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Tricholoma bonii, A New Record for Turkish Mycota and Notes on its Taxonomic Status Based on Morphological and Molecular Evidence

Tricholoma bonii, Türkiye Mikotası İçin Yeni Bir Kayıt, Morfolojik ve Moleküler Kanıtlarla Taksonomik Durumu Üzerine Notlar

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Abstract

In the present study, *Tricholoma bonii* is reported as a new record for Turkish mycota. The taxonomic status of the species is discussed by evaluating morphological and molecular evidence.

Key words: New record, *Tricholoma bonii*, Taxonomy, ITS, Turkey.

Öz

Bu çalışma ile *Tricholoma bonii*, Türkiye mikotası için yeni kayıt olarak rapor edilmiştir. Ayrıca, morfolojik ve moleküler kanıtların değerlendirilmesiyle bu türün taksonomik durumu tartışılmıştır.

Anahtar kelimeler: Yeni kayıt, *Tricholoma bonii*, Taksonomi, ITS, Türkiye.

1. Introduction

Tricholoma (Fr.) Staude is a genus of *Agaricales* Underw. including more than a thousand members according to Index Fungorum (www.indexfungorum.org, access date 23 April 2018). *Tricholoma* specimens can easily be recognized in the field by their macroscopic features such as fleshy basidiocarps, white, cream to yellowish emarginate lamellae, white spore deposit and hyaline, smooth spores (Kibby 2012; Christensen and Heilmann-Clausen 2013). While the genus can be easily recognized, species identification is not as easy, especially because of the scarcity of different morphological characters. In these perspective, *Tricholoma terreum* (Schaeff.) P. Kumm. and its allies (*T. triste* (Scop.) Quél. and *T. bonii* Basso & Candusso) form one of the most controversial group in the genus having highly similar morphological characters and this might cause misidentification.

In Turkey, more than 2000 macrofungi species have been reported (Sesli and Denchev 2008) and 53 of them belong to the genus *Tricholoma.* Recently, two new taxa, *T. anatolicum* H.H. Doğan & Intini and *T. virgatum* var. *fulvoumbonatum* E. Sesli, Contu & Vizzini were described as new species in the literature (Intini et al. 2003; Vizzini et al. 2015). But, the macrofungi biodiversity of Turkey have not been fully determined and recent surveys have contributed to Turkish mycota by adding many new records (Akata and Sesli 2017; Çolak et al. 2017; Işık and Türkekul 2018; Sesli 2018).

In the present study, we aimed to contribute to Turkish mycota with a new record and to discuss the taxonomy of *Tricholoma terreum* and its allies based on morphological and molecular evidence.

2. Material and Methods

2.1. Field Study and Phenotypic Identification

The samples were collected from the Aegean Region of Turkey during routine field trips between 2013 and 2015. In the field, morphological characters such as shape, size and color of basidiocarp, and ecological features of samples were noted and they were photographed in their habitat. The colors of the basidiocarps were observed in daylight in accordance with Kornerup and Wanscher (1978).

Microscopic characters were observed by mounting the samples in 3% KOH, 1% Congo red and 1% Creysl blue solutions and analyzing with a light microscope (Leica DM750).

The morphological approaches suggested by Galli (2005), Christensen and Heilmann-Clausen (2008, 2012, 2013) and Kibby (2012) were followed in order to identify the specimens. Dried samples were deposited in the Fungarium of Department of Biology, Muğla Sıtkı Koçman University.

2.2. Molecular Methods

Genomic DNA was extracted using Qiagen Plant Mini Kit following the instructions provided by the manufacturer. The internal transcribed spacer regions (ITS) were amplified by PCR using ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) primers which were used for sequencing as well.
The PCR products were purified using Fermentas PCR Purification Kit and sequenced by Macrogen (the Netherlands). The sequences were edited using BioEdit program (Hall 1999) and then inserted in the molecular database NCBI in order to compare with homologous ones. Afterwards, a phylogenetic analysis was performed using the Mega 6 program (Tamura et al. 2013) and the boostsrap test was applied (10000 replications) (Kimura 1980).
The sequences obtained from this study were uploaded to GenBank: their accession number is given in Tab. 1.

Table 1. *Tricholoma* collections collected from area under study.

Taxa	Collection No	Genbank No
T. terreum	Şen 507	KY121104
T. terreum	Şen 778	KY121103
T. bonii	Şen 790	KY121105
T. bonii	Şen 791	KY121106
T. triste	Şen 1324	KY121107
T. terreum	Şen 1476	KY121101
T. terreum	Şen 1504	KY121102

3. Taxonomy

Tricholomataceae Lotsy
Tricholoma (Fr.) Staude
Tricholoma bonii Basso & Candusso, Docums Mycol. 27 (no. 107): 64 (1997). **Fig. 1**

Pileus 18 – 45 mm across, first bell shaped, later convex to flat, often umbonate, surface felty to tomentose scaly, dark grey to black at centre, often marbled, paler towards to margin, rarely white to cream. **Lamellea** emerginate, white to pale grey, sometimes black punctate at the edges. **Stipe** 28 – 45 × 5 – 7 mm, cylindrical, whitish to pale greyish, fibrillose when young, later smooth. **Flesh** white to pale greyish, smell and taste indistinct. **Basidiospores** 6 – 11.3 × 3.3 – 5.5 µm, smooth, hyaline, oblong (Q value 1.61 – 2.01). **Basidia** 19 – 32 × 6.3 – 8.8 µm, 4 spored, but we observed rarely 2 spored basidia. **Cheilocystidia** present, cylindrical to clavate. **Pileipellis** a cutis, hypal elements 5 – 15 µm broad. **Clamp connections** absent.

Habitat: This species is associated with conifer trees, especially *Pinus*.

Specimens Examined: TURKEY. Afyonkarahisar – Sandıklı highway 55th kilometers, *Pinus nigra*, *Quercus* sp. and *Salix* sp. mixed forest, 11 May 2014 (Şen 790, 791); Muğla, Kavaklıdere, Salkım village, *P. brutia* ormanı, 01 December 2014 (Şen 1434).

4. Results and Discussions

In Turkey, a number of 53 species belonging to genus *Tricholoma* have so far been reported (Intini et al. 2003; Sesli and Denchev 2008). In this study, *T. bonii* is reported for the first time for Turkish mycota. The systematic of taxa was followed in accordance with Christensen and Heilmann-Clausen (2013).
The classification of the genus in question has recently been evaluated by Christensen and Heilmann-Clausen (2013) and Heilmann-Clausen et al. (2017) by using morphological investigations and molecular methods, which led to the separation of the genus into 16 clades and to place *T. bonii* within Section *Terrea*. In this study, the topology of the phylogenetic tree agrees well with that of Christensen and Heilmann-Clausen (2013) and Heilmann-Clausen et al. (2017) and is supported by different morphological characters (Fig. 2).
The taxa belonging to Section *Terrea* i.e. *T. terreum*, *T. triste* and *T. bonii* have similar morphological characters and differs from the other *Tricholoma* species by having grey to black felty cap, unchanging flesh, indistinct smell and taste (Christensen and Heilmann-Clausen 2008, 2012, 2013).
Tricholoma bonii was described by Basso and Candusso (1997) as a small white species. Christensen and Heilmann-Clausen (2013) and Heilmann-Clausen et al. (2017) reported that the color of pileus of *T. bonii* may vary from grey to black and discussed that the holotype of *T. bonii* might be albino. We found a fully white specimen (Şen 507) that matched morphologically to *T. leucoterreum*, but it was highly similar to *T. terreum* based on ITS gene sequence homology. Christensen and Heilmann-Clausen (2013) reported that *T. leucoterreum* is synonym of *T. terreum*. It may be possible that the entities belonging to these groups rarely produce albino phenotypes.
Tricholoma bonii differs from *T. terreum* by its longer spores and grey fibrillose stipe. According to Christensen and Heilmann-Clausen (2013), the spores of *Tricholoma bonii* are variable, but, its shape is predominantly subcylindrical. In our collections, we have observed similar characters and the shapes of the spores are variable (Fig. 1g). *Tricholoma triste* and *T. bonii* have similar basidiocarp but *T. triste* differs from *T. bonii* mainly by its white, woolly pileus margin (Galli 2005; Christensen and Heilmann-Clausen 2008; Kibby 2012). Furthermore, *T. bonii* possesses black spotted lamellae edges (Fig. 1e), another feature that enables distinguishing it from *T. terreum* and *T. triste* in the field. These species are identified by ITS gene sequence homologies clearly (Fig. 2) and high bootstrap values in the phylogenetic tree constructed based on ITS sequences support the morphological differences of the species.

Conflicts of Interest: No conflict of interest was declared by the authors.

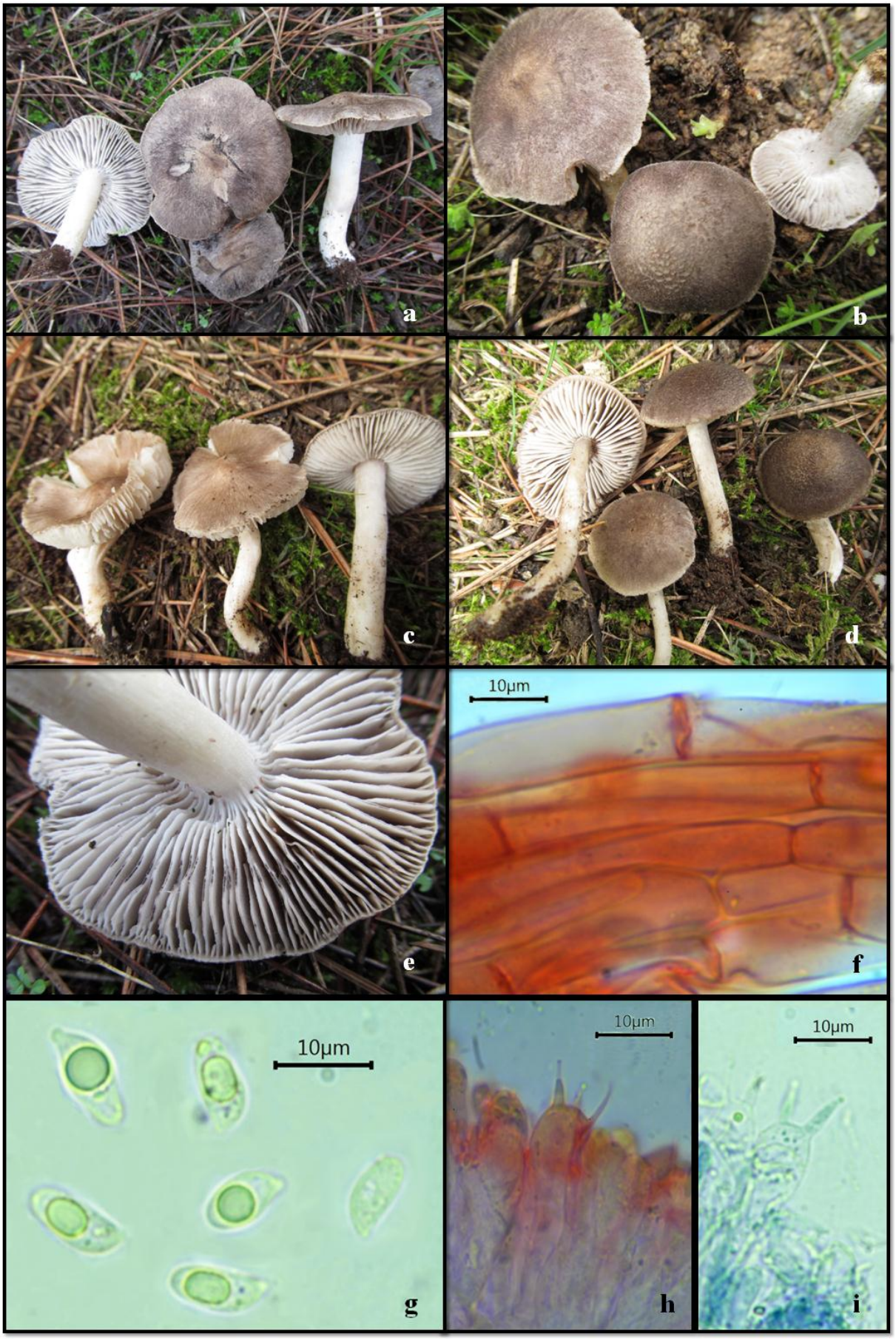

Figure 1. ***Tricholoma bonii*****,** a-e. basidiocarp, f. pileipellis (in congo red), g. basidiospore (in KOH), h. basidium (in congo red), i. basidium (in creysl blue).

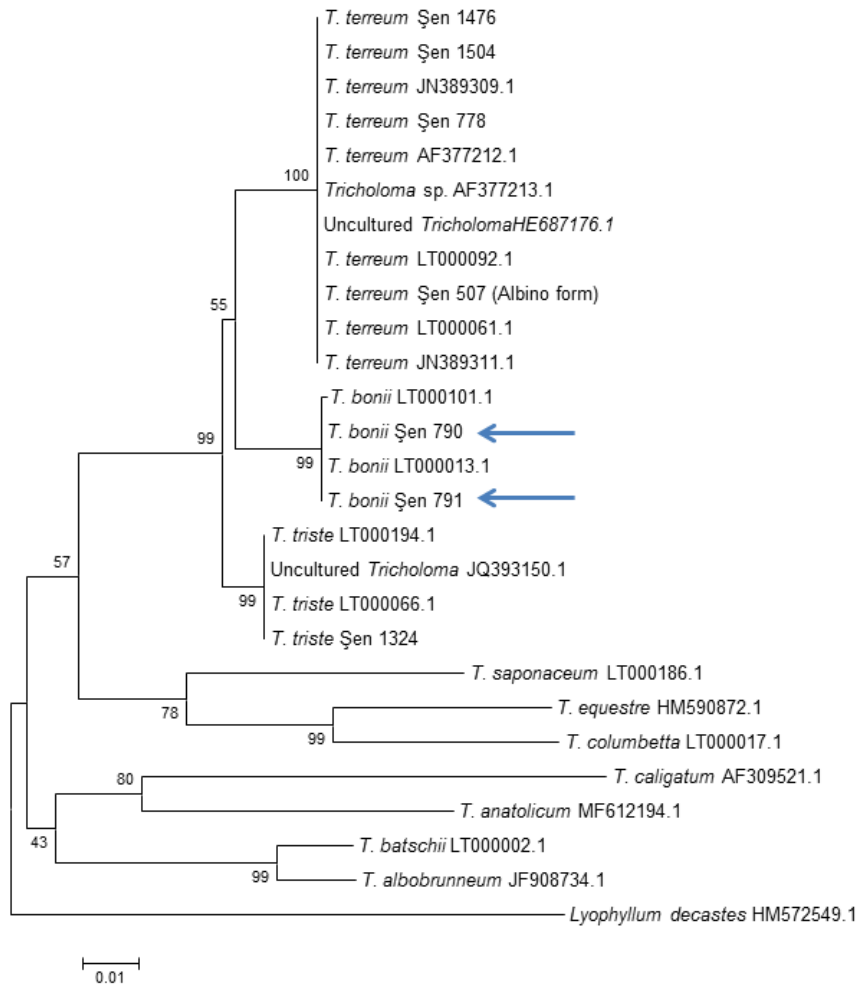

Figure 2. Phylogeny of *Tricholoma terreum* complex, generated from ITS sequences. *←New record for Turkish mycota is indicated with the arrows.

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