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# A New Lichenicolous Fungus Record from The Çamlik National Park (Yozgat, Turkey), *Tremella candelariellae* (Basidiomycota, Tremellales)

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## ABSTRACT
*Tremella candelariellae* Diederich & Etayo was reported on *Candelariella antennaria* Räsänen first time in Turkey. Morphological, anatomical and ecological characteristics of the species are presented. In addition, the sequence analysis of the ITS region was performed and the phylogenetic tree was formed by closely related species.

Research Article

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# Çamlık Milli Parkı (Yozgat, Türkiye)'ndan Yeni Bir Likenikol Mantar Kaydı, Tremella candelariellae (Basidiomycota, Tremellales)

## ÖZET
*Tremella candelariellae* Diederich & Etayo Türkiye'den ilk kez *Candelariella antennaria* Räsänen üzerinden rapor edilmiştir. Türün morfolojik, anatomik ve ekolojik özellikleri verilmiştir. Ayrıca ITS bölgesine ait dizi analizi yapılarak yakın ilişkili türlerle birlikte filogenetik ağaç oluşturulmuştur.

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Anahtar Kelimeler
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Taksonomi
*Tremella*

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## INTRODUCTION
The genus *Tremella* Pers., contains predominantly mycoparasite species growing in a wide variety of basidiomycetes and ascomycetes fungi (Chen 1998). *Tremella* species is highly variable in appearances, such as size, shape and colour. Lichenicolous species usually grow in the host's induced galls. However, they all have common features. All species within the genus have been found to be parasites and grow with them in the hymenium of other fungi (Pippola and Kotiranta, 2008). Millanes et al., 2012 studied the phylogenetic position of the genus and stated that fifty-one *Tremella* species have been identified. Two lichenicolous *Tremella* species were reported from Turkey (Halici, 2015; Kocakaya et al., 2018). In this study, morphological, anatomical, ecological and phylogenetic features of the species were given.

## MATERIAL and METHOD
Lichen sample was collected from Çamlık National Park in 2017. Morphological and anatomical investigations were performed under a stereomicroscope (Olympus SZX10) and light microscope (Olympus BX53). They were examined by standard microscopic techniques. Measurements were made in water. The new record specimen is deposited in Yozgat Bozok University Herbarium Yozgat, Turkey.

DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocols with minor modifications. ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990) and ITS1-F (CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) were used to amplify the ITS sequence. PCR-amplification was carried out, following Millanes et al. (2012). PCR products were

visualized on a 1% agarose gel. The sequence of PCR product obtained from the *Tremella* sample was performed using the Big Dye Terminator Cycle Sequencing v3.1 (Macrogen, Holland) following the manufacturer's protocol and analysed on an ABI 3730XL Genetic Analyser.

Sequence results were analyzed automatically using the Clustal W option in the BioEdit program, along with samples from Genbank. Details and GenBank accession numbers of the samples are listed in Table 1. Phylogenetic tree was constructed by Maximum Likelihood analysis using MEGA 7 program. Pairwise deletion was performed to delete and control data gaps. The tree reliability was tested with 1000 bootstrap replications. We selected the out groups used in the previous phylogenetic analysis of the genus *Tremella* (Millanes, et al., 2012).

## RESULTS and DISCUSSION

### Morphology and Anatomy

*Tremella candelariellae* Diederich & Etayo 1996

A detailed description is provided by Diederich, P. 1996

Some lichenicolous fungus is easily observed because they deform the host thallus and form large abnormally shaped galls. *T. candelariella* is an inconspicuous species that due to the small size of the host (*Candelariella* species). It is easily ignored in the herbarium, because it slightly deforms only small apothecia in the host (Lendemer, 2008). This species has been described on the thallus of *Candelariella vitellina* (Hoffm.) Müll. Arg. and *C. xanthostigma* (Pers. ex Ach.) Lettau (Diederich, 1996). In our phylogenetic studies, appeared on the apothecia of *C. antennaria* (Figure 1. A). Galls occurs on the host thallus. Galls and host thallus are the same colour. Gall surfaces are pruinose and covered with numerous yellow crystals in microscopic sections.

Basidiomata superficial, convex, bright yellow galls on the host thallus, often with a granular, pruinose surface (Figure 1.A), 0.1-0.8 mm in diam., hyphae mixed with host hyphae, basidia, hyaline, ellipsoid, 2 celled 18-20 x 10-12 µm (Figure 1.B), basidiospores ellipsoid, hyaline 6.5-7 x 5-6 µm. Conidia not seen.

Each particular species of *Tremella* is host-specific, often limited to a single fungal genus or species. Therefore, it was not seen on a different genus from *Candelariella*. The most closely related species in phylogenetic studies is *Tremella dendrographae* Diederich & Tehler. However, there are significant differences in morphological and anatomical studies. *T. dendrographae* has larger basidia and longer basidiospores. In addition, host lichens are different. *T. dendrographae* prefer to *Dendrographa* Darb. genus as a host. While *T. candelariella* formed yellow galls, *T. dendrographae* formed whitish galls on the host thallus Besides, *T. dendrographae* is a very common fungus that causes a large and visible formation(Nash et al., 2004).

Specimen examined: Turkey, Yozgat, Çamlik National Park, on *Pinus nigra* bark, 39˚48' 047" N, 34˚ 48' 498" E, alt. 1613 m, August 26, 2017 (Herb. No: CMP 0.086).

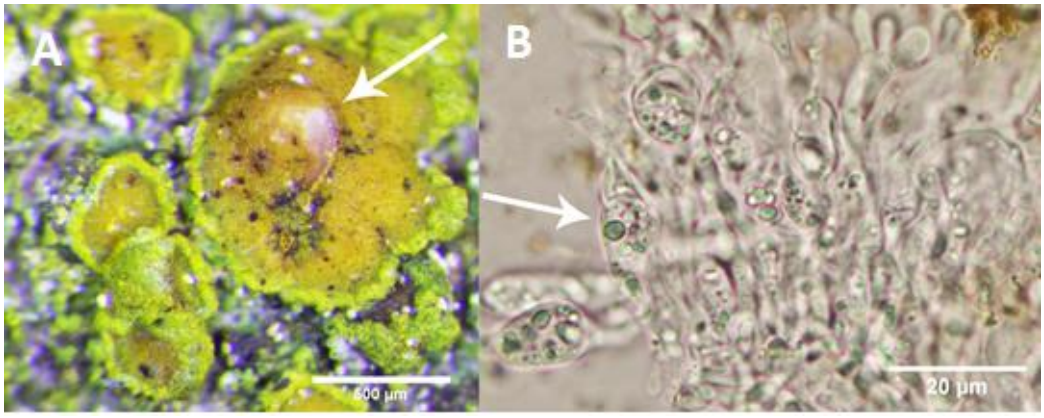


Figure 1. A) Galls on apothecia of *C. antennaria* B) Basidia
*Şekil 1. A) C. antennaria'nın apotesyumu üzerindeki gal oluşumu B) Bazidyum*

### Distribution

The species was described in Luxembourg at a altitude of 300 m on *C. vitellina* (Diederich, 1996). Later in Italy, at a altitude of 1300 m, on *C. xanthostigma* (Diederich, 1996) and in the U.S.A. In Pennsylvania on *C. xanthostigma* (Lendemer, 2008). It also known from Poland (Kukwa and Jabłońska, 2008) and Sweden (Westberg, et al., 2008). Turkish specimen was collected in central Anatolia at Çamlik National Park, 1613 m at the altitude on *C. antennaria*.

### Molecular Results

ITS sequence was successfully obtained. It was evaluated together with the sequence results from the gene bank (Table 1). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992) (Figure 2).

Table 1. Sequences downloaded from GenBank and newly produced (bold).
*Tablo 1. GenBank'tan indirilen ve yeni üretilen diziler (koyu renk).*

| Species | Locality | nrITS |
|---|---|---|
| *Tremella caloplacae* | Greenland | JN053468 |
| *T. caloplacae* | France | JN053469 |
| *T. candelariellae* | Luxembourg | JN053470 |
| ***T. candelariellae*** | **Turkey** | **MN566922** |
| *T. cetrariicola* | Finland | JN053490 |
| *T. cetrariicola* | Latvia | JN053491 |
| *T. cladoniae* | Estonia | JN053477 |
| *T. cladoniae* | France | JN053478 |
| *T. coppinsii* | UK | JN053495 |
| *T. coppinsii* | Estonia | JN053496 |
| *T. dendrographae* | USA | JN053471 |
| *T. hypogymniae* | Sweden | JN053484 |
| *T. hypogymniae* | Estonia | JN053485 |
| *T. lobariacearum* | Madeira | JN053473 |
| *T. lobariacearum* | Canary Islands | JN053474 |
| *T. phaeophysciae* | Luxembourg | JN053479 |
| *T. phaeophysciae* | Estonia | JN053480 |
| *T. umbilicariae* | Peru | KM507564 |
| *Phaeotremella pseudofoliacea* | Sweden | JN053502 |
| *Filobasidium uniguttulatum* | - | AF444302 |
| *F. floriforme* | - | AF190007 |

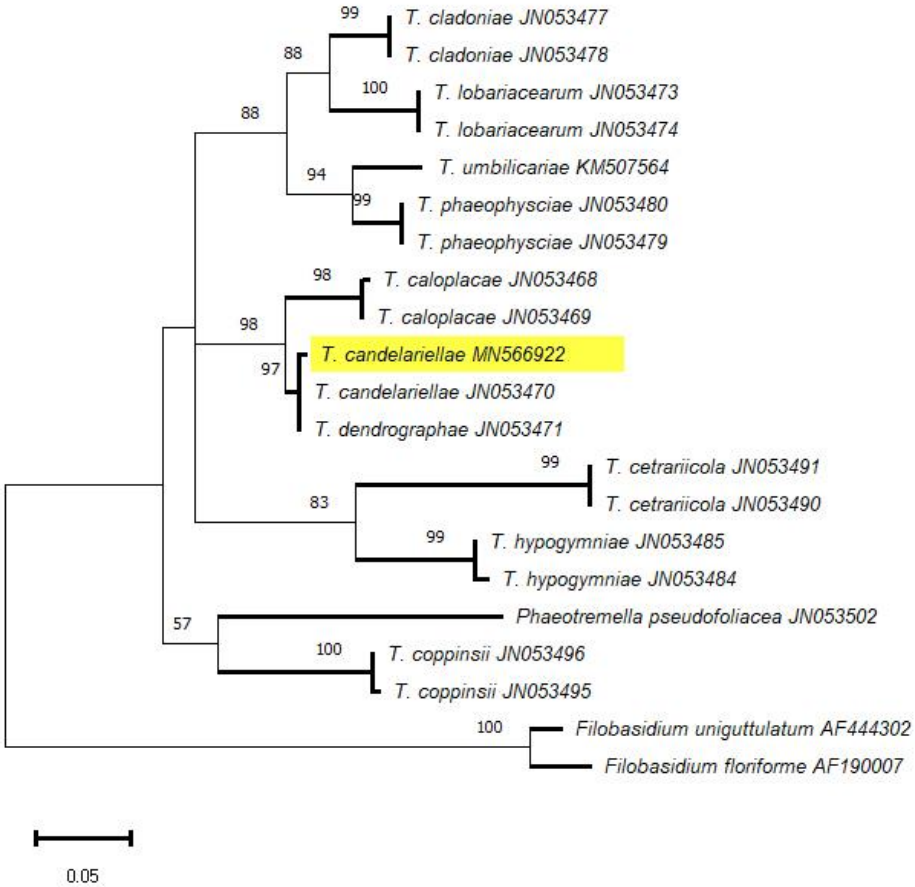


Figure 2. Maximum likelihood analysis of the ITS region of *T. candelariellae* and related species. Numbers at tree nodes indicate bootstrap values of ML (only values ≥50%).
*Şekil 2. T. candelariellae ve ilişkili türlerin ITS bölgesine ait maksimum olabilirlik analizi. Ağaç nodlarındaki sayılar, ML'nin bootstrap değerlerini gösterir (sadece ≥% 50 değerleri).*

The sequence result compared with the Luxembourg sample in the gene bank. Our sequence result is matched in the phylogenetic tree with this sample. *T. candelariella* is similar to *T. dendrographa* as the morphological and anatomical features. Also, this species are located in the same clad in phylogenetic tree.

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### Statement of Conflict of Interest

Authors have declared no conflict of interest.

### Author's Contributions

The contribution of the authors is equal.

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