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A New Record for Turkish Mycobiota from Selim (Kars) District

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Abstract: *Hebeloma cylindrosporum* (*Hymenogastraceae*, *Basidiomycota*) from Selim (Kars) district is described as a new record species for Turkish mycota. The species is assigned to the genus *Hebeloma*, section *Scabrispora*. A comprehensive description, photographs, and comparisons with related species based on morphological and phylogenetical features are provided. The phylogenetic position within the genus is provided based on the DNA sequence of nuclear ribosomal internal transcribed spacer (nrITS) region. Phylogenetic analyses show that the species is located within a well-supported section *Scabrispora*.

Key words: *Basidiomycota*, *Hebeloma*, fungal phylogeny, new record.

Selim (Kars) yöresinden Türkiye Mikobiotası için Yeni Bir Kayıt

Öz: *Hebeloma cylindrosporum* (*Hymenogastraceae*, *Basidiomycota*), Türkiye'nin Selim (Kars) ilçesinden Türk mikotası için yeni kayıt tür olarak tanımlanmıştır. Tanımlanan tür, *Hebeloma* cinsine ait *Scabrispora* seksiyonunda yer almaktadır. Detaylı deskripsiyon, fotoğraflar ve morfolojik ve filogenetik karakterlere dayalı olarak yapilan cins icindeki filogenetik pozisyonu verilmiştir. Cins içindeki filogenetik ilişkilerinin belirlenmesi transkribe edici iç aralayıcı (ITS) bölgenin dizisine dayanılarak sağlanmıştır. Yapılan filogenetik analizler türün *Scabrispora* seksiyonunda yer aldığını göstermiştir.

Anahtar Kelimeler: *Basidiomycota*, *Hebeloma*, fungal filogeni, yeni kayıt

Introduction

Hebeloma (Fr.) P. Kumm. *(Hymenogastraceae, Agaricales)* is a genus of ectomycorrhizal fungi that contains 31 species in Turkey (Sesli and et al., 2020) Beker and his colleagues (2016) divided the genus into thirteen sections (*Denudata*, *Hebeloma*, *Sinapizantia*, *Sacchariolentia*, *Velutipes*, *Theobrominum*, *Naviculospora*, *Scabrispora*, *Myxocybe*, *Pseudoamarescens*, *Duracinus*, *Porphyrospora*, *Syrjense*). Among these sections, the section *Scabrispora* is characterized by rooting basidiomes, cylindrical spores, and mostly cylindrical cheilocystidia.

In 2007, some samples of *Hebeloma cylindrosporum* Romagn. were collected from Kars province of Turkey. However, they have not been characterized until 2020. Fortunately, the collected specimens have been preserved in suitable Fungarium conditions. As a result, they were well-preserved to perform scientific analyses on them. For identification of the samples, not only microscopic and macroscopic characters but also molecular data were used. As macroscopic characters;

(pileus, lamellae, and stipe) as microscopic characters; (basidia, spores, pileipellis, hyphae, and cheilocystidia) were utilized. DNA sequences of nuclear ribosomal internal transcribed spacers (nr ITS) region including ITS1, 5.8S, ITS2 sub-regions were also used as molecular characters to determine the phylogenetic relationships and positions of *H. cylindrosporum* within *Hebeloma* genus.

The purpose of the present study is to describe *Hebeloma cylindrosporum* as a new record in Turkey using both morphological and molecular data.

Material and Metod

Taxon sampling and morphological studies

Fungal samples were collected from Selim (Kars) region of Turkey in 2007. During the fieldwork, specimens were photographed using a Canon (EOS 60D) camera equipped with Tokina 100 mm macro lens. Macroscopic characters were recorded from the fresh materials. At least 40 spores, 20 basidia, and cheilocystidia were measured under a Leica DM500 research microscope by using distilled water and Melzer's reagent solution. Measurements were made with Leica Application Suite (version 3.4.0) program and described based on the terminology of Beker et al. (2016). Dried samples were deposited in the Fungarium of Van Yüzüncü Yıl University (VANF).

Molecular studies

Genomic DNA was extracted from the dried basidiomata using the CTAB method (Doyle and Doyle 1987). The purity and quantity of the extracted DNA were determined using NanoDrop2000c UV–Vis Spectrophotometer (Thermo Scientific) and 0.8% agarose gel electrophoresis. DNA amplification was performed in a 25 µl volume mixture containing genomic DNA (10 ng/µl), 10× PCR Buffer, MgCl2 (25 mM), dNTP mixture (10 mM), selected primer pair (10 µM), Taq polymerase (5u/µl) and sterile water. Amplification of ITS region was performed using primer pair N- nc18S10 5'AGGAGAAGTCGTAACAAG3' / C26A 5'GTTTCTTTTCCTCCGCT3' (Wen and Zimmer 1996). After amplification, PCR products were run in a 1 % agarose gel and visualized by staining with Gelred dye. Positive reactions were sequenced with forward and reverse PCR primers using ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

The sequences that were taken from forward and reverse primer were assembled and edited using Alibee Multiple Alignment 3.0 software from the GeneBee website (www.genebee.msu.su/genebee.html). Ambiguous sites were checked manually and corrected. Sequence data of ITS region were deposited in GenBank and accession numbers were added to the manuscript.

Sequence alignment and phylogenetic analysis

Two sequences of *Hebeloma cylindrosporum* generated from the current study and additional sequences retrieved from NCBI software (Appendix 1) were combined and analyzed together to see phylogenetic relationship and position of the studied species within *Hebeloma* genus. *Galerina paludosa* (Fr.) Kühner was chosen as outgroup. All sequences were aligned with ClustalW program (Thompson et al., 1994) and adjusted manually where it was necessary.

Phylogenetic tree was constructed using two different methods; Maximum Likelihood (ML) and Maximum Parsimony (MP). The appropriate model of nucleotide evolution for phylogenetic analyses was determined using MEGA 6.0 and the model with the lowest BIC (Bayesian Information Criterion) score was used to describe the substitution model the best (Tamura *et al*. 2013). Tamura-Nei model (Tamura and Nei, 1993) was used for two analyses. To test branch support, bootstrap analysis was used with 1000 replicates (Felsenstein, 1985). In the ML method, initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then the topology with superior log likelihood value was selected. The Tree-Bisection-Reconnection (TBR) search method was employed with 100 random addition replications to construct the MP trees and the consensus tree inferred from 10 most parsimonious trees was used. All positions containing gaps and missing data were eliminated.

Results

Taxonomy

Basidiomycota R.T. Moore
Agaricomycetes Doweld
Agaricales Underw.
Hymenogastraceae Vittad.
Hebeloma (Fr.) P. Kumm. (TURPKOKAN)
Hebeloma cylindrosporum Romagn. **Figure 1.**

Pileus 10-55 mm; convex to planoconvex, the margin curved inward, sometimes crenulate, eroded, and wavy with age; color at center yellowish-brown to ochraceous to dark brick and color at margin cream to buff or yellowish; when young with a cortina. **Lamellae** adnexed to emarginate; the presence of tears absent or visible; cream color when young, then brown. **Stipe**: 25-80 × 3-8 mm and 4-11 mm at the base; cylindrical, usually clavate

towards the base; floccose fibrillose, pruinose, sometimes with rooting. **Spores**: 7-10.6 × 4-5.5 µm, cylindrical, occasionally ellipsoid; yellow-brown; usually guttulate; dextrinoid, with or without perispore. **Basidia**: 19-28 × 5.5-7.5 µm; cylindrical to clavate; four spored, rarely two spored. **Cheilocystidia**: 18-38 × 3-6.35(apex) × 2.5-5.6(median) × 3-6.8(basal) µm, cylindrical, occasionally clavate-lageniform. **Pileipellis:** ixocutis maximum hyphae width 5.5 µm, cylindrical, ellipsoid, and sausage-shaped (Figure 1).

Specimens examined

Turkey, Kars, Selim, N 40°27'15.76" and E 42°33'13.94", 2137 m, 10.06.2007, gregarious, under *Pinus* and *Picea* sp., Y. Uzun (Selim) VANF7881.

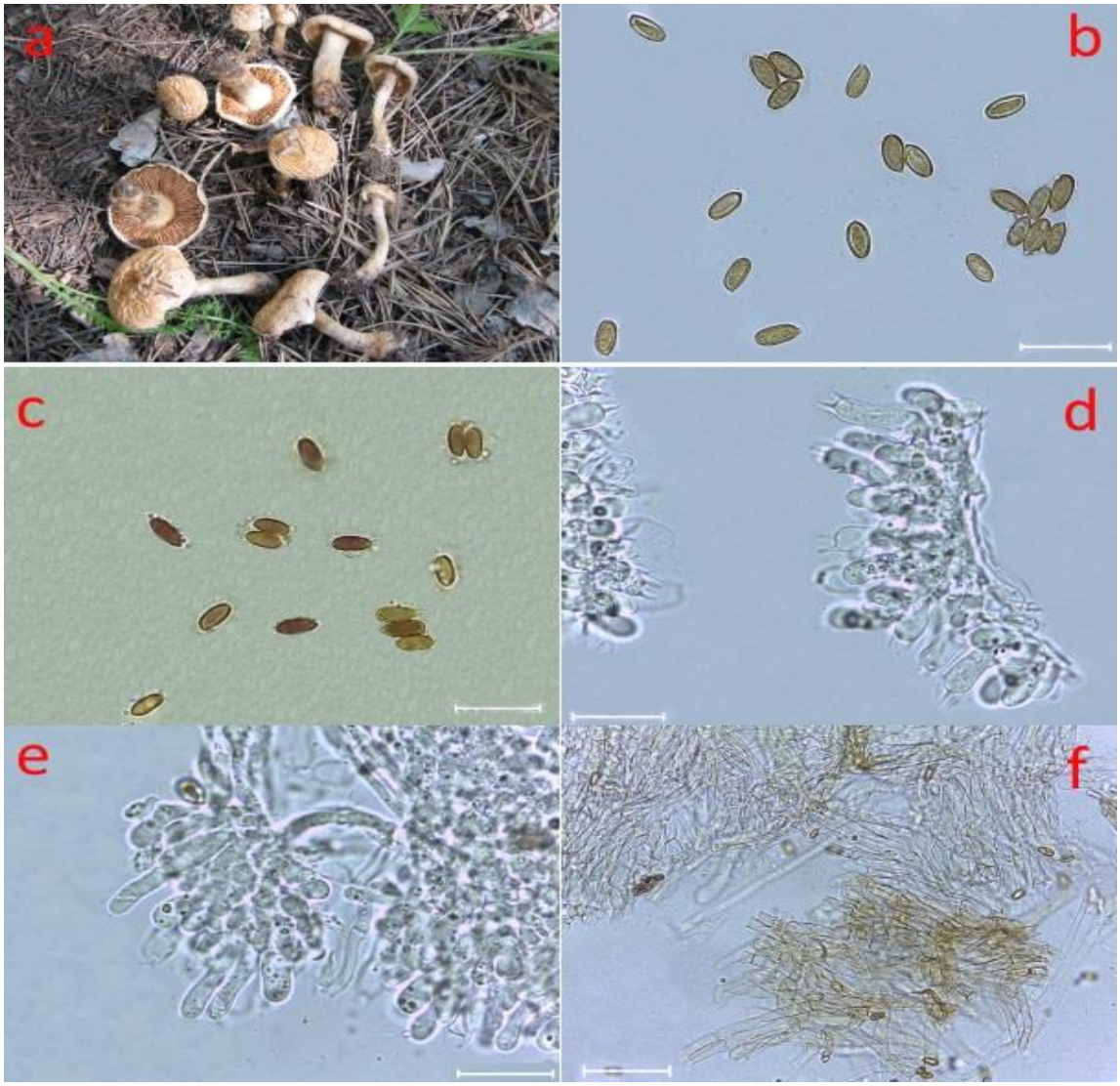

Figure 1. *Hebeloma cylindrosporum* a. Basidiomata b. Spores in distilled water c. Spores in Melzer's reagent d. Basidia e. Cheilocystidia f. Pileipellis.

Molecular analysis

The amplified DNA fragment of the ITS region was approximately 650 bp in length encompassing complete ITS1, 5.8S, and ITS2 subregions. Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997) analysis was performed using the National Center for Biotechnology Information (NCBI) database. The sequence of *Hebeloma cylindrosporum* matched their representatives with 99% identity values.

ITS data matrix comprised a total of 66 sequences including studied two samples and one outgroup sample. The aligned data included a total of 683 positions, of which 520 were conserved, and 147 were variable (77 variable sites in ITS1, 1 in 5.8S, and 70 in ITS2 subregion) nucleotides. Accession numbers of ITS region for studied

two samples were assigned as MW131677 and MW131678, respectively.

The topologies of the MP and ML phylogenetic trees had no considerable differences, so only one tree (ML) was given to indicate phylogenetic relationships and taxonomic position of studied species. Phylogenetic trees constructed based on ITS separated the species at section levels (Figure 2). The phylogenetic relations of ten sections can be observed in the constructed tree. The Scabrispora clade consists of two clusters and all *Hebeloma cylindropsorum* samples (studied and downloaded from NCBI) grouped with a 100% bootstrap value (Figure 2). Even close relationships among *H. cylindropsorum*, *H. birrus* and *H. circinans were seen in the tree, where they can be separated phylogenetically.* Similar separation among mentioned species were also detected when spore morphology was taken into account. *Hebeloma cylindropsorum* has mostly cylindrical and less ornamented spores while the others have amygdaloid spore shape.

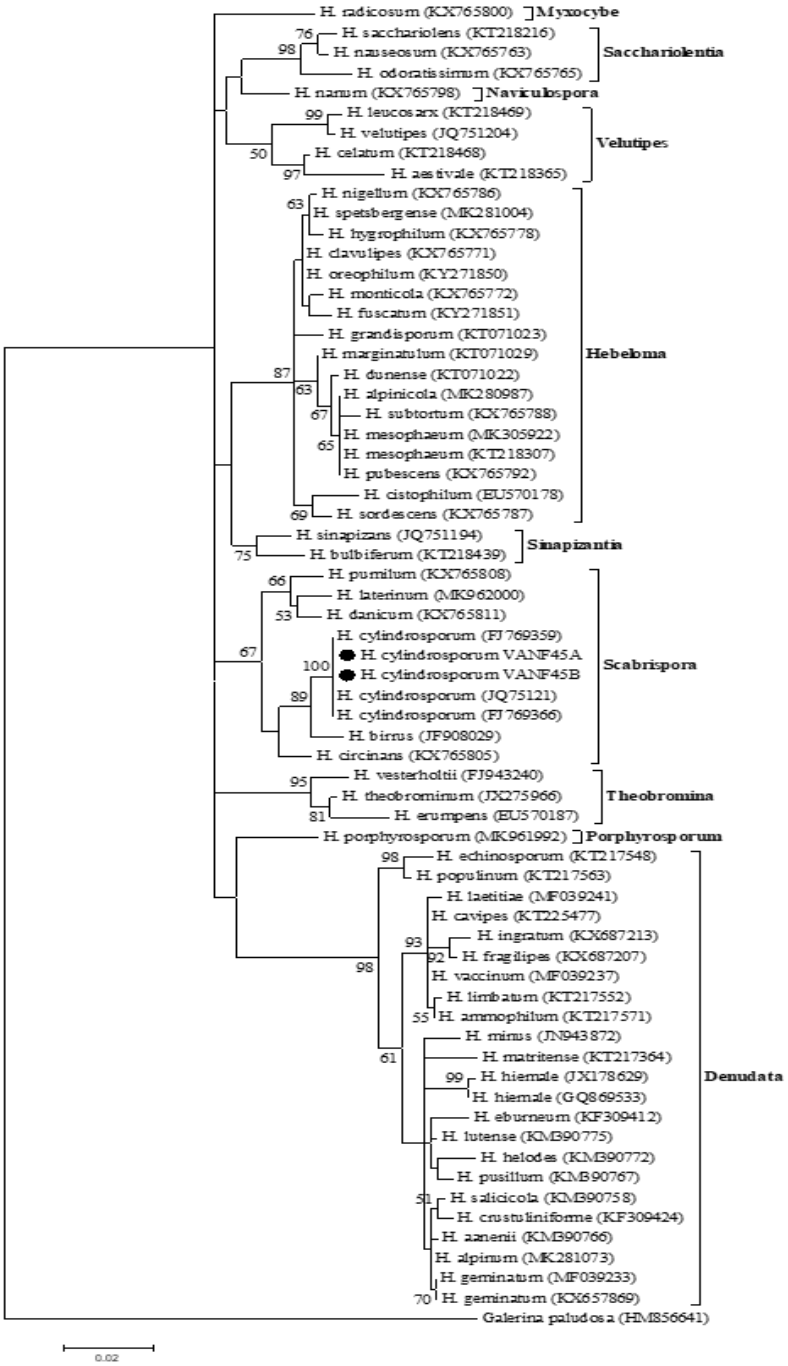

Figure 2. Phylogenetic tree of *Hebeloma* species based on ML analysis of the ITS region. The black circle indicates studied specimens. *Galerina paludosa* was used as an outgroup. Bootstrap values higher than 50% were indicated on the branches.

Discussions

Hebeloma cylindrosporum which belongs to the sect. *Scabrispora* can be distinguished from relative species by its yellow-brown, sticky pileus, thick and buff and tendency rooting stipe, emarginate to adnate lamellae, cylindrically cheilocystidia, dextrinoid mostly cylindrical spores.

The species of *Scabrispora* section are characterized by usually rooting basidiomas, cylindrically shaped spores, and cylindrically most cheilocystidia (Vesterholt 2005; Beker et al. 2016). The studied taxon that carries distinctive characters of section *Scabrispora* was introduced with the current study.

Hebeloma cylindrosporum is a mostly studied fungus in worldwide due to its superior ectomycorrhizal symbiosis feature (Laurans et al. 2001; Marmeisse et al. 2004; Aquino and Plassard, 2004; Doré et al. 2014; Becquer et al. 2018; Khullar and Reddy, 2020). The species is specifically associated with *Pinus*, even though *Quercus*. Reliable description is necessity when economic importance is considered. As observed from the results of the study, both morphological and molecular characters are very useful to describe *Hebeloma* species. The Turkish name of the genus is taken from the book "The Checklist of Fungi of Turkey" (Sesli and et al., 2020).

The current study is providing the morphological and molecular identification of the new record taxon for Turkey and the total number of *Hebeloma* species is increased from 31 to 32 by addition of *H. cylindrosporum*.

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Appendix 1

ITS sequences downloaded from NCBI database

H. aanenii (KM390766), *Hebeloma aestivale* (KT218365), *H. alpinicola* (MK280987), *H. alpinum* (MK281073), *H. ammophilum* (KT217571), *H. birrus* (JF908029), *H. bulbiferum* (KT218439), *H. cavipes* (KT225477), *H. celatum* (KT218468), *H. circinans* (KX765805), *H. cistophilum* (EU570178), *H. clavulipes* (KX765771), *H. crustuliniforme* (KF309424), *H. cylindrosporum* (FJ769359, JQ75121, FJ769366), *H. danicum* (KX765811), *H. dunense* (KT071022), *H. eburneum* (KF309412), *H. echinosporum* (KT217548), *H. erumpens* (EU570187), *H. fragilipes* (KX687207), *H. fuscatum* (KY271851), *H. geminatum* (KX657869, MF039233), *H. grandisporum* (KT071023), *H. helodes* (KM390772), *H. hiemale* (JX178629), *H. hygrophilum* (KX765778), *H. ingratum* (KX687213), *H. laetitiae* (MF039241), *H. laterinum* (MK962000), *H. leucosarx* (KT218469), *H. limbatum* (KT217552), *H. lutense* (KM390775), *H. marginatulum* (KT071029), *H. matritense* (KT217364), *H. mesophaeum* (KT218307, MK305922), *H. minus* (JN943872), *H. monticola* (KX765772), *H. nanum* (KX765798), *H. nauseosum* (KX765763), *H. nigellum* (KX765786), *H. odoratissimum* (KX765765), *H. oreophilum* (KY271850), *H. populinum* (KT217563), *H. porphyrosporum* (MK961992), *H. pubescens* (KX765792), *H. pumilum* (KX765808), *H. pusillum* (KM390767), *H. radicosum* (KX765800), *H. sacchariolens* (KT218216), *H. salicicola* (KM390758), *H. sinapizans* (JQ751194), *H. sordescens* (KX765787), *H. spetsbergense* (MK281004), *H. subtortum* (KX765788), *H. theobrominum* (JX275966), *H. vaccinum* (MF039237), *H. velutipes* (JQ751204), *H. vesterholtii* (FJ943240), *Galerina paludosa* (HM856641).

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