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研究报告

山西省蜡伞属真菌物种多样性及一新种的描述

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摘 要：【背景】蜡伞属(*Hygrophorus*)隶属于担子菌门(*Basidiomycota*)伞菌纲(*Agaricomycetes*)伞菌目(*Agaricales*)蜡伞科(*Hygrophoraceae*)，在世界分布广泛。【目的】对山西省的蜡伞属真菌物种进行资源调查，丰富该地区蜡伞属物种的多样性。【方法】采集标本并进行形态学观察及描述；提取DNA，测定内转录间隔区(internal transcribed spacer, ITS)；基于最大似然法(maximum likelihood, ML)和贝叶斯法(Bayesian inference, BI)构建分子系统发育树。【结果】结合形态学特征和分子系统发育学共鉴定出山西省蜡伞属真菌11种，包括已知种10个，新种1个。本文对新种拟褐顶蜡伞(*Hygrophorus brunneodiscoides*)的宏观、微观形态特征进行了详细的描述、绘图及拍照，并提供了这10个已知种的子实体图片和山西省蜡伞属物种检索表。【结论】本文描述了发现于中国的新种拟褐顶蜡伞(*H. brunneodiscoides*)，证实了山西存在该属物种11个，并根据野外观察对这11个物种的地理分布及其与宿主植物间的关系进行了简要讨论。

关键词：食用真菌；新种；分类学；担子菌

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Species diversity of *Hygrophorus* (*Hygrophoraceae*, *Agaricales*) in Shanxi Province and description of *Hygrophorus brunneodiscoides* sp. nov.

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Abstract: [Background] *Hygrophorus*, belonging to *Basidiomycota*, *Agaricomycetes*, *Agaricales*, *Hygrophoraceae*, is widely distributed all around the world. **[Objective]** To investigate the species of *Hygrophorus* and enrich the knowledge about the species diversity of this genus in Shanxi Province. **[Methods]** We collected fungal specimens, observed and characterized their morphology. After DNA extraction, the internal transcribed spacer (ITS) was sequenced. Phylogenetic trees were constructed based on the maximum likelihood method and Bayesian inference. **[Results]** Eleven species of *Hygrophorus* were identified based on the morphological and molecular evidence, including ten known species and a new species. This article described the macroscopic and microscopic morphological characteristics of the new species *Hygrophorus brunneodiscoides* in detail. The basidiocarp photos of ten known species and a key to the species of *Hygrophorus* in Shanxi Province were provided. **[Conclusion]** We identified a new species, *H. brunneodiscoides*, in China, confirmed 11 species of *Hygrophorus* in Shanxi Province, and briefed their geographical distribution and associations with hosts according to the field observation.

Keywords: edible mushroom; novel species; taxonomy; *Basidiomycetes*

Hygrophorus Fr. (*Hygrophoraceae*, *Agaricales*), a widely distributed genus, has been reported in six continents but not in Antarctica[1-6]. The diagnostic characteristics of this genus are: often viscid Pileus, waxy and thick Lamellae, divergent hymenial trama, slender Basidia, ellipsoid to lacrymoid, smooth hyaline basidiospores and the ectomycorrhizal habit[2,7-8]. *Hygrophorus* species predominantly establish ectomycorrhizal associations with partner trees belonging to the families *Betulaceae*, *Fagaceae* and *Pinaceae* in diverse forest ecosystems[9]. Currently, A total of 1 031 records are listed in Index Fungorum (www.indexfungorum.org/Names/Names.asp), but only about 430 are currently accepted[9-10]. In China, 90 name of *Hygrophoru*s species had been reported[5-6,8-17], but only 53 species were confirmed by molecular data[6,8-10,12-17], and the remaining species need to be reexamined and verified with molecular data.

In the last two decades, with the development of molecular phylogenetics, the classification system of this genus has been reclassified. Based on six-gene phylogenetic analysis, Lodge et al.[18] divided this genus into three subgenera, nine sections and nine subsections. Afterwards, Bellanger et al.[19] further reclassified *Hygrophorus* section *Olivaceoumbrini* s.l. into five sections by constructing ITS phylogenetic tree. Recently, a new comprehensive phylogenetic study of *Hygrophorus* has been carried out by Wang et al.[9]. Their results show that the ITS-LSU-*rpb2*-*tef1*-*α* dataset could resolve a section-level phylogeny of this genus, and nine sections are supported as monophyletic clades. They are section *Aurei*, section *Camarophylli*, section *Chrysodontes*, section *Discoidei*, section *Hygrophorus*, section *Olivaceoumbrini*, section *Picearum*, section *Pudorini* and sect. *Vividi*.

Shanxi Province is located in northern China, and the recent studies revealed the richness of macrofungi in this province[17,20-29]. There are five *Hygrophorus* species reported from this province before the present study, i.e. *H. cantharellus* (Schwein.) Fr., *H. conicus* (Schaeff.) Fr., *H. miniatus* (Fr.) Fr., *H. psittacinus* (Schaeff.) Fr., *H. viridiflavidus* L. Fan & Y.X. Zhang[17,30]. However, except for *H. viridiflavidus*, the first three species has been transferred to the genus *Hygrocybe* (Fr.) P. Kumm. and the last one has been transferred to the genus *Gliophorus* Herink by recent work[31-33]. Thus, in fact, only one species is known from this area. During the last seven years, we collected a lot of specimens of *Hygrophorus* from this province, and 11 species are recognized, including an undescribed species. Our aims of this paper are to clarify the species diversity and geographic distribution of *Hygrophorus* in Shanxi Province, and to describe and illustrate a new species.

1 Materials and Methods

1.1 Site description

The specimens studied were collected in Shanxi Province from 2017 to 2023, northern China.

The new species described in this paper collected from Wulu Mountains and Zhongtiao Mountains. Wulu Mountains is in the southwest of Shanxi Province, with a temperate continental climate. The average annual temperature is 8.7 °C, the annual precipitation is 500–560 mm, and the highest point has an elevation of 1 946.3 m. The vegetation mainly includes *Larix gmelinii* var. *principis-rupprechtii* (Mayr) Pilg., *Pinus tabuliformis* Carrière, *Pinus bungeana* Zucc.ex Endl., *Picea* spp., *Platycladus orientalis* (L.) Franco, *Quercus wutaishanica* Mayr and *Populus* spp. The soil types mainly include brown soil, cinnamon soil, meadow soil and mountain meadow soil. Zhongtiao Mountains is in the southeast of Shanxi Province, and has a warm temperate monsoonal continental climate. The average annual temperature is 12–14 °C, the annual precipitation is 600–800 mm, and the highest point is 2 358 m elevation (Shunwangping peak). The vertical band spectrum of vegetation distribution is obvious. From bottom to top, it can be divided into a cork forest band (400–1 400 m), a pine oak forest band (1 200–2 000 m), a poplar birch forest band (1 900–2 200 m), and a mountain meadow band (2 000–2 358 m). Correspondingly, the vertical soil band spectrum is mountain cinnamon soil, mountain leached cinnamon soil, mountain brown soil and mountain meadow soil. The vegetation mainly includes *Carpinus turczaninowii* Hance, *Platycladus orientalis*, *Pinus armandi* Franch., *Quercus aliena* Blume and *Q. variabilis* Blume[34].

1.2 Morphological studies

The fresh basidiomata were photographed in the field and macroscopic characteristics were recorded. Standardized color values matching the colors of the description were taken from ColorHexa (https://www.colorhexa.com/). The specimens were dried in a fruit drier at 40–45 °C and deposited in BJTC (Herbarium Biology Department, Capital Normal University, Beijing, China). Microscopic characteristics were conducted on sections from dried material, rehydrated in 75% ethanol, and subsequently mounted in 3% KOH and Congo red. All measurements were made in 3% KOH or Congo red. For each specimen at least 30 spores and 15 basidia were measured. Spore measurements exclude apiculus. Basidia measurements exclude sterigmata, which are given separately. The dimensions of basidiospores are given by (a–)b–c(–d). The range ‘b–c’ covers a minimum of 90% of the measured values. Extreme values ‘a’ and ‘d’ are given in parentheses. *Q* refers to the length/width ratio in the profile view of basidiospores, and Q_m refers to the average Q of all basidiospores±sample standard deviation.

1.3 DNA extraction, PCR amplification and DNA sequencing

Dried basidiomata were crushed by shaking (Mixer Mill MM301, Retsch, Haan, Germany) for 45 s at 30 Hz 2−4 times in a 1.5 mL tube together with 3 mm diam tungsten carbide balls, and total genomic DNA was extracted using the modified CTAB method[35]. The ITS region was amplified using the primers ITS1F/ITS4[35-36]. Polymerase chain reactions (PCR) for all regions were performed in 25 μL reaction containing 2 μL DNA template, 1 μL primer (10 μmol/L) each, 12.5 μL of 2×Master Mix [Tiangen Biotech (Beijing) Co.], 8.5 μL ddH$_2$O. PCR reactions were implemented as follows: an initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Beijing Zhongkexilin biotechnology limited company for purification, sequencing, and editing. Validated sequences were deposited in GenBank database (http://www.ncbi.nlm.nih.gov/). Other sequences of *Hygrophorus* and related species were mainly selected from those used by Huang et al.[6], Wang et al.[8], Wang et al.[9], Huang et al.[10], Huang et al.[12], Huang et al.[14], Wang & Li.[16], Lodge et al.[18], Bellanger et al.[19], Larsson & Jacobsson[37], Jacobsson & Larsson[38], Smith et al.[39-40], Morris et al.[41], Cox et al.[42], Osmundson et al.[43], Holly et al.[44], Taylor et al.[45], Castro[46], Larsson et al.[47], Pierre-Arthur et al.[48], Endo et al.[49], Hofstetter et al.[50], Naseer et al.[51], He & Yang[52], Bellanger et al.[53]. The accession numbers of new and downloaded sequences stored in the NCBI database (http://www.ncbi.nlm.nih.gov/) are provided in Table 1.

1.4 Phylogenetic analyses

The ITS dataset was used to investigate the phylogenetic positions of the *Hygrophorus* species of Shanxi. *Haasiella venustissima* (Fr.) Kotl. & Pouzar was chosen as outgroup[18]. The sequences of the markers were aligned in MAFFT v.7.110[54] under default parameters. Ambiguously aligned sites were identified by Gblocks v.0.91b[55] with default parameters. Maximum likelihood (ML) and Bayesian inference (BI) were used to infer phylogenetic analysis.

Maximum likelihood (ML) was performed using RAxML 8.0.14[56] by running 1 000 bootstrap replicates under the GTRGAMMAI model. Bayesian inference (BI) was conducted using MrBayes v3.1.2[57]. In the BI analysis, after selecting the best substitution model (GTR+I+G) determined by MrModeltest v2.3[58], we used two independent runs with four Markov chains Monte Carlo (MCMC) for 1 645 000 generations under the default settings. The average standard deviation of split frequencies (ASDSF) values was far lower than 0.01 at the end of the runs. Trees were sampled every 100 generations after burn-in (25% of trees were discarded as the burn-in phase of the analyses, set up well after convergence), and 50% majority-rule consensus trees were constructed.

Clades with bootstrap support (BS)≥70% and Bayesian posterior probability (PP)≥0.95 were considered significantly supported[59-60]. All phylogenetic trees were viewed with TreeView[61].

2 Results and Analysis

2.1 Molecular phylogenetic analyses

Twenty-five ITS sequences were newly generated from our specimens in this study. The ITS dataset contained 65 taxa, comprising 447 characters. ML and BI analyses yielded similar tree topologies and therefore only the tree inferred from the ML analysis is shown (Figure 1). According to the ITS phylogenetic tree, our specimens were resolved into eleven strongly supported branches, and ten of them were identified as known species, *i.e.*, *H. alpinus* H.Y. Huang & L.P. Tang, *H. brunneodiscus* C.Q. Wang & T.H. Li, *H. esculentus* H.Y. Huang & L.P. Tang, *H. gliocyclus* Fr., *H. hedrychii* (Velen.) K. Kult, *H. lucorum* Kalchbr., *H. orientalis* H.Y. Huang & L.P. Tang, *H. pallidoagathosmus* C.Q. Wang, Ming Zhang & T.H. Li, *H. speciosus* Peck

表 **1** 用于分子系统发育分析的 ***Hygrophorus*** 标本及其 **ITS** 序列的 **GenBank** 登录号

Table 1 *Hygrophorus* specimens used in molecular phylogenetic analyses and the GenBank accession numbers of their ITS sequence

Species	Voucher	Locality	ITS	Reference
Haasiella venustissima	Gminder971488	Italy	KF291092	Lodge et al.[18]
Ha. venustissima	E.C. 8191	Italy	JN944393	Lodge et al.[18]
Hygrophorus agathosmoides	CFSZ20158	China: Inner Mongolia	OP547738	Wang et al.[9]
H. agathosmoides	HMAS 281303	China	MZ605814	Huang et al.[14]
H. agathosmoides	HRL2823	Canada	MT981656	Bellanger et al.[19]
H. agathosmus	EL398-17	Sweden	MH656445	Larsson et al.[47]
H. agathosmus	E00905345	UK	MZ605815	Huang et al.[14]
H. agathosmus	EL191-15	Norway	MH656454	Larsson et al.[47]
H. albofloccosus	GDGM70044	USA	OP547639	Wang et al.[9]
H. albofloccosus	GDGM70063	USA	OP547642	Wang et al.[9]
H. albofloccosus	UCSC F-2171	USA	MT981691	Bellanger et al.[19]
H. alpinus	MHKMU W.H. Zhang 4631	China: Yunnan	MW762962	Huang et al.[6]
H. alpinus	MHKMU W.H. Zhang 4632	China: Yunnan	MW762963	Huang et al.[6]
H. alpinus	**BJTC FM4232**	**China: Shanxi**	**PQ113866**	**This study**
H. alpinus	**BJTC FM4278**	**China: Shanxi**	**PQ113868**	**This study**
H. alpinus	**BJTC FM4279**	**China: Shanxi**	**PQ113867**	**This study**
H. arbustivus	13486	Italy	JF908066	Osmundson et al.[43]
H. armeniacus	GDGM82364	China: Yunnan	OP547665	Wang et al.[9]
H. armeniacus	GDGM90096	China: Yunnan	OP547733	Wang et al.[9]
H. armeniacus	XHW6877	China	OP547772	Wang et al.[9]
H. atrofuscus	HKAS 54818	China: Yunnan	MZ605836	Huang et al.[14]
H. atrofuscus	HKAS 55483	China: Yunnan	MZ605835	Huang et al.[14]
H. atrofuscus	HKAS56277	China	MZ605834	Huang et al.[14]
H. aurantioluteus	SAAS191	China: Sichuan	OP547759	Wang et al.[9]
H. boyeri	HL 1113	Canada	MG882094	Moreau et al.[48]
H. boyeri	HVM 27	USA	KF879478	Holly et al.[44]
H. brunneiceps	HKAS 54804	China: Yunnan	MZ605840	Huang et al. [14]
H. brunneiceps	GDGM84682	China: Sichuan	OP547725	Wang et al.[9]
H. brunneiceps	HMAS 254315	China: Xizang	MZ605838	Huang et al.[14]
H. brunneodiscus	GDGM79240	China: Yunnan	OP547658	Wang et al.[9]
H. brunneodiscus	GDGM73213	China: Hunan	MN378318	Wang et al.[8]
H. brunneodiscus	GDGM75489	China: Hunan	MN378317	Wang et al.[8]
H. brunneodiscus	**BJTC FM1250**	**China: Shanxi**	**PQ113861**	**This study**
H. brunneodiscus	**BJTC FM1768**	**China: Shanxi**	**PQ113862**	**This study**
H. brunneodiscoides	**BJTC FM302**	**China: Shanxi**	**PQ113879**	**This study**
H. brunneodiscoides	**BJTC FM4026**	**China: Shanxi**	**PQ113880**	**This study**
H. brunneoloaurantiacus	GDGM82433	China: Yunnan	OP547672	Wang et al.[9]
H. brunneoloaurantiacus	GDGM83034	China: Yunnan	OP547677	Wang et al.[9]
H. brunneolus	SAAS510	China: Sichuan	OP547761	Wang et al.[9]

(待续)

(续表 1)

Species	Voucher	Locality	ITS	Reference
H. brunneolus	SAAS617	China: Sichuan	OP547762	Wang et al.[9]
H. brunnescens	GDGM84456	China: Sichuan	OP547694	Wang et al.[9]
H. cossus	Sowerby 1794	UK	AY242852	Larsson & Jacobsson[37]
H. cossus	Orton5038	Sweden	AY463489	Unpublished
H. deliciosus	GDGM73208	China: Xizang	MT363821	Wang & Li[16]
H. deliciosus	GDGM79208	China: Yunnan	MT363808	Wang & Li[16]
H. deliciosus	GDGM81877	China: Sichuan	OP547660	Wang et al.[9]
H. discoxanthus	Montri-243	Switzerland	MK028420	Hofstetter et al.[50]
H. discoxanthus	SJ97044	Sweden	AY242853	Larsson & Jacobsson[37]
H. eburneus	SJ97049	Sweden	AY463484	Unpublished
H. eburneus	AS94_111	Sweden	AY463485	Unpublished
H. esculentus	SAAS4747	China: Sichuan	OQ860085	Wang et al.[9]
H. esculentus	**BJTC FM3976**	**China: Shanxi**	**PQ113874**	**This study**
H. flavodiscus	GDGM70070	USA	OP547646	Wang et al.[9]
H. flavodiscus	DSH101009.2	USA	HM020691	Unpublished
H. fuligineus	GDGM70057	USA	OP547641	Wang et al.[9]
H. fuligineus	GDGM70064	USA	OP547643	Wang et al.[9]
H. fuscopapillatus	GDGM44412	China: Sichuan	MN378337	Wang et al.[8]
H. fuscopapillatus	LJW1858	China: Yunnan	MT093606	Wang et al.[8]
H. fuscopapillatus	LJW1984	China: Yunnan	OP547751	Wang et al.[9]
H. gliocyclus	GDGM84514	China: Sichuan	OP547703	Wang et al.[9]
H. gliocyclus	**BJTC FM1225**	**China: Shanxi**	**PQ113875**	**This study**
H. glutiniceps	GDGM42140	China: Guangdong	MN378310	Wang et al.[8]
H. glutiniceps	GDGM42188	China: Guangdong	MN378313	Wang et al.[8]
H. glutiniceps	GDGM42217	China: Guangdong	MN378309	Wang et al.[8]
H. glutinifer	E00218159	UK	MZ605844	Huang et al.[14]
H. glutinifer	E00101559	UK	MZ605843	Huang et al.[14]
H. glutinosus	061762	USA	MN243161	Unpublished
H. griseodiscus	GDGM84644	China: Sichuan	OP547720	Wang et al.[9]
H. griseodiscus	SAAS462	China: Sichuan	MN378338	Wang et al.[8]
H. hedrychii	GDGM44600	China: Inner Mongolia	OP547634	Wang et al.[9]
H. hedrychii	GDGM84550	China	OP547710	Wang et al.[9]
H. hedrychii	HMAS 290140	China	OK011527	Wang et al.[9]
H. hedrychii	**BJTC FM293**	**China: Shanxi**	**PQ113863**	**This study**
H. hedrychii	**BJTC FM2759**	**China: Shanxi**	**PQ113864**	**This study**
H. hedrychii	**HSA368**	**China: Shanxi**	**PQ113865**	**This study**
H. hyacinthinus	0401691	France	MT845204	Bellanger et al.[19]
H. hyacinthinus	JMM10101001	France	MT845203	Bellanger et al.[19]
H. hyacinthinus	EL326-10	Sweden	MH656470	Larsson et al.[47]
H. hypothejus	KUN-HKAS56550	Germany	MW616467	He & Yang[52]
H. hypothejus	8827	Switzerland	JF908076	Osmundson et al.[43]

(待续)

(续表 1)

Species	Voucher	Locality	ITS	Reference
H. hypothejus	781-716	UK	HM146822	Cox et al.[42]
H. limacinus	68803	Sweden	MN243166	Unpublished
H. limosus	814001	Cyprus	MT981620	Bellanger et al.[19]
H. limosus	ML811131HL	Cyprus	MT981621	Bellanger et al.[19]
H. limosus	ML91161HL	Cyprus	MT981624	Bellanger et al.[19]
H. lucorum	GDGM42148	Italy	OP547621	Wang et al.[9]
H. lucorum	GDGM79101	China: Inner Mongolia	OP547653	Wang et al.[9]
H. lucorum	**BJTC FM1994**	**China: Shanxi**	**PQ113873**	**This study**
H. lucorum	**BJTC FM1998**	**China: Shanxi**	**PQ113872**	**This study**
H. megasporus	MICH34186	USA	MT981617	Bellanger et al.[19]
H. meridionalis	LIP 0400300	Greece	MG882100	Moreau et al.[48]
H. mesotephrus	K(M)227410	UK	MT981695	Bellanger et al.[19]
H. murinidiscus	GDGM82120	China: Sichuan	OP547662	Wang et al.[9]
H. murinidiscus	GDGM82183	China: Sichuan	OP547663	Wang et al.[9]
H. murinidiscus	GDGM82186	China: Sichuan	OP547664	Wang et al.[9]
H. nemoreus	LAS85112	Sweden	EF395374	Jacobsson & Larsson[38]
H. nemoreus	—	Spain	MH703904	Castro[46]
H. occidentalis	MICH10917	USA	MZ576439	Bellanger et al.[53]
H. occidentalis	TENN10197	USA	MH656479	Larsson et al.[47]
H. orientalis	GDGM84641	China: Sichuan	OP547719	Wang et al.[9]
H. orientalis	CFSZ20884	China: Inner Mongolia	MW290182	Huang et al.[10]
H. orientalis	HKAS 75586	China: Hubei	MW290176	Huang et al.[10]
H. orientalis	**BJTC FM2049**	**China: Shanxi**	**PQ113878**	**This study**
H. orientalis	**BJTC FM2398**	**China: Shanxi**	**PQ113876**	**This study**
H. orientalis	**BJTC FM2987**	**China: Shanxi**	**PQ113877**	**This study**
H. pallidoagathosmus	CFSZ22807	China: Inner Mongolia	OP547742	Wang et al.[9]
H. pallidoagathosmus	GDGM84681	China: Sichuan	OP547724	Wang et al.[9]
H. pallidoagathosmus	GDGM84702	China: Sichuan	OP547728	Wang et al.[9]
H. pallidoagathosmus	**BJTC FM222**	**China: Shanxi**	**PQ113859**	**This study**
H. pallidoagathosmus	**BJTC FM1183**	**China: Shanxi**	**PQ113858**	**This study**
H. pallidoflavodiscus	GDGM89619	China: Yunnan	OQ860089	Wang et al.[9]
H. paludosoides	YL4339	Canada	MT981623	Bellanger et al.[19]
H. parvirussula	GDGM45208	China: Xizang	MT363822	Wang & Li[16]
H. parvirussula	GDGM85782	China: Sichuan	OP547732	Wang et al.[9]
H. parvirussula	MHKMU N.K. Zeng 2878	China: Yunnan	MH160770	Huang et al.[12]
H. pinophilus	GDGM43348	China: Sichuan	OP547622	Wang et al.[9]
H. pinophilus	Huang 878	China: Yunnan	MZ605854	Huang et al.[14]
H. pinophilus	EL289-11	Sweden	MH656468	Huang et al.[12]
H. pseudohypothejus	XHW7324	China	OP547777	Wang et al.[9]
H. pseudohypothejus	MHKMU T. Huang 447	China: Yunnan	MW762991	Huang et al.[6]
H. pustulatus	0183665	Sweden	MN243180	Unpublished

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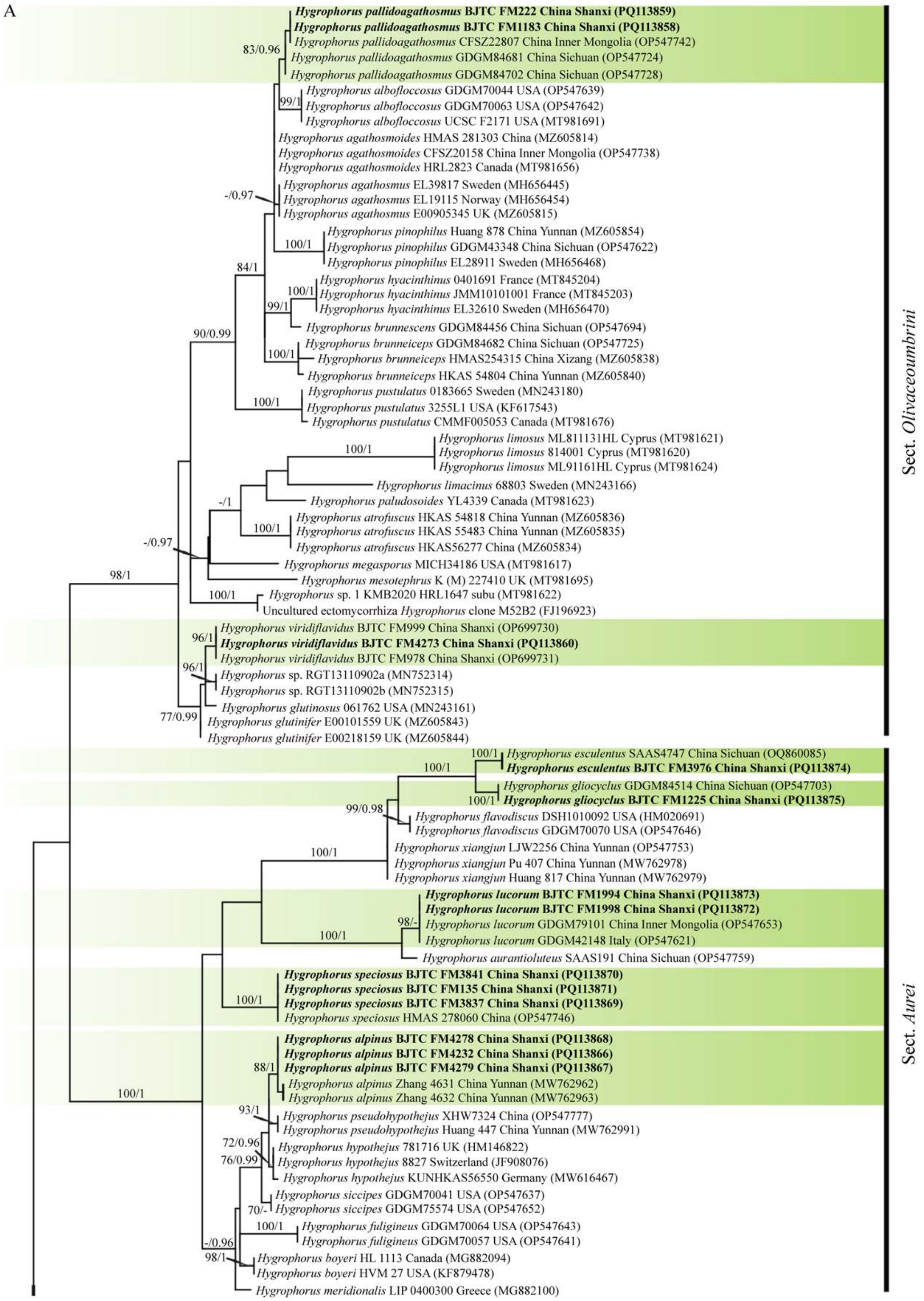
(续表 1)

Species	Voucher	Locality	ITS	Reference
H. pustulatus	CMMF005053	Canada	MT981676	Bellanger et al.[19]
H. pustulatus	3255L1	USA	KF617543	Taylor et al.[45]
H. qinggangjun	MHKMU L.P. Tang 1683	China: Yunnan	MW290150	Huang et al.[10]
H. qinggangjun	MHKMU M. Mu 464	China: Yunnan	MW290155	Huang et al.[10]
H. qinggangjun	MHKMU S.D. Yang 20	China: Yunnan	MW290149	Huang et al.[10]
H. roseobrunneus	src29	USA	DQ974727	Smith et al.[39]
H. roseobrunneus	src414	USA	EF559268	Smith et al.[40]
H. roseodiscoideus	LIP 0402283	France	MZ576440	Bellanger et al.[53]
H. roseodiscoideus	DiBo041202	France	MZ576441	Bellanger et al.[53]
H. roseoviolaceus	SAAS4509	China: Chongqing	OQ860087	Wang et al.[9]
H. russula	AH19677	Spain	MW290205	Huang et al.[10]
H. russula	AH37145	Spain	MW290206	Huang et al.[10]
H. russuliformis	MICH 10939	USA	MF399455	Unpublished
H. rutilans	XHW6661	China	OP547769	Wang et al.[9]
H. rutilans	XHW7521	China	OP547779	Wang et al.[9]
H. scabrellus	LAH35245	Pakistan	MK066234	Naseer et al.[51]
H. siccipes	GDGM70041	USA	OP547637	Wang et al.[9]
H. siccipes	GDGM75574	USA	OP547652	Wang et al.[9]
H. speciosus	HMAS 278060	China	OP547746	Wang et al.[9]
H. speciosus	**BJTC FM135**	**China: Shanxi**	**PQ113871**	**This study**
H. speciosus	**BJTC FM3837**	**China: Shanxi**	**PQ113869**	**This study**
H. speciosus	**BJTC FM3841**	**China: Shanxi**	**PQ113870**	**This study**
H. subsalmonius	4756-HRL 1323	Canada	KM248870	Unpublished
H. unicolor	SJ97046	Sweden	AY242857	Larsson & Jacobsson[37]
H. unicolor	Montri-233	Switzerland	MK028423	Hofstetter et al.[50]
H. viridiflavidus	BJTC FM978	China: Shanxi	OP699731	Zhang et al.[17]
H. viridiflavidus	BJTC FM999	China: Shanxi	OP699730	Zhang et al.[17]
H. viridiflavidus	**BJTC FM4273**	**China: Shanxi**	**PQ113860**	**This study**
H. xiangjun	LJW2256	China: Yunnan	OP547753	Wang et al.[9]
H. xiangjun	MHKMU H.Y. Huang 817	China: Yunnan	MW762979	Huang et al.[6]
H. xiangjun	MHKMU Y.J. Pu 407	China: Yunnan	MW762978	Huang et al.[6]
H. yukishiro	TUMH:61715	Japan	LC270641	Naoki et al.[49]
H. yunnanensis	MHKMU H.Y. Huang 322	China: Yunnan	MW290214	Huang et al.[10]
H. yunnanensis	MHKMU L.P. Tang 2772	China: Yunnan	MW290211	Huang et al.[10]
H. yunnanensis	MHKMU L.P. Tang 2773	China: Yunnan	MW290212	Huang et al.[10]
H. sp.1	522719	Mexico	FJ196923	Morris et al.[41]
H. sp.1	HRL1647	Canada	MT981622	Bellanger et al.[19]
H. sp.3	RGT131109/02a	Canada	MN752314	Unpublished
H. sp.3	RGT131109/02b	Canada	MN752315	Unpublished

本研究测序获得的序列用粗体表示

Newly sequenced sequences in this study are in black bold.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Hygrophorus pallidoagathosmus GDGM84702 China Sichuan (OP547728)
Hygrophorus albofloccosus GDGM70044 USA (OP547639)
99/1
Hygrophorus albofloccosus GDGM70063 USA (OP547642)
Hygrophorus albofloccosus UCSC F2171 USA (MT981691)
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Hygrophorus agathosmoides HRL2823 Canada (MT981656)
Hygrophorus agathosmus EL39817 Sweden (MH656445)
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Hygrophorus agathosmus EL19115 Norway (MH656454)
Hygrophorus agathosmus E00905345 UK (MZ605815)
Hygrophorus pinophilus Huang 878 China Yunnan (MZ605854)
100/1
Hygrophorus pinophilus GDGM43348 China Sichuan (OP547622)
84/1
Hygrophorus pinophilus EL28911 Sweden (MH656468)
Hygrophorus hyacinthinus 0401691 France (MT845204)
100/1
Hygrophorus hyacinthinus JMM10101001 France (MT845203)
99/1
Hygrophorus hyacinthinus EL32610 Sweden (MH656470)
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Hygrophorus brunneiceps HKAS 54804 China Yunnan (MZ605840)
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100/1
Hygrophorus pustulatus 3255L1 USA (KF617543)
Hygrophorus pustulatus CMMF005053 Canada (MT981676)
Hygrophorus limosus ML811131HL Cyprus (MT981621)
100/1
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98/1
Hygrophorus mesotephrus K (M) 227410 UK (MT981695)
100/1
Hygrophorus sp. 1 KMB2020 HRL1647 subu (MT981622)
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96/1
Hygrophorus viridiflavidus BJTC FM4273 China Shanxi (PQ113860)
Hygrophorus viridiflavidus BJTC FM978 China Shanxi (OP699731)
96/1
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Hygrophorus sp. RGT13110902b (MN752315)
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77/0.99
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Hygrophorus glutinifer E00218159 UK (MZ605844)
Sect. Olivaceoumbrini
100/1
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100/1
Hygrophorus esculentus BJTC FM3976 China Shanxi (PQ113874)
Hygrophorus gliocyclus GDGM84514 China Sichuan (OP547703)
100/1
Hygrophorus gliocyclus BJTC FM1225 China Shanxi (PQ113875)
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Hygrophorus xiangjun Huang 817 China Yunnan (MW762979)
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Hygrophorus lucorum BJTC FM1998 China Shanxi (PQ113872)
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Hygrophorus lucorum GDGM42148 Italy (OP547621)
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Hygrophorus speciosus HMAS 278060 China (OP547746)
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Hygrophorus alpinus BJTC FM4232 China Shanxi (PQ113866)
88/1
Hygrophorus alpinus BJTC FM4279 China Shanxi (PQ113867)
Hygrophorus alpinus Zhang 4631 China Yunnan (MW762962)
100/1
Hygrophorus alpinus Zhang 4632 China Yunnan (MW762963)
93/1
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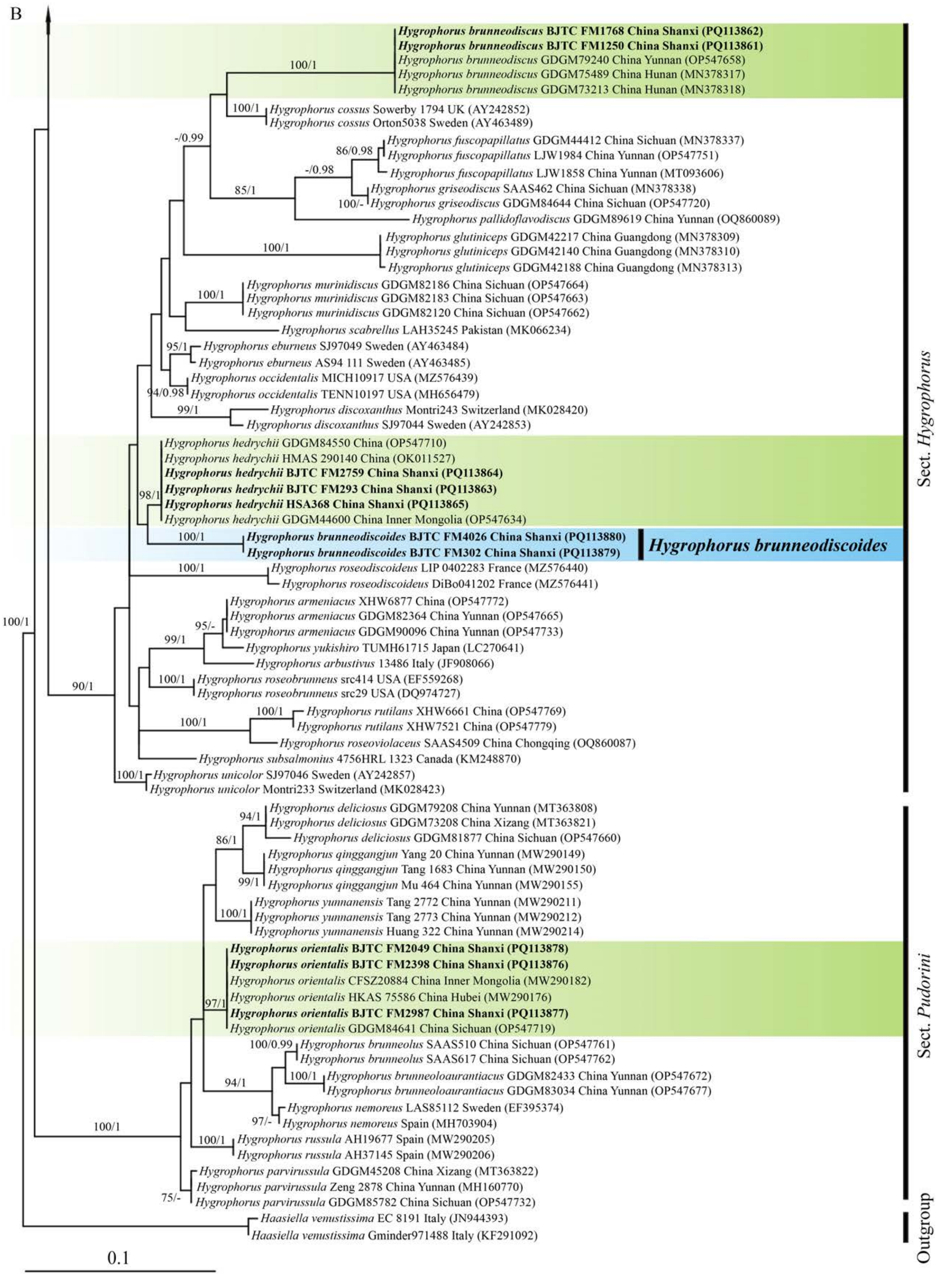
Hygrophorus pseudohypothejus Huang 447 China Yunnan (MW762991)
72/0.96
Hygrophorus hypothejus 781716 UK (HM146822)
76/0.99
Hygrophorus hypothejus 8827 Switzerland (JF908076)
Hygrophorus hypothejus KUNHKAS56550 Germany (MW616467)
Hygrophorus siccipes GDGM70041 USA (OP547637)
70/-
Hygrophorus siccipes GDGM75574 USA (OP547652)
100/1
Hygrophorus fuligineus GDGM70064 USA (OP547643)
Hygrophorus fuligineus GDGM70057 USA (OP547641)
-/0.96
98/1
Hygrophorus boyeri HL 1113 Canada (MG882094)
Hygrophorus boyeri HVM 27 USA (KF879478)
Hygrophorus meridionalis LIP 0400300 Greece (MG882100)
Sect. Aurei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Hygrophorus brunneodiscus BJTC FM1250 China Shanxi (PQ113861)
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Hygrophorus brunneodiscus GDGM75489 China Hunan (MN378317)
Hygrophorus brunneodiscus GDGM73213 China Hunan (MN378318)
100/1
Hygrophorus cossus Sowerby 1794 UK (AY242852)
Hygrophorus cossus Orton5038 Sweden (AY463489)
100/1
-/0.99
Hygrophorus fuscopapillatus GDGM44412 China Sichuan (MN378337)
Hygrophorus fuscopapillatus LJW1984 China Yunnan (OP547751)
86/0.98
Hygrophorus fuscopapillatus LJW1858 China Yunnan (MT093606)
-/0.98
85/1
Hygrophorus griseodiscus SAAS462 China Sichuan (MN378338)
100/-
Hygrophorus griseodiscus GDGM84644 China Sichuan (OP547720)
Hygrophorus pallidoflavodiscus GDGM89619 China Yunnan (OQ860089)
Hygrophorus glutiniceps GDGM42217 China Guangdong (MN378309)
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Hygrophorus glutiniceps GDGM42140 China Guangdong (MN378310)
Hygrophorus glutiniceps GDGM42188 China Guangdong (MN378313)
Hygrophorus murinidiscus GDGM82186 China Sichuan (OP547664)
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Hygrophorus murinidiscus GDGM82183 China Sichuan (OP547663)
Hygrophorus murinidiscus GDGM82120 China Sichuan (OP547662)
Hygrophorus scabrellus LAH35245 Pakistan (MK066234)
95/1
Hygrophorus eburneus SJ97049 Sweden (AY463484)
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Hygrophorus occidentalis MICH10917 USA (MZ576439)
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Hygrophorus occidentalis TENN10197 USA (MH656479)
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Hygrophorus discoxanthus SJ97044 Sweden (AY242853)
Hygrophorus hedrychii GDGM84550 China (OP547710)
Hygrophorus hedrychii HMAS 290140 China (OK011527)
Hygrophorus hedrychii BJTC FM2759 China Shanxi (PQ113864)
98/1
Hygrophorus hedrychii BJTC FM293 China Shanxi (PQ113863)
Hygrophorus hedrychii HSA368 China Shanxi (PQ113865)
Hygrophorus hedrychii GDGM44600 China Inner Mongolia (OP547634)
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Hygrophorus brunneodiscoides BJTC FM302 China Shanxi (PQ113879)
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Hygrophorus roseodiscoideus DiBo041202 France (MZ576441)
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Hygrophorus yukishiro TUMH61715 Japan (LC270641)
Hygrophorus arbustivus 13486 Italy (JF908066)
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Hygrophorus roseobrunneus src29 USA (DQ974727)
100/1
Hygrophorus rutilans XHW6661 China (OP547769)
100/1
Hygrophorus rutilans XHW7521 China (OP547779)
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Hygrophorus subsalmonius 4756HRL 1323 Canada (KM248870)
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Hygrophorus unicolor SJ97046 Sweden (AY242857)
Hygrophorus unicolor Montri233 Switzerland (MK028423)
90/1
100/1
Sect. Hygrophorus
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94/1
Hygrophorus deliciosus GDGM73208 China Xizang (MT363821)
Hygrophorus deliciosus GDGM81877 China Sichuan (OP547660)
86/1
Hygrophorus qinggangjun Yang 20 China Yunnan (MW290149)
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99/1
Hygrophorus qinggangjun Mu 464 China Yunnan (MW290155)
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100/1
Hygrophorus yunnanensis Tang 2773 China Yunnan (MW290212)
Hygrophorus yunnanensis Huang 322 China Yunnan (MW290214)
Hygrophorus orientalis BJTC FM2049 China Shanxi (PQ113878)
Hygrophorus orientalis BJTC FM2398 China Shanxi (PQ113876)
Hygrophorus orientalis CFSZ20884 China Inner Mongolia (MW290182)
97/1
Hygrophorus orientalis HKAS 75586 China Hubei (MW290176)
Hygrophorus orientalis BJTC FM2987 China Shanxi (PQ113877)
Hygrophorus orientalis GDGM84641 China Sichuan (OP547719)
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Hygrophorus brunneolus SAAS617 China Sichuan (OP547762)
100/1
Hygrophorus brunneoloaurantiacus GDGM82433 China Yunnan (OP547672)
94/1
Hygrophorus brunneoloaurantiacus GDGM83034 China Yunnan (OP547677)
Hygrophorus nemoreus LAS85112 Sweden (EF395374)
97/-
Hygrophorus nemoreus Spain (MH703904)
100/1
100/1
Hygrophorus russula AH19677 Spain (MW290205)
Hygrophorus russula AH37145 Spain (MW290206)
Hygrophorus parvirussula GDGM45208 China Xizang (MT363822)
Hygrophorus parvirussula Zeng 2878 China Yunnan (MH160770)
75/-
Hygrophorus parvirussula GDGM85782 China Sichuan (OP547732)
Sect. Pudorini
Haasiella venustissima EC 8191 Italy (JN944393)
Haasiella venustissima Gminder971488 Italy (KF291092)
Outgroup
0.1

图 1　蜡伞属的 *Aurei* 组、*Hygrophorus* 组、*Olivaceoumbrini* 组和 *Pudorini* 组物种基于 ITS 序列的 ML 树　A：系统发育树的上半部分. B：系统发育树的下半部分. 分支节点上的数字表示 ML 自举值(≥70%，左)和贝叶斯后验概率(≥0.95，右)；括号中的序号表示序列的 GenBank 号；标尺表示进化距离. *Haasiella venustissima* 作为外类群. 本研究测序获得的序列用粗体标出，新物种序列以蓝色背景标出，其余山西分布的已知种以绿色背景标出

Figure 1　The ML phylogenetic tree based on the ITS sequences of sect. *Aurei*, sect. *Hygrophorus*, sect. *Olivaceoumbrini* and sect. *Pudorini* in the genus *Hygrophorus*. A: The upper half of the phylogenetic tree. B: The lower half of the phylogenetic tree. Values at the node represent ML bootstrap (≥70% BS, left) and Bayesian posterior probability (≥0.95 PP, right) support; The sequence number in parentheses indicates the GenBank number of the sequence; The scale bar indicates the evolutionary distance. *Haasiella venustissima* is the outgroup. Our specimen sequences are printed in bold, the new species sequences are highlighted in blue, and the known species in Shanxi Province are highlighted in green.

and *H. viridiflavidus*, while the remaining clade was independent, supporting it is new to science, and it was therefore described as *Hygrophrus brunneodiscoides* sp. nov. in this paper. *H. brunneodiscoides* further clustered with the sequences of *H. hedrychii*, implying they were phylogenetically related to each other.

2.2　Taxonomy

Eleven species of *Hygrophorus* are confirmed in this study, including ten known species and the new species *Hygrophorus brunneodiscoides* sp. nov. (Figure 2, Figure 3) The known species are *H. alpinus*, *H. brunneodiscus*, *H. esculentus*, *H. gliocyclus*, *H. hedrychii*, *H. lucorum*, *H. orientalis*, *H. pallidoagathosmus*, *H. speciosus* and *H. viridiflavidus*, and the photos of basidiocarp of which are provided (Figure 4). The new species *Hygrophorus brunneodiscoides* is described and illustrated as follows. Furthermore, a key to the species of *Hygrophorus* in Shanxi Province is provided.

Hygrophorus brunneodiscoides L. Fan & Y.X. Zhang sp. nov. (Figure 2, Figure 3)

MycoBank: MB855008

Diagnosis: Basidiomata small-sized; Pileus convex to plano-convex, creamy white or light brown with dark brownish centre; Lamellae white to cream, thick and waxy, unchanging when bruised; Stipe cylindrical, creamy white to pale yellowish with white floccules at the apex; *Cystidioid elements* absent and smaller basidiospores [(5.5–)6.0–7.0(–8.5)]×[(4.0–)4.5–5.0(–6.0)] μm.

Etymology: *H. brunneodiscoides*, refer to the similarity to *H. brunneodiscus*.

Holotype: China. Shanxi Province, Pu County, Wulu Mountains, on the ground in forest of *Pinus tabuliformis*, 10 September 2017, collected by *X.Y. Yan YXY170870* (BJTC FM302 GenBank Acc. No.: ITS=PQ113879, nrLSU=PQ113856, *tef1-α*=PQ114589).

Description: **Basidiomata** small-sized, firm, fleshy, viscid, with dark brown tone. **Pileus** 20–40 mm, convex or obtusely conical with strongly involute margin at first, then becoming plano-convex, plane to slightly depressed, first rather creamy white (#f6f1eb) or light brown (#d0ac64) with dark brownish (#6b4223) to blackish brown (#544234) center, then paler especially in marginal zone towards white greyish (#f2f1f1) or light brown (#d9c99d), centre always much darker, very strongly glutinous. **Lamellae** adnate to subdecurrent, thick and waxy, white (#fbfaf9) to cream (#e4debe), unchanging when bruised. **Stipe** (35–65)×(5–12) mm, cylindrical, sometimes flexuous and tapering-rooting towards the base, creamy white (#e8e6df) to pale yellowish (#f6f1d0), smooth or with white (#f4f3f3) Floccules at the apex, most covered by unconspicuous light brownish bands of glutinous. **Context** thick, pure white (#f6f5f3) to creamy (#fff2cd). **Smell** indistinct. **Taste** not recorded. **Spore** deposit white.

图 2　拟褐顶蜡伞的担子果　A：BJTC FM302 主模式. B：BJTC FM4026 副模式. 标尺：10 mm

Figure 2　The basidiomata of *Hygrophorus brunneodiscoides*. A: BJTC FM302 holotype. B: BJTC FM4026 paratype. Scale bars: 10 mm.

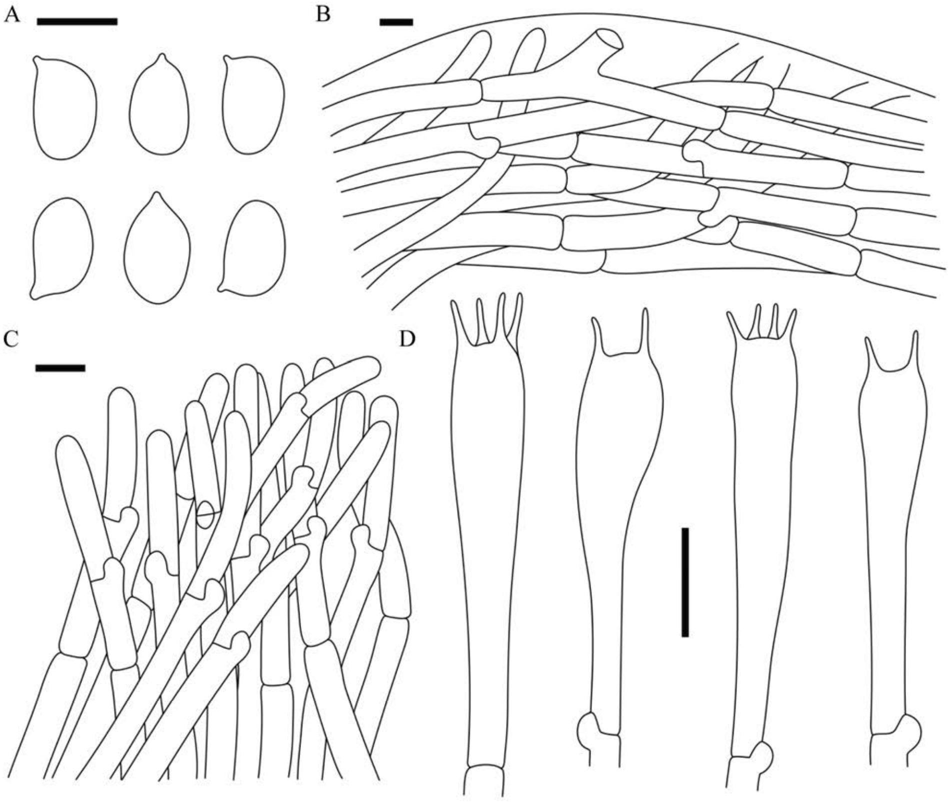

图 3　拟褐顶蜡伞的微观特征　A：担孢子. B：菌盖皮层. C：菌柄顶端絮凝物. D：担子. 标尺：A 和 B 为 5 μm；C 和 D 为 10 μm

Figure 3　Microscopic features of *Hygrophorus brunneodiscoides*. A: Basidiospores. B: Pileipellis. C: Floccules at the apex of stipe. D: Basidia. Scale bars: A and B, 5 μm; C and D, 10 μm.

Basidiospores ellipsoid to broadly ellipsoid, measuring [(5.5–)6.0–7.0(–8.5)]×[(4.0–)4.5–5.0(–6.0)] μm; Q=1.23–1.60; Q_{m}=1.40±0.09, with an oblique hilar appendage 0.5–1.0 μm long; inamyloid, thin-walled, smooth and hyaline or some have one large oleiferous guttule. **Basidia** mostly 4-spored, less 2-spored, clavate, (32.0–49.5)×(5.5–10.5) μm; sterigmata 3–7 μm long. **Cystidioid elements** absent. **Hymenophoral trama** divergent, comprised of cylindrical, interwoven, branched hyphae 3–15 μm wide. **Pileipellis** an ixotrichoderm composed of hyaline or yellow-pigmented interwoven hyphae 3–8 μm wide, sparsely branching and embedded in a gelatinous matrix; incrustations or extracellular pigment not seen. **Subpellis** composed of more or less parallel hyphae up to 13 μm wide. **Stipitipellis** an cutis, made up of branched hyphae 3.5–6.5 μm wide, with yellowish brown intracellular pigment; terminal elements rounded to attenuated, not enlarged. **Stipititrama** composed of densely packed, more or less parallelly arranged, subhyaline hyphae up to 13.5 μm wide. **Floccules at the apex** composed of compact fascicles of branched hyphae with cylindrical or slender clavate terminal elements (23–63)×(4–7) μm. **Clamp connections** present in all tissues.

Ecology & Distribution: on the ground in forest dominated by *Pinus tabuliformis* and *Quercus* sp., only known from Shanxi Province, northern China.

Additional specimen examined (paratypes): China. Shanxi Province, Yangcheng County, Zhongtiao Mountains, on the ground in mixed forest of *Pinus tabuliformis* and *Quercus* sp., elev. 1 720 m, 112°4′56″E, 35°14′4″N, 4 October 2023, CF2256 (BJTC FM4026 GenBank Acc. No.: ITS=PQ113880, nrLSU=PQ113857, *rpb2*=PQ114588).

Notes: Our molecular analysis reveals that this new species belongs to *Hygrophorus* section *Hygrophorus* and closely related to *H. hedrychii* (Figure 1) Morphologically, *H. hedrychii* has white pileus with orange-pinkish tint at centre, lamellae changing to pale ochraceous pink when mature and the host association with *Betula* sp.[8].

Amongst the members of section *Hygrophorus*, *Hygrophorus brunneodiscoides* resembles *H. brunneodiscus*, *H. fuscopapillatus* C.Q. Wang & T.H. Li, *H. griseodiscus* C.Q. Wang & T.H. Li, *H. murinidiscus* C.Q. Wang & T.H. Li and *H. occidentalis* A.H. Sm. & Hesler because their pileus all have a brown tinge. However, *H. brunneodiscus* can be differentiated from the new species by its larger basidiomata with slight fishy odour, Pileus with brownish-orange center and longer basidiospores (6.5–9.5)×(4.0–6.0) μm [*vs*. (5.5–8.5)×(4.0–6.0) μm in *H. brunneodiscoides*][8]; *H. fuscopapillatus* has pale grey pileus with brownish-grey to olive brown umbo, longer basidiospores (6.0–10.0)×(4.0–6.0) μm, smaller basidia (32–48)×(6.0–9.0) μm, and occurs in *Fagaceae*-dominated forests[8]; *H. griseodiscus* has light grey pileus with dark grey or olive grey center, larger basidiospores (7.0–10.5)×(4.0–6.5) μm and is associated with subalpine coniferous forest dominated by *Abies* and *Picea*[8]; *H. murinidiscus* has larger basidiomata, Pileus with mouse grey center, longer and narrower basidiospores (7.0–9.5)×(4.0–5.5) μm and longer basidia with *Sterigmata* up to 9 μm long[9]; *H. occidentalis* has white pileus with grayish brown center, narrower basidiospores (6.0–8.0)×(3.5–5.0) μm and is only distributed in North America[62].

2.3 Key to the species of *Hygrophorus* from Shanxi Province of northern China

1. Floccules at the stipe apex present ······· 2
1. Floccules at the stipe apex absent ········ 3
2. Basidiospores longer, (8.5–11.0)×(4.5–6.0) μm ······································ *H. viridiflavidus*
2. Basidiospores shorter, (5.5–8.5)×(4.0–6.0) μm ································· *H. brunneodiscoides*
3. Cheilocystidia and Pleurocystidia present ·· 4
3. Cheilocystidia and Pleurocystidia absent··· 8
4. Annular zone present at stipe apex ······· 5
4. Annular zone absent at stipe apex ········ 6
5. Pileus light yellow, orange white··········· *H. esculentus*
5. Pileus bright scarlet red or vivid yellow-orange ························· *H. speciosus*

图 4　山西省蜡伞属物种的担子果

Figure 4　Basidiomata of *Hygrophorus* species in Shanxi Province. A, B: *H. alpinus* (BJTC FM4232, BJTC FM4278). C: *H. brunneodiscus* (BJTC FM1768). D: *H. esculentus* (BJTC FM3976). E: *H. hedrychii* (BJTC FM293). F: *H. lucorum* (BJTC FM1994). G–J: *H. orientalis* (BJTC FM2398, BJTC FM2987). K: *H. pallidoagathosmus* (BJTC FM1183). L–M: *H. speciosus* (BJTC FM384, BJTC FM135). N–P: *H. viridiflavidus* (BJTC FM978, BJTC FM999, BJTC FM4273). Scale bars: A–P, 1 cm.

6. Habitat in broad-leaved forests ······································· *H. orientalis*

6. Habitat in conifer tree forests ············ 7

7. Pileus greyish yellow to olive brown ······································· *H. alpinus*

7. Pileus lemon yellow to chrome yellow ······································· *H. lucorum*

8. Eurasian distribution ······················ 9

8. Only distribution in China ··············· 10

9. Annular zone present at stipe apex ········ ··*H. gliocyclus*

9. Annular zone absent at stipe apex ········· ·· *H. hedrychii*

10. Habitat in broad-leaved forests ··········· ······································ *H. brunneodiscus*

10. Habitat in mixed broadleaf-conifer tree forests························· *H. pallidoagathosmus*

3 Discussion

The specificity of host association of some *Hygrophorus* species in Shanxi Province has been observed. *Hygrophorus lucorum* is a *Larix* associated species exclusively in this region; *H. brunneodiscus* is exclusively with *Quercus liaotungensis* Koidz.; *H. hedrychii* associated with *Betula* sp.; both *H. pallidoagathosmus* and *H. speciosus* grow under subalpine conifers composed of *Larix* and *Picea* only; *H. alpinus*, *H. gliocyclus* and *H. esculentus* all grow under pine, but both *H. alpinus* and *H. gliocyclus* are exclusively associated with *Pinus tabuliformis* while *H. esculentus* with *Pinus armandii* Franch. only; *H. orientalis* is observed to be associated with *Quercus liaotungensis*; both *H. viridiflavidus* and *H. brunneodiscoides* are collected from the mixed forest of *Quercus liaotungensis* and *Pinus tabuliformis*, and we are currently unsure which one is their host or maybe both are.

Our observations also showed that each species of *Hygrophorus* seems to have a distinct distribution area, and both the host tree and climate significantly shaped their geographic map. *Hygrophorus lucorum*, *H. pallidoagathosmus* and *H. speciosus* are distributed in subalpine area of northern region of Shanxi Province where has cold climate and are covered by conifers composed of *Picea*-*Larix*. All the remaining eight species are distributed in southern area of Shanxi Province where have warm climate and forest dominated by *Pinus* spp. and *Quercus* spp. *Hygrophorus orientalis* is the most popularly encountered species in south region of Shanxi Province, and it can colonize in any forested area with *Quercus liaotungensis* planted. Both *H. brunneodiscoides* and *H. hedrychii* also have a wide range of geography in south region, but less encountered. In contrast, *H. alpinus*, *H. esculentus* and *H. viridiflavidus* have been observed to limit their distribution in the Zhongtiao Mountains, the southernmost running east-west mountains in Shanxi Province where has somewhere subtropical climate.

4 Conclusion

Eleven species of *Hygrophorus* have been recognized from Shanxi Province in this study, including 10 known species and a new species, *Hygrophorus brunneodiscoides*, which suggests that Shanxi Province is actually rich in *Hygrophorus* species.

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