



Identification of some lichenized fungi species of Erciyes Mountain (Kayseri/Turkey) by using ITS (rDNA) marker

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Abstract

In this study; we analysed the ITS sequences of 9 lichenized fungi species (*Aspicilia cinerea*, *Circinaria contorta*, *Lecidea atrobrunnea*, *Lecanora rupicola*, *Physcia dubia*, *Rhizoplaca peltata*, *Rhizocarpon geographicum*, *Tephromela atra* and *Xanthoparmelia pulla*) which have a wide distribution in the alpine zone of Erciyes Volcanic Mountain in Central Anatolia. ITS sequence data obtained from those 9 species are deposited by us in GenBank for further molecular studies and Neighbor-joining dendrograms with related species obtained from GenBank are also presented here along with the macroscopic photographs of the specimens studied. Besides, morphological and ecological differences between the related species are discussed.

Key words: : Lichens, biodiversity, systematics, taxonomy

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ITS (rDNA) marker kullanılarak Erciyes Dağı'nın (Kayseri, Türkiye) bazı likenleşmiş mantar türlerinin tanımlanması

Özet

Bu çalışmada, Orta Anadolu'da yer alan Erciyes Dağı'nın alpin zonunda yayılış gösteren 9 yaygın likenleşmiş mantar türüne ait örneklerin (*Aspicilia cinerea*, *Circinaria contorta*, *Lecidea atrobrunnea*, *Lecanora rupicola*, *Physcia dubia*, *Rhizoplaca peltata*, *Rhizocarpon geographicum*, *Tephromela atra* ve *Xanthoparmelia pulla*) ITS dizi analizleri elde edilmiştir. Bu 9 türden elde edilen ITS sekans verileri ileride yapılacak moleküler çalışmalar da kullanılması için Gen Bankası'na yüklenmiştir. Ayrıca Gen Bankası'ndan temin edilen ilişkili türler ile hazırlanan Neighbor-joining dendrogramlar ve çalışılan örnekler ait makroskopik fotoğraflar da verilmiştir. Ayrıca ilişkili türler arasında morfolojik ve ekolojik farklılıklar da tartışılmıştır.

Anahtar kelimeler: Liken, biyoçeşitlilik, sistematik, taksonomi

1. Introduction

The first lichen checklist of Erciyes Mountain was published by Steiner (1905). In that study, 83 lichenized taxa were reported. In the first period of 21st century; Güvenç (2001) reported 40 taxa and John (2002) reported 2 taxa from Erciyes Mountain. Later Halıcı et. al. (2005) compiled 223 lichenized and lichenicolous fungi taxa in Erciyes Mountain. After this study 9 more lichen and lichenicolous fungi taxa were added in the checklist of Erciyes Mountain (Halıcı et. al. 2006; 2007; 2014) and the number of lichenized and lichenicolous fungus taxa reached to 232.

The taxonomic studies on lichenized fungi in Turkey based on morphological and anatomical characters recently started (Türk et. al. 2015). However, analysis of nuclear rDNA is increasingly becoming important for lichen taxonomy. Among the ribosomal regions, the internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi. In some cases, morphological and anatomical characters for identification of lichens in species level may be insufficient (Arup et. al. 2013, Vondrák et. al. 2016). For this reason; we start to analyze the ITS sequence data of lichenized fungi species previously reported in Erciyes Mountain. Here we

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present 10 ITS sequence data and related Neighbor-joining dendrograms of 9 lichenized fungi taxa from Erciyes Mountain.

2. Materials and methods

2.1. Lichen material

Lichen specimens (*Aspicilia cinerea*, *Circinaria contorta*, *Lecidea atrobrunnea*, *Lecanora rupicola*, *Physcia dubia*, *Rhizoplaca peltata*, *Rhizocarpon geographicum*, *Tephromela atra* and *Xanthoparmelia pulla*) stored in ERC (Erciyes University herbarium) were used for this study.

2.2. Extraction of DNA and PCR Amplification

Ten lichen samples were used for DNA extraction. DNA was extracted using protocol of Qiagen Dneasy Plant Kit (Cat. No: 69104). PCR was carried out in 50 µL reaction volumes using 4 µL of 10 x reaction buffer, 4 µL MgCl₂ (50 mM), 0.5 µL each primer, 1 µL dNTP (10 mM), 0.1 µL Taq DNA polymerase, 3 µL of genomic DNA and 36.9 µL dH₂O on a thermal cycler equipped with a heated lid. Primers used for PCR amplification of ITS regions were ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et. al. 1990). The PCR was performed under the following conditions: An initial denaturation 4 min at 95 °C; 7 cycles with 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C; and 33 cycles with 1 min at 94 °C, 1 min at 52 °C, and 2 min at 72 °C; a final extension step of 5 min at 72 °C was added, after which the samples were kept at 4 °C. PCR products run on agarose gel and sequence analysis of ten lichen samples from which DNA bands were obtained was done.

2.3. Phylogenetic analysis

Sequence results of the lichen samples were compared using BLAST program in NCBI website. Then, the results were edited in BioEdit program. Sequences were aligned by using Clustal w option in the BioEdit program. For phylogenetic tree, MEGA 6 (Molecular Evolutionary Genetics Analysis) program was used and genetic distances were determined by method Kimura-2-parameter. Phylogenetic trees of the lichen samples were performed by using neighbor-joining method (Tamura et. al. 2013).

Table 1. The studied specimens, herbarium numbers and GenBank accession numbers

Species name	Herbarium number	GenBank accessions number
<i>Aspicilia cinerea</i>	ERC MB 0.002	KX550108
<i>Aspicilia cinerea</i>	ERC MGH 0.018	KX550104
<i>Circinaria contorta</i>	ERC MGH 0.139	KX550106
<i>Lecidea atrobrunnea</i>	ERC MB 0.008	KX550101
<i>Lecanora rupicola</i>	ERC MB 0.010	KX550102
<i>Physcia dubia</i>	ERC MGH 0.025	KX550105
<i>Rhizoplaca peltata</i>	ERC MB 0.003	KX550109
<i>Rhizocarpon geographicum</i>	ERC MB 0.011	KX550103
<i>Tephromela atra</i>	ERC MB 0.004	KX550110
<i>Xanthoparmelia pulla</i>	ERC MB 0.001	KX550107

3. Results

Descriptions, photographs and discussions of lichenized fungus species studied for this paper are given below.

3.1. *Aspicilia cinerea* (L.) Körb.

Specimens examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.002]; Kayseri, Erciyes Mountain, Yılanlı Mountain, in the vicinity of Koyunbaba, 38° 43' N, 35° 26' E, alt. 1205 m, 29.06.2003 [ERC MGH 0.018].

Thallus cracked-areolate, 2–3.5 cm in diam; areoles usually irregular, (0.3–)0.6–1–1.4 mm in diam. Thallus surface whitish gray, sometimes yellowish white. Apothecia numerous and aspicilioid type, ranging in number from 1 to 10 per areole, (0.15–)0.2–0.25–0.3(–0.35) mm in diam. Apothecia disc black; usually pruinose. Thallus and medulla K–, C–, KC–, Pd + yellow-orange. Epihymenium greenish or brownish green. Hymenium hyaline. Paraphyses moniliform. Asci clavate, (80–)82–98–114(–120) × 14–19–24(–26) µm. Ascospores not observed. This species has a wide distribution in subarctic and

Figure 1

arctic-alpine areas in the Northern Hemisphere (Purvis 1992). It has also wide distribution in Turkey especially in subalpine and alpine areas of Turkey on hard siliceous rocks (e.g. Halıcı & Güvenç 2008, Çobanoğlu et al. 2013).

Table 2. Bant sizes and nucleotide variable sites of the specimens studied

Species name	PCR results	Sequence results	Bant size (bp)	Nucleotide variable sites
<i>Aspicilia cinerea</i> (ERC MB 0.002 and ERC MGH 0.018)	+	+	800	344
<i>Circinaria contorta</i> (ERC MGH 0.139)	+	+	750	218
<i>Lecidea atrobrunnea</i> (ERC MB 0.008)	+	+	560	182
<i>Lecanora rupicola</i> (ERC MB 0.010)	+	+	600	130
<i>Physcia dubia</i> (ERC MGH 0.025)	+	+	790	201
<i>Rhizoplaca peltata</i> (ERC MB 0.003)	+	+	589	167
<i>Rhizocarpon geographicum</i> (ERC MB 0.011)	+	+	530	196
<i>Tephromela atra</i> (ERC MB 0.004)	+	+	580	114
<i>Xanthoparmelia pulla</i> (ERC MB 0.001)	+	+	568	93

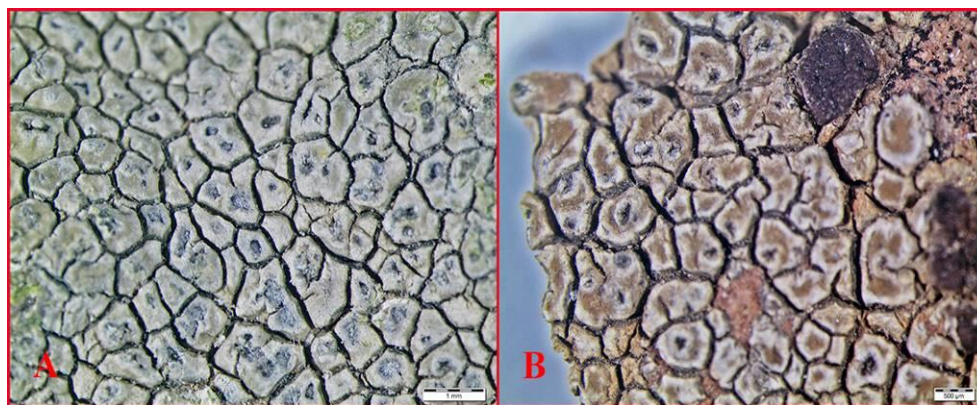


Figure 1. *Aspicilia cinerea* A. ERC MB 0.002 B. ERC MGH 0.018

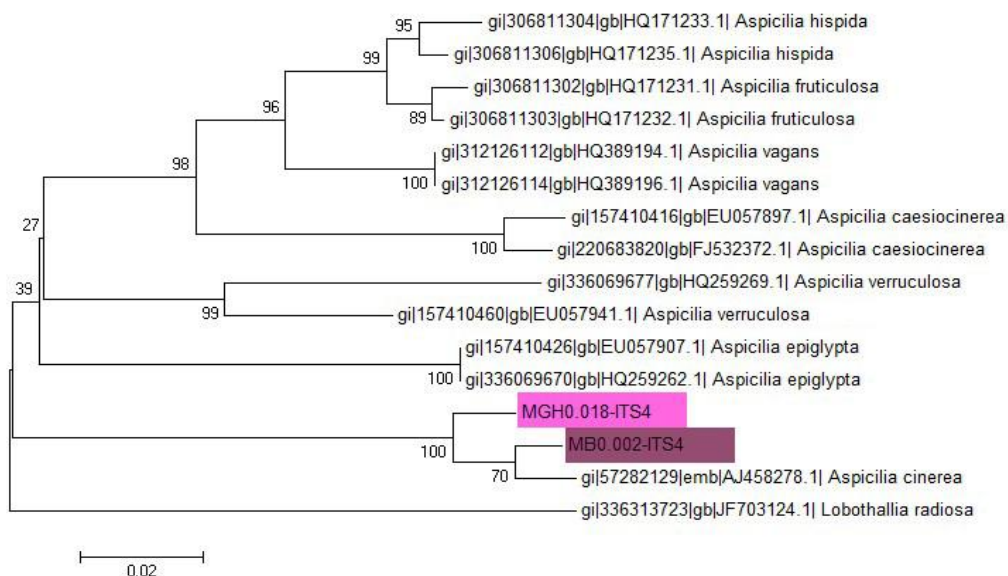


Figure 2. Neighbor-Joining (NJ) dendrogram of *Aspicilia cinerea* and related species (ERC MB 0.002 and ERC MGH 0.018)

In the NJ dendrogram (Figure 2); it is clear that our samples from Erciyes Mountain (ERC MB 0.002 and ERC MGH 0.018) is branching with *Aspicilia cinerea*. The closest branching to *A. cinerea* is *A. epiglypta* (Norrl. ex Nyl.) Hue. There are significant morphological and anatomical differences between *A. cinerea* and *A. epiglypta*. *A. epiglypta* has longer conidia than *A. cinerea*. In addition, while *A. cinerea* has a distribution in subarctic and arctic-alpine areas, *A. epiglypta* has a distribution in the coastal areas (Nordin et al. 2007). *A. cinerea* is also morphologically quite similar to *A. intermutans* but the latter species has smaller conidia and larger mature ascospores (Fryday 1997).

3.2. *Circinaria contorta* (L.) A.Nordin, Savić & Tibell

Figure 3

Specimen examined: Tukey, Kayseri, Erciyes Mountain; İncesu, Sürtme (Western part of Erciyes Mountain), 38° 35' N, 35° 36' E, alt. 1100 m, 27.05.2003 [ERC MGH 0.139].

Thallus saxicolous, pale brownish; cracked-areolate. Areoles irregular and discrete, (0.3–)0.5–0.8–1.1(–1.2) mm in diam. Prothallus not visible. Apothecia irregular, numerous and aspicilloid; blackish especially young apothecia pruinose, (0.15–)0.2–0.25–0.3(–0.4) µm in diam. All spot tests negative.

This species has a wide distribution on calcareous rocks and limestone in Northern Hemisphere (Purvis 1992). It has also very wide distribution in Turkey on siliceous and weakly calcareous rocks (e.g. Oran et al. 2007, Halıcı & Aksoy 2009).



Figure 3. *Circinaria contorta* (ERC MGH 0.139)

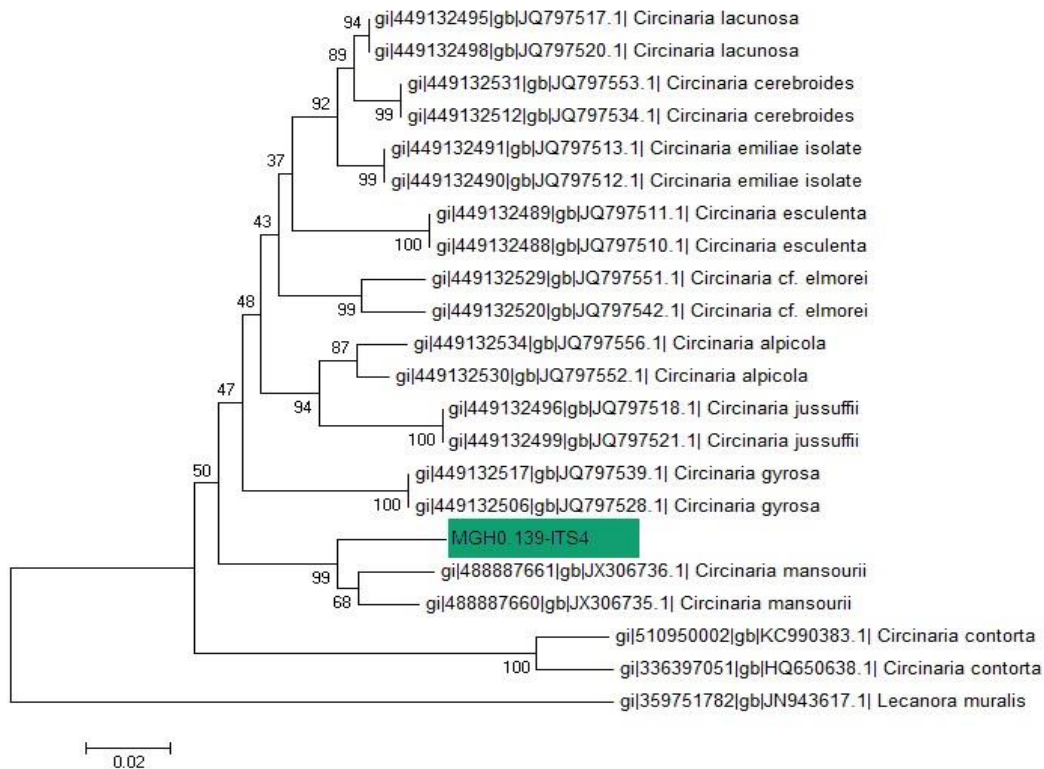


Figure 4. Neighbor-Joining (NJ) dendrogram of *Circinaria contorta* and related species (ERC MGH 0.139)

ERC MGH 0.139 numbered sample is branching with *Circinaria mansourii* in NJ dendrogram (Figure 4). But when we examine the morphology and ecology of this sample; it is obvious that it has quite important differences from *C. mansourii*. While *C. mansourii* has subsquamulose to appressed sub-fruticose thallus attached by tiny rhizomorph-like extensions on perennial grasses (Lumbsch et al. 2011); our sample has obviously crustose thallus growing on siliceous rocks. Besides *C. mansourii* is known to be sterile as no apothecia or pycnidia are known from this species (Lumbsch et al. 2011); but our sample is richly fertile. Because of all these reasons; we classified this sample under the species name *Circinaria contorta*.

3.3. *Lecanora rupicola* (L.) Zahlbr.

Figure 5

Specimen examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.010].

Thallus crustose, cracked-areolate; areoles slightly convex greyish, 3 cm in diam. Apothecia immersed to slightly raised, lecanorine, (0.5–)0.7–1–1.3(–1.5) mm in diam. Apothecia disc smooth, gray. Thallus and medulla K+ yellow, C–, KC–, Pd–; Apothecial disc C + vivid yellow. Asci clavate, 8 spored, (51–)52–56–60(–62) × (14–)16–17.5–19 μm. Ascospores hyaline, simple, (14–)14.5–15–15.5(–16) × (7–)7.1–7.5–7.9(–8) μm. Hymenium hyaline, (67–)67.5–78–88.5(–92) μm. Paraphyses slightly thickened in the apex. Subhymenium hyaline. Hypotecium hyaline.

Circumpolar from temperate to arctic in Northern Hemisphere on hard siliceous rocks from sea level to high altitudes (Purvis 1992). It has a wide distribution in Turkey (e.g. Aslan 2000, Kocakaya et al. 2009).



Figure 5. *Lecanora rupicola* (ERC MB 0.010)

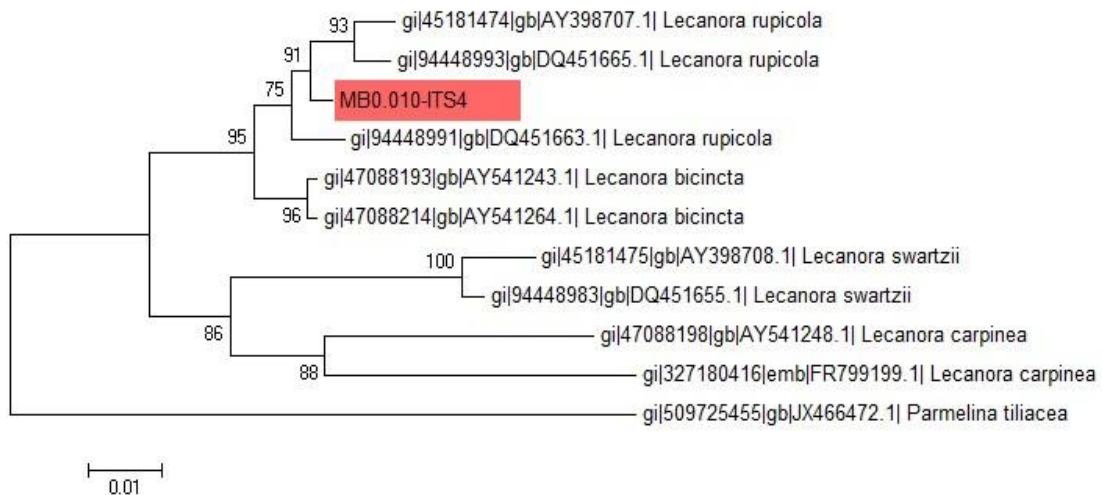


Figure 6. Neighbor-Joining (NJ) dendrogram of *Lecanora rupicola* and related species (ERC MB 0.010)

In the NJ dendrogram (Figure 6); the closest branching to *Lecanora rupicola* is *L. bicincta* Ramond. While *L. rupicola* has sessile to subimmersed apothecia and whitish or grayish pruinose apothecial disc, *L. bicincta* has slightly stalked apothecia and bluish pruinose apothecial disc. In addition, *L. bicincta* has parathecial ring which is black in apothecial discs (Lumbsch & Elix 2004). This parathecial ring is lacking in *L. rupicola*. *L. rupicola* and *L. swartzii* (Ach.) Ach. are similar species, but *L. swartzii* is reacted with C + vivid yellow to orange and has more convex areoles (Lumbsch & Elix 2004).

3.4. *Lecidea atrobrunnea* (DC.) Schaer.

Figure 7

Specimen examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.008].

Thallus areolate, 5 cm in diam. Areoles flat, irregular, dark brown to pale brown, (0.6–)0.7–0.8–0.9(–1.1) mm in diam. Surface brownish and reddish brown. Apothecia black to reddish brown, 1 per areole, (0.2–)0.25–0.3–0.35(–0.4) mm in diam. All spot tests negative. Epiphytenium brown, 14–18–22 µm. Hymenium 92–115–138(–140) µm. Asci clavate, (85–)87–100–113 × 14–15–16(–17) µm. Ascospores not observed.

Lecidea atrobrunnea has a wide distribution in the higher altitudes of mountains and alpine zones on siliceous rocks (e.g. John & Breuss 2004, Yazıcı et al. 2011).



Figure 7. *Lecidea atrobrunnea* (ERC MB 0.008)

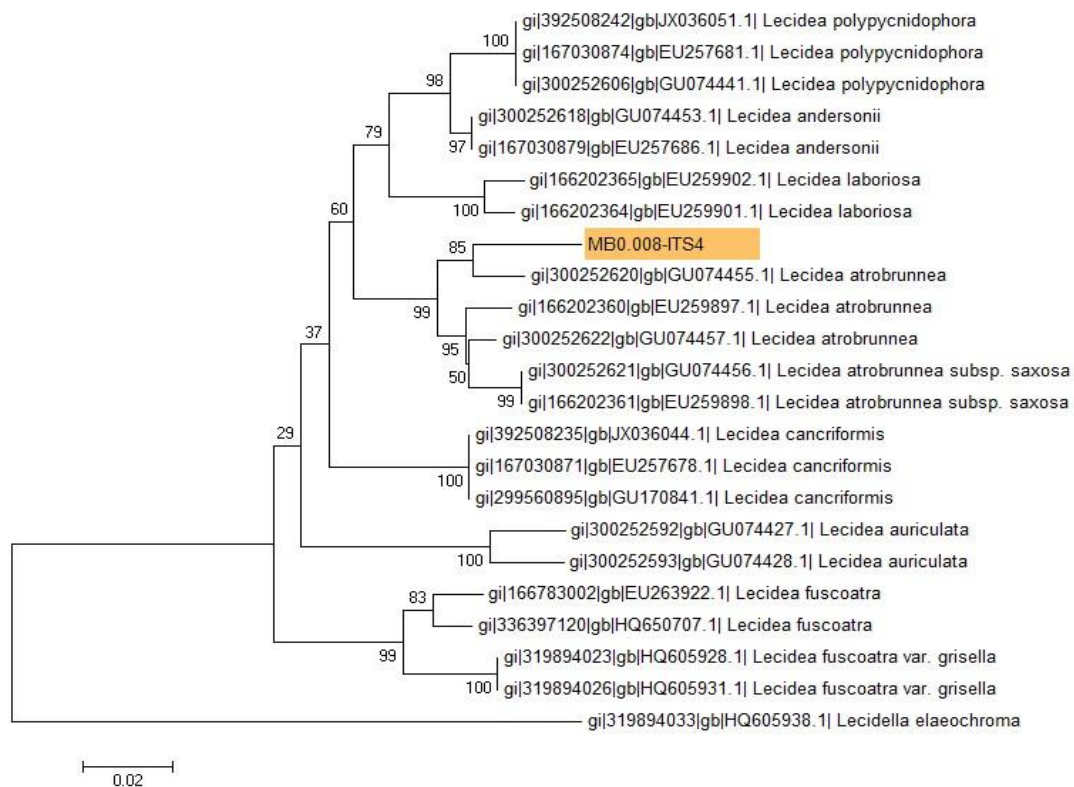


Figure 8. Neighbor-Joining (NJ) dendrogram of *Lecidea atrobrunnea* and related species (ERC MB 0.008)

Lecidea atrobrunnea and *L. atrobrunnea* subsp. *saxosa* Hertel & Leuckert are very closely related in the NJ dendrogram (Figure 8). Furthermore, *L. polypycnidophora* U. Rupr. & Türk, *L. andersonii* Filson, *L. laboriosa* Müll. Arg. and *L. cancriformis* C.W. Dodge & G.E. Baker are phylogenetically related with *L. atrobrunnea*. *L. atrobrunnea* differs with 2'-O-methylperlatolic acid from those species (Brodo et al. 2001); besides the latter species are not known from Turkey. *L. atrobrunnea* subsp. *saxosa* includes conorstictic and norstictic acids (Nash 2002). *L. polypycnidophora* includes 4-O-demethylplanaic acid (Ruprecht et al. 2010) and *L. andersonii* includes planaic acid (Ruprecht et al. 2010).

3.5. *Physcia dubia* (Hoffm.) Lettau**Figure 9**

Specimen examined: Turkey, Kayseri, Erciyes Mountain, Koç Mountain, 38° 42' N, 35° 32' E, alt. 2270 m, 16.07.2002 [ERC MGH 0.025].

Thallus irregular, gray or brownish gray, 3–4 cm in diam. Lobe tips often darker, (1.7–)2.2–2.6–3(–3.4) × (1–)1.4–1.8–2.2(–2.3) cm. Surface gray and cream-colored. Sored present, soralia lip-shaped. Apothecia few and black; semi-immersed, 0.1–0.7–1.3(–1.4) mm in diam. Thallus; K+ yellow, C–, KC–, P+ yellow.

It has a wide distribution from temperate regions to boreal regions (Seppelt *et al.* 1996). It grows on acidic rocks (e.g. Halıcı & Cansaran-Duman 2007, Candan & Özdemir Türk 2008).



Figure 9. *Physcia dubia* (ERC MGH 0.025)

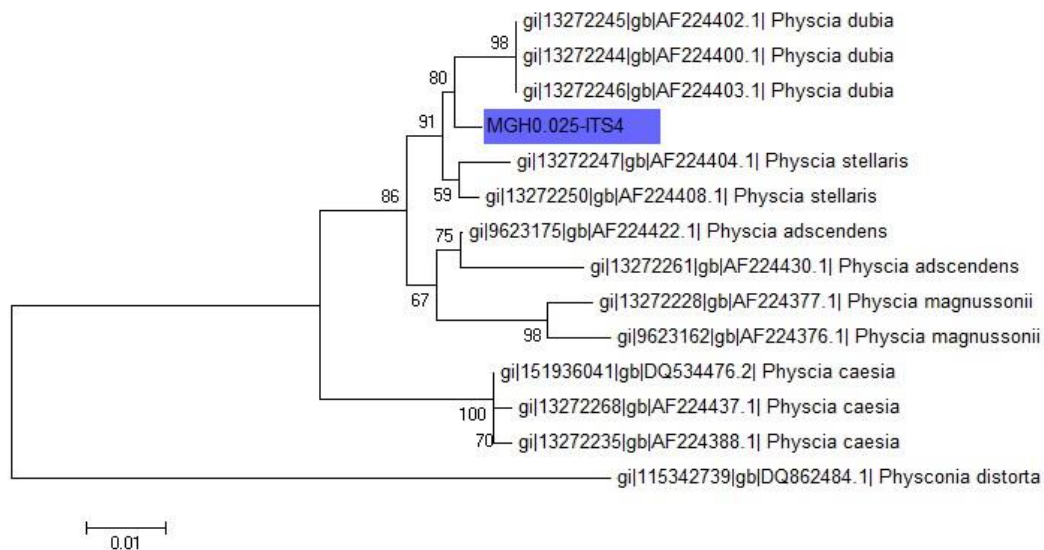


Figure 10. Neighbor-Joining (NJ) dendrogram of *Physcia dubia* and related species (ERC MGH 0.025)

In the NJ dendrogram (Figure 10), the closest branching to *Physcia dubia* is *Physcia stellaris* (L.) Nyl.. While *P. dubia* is sorediate, *P. stellaris* is not sorediate; also *P. dubia* is saxicolous while *P. stellaris* is mostly epiphytic (Brodo & Craig 2003). *P. adscendens* (Fr.) H. Olivier differs from *P. dubia* with helmet shaped soralia (Galloway & Moberg 2005). The other related saxicolous species *P. magnussonii* Frey has numerous apothecia and it is not sorediate. (Moberg & Hansen 1986). *P. dubia* is mostly known to be sterile; but interestingly the population from Erciyes Mountain which we studied was fertile with few apothecia.

3.6. *Rhizocarpon geographicum* (L.) DC.**Figure 11**

Specimen examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.011].

Thallus crustose, 4 cm in diam. Areoles scattered, (0.5–)0.8–1.2–1.6(–1.9) mm in diam. Prothallus present and black coloured. Areoles greenish yellow. Apothecia black, 0.3–0.5–0.7 mm in diam; immersed between areoles. Thallus and medulla K–, C–, KC–, Pd+ yellow. Epihymenium bluish black. Hymenium 29–68–107 µm. Paraphyses 3–3.2–3.4 µm. Asci dark green to brown, 8 spored. Ascospores muriform and ellipsoid, dark brown, (32–)33–37–41(–42) × (18–)19–20–21(–23) µm.

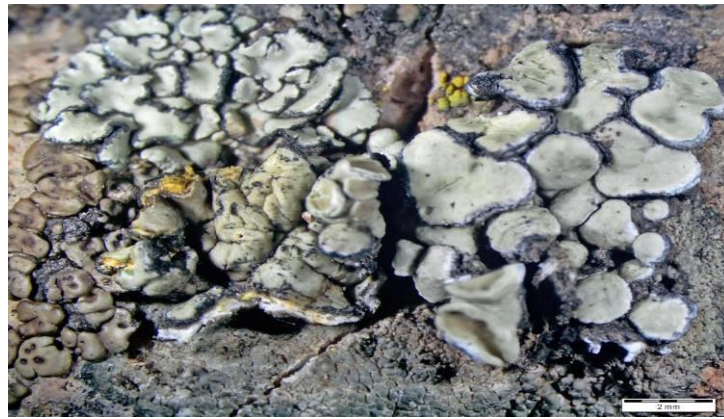


Figure 13. *Rhizoplaca peltata* (ERC MB 0.003)

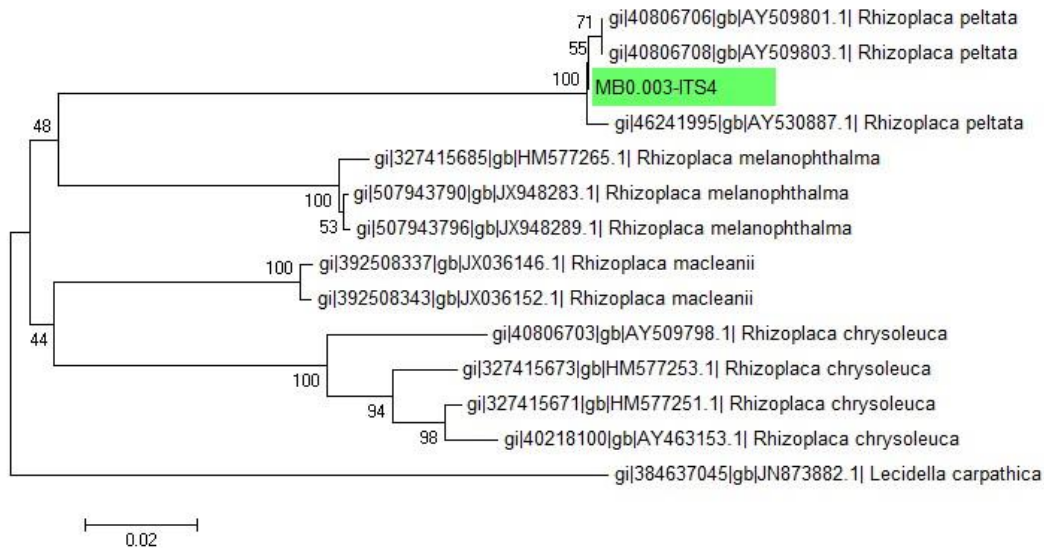


Figure 14. Neighbor-Joining (NJ) dendrogram of *Rhizoplaca peltata* and related species (ERC MB 0.003)

3.8. *Tephromela atra* (Huds.) Hafellner

Specimen examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.004].

Thallus crustose, 5 cm in diam. Areoles usually irregular. Surface grayish-white. Apothecia black, sessile, 1.1–1.3–1.5 mm in diam. Thallus and medulla K+ yellow, C–, Pd–. Epihymenium dark reddish coloured. Hymenium purplish or purplish red, 238–245–252 μm. Hypotecium 43–56–69 μm. Paraphyses branched, (2.8–)2.9–3–3.1 μm. Asci clavate, 8 spored, 38–46–54 × 15 μm. Ascospores hyaline, simple, (6–)8–10–12(–13) × (4–)5.5–6.7–7.9(–10) μm. It has a wide distribution from coastal regions to high mountain regions (e.g. Rigler 1852, Güvenç et al. 2006).

Figure 15



Figure 15. *Tephromela atra* (ERC MB 0.004)

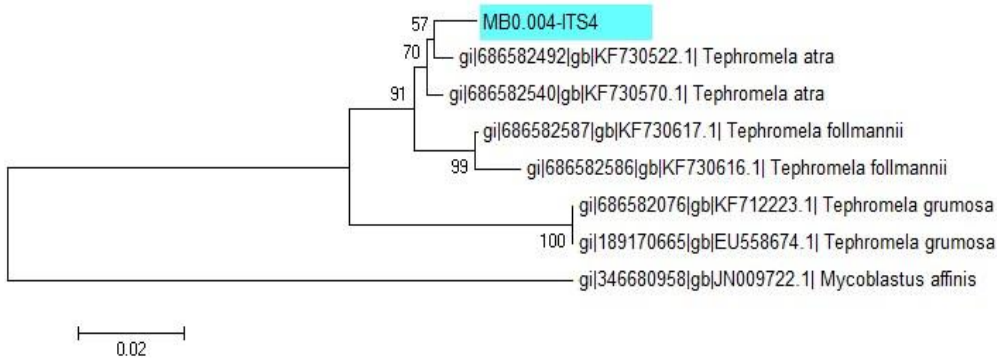


Figure 16. Neighbor-Joining (NJ) dendrogram of *Tephromela atra* and related species (ERC MB 0.004).

In the NJ dendrogram (Figure 16); the closest branching to *Tephromela atra* is *T. follmannii* Pérez-Vargas, Hern.-Padr. & Elix. *T. follmannii* differs from *T. atra* with areolate-bullate to verrucose thallus (Pérez-Vargas et al. 2010). Another species of the genus *T. grumosa* (Pers.) Hafellner & Cl. Roux is sorediate, but *T. atra* is not (Muggia et al. 2008). While *T. atra* includes α -collatolic and α -alectoronic acid, *T. grumosa* includes lichesterinic acid (Westberg et al. 2015, Millot et al. 2008, Hesbacher et al. 1996). *T. atra* is very similar morphologically with *Lecanora gangaleoides* but it can be easily distinguished with its purple hymenium (Millot et al. 2008).

3.9. Xanthoparmelia pulla (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

Figure 17

Specimen examined: Turkey, Kayseri, Talas; Erciyes Mountain, Ali Mountain, 38° 40' 476" N, 35° 32' 993" E, alt. 1380 m, 06.04.2015 [ERC MB 0.001].

Thallus foliose, 6 cm in diam. Surface dark green and brownish green. Lobes (6–)6.1–6.3–6.5 × 3.4–3.5–3.6 mm. Apothecia absent. All spot tests negative.

It has a wide distribution in Africa, Australia, New Zealand and Europe. It is mostly found on siliceous soil and siliceous, acidic and volcanic rocks (e.g. Nimis & John 1998, Kınalıoğlu & Aptroot 2012).



Figure 17. *Xanthoparmelia pulla* (ERC MB 0.001)

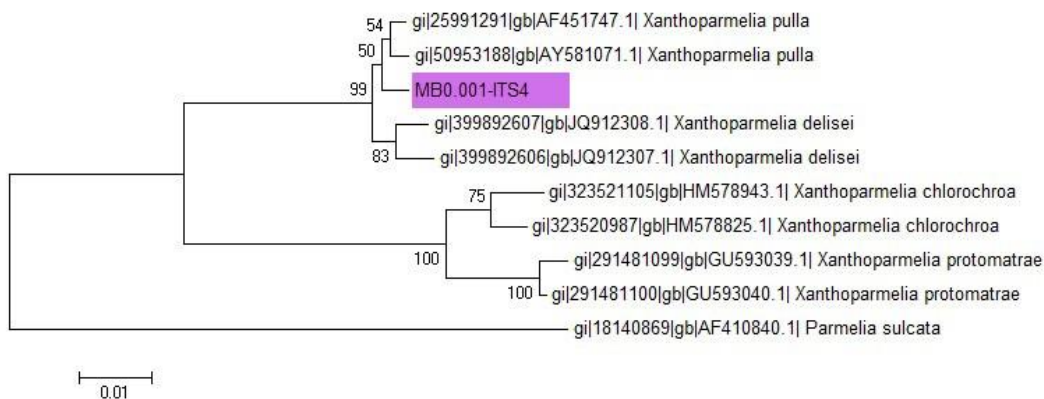


Figure 18. Neighbor-Joining (NJ) dendrogram of *Xanthoparmelia pulla* and related species (ERC MB 0.001)

In the NJ dendrogram (Figure 18); the closest branching to *X. pulla* is *X. delisei* (Duby) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch. Morphologically these two species are very similar to each other but *X. delisei*

contains glomelliferic and glomellic acids (de Paz et al. 2012, Szczepańska & Kossowska 2014). Other related species *X. protomatrae* contains fumarprotocetraric acid (Giordani et al. 2002), but *X. pulla* contains stenosporic, divaricatic and 4-O-demethylstenosporic acid (de Paz et al. 2012, Szczepańska & Kossowska 2014).

4. Conclusions and discussion

In this study, the ITS sequences of 9 common lichenized fungi species (*Aspicilia cinerea*, *Circinaria contorta*, *Lecidea atrobrunnea*, *Lecanora rupicola*, *Physcia dubia*, *Rhizoplaca peltata*, *Rhizocarpon geographicum*, *Tephromela atra* and *Xanthoparmelia pulla*) which has a wide distribution in the alpine zones of volcanic Erciyes Mountain in Central Anatolia are provided. Sometimes morphological characters used in lichen classification lead to misidentifications. Because of this, it is also important to make molecular studies in lichen taxonomy. Those ITS sequences provided for this study are now in GenBank and can be used in the future phylogenetic studies of lichens..

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