A new species of *Hygrophorus*, *H. yadigarii* sp. nov. (Hygrophoraceae), with an isolated systematic position within the genus from the Colchic part of Turkey

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Abstract: *Hygrophorus yadigarii* (Hygrophoraceae/Basidiomycota) is described as a new species for science based on basidiomata collected from Maçka, Trabzon, Turkey. The new taxon is quite different even from the closest relatives, easily distinguished by the other species because of its grayish to ash-colored, gregarious to subcaespitose, sticky basidioma; a slightly umbonate to depressed pileus; a cylindrical to clavate, grayish stipe; ellipsoid and smooth basidiospores; quite long basidia; clavate, cylindrical or narrowly utriform, apically pyriform or strangulated cheilocystidia; and gelatinous pileipellis. A description with field and micromorphological illustrations, a phylogenetic tree, a simple key, a comparison chart including similar species, and a short discussion are provided.

Key words: Colchic, *Hygrophorus*, Maçka, new species, Trabzon

1. Introduction

The genus *Hygrophorus* belongs to the family Hygrophoraceae, proposed by Roze (1876). Singer (1986) recognized seven genera in that family: *Hygrophorus*, *Neohygrocybe* Singer (= *Pseudoomphalina*), *Hygrotrama* Singer (= *Camarophyllopsis*), *Camarophyllus* (Fr.) P.Kumm., *Hygrocybe* (Fr.) P.Kumm., *Hygroaster* Singer (= *Hygrocybe*, see below), and *Omphaliaster* D.Lam.

Recent molecular studies have shown that many known entities can be classified in Hygrophoraceae such as *Acantholichen* P.M.Jørg.; *Ampulloclitocybe* Redhead, Lutzoni, Moncalvo & Vilgalys; *Arrhenia* Fr.; *Cantharellula* Singer; *Cantharocybe* H.E.Bigelow & A.H.Sm.; *Chromosera* Redhead, Ammirati & Norvell; *Chrysomphalina* Clémence; *Corylophus* (Donk) Bon; *Cyphellostereum* D.A.Reid; *Dictyoenema* Reinsch; *Eonema* Redhead, Lücking & Lawrey; *Gliophorus* Herink; *Haasiella* Kotl. & Pouzar; *Humidicutis* (Singer) Singer; *Hygroaster* Singer; *Hygrocybe* (Fr.) P.Kumm.; *Hygrocybe* (Fr.) P.Kumm., *Lichenomphalia* Redhead, Lutzoni, Moncalvo & Vilgalys; *Neohygrocybe* Herink; *Porporolomopsis* Bresinsky; and *Pseudoarmillariella* (Singer) Singer (Boertman, 1995; Lodge et al., 2014).

*Hygrophorus* Fr. is a large genus with more than 900 records worldwide (Kirk et al., 2008), represented by 30 species in Turkey (Demirel et al., 2003; Doğan et al., 2007; Sesli and Denchev, 2008; Akata and Doğan, 2015; Uzun et al., 2017), and it is characterized by its tricholomatoid, collybioid, clitocyboid, or omphalinoid small to large, thin to fleshy, dry to very glutinous or viscid basidioma; a whitish, dull-colored, gray, brownish, yellowish, orange, or reddish dry to fairly sticky pileus; closely or widely spaced, adnate to decurrent, typically thick and waxy, generally whitish, sometimes yellowish or pinkish lamellae; a dry to glutinous, glabrous or fibrillose, generally pruinose or granulose stipe; an often thick, sometimes reddening or yellowing whitish content; and long, narrowly clavate basidia and smooth, hyaline, nonamyloid basidiospores (Singer, 1986; Boertmann, 1995; Young, 2005; Kovalenko, 2012).

According to the monograph by Hesler and Smith (1963), section *Hygrophorus* is divided into 8 subsections due to the macromorphology of the basidioma: subsect. *Chrysodontini* Singer (with yellow granules on the basidioma), subsect. *Pallidini* A.H.Sm. & Hesler (basidioma slightly greasy), subsect. *Hygrophorus* (basidioma distinctly slimy), subsect. *Discoidei* (Bataille) Konrad & Maubl. (basidioma yellowish, yellow-brown to reddish), subsect. *Fulvoincarnati* A.H.Sm. & Hesler (basidioma more or less orange colored), subsect. *Discoidi* (Bataille) Konrad & Maubl. (basidiomata yellowish, yellow-brown to reddish), subsect. *Olivaceombrini* Bataille (basidioma partly slimy and usually shiny), and subsect.

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In this paper, a new species belonging to the genus Hygrophorus collected in Turkey, *Hygrophorus yadigarii*, sp. nov., is fully described and discussed.

2. Materials and methods

The basidiomata of *Hygrophorus yadigarii* were collected from the Sevinç neighborhood of Maçka, Trabzon, Turkey, on 3 November 2016. Color slides of the basidiomata were taken at the collection site. The pileus of one basidioma was used to obtain a spore print and the others were dried for future microscopic studies. Very thin microscopic sections were obtained from the basidiomata, treated in concentrated ammonia solution and subsequently in Congo red, and finally investigated under a Zeiss Axio Imager A2 trinocular research microscope (Clémonçon, 2009). Microscopic structures such as basidiospores, basidia, and cystidia were measured with Axio Imager software. The holotype materials are kept at the herbarium of the Fatih Faculty of Education at Karadeniz Technical University (KATO), Trabzon, Turkey.

2.1. DNA extraction, PCR amplification, and DNA sequencing

Total DNA was extracted from the dry specimens employing a modified protocol based on Murray and Thompson (1980). A portion of each sample was blended with the aid of a micropestle in 600 µL of CTAB buffer (CTAB 2%, NaCl 1.4 M, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform and isooamyl alcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13,000 × g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifuged again for 2 min, and dried. It was finally resuspended in 200 µL of ddH₂O. PCR amplification was performed with primers ITS1F and ITS4 (White et al., 1990; Gardes and Bruns, 1993) for the ITS region, while LR0R and LR5 (Vilgalys and Hester, 1990; Cubeta et al., 1991) were used to amplify the 28S rDNA region. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C, and 72 °C (45, 30, and 45 s, respectively) and a final 72 °C step for 10 min. PCR products were checked on 1% agarose gels, and positive reactions were sequenced with one or more PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

2.2. Sequence alignment and phylogenetic analysis

BLAST (Altschul et al., 1997) was used to select the most closely related 28S rDNA sequences from INSD public databases. Sequences came mainly from Lutzioni (1997), Larsson et al. (2004), Matheny et al. (2006), Geml et al. (2012), Lucking et al. (2013), and Lodge et al. (2014), among others. Sequences were first aligned in MEGA 5.0 software (Tamura et al., 2011) with its ClustalW application and then corrected manually. The final alignment included 352/700 variable sites. Final alignment was subjected to MrModeltest 2.3 (Nylander, 2004) in PAUP* 4.0b10. Model GTR+I+G was selected and implemented in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), where a Bayesian analysis was performed (data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 3.38M generations, standard deviation having fallen below 0.01. The first 25% of trees were discarded as burn-in. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis, 2006) using the standard search algorithm (2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

3. Results

3.1. Molecular analysis

Topology obtained with 28S rDNA failed to resolve only the most suprageneric relationships among the Hygrophoraceae genera included in the analysis, in contrast with the 4-gene analysis by Lodge et al. (2014), although it was enough to support the monophyletic status of most of these genera. *Hygrocybe* and *Hygroaster* were not discriminated by 28S rDNA analysis, while no significant support for the monophyletic status of the Lichenomphaloideae genera *Arrhenia* and *Omphalina* was found, and only partial support for *Dictyonema* and *Lichenomphalia* was recovered, in agreement with the multigeneric analysis of Lodge et al. (2014). The 28S rDNA sequence obtained from the sample KATO - Fungi 3843 (holotype of the new species *Hygrophorus yadigarii*) was found to be significantly related to the genus *Hygrophorus*, but no significant similarity with the sections delimited by Lodge et al. (2014) could be found, suggesting that it might represent a new one.

3.2. Taxonomy

*Hygrophorus yadigarii* E. Sesli, Antonín & Contu, sp. nov. (Figures 1–5)

- GenBank Acc. No: MF370228
- MycoBank No: MB 821862

**Diagnosis:** Basidiomata tricholomatoid, gregarious to subcaespitose in mixed forests, medium sized, fairly sticky; the surface of pileus grayish, slightly yellowish at some
places (probably slime layer leaving yellowish stains in age), margin often cleft; center usually slightly umbonate; stipe grayish and lamellae slightly bluish white.

**Holotype:** Turkey, Trabzon, Maçka, Sevinç neighborhood, 40°50′51.06″N, 39°37′41.07″E, 751 m alt., 03.11.2016, leg. E. Sesli (KATO - Fungi 3843).

**Etymology:** The specific epithet honors the father of the first author (Yadigar Sesli), who died in 2016.

**Pileus** 20–60 mm wide; hemispherical when young, later convex to plane; slightly depressed in the center; somewhat irregular, sometimes cleft and undulating; umbo indistinct or broadly hill-shaped; surface very slimy-lubricous when moist, viscid when dry; the edges are raised up in maturity; margin incurved for a long time; the surface grayish, ash-colored, slightly yellowish at some places (probably slime layer leaving yellowish stains in age); slightly fibrillose to floccose. **Lamellae** adnate; becoming subdistant or subdecurrent in age; whitish to creamy, slightly bluish; broad; thick; waxy; \( L = 35–45, I = 1–3 \). **Stipe** 40–70 mm long and 5–10 mm wide; cylindrical to clavate; grayish; longitudinally fibrous; somewhat larger toward the base; sometimes flattened; base coated with white mycelium; sometimes twisted or compressed and grooved. **Context** slightly bluish white and soft. **Odor** and **taste** not distinctive.
Figure 2. The 50% majority rule consensus 28S rDNA phylogram of the Lichenomphalioideae, Hygrophoroideae, and Hygrocyboideae lineages of the Hygrophoraceae obtained in MrBayes from 25,350 sampled trees. Nodes supported by 0.95 Bayesian PP or 70% ML BP are shown annotated.
Basidiospores (6.5–)7.5–8.5(–9.5) × (4.3–)5.5(–6.3) µm (n = 54 and Q = 1.5–1.7), on average 8.1 × 5.2 µm (basidiospores rarely very large, e.g., 16 × 9 µm from 2-spored basidia); ellipsoid; hyaline; smooth; always with drops; with a distinct apiculus; medium to thin-walled; greenish in water and pale yellow in Congo red. Spore deposit white. Basidia slenderly clavate; 55–70(–72) × (7.5–)8–9(–9.5) µm (n = 15); 2–4-spored; some with siderophilous granules; sterigmata long (5–7.5 µm). Basidioles 45–60(–62) × 5.5–7.5(–7.7) µm. Cheilocystidia present; 30–60(–70) × (3.9–)4.5–7.5(–9) µm; variable; consisting of clavate, cylindrical or narrowly utriform, apically pyriform, strangled, sinuous clavate; slightly thick- to thin-walled; hyaline elements (n = 15). Pleurocystidia absent. Pileipellis made up of irregular to parallel hyphae; (2.8–)4–8(–12.3) µm across; occasionally branched; some hyphal ends ascending and embedded in a gelatinous matter. Subpellis consists of elongate hyphae 8.5–20 µm wide. Clamp connections present in all tissues.

Ecology and distribution: Gregarious to subcaespitose in hornbeam-spruce dominated forest, on soil, in debris, under hornbeam. Autumn. Known only from the Colchic part of Turkey.

4. Discussion
Hygrophorus yadigarii can be distinguished from other closely related species because of its tricholomatoid, grayish to ash-colored, gregarious to subcaespitose, fairly sticky basidioma; a slightly umbonate to depressed, somewhat irregular, 20–60 mm wide pileus; a cylindrical to clavate, grayish, longitudinally fibrillose, 40–70 mm stipe; ellipsoid, hyaline, smooth, 8.1 × 5.2 µm basidiospores; slenderly clavate and very long (55–70 µm) basidia; clavate, cylindrical or narrowly utriform, apically pyriform

Figure 3. Hygrophorus yadigarii: a, b, and c- cross-sections through the lamella (scale bars: 10 µm). Photos by E Sesli.
or strangulated cheilocystidia; and a gelatinous pileipellis (Table).


*Hygrophorus tephroleucus* differs from *H. yadigarii* by the gray brown to dark brown pileus, decurrent lamellae, a whitish stipe, larger (9–11 × 5–6 µm) basidiospores, and smaller basidia (40–60 × 6–8 µm). *Hygrophorus mesotephrus* differs by a smaller (20–30 mm), bell-shaped or convex, pale grayish brown pileus; decurrent and white lamellae; larger (8.5–12 × 5.5–7.5 µm), ellipsoid to oblong ovoid basidiospores (Bon, 1990; Kovalenko, 2012).

The other morphologically similar species from Europe, *H. pustulatus*, has a smaller (15–35 mm), slightly viscid, scaly, gray brown pileus; subdecurrent lamellae; smaller (50–65 × 7–9 µm) basidia; and lacks cheilocystidia (Bon, 1990; Breitenbach and Kränzlin, 1991).

*Hygrophorus fuligineus* has a larger (40–120 mm), blackish, clove brown to brownish olive, grayish brown or olive-gray pileus; a longer stipe (40–100 mm); and lacks cheilocystidia. *Hygrophorus occidentalis* is different from our new species by its slightly larger (20–80 mm) and variably colored (hairs brown to fuscous, yellowish or smoky, white to pale cinereous) pileus; smaller (6–8 × 3.5–

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**Figure 4. Hygrophorus yadigarii**: a- basidiospores, b and c- pileipellis (scale bars: a = 10 µm, b and c = 20 µm). Photos by E Sesli.
5 µm) basidiospores; and the absence of cheilocystidia. Another close species from North America, *H. virgatulus*, has a smaller (25–50 mm), whitish-brownish pileus; narrower (3.5–5 µm) basidiospores; and smaller basidia (32–54 × 5–7 µm) (Hesler and Smith, 1963).

Genetically, 28S rDNA data suggest that *H. yadigarii* is different from all records in public databases. BLAST top matches are *Hygrophorus hyacinthinus*, *H. pustulatus*, and *H. agathosmus* (95% similarity), although ITS data are almost 20% different from all other *Hygrophorus* ITS sequences available.

*Hygrophorus hyacinthinus* has a larger (20–100 mm), grayish to dirty white pileus; a taller (50–100 mm), dry, smooth, naked, dirty white or light gray stipe; larger (7.5–11 × 4.5–6.2 µm) basidiospores; and grows in coniferous forests (Hesler and Smith, 1963; Bon, 1990; Kovalenko, 2012). Another molecularly close species, *H. pustulatus*, has a smaller (20–45 mm), convex, at times papillate, ashy to darker brownish, fibrillose pileus; somewhat decurrent, bluntly adnate, close to subdistant, pure white lamellae; a dark-gray punctate stipe; and lacks cheilocystidia (Hesler and Smith, 1963; Bon, 1990). *Hygrophorus agathosmus* differs from *H. yadigarii* with its larger (40–80 mm), ashy gray, glutinous to viscid, glabrous pileus; white to sordid grayish lamellae; a whitish to pale ashy, solid, dry or moist, evenly fibrillose pruinose stipe; larger (8–10.5 × 4.5–5.5 µm) basidiospores; smaller basidia (48–65 × 6–8 µm); and lack of cheilocystidia (Hesler and Smith, 1963; Bon,
According to some studies, *Hygrophorus agathosmus* (Fr.) Fr. and *Hygrophorus hyacinthinus* Quél. are the same taxon, but this needs to be confirmed with further studies (Kirk et al., 2008).

Phylogenetic analysis of 28S rDNA did not recover a significant relationship between *H. yadigarii* and any of the other *Hygrophorus* sections as defined by Lodge et al. (2014), maybe because of the monogenic approach employed here, or else because *H. yadigarii* actually represents a different, independent section within *Hygrophorus*. To test this hypothesis, a thorough multigenic review of *Hygrophorus* should be conducted.

### 4.1. A simple key to the *Hygrophorus* species close to *H. yadigarii*

We prepared a simple key below including the close relatives of *Hygrophorus yadigarii* to date. The key is in accordance with those of Hesler and Smith (1963), Bon (1990), Breitenbach and Kränzlin (1991), Boertman (1995), and Kovalenko (2012).

<table>
<thead>
<tr>
<th>Character</th>
<th><em>H. fuliginous</em></th>
<th><em>H. mesoteaphrus</em></th>
<th><em>H. occidentalis</em></th>
<th><em>H. pastulatus</em></th>
<th><em>H. tephroleucus</em></th>
<th><em>H. virgutalus</em></th>
<th><em>H. yadigarii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pileus</td>
<td>Blackish brownish, grayish brown or olive-gray</td>
<td>Pale drab, cinereous, buff, avellaneous</td>
<td>Hair brown, fuscous, yellowish, smoky, white</td>
<td>Ashy with a darker brownish disc</td>
<td>Pulwod-cinereous, dark ash, gray, pale ash gray</td>
<td>Whitish with a brownish disc</td>
<td>Grayish, ash-colored</td>
</tr>
<tr>
<td>Stipe</td>
<td>White</td>
<td>White</td>
<td>Concolorous with the pileus</td>
<td>Whitish</td>
<td>White</td>
<td>White</td>
<td>Grayish</td>
</tr>
<tr>
<td>Spores</td>
<td>7–9 × 4.5–5.5 μm</td>
<td>8–11 × 5–6 μm</td>
<td>6–8 × 3.5–5 μm</td>
<td>7–9 × 4–5 μm</td>
<td>8–10 × 4–5 μm</td>
<td>7–9 × 3.5–5 μm</td>
<td>7.5–8.5 × 4.5–5.5 μm</td>
</tr>
<tr>
<td>Basidia</td>
<td>4-spored, 38–38 × 8–10 μm</td>
<td>4-spored, 45–60 × 9–10 μm</td>
<td>4-spored, 29–43 × 6–8 μm</td>
<td>4-spored, 46–61 × 6–8 μm</td>
<td>4-spored, 40–58 × 6–8 μm</td>
<td>4-spored, 32–54 × 3–7 μm</td>
<td>2- and 4-spored, 55–70 × 8–9 μm</td>
</tr>
<tr>
<td>Habitat</td>
<td>In coniferous and mixed woods</td>
<td>Under oak</td>
<td>In mixed oak-pine</td>
<td>Under fir and redwood</td>
<td>Under conifers</td>
<td>In open woods</td>
<td>In debris under hornbeam</td>
</tr>
</tbody>
</table>

| 1 Pileus up to 100–120 mm | 2 |
| 1* Pileus smaller | 4 |
| 2 Under beech, stipe whitish to pale ash…*H. agathosmus* | 3 |
| 2* Under other trees | 3 |
| 3 Stipe white, 40–100 mm | 4 |

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