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Morphological and Molecular Characterization of *Hebeloma subtortum* (Hymenogastraceae), a New Record Macrofungus from Bingöl Province, Turkey

Ayten DİZKIRICI[1*], İsmail ACAR[2], Ayşenur KALMER[1], Yusuf UZUN[2]

[1]Department of Molecular Biology and Genetics, Van Yüzüncü Yıl University, 65080, Van, Turkey
[2]Department of Organic Agriculture, Başkale Vocational High School, Van Yüzüncü Yıl University, 65080, Van, Turkey
[3]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Van Yüzüncü Yıl University, 65080, Van, Turkey
*Corresponding author: aytendizkirici@gmail.com
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Abstract

Aim of Study: The specimen identified as *Hebeloma subtortum* based on both morphological and molecular characterizations.

Area of Study: Samples were collected from Bingöl province and the study was conducted at the Department of Molecular Biology and Genetics in Van Yüzüncü Yıl University.

Material and Methods: Characters of pileus, stipe, lamellae and basidia, cystidia, spores were used as macroscopic and microscopic features, respectively. DNA sequences of two loci including the internal transcribed spacer (ITS) region and the large subunit (LSU) of nuclear ribosomal RNA gene were used to show the evolutionary relationship and taxonomic position of the species within *Hebeloma* genus. The DNA sequences of the above-mentioned regions of *H. subtortum* were compared to those of the same and different species of the genus downloaded from NCBI.

Main results: In phylogenetic analyses, *H. subtortum* distinctly clustered with its representatives retrieved from NCBI with high bootstrap value. The ITS tree was more informative compared to LSU. *Hebeloma subtortum* closely grouped with *H. mesophaeum* in the ITS tree.

Research highlights: *Hebeloma subtortum* has been described and illustrated as a new record from Turkey.

Keywords: *Hebeloma*, ITS, mycogenetics, phylogeny

Bingöl ilinden yeni bir kayıt olarak *Hebeloma subtortum* (Hymenogastraceae) türünün morfolojik ve moleküler karakterizasyonu

Öz

Çalışmanın Amacı: *Hebeloma subtortum* türü hem morfolojik hem de moleküler karakterizasyonlara dayanarak tanımlanmıştır.

Çalışma Alanı: Örnekler Bingöl ilinden toplanmış ve çalışma Van Yüzüncü Yıl Üniversitesi Moleküler Biyoloji ve Genetik Bölümü'nde yapılmıştır.

Gereç ve Yöntemler: Şapka, sap, lamel yapıları ve bazidya, sistidya, spor yapıları sırasıyla makroskopik ve mikroskopik özellikler olarak kullanılmıştır. Nükleer ribozomal iç aralayıcı bölge (nrITS) ve ribozomal en büyük alt birim (LSU) olmak üzere iki DNA bölgesi çalışılan türün evrimsel ilişkisini ve *Hebeloma* cinsi içindeki taksonomik konumunu göstermek için kullanılmıştır. Çalışılan türün DNA dizileri, NCBI very tabanından indirilen cinse ait aynı ve farklı türlerle karşılaştırılmıştır.

Ana sonuçlar: Filogenetik analizlerde, *H. subtortum*, yüksek bootstrap değeri ile NCBI'den alınan temsilcileri ile belirgin bir şekilde kümelenmiştir. ITS ağacı, LSU'ya kıyasla daha bilgilendirici olmuştur. *H. subtortum*, ITS ağacında *H. mesophaeum* ile yakın gruplanmıştır.

Araştırma konuları: *Hebeloma subtortum*, Türkiye'den yeni bir kayıt olarak tanımlanmış ve gösterilmiştir.

Anahtar kelimeler: *Hebeloma*, ITS, mikogenetik, filogeni

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Introduction

Hebeloma (Fr.) P. Kumm. (Hymenogastraceae Vittad., Agaricales Underw.) is a genus of ectomycorrhizal fungi comprising approximately 530 worldwide published species names with its main distribution in the temperate zones of the northern hemisphere (Mycobank.org). In the field, *Hebeloma* is easily separated from related genera based on several special characters such as the chocolate colored lamellae, the occasional presence of a fibrillose veil, often scanty, that when broken usually leaves remnants on the margin of the pileus and/or on the stipe and the presence of distinct sterile, often narrow (sometimes fusoid-ventricose) or greatly elongated cells among those along the edges of the gills (the cheilocystidia) (Smith, Evenson & Mitchel,. 1983). Beker, Eberhardt & Vesterholt (2016) divided the genus into thirteen sections (*Denudata*, *Hebeloma*, *Sinapizantia, Sacchariolentia, Velutipes, Theobrominum, Naviculospora, Scabrispora, Myxocybe, Pseudoamarescens, Duracinus, Porphyrospora, Syrjense*) using different characters such as habitat, smell, number of full-length lamellae, presence of cortina, shape of cheilocystidia and dextrinoid reaction of the spore.

In Turkey, 28 species of *Hebeloma* have been reported so far (Sesli and Denchev, 2014; Güngör, Solak, & Kalmış, 2015; Sesli, Contu, Vila, Moreau & Battistin, 2015; Solak, Işıloğlu, Erbil & Allı, 2015; Beker et al. 2016; Doğan and Kurt, 2016; Sesli, Örtücü & Aytaç, 2018). In Beker et al. (2016), seven *Hebeloma* records were cited for Turkey; these citations were based on morphological and molecular characters, but no morphological details were provided for these particular collections. They were: *H. celatum* Grilli (1), *H. cistophilum* Maire (1), *H. dunense* L. Corb. & R. Heim (2), *H. laterinum* (Batsch) Vesterh. (1), *H. mesophaeum* (Pers.) Quél. (1), *H. subtortum* P. Karst. (1). We have reviewed the content of all these papers but not examined all cited collections. If we remove synonyms (in brackets) and use the names accepted within Beker et al. (2016), then the following species have been reported for Turkey (names in bold are names as reported): *H. aestivale* Vesterh., *H. alpinum* (J. Favre) Bruchet, *H. avellaneum* Kauffman, *H. birrus* (Fr.) Gillet, *H. cavipes* Huijsman (*H. album* Peck, *H. vejlense* Vesterh.), *H. celatum* Grilli, *H. circinans* (Quél.) Sacc., *H. cistophilum* Maire, *H. clavulipes* Romagn. (*H. candidipes* Bruchet), *H. crustuliniforme* (Bull.) Quél., *H. dunense* L. Corb. & R. Heim (*H. collariatum* Bruchet), *H. eburneum* Malençon, *H. fragilipes* Romagn., *H. laterinum* (Batsch) Vesterh., *H. leucosarx* P.D. Orton, *H. mesophaeum*, *H. nauseosum* Sacc. (*H. fusipes* Bres., *H. gigaspermum* Gröger & Zschiesch.), *H. populinum* Romagn., *H. porphyrosporum* Maire (often reported as *H. sarcophyllum* (Peck) Sacc. which was incorrectly synonymised and is a north American species that as far as we are aware has never been confirmed in Europe), *H. pusillum* J.E. Lange, *H. radicosum* (Bull.) Ricken, *H. sacchariolens* Quél. (*H. pallidoluctuosum* Gröger & Zschiesch.), *H. sinapizans* (Paulet) Gillet, *H. sordescens* Vesterh., *H. subtortum* (*H. mesophaeum* var. *lacteum* Vesterh., *H. sordidum* Maire), *H. syrjense* P. Karst., *H.* theobrominum Quadr., *H. velutipes* Bruchet (*H. stenocystis* J. Favre ex Quadr.). Apart from the collections cited by Beker et al. (2016), *Hebeloma subtortum* has been previously reported under the two names *H. mesophaeum* var. *lacteum* (Yılmaz Ersel, 2005) and *H. sordidum* (Doğan and Kurt, 2016). The latter description is unlikely to represent this taxon; while the description fits reasonably well, the figure (Fig. 6 in Doğan and Kurt, 2016) showing spores and cheilocystidia, appears to be more likely to represent a species from *Hebeloma* sect. *Scabrispora*, perhaps *H. pumilum*. The former description and figure (Fig. 1 in Yılmaz Ersel, 2005) are representative of this taxon. The present study contributes to the documentation of a new record of *Hebeloma subtortum*, supported by a full description and phylogenetic results. The species is found in section *Hebeloma* which is one of the largest sections of the genus. This section is differentiated from others by the presence of a partial veil and cheilocystidia which are always lageniform or ventricose. Deciding the section where the sample is classified, depends on a few characters such as smell, number of full length lamellae, habitat, the

presence of remnants of veil, the shape and dextrinoidity of the spores and the shape of the cheilocystidia (Beker et al. 2016). Since a raphanoid smell is common to several sections of the genus, this character is insufficient to determine the section to which a collection may belong. Therefore, a few characters should be used together to properly decide the section where the sample is found. For instance, if a raphanoid smell is accompanied by the presence of a cortina and ventricose cheilocystidia, it is true to say that the specimen belongs to the section *Hebeloma* (Beker et al. 2016).

Though identification of the genus in the field is not difficult, determination at the species level can be confusing because of similarities in morphological characters. Therefore, in the current study, not only microscopic and macroscopic characters, but also molecular techniques were used to eliminate contradictory species descriptions and be sure that studied specimen is a confirmed record for Turkey. To address the morphology, given the similarity of many species, both macroscopic (pileus, lamellae and stipe) and microscopic (basidia, spores, pileipellis, hyphae and cheilocystidia) features were used. In addition to these characters, the ribosomal internal transcribed spacers (ITS) comprising ITS1, 5.8S rDNA, and ITS2 sub-regions and the large subunit nuclear ribosomal RNA gene (nrLSU) sequences were also used to be sure of species delimitation and to determine the phylogenetic perspective of the local species with respective to the other species within the genus. Recent phylogenetic studies (Aanen, Kuyper, Boekhout & Hoekstra, 2000; Aanen, Kuyper & Hoekstra, 2001; Vesterholt, Eberhardt & Beker, 2014; Eberhardt, Beker & Vesterholt 2015a; Eberhardt, Ronikier, Schütz & Beker, 2015b) demonstrate that ITS region is very useful marker for studying infrageneric classification of *Hebeloma* genus and some mycologists (Shimono, Kato & Takamatsu, 2004; Vizzini, Ercole & Contu, 2012; Demirel, 2016) favoured LSU as a phylogenetic marker since it is easily amplified, sequenced, and aligned. Therefore, sequences of these two regions were selected to study the phylogeny.

The aim of the present study is to demonstrate *Hebeloma subtortum* as a confirmed record for Turkey based on microscopic, macroscopic and molecular characters.

Material and methods

Sampling, macroscopic and microscopic studies

During field work in 2017, samples were collected from Bingöl province of Turkey and identified based on microscopic, macroscopic and molecular characters. Collected samples were associated with conifer in the field. Basidiocarps were photographed, dug out from the soil with a sharp knife, and carried to the laboratory. Collected materials were characterized based on microscopic and macroscopic characters. Measurements for basidia, basidiospores and cheilocystidia were made by using a Leica DM500 research microscope. For microscopic studies, thin sections from lamellae and surface of the basidiomata were obtained with new razor blades, and treated in distilled water, potassium hydroxide and Melzer's reagent. All measurements (spore, basidium, cheilocystidia, pileipellis, hyphae) were done with a Leica EZ4 stereo microscope with the Leica Application Suite (version 3.2.0) programme. In particular, the dextrinoid reaction of the spores is accepted to be an invaluable character for the genus (Beker et al. 2016). The colour of the endospore treated with Melzer's reagent has been observed after 1–2 minutes or more in spores floating around the hymenium, avoiding spores sitting on or very close to the lamella that are likely to show a less distinct dextrinoid reaction or none at all. The identified sample is stored at the Fungarium of Van Yüzüncü Yıl University (VANF).

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from dry specimen employing a modified protocol based on CTAB method (Doyle and Doyle, 1987). A small portion of pileus was crushed with the aid of a micropestle in 600 µL CTAB buffer. The suspension was incubated for 10–20 min at 65ºC and a similar volume of chloroform: isoamilalcohol (24:1) was added. The mixture was then centrifuged for 10 min

at 13,000 g, and total DNA in the supernatant was precipitated with a volume of isopropanol. Centrifugation was repeated for 15 min at the same speed to take DNA as pellet. 70% and 90% cold ethanol were used to wash DNA and the sample was dried at room temperature. For further studies, total DNA was suspended in 50 µL TE. The purity and quantity of extracted DNA were determined with NanoDrop2000c UV–Vis Spectrophotometer (Thermo Scientific) and 0.8% agarose gel electrophoresis. Isolated stock DNA was stored at -20°C. Primer pairs N-nc18S10(F)/C26A(R) (Wen and Zimmer, 1996) and LR0R(F)/LR5(R) (Vilgalys and Hester, 1990) were used to amplify the ITS and LSU region, respectively. PCR was performed in 25 µl reaction volume following the protocol; a hot start at 94 ºC for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 sec, annealing at 54° C for 1 min, extension at 72 °C for 1 min, and a final 72 ºC step 10 min. Amplicons were checked in 1% TAE agarose gels staining with Gelred dye, and positive reactions were sequenced with forward and reverse PCR primers using ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

Sequence alignment and phylogenetic analysis

All sequence *chromatograms* were opened using *Finch TV* (http://www.geospiza.com/*finchtv*/) and checked for putative base calling errors. Quality (Q) value was used to assess the accuracy of each base in the sequences. For initial comparison, Basic Local Alignment Search Tool (BLAST) (Altschul, Gish, Miller, Myers & Lipman, 1997) analysis was performed using the National Center for Biotechnology Information (NCBI) database. To clarify the phylogenetic position of the sample used in the current study, related sequences were retrieved from the database. A total of 62 sequences, representing different sections of genus *Hebeloma,* were included in the phylogenetic analysis of ITS regions (Appendix 1). *Galerina pruinatipes* and *Galerina pseudocamerina* were chosen as outgroup samples (Boyle, Zimdars, Renker & Buscot, 2006). The LSU region was less studied compared to ITS for *Hebeloma* so 24 LSU sequences were downloaded from NCBI and analysed with our sample. The constructed phylogenetic tree was less informative compared to the ITS tree and so is not included in this paper.

The sequence alignments and phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis (MEGA 6) software (Tamura, Stecher, Peterson, Filipski & Kumar, 2013). The Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura and Nei, 1993) were used to construct phylogenetic tree. Initial tree(s) for the heuristic search were automatically obtained by using Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. After analysis, the tree with superior log likelihood value was selected. The confidence of branching was assessed using 1000 bootstrap resamplings. All positions containing gaps and missing data were eliminated.

Results

Taxonomy

Hebeloma subtortum P. Karst., 470, Bidrag till Kännedom av Finlands Natur och Folk 48:466, (1889)

Specimens examined: —TURKEY, Bingöl, Genç Conifer Forest, 1076 m, 38°44'09550" N−40°34'035" E, 18.05.2017, Uzun Y. 1242, (VANF).

Macroscopic description

Pileus 20-80 mm across, hemispherical to planoconvex, convex to expanded spherical with a broad umbo, whitish, cream to clay-buff, reddish-yellow or almost light brown in the center. Young specimens *have cortina*. The edges of pileus carry the velar residue. Lamellae adnate, sometimes subdeccurent, whitish when young then cream to light brownish, flocculose. Stipe 30–130 × 5–12 mm, cylindrical, floccosity pruinose-fibrillose, widened towards to base. Taste not recorded. Spore print not recorded (Figure 1).

Figure 1. a-b) Basidiocarps of *Hebeloma subtortum*

Microscopic description

Spores 7–11(12) × 5–7 µm, mainly ovoid, elliptical, pale yellowish to grey yellow, indextrinoid, usually guttulate. Basidia 25-29 × 5.8 - 7.8 µm, 4-spored, rarely 2-spored. Basidioles 20-29 × 5.5 - 7.6 µm. Cheilocystidia 25-75 µm in length, median width 4-6 µm, basal width 6.5-12 µm, lageniform, ventricose, occasionally cylindrical, sometimes septate. Pleurocystidia none. Caulocystidia similar to cheilocystidia, up to 120 µm. Pileipellis up to 14 µm, clamp connection abundant. Hyphae up to 14 µm across clamp connection present (Figure 2-4). NCBI numbers: MG914656 (ITS region), MG914657 (LSU region).

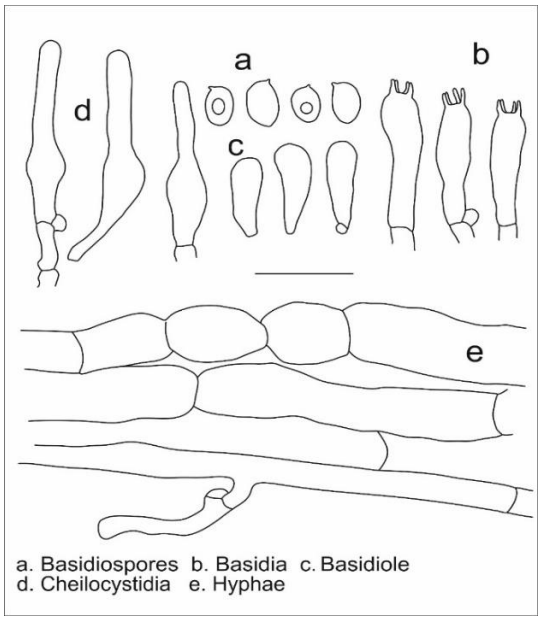

Figure 2. Microcharacters of *Hebeloma subtortum* (Scale: 20 µm)

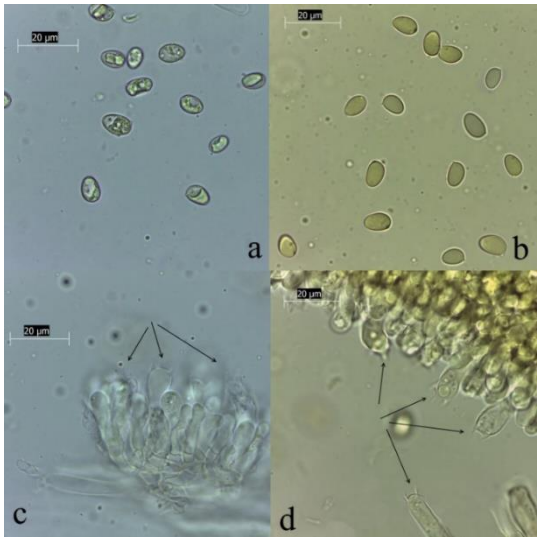

Figure 3. *Hebeloma subtortum* a) spores (distilled water), b) spores (Melzer's reagent), c) basidia (distilled water), d) basidia (Melzer's reagent)

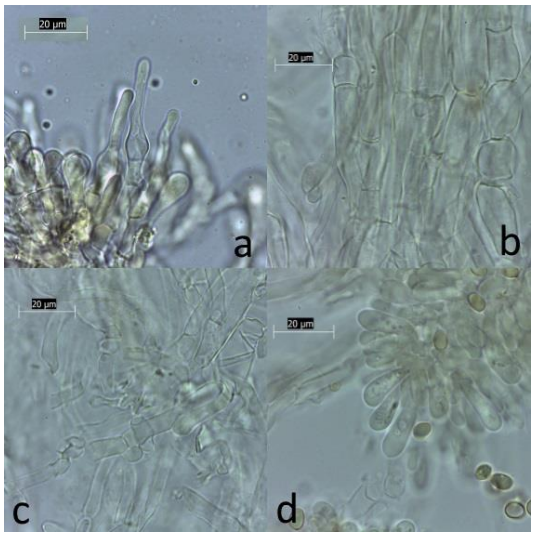

Figure 4. *Hebeloma subtortum* a) cheilocystidia (distilled water), b) hyphae (distilled water), c) pileipellis (distilled water), d) basidioles (KOH)

Molecular analysis

The length of the ITS region was 690 bp, from which 662 characters were used in final analysis after trimming the alignment from both 5' and 3' sides. BLAST analysis revealed that ITS sequence of used sample matched with those of *Hebeloma subtortum* (KX765788, KX765789, KX765790, and KX765791) with the maximum identity (100%). Only one substitution (T/A) was observed between our sample and *Hebeloma subtortum* samples retrieved from NCBI. 150 variable sites were observed in the aligned data and sequences of the 5.8S rDNA region were identical among 62 sequences. ITSl appeared to be slightly more variable than ITS2 sub-region (82 variable sites in ITSl and 68 in ITS2). Base composition of the entire region was as follow; A 0.24, C 0.23, G 0.22, and T 0.32. LSU region has 930 bases and after trimming 847 characters were used for the analysis. 26 variable sites were observed in the aligned data. Our sample blasted with sequences of *H. collariatum*, *H. affine* and *H. mesophaeum* species 100% identity value.

The tree constructed based on ITS region obtained significant support for all *Hebeloma* sections except *Naviculospora*. The *Hebeloma* clade consists of three clusters and all *Hebeloma subtortum* samples grouped together with a bootstrap value of 89 % (Figure 5). The DNA sequence of our studied sample was identical with those of *H. subtortum* downloaded except 464. base which is found in ITS2 sub-region. This base was thymine in the studied sample while adenine in each downloaded sample. Because of this substitution, the studied *H. subtortum* sample bound the representatives externally in the phylogenetic tree (Figure 5). *Hebeloma mesophaeum* samples clustered close to *H. subtortum.* Although these two species are morphologically similar, they can be distinguished from each other based on macroscopic and microscopic characters (Table 1). *Hebeloma subtortum* and *H. mesophaeum* have almost similar spore length and width but *H. subtortum* is differentiated by many ovoid-pale yellow spores, more than 50 full length lamellae and stipe width of mature basidiomes ≥ 4 mm. Even though the LSU tree was less informative, it still showed the close relation between our sample and some sequences of *H. mesophaeum* and *H. dunense* (*H. collariatum*), which are also found in *Hebeloma* section *Hebeloma*.

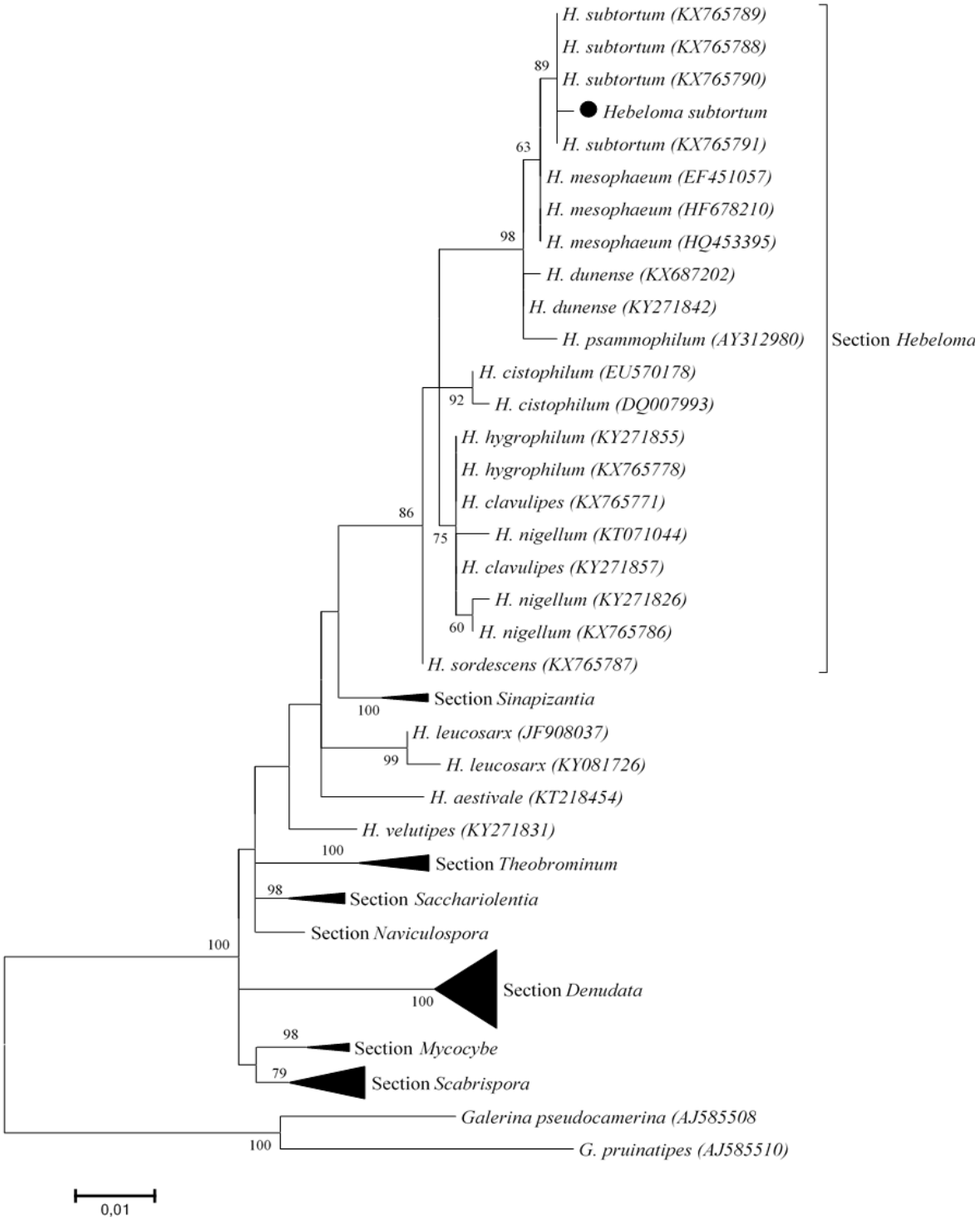

Figure 5. Phylogenetic relationships of *Hebeloma* species based on the ITS region. The black circle indicates studied specimen. *Galerina pruinatipes* and *Galerina pseudocamerina* are used as outgroup. Bootstrap analyses are based on 1000 replicates and values higher than 50% are indicated on branches.

Table 1. Comparison of *H. subtortum* and *H. mesophaeum* based on macroscopic and microscopic characters.

Species	Pileus	Lamellae	Stipe	Basidia	Spores	Cheilocystidia	Ecology
H. subtortum	2-8 cm, convex to expanded spherical with a broad umbo	adnate, sometimes subdeccurent	3-13 × 0.5-1.2 cm, cylindrical, floccosity pruinose, widened towards base	25-29 × 5.8 - 7.8 µm	7–11(12) × 5–7 µm, mainly ovoid, elliptical	25-75 × 4-6 µm, basal width 6.5-12 µm, lageniform, ventricose	Under *Abies* Mill, *Betula* L., *Castenea* Miller, *Cedrus* Trew, *Fagus* L., *Picea* A. Dietr., *Pinus* L., *Quercus* L. and *Tilia* L.
H. mesophaeum	2-7 cm, broadly convex, broadly bell-shaped	adnexed to notched	2-9 × 1 cm, more or less equal, silky	30-40 × 7-10 µm	8-11 x 5-7 µm, elliptical	30-70 × 7-12 µm, lageniform, ventricose	Scattered or in small groups under conifers

Discussion

The identification of *Hebeloma* species is really difficult not only macroscopically and microscopically but also in many cases molecularly due to recent speciation, morphological plasticity within species, reticulate evolution or other discrepancies between gene and species evolution. Therefore, determination of the correct section is the first step to identy a specimen. Understanding of sections is relatively straightforward with a small amount of microscopy, and a certain amount of experience. For instance, section *Hebeloma* is characterised by a raphanoid smell accompanied by the presence of a cortina and ventricose cheilocystidia.

Hebeloma subtortum (Sect. *Hebeloma*) is a species associating with conifers and other trees such as *Castenea, Fagus*, *Quercus* and *Tilia.* In the constructed ITS tree, *H. subtortum* clustered with its representatives retrieved from NCBI with high bootstrap value and within the same clade as *H. mesophaeum* collections. All of these species are found in *Hebeloma* section that is characterized by a cortina and the ventricose or lageniform cheilocystidia. Especially, *H. subtortum* and *H. mesophaeum* are morphologically very similar so a close relationship is expected in the tree, as well. However, *H. subtortum* can be separated based on several features. For example, characters of spores, lamellae, stipe, and basidia are important characters for identification of this species. *Hebeloma subtortum* has mainly ovoid spores; adnate, occasionally subdeccurent lamellae; a pruinose stipe, widened towards the base and smaller basidia. *Hebeloma mesophaeum* has pileus; dark wine to brown in the center, spores; indextrinoid ovoid and ellipsoid (Beker et al. 2016). *Hebeloma subtortum* was identified by Beker et al. (2016) and the species was firstly recorded for Turkey in this study. Macroscopic and microscopic characters were compared for Turkish specimens of *Hebeloma subtortum* and data of another authors (Table 2).

Table 2. Comparison of macroscopic and microscopic characters for Turkish specimens of *Hebeloma subtortum* and data of another authors.

Pileus	Lamellae	Stipe	Basidia	Cheilocystidia	Spores	References
20-80 mm, convex to expanded spherical with a broad umbo	adnate, sometimes subdeccurent	30-130 × 5-12 mm, cylindrical, floccosity pruinose, widened towards base	25-29 × 5.8 - 7.8 µm	25-75 × 4-6 µm, basal width 6.5-12 µm, lageniform, ventricose	7–11(12) × 5–7 µm, mainly ovoid, elliptical	This study
40-60 mm, convex, smooth, universal veil	adnate, presence of tears	70-90 × 5-10 mm, cylindrical, pruinose	16.6-19.4 × 5.5-5.7 µm, four spored	36-60 × 4.1-5.6 × 6.5-12 µm, mainly lageniform, ventricose	8-10 × 5.3-6.3 µm, mainly ovoid, elliptical	Beker et al. 2016

The number of *Hebeloma* species present in Turkey was recorded as 28 (Sesli and Denchev, 2014) and this number increased to 32 based on further recent studies (Güngör et al. 2015; Sesli et al. 2015; Solak et al. 2015; Doğan and Kurt, 2016; Sesli et al. 2018). Beker et al. (2016) added one more species to this list. However, when synonyms are taken into consideration the total number is reduced to 28. While, as discussed earlier, *Hebeloma subtortum* has already been recorded in Turkey (under the names *Hebeloma mesophaeum* var. *lacteum* and *H. sordidum*) this appears to be the first published record which is further confirmed by phylogeny. The *Hebeloma* sample collected from Bingöl-Genç in 2017 was identified as *Hebeloma subtortum* based on both morphological and molecular studies.

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Appendix

ITS sequences downloaded from NCBI database

H. aestivale (KT218454), *H. aanenii* (KX657845), *H. atrobrunneum* (AY308586), *H. birrus* (JF908026), *H. bulbiferum* (KT218439), *H. calyptrosporum* (AY309961), *H. cavipes* (KX687193), *H. cistophilum* (EU570178, DQ007993), *H. clavulipes* (KY271857, KX765771), *H. crustuliniforme* (KX657847), *H. cylindrosporum* (KX687197), *H. dunense* (KX687202, KX687202, KX687202), *H. eburneum* (KF309412), *H. fragilipes* (KX687207), *H. geminatum* (KM390732), *H. helodes* (AF124703), *H. hiemale* (JX178629), *H. hygrophilum* (KY271855, KX765778), *H. ingratum* (KX687213), *H. laterinum* (KX687214), *H. leucosarx* (KY081726), *H. limbatum* (KT217552), *H. lutense* (KM390775), *H. mesophaeum* (HF678210, HQ453395), *H. nanum* (KX765798), *H. nauseosum* (KX765763), *H. nigellum* (KY271826, KY271826, KT071044), *H. ochroalbidum* (KM390610), *H. oculatum* (AY311525), *H. odoratissimum* (KX687216), *H. pallidoluctuosum* (AY311526), *H. psammophilum* (AY312980), *H. populinum* (EF644107), *H. pumilum* (KX765808), *H. pusillum* (KM390767), *H. pseudofragilipes* (KT217551), *H. radicosum* (KX765800, AY278767, FJ168582), *H. sacchariolens* (KX449205), *H. salicicola* (KM390758), *H. sinapizans* (KT218484), *H. sordescens* (KX765787), *Hebeloma subtortum* (KX765791, KX765790, KX765789, KX765788), *H. theobrominum* (JQ751213, EU570182), *H. vaccinum* (KT217576), *H.*

velutipes (KY271831), *H. vesterholtii* (FJ943240).

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