

A New Lichenicolous Fungus Record From The Turkey, *Tremella macrobasidiata* (Basidiomycota, Tremellales)

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Abstract: Despite the recent increase in studies on lichenicolous fungi in Turkey, there is still no checklist. A key to lichenicolous fungus prepared by Halıcı (2008) were published 118 taxa from the Turkey. So far, the numbers of lichenicol fungi known in Turkey is 193. Generally, lichenicol Ascomycota and mitosporic fungi records are reported, whereas lichenicol Basidiomycota recording is rare. *Tremella macrobasidiata* is reported on *Lecanora chlarotera* from Turkey for the first time. The morphological, anatomical and ecological characteristics of the species are given. In addition, a phylogenetic tree was constructed by comparing the sequence data of the ITS region of closely related species.

Türkiye'den Yeni Bir Likenikol Mantar Kaydı, *Tremella macrobasidiata* (Basidiomycota, Tremellales)

Anahtar Kelimeler

Basidiomycota,
Tremellales,
Tremella,
Likenikol mantar,
nrITS

Özet: Türkiye'de likenikol mantarlar üzerine yapılan çalışmalar son yıllarda artmasına rağmen hala bir kontrol listesi mevcut değildir. Halıcı (2008) tarafından hazırlanan likenikol fungus anahtarında Türkiye'den 118 takson yayınlanmıştır. Şu ana kadar Türkiye'den bilinen likenikol mantar sayısı 193'tür. Genellikle likenikol Ascomycota ve mitosporik fungus kayıtları bildirilirken, likenikol Basidiomycota kaydı nadirdir. *Tremella macrobasidiata*, ilk kez Türkiye'den *Lecanora chlarotera* üzerinden bildirilmiştir. Türün morfolojik, anatomik ve ekolojik özellikleri verilmiştir. Buna ek olarak, yakın ilişkili türlerin ITS bölgelerine ait dizi analizleri ile karşılaştırılarak bir filogenetik ağaç oluşturulmuştur.

1. Introduction

The genus *Tremella* Pers. includes mostly mycoparasitic species. Many of these are host specific and limited to a single genus or fungal species. More than 50 lichenicolous species have been identified that grow up in lichenized fungi until now [1-8]. In the Turkey, only one *Tremella* lichenicolous species have been reported so far [9]. Lichenicolous *Tremella* species that development in the hymenium of the host inconspicuous at first but then the formation of waxy to gelatinous galls is observed on the thallus or apothecium [2, 6]. The phylogenetic position of the genus has been determined by Millanes [10]. In this study, phylogenetic, morphological and ecological features of the species are given.

2. Material and Method

Morphological and anatomical examinations were all performed under stereomicroscopes (Olympus SZX10) and light microscope (BX53). Sections for the

anatomical examinations were taken directly under the stereo microscope using manual razors and examined 10% KOH, Congo-red, and water. DNA isolation was performed with DNeasy Plant Mini Kit (Qiagen). PCR-amplification was carried out in 50 µL reaction volumes using 3 µL of 10 x reaction buffer, 3 µL MgCl₂ (50 mM), 0.5 µL each primer (ITS1F and ITS4), 1 µL dNTP (10 mM), 0.1 µL Taq DNA polymerase, 3 µL of genomic DNA and 38.9 µL dH₂O on a thermal cycler equipped with a heated lid. [11] and ITS1-F [12] were used to amplify the ITS sequence. Polymerase chain reaction (PCR) cycling parameters included an initial hold at 94°C for 5 min, then denaturing at 94°C for 1 min, annealing at 56°C for 1 min., decreasing 1°C per cycle for the first 6 of the 39 cycles, and extension at 72°C for 3 min. PCR products obtained from lichen samples were sent to BMLabosis for sequence analysis. ITS sequence results were analyzed manually using samples from Genbank using the Clustal W option in the BioEdit program. For phylogenetic tree, MEGA 7 (Molecular Evolutionary Genetics Analysis) program was used.

Phylogenetic tree was constructed by Maximum Likelihood analysis using Kimura 2-parameter model. Pairwise deletion was performed for deleting data gaps and checking. The tree reliability was tested with 1000 bootstrap replications. *Phaeotremella pseudofoliacea* JN053502 was used as the outer group. The specimens are deposited in Bozok University Herbarium, Yozgat, Turkey, (DK 0.079, CMP 0.007).

3. Results and Discussion

3.1. Morphology and anatomy

3.1.1. *Tremella macrobasidiata*

A detailed description is provided by Zamora et al. [6].

A pathogenic lichenicolous fungus that infects apothecia in the host, for this reason, it has prevented the production of ascospores. Basidiomata does not appear obvious because into hymenium. Basidia subglobose, $20\text{--}51 \times 12\text{--}24 \mu\text{m}$, Basidiospores $7\text{--}15 \times 7.5\text{--}15 \mu\text{m}$, globose to subglobose.

Because lichenicolous *Tremella* species are inconspicuous species, they are overlooked by both micologists and lichenologists [7]. The species not observed on *Lecanora chlarotera* Nyl., during our morphological studies was noticed as a result of molecular studies (Figure 1). Subsequent detailed anatomical examinations showed that *L. chlarotera* developed in apothecia. According to the literature, the species grows in the apothecia of epiphytic lichen *L. chlarotera*, first formed hymenium and later formed gall and is apparently common [6].

Specimen examined: **TURKEY, Çorum**, Laçın, in the hymenium of *L. chlarotera* on *Quercus* bark, $40^{\circ} 45' 692''$ N, $34^{\circ} 52' 169''$ E, alt. 1020 m, (Herb. No: DK 0.079).

3.2. Ecology and distribution

Tremella macrobasidiata is known from Spain and appears to be host-specific in *L. chlarotera*. The distribution is mainly related to a cosmopolitan epiphytic lichen with a wide ecological diversity [13,14]. For this reason, it is stated that new records are expected [6]. Spanish specimens were collected at altitudes between 590 and 1340 m, extends from the meso-ordination of the Mediterranean Region by Zamora [6]. The Turkish sample was collected at a height of 1020 m from the Black Sea Region.

3.3. Molecular results

Sequence results were checked in GenBank by blastn similarity search (Table 1). The ITS sequences of *T. macrobasidiata* collected from the Turkey were blasted with sequences of species from Spain and

Portugal. It is seen that these species are matched in phylogenetic tree (Figure 2).



Figure 1. Thallus of *L. chlarotera*

Table 1. Sequences used for analysis were downloaded from the gene bank, the new product is indicated by bold .

Species	Locality	nrITS
<i>Tremella caloplacae</i>	France	JN053469
<i>Tremella celata</i>	Spain	KT334576
<i>Tremella celata</i>	Spain	KT334577
<i>Tremella endosporogena</i>	Spain	KT334578
<i>Tremella endosporogena</i>	Spain	KT334579
<i>Tremella diderichiana</i>	Spain	KT334580
<i>Tremella diderichiana</i>	Spain	KT334581
<i>Tremella lobariacearum</i>	Madeira	JN053473
<i>Tremella macrobasidiata</i>	Spain	KT334582
<i>Tremella macrobasidiata</i>	Portugal	KT334583
<i>Tremella macrobasidiata</i>	Turkey	MG209530
<i>Tremella phaeophysciae</i>	Luxembourg	JN053479
<i>Tremella tuckerae</i>	Mexico	JN053482
<i>Tremella tuckerae</i>	Spain	KT334584
<i>Tremella tuckerae</i>	Spain	KT334585
<i>Tremella variae</i>	Spain	KT334586
<i>Tremella variae</i>	Spain	KT334587
<i>Phaeotremella pseudofoliacea</i>	Sweden	JN053502

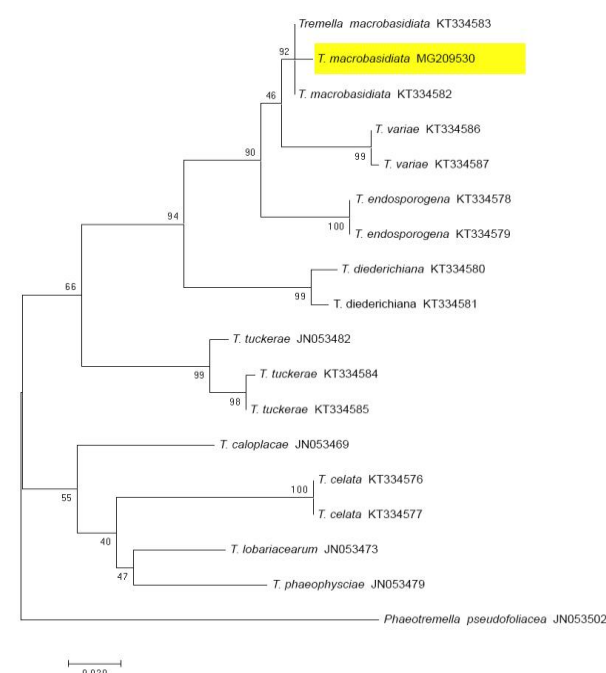


Figure 2. Maximum Likelihood (ML) analysis of the ITS region of *T. macrobasidiata* and related species

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