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New records of lichenized and non-lichenized fungi from Lifos Mountain (Hacılar, Kayseri, Türkiye): An integrative taxonomic approach using morphological and ITS sequence data

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Abstract

Purpose: Lifos Mountain is one of the significant but relatively understudied regions with volcanic geology, elevational range and diverse microhabitats in the northwestern slopes of Mount Erciyes, Central Anatolia (Türkiye) in terms of fungal and lichen diversity. The aim of this study was to explore the diversity of fungi found on Lifos Mountain by an integrative taxonomic approach that integrates morphological, anatomical, ecological, chemical and molecular data. Another objective of the study was to record new taxa not previously found and to add to the knowledge of Turkish fungal and lichen biodiversity.

Method: Fungi and lichens collected from various habitats on Lifos Mountain were studied by detailed macro and micro-morphological characteristics. Stereomicroscopic and light microscopic observations were conducted for anatomical purposes and in case of need, standard lichen spot tests were performed. Dried specimens were used for genomic DNA extraction, amplification and sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region. Sequences were obtained and compared with the reference sequences found in the GenBank using BLAST, aligned using ClustalW and further analysed using Maximum Likelihood Phylogenetic analyses in MEGA 7. All of the morphological, ecological, chemical and molecular data were checked together for reliable species identification.

Findings: The fungi identified were *Camarosporidiella astragalicola* Jaklitsch & Voglmayr, *Exidia inflata* Cazenave & G. Gruhn and *Rhizoplaca porteri* S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair. Maximum likelihood phylogenetic analyses showed that all specimens examined clustered within their respective species-level clades and morphological species assignments were correct. Turkish specimens grouped with their respective taxa and were significantly different from morphologically related species. *Camarosporidiella astragalicola* was found growing on the endemic plant *Astragalus argaeus*, and *Exidia inflata* was found on the bark of deciduous trees. Exposure high elevation, with volcanic siliceous rocks was the habitat in which *Rhizoplaca porteri* were found, consistent with their known ecological preferences. The results are the first records of all three taxa for Türkiye, supported by both morphological and molecular data.

Conclusion: The current study is the first attempt to assess the fungal and lichen diversity of Lifos Mountain in an integrated manner, both with traditional taxonomic approach and DNA barcoding methods. The obtained results show the utility of using both morphological and molecular data for proper species delimitation and identification. In addition, three new national records were established, indicating the significance of Lifos Mountain as an important hotspot of new fungal and lichen diversity in Central Anatolia. The results significantly increase the distribution range of the recorded taxa and add to the global mycobiota valuable taxonomic, phylogenetic, ecological and biogeographical information.

Keywords: ITS rDNA, DNA barcoding, fungal diversity, lichenized fungi, new records, Lifos Mountain.

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Lifos Dağı'ndan (Hacılar, Kayseri, Türkiye) likenleşmiş ve likenleşmemiş mantarlar için yeni kayıtlar: Morfolojik ve ITS dizi verilerine dayalı bütüncül bir taksonomik yaklaşım

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Özet

Amaç: Orta Anadolu'da (Türkiye), Erciyes Dağı'nın kuzeybatı yamaçlarında yer alan Lifos Dağı, volkanik jeolojisi, yükselti gradyanları ve heterojen mikrohabitatları nedeniyle fungal ve liken çeşitliliği açısından önemli ancak yeterince araştırılmamış bir alanı temsil etmektedir. Bu çalışmanın amacı, morfolojik, anatomik, ekolojik, kimyasal ve moleküler verileri birleştiren bütüncül bir taksonomik yaklaşım kullanarak Lifos Dağı'nın fungal çeşitliliğini araştırmaktır. Ayrıca çalışma, daha önce kaydedilmemiş taksonları ortaya koymayı ve Türkiye fungal ile liken biyolojik çeşitliliğinin anlaşılmasına katkı sağlamayı hedeflemiştir.

Yöntem: Fungal ve liken örnekleri Lifos Dağı'ndaki farklı habitatlardan toplanmış ve ayrıntılı makro- ve mikromorfolojik özellikleri bakımından incelenmiştir. Anatomik gözlemler stereomikroskop ve ışık mikroskobu teknikleri kullanılarak gerçekleştirilmiş, gerekli durumlarda standart liken spot testleri uygulanmıştır. Genomik DNA kurutulmuş örneklerden ekstrakte edilmiş ve nükleer ribozomal internal transcribed spacer (ITS) bölgesi çoğaltılarak dizilenmiştir. Elde edilen diziler, GenBank veri tabanındaki referans verilerle BLAST kullanılarak karşılaştırılmış, ClustalW ile hizalanmış ve MEGA 7 programında gerçekleştirilen Maksimum Olabilirlik (Maximum Likelihood) filogenetik analizlerine tabi tutulmuştur. Güvenilir tür teşhisi sağlamak amacıyla morfolojik, ekolojik, kimyasal ve moleküler veri setleri birlikte değerlendirilmiştir.

Bulgular: Üç fungal tür tanımlanmıştır: *Camarosporidiella astragalicola* Jaklitsch & Voglmayr, *Exidia inflata* Cazenave & G. Gruhn ve *Rhizoplaca porteri* S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair. Maksimum olabilirlik filogenetik analizleri, incelenen tüm örneklerin ilgili tür düzeyindeki kladlar içerisinde konumlandığını ortaya koymuş ve morfolojik teşhislerin doğruluğunu doğrulamıştır. Türkiye'den elde edilen örnekler, ilgili taksonların doğrulanmış referans dizileriyle birlikte kümelenmiş ve morfolojik olarak benzer türlerden açık şekilde ayrılmıştır. *Camarosporidiella astragalicola*, endemik bir bitki olan *Astragalus argaeus* üzerinde; *Exidia inflata* ise yaprak döken ağaçların kabuklarında tespit edilmiştir. *Rhizoplaca porteri* örnekleri, bilinen ekolojik tercihleriyle uyumlu olarak yüksek rakımlardaki açıkta bulunan volkanik silisli kayalar üzerinde bulunmuştur. Tüm üç takson, birleşik morfolojik ve moleküler kanıtlara dayanılarak Türkiye'den ilk kez rapor edilmektedir.

Sonuç: Bu çalışma, Lifos Dağı'nın fungal ve liken çeşitliliğinin hem geleneksel taksonomik yöntemler hem de DNA barkodlama yaklaşımları kullanılarak gerçekleştirilen ilk bütüncül değerlendirmesini temsil etmektedir. Elde edilen sonuçlar, doğru tür sınırlandırması ve teşhisi için morfolojik ve moleküler verilerin birlikte kullanılmasının etkinliğini göstermektedir. Ayrıca, Türkiye için yeni dört kaydın ortaya konulması, Lifos Dağı'nın Orta Anadolu'daki daha önce belgelenmemiş fungal ve liken çeşitliliği açısından önemli bir sıcak nokta olduğunu ortaya koymaktadır. Bu bulgular, kayıt altına alınan taksonların bilinen yayılış alanlarını önemli ölçüde genişletmekte ve küresel mikobiyotayadeğerli taksonomik, filogenetik, ekolojik ve biyocoğrafik veriler sağlamaktadır.

Anahtar Kelimeler: ITS rDNA, DNA barkodlama, fungal çeşitlilik, likenleşmiş mantarlar, yeni kayıtlar, Lifos Dağı.

1. Introduction

Lichens are self-sustaining symbiotic associations formed primarily between a fungal partner (mycobiont) and one or more photosynthetic partners (photobionts), usually green algae and/or cyanobacteria. They represent one of the most successful life forms in terrestrial ecosystems and occur in a wide variety of habitats ranging from arid deserts to polar regions and alpine environments [1]. Owing to their remarkable ecological adaptability, lichens contribute significantly to nutrient cycling, rock weathering, soil formation, and ecosystem functioning.

Traditionally, lichen taxonomy has been based on morphological, anatomical, ecological, and chemical characteristics. For species identification the diagnostic characters have been traditionally used for such a purpose as the morphology of thallus, morphology of the reproductive structures, morphology of the ascospores and secondary metabolites [2]. But, species delimitation can be complicated by morphological plasticity, cryptic speciation, and environmental influences on phenotype, and convergence of morphological traits. So, morphology is often not enough for species identification, especially in species complexes and closely related taxa [3-5].

Molecular techniques have greatly enhanced the systematics of lichens and fungi. The Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) has been adopted as the official fungal DNA barcode thanks to its excellent species-level discriminatory power [6]. ITS-based DNA barcoding has been a powerful tool for discovering numerous cryptic taxa and has significantly sharpened the species concept in many groups of fungi forming lichining fungi, including *Rhizoplaca*, and lich-forming fungi [7,8]. Hence, with recent advances in taxonomy the use of molecular analysis has become more common among researchers in order to obtain a reliable species identification.

With its total height of 3917 m a.s.l., Mount Erciyes is the highest mountain in the Central Anatolia, and is one of the most important volcanic massifs in Türkiye. Lifos Mountain, which is on the northwestern slopes of Erciyes, is composed of mostly volcanic substrate such as andesitic and basaltic rocks (Figure 1). It includes a variety of habitats including steppe, alpine grassland and open woodland communities. A wide variety of lichen forming and non-lichen forming fungi occurs under these environmental conditions. The volcanic geology, elevational gradients and continental climatic conditions result in a diversity of microhabitats that are only poorly studied from a mycological point of view.

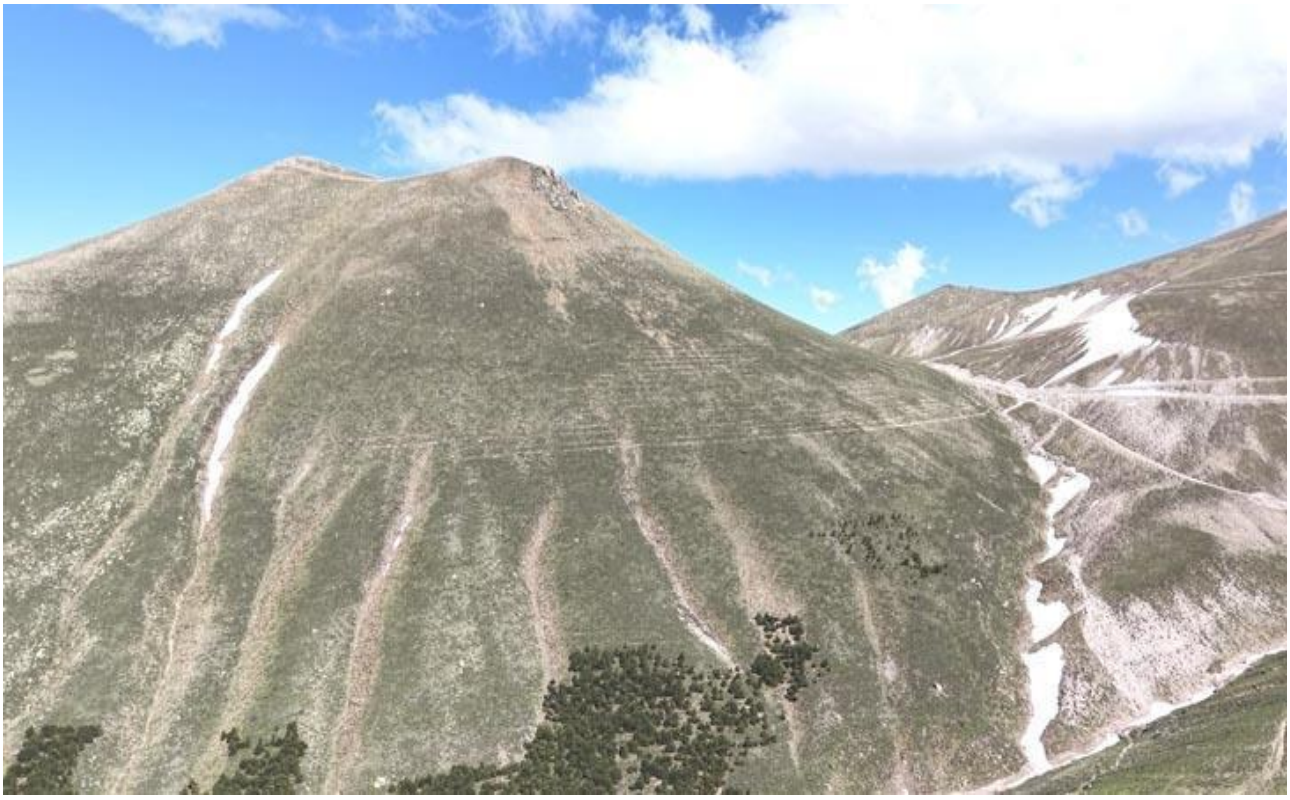

Figure 1. General view of Lifos Mountain (Mount Erciyes), Kayseri Province, Central Anatolia, Türkiye, showing the volcanic landscape and alpine habitats of the study area [9]

The diversity of lichen flora of Erciyes Mountain and its surrounding areas has been previously reported. Central Anatolian volcanic mountain ecosystems are important sources of lichen diversity, with many taxa reported by Halıcı and co-workers and emphasis placed on the importance of these ecosystems in the conservation of the lichen flora of Central Anatolia [10,11]. Research into molecular studies in the area is still relatively limited. One of the first studies using a marker of DNA-based identification methods on Erciyes Mountain was carried out in the study of Barak et al. (2016) which identified some lichenized fungi using the ITS (rDNA) marker and proved that DNA-based methods are effective in resolving taxonomic uncertainties in lichen-forming fungi [12]. The study yielded valuable baseline information on lichen biota of Erciyes Mountain in terms of molecular level. Despite the significant efforts, molecularly supported taxonomic studies in particular for Lifos Mountain are still very limited. To date, no comprehensive investigation integrating detailed morphological examinations with ITS-based phylogenetic analyses has been conducted for the fungal and lichenized fungal diversity of this locality. Therefore, the fungal biodiversity of Lifos Mountain remains insufficiently explored, particularly from a molecular systematic perspective.

Several studies have expanded the known diversity of lichenized and non-lichenized fungi in Türkiye through the documentation of new national records. Among lichenized fungi, Yazıcı et al. (2005) reported *Adelolecia pilati*, *Dermatocarpon arnoldianum*, *Xanthoparmelia protomatrae*, and *Verrucaria ochrostoma* as new records for the country [13] . Karagöz et al. (2011) subsequently added *Diplotomma hedinii*, *Lecanora valesiaca*, and *Xanthoria weberi* from the Kemaliye district of Erzincan [14]. More recently, Kocakaya et al. (2023) reported the lichenized species *Gyalolechia klementii* and *Parvoplaca servitiana*, together with four lichenicolous fungal taxa, from Üzümdere Nature Park. New national records have also been documented among non-lichenized fungi [15]. Akata et al. (2011) reported four macrofungal taxa from Trabzon, while Türkoğlu and Castellano (2014) added six hypogeous ascomycete taxa to the Turkish mycobiota [16]. Akçay et al. (2023) recorded *Hymenoscyphus imberbis*, *H. vitigenus*, and *Gemmina gemmarum* for the first time from Türkiye [17]. In Central Anatolia, Güllü and Halıcı (2025) combined morphological examinations with ITS-based molecular analyses to document new macrofungal records from Koramaz Valley [18]. Collectively, these studies indicate that the national fungal inventory continues to expand and provide a relevant context for investigating both lichenized and non-lichenized fungi on Lifos Mountain.

The present study combines detailed morphological examinations with ITS-based molecular analyses to investigate fungal diversity on Lifos Mountain. As a result, three species, namely ***Camarosporidiella astragalicola*** Jaklitsch & Voglmayr, ***Exidia inflata*** Cazenave & G. Gruhn, Lynge and ***Rhizoplaca porteri*** S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair were identified and are reported here for the first time from Türkiye. The published geographical records of these taxa provide a broader context for their occurrence in Central Anatolia. *Camarosporidiella astragalicola* was described from Crete, Greece, on stems of *Astragalus angustifolius*, with additional material reported

from Mount Etna, Sicily, Italy, on A. siculus [19]. *Exidia inflata* was documented from France, where it was collected on terminal branches of *Populus tremula* [20]. *Rhizoplaca porteri* was reported from western North America in earlier molecular studies of the *R. melanophthalma* species complex [21]. The Lifos Mountain collections therefore add geographical information for these taxa and contribute to the documentation of fungal and lichen biodiversity in Central Anatolia, while emphasizing the value of integrating morphological, ecological, and molecular evidence in taxonomic research. These findings contribute to the knowledge of fungal and lichen biodiversity in Central Anatolia and emphasize the importance of integrating morphological and molecular approaches in contemporary taxonomic research.

2. Materials and methods

2.1. Study Area and Specimen Collection

Specimens were collected during field surveys conducted on Lifos Mountain, located on the slopes of Mount Erciyes in Kayseri Province, Central Anatolia, Türkiye. The study area has volcanic geological formations with a preponderance of andesitic and basaltic rocks, continental climate and a wide variety of habitats from steppe vegetation to alpine communities. Carefully the collected specimens were taken off their substrates and deposited in the herbarium after labelling them.

2.2. Morphological and Anatomical Studies

The macromorphological characters were studied under stereomicroscope. Light microscopy and micromorphological and anatomical observations were done. Hand-cut sections of the reproductive structures were prepared and mounted in water, 10% KOH, Lugol's iodine solution (I) and other suitable mounting media as required. Multiple measurements were made per specimen of anatomical structures.

2.3. Chemical Analyses

The standard lichen spot tests (K, C, KC and P) were carried out by standard methods [2]. The response of the cortex and medulla were noted and compared with published description. Chemical characters were employed as add-on taxonomic data for species identification.

2.4. Molecular Characterization

2.4.1. DNA Extraction, PCR Amplification and Sequencing

DNA was isolated from dried fungus and lichen thalli by using NucleoGene Plant Mini Kit following the manufacturer's protocol. The nuclear ribosomal ITS region was PCR-amplified based on universal fungal primers. Polymerase chain reaction (PCR) amplifications were performed with a 2× commercial PCR Master Mix, primers, template DNA and distilled water.

PCR amplification was performed under the following thermal cycling conditions: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52–55°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min.

Amplification products were visualized on 1.5% agarose gels stained with an appropriate nucleic acid stain and photographed under ultraviolet illumination. Successfully amplified PCR products were purified and sequenced commercially by Oligomer Biotechnology (Ankara, Türkiye).

2.4.2. Phylogenetic Analyses

The ITS sequences were first compared against the reference sequences available in the GenBank with the help of the BLAST algorithm. Highly similar sequences were retrieved and used to build datasets for phylogenetic analyses. The sequence alignments were created using the ClustalW algorithm in BioEdit 7.2.5 and manually edited as required [22].

Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method implemented in MEGA version 7.0 [23]. Node support was estimated using 1000 bootstrap replicates. Resulting phylogenetic trees were visualized and edited prior to figure preparation.

3. Results

3.1. *Camarosporidiella astragalicola* Jaklitsch & Voglmayr

Morphological Description

Ascomata semi-immersed to superficial, subglobose, papillate, grey to black, with a finely to distinctly verruculose outer surface, developing on a compact blackish-brown subiculum. Peridium 30–110 µm thick, pseudoparenchymatous, composed of dark brown cells and becoming thicker towards the apex and basal regions. Hamathecium consisting of numerous branched and anastomosing pseudoparaphyses (Figure 2).

Asci 8-spored, bitunicate, fissitunicate, cylindrical, shortly stipitate, with a furcate or nodulose base. Ascospores 22–24 × 13–15 µm, ellipsoid to subfusiform, slightly constricted at the median septum, usually broader in the upper half, straight to slightly curved, yellowish-brown to medium brown, becoming dark brown in 3% KOH. Spores predominantly (3–)5–6(–7)-transversely septate with 1–2 longitudinal septa, occasionally lacking longitudinal septa when immature. Ascospore wall smooth in water but becoming faintly punctate in KOH (Figure 2).

Asexual morph present. Conidia (20–)23.5–33(–41.7) × (8.8–)10–15(–20) µm, oblong to ellipsoid, dark brown, smooth-walled, straight to slightly curved, with 3–7(–8) transverse septa and 1–2(–3) longitudinal septa, becoming muriform at maturity.

Habitat and distribution: On stems of *Astragalus argaeus* Boiss. (Fabaceae), Lifos Mountain (Erciyes Mountain), Kayseri Province, Central Anatolia, Türkiye, at 2180 m a.s.l. (38°35′10″ N, 35°28′16″ E), 27 April 2025, leg. Ş. Karakaya and M. Güllü, voucher ERCH LIF 0.048.

Taxonomic Notes

The Turkish specimen examined in this study agrees well with the current concept of *Camarosporidiella astragalicola* presented by Jaklitsch et al. (2025). The species is characterized by semi-immersed to superficial papillate ascomata, a thick dark pseudoparenchymatous peridium, cylindrical bitunicate asci, and brown ellipsoid to subfusiform ascospores measuring 22–24 × 13–15 µm with predominantly 5–6 transverse and 1–2 longitudinal septa. The dimensions and septation pattern of the ascospores observed in the Turkish material fall within the range reported for the species by Jaklitsch et al. (2025) [19].

Among morphologically related taxa, *Camarosporidiella celtidis* (Shear) Thambug., Wanas. & K.D. Hyde and *C. laburni* (Pers.) Wanas., Bulgakov, Camporesi & K.D. Hyde share similar pseudo-camarosporium-like characteristics, including brown multi-septate ascospores and muriform conidia. However, *C. astragalicola* differs by its combination of ascospore dimensions, septation pattern and host association. The examined specimen was collected on stems of *Astragalus argaeus*, an endemic species restricted to Central Anatolia, which corresponds well with the host preference reported for *C. astragalicola* [24]. In addition, the relatively broad ascospores and large muriform conidia observed in the Turkish material are consistent with the diagnostic features of the species.

Ecologically, the occurrence of *C. astragalicola* on volcanic mountain slopes of Lifos Mountain at an elevation of 2180 m expands the known geographical distribution of the species and demonstrates its adaptation to high-altitude continental habitats. The morphological features observed in the Turkish specimen, together with its host association and ecological characteristics, support its identification as *Camarosporidiella astragalicola*. To the best of our knowledge, this study represents the first record of *C. astragalicola* from Türkiye and extends the known distribution range of the species to Central Anatolia.

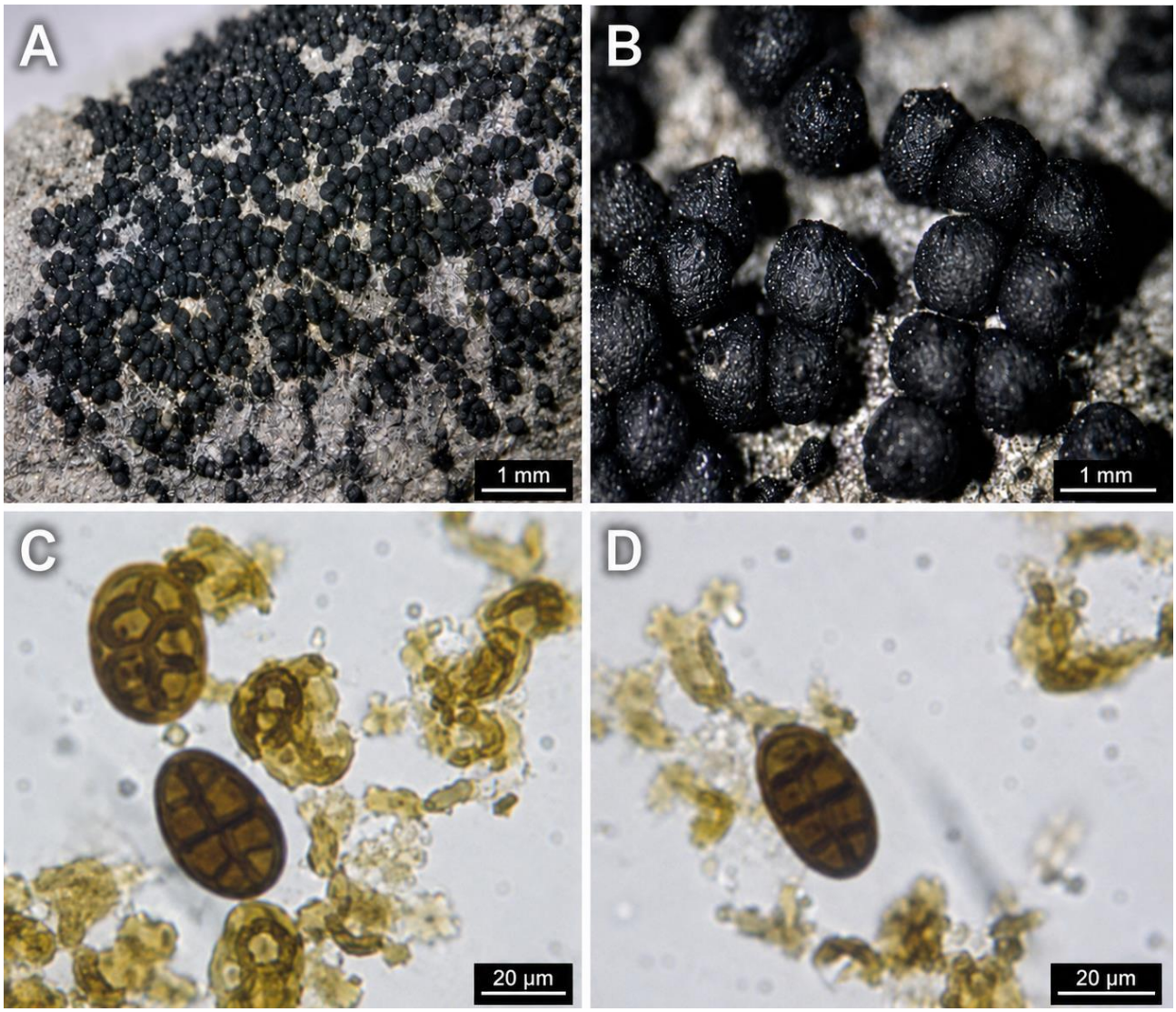

Figure 2. Morphological and anatomical characteristics of the examined lichen species. (A) General view of the thallus showing numerous black, perithecioid ascomata distributed across the thallus surface. (B) Close-up of ascomata, showing their rounded to slightly irregular shape and rough surface. (C) Muriform ascospores with distinct transverse and longitudinal septa. (D) Detail of a muriform ascospore showing the septation pattern. Scale bars: A-B:1 mm, C–D = 20 µm.

Phylogenetic analyses based on ITS sequence data placed the Turkish specimen ERCH LIF 0.048 within the *Camarosporidiella astragalicola* clade. The specimen clustered together with authenticated reference sequences of *C. astragalicola* (PQ863529.1, PQ863530.1 and PQ863531.1) and formed a well-defined lineage within the genus *Camarosporidiella*. The closest phylogenetic relative of the Turkish specimen was *C. astragalicola* accession PQ863531.1, with which it shared an identical phylogenetic placement (Figure 3). The *C. astragalicola* lineage was recovered in close association with *C. laburni*, although both species remained clearly separated as distinct taxa. Morphologically, *C. laburni* differs from *C. astragalicola* by possessing narrower ascospores and differences in ascospore septation and host association [19]. The Turkish specimen exhibited the characteristic features of *C. astragalicola*, including semi-immersed papillate ascomata, cylindrical bitunicate asci, and ellipsoid to subfusiform ascospores measuring 22–24 × 13–15 µm with predominantly 5–6 transverse and 1–2 longitudinal septa. Ecologically, the specimen was found on stems of *Astragalus argaeus* that grow on the volcanic slopes of Lifos mountain at an altitude of 2180 m, which is consistent with the host preference and ecological properties as reported for the species [19]. The combination of morphological data and phylogenetic position is a strong support for the identification of ERCH LIF 0.048 as *Camarosporidiella astragalicola*. To our knowledge this is the first record of the species in Türkiye and adds the Central Anatolia as a new distribution area.

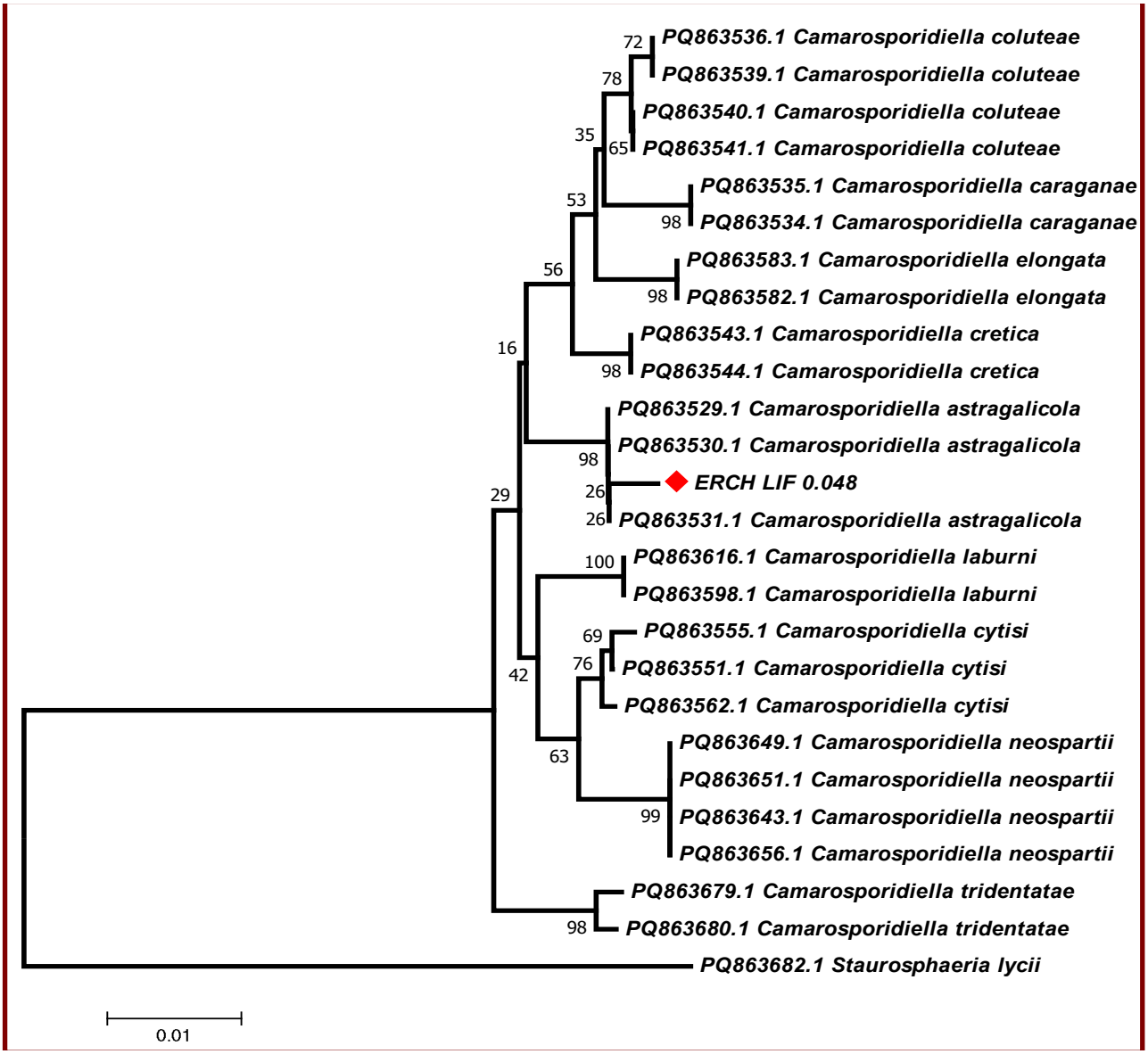

Figure 3. Maximum likelihood phylogenetic tree inferred from ITS sequence data showing the placement of *Camarosporidiella astragalicola* (ERCH LIF 0.048) collected from Lifos Mountain (Erciyes Mountain), Kayseri, Türkiye. Bootstrap values are indicated above branches. *Staurosphaeria lycii* (PQ863682.1) was used as the outgroup

3.2. *Exidia inflata* Cazenave & G. Gruhn

Morphological Description

Basidiomata gelatinous, resupinate to effused, firmly attached to the substrate, with only the hymenial surface visible. Individual basidiomata irregularly confluent, forming dark brown to blackish patches on the bark surface. Lower surface absent or strongly reduced. Fertile surface smooth or bearing a few coarse, irregularly distributed wart-like projections, lacking spinules. Texture soft and gelatinous when fresh, becoming contracted upon drying (Figure 4).

Hymenial surface dark brown to blackish, dull to slightly shiny. Context gelatinous and homogeneous. Hyphal system monomitic, composed of thin-walled, hyaline, septate hyphae.

Basidia longitudinally septate, typical of the genus *Exidia*. Basidiospores allantoid, smooth, hyaline, thin-walled, measuring 13–15 × 3–4 µm in the examined material. The measured spores correspond closely to the dimensions reported for the species [(10–)11–14 × 3–4 µm] (Figure 4).

Habitat and distribution: Growing corticolously on the trunk of a deciduous tree in a forested area of Lifos Mountain (Erciyes Mountain), Kayseri Province, Central Anatolia, Türkiye (38°35′36″ N, 35°29′40″ E), at 1880 m, 27 April 2025, leg. Ş. Karakaya and M. Güllü, voucher ERCH LIF 0.001.

Taxonomic Notes

The Turkish specimen agrees well with the current concept of *Exidia inflata* in possessing dark brown to blackish resupinate basidiomata, a smooth to weakly verrucose hymenial surface lacking spinules, and narrow allantoid basidiospores measuring 13–15 × 3–4 µm [25]. The ascospore dimensions observed in the present material closely match those reported for the species and fall within the known range of variation.

Among morphologically similar taxa, *Exidia nigricans* and *Exidia glandulosa* are the species most likely to be confused with *E. inflata*. However, both taxa generally develop more distinctly pulvinate or cerebriform basidiomata and possess broader spores than those observed in the Turkish specimen [25]. Furthermore, *E. glandulosa* is characterized by conspicuous sterile peg-like structures and a more strongly warted hymenial surface, whereas *E. inflata* typically exhibits a smooth to weakly verrucose fertile surface lacking such structures [25].

Ecology: *Exidia inflata* is known to grow on bark and fallen wood of deciduous trees in temperate forested environments. The finding of the species on a corticolous substrate at 1880 m on Lifos Mountain is in line with its known ecology. The dark basidiomata with resupinate shape, smooth hymenial surface, narrow allantoid basidiospores and deciduous-host association clearly suggest that the Turkish material is *Exidia inflata*. To our knowledge this is the first record of *E. inflata* from Türkiye and the range of the species is now extended to Central Anatolia.

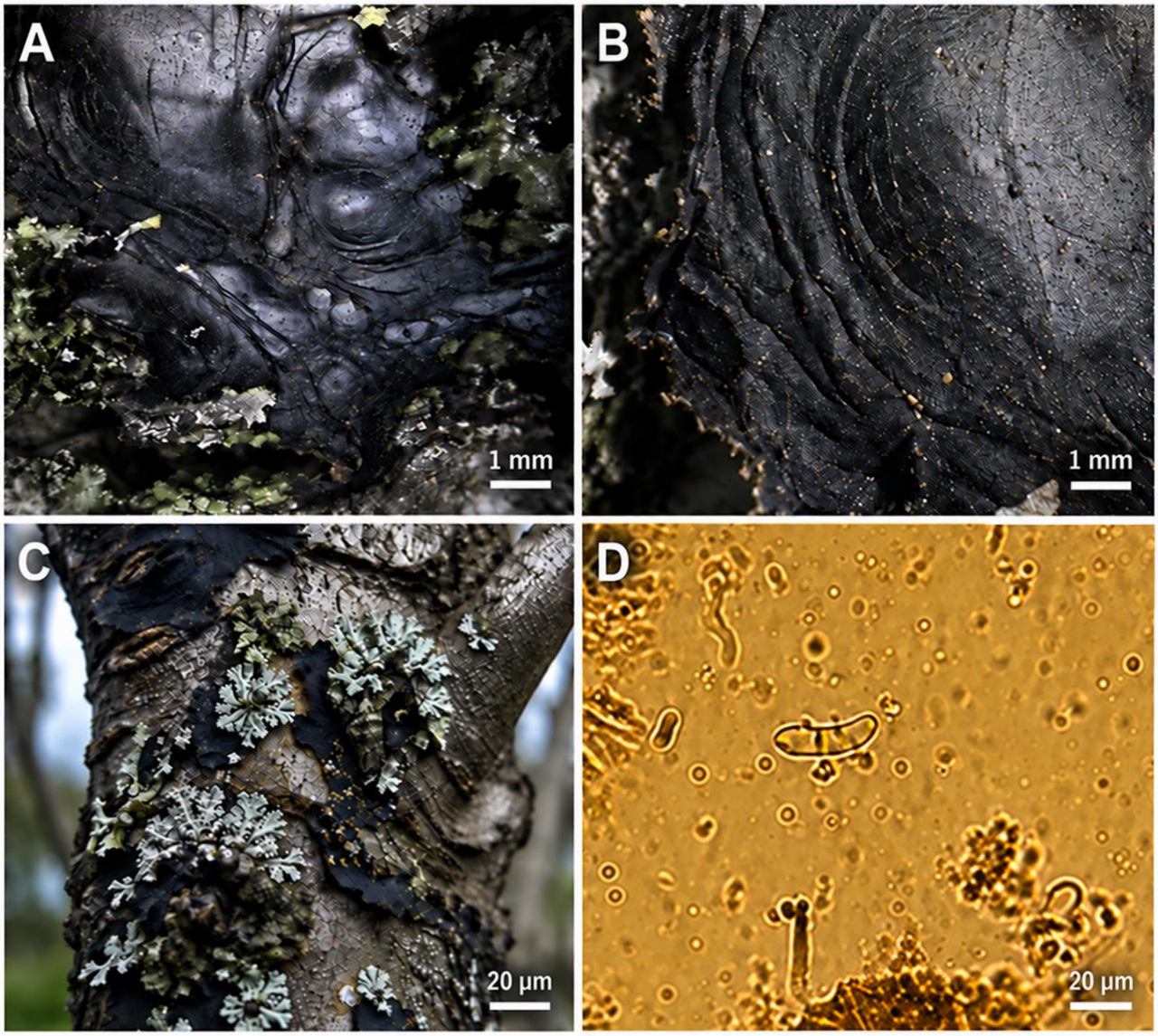

Figure 4. Morphological characteristics of *Exidia inflata*. (A) General view of the basidiomata showing dark brown to black, gelatinous and strongly inflated lobes with a distinctly wrinkled surface. (B) Close-up view of the upper surface illustrating the folded, undulate and cerebriform structure of the basidiomata. (C) Habit of *E. inflata* growing on the bark of a living tree in association with epiphytic lichens. (D) Microscopic view of hyaline, ellipsoid to allantoid basidiospores observed in the hymenial preparation. Scale bars: Scale bars: A-B:1 mm, C–D = 20 µm

Phylogenetic analyses based on ITS sequence data placed the Turkish specimen ERCH LIF 0.001 within the *Exidia inflata* Cazenave & G. Gruhn clade. The specimen clustered together with authenticated *E. inflata* sequences (PQ862236.1, PQ862238.1 and PQ862240.1), confirming its taxonomic placement. The Turkish specimen showed the closest affinity to accession PQ862236.1 and formed part of a moderately supported subclade (BS = 79), whereas the broader *E. inflata* lineage received stronger support (BS = 93) (Figure 5). The resulting topology clearly separated *E. inflata* from morphologically similar species including *E. reflexa* F. Wu, L.F. Fan & S.Y. Ye and *E. glandulosa* (Bull.) Fr.. Among these, *E. reflexa* was recovered as the closest phylogenetic relative of *E. inflata*. Morphologically, however, *E. reflexa* differs by developing more distinctly reflexed basidiomata and broader basidiospores, whereas *E. inflata* is characterized by appressed resupinate basidiomata and narrow allantoid spores usually measuring 11–14 × 3–4 µm [25]. Likewise, *E. glandulosa* differs by possessing more prominently warted basidiomata and conspicuous sterile peg-like structures on the hymenial surface [25]. The Turkish specimen exhibited the characteristic morphology of *E. inflata*, including dark brown to black resupinate basidiomata and allantoid basidiospores measuring 13–15 × 3–4 µm. Ecologically, the specimen was collected from the bark of a deciduous tree in a montane forest habitat on Lifos Mountain at 1880 m elevation, which is consistent with the known ecology of the species. The congruence between morphological observations and phylogenetic placement strongly supports the identification of ERCH LIF 0.001 as *Exidia inflata*. To the best of our knowledge, this represents the first record of *E. inflata* from Türkiye.

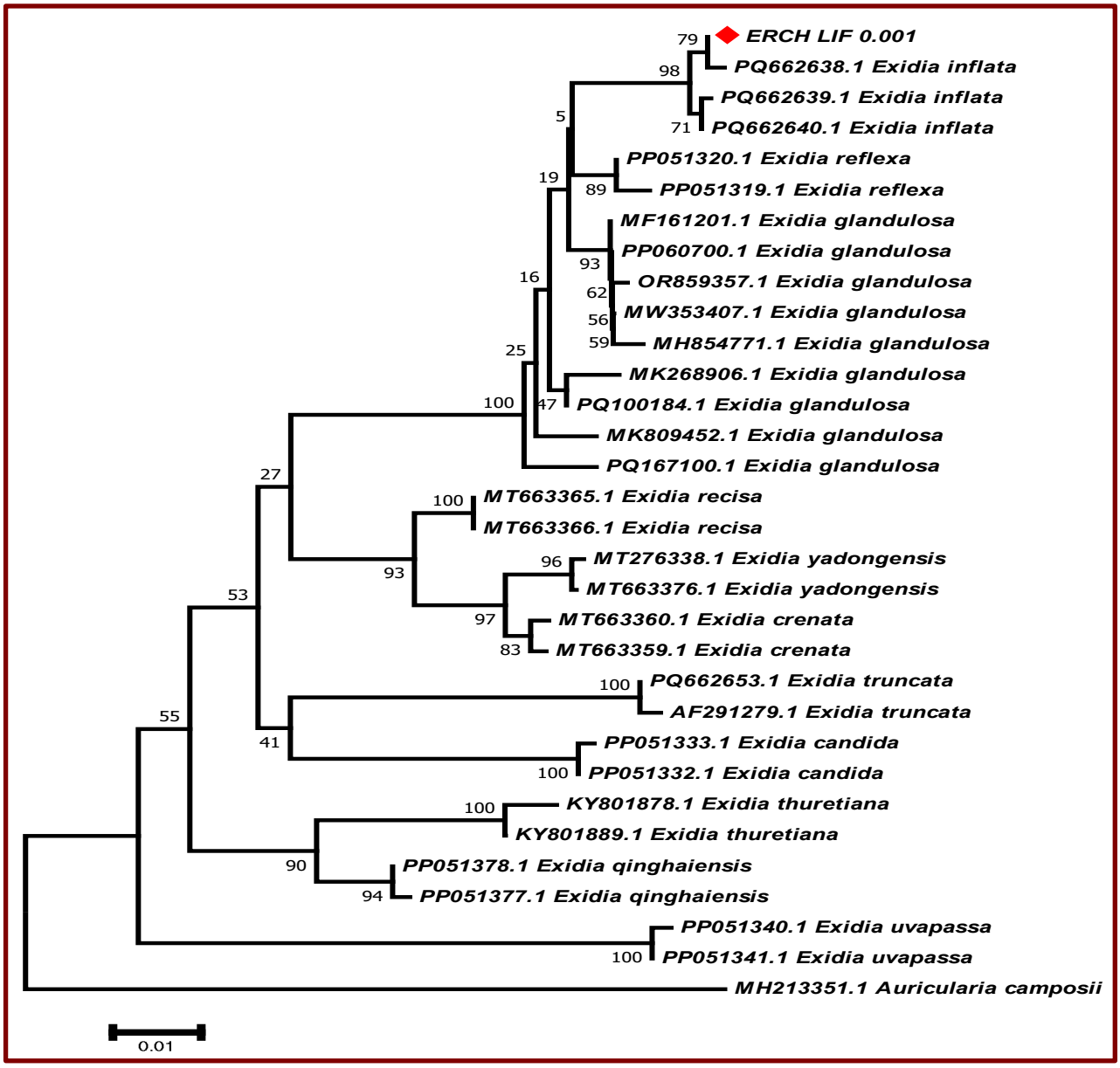

Figure 5. Maximum likelihood phylogenetic tree inferred from ITS sequence data showing the placement of *Exidia inflata* (ERCH LIF 0.001) collected from Lifos Mountain (Erciyes Mountain), Kayseri, Türkiye. Bootstrap values are indicated above branches. *Auricularia cornea* (MN213351.1) was used as the outgroup

3.3. *Rhizoplaca porteri* S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair

Morphological Description

Thallus saxicolous, foliose to subfruticose, forming irregular rosettes up to several centimetres in diameter. Lobes densely crowded, strongly convoluted and often intricately branched, 0.5–2.0 mm wide, ascending to somewhat erect, pale yellowish-green to yellow-grey when dry. Upper surface smooth to weakly rugose, lacking conspicuous pruina. Margins rounded, frequently overlapping and forming compact cushions. Lower surface not distinctly differentiated, attached to the substrate by a narrow basal holdfast (Figure 6).

Photobiont chlorococcoid green algae. Cortex well developed; medulla white. Apothecia not observed in the examined specimen. Pycnidia not observed. Asci not observed. Ascospores hyaline, simple, ellipsoid, measuring 9–13 × 7 µm (based on examined material), consistent with the spore dimensions reported for the species in the literature. Conidia not observed.

Ecology and Distribution: The specimen was collected from volcanic rocks on Lifos Mountain (Erciyes Mountain), Kayseri Province, central Türkiye, at 38°35′08″ N, 35°28′40″ E. The species occurred directly on exposed andesitic or basaltic rock surfaces. The occurrence of *R. porteri* on volcanic substrates agrees with its known preference for acidic to neutral siliceous rock habitats in montane and arid environments. The examined material was found in open habitats exposed to high solar radiation and periodic drought stress.

Specimen Examined: Türkiye. Kayseri Province, Lifos Mountain (Erciyes Mountain), 38°35′08″ N, 35°28′40″ E, on volcanic rock, saxicolous, 27 April 2025 leg. Ş. Karakaya and M. Güllü, voucher ERCH LIF 0.064.

Taxonomic Notes

Rhizoplaca porteri S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair is a member of the *Rhizoplaca melanophthalma* species complex and can be distinguished by its densely aggregated thallus composed of narrow, strongly convoluted and ascending lobes forming compact pulvinate cushions. The Turkish specimen examined in this study agrees well with the circumscription provided by Leavitt et al. (2013), particularly in its yellowish-grey to pale yellow-green thallus, saxicolous habit, and simple ellipsoid ascospores measuring 9–13 × 7 µm [5].

Another superficially similar species is *Rhizoplaca chrysoleuca* (Sm.) Zopf is distinguished from by its typically larger thallus, its typically broad lobes, its brighter yellow-green color, and its preference for more exposed xeric habitats. The densely crowded, highly contorted lobe morphology of the present specimen is more in keeping with the morphology of *R. porteri*.

It can be confused with *R. porteri* in general growth form, which is also widespread across North America, but *R. haydenii* tends to have thicker, more inflated lobes and to form larger rosettes; overall it grows in somewhat different habitats. Molecular data have also shown that despite their morphological similarity these taxa represent distinct evolutionary lineages.

The presence of the species *R. porteri* on volcanic andesitic or basaltic rocks of Lifos Mountain is in line with its ecological preferences as known from the North American and Eurasian populations. Morphological characters found in the Turkish specimens in combination with its occurrence on substrate and spore size suggest that it *is Rhizoplaca porteri*.

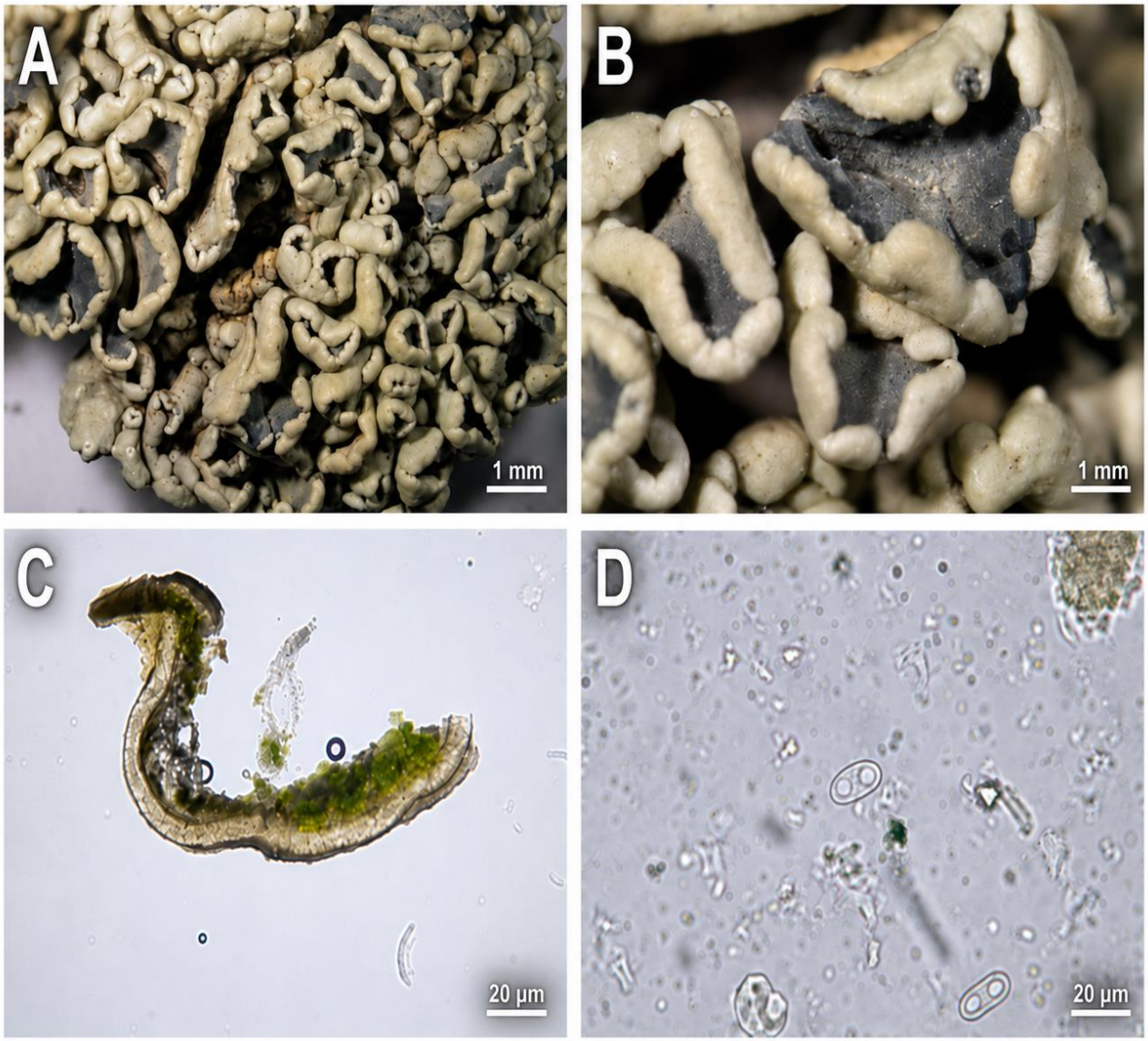

Figure 6. Morphological and anatomical features of *Rhizoplaca porteri* collected from Türkiye. (A) General habit of the thallus with densely aggregated, convoluted lobes. (B) Close-up view of the lobe surface and morphology. (C) Anatomical section of the thallus showing cortex, algal layer, medulla and lower cortex. (D) Microscopic view of ascospores. Scale bars: Scale bars: A-B:1 mm, C–D = 20 µm

Phylogenetic analysis based on ITS sequences placed the Turkish specimen ERCH LIF 0.064 firmly within the *Rhizoplaca porteri* clade, clustering with authenticated reference sequences of *R. porteri* from GenBank (KU934758.1, KU934823.1, KU934812.1, NR111853.1 and HM577330.1) (Figure 7). Within the inferred topology, the closest related taxon to the *R. porteri* clade was *Rhizoplaca polymorpha* S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi & St. Clair, although the two species formed distinct and well-separated lineages. The plant morphology of *R. porteri* differs from *R. polymorpha* in that the former produces narrower foliose lobes, strongly convoluted and densely aggregated in compact pulvinate thalli, while the latter usually forms broader lobes arranged in a regular manner and with less convoluted growth form [7]. Anatomically, *R. porteri* tends to have smaller ascospores and smaller thalline organization, while *R. polymorpha* may have larger and stouter thalli and broader lobes. Ecologically, *R. porteri* is often found on exposed siliceous volcanic rocks in arid and montane areas, while R. polymorpha is more widespread over rocky substrates and ecological conditions in temperate areas [7]. Chemically, they may share the presence of usnic acid as one of the main compounds in the cortex, but the secondary metabolite profile of the members of the *Rhizoplaca melanophthalma* complex suggests that medullary compounds may differ and this may help in distinguishing these taxa [7]. The colour, structure, distribution pattern of ascospores, the presence of the yellowish-brown prothallus, and the presence of a marginal yellowish-brown zone are all characteristic features of *Rhizoplaca porteri* which place ERCH LIF 0.064 into the *R. porteri* lineage, thus confirming the taxonomic affinity of the Turkish material with populations documented so far.

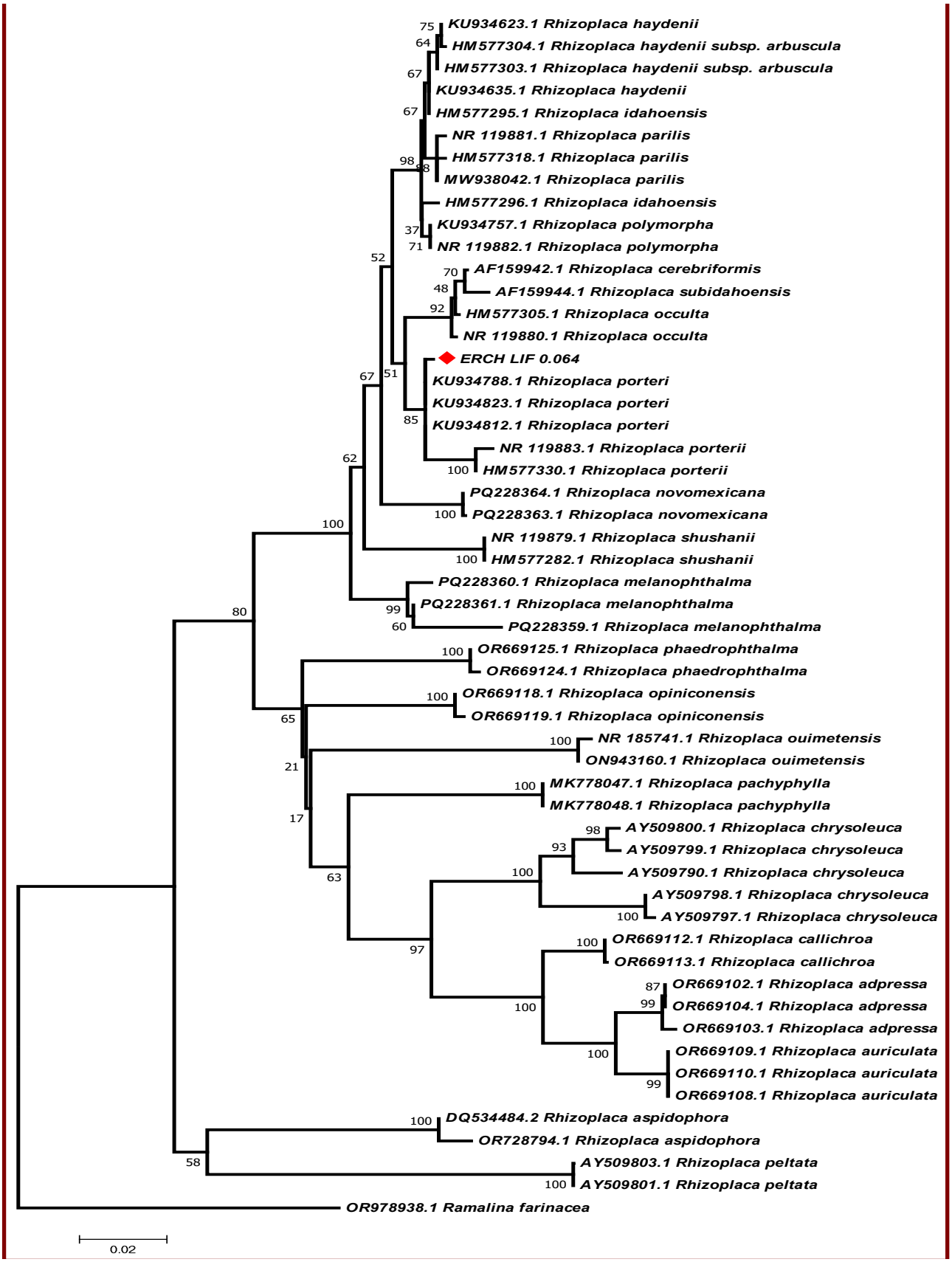

Figure 7. Maximum likelihood phylogenetic tree inferred from ITS sequences showing the position of *Rhizoplaca porteri* (ERCH LIF 0.064) collected from Lifos Mountain, Türkiye. Bootstrap values are indicated above branches. The Turkish specimen clusters with reference sequences of *R. porteri* obtained from GenBank. *Ramalina farinacea* (OR978938.1) was used as the outgroup

4. Discussion

ITS-based phylogenetic analyses combined with detailed morphological examination were helpful in the reliable identification of three fungal taxa collected from Lifos Mountain, *Camarosporidiella astragalicola*, *Exidia inflata* and *Rhizoplaca porteri*. In each case, the morphological data confirmed the molecular data and the Turkish specimens grouped with the reference sequences with authentication from GenBank. The results presented in this study indicate the usefulness of the integration of traditional taxonomic approaches and DNA barcoding methods in modern fungal systematics.

The result of the current study places emphasis on the need for ITS sequence data for species identification, especially for species where morphological convergence and phenotypic plasticity may blur the separation between species. Previous studies have demonstrated poor accuracy in species delimitation based on morphology alone in a number of fungal and lichenized fungal genera, notably in species complexes like *Rhizoplaca melanophthalma* or the *Rhizocarpon geographicum* group [3-5]. ITS phylogenies in the present study showed significant separation of morphologically similar taxa and were also a valuable aid to the identifications made in the morphological and anatomical studies.

It is especially interesting that both species grow upon siliceous volcanic hosts and that they are found at high elevations, where *Rhizoplaca porteri* has been discovered. The ecological conditions available in Lifos Mountain with its abundance of andesitic and basaltic formations are comparable to those reported for the species in other parts of the world. Results of these taxa occurring on volcanic substrates confirm previous observations that mountain ecosystems with different lithological conditions can host specialized lichen communities [7,8].

In the same way, *Camarosporidiella astragalicola*, a new fungus species found on *Astragalus argaeus*, is another fungal species endemic to Central Anatolia which shows host associated fungal diversity. It is thought that many endemic vascular plant species inhabiting the volcanic slopes of the Mount Erciyes could generate the special habitats for fungi. The present record is an extension of the distribution area of *C. astragalicola*, and shows that host-specific fungi are underutilized in the Anatolian mountain ecosystems.

The finding of *Exidia inflata* has highlighted the underrated biodiversity of corticolous basidiomycetes in the area. Even though gelatinous fungi make up a normal part of temperate forest ecosystems, they rarely get focus in biodiversity surveys, mainly because their basidiomata are short-lived and they appear only at certain times of the year. That *E. inflata* occurred on the bark of deciduous trees at a high elevation suggests that the montane forests of Lifos Mountain harbor a more diverse fungal community than had been acknowledged so far.

Mount Erciyes and its surrounding areas were previously studied from a floristic and lichenological point of view [10-12]. However, molecularly supported studies focusing on Lifos Mountain have remained very few. So, this paper is among the first major efforts to integrate morphology and ITS-based phylogenetics for assessing fungal biodiversity in this area. Finding three species that have never been recorded in Türkiye before proves that the fungal diversity of Central Anatolian volcanic mountains is still not fully known and that more surveys will probably uncover more new taxa.

5. Conclusions

In conclusion, results indicate that the Lifos Mountain may be an important biodiversity hotspot for lichenized and non-lichenized fungi. The combination of volcanic geology, elevational differences, continental climate and the variety of vegetation types appear conducive to the occurrence of specialized fungal communities. A more comprehensive sampling and the application of multilocus phylogenetic approach will certainly contribute to the understanding of the diversity and biogeography of the fungi in Anatolia.

To sum up, in this study, *Camarosporidiella astragalicola*, *Exidia inflata* and *Rhizoplaca porteri* were documented for the first time in Türkiye with genetic data. Extensive morphological, anatomical, ecological and phylogenetic characterizations have strongly supported the identification of the three species. The unambiguous grouping of Turkish collections with verified reference sequences was achieved by ITS sequence comparisons, thus validating their taxonomy.

The findings underline the need to combine classical taxonomy methods with DNA barcoding techniques for correct species identification and biodiversity evaluations. Besides, finding three species that have not been recorded before shows that the fungal diversity of Lifos Mountain and the wider Erciyes Mountain area is extensive but still largely unknown.

Lifos Mountain is a volcanic area. In this region, different types of plants, including plant species found only here, grow at various heights. Because of this, the mountain acts as a rich source of fungi and lichen diversity in Central Anatolia. Further investigation through both the examination of physical features and genetic analysis of fungi will lead to the discovery of new species and greatly help us in understanding where fungi live in different parts of Türkiye. Thus, this research significantly adds to the Turkish mycobiota database and also draws attention to the necessity of conducting more detailed taxonomic studies combining multiple approaches in Anatolian mountain ecosystems.

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(x) This article does use artificial intelligence. *For example:* "During the preparation of this manuscript, the authors used [Name of Tool/Service] to [describe how the tool was used, e.g., improve English phrasing and grammar]. The authors reviewed and edited the content as needed and take full responsibility for the framework and validity of the final paper."

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Author contributions: All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Ethical statement: This study does not require ethical approval.

Data Availability Statement (Please see the Data Availability Policy section for details.)
( ) Option A (Public Repository): "The research data supporting this study are publicly available in [Name of Repository: e.g., Zenodo, Figshare, Dryad] with the permanent DOI link [URL/DOI]."
(x) Option B (Upon Request): "The raw data supporting the findings of this study are available from the corresponding author [Author Initials] upon reasonable request and within ethical boundaries."
( ) Option C (Third-Party Data): "The data used in this study were obtained from [Name of Institution/Source] and are subject to third-party restrictions regarding redistribution."
( ) Option D (No Data): "No new data were created or analyzed in this study. Data sharing is not applicable to this article."

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