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Hymenoscyphus conscriptus & *H. fucatus,* newly recorded from Turkey

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Abstract—*Hymenoscyphus conscriptus* and *Hymenoscyphus fucatus* are described as new records from Turkey based on morphological characters and DNA sequence data. Phylogenetic analyses of internal transcribed spacer nuclear ribosomal DNA sequences (nrITS) show that *H. conscriptus* and *H. fucatus* form well-supported clades. *Hymenoscyphus conscriptus* and *H. fucatus* cluster as a highly supported group, closely related to *H. yui* and *H. serotinus,* but represent independent species in the phylogenetic tree; the synonymy of *H. conscriptus* under *H. calyculus* is not supported phylogenetically. Detailed descriptions, illustrations, and discussions concerning morphologically similar and phylogenetically closely related species are provided for each species.

Keywords—*Helotiaceae, Helotiales,* taxonomy

Introduction

The current classification of fungi is still largely based on morphological characters, but DNA sequence analyses of a specific region also help to identify species correctly and determine phylogenetic relationships. Morphological characters (such as apothecial shape and color) are important for distinguishing the families in *Helotiales* (Korf 1973; Wang & al. 2006). However, classifications based on apothecial morphology are not always sufficient, so more studies are required for specifying the taxonomy within the *Helotiales*.

Hymenoscyphus, one of the largest genera of *Helotiaceae,* encompasses some saprotrophic and parasitic species with highly divergent morphological, ecological, and biological characters (Wang & al. 2006; Kirk & al. 2008). *Hymenoscyphus* was established by Gray (1821) and lectotypified with *H. fructigenus* (Bull.) Gray (Dennis 1964). Most *Hymenoscyphus* species have been reported from Europe (Phillips 1887, Dennis 1956, Lizoň 1992), America (White 1943; Dumont 1981a,b; Dumont & Carpenter 1982), and Asia (Thind & Sharma 1980; Sharma 1991; Zhang & Zhuang 2002a,b). About 155 species have been identified in the genus (Kirk & al. 2008) with numerous species recently added (Queloz & al. 2011; Baral & Bemmann 2013; Zheng & Zhuang 2013a,b,c, 2014; Gross & Han 2015; Gross & al. 2015; Kowalski & Bilański 2019; Baral 2019). *Hymenoscyphus* species are normally saprophytic on plant debris, such as wood, twigs, fruits, leaves, and herbaceous stems. They are morphologically characterized by stipitate-cupulate to discoid apothecia; white to yellowish or light-colored hymenial surfaces; more or less long stipes; ectal excipulum typically of textura prismatica (sometimes mixed with textura angularis); fusoid, ellipsoid to scutuloid or ciboroid ascospores; with or without setulae and existence of the remarkable refractive vacuolar bodies in the paraphyses (Dennis 1964, Lizoň 1992, Sharma 1991, Zhang 2002, Kowalski & Bilański 2019).

Some *Hymenoscyphus* species are morphologically difficult to distinguish. making molecular approaches often necessary for taxonomic identification. The internal transcribed spacer of nuclear ribosomal DNA (nrITS) region and a few additional useful regions have been widely used as reliable phylogenetic approaches to determine *Hymenoscyphus* at the species level (Han & Shin 2008; Queloz & al. 2011; Zheng & Zhuang 2013a, 2014, 2015; Baral & Bemmann 2014; Gross & Han 2015; Gross & al. 2015; Kowalski & Bilański 2019; Pastirčáková & al. 2020). For the current study, the ITS region was selected because of availability of universal primers, high PCR success rate, a high percent of correct identification, the considerable number of sequences available in GenBank, and its superior resolution at the infrageneric classification level for the genus. After a macro- and micro-morphological study of the collections and our molecular phylogenetic analyses based on ITS sequences, we concluded that the specimens represent new records for Turkish mycobiota. Sesli & al. (2020) and Çetinkaya & Uzun (2021) reported 14 *Hymenoscyphus* species from Turkey, and *H. conscriptus* and *H. fucatus* are new Turkish records.

Materials & methods

Sampling, macroscopic, microscopic studies

Fresh *Hymenoscyphus* ascomata were sampled from the Bingöl province of Turkey in 2019. The samples were photographed in the field with a Canon (EOS 60D) camera equipped with Tokina 100 mm macro lens. Microscopic characters were observed in both distilled water and IKI solution with a Leica DM500 research microscope under oil immersion. At least 20 ascospores, 20 paraphyses and 15 asci from each studied sample were measured using the Leica Application Suite (version 3.4.0) programme and described based on Hengstmengel (1996), Baral & Bemmann (2013), and Anonymous (2021). The specimens are stored in the Fungarium of Van Yüzüncü Yıl University, Van, Turkey (VANF).

Molecular studies

Fungal genomic DNA was extracted from dried apothecia by following a slightly modified CTAB protocol (Doyle & Doyle 1987). Total genomes were isolated from two specimens as representatives for species *H. conscriptus* and *H. fucatu*s. The primer pair N-nc18S10(F) / C26A(R) was used to amplify the ITS region (Wen & Zimmer 1996). All PCR reactions were performed with Thermalcycler (ThermoScientific) under a program consisting of a hot start at 95°C for 4 min, followed by 30 cycles of denaturation at 94 for 1 min, annealing at 54°C for 50 sec, and extension at 72°C for 1 min, with a final elongation step for 5 min. Every reaction was conducted with a negative control containing the mix without template DNA. After checking amplicons in 1% TAE agarose gels staining with Gelred dye, positive reactions were sequenced with forward and reverse primers. The PCR was purified and DNA sequenced in an Applied Biosystems ABI 3730XL automated sequencer. The sequences generated in the study were uploaded to NCBI GenBank Sequence Database.

Finch TV software was used to observe sequence chromatograms. Forward and reverse sequences were aligned to get the correct and reliable sequence for each studied sample. The ITS sequences were trimmed and edited using Molecular Evolutionary Genetics Analysis (MEGA v. 6; Tamura & al. 2013). The obtained DNA sequences were compared by Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) against ITS sequences in the NCBI. Then a combined dataset was prepared comprising the sequences based on the highest scored hits of BLAST search. The ITS sequences of our four *Hymenoscyphus* strains were compared with ITS sequences of 34 other *Hymenoscyphus* strains (from GenBank), which allowed the exact positioning of the new records within the genus *Hymenoscyphus*. The tree was rooted with *Hyaloscypha fuckelii* Nannf. (EU940230) and *Hyaloscypha aureliella* (Nyl.) Huhtinen (EU940228).

The dataset containing multiple sequences was aligned using MEGA. The appropriate model of nucleotide evolution for phylogenetic analyses was determined using the same program, and the model with the lowest BIC (Bayesian Information

Criterion) score was used to describe the best substitution model. Phylogenetic analyses were performed using Maximum Parsimony (MP) in MEGA, and Bayesian Inference (BI) in MrBayes v.3.2 (Ronquist & al. 2012). All positions containing gaps and missing data were eliminated. Initial tree(s) for the heuristic search were automatically obtained by 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 Tree-Bisection-Reconnection (TBR) search method was employed with 100 random addition replications to construct the MP trees and the consensus tree was inferred from the 5 most parsimonious trees. Bootstrap analysis with 1000 repetitions was included to test branch topology (Felsenstein 1985). Bootstrap values lower than 50 % were not given in phylogenetic trees.

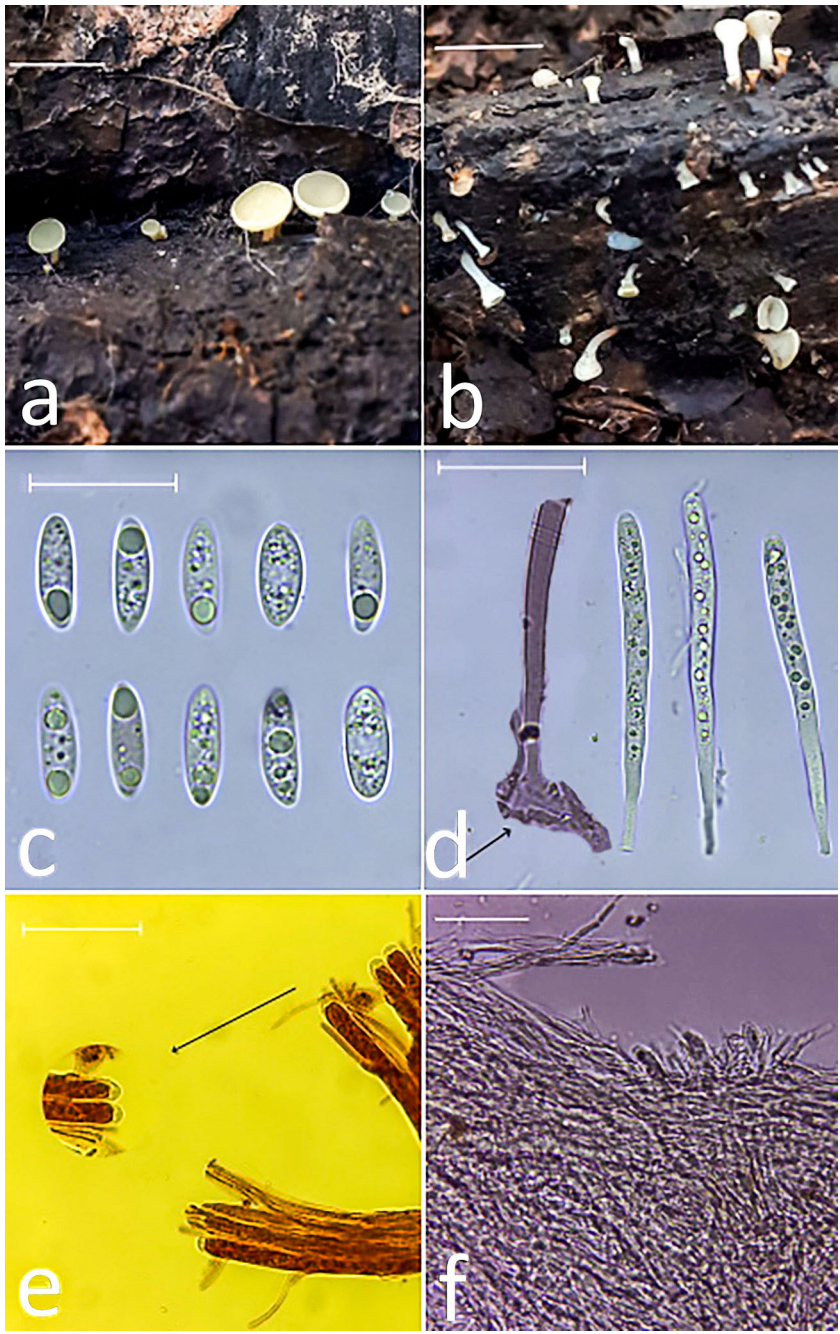
The model test implemented in MEGA showed that the K2+G was the most suitable analysis method. MrBayes was employed to conduct Bayesian phylogenetic analysis using the Markov chain Monte Carlo (MCMC) method under a K2+G model with all the remaining settings set to default (incremental heating scheme of chains, unconstrained branch length, and uninformative topology priors). Markov chains were run for 1,000,000 generations, saving one tree each 1000 generations. A conservative burn-in (25%) was applied after checking for stability on the log-likelihood curves and split variances being <0.01. A majority rule consensus tree of the remaining trees was calculated. Branch support was determined by Bayesian Posterior Probabilities (BPP). The trees were viewed in Figtree v1.3.1. (Rambaut 2010).

Taxonomy

Hymenoscyphus conscriptus (P. Karst.) Korf ex Kobayasi & al.,
Ann. Rep. Inst. Ferment. Res., 1965-66, 3: 55 (1967) Fig. 1

Apothecia 2–3 mm wide, disc or saucer-shaped, individually or in clusters, whitish to yellowish, sometimes blackened at the base. Stipe 2–6 mm long when rehydrated, cylindrical to obconical. Asci [n:15] 110–150 × 7.3–10 µm, cylindric-clavate to cylindric-obconical, 8 spored, apex truncated conical; annulus turning medium blue in IKI, ascus base with croziers. Ascospores [n:20] 13–17 × 4.4–6.2 µm, ellipsoid-cylindrical to obovoid-fusiform, without septa, with several small drops near each end when alive, in dead state with 1-2 large drops due to confluence, without setulae. Paraphyses [n:20] 1.5–4.3 µm wide, cylindrical, uninflated, or slightly lanceolate, never septate

Fig. 1. *Hymenoscyphus conscriptus* (VANF – Acar 1138). a, b. Ascocarp; c. Ascospores in distilled water; d. Asci in distilled water; e. Asci and paraphyses in IKI; f. Ectal excipulum in distilled water. All in dead state, except for apothecia. Scale bars: a, b = 5 mm; c = 20 µm; d–f = 50 µm.


a
b
c
d
e
f

at the apex but are always septate downwards. Ectal excipulum consisting of textura prismatica; hyphae 4–12 µm wide.

Specimen examined: —**TURKEY, Bingöl, Çapakçur stream,** 38.8942°N 40.4792°E, 1150 m, garden area, on branches of *Salix* and *Populus* spp., 24.10.2019, I. Acar 1138 (VANF; GenBank MW959789, MW959790).

Hymenoscyphus fucatus (Cooke & W. Phillips) Baral, Beih. Z. Mykol. 6: 128 (1985) Fig. 2

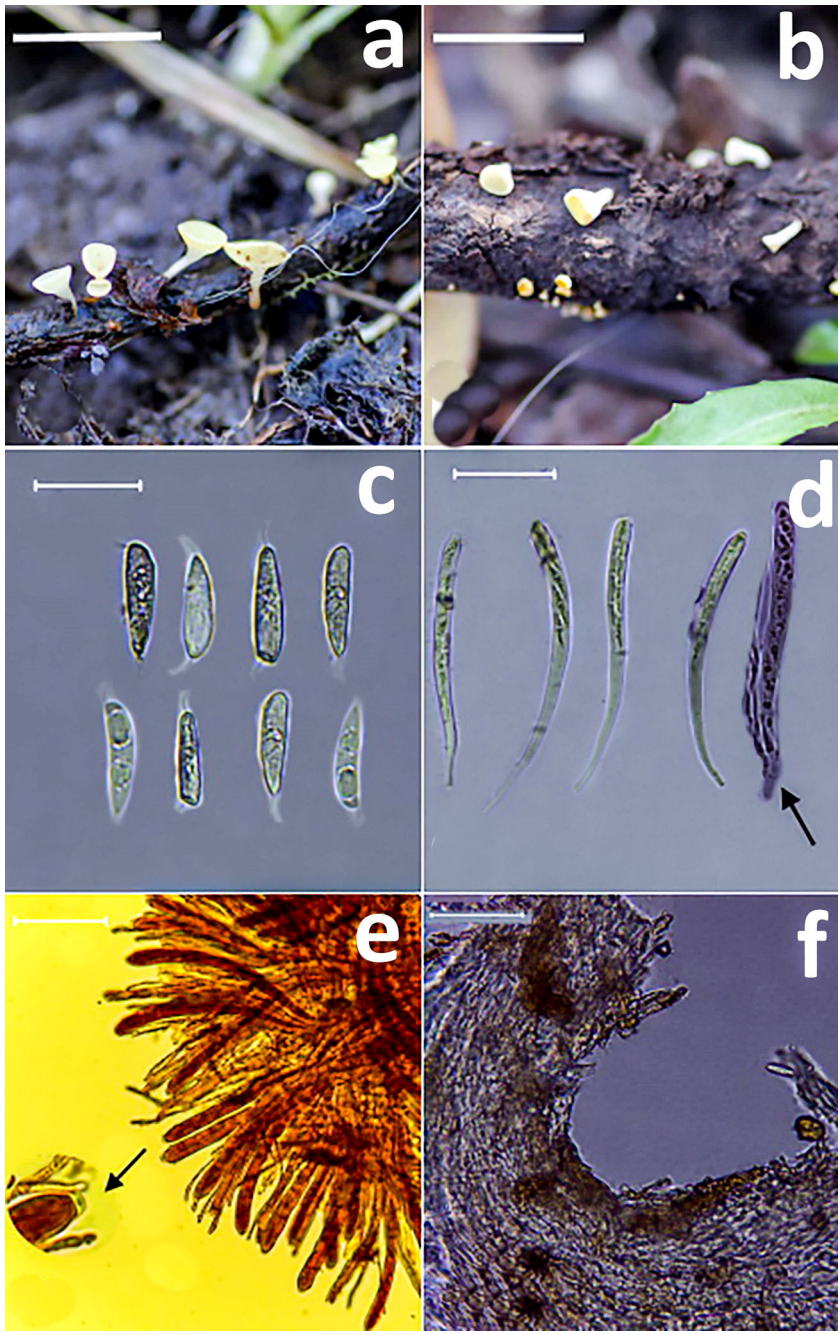
Apothecia 1–2 mm wide, stipitate-cupulate, saucer-shaped, slightly clustered, sparsely mutually grown together at the base, whitish to yellowish, erumpent through (sometimes blackened) epidermis or superficial on delaminated parts of the substratum. Stipe 2–7 mm long when rehydrated, cylindrical to obconical. Asci [n:15] 120–150 × 7–12 µm, cylindric-clavate to cylindric-obconical, 8 spored, ascus base with croziers, apex truncated conical; annulus turning medium blue in IKI. Ascospores [n:20] 19–30 × 4–6.2 µm, ellipsoid-fusiform to obovoid-fusiform, sometimes cylindrical, smooth or slightly curved, guttulate, obliquely biseriate, at apex and base mostly provided with 1–2(–3) µm long, straight or slightly curved setulae. Paraphyses [n:20] 1.5–3 µm diam (slightly wider at the apex), subcylindrical. Ectal excipulum a textura prismatica, hyphae 4–l0 µm diam:

Specimen examined—**TURKEY, Bingöl, Haserek village**, 38.9139°N 40.3133°E, 1624 m, garden area, on stem of plant, 6.11.2019, I. Acar 1149 (VANF; GenBank MW959791, MW959792).

Phylogenetic analysis

The ITS alignment included 40 sequences with 502 nucleotides representing our four new *Hymenoscyphus* strains and 34 *Hymenoscyphus* strains downloaded from GenBank, with two *Hyaloscypha* strains as outgroup. After deleting the unaligned and ambiguously aligned sites, 493 characters remained for analyses, including 92 variants and 72 parsimony informative characters. The MP and BI analyses inferred from the ITS dataset produced similar tree topologies except for minor differences in the arrangement of a few terminal branches. The MP analysis produced five most parsimonious trees (tree length: 294; consistency index: 0.61; retention index: 0.81; rescaled consistency index: 0.53). The phylogram inferred from MP analysis is shown in Fig. 3. Statistical supporting values >50% or >0.50 were shown at the nodes.

Fig. 2. *Hymenoscyphus fucatus* (VANF – Acar 1149). a, b. Ascocarp; c. Ascospores in distilled water; d. Asci in distilled water; e. Asci and paraphyses in IKI; f. Ectal excipulum in distilled water. All in dead state, except for apothecia. Scale bars: a, b = 5 mm; c = 20 µm; d–f = 50 µm.


a
b
c
d
e
f

The phylogenetic tree was divided into three main clades (A, B, C) with moderate bootstrap support and few scattered species. In the tree, our sequences of *Hymenoscyphus conscriptus* and *Hymenoscyphus fucatus* formed moderately supported clusters in clade C with their representatives downloaded from NCBI database (Fig. 3). The DNA sequences from our *Hymenoscyphus conscriptus* specimen showed very high homology with the sequence of MK163877 (an unidentified *Hymenoscyphus* sp.; Pedersen & al. 2020), so that they clustered together without any genetic distance in the phylogenetic tree with 100% bootstrap value (Fig. 3). *Hymenoscyphus conscriptus* appeared to be sister to *H. yui* (KJ472303, KJ472304; Zheng & Zhuang 2015) and *H. serotinus* (DQ431168; Baral & al. 2006, and KM114541; Gross & al. 2015). No nucleotide substitution or indel was detected between *H. conscriptus* and MK163877, but more than twenty-five substitutions were seen among *H. conscriptus, H. yui,* and *H. serotinus*. All of these nucleotide variations were observed in ITS1 and ITS2 subregions. DNA sequences of our *Hymenoscyphus fucatus* specimen presented high homology with the sequence of *Hymenoscyphus fucatus* (JX977147, JX977148; Zheng & Zhuang 2013a). Turkish sequences clustered together with moderate bootstrap and posterior probability values (Fig. 3). Nine nucleotide variations (ITS1 and ITS2) were detected between the Turkish and downloaded *H. fucatus* sequences.

Discussion

Our study shows that combining morphological characters with molecular data is important in identifying a fungus sample to species. The samples collected from Bingöl, Turkey, were determined as *Hymenoscyphus conscriptus* and *Hymenoscyphus fucatus* based on both morphological and molecular characters.

Hymenoscyphus conscriptus and *H. yui* H.D. Zheng & W.Y. Zhuang share very similar macroscopic/microscopic characteristics and sequence data. Apothecia are similar in shape, color, size, asci arising from croziers, the size of its spores. Even though they have very similar characters these two species can be morphologically distinguished the non-guttulate (eguttulate) spores and the ascus apex that is nonamyloid in IKI in *H. yui* (Zheng & Zhuang 2015). *Hymenoscyphus conscriptus* bears guttulate spores, and the apices of its asci are amyloid in IKI. These two species were separated, therefore, based on both macroscopic/microscopic characteristics and sequence data.

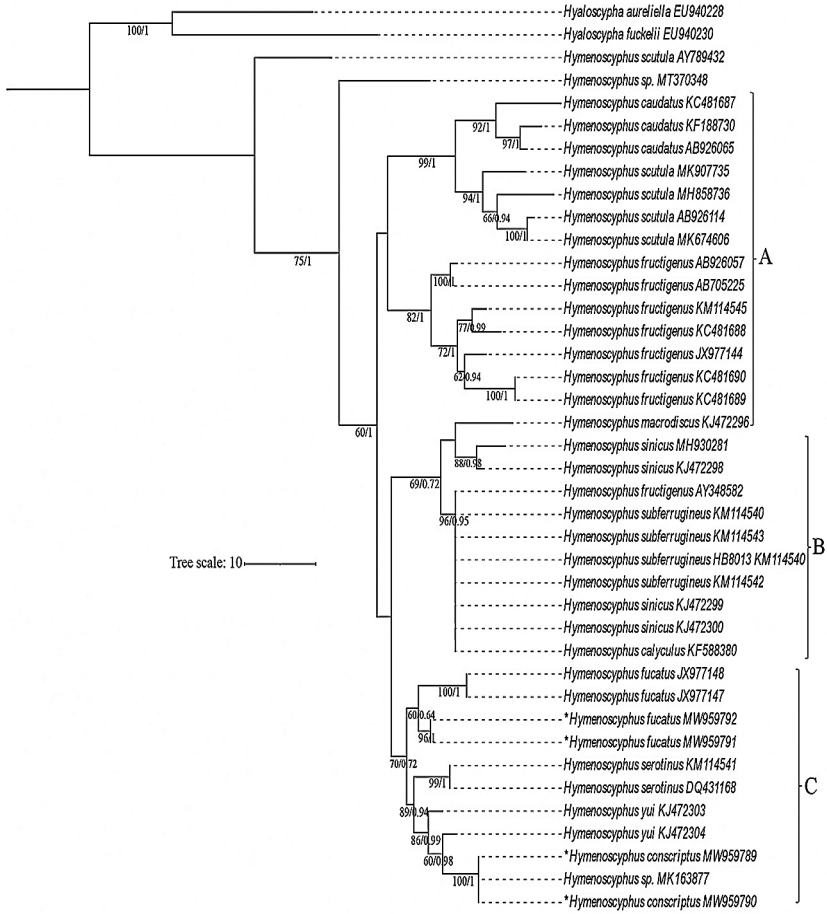


Fig. 3. Maximum Parsimony tree inferred from internal transcribed spacer nuclear ribosomal DNA sequences showing the relationships among *Hymenoscyphus conscriptus*, *Hymenoscyphus fucatus* and related *Hymenoscyphus* species. Statistical support values at nodes are bootstrap values >50% and Bayesian Posterior Probabilities >0.50. Turkish sequences are marked with *.

Hymenoscyphus fucatus shares some similar morphological and molecular characters with *H. serotinus* (Pers.) W. Phillips. Both have similar apothecial shape and color and excipulum structure, but their distinctive stipe and spore structures distinguish them: *Hymenoscyphus serotinus* has an overall pubescent stipe and more scutuloid-curved spores that lack setulae (Baral & Bemmann 2013) while *H. fucatus* lacks pubescence and has setulate spores.

Hymenoscyphus calyculus (Fr.) W. Phillips and *H. scutula* (Pers.) W. Phillips macroscopically similar species could not be easily distinguished when only apothecia and stipe structures were examined. However, paraphyses, habitat, and ascospores are useful and distinctive characters to differentiate them. *Hymenoscyphus calyculus* has septate paraphyses, spores 15–24 × 3–4 µm and it is saprobic on sticks, twigs of hardwoods and broadleaves trees (Breitenbach & Kränzlin 1984; Kuo 2008) while *H. conscriptus* has non-septate paraphyses and smaller spores and grows exclusively on branches and trunks of *Salix* species. *Hymenoscyphus conscriptus* and *H. calyculus* were also distinguished by their ITS sequences and lie within different clades in the phylogenetic tree (Fig. 3).

Hymenoscyphus fucatus and *H. scutula* (Pers.) W. Phillips were considered separate species by Baral (Baral & Krieglsteiner 1985) and Hengstmengel (1996). *Hymenoscyphus fucatus* and *H. scutula* are microscopically similar in apothecial shape and color and ascospore morphology. However, dimensions of apothecia and asci can be used to distinguish the two species. *Hymenoscyphus scutula* has apothecia that are 1–4 mm diam and asci that measure 90–110 × 8–10 µm and lack croziers (Breitenbach & Kränzlin 1984; Uzun & al. 2010); *H. fucatus* has apothecia 1–2 mm and asci measuring 120–150 × 7–12 µm and arising from croziers. The phylogenetic distance *Hymenoscyphus fucatus* is phylogenetically quite distant from *H. scutula* so these species cannot be accepted as synonyms.

Determination of fungi at the species level is still a matter of intensive debate. Limited morphological features and inaccurate description or identification of some taxa are, as in other fungal groups, main problems in the taxonomic studies of *Hymenoscyphus* genus. Future work will improve our knowledge of fungal species diversity and firmly establish a logical generic circumscription. With the aid of integrated studies combining morphology and sequence data, *Hymenoscyphus conscriptus* is supported as an independent species, and it and *H. fucatus* have been confirmed as new records for Turkey.

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Literature cited

Anonymous. 2021. *Hymenoscyphus conscriptus* (Karst.) Korf. https://smd38.fr/documents/fiches_techniques/toutes_especes/Hymenoscyphus%20conscriptus.pdf [Access date: 08.04.2021].

Baral HO. 2019. *Hymenoscyphus reynoutriae* sp. nov. (= *H. dearnessii* s. auct.), a neomycete in Europe, and *H. phalaridis* sp. nov., specific to monocots (*Helotiaceae*, *Helotiales*). Mycologia Montenegrina 20: 11–40.

Baral HO, Bemmann M. 2013. *Hymenoscyphus serotinus* and *H. lepismoides* sp. nov., two lignicolous species with a high host specificity. Ascomycete.org 5(4): 109–128. https://doi.org/10.25664/art-0086

Baral HO, Bemmann M. 2014. *Hymenoscyphus fraxineus* vs. *Hymenoscyphus albidus* – a comparative light microscopic study on the causal agent of European ash dieback and related foliicolous, stroma-forming species. Mycology 5(4): 228–290. https://doi.org/10.1080/21501203.2014.963720

Baral HO, Krieglsteiner GJ. 1985. Bausteine zu einer Askomyzeten-Flora der Bundesrepublik Deutschland: in Suddeutschland gefundene inoperkulate Diskomyzeten – mit taxonomischen, ökologischen, chorologischen Hinweisen und einer Farbtafel. Beihefte zur Zeitschrift für Mykologie 6. 160 p.

Baral HO, Galan CHO, Lopez R, Arenal J, Villarreal F, Rubio M, Collado V, Platas J, Peläez GF. 2006. *Hymenoscyphus crataegi* (*Helotiales*), a new species from Spain and its phylogenetic position within the genus *Hymenoscyphus*. Sydowia 58(2): 145–162.

Breitenbach J, Kränzlin F. 1984. Fungi of Switzerland. Vol. 1, Verlag Mykologia Lucerne, Switzerland.

Çetinkaya A, Uzun Y. 2021. *Hymenoscyphus caudatus*, a new ascomycete record for the mycobiota of Turkey. Anatolian Journal of Botany 5(1): 19–22. https://doi.org/10.30616/ajb.826640

Dennis RWG. 1956. A revision of the British *Helotiaceae* in the Herbarium of the Royal Botanic Garden, Kew, with notes on related European species. Mycological Papers 62. 216 p.

Dennis RWG. 1964. Remarks on the genus *Hymenoscyphus* S.F. Gray, with observation on sundry species referred by Saccardo and others to the genera *Helotium*, *Pezizella* or *Phialea*. Persoonia 3: 29–80.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Dumont KP. 1981a. *Leotiaceae* II. A preliminary survey of the neotropical species referred to *Helotium* and *Hymenoscyphus*. Mycotaxon 12: 313–371.

Dumont KP. 1981b. *Leotiaceae* III. Notes on selected temperate species referred to *Helotium* and *Hymenoscyphus*. Mycotaxon 13: 59–84.

Dumont KP, Carpenter SE. 1982. Los hongos de Colombia—VII: *Leotiaceae*—IV: *Hymenoscyphus caudatus* and related species from Colombia and adjacent regions. Caldasia 13: 567–602.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Gray SF. 1821. A natural arrangement of British plants, vol. 1. C. Baldwin Printer, London.

Gross A, Han JG. 2015. *Hymenoscyphus fraxineus* and two new *Hymenoscyphus* species identified in Korea. Mycological Progress 14(19): [13 p.]. https://doi.org/10.1007/s11557-015-1035-1

Gross A, Hosoya T, Zhao YJ, Baral HO. 2015. *Hymenoscyphus linearis* sp. nov.: another close relative of the ash dieback pathogen *H. fraxineus*. Mycological Progress 14(20): [15 p.]. https://doi.org/10.1007/s11557-015-1041-3

Han JG, Shin HD. 2008. *Hymenoscyphus ginkgonis* sp. nov. growing on leaves of *Ginkgo biloba*. Mycotaxon 103: 189–195.

Hengstmengel J. 1996. Notes on *Hymenoscyphus* II – on three non-fructicolous species of the 'fructigenus group' with croziers. Persoonia 16(2): 191–207.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Dictionary of the fungi. 10th ed. Wallingford: CABI.

Korf RP. 1973. *Discomycetes* and *Tuberales*. 249–319, in: GC Ainsworth & al. (eds). The Fungi: an Advanced Treatise, Vol. 4A. Academic press, London and New York.

Kowalski T, Bilański P. 2019. *Hymenoscyphus pusillus*, a new species on leaves of *Fraxinus pennsylvanica* in Poland. Forest Pathology 49: 1–16. https:/doi.org/10.1111/efp.12481

Kuo M. 2008. *Hymenoscyphus calyculus*. MushroomExpert.Com Web site: http://www.mushroomexpert.com/hymenoscyphus_calyculus.html [Accession date: 22.02.2023].

Lizoň P. 1992. The genus *Hymenoscyphus* (*Helotiales*) in Slovakia, Czechoslovakia. Mycotaxon 45: 1–59.

Pastirčáková K, Adamčíková K, Barta M, Pažitný J, Hoťka P, Sarvašová I, Kádasi-Horáková M. 2020. Host range of *Hymenoscyphus fraxineus* in Slovak arboreta. Forests 11(5): 1-18. https://doi.org/10.3390/f11050596

Pedersen NB, Matthiesen H, Blanchette RA, Alfredsen G, Held BW, Westergaard-Nielsen A, Hollesen J. 2020. Fungal attack on archaeological wooden artefacts in the Arctic—implications in a changing climate. Scientific Reports 10(14577): [11 p.]. https://doi.org/10.1038/s41598-020-71518-5

Phillips WA. 1887. A manual of the British discomycetes. London: Kegan Paul, Trench & Co.

Queloz V, Grünig CR, Berndt R, Kowalski T, Sieber TN, Holdenrieder O. 2011. Cryptic speciation in *Hymenoscyphus albidus*. Forest Pathology 41: 133–142. https://doi.org/10.1111/j.1439-0329.2010.00645.x

Rambaut, A. 2010. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Ronquist F, Teslenko M, Mark PVD, Ayres DL. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sesli E, Asan A, Selçuk F (eds). 2020. Türkiye mantarları listesi (The checklist of fungi of Turkey). Ali Nihat Gökyiğit Vakfı Yayını. İstanbul. 1177 p.

Sharma MP. 1991. Diversity in the Himalayan *Hymenoscyphus* S.F. Gray: an overview. 107–211, in: SP Khullar, MP Sharma (eds). Himalayan Botanical Researches. New Delhi: Ashish Publishing House.

Tamura K, Stecher G, Peterson D, Filipski AM, Kumar S. 2013. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Thind KS, Sharma MP. 1980. The genus *Hymenoscyphus* S.F. Gray in Indian. Nova Hedwigia 32: 121–132.

Uzun Y, Kaya A, Akçay ME, Demirel K. 2010. New additions to the Turkish macromycota from Bingöl province (Turkey). Turkish Journal of Botany 34: 63–66. https://doi.org/10.3906/bot-0910-187

Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, Hibbett DS. 2006. Evolution of helotialean fungi (*Leotiomycetes*, *Pezizomycotina*): a nuclear rDNA phylogeny. Molecular Phylogenetics and Evolution 41: 295–312. https://doi.org/10.1016/j.ympev.2006.05.031

Wen J, Zimmer EA. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, *Araliaceae*): inferences from ITS sequences of nuclear ribosomal DNA. Molecular Phylogenetics and Evolution 6: 167–177. https://doi.org/10.1006/mpev.1996.0069

White WL. 1943. Studies in the genus *Helotium*, III. History and diagnosis of certain European and North American foliicolous species. Farlowia 1: 135–170. https://doi.org/10.5962/p.315976

Zhang YH. 2002. Taxonomy of *Hymenoscyphus* (*Helotiales*) in China and a phylogenetic approach to the genus [in Chinese]. Dissertation for Doctoral Degree. Beijing: Institute of Microbiology, Chinese Academy of Sciences.

Zhang YH, Zhuang WY. 2002a. Re-examinations of *Helotium* and *Hymenoscyphus* (*Helotiales*, *Helotiaceae*): specimens on deposit in HMAS. Mycotaxon 81: 35–43.

Zhang YH, Zhuang WY. 2002b. New species and new Chinese records of *Hymenoscyphus* (*Helotiales*). Mycosystema 21: 493–496.

Zheng HD, Zhuang WY. 2013a. Four new species of the genus *Hymenoscyphus* (*Fungi*) based on morphology and molecular data. Science China Life Sciences 56: 90–100. https://doi.org/10.1007/s11427-012-4429-1

Zheng HD, Zhuang WY. 2013b. Four species of *Hymenoscyphus* (*Helotiaceae*) new to China. Mycosystema 32(Supp. 1): 152–159.

Zheng HD, Zhuang WY. 2013c. Three new species of *Hymenoscyphus* from tropical China. Mycotaxon 123: 19–29. https://doi.org/10.5248/123.19

Zheng HD, Zhuang WY. 2014. *Hymenoscyphus albidoides* sp. nov. and *H. pseudoalbidus* from China. Mycological Progress 13: 625–638. https://doi.org/10.1007/s11557-013-0945-z

Zheng HD, Zhuang WY. 2015. Five new species of *Hymenoscyphus* (*Helotiaceae, Ascomycota*) with notes on the phylogeny of the genus. Mycotaxon 130: 1017–1038. https://doi.org/10.5248/130.1017