This article is cited as: Aldemir Terman, Ş., Akçay, M.E., Dizkırıcı, A. (2024). Expanding the *Tricharina* Diversity in Türkiye: The Identification of *Tricharina cretea* from Nemrut Mountain, *Mantar Dergisi*, 15(Special issue) 29-36.

Geliş(Recevied) :14.10.2024 ***Research Article***
Kabul(Accepted) :21.11.2024 **Doi: 10.30708/mantar.1566971**

Expanding the *Tricharina* Diversity in Türkiye: The Identification of *Tricharina cretea* from Nemrut Mountain

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Abstract: The genus *Tricharina* (*Pezizales*) presents taxonomic challenges due to the morphological similarities among its species. Currently, 21 species are recognized globally, with three previously reported from Türkiye. This study identifies *Tricharina cretea* as a new record from Nemrut Mountain (Bitlis/Tatvan), Türkiye, based on morphological and molecular analyses. The species is characterized by shallow, disc-shaped apothecia. Molecular phylogenetics, incorporating internal transcribed spacer (ITS) and large subunit (LSU) regions, were analyzed using Bayesian inference (BI). Phylogenetic analysis revealed that *T. cretea* is closely related to *T. praecox*. Concatenated ITS+LSU data facilitated the clear delimitation of *T. cretea* from related taxa. Detailed morphological descriptions, illustrations, and comparisons with morphologically and phylogenetically similar species are provided.

Keywords: *Tricharina*, ITS, LSU, New record, Phylogeny

Türkiye'deki *Tricharina* Çeşitliliğinin Genişletilmesi: Nemrut Dağı'ndan *Tricharina cretea* Türünün Tanımlanması

Öz: *Tricharina* cinsi (*Pezizales*), türleri arasındaki morfolojik benzerlikler nedeniyle taksonomik açıdan zorlukların olduğu bir cinstir. Günümüzde dünya genelinde 21 tür tanımlanmış olup, Türkiye'den daha önce üç tür rapor edilmiştir. Bu çalışma, Türkiye'nin Nemrut Dağı (Bitlis/Tatvan) bölgesinden *Tricharina cretea*'yı morfolojik ve moleküler analizlere dayalı olarak yeni bir kayıt olarak tanımlamaktadır. Tür, sığ ve disk şeklindeki apotezyum yapısı ile karakterize edilmiştir. Moleküler filogenetik analizlerde transkribe edilen aralayıcı bölgeler (ITS) ve büyük alt ünite (LSU) bölgeleri kullanılmış ve analizler Bayes çıkarımı (BI) yöntemleriyle gerçekleştirilmiştir. Filogenetik analizler, *T. cretea*'nın *T. praecox* ile yakından ilişkili olduğunu ortaya koymuştur. ITS+LSU birleşik verileri, *T. cretea*'nın ilişkili taksonlardan net bir şekilde ayrılmasını sağlamıştır. Morfolojik olarak benzer ve filogenetik olarak ilişkili türlerle karşılaştırmaların yanı sıra detaylı tanımlar ve görseller sunulmuştur.

Anahtar kelimeler:, *Tricharina*, ITS, LSU, Yeni kayıt, Filogeni

Introduction

Pyrophilic fungi, commonly known as post-fire fungi, constitute a distinctive and ecologically specialized group of organisms adapted to transient and unstable habitats, particularly those shaped by fire events (Dix and Webster, 1995). These fungi are predominantly observed in burned environments, where they benefit from reduced competition and demonstrate remarkable adaptations to the altered physical and chemical conditions following fire. One key factor favoring their proliferation is their ability to thrive under elevated pH levels typically associated with post-fire soils (El-Abyad and Webster, 1968).

Tricharina is a genus of pyrophilic fungi within the order *Pezizales*, encompassing approximately 21 species globally (indexfungorum.org). These fungi are well-adapted to fire-influenced habitats, where they play a critical role in nutrient cycling and ecosystem recovery. Members of the genus are characterized by their shallow, disc-shaped apothecia; cylindrical, 8-spored asci; uniseriate, ellipsoid ascospores; and distinctive reproductive structures tailored to transient environments. *Tricharina* is predominantly distributed across temperate regions, with numerous species reported from Europe, North America, and Asia (Van Vooren et al., 2023). In Türkiye, three species have been previously recorded (Sesli et al., 2020; Uzun, 2023), but the diversity and ecological roles of *Tricharina* within the region remain poorly explored.

Recent advancements in molecular techniques, particularly DNA sequencing of the internal transcribed spacer (ITS) and large subunit (LSU) regions, have significantly improved our understanding of phylogenetic relationships within the genus. These approaches enable more precise differentiation among closely related species.

The present study aims to expand the understanding of *Tricharina* diversity in Türkiye by documenting *Tricharina cretea* as a new record from Nemrut Mountain (Bitlis/Tatvan). Through a combination of morphological descriptions and molecular phylogenetic analyses, this research seeks to clarify the phylogenetic relationships of *T. cretea* with other species within the genus, thereby contributing to a broader understanding of *Tricharina*'s diversity and ecological roles.

Material and Method

Taxon sampling and morphological studies

Macrofungus samples were collected in 2022 from the Tatvan district of Bitlis province, Türkiye, and were deposited in the Fungarium of Van Yüzüncü Yıl University (VANF). Morphological examinations and measurements were performed on field-collected specimens. For ascospore measurements, thirty mature spores ejected from a single specimen were analyzed. All measurements and descriptions were made on specimens mounted in distilled water. Microscopical observations were carried out using a Leica DM500 research microscope, and measurements were recorded using the Leica Application Suite software (version 3.2.0). The morphological analyses followed the protocols established by Van Vooren et al. (2017, 2019, 2023).

DNA extraction, PCR amplification and Sequencing

DNA was extracted from dried fungarium collections using a slightly modified protocol based on Doyle and Doyle (1987). To amplify the ITS and LSU regions, primer pairs ITS1/ITS4 (White et al., 1990) and LROR/LR5 (Vilgalys and Hester, 1990) were employed, respectively. The PCR reaction was conducted in a 25 µl mixture containing genomic DNA (10 ng/µl), 10X PCR Buffer, $MgCl_2$ (25 mM), dNTP mixture (10 mM), the selected primer pair (10 µM), Taq polymerase (5 u/µl), and sterile water. PCR products were analyzed on a 1.0% agarose gel and visualized using GelRed dye. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 2 minutes; 30 cycles of denaturation at 94 °C for 1 minute, annealing at 54 °C for 45 seconds (53 °C for LSU), and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 5 minutes. Positive PCR reactions were sequenced using forward and reverse primers with an ABI 3730XL automated sequencer (BM Labosis, Ankara, Türkiye). Alongside the sequences obtained in this study, a representative selection of sequences from *Tricharina* species was retrieved from NCBI database for comparative analysis. The accession numbers for studied and retrieved sequences are given as an Appendix.

Pylogenetic analysis

DNA sequences were assembled and edited using Mesquite 3.7 software (Maddison and Maddison, 2009) in conjunction with Mafft 7.311 (Katoh and Standley, 2013). Chromatograms were meticulously examined and corrected for potential sequencing errors. The sequences generated in this study were deposited in NCBI databse under the following accession numbers: ITS (PQ198377) and LSU (PQ198414). For preliminary phylogenetic comparison, a Basic Local Alignment Search Tool (BLAST, Altschul et al., 1990) analysis was conducted to incorporate related sequences, facilitating the clarification of the phylogenetic positions of the samples within the genus. Separate alignments for the ITS and LSU regions, as well as a concatenated dataset (ITS+LSU), were prepared for subsequent phylogenetic analyses. A total of 15 sequences, including those from the current study, were analyzed across the ITS, LSU, and concatenated

datasets. *Geopora cercocarpi* (NR_121491), *G. cercocarpi* (HQ283091), and *G. cercocarpi* (SOC1590) were selected as outgroup samples for the respective datasets.

Phylogenetic inference was performed using Bayesian inference (BI) methods with MrBayes v.3.2.6 (Ronquist et al. 2003). The analysis utilized the Markov Chain Monte Carlo (MCMC) method, with all settings left at default, including the incremental heating scheme for chains, unconstrained branch lengths, and uninformative topology priors. The MCMC was run for 3 million generations until the average standard deviation of split frequencies fell below 0.01, with the first 25% of generations treated as burn-in. A majority rule consensus tree was generated from the remaining trees, and branch support was assessed using Bayesian Posterior Probabilities (BPP). The trees were visualized using stree 1.4.3 (Rambaut, 2010).

Results

Tricharina cretea (Cooke) K.S. Thind and Waraitch, Panjab University Research Journal (Science), New Series 21: 154 (1971)

Synonymy:

Ascorhizoctonia cretea Chin S. Yang and Korf, Mycotaxon 23: 470 (1985)

Lachnea cretea (Cooke) W. Phillips, Man. Brit. Discomyc. (London): 228 (1887)

Peziza cretea Cooke, Mycogr., Vol. 1. Discom. (London)(no. 5): 214, fig. 362 (1878)

Scutellinia cretea (Cooke) Kuntze, Revis. gen. pl. (Leipzig) 2: 869 (1891)

Tricharia cretea (Cooke) Boud., Hist. Class. Discom. Eur. (Paris): 58 (1907)

Tricharina praecox var. *cretea* (Cooke) Chin S. Yang and Korf, Mycotaxon 24: 505 (1985)

Position in classification: *Pyronemataceae* Corda, Pezizales, *Pezizomycetidae*, *Pezizomycetes*, *Pezizomycotina*, *Ascomycota*, Fungi

Macroscopic features: Apothecia (fruiting body) 2-7 mm across, cup shaped, forming shallow discs, dirty white to yellowish brown, only in older apothecia of beige tint, outer surface in same color, stalkless. Margin covered with short whitish to cream hairs, slotted in mature specimens (Fig 1a).

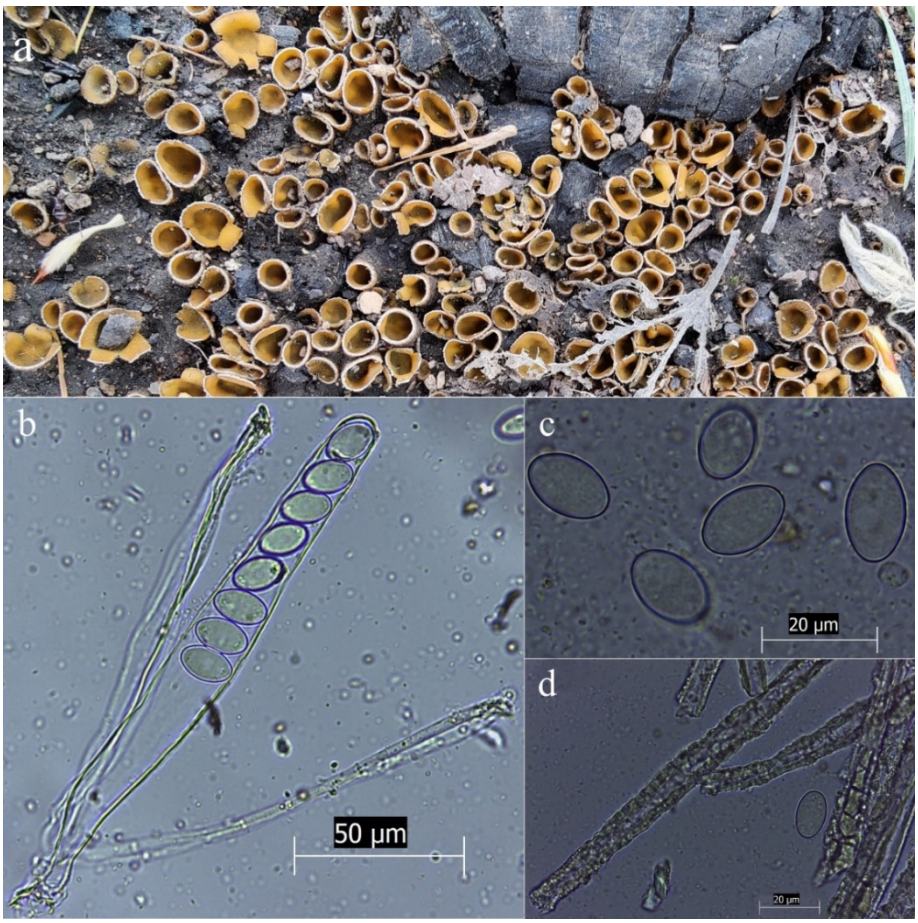

Figure 1. a. Fresh ascocarp of *Tricharina cretea* in natural habitat. b. asci in distilled water. c. ascospores in distilled water. d. hairs in distilled water (Photos by Dr. M. Emre Akçay).

Microscopic features: Ascospores 14–17 × 8–10 µm, ellipsoid-fusoid or ellipsoid with tapering ends, uniseriate, sometimes asymmetrical, hyaline, without oil drops or polar granules, smooth, rather thick-walled Fig 1b). Asci 130-160 × 13–16 µm, operculate, cylindrical, eight-spored, inamyloid, probably with crozier -hard to see because asci are collapsed- (Fig 1b). Paraphyses filiform, hyaline, without vacuole nor guttules, not enlarged at the apices, 3–4 µm wide. Excipular hairs 150–310 × 7–9 µm, superficial, more or less flexuous, with a simple enlarged to bulbous base (up to 30 µm wide), pale brownish, septate, thick-walled (up to 4 µm). Marginal hairs 50–235 × 10–13 µm, similar but straight and not bulbous, often clustered, mostly pointed at the top, brownish, few septate (Fig 1d). Excipulum consists of globose to prismatic cells of "textura globulosa" to "t. angularis".

Habitat features: Cespitose or clustered together on bare or mossy burned ground (Van Vooren et al., 2017).

Molecular phylogeny

The ITS, LSU, and concatenated data matrices comprised 15 sequences representing the genus *Tricharina*, along with one outgroup from the genus *Geopora* (Table 1). The aligned ITS dataset reached a length of 605 bp after the exclusion of poorly aligned sites, containing 102 variable sites and 84 parsimony-informative sites (Fig. 2). The aligned LSU dataset was 805 bp long, with 50 variable sites and 42 parsimony-informative sites (Fig. 3). The concatenated dataset had a total aligned length of 1410 bp, incorporating 152 variable sites and 126 parsimony-informative sites (Fig. 4). Phylogenetic relationships were most effectively resolved using the concatenated tree, which revealed two primary clades. Notably, within this tree, *Tricharina cretea* was found to be closely related to its representative downloaded from database and *Tricharina praecox (*var. *praecox)* (Fig. 4).

Table 1. ITS and LSU sequences downloaded from the NCBI database (Taxa highlighted in bold indicate the specific specimens examined in this study)

Taxa	NCBI accession no ITS	Country/Voucher	NCBI accession no LSU	Country/Voucher
Tricharina cretea	**PQ198377**	**Türkiye/VANF52**	**PQ198414**	**Türkiye/VANF52**
	MH861871	Sweden/CBS 235.85	MH873559	Sweden/CBS 235.85
T. praecox var. praecox	NR_103690	China/CBS 240.85	NG_057955	Sweden/O-253286 (O)
T. praecox	MW205788	India/ ES1	DQ646525	USA/KH.03.101 (FH)
T. gilva	MW447093	Poland/ FeC100	NG_064139	Sweden/CBS 236.85
T. groenlandica	MH861873	Greenland/CBS:237.85	U38576	Canada/CSY 104
T. ochroleuca	OP730559	Norway/ALV25099	OP730559	Norway/ALV25099
T. hiemalis	MH857979	Netherlands/CBS 263.60	OL832172	France/ALV31245
T. aethiopica	NR_166559	Ethiopia /MSTR P-19994	NG_068790	Ethiopia /MSTR P-19994
T. glabra	KY364024	Germany/MSTR P-19995	KY364067	Germany/MSTR P-19995
T. striispora	JQ836556	Argentina/BCCM/ MUCL 41297	JQ836560	Argentina/BCCM/MUCL 41297
T. indica	MN385976	India/BPI 571761	MN386004	India/BPI 571761
T. pallidisetosa	OP747472	Spain/ALV25098	OP730557	Spain/ALV25098
T. tophiseda	OP747482	Portugal/ALV36260	OP730566	Portugal/ALV36260
Geopora cercocarpi	NR_121491	USA/ SOC1590	HQ283091	USA/ SOC1590

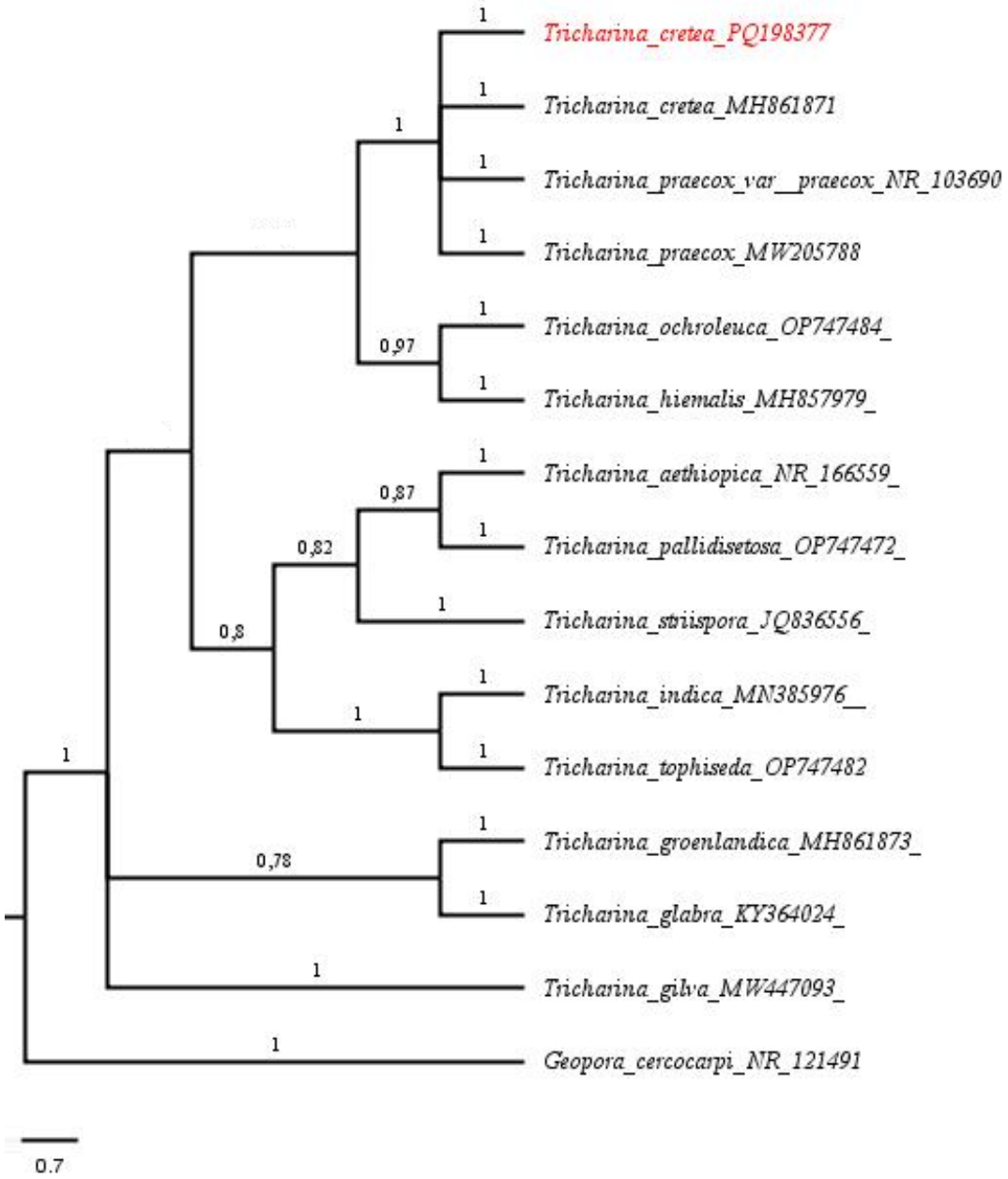

Figure 2. Majority Rule Consensus tree of ITS region conducted by Bayesian analysis. Posterior probability are indicated above the nodes. The specimen of the current study, *Tricharina cretea,* is given in red.

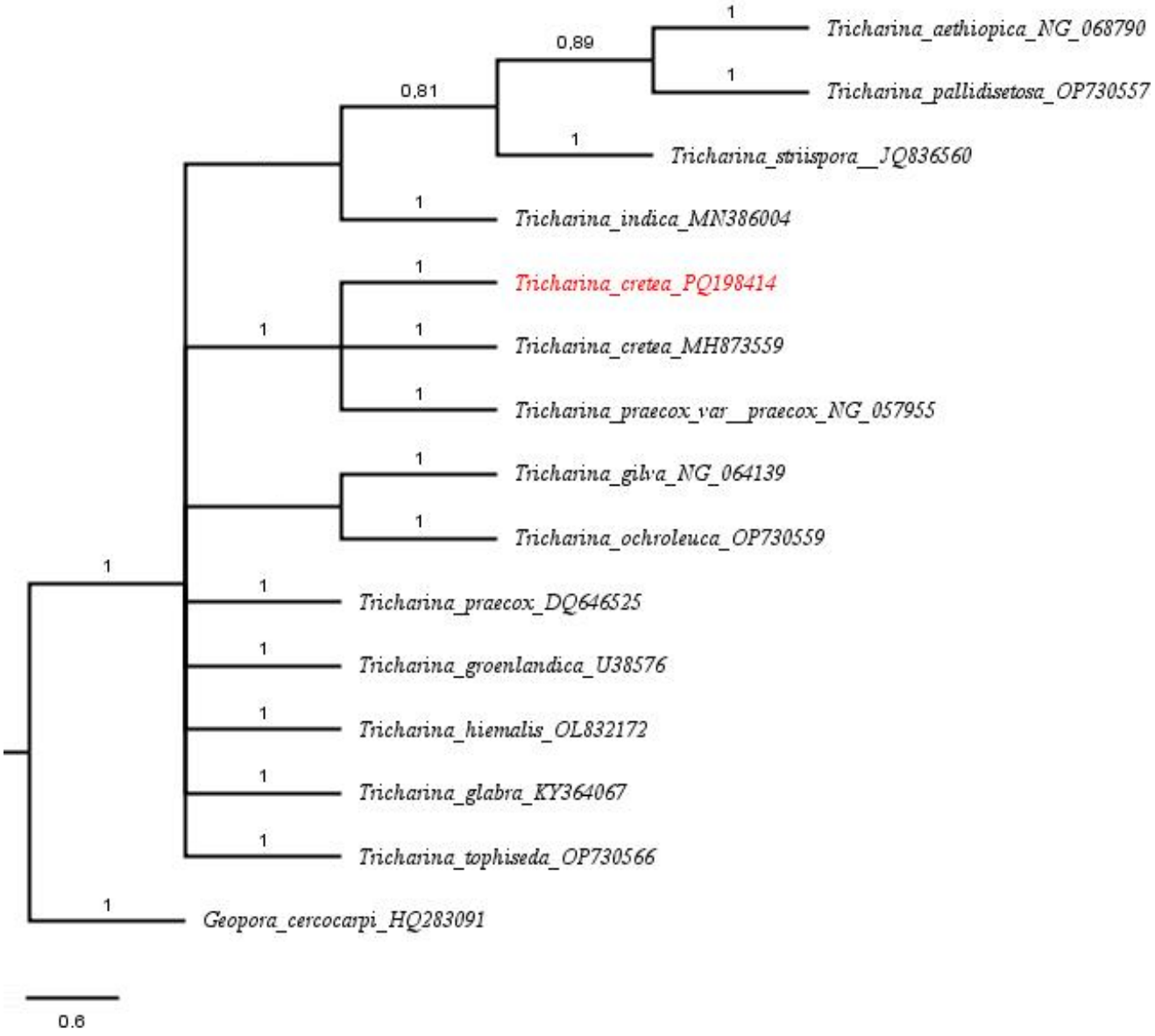

Figure 3. Majority Rule Consensus tree of LSU region conducted by Bayesian analysis. Posterior probability are indicated above the nodes. The specimen of the current study, *Tricharina cretea,* is given in red.

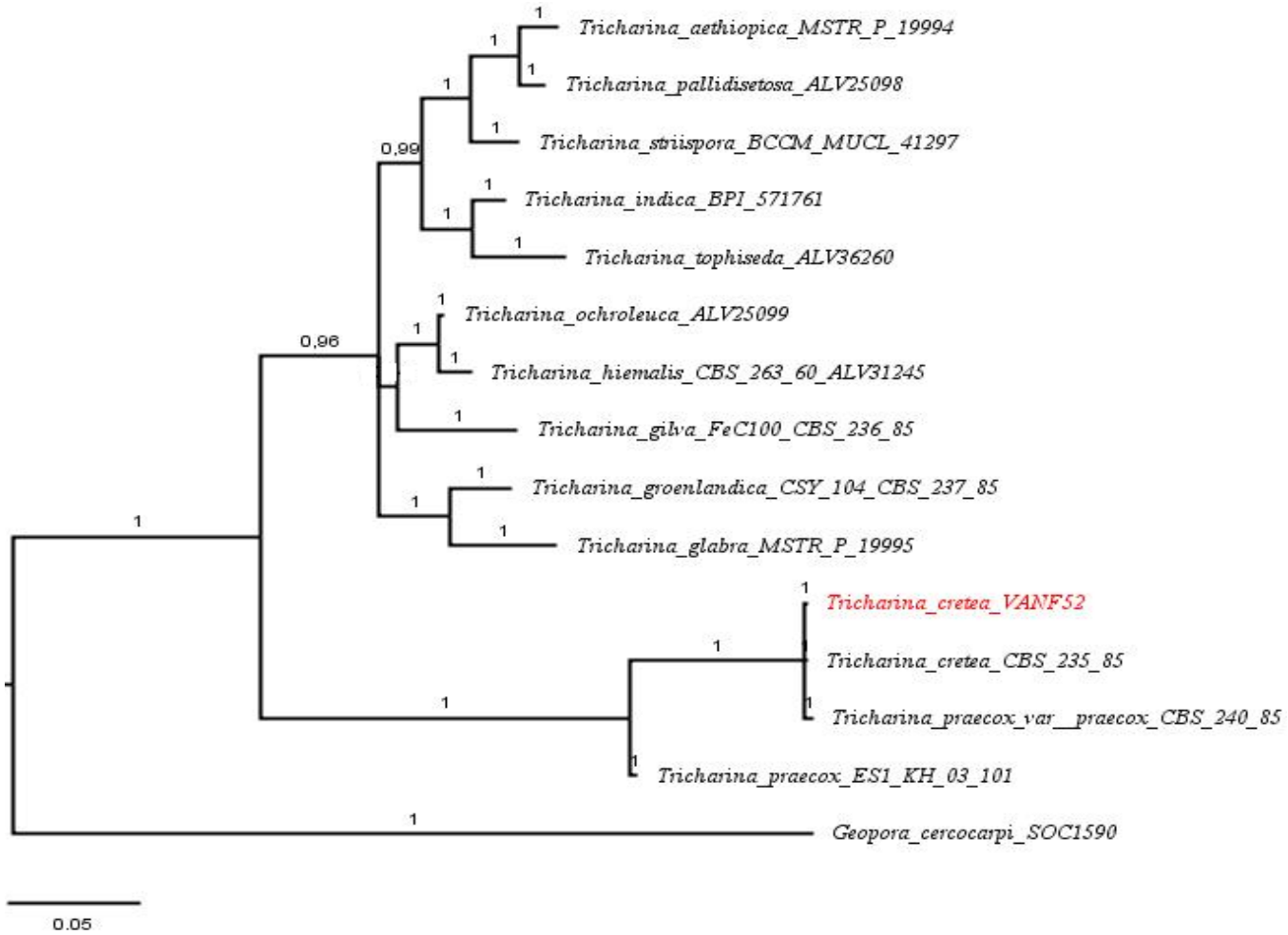

Figure 4. Majority Rule Consensus tree of concatenated (ITS+LSU) data conducted by Bayesian analysis. Posterior probability are indicated above the nodes. The specimen of the current study, *Tricharina cretea*, is given in red.

Discussions

Tricharina is a complex genus characterized by morphological similarities that often cause difficulties in species identification (Van Vooren et al., 2017). This study not only indicates the morphological characteristics that distinguish *T. cretea*, such as its shallow disc-shaped apothecia and distinctive microscopic features, but also reinforces the utility of molecular techniques to resolve taxonomic ambiguities within closely related species.

Phylogenetic analysis using Bayesian inference revealed that *Tricharina cretea* is closely related to *T. praecox* and *T. praecox* var. *praecox.* Morphologically, *Tricharina cretea* exhibits unique features such as its shallow disc-shaped apothecia, which change from dirty-white to beige in older specimens, and its characteristic surface adorned with short brownish hairs. The detailed description of the excipulum and asci reinforces the necessity for careful morphological examination when classifying fungi in this genus. The concatenated data set (ITS+LSU) confirmed the importance of comprehensive molecular approaches by increasing phylogenetic resolution. The tree constructed based on concatenated data supports the validity of *T. cretea* as a distinct species and helps to clarify the evolutionary relationships within the genus.

Identification of *Tricharina cretea* expands the known diversity of the genus in Türkiye, and the total number of recognized *Tricharina* species has increased from three to four with this addition. This study also emphasizes the importance of combining morphological and molecular approaches to figure out the complexities of fungal diversity.

Author contributions

The authors have equal contribution.

Conflicts of interest

The authors declare no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Şuheda ALDEMİR TERMAN, Mustafa Emre AKÇAY, Ayten DİZKIRICI)

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