



Taxonomy and phylogenetic appraisal of *Hypomyces iranica* sp. nov. (*Hypocreaceae*, *Hypocreales*)

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Abstract

Hypomyces is an important genus of fungicolous fungi associated with different ascomycetes and basidiomycetes. Numerous researchers have conducted morphological and molecular studies on this genus. In this study, we collected submerged branches of *Fagus* sp. from Mazandaran Province in Iran, from which a fungal isolate was obtained. Morphology coupled with maximum likelihood and Bayesian inference analyses of the combined ITS, LSU, and *rpb2* sequence data showed it is a novel taxon (*Hypomyces iranica*). The morphology of the newly described species is compared with its sister taxa, and a comprehensive description and micrographs are provided.

Keywords – 1 new species – Mazandaran – morphology – multilocus phylogeny – saprobes

Introduction

Hypomyces (Fr.) Tul. & C. Tul is an important genus in *Hypocreaceae* (*Hypocreales*) (Yu et al. 2020). The genus was introduced as a subgenus of *Hypocrea* Fr. and elevated to a genus with *H. lactifluorum* (Schwein.) Tul. & C. Tul. as the type species (Tulasne & Tulasne 1860). The first detailed taxonomic study of this group was carried out by Arnold (1971), who distinguished *Hypomyces* from the related genera *Arachnocrea*, *Apiocrea*, and *Peckiella*. Several asexual morphs (Arnold 1969, 1970, Pöldmaa 2000, Zare & Games 2016) of *Hypomyces* were reported, mostly cladobotryum-like asexual morph with verticillate conidiophores and conidia produced in a basipetal succession. The sexual morph name is predominant over the asexual name. Thus, the International Code of Nomenclature for algae, fungi, and plants (ICN) recommended *Hypomyces* over *Cladobotryum* (McNeill et al. 2012; Rossman et al. 2013, Hyde et al. 2020). Following Arnolds' classification for *Hypomyces* (1971), subsequent comprehensive taxonomic studies were conducted on classifying this genus (Rogerson & Samuels 1985, 1989, 1993, 1994, Pöldmaa 1996, 2000, 2003, 2011, Pöldmaa et al. 1997, 1999). The members of this genus are distributed in different regions, *i.e.*, Australia, Asia, Europe, and North America (Zeng & Zhuang 2016, Lechat et al. 2017, Zare & Gams 2016, Rogerson & Samuels 1989), and currently, 118 *Hypomyces* species are listed in the Index fungorum (2022).

Host range and morphological diversity are key determinants of *Hypomyces* (Kim et al. 2017). It is characterized by perithecia in a concolorous subiculum with brightly or lightly colored,

cylindrical asci with fusiform thickened apex and septate (usually two-celled) or aseptate ascospores that are biapiculate and warted (Pöldmaa 2000, Lechat et al. 2017, Hyde et al. 2020). Phylogenetic analyses demonstrated that this group is monophyletic and supported by subsequent phylogenetic analyses of the nuclear large subunit rDNA (LSU) data (Pöldmaa et al. 1999, Pöldmaa 2000, Pöldmaa & Samuels 2004). In addition to the morphological analyses, phylogenetic analyses using the internal transcribed spacer (ITS), nuclear large subunit rDNA, (LSU), RNA polymerase II subunit 1 (*rpb1*), RNA polymerase II subunit 2 (*rpb2*), and translation elongation factor 1 α (*tef1*) markers have been used to identify species in this genus (Rehner & Samuels 1995, Pöldmaa 2000, Pöldmaa et al. 2000, Pöldmaa 2011, Zare & Gams 2016). Some *Hypomyces* species and their sexual morphs were reported from Iran (Asef & Mohammadi Goltapeh 2002, Zare & Khabbaz-Jolfaei 2005, Asef & Zare 2006), but their diversity in Iran has not been explicitly considered. In this study, a new species of *Hypomyces* was isolated from submerged plant material in Mazandaran Province, Iran, and is here described, illustrated, and compared with other taxa.

Materials & Methods

Sample collection, isolation, and morphological study

Fresh samples were collected from submerged branches of *Fagus* sp. in Sangdeh forest, Mazandaran Province, Iran, in July 2017 and taken to the laboratory in zip-lock plastic bags. Fungal fruiting bodies were examined using SMZ 168 Series stereomicroscope (Motic, Xiamen, China). Isolation of single spores was performed to obtain pure culture, and germinated spores were cultivated on potato dextrose agar (PDA) media at 25°C for one week. Pure cultures were inoculated on malt extract agar (MEA), PDA, and potato carrot agar (PCA) and incubated for ten days under darkness at 25 °C to observe the macro and micro-morphologies, including colony features, pigmentation, conidiophores structure, branching, phialides, conidia, and resting structures. Morphological characters were photographed using a digital camera (Nikon DS-Ri2, Tokyo, Japan) fitted to a compound microscope (Nikon ECLIPSE Ni, Tokyo, Japan), and the measurements were obtained using Tarosoft (R) Image Frame Work version 0.9.7. The living culture was deposited at the Microbial of Agriculture Biotechnology Research Institute of Iran Culture Collection, Karaj, Iran (ABRIICC), and the herbarium specimen at the Ferdowsi University of Mashhad Microorganisms Collection (FUM). Facesoffungi and Index Fungorum numbers were obtained as explained in Jayasiri et al. (2015) and Index Fungorum (2022).

Fungal biomass preparation, DNA extraction, PCR amplification, and sequencing

Fungal biomass and DNA extractions were performed according to the protocol described by Rapley (2000). The quality of the DNA was examined in 1% agarose gel visualized in a spectrophotometer (Biochrom™ WPA Biowave II UV-Vis). DNA samples were subjected to polymerase chain reaction (PCR) using the primers and conditions described in Table 1. The LSU, ITS, and *rpb2* genomic regions were amplified. The PCR mixture was composed of PCR Buffer 10 x 2.5 μ l, MgCl₂ 1 μ l (1.5 mM), dNTPs (mix) 1 μ l (0.2 mM), 1 μ l of each primer, 0.3 U of Taq DNA polymerase enzyme (5 units/ μ l, Genet Bio, South Korea), 2 μ l of DNA template (20–30 ng/ μ l), and deionized water to adjust the final volume to 25 μ l. The PCR products were visualized on 1% agarose gels containing the Safeview DNA stain (Bioneer, South Korea) and sequenced by Bioneer Company (Bioneer, South Korea). The newly generated sequences were deposited in the GenBank database.

Phylogenetic analysis

The obtained sequences of ITS, LSU, and *rpb2* were assembled and submitted to BLASTn searches against the NCBI non-redundant database for identifying closely related taxa. Sequences data of related taxa were obtained from the literature (Pöldmaa et al. 2000, 2019, Tokiwa & Okuda 2005, Jaklitsch et al. 2008, Pöldmaa 2011, Taam & Pöldmaa 2013, Zare & Gams 2016, Sun et al. 2019a, Vu et al. 2019, Zeng & Zhuang 2019) and downloaded from the GenBank database (Table

2). The sequences were aligned using MAFFT version 7 (Kato et al. 2019) and manually edited via BioEdit version 7.0.9 (Hall 1999). Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were run for both individual (ITS, LSU, and *rpb2*) and combined gene datasets (ITS-LSU-*rpb2*) on the CIPRES Science Gateway portal (CIPRES) (Miller et al. 2012). The ML tree analysis was carried out using RAxMLHPC2 on the XSEDE (v. 8.2.10) tool (Stamatakis 2014) under the GTRCAT substitution model and 1,000 bootstrap iterations. For BI, the optimal substitution model for each region was determined using jModelTest2 on the CIPRES, under the Akaike information criterion (AIC) (Darriba et al. 2012). The TIM2+I+G model (-lnL = 10235.4969) was selected as the optimal for ITS gene region, while TIM2+I+G (-lnL = 6918.4799) and SYM+I+G (-lnL = 10643.7712) were selected for LSU and *rpb2*, respectively. Bayesian analysis was performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES with four simultaneous Markov Chains run for 5,000,000 generations. Phylogenetic trees were visualized with FigTree version 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) and reorganized in Microsoft PowerPoint (2016).

Table 1 Gene regions used in this study with primers, PCR conditions, and references.

Gene Regions	Primers	PCR conditions	References
LSU	LR0R/LR5	94 °C for 3 min, 94 °C for 30 s, 50 °C for 45 s, 72 °C for 2 min (40 cycles), 72 °C for 10 min	Vilgalys & Hester (1990), Rehner & Samuels (1994)
ITS	ITS5/ITS4	95°C for 4 min, 94 °C for 45 s, 56 °C for 1min, 72 °C for 2 min (40 cycles), 72 °C for 10 min	White et al. (1990)
<i>rpb2</i>	fRPB2-5f/fRPB2-7cr	95 °C for 5 min, 95 °C for 1 min, 55 °C, 1.25 min, 72 °C for 2 min (35 cycles), 72 °C for 10 min	Liu et al. (1999)

Results

BLASTn searches and Phylogenetic analysis

The BLASTn similarity searches of the ITS sequence resulted in *H. tubariicola* strains, CBS 386.73 (MH860710), CBS 115.79 (NR_158483), and 115.79 (KU382164) as the closest species, all with 96% of identity. For the LSU, the highest sequence identities to our isolate were *H. tubariicola* strains NIBRFG0000501019 (KY783351) and CBS 115.79 (MH872953), with 98% similarities for each, and the *rpb2*, *H. hubeinsis* strain 9791 (MK484605) and *Sepedonium chalcipori* strain KSH558 (KU041495) resulted with 87% and 86% similarities, respectively.

The combined ITS, LSU, and *rpb2* dataset consisted of 122 sequences (Table 2), with *Trichoderma harzianum* (CBS 226.95), *T. hamatum* (DAOM 167057), and *T. viride* (CBS 119325) as outgroup taxa. The combined dataset contained 3,395 characters (LSU=1,168, ITS=656, *rpb2*=1,571) after alignments, including gaps. The final ML optimization likelihood value of the best RAxML tree was -34575.353885, and the matrix had 1,660 distinct alignment patterns, with 51.55% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.240306, C = 0.276734, G = 0.269899, T = 0.213061; substitution rates AC = 1.488201, AG = 3.029751, AT = 1.349401, CG = 0.844580, CT = 6.404318, GT = 1.0; gamma distribution shape parameter α = 0.191465. The ML and BI analyses yielded a similar tree topology (Fig 1). In our phylogenetic analyses, the new species *Hypomyces iranica* (ABRIICC 10210) formed a phylogenetically distinct lineage with 79% ML bootstrap and 0.97 posterior probability support. The closest relative species were *H. tubariicola* (CBS 225.84), *H. tubariicola* (CBS 115.79), *H. hubeiensis* (HMAS 254597) and *H. subglobosus* (CBS 484.83) (Fig 1).

Base pair comparisons of the ITS, LSU, and *rpb2* sequences of *H. iranica* (ABRIICC 10210) with *H. hubeiensis* (HMAS 254597) strain showed 15/650 (2.30%), 4/800 (0.50%) and 133/1060 (12.54%) base pairs differences, respectively. The comparisons of the ITS and LSU sequences of *H. iranica* (ABRIICC) with *H. tubariicola* (CBS 225.84) strain showed 19/650 (2.92%) and 4/680 (0.58%) differences, respectively.

Table 2 GenBank accession numbers of the taxa used in the phylogenetic analysis.

Species	Strain No.	GenBank accession numbers			References
		ITS	LSU	<i>rpb2</i>	
<i>Cladobotryum amazonense</i>	CBS 470.80	MH861285	MH873051	-	Vu et al. (2019)
<i>C. apiculatum</i>	CBS 174.56	MH857566	MH869109	-	Vu et al. (2019)
<i>C. apiculatum</i>	CBS 829.69	MH859447	MH871222	-	Vu et al. (2019)
<i>C. asterophorum</i>	CBS 676.77	FN859395	MH872869	FN868649	Pöldmaa (2011)
<i>C. asterophorum</i>	CBS 118999	FN859408	DQ376085	FN868662	Pöldmaa (2011)
<i>C. croceum</i>	CBS 231.95	MH862511	MH874154	-	Vu et al. (2019)
<i>C. cubitense</i>	CBS 416.85	FN859396	-	FN868650	Pöldmaa (2011)
<i>C. cubitense</i>	CBS 127528	-	MH877928	-	Vu et al. (2019)
<i>C. cubitense</i>	G.A. m643.w	FN859397	-	FN868651	Pöldmaa (2011)
<i>C. indoafricum</i>	TFC 201295	FN859403	FN859403	FN868657	Pöldmaa (2011)
<i>C. indoafricum</i>	TFC 201286	FN859402	-	FN868686	Pöldmaa (2011)
<i>C. indoafricum</i>	FSU 5807	FN859399	-	FN868654	Pöldmaa (2011)
<i>C. multiseptatum</i>	CBS 472.71	FN859405	MH871991	FN868659	Pöldmaa (2011)
<i>C. multiseptatum</i>	TFC:99 177	-	-	HF911532	Taam & Pöldmaa (2013)
<i>C. multiseptatum</i>	TFC:99 82	-	-	HF911533	Taam & Pöldmaa (2013)
<i>C. obconicum</i>	CBS 528.81	MH861373	MH873126	-	Vu et al. (2019)
<i>C. paravirescens</i>	TFC 97 23	FN859406	FN859406	FN868660	Pöldmaa (2011)
<i>C. penicillatum</i>	CBS 407.80	FN859407	MH873046	FN868661	Pöldmaa (2011)
<i>C. protrusum</i>	CBS 118999	FN859408	FN859408	FN868662	Pöldmaa (2011)
<i>C. protrusum</i>	CBS 127531	-	MH877929	-	Vu et al. (2019)
<i>C. protrusum</i>	CGMCC 3.19064	MH459156	MH459167	MH464784	Sun et al. (2019b)
<i>C. purpureum</i>	CBS 154.78	FN859415	-	FN868669	Pöldmaa (2011)
<i>C. rubrobrunnescens</i>	CBS 176.92	FN859416	MH874016	FN868670	Pöldmaa (2011)
<i>C. rubrobrunnescens</i>	CBS 118756	-	DQ376086	HF911609	Kirschner et al. (2007)
<i>Cladobotryum</i> sp.	FSU 5046	FN859421	-	FN868675	Pöldmaa (2011)
<i>C. stereicola</i>	CBS 457.71	MH860217	MH871984	-	Vu et al. (2019)
<i>C. stereicola</i>	CBS 831.69	MH859448	MH871224	-	Vu et al. (2019)
<i>C. tchimbелense</i>	TFC 201146	FN859419	FN859419	FN868673	Pöldmaa (2011)
<i>C. tchimbелense</i>	CBS 127166	MH864455	MH875895	-	Vu et al. (2019)
<i>C. tenue</i>	CBS 152.92	FN859420	FN859420	FN868674	Pöldmaa (2011)
<i>C. tenue</i>	CBS 678.71	-	-	HF911617	Taam & Pöldmaa (2013)
<i>Hypomyces aconidialis</i>	TFC 201334	FN859457	FN859457	FN868711	Pöldmaa (2011)
<i>H. aconidialis</i>	TFC 201215	FN859456	FN859456	FN868710	Pöldmaa (2011)
<i>H. albidus</i>	CBS 460.71	MH860220	MH871987	-	Vu et al. (2019)
<i>H. ampullaris</i>	CGMCC 3.20157	MW03719 8	MW03720 2	MW14258 7	Gu et al. (2021)
<i>H. ampullaris</i>	CGMCC 3.20158	MW03719 9	MW03720 3	MW14258 7	Gu et al. (2021)
<i>H. armeniacus</i>	TFC 02-862	FN859424	FN859424	FN868678	Pöldmaa (2011)
<i>H. armeniacus</i>	CBS 822.69	MH859444	MH871220	-	Vu et al. (2019)
<i>H. aurantius</i>	TFC 95 171	FN859425	FN859425	FN868679	Pöldmaa (2011)

Table 2 Continued.

Species	Strain No.	GenBank accession numbers			References
		ITS	LSU	<i>rpb2</i>	
<i>H. aurantius</i>	HMAS 279804	MH459151	MH459169	MH464786	Sun et al. (2019b)
<i>H. australasiaticus</i>	TFC 03-8	FN859428	FN859428	FN868681	Pöldmaa (2011)
<i>H. australasiaticus</i>	CBS 127152	MH864443	MH875883	-	Vu et al. (2019)
<i>H. australasiaticus</i>	TFC 99-95	FN859427	-	FN868680	Pöldmaa (2011)
<i>H. australis</i>	TFC 2007-18	AM779860	AM779860	-	Pöldmaa (2007)
<i>H. boletiphagus</i>	MFLU 17-1391 ^T	MH459152	MH459168	MH464785	Sun et al. (2019b)
<i>H. boletus</i>	MFLU 17-1392	MH459153	MH459170	MH464787	Sun et al. (2019b)
<i>H. chlorinigenus</i>	KSH511	KT946843	-	KU041493	Otto et al. (2016)
<i>H. chlorinigenus</i>	KSH512	KT946844	-	KU041494	Otto et al. (2016)
<i>H. completus</i>	KSH411 (S172)	KT946842	-	KU041492	Otto et al. (2016)
<i>H. completus</i>	KSH410 (S171) ^T	KT946841	-	KU041491	Otto et al. (2016)
<i>H. corticiicola</i>	CBS 137.71	MH860037	MH871817	-	Vu et al. (2019)
<i>H. dactylarioides</i>	CBS 141.78	FN859429	MH872879	FN868683	Pöldmaa (2011)
<i>H. dactylarioides</i>	GJS 75 79	-	-	HF911530	Taam & Pöldmaa (2013)
<i>H. ellipsosporus</i>	CBS 696.86	KU382158	-	-	Zare & Gams (2016)
<i>H. fistulina</i>	HMAS 279800 ^T	MH459154	MH459171	-	Sun et al. (2019b)
<i>H. gabonensis</i>	TFC 201156	FN859430	FN859430	FN868684	Pöldmaa (2011)
<i>H. gabonensis</i>	CBS 127154	MH864445	MH875885	-	Vu et al. (2019)
<i>H. heterosporus</i>	CBS 719.88	FN859398	MH873844	FN868653	Pöldmaa (2011)
<i>H. hubeiensis</i>	HMAS 254597 ^T	MK478467	MN044762	MK484605	Zeng & Zhuang (2019)
<i>H. iranica</i>	ABRIICC 10210	OP738997	OP748942	OP750377	This study
<i>H. khaoyaiensis</i>	G.J.S. 01 304 ^T	FN859431	AJ583483	FN868685	Pöldmaa (2011)
<i>H. lactifluorum</i>	TAAM 170476 ^T	FN859432	EU710768	EU710773	Pöldmaa (2011)
<i>H. laeticolor</i>	JCM 10758 ^T	LC228655	LC228712	-	Unpublished
<i>H. luteovirens</i>	CBS 128483	MH864958	MH876402	-	Vu et al. (2019)
<i>H. luteovirens</i>	OSC 108826	EU652350	EU652380	-	Vu et al. (2019)
<i>H. microspermus</i>	CBS 141557	-	MH878229	-	Vu et al. (2019)
<i>H. microspermus</i>	S177	AF054852	-	-	Sahr et al. (1999)
<i>H. mycophilus</i>	CBS 175.56	MH857567	MH869110	-	Vu et al. (2019)
<i>H. mycophilus</i>	strain 10583	KU937111	KU937109	-	Zeng & Zhuang (2019)
<i>H. odoratus</i>	G.A. m329	FN859434	FN859434	FN868688	Pöldmaa (2011)
<i>H. odoratus</i>	TFC 200887	FN859439	-	FN868693	Pöldmaa (2011)
<i>H. odoratus</i>	CBS 820.69	MH859442	MH871219	-	Vu et al. (2019)
<i>H. orthosporus</i>	HMAS 279649	MK478468	MN044763	-	Pöldmaa (2011)
<i>H. orthosporus</i>	TFC 97-130	-	AF160241	-	Pöldmaa (2011)
<i>H. penicillatus</i>	NBRC 100524	LC146740	LC146740	-	Sun et al. (2019b)
<i>H. pseudolactifluorum</i>	MFLU 20-0265 ^T	MT260402	MT260399	MT259359	Yu et al. (2020)
<i>H. pseudolactifluorum</i>	MFLU 20-0266	MT260403	MT260400	MT259360	Yu et al. (2020)
<i>H. polyporinus</i>	ATCC 76479	-	AF543793	-	Currie et al. (2003)
<i>H. peltigericola</i>	CBS 141848 ^T	KY088202	-	-	Lechat et al. (2017)
<i>H. pseudocorticiicola</i>	JCM 12654	LC228663	LC228721	-	Sun et al. (2019b)
<i>H. robledoii</i>	CBS 121664	MH863136	MH874687	-	Vu et al. (2019)
<i>H. rosellus</i>	TFC 201071	FN859443	FN859443	FN868697	Pöldmaa (2011)
<i>H. samuelsii</i>	CBS 536.88	FN859444	-	FN868698	Pöldmaa (2011)
<i>H. samuelsii</i>	TFC 2007 23	FN859451	FN859451	FN868705	Pöldmaa (2011)

Table 2 Continued.

Species	Strain No.	GenBank accession numbers			References
		ITS	LSU	<i>rpb2</i>	
<i>H. semicircularis</i>	CBS 705.88	FN859417	MH873843	FN868671	Pöldmaa (2011)
<i>H. semitranslucens</i>	CBS 458.71	MH860218	MH871985	-	Vu et al. (2019)
<i>H. semitranslucens</i>	CBS 821.70	MH859960	MH871759	-	Vu et al. (2019)
<i>H. sibirinae</i>	CBS 744.88	MH862151	AJ459304	-	Vu et al. (2019)
<i>H. sibirinae</i>	10280	KU937112	KU937110	-	Zeng et al. (2017)
<i>H. sichuanensis</i>	CGMCC 3.20159	MW03720 0	MW03720 4	MW14258 9	Gu et al. (2021)
<i>H. sichuanensis</i>	CGMCC 3.20160	MW03720 1	MW03720 5	MW14259 0	Gu et al. (2021)
<i>H. sinicus</i>	HMAS 251317 ^T	JQ814696	MN044986	-	Zhuang et al. (2012)
<i>H. stephanomatis</i>	GJS 88 50	-	AF160243	AF545566	Pöldmaa (2011)
<i>H. stephanomatis</i>	CBS 446.64	MH858481	MH870114	-	Vu et al. (2019)
<i>H. subglobosus</i>	CBS 543.86	KU382160	-	-	Zare & Gams (2016)
<i>H. subiculosus</i>	TFC 97.166	FN859452	-	FN868706	Pöldmaa (2011)
<i>H. subiculosus</i>	CBS 274.61	MH858053	MH869620	-	Vu et al. (2019)
<i>H. tremellicola</i>	CBS 441.65	KU382166	-	-	Zare & Gams (2016)
<i>H. tubariicola</i>	CBS 115.79	KU382164	MH872953	-	Vu et al. (2019)
<i>H. tubariicola</i>	CBS 225.84	KU382162	KU382220	-	Zare & Gams (2016)
<i>H. virescens</i>	G.A. i1906 ^T	FN859454	-	FN868708	Pöldmaa (2011)
<i>H. virescens</i>	G.A. i1899	FN859453	-	FN868707	Pöldmaa (2011)
<i>Sepedonium ampullosporum</i>	CBS 392.52	MH857094	MH868629	-	Vu et al. (2019)
<i>S. boletiphagum</i>	S179	AF054867	-	-	Sahr et al. (1999)
<i>S. brunneum</i>	S171	AF054861	AF213028	-	Otto et al. (2016)
<i>S. chalcipori</i>	CBS 278.92	MH862358	MH874023	-	Vu et al. (2019)
<i>S. chalcipori</i>	CBS 148.92	MH862347	MH874014	-	Vu et al. (2019)
<i>S. chalcipori</i>	KSH558	KT946845	-	KU041495	Otto et al. (2016)
<i>S. aff. chalcipori</i>	KSH883	KT946848	-	KU041498	Otto et al. (2016)
<i>S. laevigatum</i>	CBS 101645	AF054858	-	-	Sahr et al. (1999)
<i>S. laevigatum</i>	S176	AF054855	-	-	Sahr et al. (1999)
<i>S. laevigatum</i>	S88	AF054857	-	-	Sahr et al. (1999)
<i>S. tulasneanum</i>	CBS 940.69	MH859489	MH871270	-	Vu et al. (2019)
<i>S. tulasneanum</i>	S165	AF054860	-	-	Sahr et al. (1999)
<i>S. tulasneanum</i>	S29	AF054859	-	-	Sahr et al. (1999)
<i>Mycogone calospora</i>	CBS 504.82	-	AF213030	-	Pöldmaa (2000)
<i>M. perniciososa</i>	CBS 648.82	FJ904634	-	-	Meyer & Korsten (2008)
<i>M. perniciososa</i>	WB17	FJ904632	-	-	Meyer & Korsten (2008)
<i>M. perniciososa</i>	WB14	FJ904631	-	-	Meyer & Korsten (2008)
<i>M. rosea</i>	TFC 96-62	-	AF213031	-	Pöldmaa (2000)
<i>M. rosea</i>	CBS 488.67	-	MH870753	-	Vu et al. (2019)
<i>Trichoderma harzianum</i>	CBS 226.95	AF057606	HM466680	AF545549	Ospina-Giraldo et al. (1998)
<i>T. hamatum</i>	DAOM 167057 ^T	EU280124	HM466686	AF545548	Hoyos-Carvajal et al. (2009)
<i>T. viride</i>	CBS 119325 ^T	DQ677655	-	EU711362	Jaklitsch et al. (2008)

Note: ^T indicates the type strains. The newly generated sequences are indicated in bold.

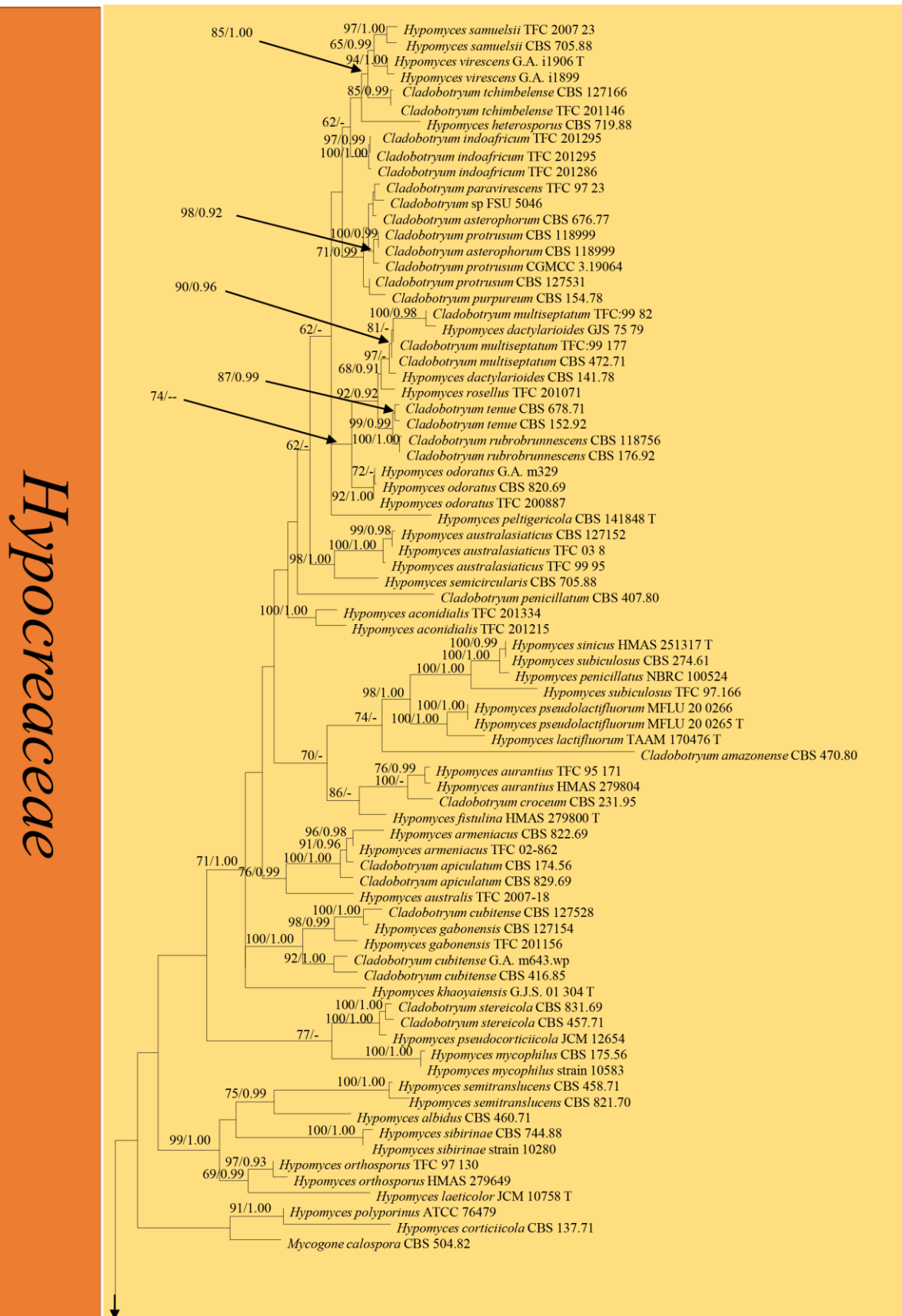


Fig 1 – Maximum likelihood tree generated from RAxML using ITS, LSU, and *rpb2* combined dataset. The tree is rooted with *Trichoderma harzianum* (CBS 226.95), *T. hamatum* (DAOM 167057) and *T. viride* (CBS 119325). The ML bootstraps $\geq 60\%$ and Bayesian posterior probabilities ≥ 0.9 are noted at the nodes. The new species is indicated in blue, and type strains are symbolized with T.

Hypocreaceae

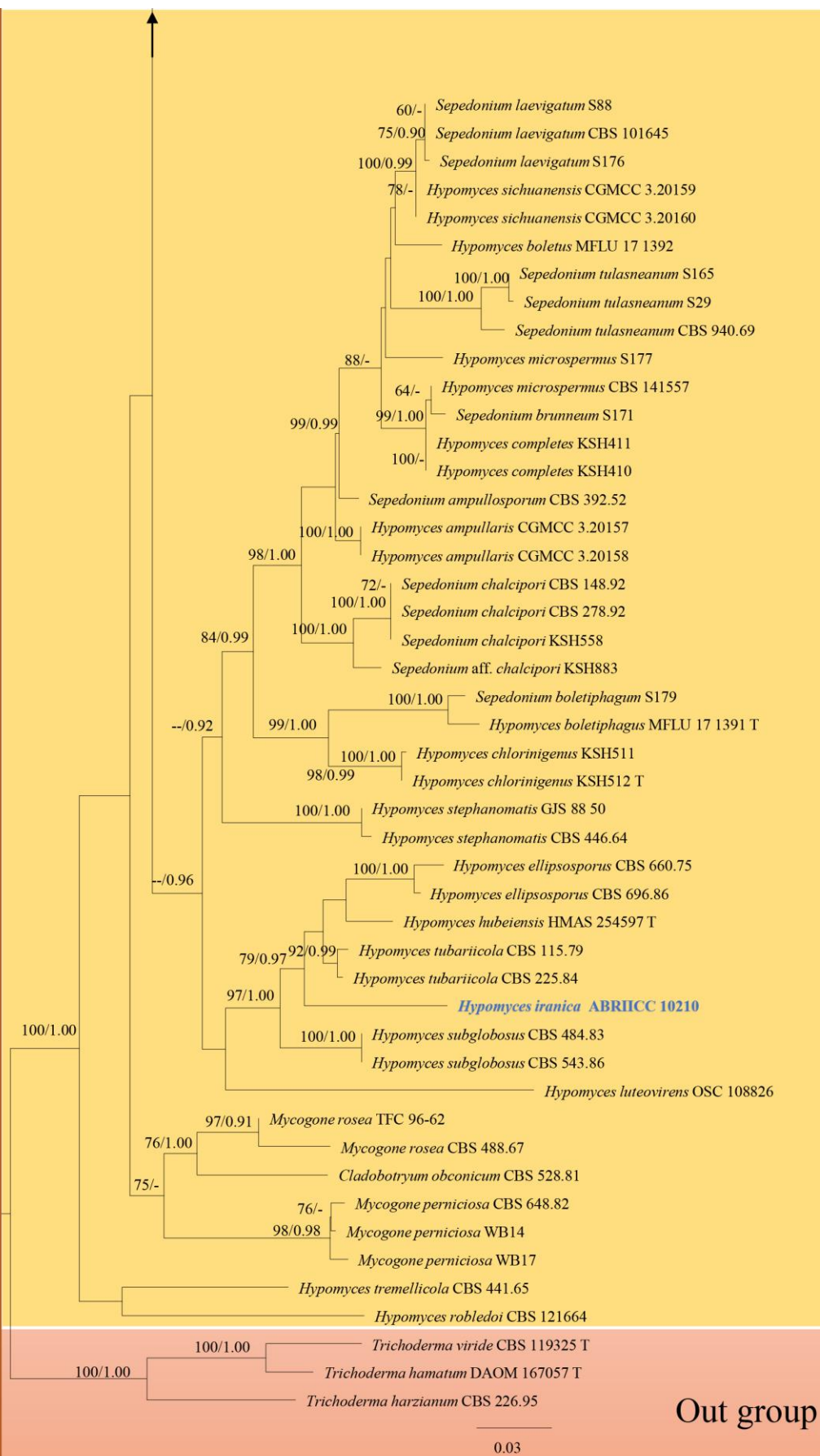


Fig 1 – Continued.

Taxonomy

Hypomyces iranica O. Karimi sp. nov.,

Fig 2

Index Fungorum number: IF 558186; Facesoffungi number: FoF 12770

Etymology – In reference to Iran, where the fungus was collected.

Holotype – FUM 1455

Saprobic on a submerged branch of *Fagus* sp. Asexual morph: *Vegetative hyphae* 1.0–2(–4) μm wide. *Conidiophores* arising from aerial hyphae, hyaline, septate, branched, erect, up to over 320 μm tall, to 5 μm wide at the base, bearing 1–4 whorls of 1–6 phialides. *Phialides* 15–30 \times 1–3 μm (\bar{x} = 21.2 \times 2.8 μm , n = 70), subulate, tapering toward the apex, hyaline, smooth-walled. *Conidia* 4–9 \times 1–3 μm (\bar{x} = 4.9 \times 2 μm , n = 120), forming globose heads, obovoid, rod-shaped to ellipsoidal, sharp-edged base, cyanophilic, aseptate, hyaline, smooth-walled. *Chlamydospores* 5.5–17 μm in diameter, intercalary, arrange singly, in chains or clusters, globose, hyaline, thick, smooth-walled. Sexual morph: Undetermined.

Culture characteristics – *Colonies* on PDA, reached 35–40 mm in diameter in 10 days at 25 °C, cottony and floccose, initially white to grey and later on dark grey, producing pigments in the media. The media turned yellowish to dark orange after two weeks. Mycelium often superficial to immersed in media. On MEA, 25–30 mm in diameter in 10 days at 25 °C, slightly, cottony, white to grey, reverse yellowish to brown with black spots. On PCA, reaching 30–36 mm in diameter in 10 days at 25°C, superficial, white to grey, reverse pale brown. Crystals absent.

Material examined – Iran, Mazandaran Province, Sari. Sangdeh forest, Flord series, on dead branches of *Fagus* sp., 16 July 2017, O. Karimi (FUM 1455, holotype), ex-type living culture (ABRIICC 10210).

Notes – Among the known *Hypomyces* species, *H. iranica* is morphologically similar to *H. ellipsosporus* Zare & W. Gams, *H. subglobosus* Zare & W. Gams, *H. hubeiensis* Z.Q. Zeng & W.Y. Zhuang, and *H. tubariicola* (W. Gams) Zare & W. Gams. *Hypomyces ellipsosporus* differs from our species in having mostly ellipsoidal, 5–7 \times 3–4 μm conidia with apiculate base, cyanophilic, rather shorter conidiophores producing whorls of up to 3–5 phialides and longer phialides (20–40 \times 1.5–2.5 μm) (Zare & Gams 2016); *H. subglobosus* differs in having subglobose to short-cylindrical 4–5 \times 3–3.5 μm conidia, slightly apiculate at the base, strongly cyanophilic, no chlamydospores (Zare & Gams 2016); *H. hubeiensis* differs by the smaller conidia (3–6 \times 1–2.3 μm) and smaller phialides (8–20 \times 2–3 μm) sizes, and no chlamydospores (Zeng & Zhuang 2019); *H. tubariicola* differs by having wider conidia (6–8.5 \times 3.5–4.5 μm) with one or two guttules, and chlamydospores scarce in old cultures, single or short chains, globose, hyaline, 7.5–12 μm in diameter. The comparison between our new species and the most related species is tabulated in Table 3.

Table 3 Synopsis of morphological characters between *H. iranica* and *H. ellipsosporus*, *H. hubeiensis*, *H. tubariicola*, and *H. Subglobosus*.

Species	<i>H. ellipsosporus</i>	<i>H. hubeiensis</i>	<i>H. tubariicola</i>	<i>H. subglobosus</i>	<i>H. iranica</i>
Colony on PDA	White to greyish white	Floccose, surface grey white.	White to greyish white.	Moderately fast, white, buff to dark brick, looking powdery.	Cottony and floccose, initially white to grey and later on dark grey producing pigments.

Table 3 Continued.

Species	<i>H. ellipso sporus</i>	<i>H. hubeiensis</i>	<i>H. tubariicola</i>	<i>H. subglobosus</i>	<i>H. iranica</i>
Conidiophores	Rather short, erect, producing whorls of up to 3–5 phialides.	Arising from aerial hyphae, branched, septate, 1–2 verticillate, with terminal whorl of 2–6 phialides.	Usually arising from felty aerial hyphae, erect, up to over 220 µm tall, to 4 µm wide at the base, bearing 1–3 whorls of 3–4(–7) phialides.	Bears whorls of 1–5 phialides, mainly at or near the end of the conidiophores.	Arising from aerial hyphae, septate, branched, erect, up to over 320 µm tall, to 5 µm wide at the base, bearing 1–4 whorls of 1–6 phialides.
Phialides	Rather long, measuring 20–40 × 1.5–2.5 µm, producing conidia in dry globose heads.	Subulate, tapering toward apex, smooth, 8–20 × 2–3 µm.	22–35(–45) µm long, from 1.7–2.7 µm, gradually tapering to 0.8–1.3(–1.7) µm.	Rather long, measuring 16–36 × 1.5–2.2 µm, gradually tapering from the base to the apex.	Subulate, tapering toward apex, hyaline, 15–30 (21.17) × 1–3 (2.82) µm.
Conidia	Cyanophilic, ellipsoidal with symmetrically rounded ends or slightly apiculate at the base, 5.5–6.5(–8) × 3–3.5(–4) µm.	Rod-shaped to narrowly ellipsoidal, aseptate, hyaline, smooth, 3–6 × 1–2.3 µm.	Forms globose heads, obovoid, with slightly truncate base, smooth-walled, 6–8.5 × 3.5–4.5 µm, moderately cyanophilic, with one or two conspicuous guttules.	Forms dry globose heads, globose, subglobose to ellipsoidal, some also short-cylindrical, 4–5 × 3–3.5 µm.	Forms globose heads, obovoid, rod-shaped to ellipsoidal having sharp-edged base, cyanophilic, aseptate, hyaline 4–9 (4.9) × 1–3 (2.03) µm, no guttules.
Chlamydospores	In old colonies: subglobose, 5–5.5(–8) µm in diameter.	No chlamydospores	Chlamydospores scarce in old cultures, single or in short chains, globose, hyaline, 7.5–12 µm in diameter.	No chlamydospores	intercalary, single, chains or clusters, globose and hyaline 5.5–17 µm in diameter.
Hosts	decaying wood	<i>Agaricus</i> sp.	<i>Tubaria furfuracea</i>	<i>Crepidotus mollis</i>	<i>Fagus</i> sp. (Submerged wood)
References	Zare & Gams (2016)	Zeng & Zhuang (2019)	Zare & Gams (2016)	Zare & Gams (2016)	This study

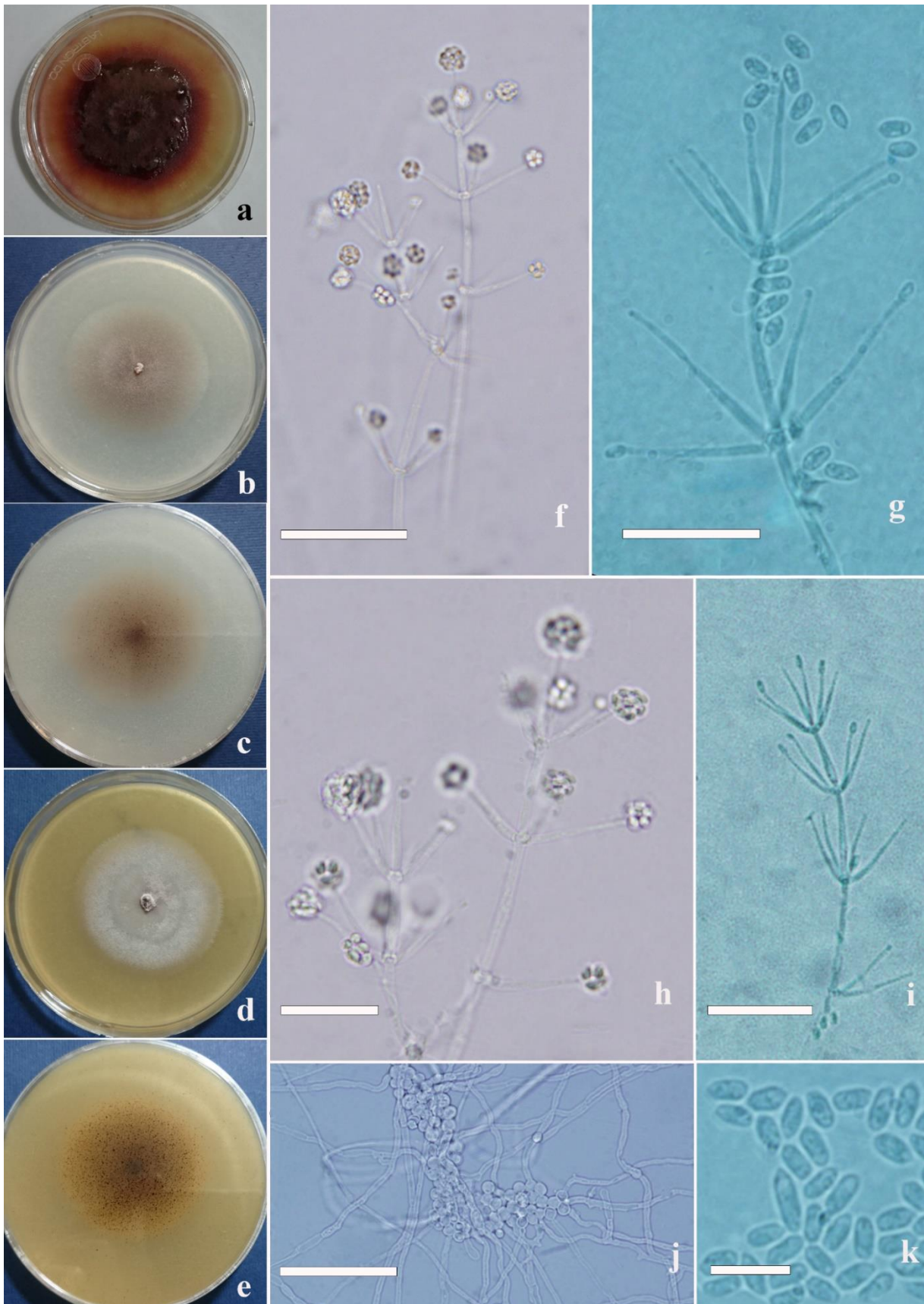


Fig 2 – *Hypomyces iranica* (ABRIICC 10210). a Colony on PDA (above). b, c Colony on PCA (above and below), d, e Colony on MEA (above and below). f–i Conidiophores and phialides. j Chlamydospore. k Conidia. Scale bars: f = 200 μm, g = 50 μm, h = 200 μm, i = 100 μm, j = 100 μm, k = 10 μm. (j, i and k stained with Lacto-phenol cotton blue).

Discussion

Species of *Hypocreaceae* are usually found on plants, fungi, myxomycetes, and lichens as biotrophic, hemibiotrophic, saprobic, hypersaprobic, and mycoparasites in terrestrial and aquatic habitats worldwide (Rossman et al. 1999, Põldmaa 2000, Põldmaa 2011, Zeng & Zhuang 2016). *Hypomyces* species are commonly known as fungicolous and have been isolated from various hosts such as agarics, boletes, Aphyllophorales, discomycetes, and decaying wood (Rogerson & Samuels 1985, 1989, 1993, 1994, Põldmaa 1996, 2000, 2003, 2011, Tamm & Poldmaa 2013, Zare & Gams 2016, Zeng & Zhuang 2016, Lechat et al. 2017, Sun et al. 2019a). Host range and morphological features are the key characteristics of this genus (Kim et al. 2017). *Hypomyces* species are important as mushroom pathogens, and in some cases, it causes significant economic damage, such as in the case of cobweb or wet bubble disease caused by *H. aurantius*, *H. perniciosus*, *H. rosellus*, and *H. odoratus* (Mohammadi Goltapeh et al. 2000, Li et al. 2019). Asexual morphs of fungicolous fungi species usually have a wide range of hosts and distribution, but the appearance and observation of their sexual morphs are rare and host-specific.

There are limited studies on the identification and diversity of *Hypomyces* species in Iran, and most were reported on fungal hosts. Mohammadi Goltapeh et al. (2000) isolated *Cladobotryum dendroides* as a pathogen of cobweb disease of white button mushroom (*Agaricus bisporus*). Asef & Mohammadi Goltapeh (2002) isolated four *Cladobotryum* species (*Cladobotryum dendroides*, *C. polypori*, *C. varium* and *C. verticillatum*) from the basidiocarp of *Armillaria mellea*, *Agaricus bisporus*, *Lactarius vellereus* and *carpophore* of *Russula* sp. Three species of them (*C. polypori*, *C. varium* and *C. verticillatum*) were reported as new records for Iran but the sexual morph was not identified in any of them. In another study, Asef & Zare (2006) recorded *H. aurantius*, *H. rosellus* and *H. polyporinus* and also reported *C. clavisporem* as the anamorph for *H. polyporinus*. Zare & Asef (2008) also reported *Sepedonium microspermum* and *Sepedonium* sp. on mushrooms. In this study, we examined woody specimens to investigate the appearance of *Hypomyces* on them, and we introduced *H. iranica*, isolated from submerged wood of *Fagus* sp., from Sangdeh forest, Mazandaran Province, Iran, as a novel taxon.

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