



New locality records of *Trichoglossum hirsutum* (Geoglossales: Geoglossaceae) based on molecular analyses, and prediction of its potential distribution in Turkey

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Kaygusuz O, Eser Y, Çolak ÖF 2020 – New locality records of *Trichoglossum hirsutum* (Geoglossales: Geoglossaceae) based on molecular analyses, and prediction of its potential distribution in Turkey. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 10(1), 443–456, Doi 10.5943/cream/10/1/34

Abstract

Trichoglossum hirsutum, a rare and noteworthy species in the genus *Trichoglossum*, has been previously collected only from Black Sea Region in Turkey. This work presents new findings on distribution of four specimens identified as *T. hirsutum*, the second record from Aegean and Mediterranean Region of Turkey, using morphology and nrITS sequence analysis. In addition, *T. hirsutum* was identified within forest dominated by the relict endemic *Liquidambar orientalis*, the first reported association with this host. Detailed morphological description, macro photographs and drawings of micro structures of the species studied have been presented. The potential distribution map for *T. hirsutum* was produced by maximum entropy (MaxEnt) analyses. Twenty environmental variables were used to model its distribution and potential habitat. The relative importance of the environmental variables for modeling was evaluated using the Jackknife test. Model performance was evaluated using repeated 90/10 training/test data partitions using the area under curve (AUC). The obtained model was strongly supported by measurements. Our study found that solar lighting index, diurnal temperature range and ruggedness played a key role in the distribution of *T. hirsutum*.

Key words – Ascomycota – biogeographic distribution – fungal diversity – MaxEnt – Mediterranean fungi – phylogeny

Introduction

Fungi are the second largest group of eukaryotic organisms within the world, with about one hundred thousand species described (Kirk et al. 2008). The total number of species is expected to be between 1.5 and 5.1 million (Hawksworth 1991, 2001, O'Brien et al. 2005, Blackwell 2011). According to various studies, the Ascomycetes constitute about half of all fungal species (Bass & Richards 2011). Environmental conditions, especially temperature, precipitation, and geographic location, are the main known exogenous factors which influence the production of fruit bodies of fungi (Ammirati et al. 1985, Eveling et al. 1990, Kausserud et al. 2008).

It is estimated that the global temperature rise in the next century will be in the range of 1 to 5°C (Bidartondo et al. 2018). It is foreseen that this larger-than-average increase in temperature will cause changes in Subarctic (boreal) and Arctic ecosystems, increase hot days, decrease cold days, change precipitation and snowfall patterns and increase temperatures in temperate mountain zones (Bidartondo et al. 2018, Mucha et al. 2018). This climate change will result in changes in the timing of phenological events in many organisms including fungi (Kausserud et al. 2010).

The genus *Trichoglossum* Boud. in the family Geoglossaceae Corda was originally described by Boudier (Boudier 1885), and contains twenty species, commonly known as “dark earth tongues” (Schoch et al. 2009, Ekanayaka et al. 2017, Wijayawardene et al. 2017). *Trichoglossum hirsutum* (Pers.) Boud. has worldwide distribution and occurs in almost all climatic zones (Korf 1981, Mráz 1997, Douanla-Meli & Langer 2005). However, it is listed as critically endangered species for Czech Republic, Slovakia (Antonín et al. 1995, Kučera et al. 2008) and Poland (Wojewoda & Lawrynowicz 1992), as endangered for Austria (Krisai 1986), Finland (Anonymous 1987), Netherlands (Arnolds 1989), Germany (Benkert 1992), and Lithuania (Anonymous 2020). It has been selected as a woodland indicator species for Sweden and a grassland indicator species for Ireland (Kučera et al. 2008).

Trichoglossum species are characterized by black or dark brown club-shaped apothecia, a smooth or velvety stipe, hymenial setae, apically curved or coiled and paraphyses with septa, inoperculate and amyloid asci, and straight, fusoid, dark ascospores typically with 7–15 septa (Arora 1986, Hansen & Knudsen 2000, Ekanayaka et al. 2017). *Trichoglossum* species grow on grassy, forested or swampy ground and are known to be saprobics in the soil (Hansen & Knudsen 2000, Kučera et al. 2008, Sesli & Denchev 2008, Hladki & Romero 2009, Akata & Kaya 2013, Solak et al. 2015, Ekanayaka et al. 2017, Hubregtse 2019).

Trichoglossum hirsutum was previously recorded from only Trabzon province from Black Sea Region in Turkey (Akata & Kaya 2013). Our collections of *T. hirsutum* are given from Burdur and Muğla (Mediterranean Region), and Denizli (Aegean Region) provinces which are new locality records of this fungi. The main purpose of this study is to provide up-to-date information on rare and noteworthy *T. hirsutum*, to present its molecular phylogenetic tree with the first sequences data of new collections and to predict and illustrate its potential distribution in Turkey.

Materials & Methods

Morphological studies

Trichoglossum hirsutum samples were collected in different geographical areas of Turkey between 2015 and 2019. The photographs of the specimens were taken in their natural habitats. The macroscopic descriptions and images of the ascomata were based on fresh or dried specimens. For micro-morphological analyses, the dried materials were rehydrated in 5% KOH, or stained with Melzer’s reagent or Congo red. A total of 40 mature ascospores from each ascoma were measured. The collected specimens have been deposited at the fungarium of Isparta University of Applied Sciences (in GUL).

DNA extraction, PCR and sequencing

DNA was isolated from dried specimens using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, Irvine, CA, USA). Methods for DNA extraction, PCR and sequencing were the same as those outlined in Kaygusuz et al. (2019). PCR and sequencing were carried out to obtain sequences of internal transcribed spacer (ITS) region from nuc-rDNA using the primer pair ITS1-F and ITS4 (White et al. 1990, Gardes & Bruns 1993). Products were sequenced using Sanger DNA sequencing service Source Bioscience (Berlin, Germany). Resulting sequencing products were processed, edited and assembled using Chromas Lite 2.1.1 (<http://technelysium.com.au/wp/chromas/>) and BioEdit 7.2.5 (Hall 1999). All ITS sequences have been deposited in GenBank (accessions MF228807, MT041772, MT041773 and MT041774).

Phylogenetic analyses

Four new sequences of nrITS were generated and additional thirty-one sequences used in phylogenetic analysis were downloaded from the GenBank and UNITE (Nilsson et al. 2018) databases. Sequences were aligned automatically using MAFFT version 7.110 (Kato & Standley 2013), and final alignments were corrected via BioEdit and MEGA X v.10.0.5 (Kumar et al. 2018). Phylogenetic analyses were performed for the ITS dataset by both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML analysis was run in the Cipres Science Gateway v.3.3 interface (<http://www.phylo.org/portal2/>) (Miller et al. 2010) by RAxML v.8.2.10 (Stamatakis 2014). The BI was undertaken using MrBayes version 3.2.2 (Ronquist et al. 2012). The phylogenetic tree was presented with FigTree v.1.4.3 (Rambaut 2016).

Environmental variables and data matrix

The ASTER Global Digital Elevation Model Version 3 (GDEM v3) data to be used to create the Digital Elevation Model (DEM) of the area was obtained from NASA Earthdata service (<http://earthdata.nasa.gov/>). Slope and aspect maps were obtained primarily from DEM created using ArcMap v.10.2 software (Mert & Kıraç 2017). The topographic position index, landform index, topographic wetness index, roughness index, ruggedness index, shading index, solar lighting index and solar radiation index of the area were created using the topography tools plugin prepared by Jenness (2006). The topographic position index (TPI) is calculated to measure topographic slope positions and to automate landform classifications. Landform index divides the area into ten different landform classes, such as canyons, mid-slope drainages, local ridges, U-shaped valleys, plains, foot slopes, upper slopes, upland drainages, mid-slope ridges and mountain tops (Tagil & Jenness 2008). Topographic wetness index (TWI) is applied to quantify topographic control on hydrological processes (Pourghasemi et al. 2012, 2013). The roughness index classifies the area in seven different ways according to its slope percentage (lowland 0%), near flat (1-3%), slightly rough (3-5%), moderately rough (5-10%), rough (10-20%), quite rough (20-50%) and extremely rough (> 50%). The ruggedness index gives the ratio of the steepness of the slope. The shading index shows which areas and for how long will remain in the shade due to the structure of the land. The solar lighting index calculates which parts of the land will benefit from the sun at 8 different hours (06:00-08:00-10:00-12:00/midday-14:00-16:00-18:00-20:00-total) of the day according to the angle of incidence of the sun (Table 1). The solar radiation index calculates the hours of sunshine in the areas and how much this heat will be retained according to the slope, aspect and altitude.

Table 1 Code names and description used for the solar lighting variables.

Abbreviation	Description
6 am	Solar lighting index 06:00
8 am	Solar lighting index 08:00
10 am	Solar lighting index 10:00
midday	Solar lighting index 12:00
2 pm	Solar lighting index 14:00
4 pm	Solar lighting index 16:00
6 pm	Solar lighting index 18:00
8 pm	Solar lighting index 20:00
Total	Total solar lighting index

Bioclimatic data were obtained from the WorldClim database (Hijmans et al. 2005) that provides a set of global climate layers for nineteen bioclimatic variables (www.worldclim.org, Table 2).

Predictive analysis with MaxEnt

Environmental variables and bioclimatic data showing high correlation with each other were

evaluated by Pearson's correlation coefficient ($r^2 > 0.85$) and factor analysis (Hinkle et al. 1994). With these analyses, the variables that best represent each other were determined and the multiple connection problem that could arise was prevented. The predictive modeling of the geographical distribution of *T. hirsutum* was analyzed by the maximum entropy model (MaxEnt software v.3.3.3k.) using thirty-two environmental variables and five locality data from *T. hirsutum* samples collected in this study (Phillips et al. 2006, Mert & Kıraç 2017). In MaxEnt, presences were split into training (90%) and test (10%) datasets, a process that was repeated to build 10 new partitioned datasets (Mert & Kıraç 2017). In order to determine the performance measures of the models obtained as a result of all these analyses, the receiver-operating characteristic (ROC) curve and area under the curve (AUC) values of the models were assessed (Phillips et al. 2004, Elith et al. 2006). AUC values are evaluated as very good discrimination test if they are close to 1, good test if close to 0.7, and informative or chance test if close to 0.5 (Swets 1988, Baldwin 2009).

The jackknife analysis was used in questioning and evaluating the bioclimatic variables affecting the models (Krebs 1999, Phillips 2010). As a result of this analysis, the effect of environmental variables on the potential distribution models of the species and the relationship states are defined.

Table 2 Code names and description used for the nineteen bioclimatic variables.

Abbreviation	Description
Bio1	Annual mean temperature (°C)
Bio2	Mean diurnal range (Mean of monthly (max temp – min temp)) (°C)
Bio3	Isothermality (Bio2/Bio7 * 100)
Bio4	Temperature seasonality (Standard deviation * 100)
Bio5	Maximum temperature of warmest month (°C)
Bio6	Minimum temperature of coldest month (°C)
Bio7	Temperature annual range (°C) (Bio5 – Bio6)
Bio8	Mean temperature of wettest quarter (°C)
Bio9	Mean temperature of driest quarter (°C)
Bio10	Mean temperature of warmest quarter (°C)
Bio11	Mean temperature of coldest quarter (°C)
Bio12	Annual precipitation (mm)
Bio13	Precipitation of wettest month (mm)
Bio14	Precipitation of driest month (mm)
Bio15	Precipitation seasonality (Coefficient of variation)
Bio16	Precipitation of wettest quarter (mm)
Bio17	Precipitation of driest quarter (mm)
Bio18	Precipitation of warmest quarter (mm)
Bio19	Precipitation of coldest quarter (mm)

Results

Molecular phylogeny

Our phylogenetic analysis consisted of thirty-five total nucleotide sequences, containing 1256 nucleotide sites. The resulting phylogram with the lowest BIC (Bayesian Information Criterion) value (–13618.13) and highest log likelihood (–6569.26) are presented. We selected the topology resulting from the first iteration to present here (Fig. 1, –lnL = 6457.78). The phylograms obtained by both methods (BI and ML) showed similar topology. Therefore, only the ML phylogenetic tree with both Maximum likelihood bootstrap (MLB) values and Bayesian posterior probability (BPP) have been shown in Fig. 1.

In the ML phylogram of *Trichoglossum* two major clades can be recognized (Fig. 1): (1) clade 1 (MLB = 100%, BPP = 1.0) formed by *T. walteri* (Berk.) E.J. Durand and *T. farlowii* (Cooke) E.J. Durand, and (2) clade 2 (MLB = 75%, BPP = 0.95) composed of *T. hirsutum* (Pers.) Boud., *T. octopartitum* Mains, *T. rasum* Pat., *T. septatum* Ekanayaka, Q. Zhao & K.D. Hyde, *T.*

variabile (E.J. Durand) Nannf. and provisionally undescribed taxa (*Trichoglossum* sp., Uncultured Ascomycota, Envir Fungi). In the evolutionary tree, clade 1 grouped with strong support (MLB = 100%, BPP = 1.0) and contained *T. walteri* and *T. farlowii*, sister to clade 2. *T. hirsutum* grouped in clade 2 with collections from Turkey, Spain, Estonia, England and USA, forming a well-supported branch (MLB = 75-77%, BPP = 0.88-0.95). In the phylogenetic analysis *T. hirsutum* is placed as sister to all other members within the clade 2 (Fig. 1).

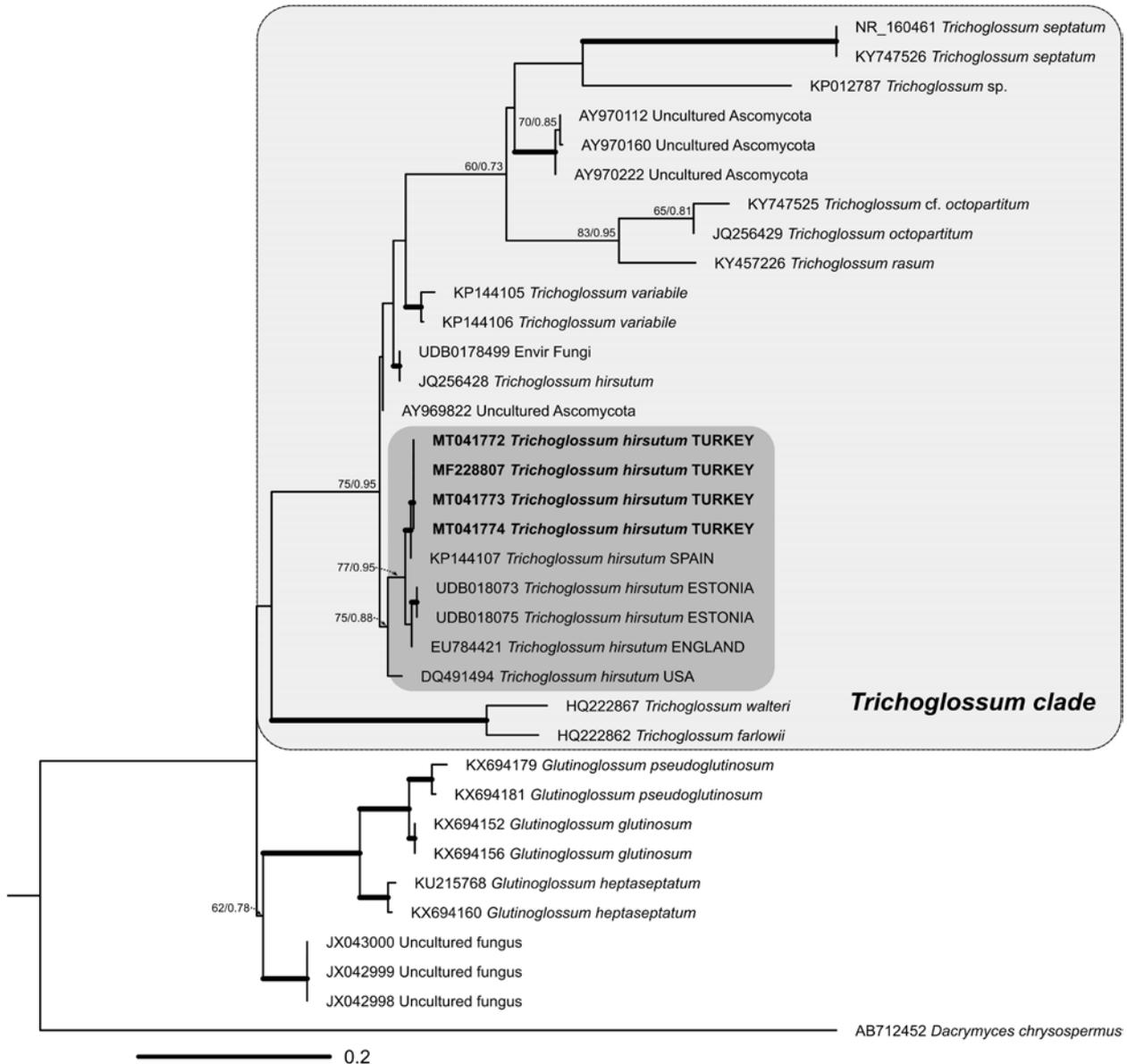


Fig. 1 – Phylogenetic tree obtained from the maximum likelihood analysis of the ITS-rDNA dataset. *Dacrymyces chrysospermus* (AB712452) was used as outgroup. Support values (Maximum likelihood bootstrap - MLB \geq 60% / Bayesian posterior probability - BPP \geq 0.70) are presented above individual branches. The branches are bold when MLB \geq 90% and BPP \geq 0.95. Sequences generated from Turkey are indicated in bold.

Taxonomy

Trichoglossum hirsutum (Pers.) Boud., Hist. Class. Discom. Eur. (1907: 86)

Facesoffungi number: FoF 06142

Basionym – *Geoglossum hirsutum* Pers., Commentatio de Fungis Clavaeformibus: 37. 1797.

Synonyms – *Clavaria atra* J.F. Gmel., Systema Naturae 2(2): 1443. 1792.

Fig. 2

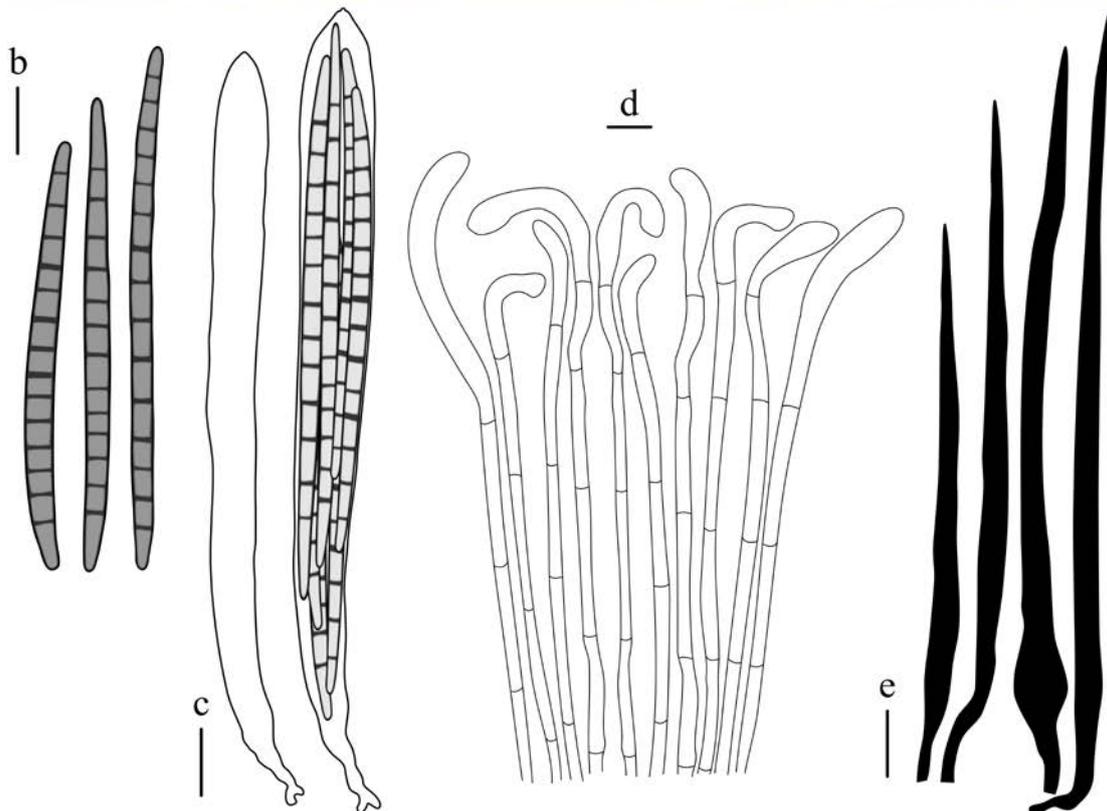


Fig. 2 – *Trichoglossum hirsutum* (OKA2110). a Apothecia. b Ascospores. c Asci. d Paraphyses. e Hymenial setae. Scala bars: a = 20 mm; b, c, e = 20 μ m; d = 10 μ m.

Description – Ascocarp (55–)65–80(–90) mm high, an inflated head that tapers into a thin stipe, typically clavate, very often spatulate; fertile part 25–35 \times 5–8 mm, clavate to lanceolate or fusiform, often compressed or flattened, usually vertically grooved, irregularly twisted and furrowed, black to blackish brown; surface dry, densely setose, minutely velvety. Stipe up to 50 mm tall and about 3 mm thick, cylindrical, tapering towards the base, sometimes slightly

compressed, thin, terete; surface densely setose and velvety, rough; dark brown to black. Context tough and brown, not glutinous. Smell and taste indistinct. Spore print dark black.

Ascospores $90\text{--}160 \times 6\text{--}7 \mu\text{m}$, cylindrical to cylindrical-clavate, mostly regularly 13–15 septate, straight or slightly curved, arranged in parallel fashion within the ascus, smooth, non-amyloid, brown. Asci $200\text{--}270 \times 20\text{--}25 \mu\text{m}$, narrowly cylindrical to broadly clavate, narrowed below, apex rounded, with an apical amyloid pore, eight-spored. Paraphyses $5\text{--}7 \mu\text{m}$ in diameter, enlarged and straight to curved to hooked towards the apices, numerous, filiform, hyaline, septate, light brown at the base and darker at the apex. Hymenial setae $170\text{--}265 \times 6\text{--}12 \mu\text{m}$, abundant, needle-like, slender, acuminate, dark brown to black, non-septate.

Specimens examined – TURKEY, Muğla Province, Köyceğiz district, near Döğüşbelen town, under or in the close vicinity of *Liquidambar orientalis* Miller, on wet soil among mosses, elev. 15 m, 22.03.2015, coll. and det. by O. Kaygusuz & Ö.F. Çolak, OKA2110 in GUL (nrITS = MF228807, OKA-110); Denizli Province, Acıpayam district, near Gölcük village, under of *Pinus nigra* Arn in calcareous grassland, elev. 570 m, 24.04.2016, coll. and det. by O. Kaygusuz, OKA-TR-112 in GUL (nrITS = MT041772, OKA-112); Muğla Province, Fethiye district, near Yanıklar town, in the close vicinity of *L. orientalis*, elev. 10 m, 20.03.2019, coll. and det. by O. Kaygusuz, OKA-TR-114 in GUL (nrITS = MT041774, OKA-114); Burdur Province, Bucak district, near Kargı village, under or in the close vicinity of *L. orientalis*, on wet soil among mosses, elev. 112 m, 09.04.2019, coll. and det. by O. Kaygusuz, OKA-TR-113 in GUL (nrITS = MT041773, OKA-113).

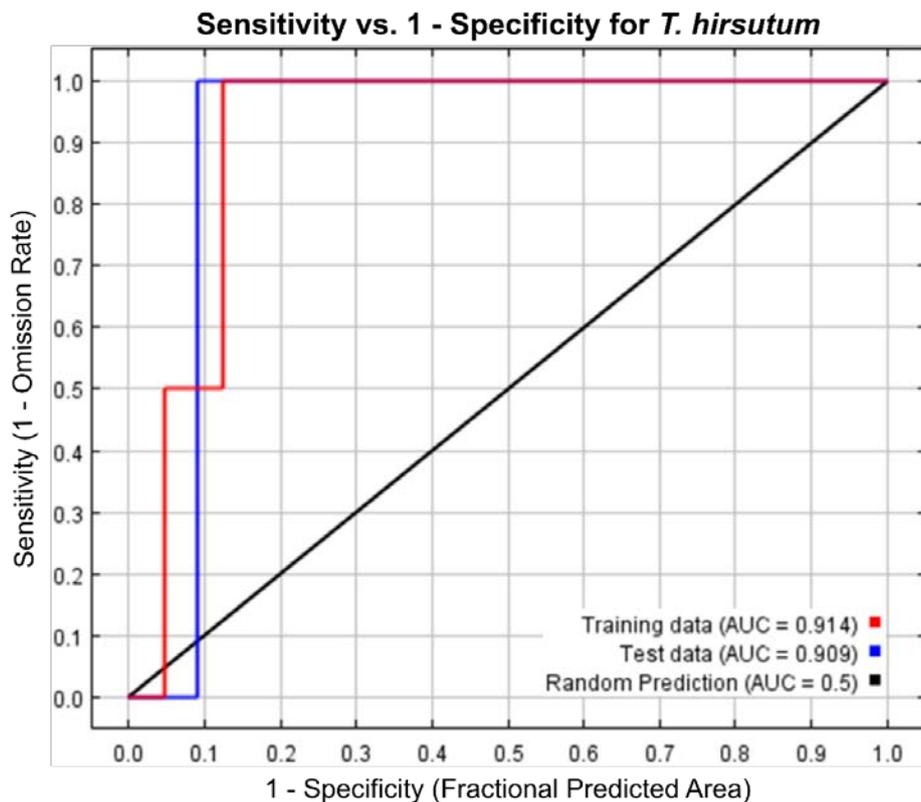


Fig. 3 – ROC curve of Sensitivity versus Specificity for the potential distribution model performance of *Trichoglossum hirsutum*.

Ecology – Mainly present at under 600 m of elevation, fruiting body isolated solitary or gregarious, on the ground amongst moss or grassland, in places continuously damp and slightly dark, in deciduous or coniferous forests, mostly on soils that are rich in humus, clayey-loamy, low in lime, fruiting in warm periods between late March and early April. Reported under or in the close vicinity of *Liquidambar orientalis* and *Pinus nigra*.

Potential Distribution

As a result of statistical analysis, 32 of 41 environmental variables were used in modeling studies. Environmental variables such as solar lighting (6 am, 8 am, 10 am, midday, 2 pm, 4 pm, 6 pm, 8 pm and total solar lighting), bioclimatic (Bio1 – Bio19), and other environmental variables (aspect, aspect suitability index, topographic wetness index, slope, roughness index, ruggedness index, shading index, radiation index, temperature index, solar radiation index, topographic position index, elevation, landform index) were used in statistical analysis. As a result of the analysis, the training data AUC value was 0.914 and the test data AUC value was 0.909 (Fig. 3). According to this result, it is seen that the model has a rather close value to very good model success.

In addition, the environmental variables affecting the potential distribution of the *T. hirsutum* were determined to be 8 pm, Bio2 (Mean Diurnal Range - mean of monthly (max temp – min temp °C) and ruggedness (Fig. 4).

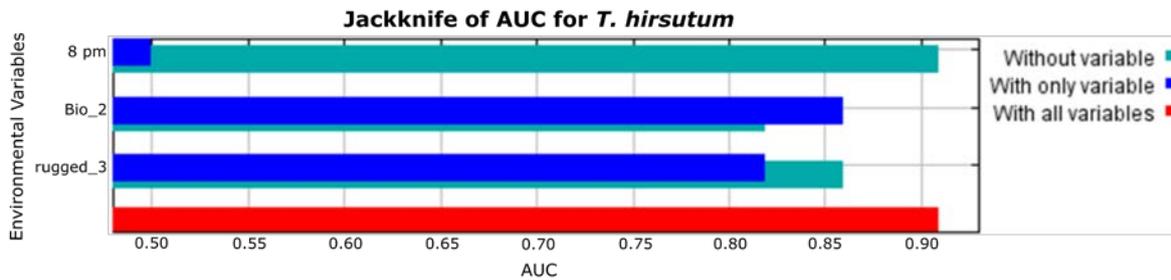


Fig. 4 – Jackknife analysis of relative importance of predictor variables for *Trichoglossum hirsutum*. Dark blue bars point out the gain achieved in the jackknife results of models when including only that variable and excluding the remaining variables. Green bars show how much the gain is diminished without the given predictor variable. Red bar indicates the gain achieved when including all predictors.

As a result, these environmental variables that affect the potential distribution of *T. hirsutum* have shaped the potential distribution map we want to create within the scope of the present study (Fig. 5). The areas shown in red on the map are current and potential areas where *T. hirsutum* is most likely to exist. The areas shown in blue represent areas that are not suitable for the species or that the species does not prefer.

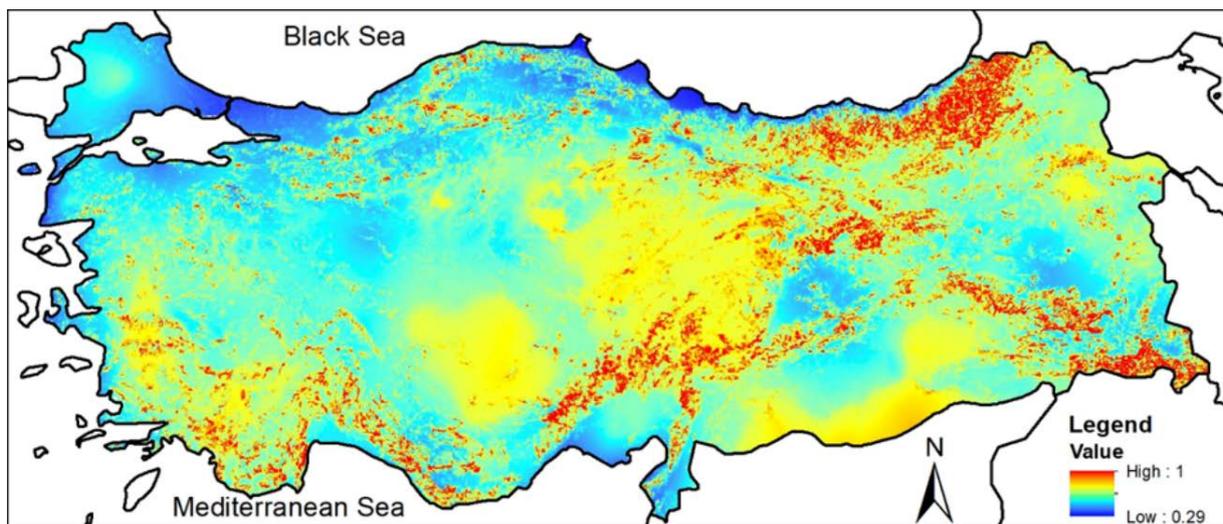


Fig. 5 – Map showing predicted potential distribution/habitat suitability for *Trichoglossum hirsutum*, as analyzed by the maximum entropy model (MaxEnt). The different colours is depicted

the number of predicted probability of presence models, with values ranging from 0 to 1. Using the MaxEnt logistic output, the red colours indicates areas with a higher probability of occurrence while the blue colours represents lower probabilities of occurrence.

Discussion

The darker-spored species of *Trichoglossum* and *Geoglossum* Pers. are morphologically very similar to each other. However, while members of *Trichoglossum* have short hairs covering the surface of the fruiting body, *Geoglossum* species have a smooth surface. In addition, *Trichoglossum* species can be easily distinguished from *Geoglossum* species by microscopic features like length of ascospores and number of septa, the number of spores on each ascus, and the shape of the paraphyses.

Trichoglossum hirsutum is morphologically closely related to *T. variabile*, *T. octopartitum*, *T. septatum*, *T. farlowii* and *T. walteri*. However, *T. hirsutum* differs from these species in ascus and ascospore sizes, number of septa in ascospores, paraphyses width, setae, and ascomata sizes (Table 3). As shown in Fig. 1, *T. hirsutum* is phylogenetically closely related to but distinct from *T. variabile* and *T. octopartitum* based on the ITS data. According to the present phylogenetic tree, *T. hirsutum* separates from *T. farlowii* and *T. walteri* into a different clade recovered by the analyses of the ITS data set. Morphologically, *T. farlowii* and *T. walteri* differ from all the other species in the genus by having light brown ascospores (Ekanayaka et al. 2017). Phylogenetically, the result that the Turkish collections of *T. hirsutum* clustered with five collections of *T. hirsutum* from Spain, Estonia, England and USA as a whole gets strong statistical support, 75% of bootstrap and 0.88 bayesian PP support respectively. In the phylogenetic analysis *T. hirsutum* is placed as sister to all other members within the clade 2. The results of a first molecular phylogenetic analysis of *T. hirsutum* from Turkey are in agreement with Hustad et al. (2013), Ekanayaka et al. (2017) and Prabhugaonkar & Pratibha (2017).

Trichoglossum species grow on grassy, forested or swampy ground in North and Central America, Europe, Asia, Australia, India, South America and New Zealand and are known to be saprobics in the soil (Hansen & Knudsen 2000, Kučera et al. 2008, Sesli & Denchev 2008, Hladki & Romero 2009, Akata & Kaya 2013, Arouzo & Iglesias 2014, Solak et al. 2015, Ekanayaka et al. 2017, Hubregtse 2019). In particular, *T. hirsutum* grows in deciduous or coniferous forests, typically on very rotten wood or soil and is associated with wet to marshy meadows, peat-bogs and moors, often amongst *Molinia caerulea* (L.) Moench, *Sphagnum flexuosum* Dozy & Molk., *Sphagnum* sp. (Breitenbach & Kränzlin 1984, Antonín et al. 1995, Mráz 1997, Hansen & Knudsen 2000, Kučera et al. 2008, Hladki & Romero 2009, Akata & Kaya 2013, Arouzo & Iglesias 2014, Ekanayaka et al. 2017). The relict endemic *Liquidambar orientalis* is stated here for the first time as a host of *T. hirsutum*.

Table 3 Comparison of morphological characteristics of some *Trichoglossum* species (L = length, W = width) (¹Kučera et al. 2010, ²Beug et al. 2014, ³Ekanayaka et al. 2017, ⁴Prabhugaonkar & Pratibha 2017, ⁵This study).

Taxon	Ascomata size (L × W mm)	Setae size (L × W μm)	Paraphyses size (W μm)	Asci size (L × W μm)	Ascospores size (L × W μm)	Number of septa in ascospores
<i>T. farlowii</i> ^{2,3,4}	30–80 × 3–15	-	2–3	150–180 × 15–20	30–90 × 4.5–10	3–5
<i>T. hirsutum</i> ⁵	15–90 × 5–8	170–260 × 6–12	5–7	200–270 × 20–25	90–160 × 6–7	13–15
<i>T. octopartitum</i> ^{1,4}	10–45 × 1–6	75–256 × 5–10	2–4	175–200 × 18–20	80–150 × 4–5.5	7
<i>T. variabile</i> ^{2,4}	20–40 × 2–5	79–177 × 5–8	6–8	150–226 × 18–20	80–150 × 4.5–6	4–16
<i>T. walteri</i> ^{2,4}	34 × 2.5	78–200 × 6–10	3–6	120–217 × 18–24	45–108 × 5–8	7

Although many studies have predicted that the performance of species distribution models may be poor when few records are used (Pearce & Ferrier 2000, Kadmon et al. 2003, Hernandez et al. 2006), MaxEnt is reported to provide acceptable power to predicting distributions even with very few records, such as <10 (Wisniewski et al. 2008, Shcheglovitova & Anderson 2013, Yuan et al. 2015). Thus, we used the MaxEnt approach to predict distributions of *T. hirsutum* and obtained a high AUC value (0.914), so that large areas with very good probability of the species' occurrence was predicted.

Modeling results found out the species-environment relationship and the contribution rate of each variable in the model. According to the result of the Jackknife analysis, bioclimatic variables were the most important predictors in *T. hirsutum*, so we analyzed MaxEnt with just the 19 bioclimatic variables. Climate variables, particularly species-specific physiological thresholds of temperature and precipitation tolerance, are reported to be important in determining the distribution of species at regional or large scales (Gaston 1994, Lomba et al. 2010, Yuan et al. 2015). According to the solar lighting variables that shapes the potential distribution map obtained for the *T. hirsutum*, it is concluded that the target samples prefers areas that receive sun only after 8 pm. Light is very important for many physiological and morphological responses in fungi. Fungi can adapt to environmental conditions by detecting ultraviolet, blue, green and red light using up to 11 photoreceptors (Yu & Fischer 2019). In addition, some fungi can sense differences in the color (wavelength) and intensity of the light, as well as determine the direction of the solar lighting (Cohen & Delbrück 1959, Corrochano & Galland 2016, Yu & Fischer 2019). Finally, it has been suggested that the day length is as important as the temperature and precipitation in the formation of the fruiting bodies of the fungi (Cooke 1958). In our study, it was determined that *T. hirsutum* preferred areas that can receive daylight after 8 pm, that is, areas where the length of daylight is maximum. It was also proposed that these areas have low intensity of the solar light, consequently lower temperature and higher moisture in comparison with other solar lighting variables.

In addition, it is observed that other environmental factors affecting the potential distribution of *T. hirsutum* are areas where daily temperature difference increases (Bio2) and areas that low suddenly (roughness). The diurnal temperature range and altitude can indirectly affect the distribution of *T. hirsutum* as it has a direct impact on the climatic conditions of a specific area.

Daily mean temperature is used as an important indicator for climate change study (Qu et al. 2014). However, according to our results, it was determined that the length of daylight after 8 pm was a more effective factor in the distribution of *T. hirsutum* compared to the diurnal temperature range and ruggedness. If global climate change will lead to more extreme climatic conditions in the future, it may affect the potential distribution of *T. hirsutum*.

In conclusion, our survey presents up-to-date information on rare and noteworthy *T. hirsutum* identified from Aegean and Mediterranean Regions, gives important distributional records along with first molecular phylogenetic placement of the species and predicts its future potential distribution in Turkey. In addition, *Liquidambar orientalis* is found as the new host for the *T. hirsutum* discovered here. Our study provides information on the existence of certain rare and important fungi in Turkey, and contribute to future studies on the phylogeny, morphology, taxonomy and potential distribution of the species.

Acknowledgements

The authors would like to acknowledge the financial support of General Directorate of Agricultural Research and Policies, the Ministry of Agriculture and Forestry of the Republic of Turkey (TAGEM) (Project number: TAGEM14 /AR-GE/40).

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