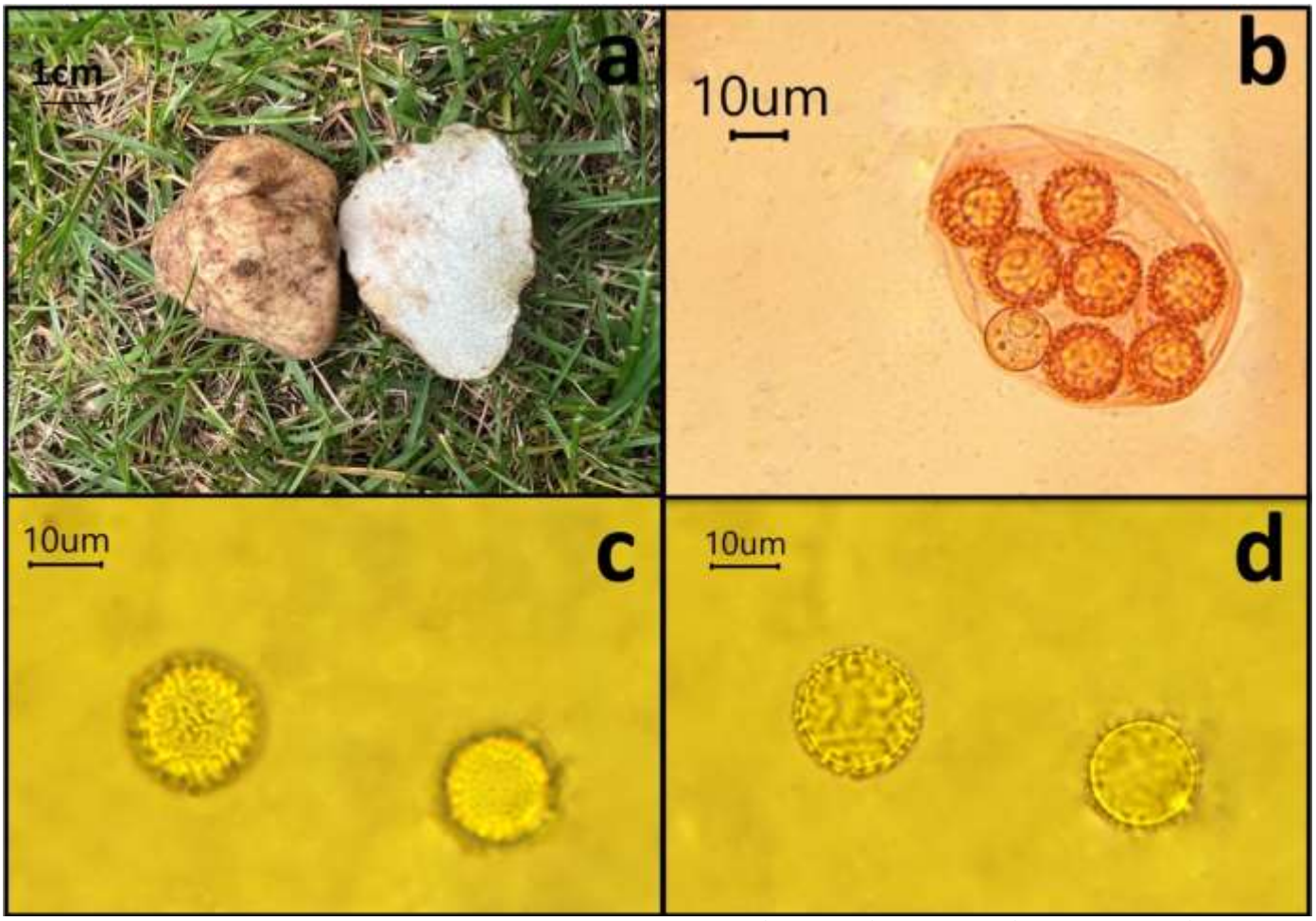


Figure 1. a. *Terfezia albida* fruiting body, b. ascospores within the ascus (Congo red), c-d. spores (Melzer's reagent).
Şekil 1. a. Terfezia albida fruktifikasyon yapısı, b. askus içindeki askosporlar (Kongo kırmızısı), c-d. sporlar (Melzer ayıracı).

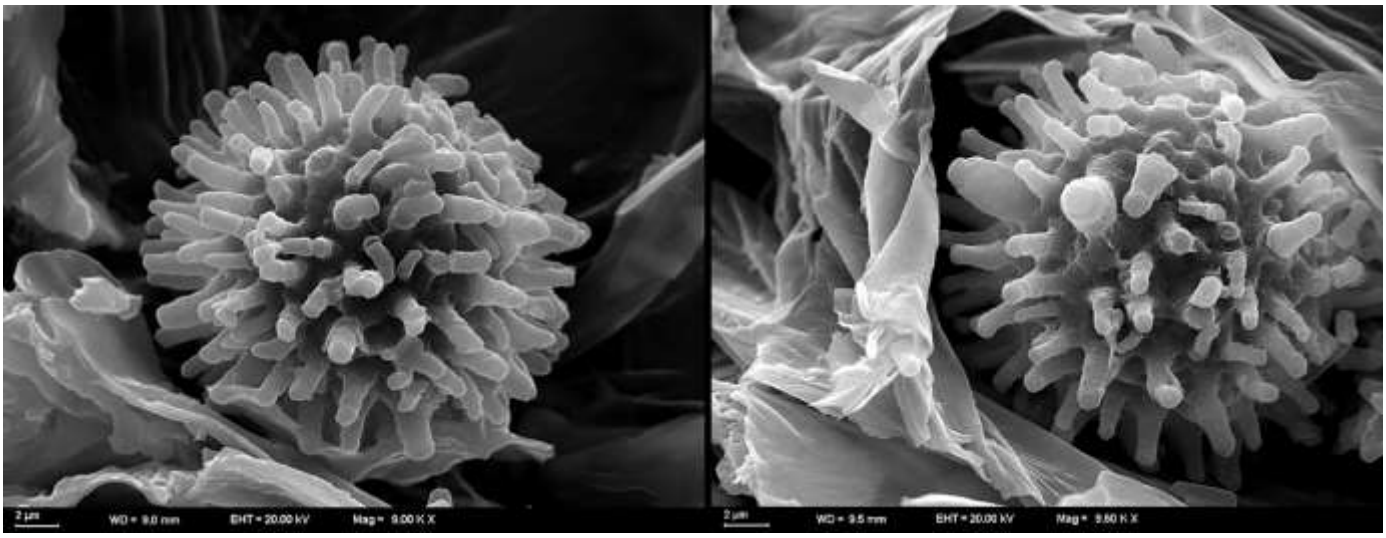


Figure 2. *Terfezia albida* ascospore images captured by SEM.
Şekil 2. SEM ile çekilmiş Terfezia albida askospor görüntüleri.

Evolutionary History of *T. albida* Specimens

The phylogenetic placement of specimens ANK Akata 8786 and ANK Akata 8787 was examined by analyzing their nuclear ribosomal nrITS rDNA sequences, which were amplified using standardized molecular protocols and deposited in the NCBI GenBank under accession numbers OR398194 and OR398208, respectively. To contextualize their evolutionary relationships, a comparative dataset of nrITS sequences representing a

taxonomically diverse subset of the genus *Terfezia* was compiled. Details of all sequences included in the phylogenetic analyses, including taxonomic identities, voucher specimen numbers, geographic origins, and GenBank accession numbers, are provided in Table 1. To root the phylogenetic tree and establish a broader evolutionary context, the nrITS sequence of *Peziza nordica* Kristiansen, LoBuglio & Pfister, a species from the same family, *Pezizaceae*, was selected as the outgroup.

Molecular phylogenetic reconstruction, conducted using Maximum Likelihood analysis, revealed eight well-supported clades within *Terfezia*. Among them, Clade 6 consisted exclusively of *Terfezia albida* samples, within which both ANK Akata 8786 and 8787 were robustly nested. The assignment of these two specimens to *Terfezia albida* was strongly supported by high bootstrap values, underscoring the reliability of their phylogenetic grouping. Complementary BLASTn analyses further substantiated this classification, showing >99% sequence identity between the nrITS regions of the ANK Akata specimens and previously characterized *Terfezia albida* accessions. Taken together, these molecular data corroborate the identification of ANK Akata 8786 and ANK Akata 8787 as members of *Terfezia albida* and affirm their close evolutionary affinity within the genus. The use of *Peziza nordica* as an early-diverging outgroup taxon provided a stable root for the tree and supported the monophyletic grouping of *Terfezia* (Figure 3).

Table 1. Taxonomic identities, voucher specimen numbers, collection localities, and corresponding GenBank accession numbers for the nrITS xsequences included in the phylogenetic analyses. Unpublished refers to sequences deposited in GenBank without an associated peer-reviewed publication.

Çizelge 1. Filogenetik analizlere dahil edilen nrITS dizilerine ait taksonomik tanımları, referans örnek numaraları, toplama lokaliteleri ve ilgili GenBank erişim numaraları. Yayımlanmamış (Unpublished), GenBank'ta depolanan ve ilgili hakemli yayını bulunmayan dizileri ifade eder.

Species	Specimen Voucher/Isolate/Strain	nrITS GenBank Accession Number	Geographical origin	Reference
Terfezia cistophila	j377	KP728825	Greece: Artemida Attica	Bordallo et al., 2015
	j384	KP728826	Greece: Rafina Attica	Bordallo et al., 2015
	j392	KP728827	Greece: Zagora Magnesia	Bordallo et al., 2015
	MUB Fung-j477 (Type Material)	NR_160445	Greece: Nea Makri Attica	Bordallo et al., 2015
	j479	KP728829	Greece: Zagora Magnesia	Bordallo et al., 2015
	MUB:Fung-1080	PQ523466	Spain: Badajoz, Quintana de la Serena	Unpublished
	2004865	MW508653	Portugal: Portalegre	Santos-Silva et al., 2021
Terfezia lusitanica	MUB:Fung-j682	NR_159059	Spain: Caceres	Bordallo et al., 2018
	MUB:Fung-1131	PV247034	Spain: Caceres, Valdehuncar	Unpublished
	fruitbody(123)	MW508553	Portugal: Évora	Santos-Silva et al., 2021
	2004873	MW508554	Portugal: Évora	Unpublished
	j469	MG818752	Portugal: Montemor	Bordallo et al., 2018
	j817	MG818754	Portugal: Montemor	Bordallo et al., 2018
Terfezia fanfani	MUB:Fung-1116	PQ824695	Spain: Badajoz, Quintana de la Serena	Santos-Silva et al., 2021
	2004884	MW508573	Portugal: Portalegre	Santos-Silva et al., 2021
	2004726i	MW508574	Portugal: Portalegre	Santos-Silva et al., 2021
	2004845	MW508575	Portugal: Évora	Santos-Silva et al., 2021
	2004726c	MW508576	Portugal: Portalegre	Santos-Silva et al., 2021
	2004726e	MW508584	Portugal: Portalegre	Santos-Silva et al., 2021
	2003461	MW508590	Portugal: Évora	Santos-Silva et al., 2021
	2004856	MW508597	Portugal: Évora	Santos-Silva et al., 2021
	2004880	MW508601	Portugal: Portalegre	Santos-Silva et al., 2021
	2003467	MW508607	Portugal: Portalegre	Santos-Silva et al., 2021

Terfezia honrubiae	AH19619	MN512331	Spain: Salamanca, Guijuelo	Moreno et al., 2019
	AH45976	MN512332	Spain: Segovia, Revenga	Moreno et al., 2019
	AH46458	MN512334	Spain: Avila, Medinilla	Moreno et al., 2019
	AH 49385	OR528634	Spain: Segovia, La Atalaya	Moreno & Alvarado, 2023
	MUB:Fung-1120	PV030235	Spain: Caceres, Valdehuncar	Unpublished
	MUB:Fung-1127	PV030242	Spain: Badajoz, Quintana de la Serena	Unpublished
Terfezia pini	MUB:Fung-0027	NR_164515	Spain: Burgos	Bordallo et al., 2013
	2004623	MW508546	Portugal: Évora	Bordallo et al., 2013
	2004621	MW508544	Portugal: Évora	Bordallo et al., 2013
	2004640	MW508545	Portugal: Évora	Bordallo et al., 2013
	2004841	MW508542	Portugal: Évora	Bordallo et al., 2013
	fruitbody(315)	MW508541	Portugal: Évora	Bordallo et al., 2013
	2004863	MW508540	Portugal: Évora	Santos-Silva et al., 2021
	ANK Akata 8786	**OR398194**	**Türkiye: Konya**	**Current study**
	ANK Akata 8787	**OR398208**	**Türkiye: Konya**	**Current study**
	MUB:Fung-1000	PQ380240	Spain: Albacete, El Ballestero	Unpublished
Terfezia albida	MUB:Fung-1064	PQ380246	Spain: Albacete, Tarazona de la Mancha	Unpublished
	MUB:Fung-1069	PQ380251	Spain: Albacete, El Ballestero	Unpublished
	j574	OP458226	Spain: Albacete, Lezuza	Unpublished
	tO16	HM056221	Spain: Albacete	Bordallo et al., 2013
	MUB:Fung-0029 (Type material)	NR_137053	Spain: Albacete	Bordallo et al., 2013
Terfezia extremadurensis	MUB:Fung-0026 (Type material)	NR_137050	Spain: Caceres	Bordallo et al., 2013
	MUB:Fung-1100	PQ807698	Spain: Caceres, Valdecanas	Unpublished
	MUB:Fung-1106	PQ807704	Spain: Jaen, Pontones	Unpublished
	MUB:Fung-1107	PQ807705	Spain: Badajoz, Quintana de la Serena	Unpublished
	MUB:Fung-1109	PQ807707	Spain: Badajoz, Quintana de la Serena	Unpublished
	tL10	HM056204	Spain: Caceres	Bordallo et al., 2013
	tO17	HM056202	Spain: Caceres	Bordallo et al., 2013
	2003831	MW508663	Portugal: Portalegre	Santos-Silva et al., 2021
	2004273	MW508655	Portugal: Castelo Branco	Santos-Silva et al., 2021
Terfezia grisea	MUB:Fung-j388 (Type material)	NR_160444	Greece: Schinias Attica	Bordallo et al., 2015
	j327	KP189328	Spain: Burgos	Bordallo et al., 2015
	j386	KP189329	Greece: Schinias Attica	Bordallo et al., 2015
	j389	KP189331	Greece: Schinias Attica	Bordallo et al., 2015
	j476	KP189332	Greece: Schinias Attica	Bordallo et al., 2015
	j485	KP189333	Spain: Burgos	Bordallo et al., 2015
	MUB:Fung_1130	PV030245	Spain: Caceres, Valdehuncar	Unpublished
	2004725e	MW508537	Portugal: Setúbal	Santos-Silva et al., 2021
	2004725h	MW508539	Portugal: Setúbal	Santos-Silva et al., 2021
Peziza nordica	FH:00304781 (Type material)	NR_148104	Norway	Pfister et al., 2016

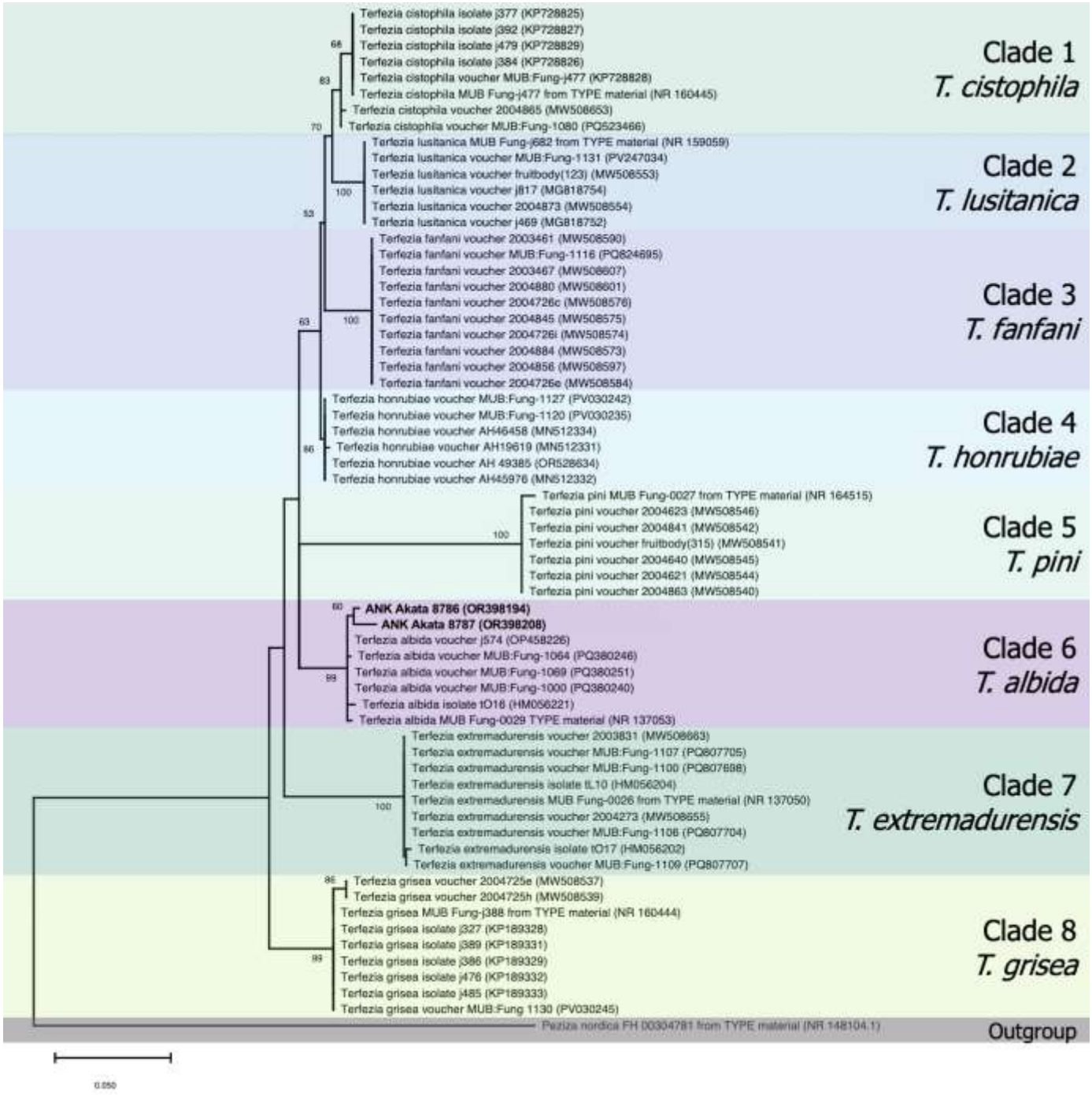


Figure 3. Phylogenetic relationships among 62 *Terfezia* specimens were inferred using the nrITS region and the Maximum Likelihood (ML) method with the T92+G model. Bootstrap support values (≥50%) are indicated above branches. Sequences used in the analysis were retrieved from the NCBI GenBank database; corresponding accession numbers are provided next to each taxon. Eight major clades corresponding to currently accepted species, *Terfezia cistophila* (Clade 1), *T. lusitanica* (Clade 2), *T. fanfani* (Clade 3), *T. honrubiae* (Clade 4), *T. pini* (Clade 5), *T. albida* (Clade 6), *T. extremadurensis* (Clade 7), and *T. grisea* (Clade 8) are highlighted in different colors. Two newly obtained sequences from ANK Akata fungarium collections (8786 and 8787) are clustered within Clade 6, representing *Terfezia albida*. *Peziza nordica* was used as the outgroup. Scale bar represents 0.050 genetic distance.

Şekil 3. nrITS bölgesi kullanılarak ve T92+G modeli ile Maksimum Olabilirlik (ML) yöntemi uygulanarak elde edilen 62 Terfezia örneği arasındaki filogenetik ilişkiler. Bootstrap destek değerleri (≥%50) dalların üzerinde gösterilmiştir. Analizde kullanılan diziler NCBI GenBank veritabanından alınmış olup, ilgili erişim numaraları her bir taksonun yanında verilmiştir. Güncel olarak kabul edilen türlere karşılık gelen sekiz ana klad; Terfezia cistophila (Klad 1), T. lusitanica (Klad 2), T. fanfani (Klad 3), T. honrubiae (Klad 4), T. pini (Klad 5), T. albida (Klad 6), T. extremadurensis (Klad 7) ve T. grisea (Klad 8) farklı renklerle vurgulanmıştır. ANK Akata fungaryumu koleksiyonlarından yeni elde edilen iki dizi (8786 ve 8787), klad 6 içerisinde kümelenmiş olup Terfezia albida'yı temsil etmektedir. Peziza nordica dış grup olarak kullanılmıştır. Ölçek çubuğu 0.050 genetik uzaklığı göstermektedir.

DISCUSSION and CONCLUSION

Within the spiny-spored *Terfezia* complex, *T. albida* stands out as a morphologically and ecologically distinct species, characterized by larger ascomata, a consistently white peridial surface, a grayish-green gleba, and a notable spermatic odor. It strongly prefers alkaline clayey soils and forms ectomycorrhizal associations with *Helianthemum* species (Bordallo et al., 2013). In contrast, *Terfezia olbiensis* (Tul. & C. Tul.) Sacc. inhabits limestone and clayey woodlands dominated by *Pinus* and *Quercus* species, without a symbiotic relationship with *Helianthemum*. It can be distinguished morphologically by shorter spines (<2.5 µm) and a mild rotting odor (Bordallo et al., 2013; Akata et al., 2025a). Although *Terfezia grisea* Bordallo, Kaounas & Ant. Rodr. also associates with *Helianthemum* species and prefers alkaline soils; it differs from *T. albida* by its ochraceous to blackish peridial surface, dark gray gleba, and absence of a strong spermatic odor (Bordallo et al., 2015). *Terfezia cistophila* Ant. Rodr., despite sharing a spermatic odor, grows in acidic soils, associates with *Cistus* species, and is easily distinguished by its smaller ascomata, a peridium that blackens rapidly with age, and a pale ochre gleba (Díez et al., 2002; Akata et al., 2025a). Similarly, *Terfezia pini* Bordallo, Ant. Rodr. & Muñ.-Moh. shows significant ecological and microscopic differences from *T. albida*. It is typically found in burned forest environments beneath *Pinus* and *Quercus* species, without forming associations with cistaceous plants. Microscopically, it is characterized by spores exhibiting pseudo-reticulate ornamentation formed by elongated spines that fuse at their bases, a diagnostic feature absent in *Terfezia albida* (Bordallo et al., 2013).

Further distinctions are evident among species inhabiting acidic grasslands dominated by *Tuberaria guttata* (L.) Fourr. (Bordallo et al., 2013, 2018). *Terfezia honrubiae* G. Moreno, Manjón, Morte & P. Alvarado is distinguished by its brown gleba, pseudoparenchymatous peridial structure, and fruiting habit, which ranges from solitary to gregarious. This contrasts with *Terfezia albida*, characterized by a grayish-green gleba, a non-pseudoparenchymatous peridium, and a consistent preference for alkaline substrates (Bordallo et al., 2013; Moreno et al., 2019). *Terfezia fanfani* Mattir. produces reddish ascomata and smaller spores with slender ornamentation, differing markedly from the larger, whitish ascomata and thick-spored morphology of *T. albida* (Bordallo et al., 2013). Similarly, *Terfezia lusitanica* Bordallo, Ant. Rodríguez, Louro & Muñ.-Moh. can be distinguished by its ochre-colored peridium and unique spore dimensions, lacking the white peridial coloration and globular characteristics of *T. albida* (Bordallo et al., 2018). *Terfezia pseudoleptoderma* Bordallo, Ant. Rodríguez, Louro & Muñ.-Moh. It is further distinguished by its small ascomata (<20 mm diameter), spores with pseudo-reticulate ornamentation, and a strict fruiting period limited to late winter and early spring. Lastly, *Terfezia extremadurensis* Muñ.-Moh., Ant. Rodr. & Bordallo is especially notable for its tuber-like glebal structure, in which meandering veins do not form enclosed pockets around the fertile tissue, unlike the fully compartmentalized gleba seen in *T. albida* (Bordallo et al., 2013).

Morphological similarities among fungal species make species-level distinctions difficult, especially in groups such as *Terfezia*, which are among the hypogeic genera that form underground sporocarps. This underscores the need for molecular data in identification. In this context, the nuclear ribosomal ITS region is widely recognized as a standard marker for fungal molecular taxonomy because of its high species-discriminatory power and the availability of extensive reference databases (White et al., 1990; Schoch et al., 2012; Raja et al., 2017). In this study, ITS rDNA sequences were used to identify ANK Akata 8786 and ANK Akata 8787; phylogenetic analyses revealed that both samples were closely related to the species *Terfezia albida*. The fact that these specimens show over 99% sequence similarity with *Terfezia albida* samples deposited in GenBank and are placed in the same clade in phylogenetic analyses strongly supports their molecular-level identification as *T. albida*. These findings once again demonstrate the indispensable role of molecular markers in resolving taxonomic confusion within the genus *Terfezia* and show that diagnoses based solely on morphological features are often insufficient. Future multi-gene phylogenetic studies and, where feasible, whole-genome sequencing are expected to provide higher-resolution insights into the evolutionary relationships between *Terfezia albida* and closely related species.

Previous reports of *Terfezia albida* in Türkiye (Uzun & Kaya, 2016) relied solely on morphological observations. The earlier study provided brief macroscopic and microscopic descriptions but lacked a detailed ecological context. In contrast, this study employs an integrative approach, combining macro- and micromorphological analyses with molecular phylogenetic methods based on ITS rDNA sequences. Our findings confirm the species' taxonomic status in Türkiye and enhance understanding of its ecology and distribution. These results complement earlier morphological records, providing a more robust framework for species delimitation.

Although the present study is based on a limited number of specimens, the integrative use of morphological characters and nrITS-based molecular data is sufficient for reliable species-level taxonomic verification. Nevertheless, future studies incorporating broader sampling and analyses spanning multiple gene regions, as well as genomic approaches, will be necessary to elucidate population-level genetic diversity and phylogeographic patterns of *Terfezia albida* in Türkiye.

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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.

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