Anatolian Journal of Botany
8(2): 176-180 (2024)
Research article *doi:10.30616/ajb.1530301*

First record of *Hygrophoropsis flavida* (*Hygrophoropsidaceae, Agaricomycetes*) from Türkiye

Oğuzhan KAYGUSUZ
Isparta University of Applied Sciences, Atabey Vocational School, Department of Plant and Animal Production, Isparta, Türkiye
oguzhankaygusuz@isparta.edu.tr

Received : 08.08.2024
Accepted : 06.09.2024
Online : 11.09.2024

Hygrophoropsis flavida (*Hygrophoropsidaceae, Agaricomycetes*)'nın Türkiye'den ilk kaydı

Abstract: *Hygrophoropsis flavida* is reported for the first time from southern Türkiye, based on morphological and multi-molecular phylogenetic analyses, including nucleotide sequences of the nuclear ribosomal internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) and large subunit (LSU) of the ribosomal RNA (rRNA) gene. In addition, the species was found on the rotten wood of relict endemic *Liquidambar orientalis* trees, which are presented here for the first time as a new substrate for this genus. A description of this species, based on macro- and micromorphological characteristics, is provided, along with colour photographs and line drawings.

Key words: *Agaricales*, multi-locus phylogeny, new record, nrITS, nrLSU, taxonomy, Türkiye

Özet: *Hygrophoropsis flavida*, morfolojik ve nükleer ribozomal iç transkripsiyonlu aralığının (ITS1-5.8S-ITS2 = ITS) ve ribozomal RNA (rRNA) geninin büyük alt biriminin (LSU) nükleotid dizilerini içeren moleküler filogenetik analizlere dayanarak Türkiye'nin güneyinden ilk kez rapor edilmiştir. Ayrıca, söz konusu tür, ilk kez bu cins için yeni bir konukçu olarak sunulan relikt endemik *Liquidambar orientalis* ağaçlarının çürümekte olan odun kalıntıları üzerinde bulunmuştur. Türün, makro ve mikromorfolojik özelliklerine dayanan tanımı, renkli fotoğrafları ve çizgi çizimleri ile birlikte verilmiştir.

Anahtar Kelimeler: *Agaricales*, çok lokuslu filogeni, yeni kayıt, nrITS, nrLSU, taksonomi, Türkiye

Citation: Kaygusuz O (2024). First record of *Hygrophoropsis flavida* (*Hygrophoropsidaceae, Agaricomycetes*) from Türkiye. Anatolian Journal of Botany 8(2): 176-180.

1. Introduction

Hygrophoropsis (J. Schröt.) Maire ex Martin-Sans is one of the small genera in the *Boletales* E.-J. Gilbert, (*Hygrophoropsidaceae*), with its type species *H. aurantiaca* (Wulfen) Maire ex Martin-Sans, and is primarily characterised by its lamellate hymenophore; thick, narrow, often forked, dichotomously branched and decurrent lamellae; a central stipe that is often reduced and tapering; hyaline, thin- to somewhat thick-walled, dextrinoid basidiospores; and a white basidiospore print (Kuyper, 1995; Knudsen and Vesterholt, 2008; Kibby, 2012; Holec and Kolařík, 2013). The species of the genus occurs widely in areas across both the northern and southern hemispheres (Kuyper, 1995; Knudsen and Vesterholt, 2008). *Hygrophoropsis aurantiaca* is the most widely dispersed species, occurring on multiple continents (Kuyper, 1995; Roberts and Evans, 2011). In contrast, other species within the genus are less well-known and tend to have more restricted geographic distributions (Kibby, 2012).

Hygrophoropsis is closely related to *Coniophora* DC., and *Leucogyrophana* Pouzar (Binder and Hibbett, 2006; Zmitrovich et al., 2019). Unlike most *Boletales*, species of *Hygrophoropsis* are saprotrophic, causing brown rot, and are non-ectomycorrhizal (Watling, 2008). This distinguishes them from the family *Paxillaceae* Lotsy, where the genus was previously classified (Kibby, 2012).

Hygrophoropsis includes over 18 species globally (www.catalogueoflife.org/annual-checklist/, Catalogue of Life 2024). According to the Index Fungorum database (www.indexfungorum.org, accessed 7 August 2024), 42 taxa of *Hygrophoropsis* have been documented, though some of these are considered illegitimate names or synonyms. In Türkiye, two species, *Hygrophoropsis aurantiaca* and *H. macrospora* (D.A. Reid) Kuyper, have been previously reported (Sesli et al., 2020; Solak and Türkoğlu, 2022). This study, part of ongoing research to explore macrofungal biodiversity in Türkiye, presents the first record of *Hygrophoropsis flavida* Testoni & Setti in the country, supported by both morphological and phylogenetic analyses.

2. Materials and Method

2.1. Collections and morphological analyses

Hygrophoropsis specimens were gathered during field expeditions to the Burdur and Muğla Provinces in southern Türkiye in 2015 and 2017. The characteristics of fresh specimens were observed, and morphological features were documented following the methodology described by Vellinga (1988) and Kuyper (1995). For microscopic analysis, dried samples were mounted using cotton blue, 3% KOH or Melzer's reagent. A Leica DM750 microscope was used to examine microscopic features at magnifications of up to 1000×. Measurements were taken from a minimum of 30 basidiospores, and the Q value

represents the average length-to-width ratio derived from these measurements. All specimens have been stored in the fungarium at Isparta University of Applied Sciences (ISUF).

2.2. DNA analyses techniques

The ZR Fungal/Bacterial DNA MiniPrep kit was employed to extracted from small dried specimens. To amplify DNA through Polymerase Chain Reaction (PCR), two sets of barcodes and primers were utilized. ITS1F (White et al., 1990) and ITS4 (Gardes and Bruns, 1993) were used to amplify a fragment of the Internal Transcribed Spacer (ITS) region, while LR0R (Vilgalys and Hester, 1990) and LR5 (Rehner and Samuels, 1994) were specific for nuclear ribosomal Large Subunit (28S) gene.

A total of 25 µL was used to set up the PCR reaction mixture, which contained 12.5 µL of 2× PCR Master Mix, 8.5 µL of distilled water, 2 µL of DNA template, and 1 µL of each primer. A preliminary denaturation at 95°C for 7 minutes prepared the mixture for the amplification of the rDNA ITS and LSU regions. Following this, there were 35 cycles of denaturation (for one minute) at 93°C, annealing (for 45 seconds) at 55°C, and extension (for one minute) at 72°C. The final cycle involved maintaining the temperature at 72°C for ten minutes. A 1.0% agarose gel stained with ethidium bromide was used to visualize the final PCR products.

The selection of sequences for phylogenetic analyses was guided by BLAST results obtained from both GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the UNITE database (https://unite.ut.ee/analysis.php) (Nilsson et al., 2019). The combined nrITS and nrLSU dataset was aligned using the MAFFT v7 software for multiple sequence alignment (Katoh et al., 2019) and adjusted manually in BioEdit v7.0 when necessary (Hall, 2004). Phylogenetic relationships among taxa were determined through Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. The ML analysis utilized RAxML v7.2.6 (Stamatakis, 2014) with 1,000 bootstrap replicates and the GTRGAMMA substitution model. For the BI analysis, MrBayes was used on the XSEDE platform via the CIPRES Science Gateway. This involved Markov Chain Monte Carlo (MCMC) sampling with six parallel chains running for 1 million generations, sampling trees every 1,000 generations. Branches with Maximum Likelihood Bootstrap (MLB) values of 80% or higher and Bayesian Posterior Probabilities (BPP) values of 0.90 or higher were indicated. The resulting phylogenetic tree was visualized using FigTree v1.4.2 (Rambaut, 2012).

3. Results

3.1. Phylogeny

Three sequences of *Hygrophoropsis* specimens were generated and deposited in GenBank (PQ148172-

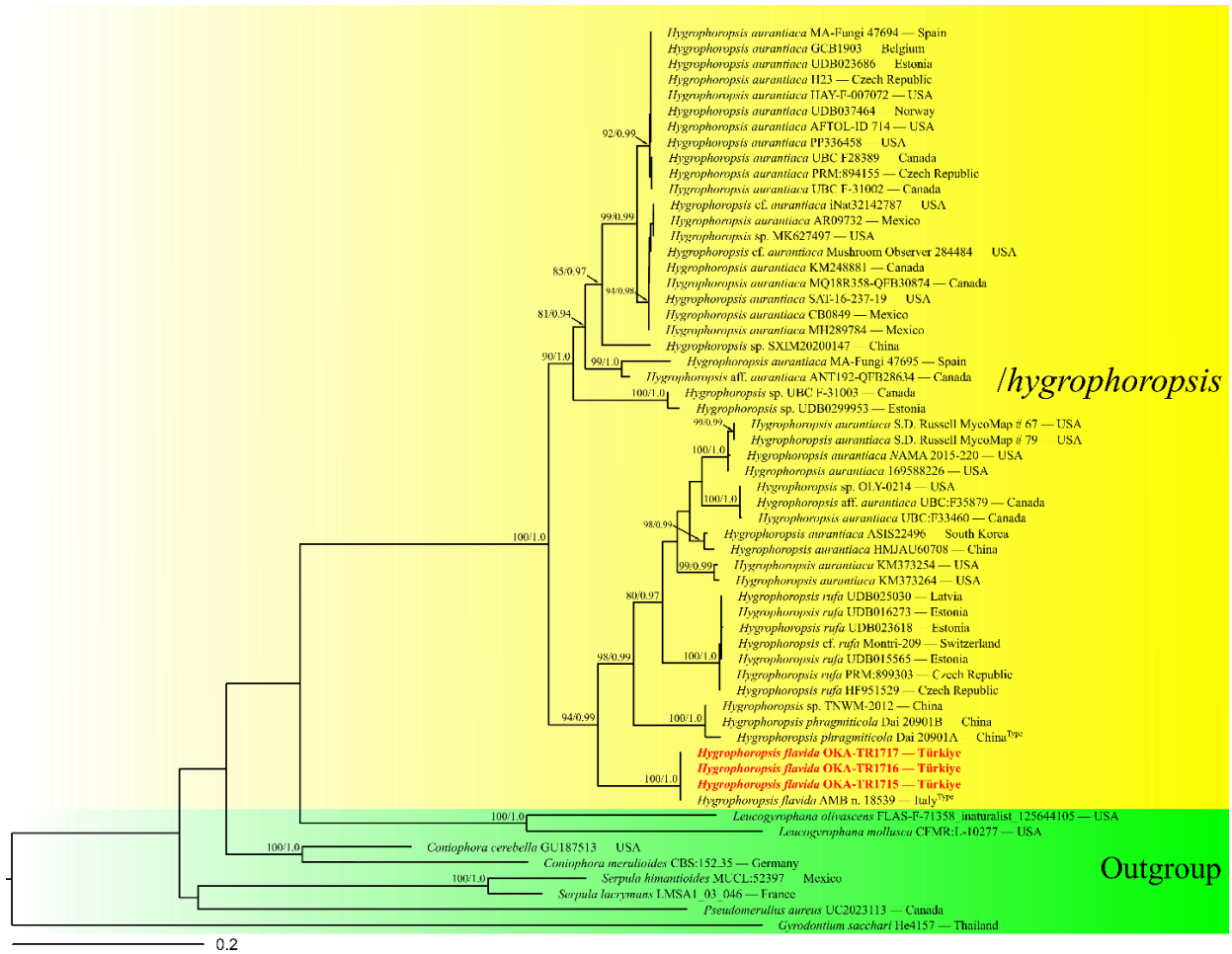

Figure 1. Phylogram generated from Maximum Likelihood (ML) analysis based on combined nrITS and nrLSU sequence data of *Hygrophoropsis*. Taxa used as outgroups in the phylogenetic analyses are highlighted in green. Newly generated sequences are shown in red.

PQ148174, PQ148178-PQ148180) as part of this study. According to the phylogenetic analyses based on rDNA ITS/LSU, the collections of *Hygrophoropsis flavida* (OKA-TR1715, OKA-TR1716, and OKA-TR1717) presented from Türkiye match the type sequence of *H. flavida* (AMB n. 18539). In addition, the collections of *Hygrophoropsis flavida* form a clade well supported by high bootstrap values (MLB = 100%, BPP = 1.0, Fig.1) and show strong statistical support for its genetic separation from other species within the genus *Hygrophoropsis.*

3.2. Taxonomy

Hygrophoropsis flavida Testoni & Setti, Riv. Micol. 64(3): 242 (2022) (Fig. 2)

Descriptions: Pileus 60–80 mm in diameter, initially convex to plano-convex, then expanding to applanate with a depressed at the centre; involute margin when young, becomes more or less inflexed, with a wavy-undulating margin when old; surface felty, creamy-whitish to yellow-cream or pale-ochraceous, slightly paler towards the margin. Lamellae crowded, decurrent to deeply decurrent, with about 10–25 mm connected to the stipe, forked and dichotomously branched, cream-coloured, then distinctly orange, with an entire, concolorous edge. Stipe 25–55 × 3.0–9.0 mm, curved and lateral, leathery or cartilaginous; surface hairy, whitish-orange, then yellowish-orange. Taste mushroom-like and odor mild. Spore print whitish to whitish-cream.

Basidiospores 5.5–6.5× 3.5–4.0 µm, Q = 1.6, ellipsoid, with a hilar appendage, smooth, thin- or slightly thick-walled, hyaline. Basidia 30–35 × 5.0–7.0 µm, clavate, 4–spored, thin-walled, hyaline. Basidioles 15–20 × 4.0–6.5 µm, narrowly clavate, thin-walled, hyaline. Pleurocystidia 35–45 × 6.0–12.0 µm, clavate to lageniform, sometimes moniliform, thin-walled, hyaline. Pileipellis a cutis of variously interwoven hyphae, branched, slightly gelatinous, 4.0–10.0 µm wide, cylindrical, hyaline; terminal hyphae 5.0–12.0 µm wide, narrowly clavate to clavate, thin-walled, hyaline. Trama of gills made up of parallel to slightly interwoven hyphae, 5.0–10.0 µm wide, cylindrical, thin-walled, hyaline. Hyphae of stipe consisting of cylindrical cells, 3.0–5.0 µm wide, thin-walled, hyaline. In all tissues, clamp connections are present.

Ecology: Saprotrophic, occurring on rotten branches or buried wood of *Liquidambar orientalis* Mill. during autumn.

Additional materials examined: Türkiye, Muğla Province, Fethiye district, in Yanıklar town, on decayed wood of *Liquidambar orientalis*, elev. 9 m a.s.l., 20 October 2015, leg. O. Kaygusuz (OKA-TR1715, GenBank nrITS: PQ148172, nrLSU: PQ148178); ibid., Köyceğiz district, in Döğuşbelen town, on *L. orientalis*, elev. 3 m a.s.l., 1 November 2016, leg. O. Kaygusuz (OKA-TR1716, GenBank nrITS: PQ148173, nrLSU: PQ148179). Burdur Province, Bucak district, close to Karacaören, in Sweetgum Forest Nature Protection Area, on wood of *L. orientalis*, elev. 255 m a.s.l., 25 October 2017, leg. O. Kaygusuz (OKA-TR1717, GenBank nrITS: PQ148174, nrLSU: PQ148180).

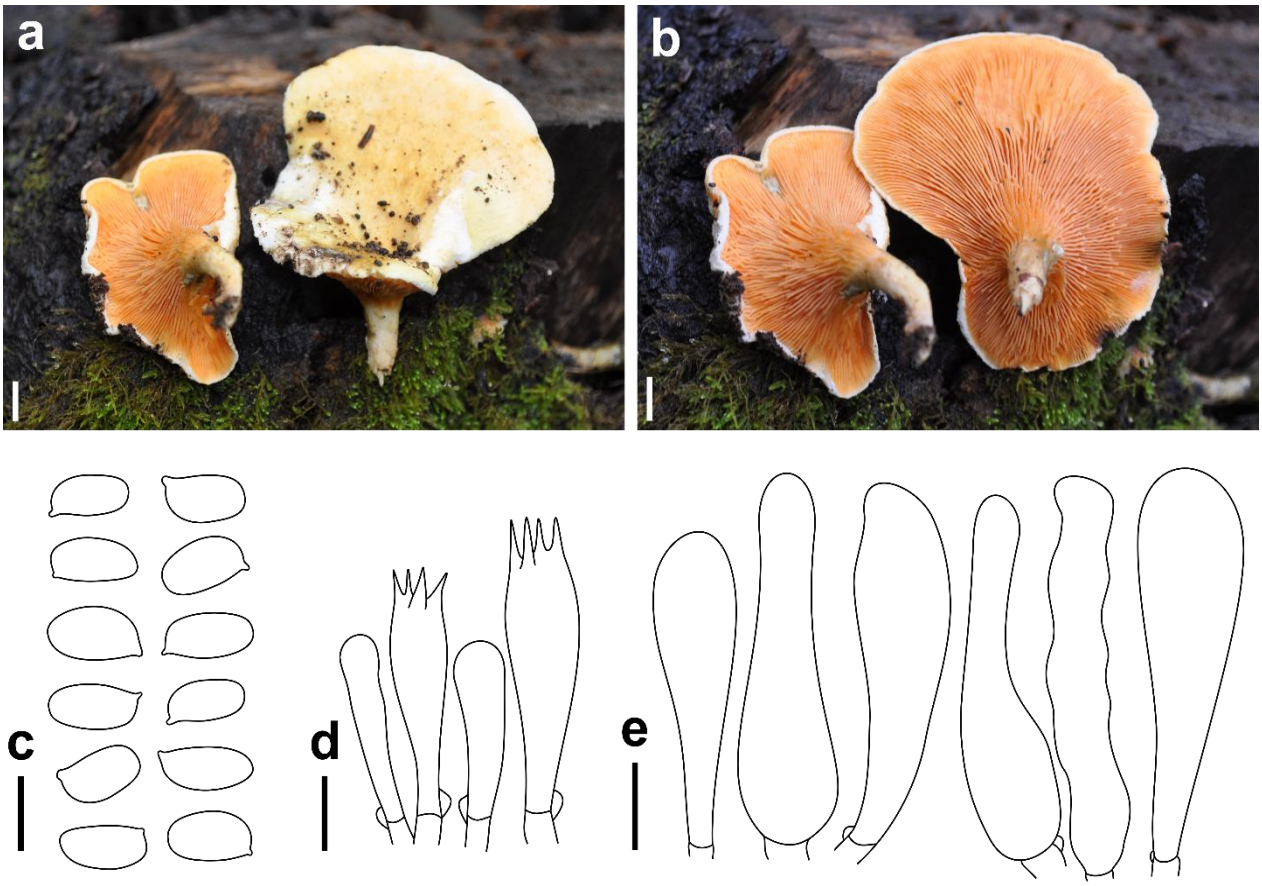

Figure 2. *Hygrophoropsis flavida*. (a-b) Basidiomata. (c) Basidiospores. (d) Basidia and basidioles. (e) Pleurocystidia. Scale bars: (a-b) = 10 mm, (c) = 5 µm, (d-e) = 10 µm.

4. Discussions

Hygrophoropsis flavida was recently described from Italy by Testoni and Setti (2022) from dead stems of *Populus* sp. Specimens of *Hygrophoropsis flavida* collected in Türkiye are reported on decaying wood of the relict endemic *Liquidambar orientalis*, representing a novel substrate for the genus *Hygrophoropsis*.

The phylogenetic results indicated that *Hygrophoropsis flavida* has a close affinity with *H. aurantiaca*, *H. phragmiticola* L.T. Ban & Meng Zhou, and *H. rufa* (D.A. Reid) Knudsen. However, morphologically, *Hygrophoropsis aurantiaca* differs from *H. flavida* by a bright to pale orange pileus with a felty-tomentose surface, a slender and darker orange stipe, and slightly longer basidiospores (5.5–7.0 × 3.0–4.0 µm) (Kuyper, 1995; Knudsen and Vesterholt, 2008; Kibby, 2012). *Hygrophoropsis phragmiticola*, originally described from China, can be distinguished by its creamy-whitish to pale-ochraceous pileus, white to cream lamellae, notably longer basidiospores (6.0–10.0 × 4.0–4.5 µm), and its growth on *Phragmites* sp. (Hongpeng et al., 2022). *Hygrophoropsis rufa* differs by having larger basidiomata (up to 100 mm in diameter), orange-brown to dark brown pileus, and usually grows on stumps or trunks of coniferous trees (Knudsen and Vesterholt, 2008; Holec and Kolařík, 2013).

Hygrophoropsis flavida also resembles some European species, such as *H. fuscosquamula* P.D. Orton and *H. macrospora*. *Hygrophoropsis fuscosquamula* differs from *H. flavida* by its dark-brownish hairs on the pileus surface, slightly longer basidiospores (6.0–8.0 × 3.5–4.5 µm), and cylindric-clavate pileipellis cells with brownish contents (Orton, 1960). *Hygrophoropsis macrospora* differs by having a pale pileus and lamellae, and distinctly longer basidiospores (up to 13 µm) (Reid, 1972; Kuyper, 1995; Krieglsteiner, 2001; Kibby, 2012; Glejdura, 2013; Gyosheva and Stoykov, 2017).

Conflict of Interest

The author has declared no conflict of interest.

Acknowledgement

The author would like to acknowledge the financial support of the Scientific and Technical Research Council of Türkiye (TUBITAK) in the 2219 International Postdoctoral Research Fellowship Programme (Grant No. 1059B192202880).

References

Binder M, Hibbett DS (2006). Molecular systematics and biological diversification of *Boletales*. Mycologia 98(6): 971-981.

Catalogue of Life (2024). Available at http://www.catalogueoflife.org/annual-checklist/ [accessed 07 August 2024].

Gardes M, Bruns TD (1993). ITS primers with enhanced specificity for *Basidiomycetes*-Application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Glejdura S (2013). New records of *Basidiomycetes* and *Ascomycetes* in the Stolické vrchy Mts. (Slovakia). Mykol. Listy 124: 15-40.

Gyosheva MM, Stoykov D (2017). *Hygrophoropsis macrospora* (*Basidiomycota*, *Boletales*): A new record for the Balkan Mycota. Phytologia Balcanica 23(2): 153-156.

Hall TA (2004). BioEdit. Ibis Therapeutics, Carlsbad, CA, 92008, USA. Website: http://www.mbio.ncsu.edu/BioEdit/bioedit.html / [accessed 07 August 2024].

Holec J, Kolařík M (2013). Notes on the identity of *Hygrophoropsis rufa* (*Basidiomycota*, *Boletales*). Czech Mycology 65(1): 15-24.

Hongpeng Y, Meng Z, Gang W, Litong B (2022). A new and edible species of *Hygrophoropsis* (*Boletales*, *Basidiomycota*) from North China. Mycosystema 41(2): 181-189.

Index Fungorum (2024). Search Index Fungorum. http://www.indexfungorum.org/names/Names.asp/ [accessed 07 August 2024].

Katoh K, Rozewicki J, Yamada KD (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160-1166.

Kibby G (2012). The *Hygrophoropsis aurantiaca* complex. Field Mycology 13(2): 43-50.

Knudsen H, Vesterholt J (2008). Funga Nordica: Agaricoid, boletoid and cyphelloid genera. Copenhagen: Nordsvamp.

Krieglsteiner GJ (2001). Die Groβpilze Baden-Würtenbergs. Band 3. Ständerpilze: Blätterpilze I. Stuttgart: Eugen Ulmer Verlag.

Kuyper TH (1995). Genus *Hygrophoropsis*. In: Bas C, Kuyper TW, Noordeloos ME, Vellinga EC (eds.) Flora Agaricina Neerlandica. vol. 3. Boca Raton, USA: CRC Press.

Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47(D1): 259-264.

Orton PD (1960). New check list of British agarics and boleti: Part III. Notes on genera and species in the list. Transactions of the British Mycological Society 43(2): 159-384.

Rambaut A (2012). FigTree, version 1.4.2. Edinburgh: University of Edinburgh.

Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625-634.

Reid DA (1972). Fungorum rariorum icones coloratae. Lehre: Pars VI.

Roberts P, Evans S (2011). The book of fungi. Chicago: University of Chicago Press.

Sesli E, Asan A, Selçuk F (eds.) Abacı Günyar Ö, Akata I, Akgül H, Aktaş S, Alkan S, Allı H, Aydoğdu H, Berikten D, Demirel K, Demirel R, Doğan HH, Erdoğdu M, Ergül C, Eroğlu G, Giray G, Halikî Uztan A, Kabaktepe Ş, Kadaifçiler D, Kalyoncu F, Karaltı İ, Kaşık G, Kaya A, Keleş A, Kırbağ S, Kıvanç M, Ocak İ, Ökten S, Özkale E, Öztürk C, Sevindik M, Şen B, Şen İ, Türkekul, İ, Ulukapı M, Uzun Ya, Uzun Yu, Yoltaş A (2020). The checklist of fungi of Turkey. İstanbul: Ali Nihat Gökyiğit Vakfı Yayını.

Solak MH, Türkoğlu A (2022). Macrofungi of Turkey, Checklist vol. III. İzmir: Kanyilmaz Matbaacılık Kağıt ve Ambalaj Sanayi Ticaret Ltd. Şti.

Stamatakis A (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312-1313.

Testoni A, Setti L (2022). *Hygrophoropsis flavida*: Una nuova specie trovata nel Parco del Delta del Po. Rivista di Micologia 64(3): 239-452.

Vellinga EC (1988). Glossary. In: Bas C, Kuyper Th.W, Noordeloos ME, Vellinga EC (eds.) Flora Agaricina Neerlandica, vol. 1. Rotterdam: A.A. Balkema.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246.

Watling R (2008). A manual and source book on the boletes and their allies. Synopsis Fungorum. vol. 24. Oslo: Fungiflora.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) PCR protocols: a guide to methods and applications. New York: Academic Press.

Zmitrovich IV, Kalinovskaya NI, Myasnikov AG (2019). Funga photographica. *Boletales* I: *Coniophoraceae*, *Hygrophoropsidaceae*, *Paxillaceae*, *Serpulaceae*, *Tapinellaceae* boreales. Folia Cryptogamica Petropolitana 7: 58.