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Ramalina digitellata (Ramalinaceae), A New Lichen Record for Türkiye

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Abstract

Ramalina digitellata is reported for the first time from Türkiye. Ecological and distribution information and photos of the species were given. *Ramalina digitellata* can be easily distinguished from other species belonging to the *Ramalina* genus due to the fact that the apical parts of the branches have finger-like protrusions. Besides the morphological and anatomical features, the phylogenetic tree obtained by comparing the sequence analyzes of the ITS regions was also given. The phylogenetic tree is consistent with the morphological and anatomical results.

Keywords: Lichen, Phylogenetic, *Ramalina*, Sinop, Türkiye

Türkiye İçin Yeni Bir Liken Kaydı, *Ramalina digitellata* (Ramalinaceae)

Özet

Ramalina digitellata türü Türkiye'den ilk kez rapor edilmektedir. Türe ait ekolojik bilgiler, dağılış bilgileri ve türe ait fotoğraflar verilmiştir. *Ramalina digitellata, Ramalina* cinsine ait olan diğer türlerden dalların uç kısımlarının parmak şeklinde çıkıntılara sahip olması ile kolayca ayırt edilebilmektedir. Morfolojik ve anatomik özelliklerinin yanı sıra ITS bölgelerinin sekans analizlerinin karşılaştırılması ile elde edilen filogenetik ağaç da verilmiştir. Filogenetik ağaç sonuçları, morfolojik ve anatomik sonuçlarla uyumluluk göstermektedir.

Anahtar kelimeler: Filogenetik, Liken, *Ramalina*, Sinop, Türkiye

INTRODUCTION

Approximately 2000 lichen and lichenicolous fungus taxa are known in Türkiye so far (John & Türk 2017; John et al. 2020). Studies on lichens and lichenicol fungi in our country increased in the late 20th and early 21st centuries. These studies are mostly floristic studies covering certain regions (Aslan et al. 2002; Yazıcı et al. 2008; Öztürk & Güvenç 2010; Çobanoğlu et al. 2013; Kocakaya et al. 2014; Koç et al. 2017; Yavuz & Çobanoğlu 2018; Oran 2021). As a result of increasing lichen studies in recent years, more than 100 lichen and lichenicolous fungus species have been identified in our country by researchers (John et al. 2020).

The genus *Ramalina* Ach. is a fruticose lichen. Thallus fruticose, bushy shaped, rigid and erect, pendent, greenish and yellowish green, usually attached to the substrate at one point. Branched straight or channeled, rigid and flattened. Some species have soredia and pseudocyphellae. Without isidia. Medulla white, loosed, compact. Apothecia is located in different parts of the branches. Asci

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8-spored. Ascospores hyaline and 1-septate. On bark, rock (Egea Fernández & Rowe 1987; Purvis et al. 1992).

MATERIAL AND METHOD

The lichenized fungi taxon *Ramalina digitellata* Nyl. was collected from Sinop province in 2020. Thallus shrubby, rigid and erect. The thallus surface hollow. Branches 0.7-1.3 cm long, with pseudocyphellae. The most distinctive feature that distinguishes it from other species belonging to the *Ramalina* genus is that the apical parts of the branches have distinctive finger-like lobules (digitiform). Apothecia 0.2-0.5 mm diam. Asci 8-spored. Ascospores 1-septate, hyaline, 9-13.5 × 4-6 µm. Spot tests: all reactions negative, C-, K-, KC-, P-. Epihymenium I+ blue. *R. digitellata* was detected for the first time in our country on siliceous rocks from the Sinop province (Figure 1).

Specimen examined

Türkiye: Sinop, on siliceous rocks, 42°02'09"N, 35°11'23"E, 140 m, Barak & Kocakaya, MUB 0.582.

Figure 1. Thallus of *Ramalina digitellata* MUB 0.582

Freshly collected samples for molecular analysis were crushed in a sterile pestle. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). The manufacturer's protocol was used with some minor modifications. ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATT GATATGC) primers were used to amplifying ITS sequencing of the obtained total genomic DNA (White et al. 1990; Gardes & Bruns 1993). The PCR reaction was carried out in the steps given below: 95 °C 5 min for initial denaturation; 30 cycles following steps: 94 °C 1 min for denaturation, 55 °C 1 min for annealing, 72 °C 90 sec for extension; 72 °C 5 min for final extension. PCR product was run on 1% agarose gel and sequence analysis was performed.

ITS sequence analysis results were analyzed with Clustal W clustering analysis process in BioEdit program by using the sequence data of our species and closely related species from Genbank. The morphologically similar species *Evernia prunastri* (L.) Ach. was used as the outgroup. The information and accession numbers of the sequences obtained from Genbank are given in Table 1. MEGA 11 (Molecular Evolutionary Genetics Analysis) program was used to construct a phylogenetic tree (Tamura et al. 2021). The phylogenetic tree was constructed by Maximum Likelihood analysis

using the Tamura-Nei model (Figure 2). Tree reliability tested with 1000 bootstrap replications. The specimen is preserved in Yozgat Bozok University Lichen Herbarium.

RESULTS AND DISCUSSION

Ramalina digitellata was determined for the first time in our country on siliceous rocks from Sinop province at an altitude of 150 m. *R. digitellata* is morphologically similar to *R. farinacea* (L.) Ach. and *R. capitata* (Ach.) Nyl. species. *Ramalina digitellata* is distinguished from *R. farinacea* by having branches with a rigid, erected and finger-like lobules, and having apothecia and pseudocyphellae. *R. capitata* is easily distinguished by having capitate soredia at apical parts of the branches.

Table 1. Informations and Genbank accession number of the species.

Taxa	**Genbank number**	**Locality/Source**
Ramalina capitata	MK811626	Norway/Genbank
Ramalina capitata	MK812398	Norway/Genbank
Ramalina capitata	MK811917	Norway/Genbank
Ramalina digitellata	MN959920	Algeria/Genbank
Ramalina digitellata	GU827326	France/Genbank
Ramalina digitellata	**MUB 0.582**	**Türkiye**
Ramalina farinacea	GU593042	Korea/Genbank
Ramalina farinacea	JF414694	USA/Genbank
Ramalina farinacea	MN387156	Poland/Genbank
Ramalina fastigiata	MN811245	France/Genbank
Ramalina fastigiata	OL622020	UK/Genbank
Ramalina fastigiata	ON088597	UK/Genbank
Ramalina fraxinea	KC960763	Türkiye/Genbank
Ramalina fraxinea	MN811371	Italy/Genbank
Ramalina fraxinea	MK812489	Norway/Genbank
Ramalina pollinaria	MT883392	South Korea/Genbank
Ramalina pollinaria	MT883393	South Korea/Genbank
Ramalina sinensis	MT883409	South Korea/Genbank
Ramalina sinensis	MT883410	South Korea/Genbank
Evernia prunastri	MN387119	Poland/Genbank
Evernia prunastri	MN387120	Poland/Genbank

Ramalina digitellata species was examined in terms of morphological, anatomical and ecological characters. ITS sequence of the species was successfully obtained. It was evaluated together with similar sequence data from Genbank. Analysis was performed using the 21 nucleotide sequence with the outgroup. It is seen that the results of the phylogenetic tree are compatible with the morphological and anatomical characters (Figure 2).

Ramalina digitellata is known from Spain, Italy, France, Portugal in Europe (Egea Fernández & Llimona 1983; Maheu 1909; Sérusiaux et al. 2010; Roux 2012).

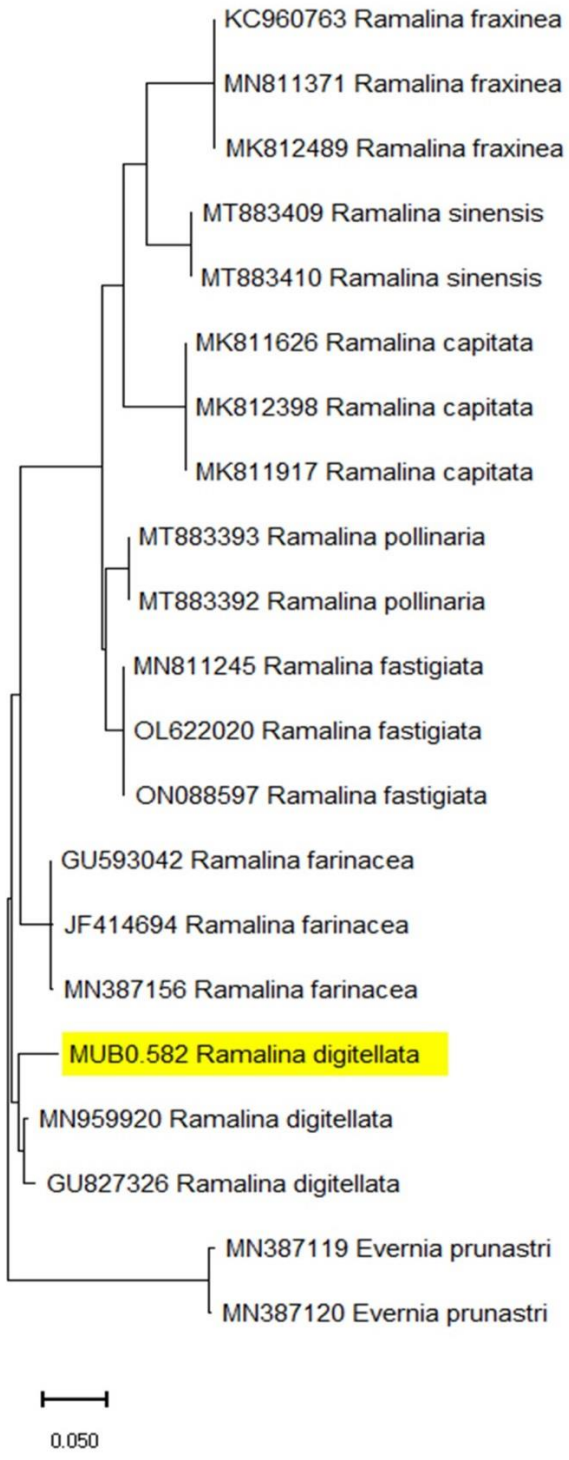

Figure 2. ML analysis (Maximum Likelihood) of the ITS regions of *Ramalina digitellata* and related taxa.

AUTHOR CONTRIBUTION STATEMENT

In this study; study idea and design, data collection, analysis and interpretation of results, writing the article draft were made by Mustafa Kocakaya, Mehmet Ünsal Barak and Zekiye Kocakaya.

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