

Mycobiology



ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tmyb20

Unveiling the Diversity of Hydnum in the Republic of Korea with One New Species, Hydnum paucispinum

Ji Seon Kim, Wonjun Lee, Changmu Kim, Hanna Park, Chang Sun Kim & Young Woon Lim

To cite this article: Ji Seon Kim, Wonjun Lee, Changmu Kim, Hanna Park, Chang Sun Kim & Young Woon Lim (28 Oct 2023): Unveiling the Diversity of *Hydnum* in the Republic of Korea with One New Species, Hydnum paucispinum, Mycobiology, DOI: 10.1080/12298093.2023.2265137

To link to this article: https://doi.org/10.1080/12298093.2023.2265137

6 © 2023 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.

|--|

View supplementary material 🖸



Published online: 28 Oct 2023.



🧭 Submit your article to this journal 🕑

Article views: 1



View related articles 🗹

View Crossmark data 🗹

RESEARCH ARTICLE

OPEN ACCESS

Taylor & Francis

Unveiling the Diversity of *Hydnum* in the Republic of Korea with One New Species, *Hydnum paucispinum*

Ji Seon Kim^a (b), Wonjun Lee^a (b), Changmu Kim^b, Hanna Park^a (b), Chang Sun Kim^c (b) and Young Woon Lim^a (b)

^aSchool of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul, Korea; ^bSpecies Diversity Research Division, National Institute of Biological Resources, Incheon, Korea; ^cForest Biodiversity Division, Korea National Arboretum, Pocheon-si, Korea

ABSTRACT

Hydnum is a genus of ectomycorrhizal fungi belonging to the Hydnaceae family. It is widely distributed across different regions of the world, including North America, Europe, and Asia; however, some of them showed disjunct distributions. In recent years, with the integration of molecular techniques, the taxonomy and classification of *Hydnum* have undergone several revisions and advancements. However, these changes have not yet been applied in the Republic of Korea. In this study, we conducted an integrated analysis combining the morphological and molecular analyses of 30 specimens collected over a period of approximately 10 years in the Republic of Korea. For molecular analysis, the sequence data of the internal transcribed spacer (ITS) region, the large subunit of nuclear ribosomal RNA gene (nrLSU), and a portion of translation elongation factor $1-\alpha$ (*TEF1*) were employed as molecular markers. Through this study, we identified eight species that had previously not been reported to occur in the Republic of Korea, including one new species, *Hydnum paucispinum*. A taxonomic key and detailed descriptions of the eight *Hydnum* species are provided in this study.

ARTICLE HISTORY

Received 12 July 2023 Revised 26 September 2023 Accepted 26 September 2023

KEYWORDS Hydnum; Hydnaceae; ectomycorrhiza; sequencebased identification

1. Introduction

The genus *Hydnum* L. belongs to the family Hydnaceae [1,2], and its type species is *H. repandum* [3]. This genus is characterized by pileate to stipitate basidiomata; aculeate hymenophores; cylindrical to clavate basidia; and subglobose to ovoid, elliptical, colorless basidiospores. The presence of an aculeate hymenophore is the most distinctive characteristic of *Hydnum* [4–6]. *Hydnum* plays an important role in forest ecology, as it forms ectomycorrhizal relationships with certain trees, such as Pinaceae and Fagaceae [7–9].

In the early fungal taxonomic generation, terrestrial agaric mushrooms with aculeate hymenophores were assigned to the genus *Hydnum* [10–12]. As a result, there are currently 978 records of *Hydnum* species registered in the Index Fungorum (http:// www.indexfungorum.org/, accessed on May 26, 2023). Sequence-based classification (SBC) and identification (SBI) methods have been widely used to overcome the limitations of morphology-based identification of fungi [13]. Molecular phylogenetic analysis has shown that the aculeate hymenophore has evolved independently several times in distantly related taxa [14]. Consequently, many species formerly classified as *Hydnum* have been reclassified into other genera such as *Hydnellum* and *Sarcodon* [15,16]. The SBC of *Hydnum* has undergone continuous revisions [2,17,18], leading to the reestablishment of the Hydnaceae family, including the genus *Hydnum* [19]. Furthermore, the SBI has revealed numerous misidentifications of species within the genus *Hydnum* [20–22].

Currently, only one *Hydnum* species (*H. repandum*) and its varieties (*H. repandum* var. *album*) have been described in the Republic of Korea [23]. Previously, six *Hydnum* species and one variety were reported (*H. aspratum*, *H. caput-medusae*, *H. crinaceus*, *H. imbricatum*, *H. repandum*, *H. repandum* var. *album*, *H. septentrionale*). However, many of these species were subsequently reclassified into other genera. Only reports of *H. repandum* and its variety *H. repandum* var. *album* have persisted in the Republic of Korea for over 80 years [24]. Recent studies based on the SBC and SBI have revealed that many Asian mycorrhizal species have been

CONTACT Young Woon Lim 🔯 ywlim@snu.ac.kr

B Supplemental data for this article can be accessed online at https://doi.org/10.1080/12298093.2023.2265137.

^{© 2023} The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

misidentified, often using the names of European and American species [25–27]. *Hydnum repandum* and *H. repandum* var. *album* are ectomycorrhizal species named after European specimens; however, the applicability of these names has not been clarified through molecular analyses. Additionally, a previous analysis of global *Hydnum* diversity indicated clear differences between East Asian species and those from other continents [17]. Therefore, taxonomic studies based on molecular phylogeny are necessary to elucidate mycorrhizal *Hydnum* species in the Republic of Korea.

To address taxonomic uncertainties and uncover the true diversity of *Hydnum* species in the Republic of Korea, we investigated hydnoid specimens preserved in three main Korean Fungariums. We employed three molecular markers: the sequences of internal transcribed spacer (ITS), nuclear ribosomal large subunit RNA (nrLSU), and the translation elongation factor 1- α (*TEF1*) gene. As a result, a total of eight *Hydnum* species were identified in the Republic of Korea. Among these, one species is potentially new to science, emphasizing the significance of this research in revealing the previously unrecognized *Hydnum* diversity in the Republic of Korea.

2. Materials and methods

2.1. Samples and morphological observations

Thirty *Hydnum* specimens, collected between 2009 and 2021, were examined (Table 1). The specimens were sourced from three institutions: the Seoul National University Fungus Collection (SFC), the Korea National Arboretum (KA), and the National Institute of Biological Resources of Korea (NIBR). Most specimens were collected within the Republic of Korea, while a few were obtained from Central Siberia, Russia and Lac Dương District, Vietnam. The detailed collection information for each specimen is presented in Table 1. Most of the specimens were temporarily identified as *H. repandum* based on their morphology. Specimen information such as locality, habitat, and photographs was obtained from each fungarium.

Macromorphological features of fresh basidiomata were described based on photographs of fresh specimens taken in the field. Color names and alphanumeric codes were rated using the Methuen Handbook of Colour [28]. Micromorphological features, including basidiospores, basidia, and hyphae, were observed and measured in dried specimens using an Eclipse 80i compound light microscope (Nikon, Japan) and NIS ELEMENT BR software v3.2 (Nikon, Japan). Small pieces of spinose structures from each specimen were mounted in 5% KOH and stained with 1% Congo red. A minimum of 20 basidia, 30 basidiospores, and 20 other microstructures were measured for each specimen. The measurements were taken at $400 \times$ magnification using ImageJ software [29]. The *E* value represents the length-to-width ratio of basidiospores, and the *Q* value indicates the average *E* value. The *Q* value was used to determine the shape of the spore morphology, and the range of *E* values and the photo taken during microscopic observation were used to confirm this using A Glossary of Mycology [30]. Melzer's reagent was used to assess the amyloid reaction [31].

2.2. Molecular approaches: DNA extraction, PCR, sequencing, and phylogenetic analysis

The tissue samples were placed in CTAB buffer and homogenized using a bead ruptor elite homogenizer (OMNI International, Kennesaw, GA, USA). Genomic DNA was extracted using the AccuPrep Genomic DNA Extraction Kit (Bioneer Co., Daejeon, Korea) according to the manufacturer's protocol. The ITS region was amplified using the primer set ITS1F/ITS4 [32,33] and the nrLSU region was amplified using three different primer sets (LR0R/LR3, LR5, and LR7) [33]. For the TEF1 region, Hydnum-specific primers from previous studies were used (HEF1F/HEF1R and HydTEF1-F/ HydTEF1-R) [17,34]. Polymerase chain reaction was performed using the AccuPower PCR premix (Bioneer Co., Daejeon, Korea) in a C1000 thermal cycler (Bio-Rad, Richmond, CA, USA), according to the PCR conditions described by [26]. The PCR amplicons were verified by electrophoresis on a 1% agarose gel and purified using an Expin PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea). The same primer pairs were used for sequencing, which was performed using an ABI Prism 3700 Genetic Analyzer (Life Technologies, Gaithersburg, MD, USA) by Macrogen (Seoul, Republic of Korea).

The generated sequences were manually proofread and edited using MEGA7 [35] and deposited in GenBank. The aligned sequences were checked and edited manually using MEGA7 [35]. For the phylogenetic analysis, reference sequences were retrieved from GenBank and UNITE and analyzed using our sequences (Table 2). Although the *TEF1* region has recently been used in *Hydnum* phylogenetic studies [17,19], there are currently not enough *TEF1* region sequences available for phylogenetic analysis. Therefore, this study provided supplemental *TEF1* sequence information for Korean specimens to aid future research. All sequences of the ITS and nrLSU regions were

Table 1. Summary of information information of the Hydnum specimens collected for the present study.

Species	Specimen NUM	Country	Site	Collection date	ITS	nrl SI I	TEE1
Hydnum albonallidum	KA16-0626	Republic of	Forimok Valley, Haean-dong	2016-07-18	OR211374	OR211385	
	KA16 1002	Korea	Jeju-si, Jeju island	2010 07 10	01/2113/4	01/211303	00000040
	KA 16-1082	Kepublic of Korea	Eorimok Valley, Haean-dong, Jeju-si, Jeju island	2016-09-26	ON907799	ON907770	OR220049
H. cremeoalbum	SFC20181012-04	Republic of Korea	Hong island, Hongdo-ri, Heuksan-myeon, Sinan-gun, Joollanam do	2018-10-12	ON907782	ON907775	
H. minus	KA13-1475	Republic of Korea	Udu Mountain, Suwol-ri, Gajo- myeon, Geochang-gun,	2013-10-17	ON907783	ON907767	
	KA16-1189	Republic of Korea	Gyeongsangnam-do Sogwang-ri, Seo-myeon, Ulleung-gun,	2016-10-05	ON907784	OR211387	
	KA17-0894	Republic of Korea	Gyeongsangbuk-do Sogwang-ri, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	2017-09-14	ON907785	OR211388	
	KA20-0486	Republic of Korea	Dongmyeon-ri, Jaesan-myeon, Bonghwa-gun, Gyeongsangbuk-do	2020-08-13	ON907786	ON907771	OR220054
	KA20-0682	Republic of Korea	Dongmyeon-ri, Jaesan-myeon, Bonghwa-gun, Gyeongsanghuk-do	2020-09-14	ON907787	OR211389	OR220056
	KA20-0770	Republic of	Doota Mountain, Miro-myeon,	2020-09-16	ON907788	ON907766	
	KA20-0815	Republic of Korea	1610, Guryongnyeong-ro, Seo- myeon, Yangyang-gun, Gangwon-do	2020-09-17	ON907789	OR211390	OR220055
H. orientalbidum	NIBRFG0000122620	Republic of	Daeam Mountain, Yanggu-gun,	2012-09-27	OR211377	OR211393	
	NIBRFG0000109936	Republic of	Hakgok-ri, Socho-myeon,	2009-08-15	OR211375	OR211391	
	SFC20150902-97	Korea Republic of	Wonju-si, Gangwon-do 760, Jajangnamusup-gil, Inje- eun, Inje-gun, Gangwon-do	2015-09-02	OR211381	OR211399	OR220060
	NIBRFG0000113465	Republic of Korea	Unak Mountain, Hwahyeon- myeon, Pocheon-si,	2009-09-18	OR211376	OR211392	
H. pallidomarginatum	KA16-0264	Republic of Korea	Gyeonggi-do Bakji island, 209, Bakjido-gil, Anjwa-myeon, Sinan-gun, Jeollanam-do	2016-06-28	ON907790	ON907765	OR220046
	KA16-0511	Republic of Korea	Bakji island, 209, Bakjido-gil, Anjwa-myeon, Sinan-gun,	2016-07-12	ON907791	ON907763	OR220047
	SFC20180705-81	Republic of Korea	Duryunsan, Samsan-myeon, Haenam-gun, Jeollanam-do	2018-07-05	ON907792	ON907764	OR220048
H. pinicola	KA13-1480	Republic of Korea	Udu Mountain, Suwol-ri, Gajo- myeon, Geochang-gun, Gyeongsangnam-do	2013-10-17	OR211373	OR211384	
	SFC20180928-18	Republic of	Deokgye-ri, Yeongdeok-gun, Gyeongsangbuk-do	2018-09-28	OR211383	OR211401	OR220059
	SFC20170811-18	Russia	Ordzhonikidzevskiy Rayon,	2017-08-11	OR211382	OR211400	OR220058
H. paucispinum	NIBRFG0000503828	Vietnam	Dạ Chais, Lạc Dương,	2018-06-16	OR211379	OR211395	
sp. 1100	NIBRFG0000507153	Vietnam	Xã Đạ Nhim, Lạc Dương, Lâm Đồng	2019-07-01	OR211380	OR211396	OR220063
	NIBRFG0000508573	Republic of Korea	Gaya Mountain, Gaya-myeon, Hapcheon-gun,	2019-07-01	ON907801	ON907774	OR220057
H. ventricosum	NIBRFG0000502952	Russia	Gyeongsangnam-do Sovetskaya Ulitsa, Tsukanovo, Primorskiy, kray	2018-08-07	OR211378	OR211394	
	NIBRFG0000508910	Republic of	Taebaek Mountain, Gohan-eup,	2020-07-30	ON907795	OR211397	OR220050
	NIBRFG0000508951	Republic of	Taebaek Mountain, Gohan-eup,	2020-07-30	ON907796	OR211398	OR220053
	NIBRFG0000509012	Republic of Korea	Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do	2020-09-10	ON907797	ON907769	OR220052
	SFC20210902-27	Republic of	Taebaek Mountain, Gohan-eup,	2021-09-02	ON907798	ON907768	OR220051
	KA16-0826	Republic of Korea	415, Gwangneungsumogwon- ro, Soheul-eup, Pocheon-si, Gveonggi-do	2016-07-28	ON907794	OR211386	
Hydnum cf. tomaense	KA20-0732	Republic of Korea	191, Geommasan-gil, Subi- myeon, Yeongyang-gun, Gyeongsangbuk-do,	2020-09-15	ON907793	ON907772	OR220061

Subgenus	Section	Species	Voucher	Country	ITS	nrLSU
Alba		Hydnum albomagnum	RAS231	United States	MH379943	
		, ,	AFTOL-ID 471	United States	DQ218305	AY700199
			Wei 10247	China	MW980551	MW979537
			Wei 10194	China	MW980550	MW979536
		H. cremeoalbum	TUMH 40462	Japan	AB906674	
			HKAS92345	China	KU612619	KU612676
		H pipicola		Japan	AB906678	
		н. рипсона	TUMH 64003	Japan	LC021813	
		H. subcremeoalbum	TU110688	Papua New Guinea	UDB013289	
Alba s. lato		H. albidum	RAS058	United States	MH379873	
			10640TJB	United States	MH379883	
		H. alboaurantiacum	TENN:073548	United States	MH379904	
			TFB12761	United States	MH379855	
		H. alboluteum	TUMH 63988	Japan	LC621802	
		H flavido canum	10MH 03983	Japan	LC021/98	
		H. Haviaocanum	Yuan 13900a	China	MW980550	MW979546
		H. minus	IFP 019483	China	MW980558	MW979544
			IFP 019482	China	MW980557	MW979543
			TUMH:60737	Japan	AB906675	
		H. orientalbidum	TUFC 101371	Japan	LC377875	
			SuR20170915-3	Japan	LC377873	
		H. tomaense	TUMH:64086	Japan	LC621885	
			TUMH:64085	Japan Danan Nawa Calinaa	LC621884	
		H. treui	10110403	Papua New Guinea	UDB013043	
Brovisning		H. Zongoličense H. bravisninum	GO-2010-142a Wai 10258	China	NC152121 MW/080570	MW070560
brevispina		n. orevispinum	Wei 10238	China	MW980578	MW979559
		H. tenuistipitum	Wei 10211	China	MW980576	MW979557
		· · · · · · · · · · · · · · · · · · ·	Wei 10417	China	MW980577	MW979558
Hydnum		H. boreorepandum	TUMH 64005	Japan	LC621814	
			H T. Niemela 1679	Finland	KX388658	
			H 6003711	Finland	KX388657	
		H. olympicum	SAT-10-208-05	United States	MT955159	
			H I NISKANEN 09-134	United States	KX388661	
		H. Slovenicum		Slovenia	AJ547884 A 1547870	
		H sphaericum	IFP 019471	China	MW980565	MW979551
		n. sprachcum	IFP 019470	China	MW980563	MW979549
			IFP 019485	China	MW980564	MW979550
		H. subolympicum	DAOM744368	Canada	MH174257	
		H. neorepandum	H T. Niskanen 10-086	Canada	KX388660	
			H T. Niskanen 10-095	Canada	KX388659	
		H. repando-orientale	TUMH60743	Japan	AB906684	
		H ronandum	IUMH60/45	Japan Finland	AB906683	
		H. repandum H. vegabundum	H 0003710 CL 04985	Finiand United States	MH370000	
		n. vegabanaam	10782TIB	United States	MH379949	
		H. washinatonianum	UBC F-32538	Canada	MF954990	
		5	strain 214	Canada	MH379846	
		Hydnum sp.	HKAS55410	China	KU612596	
Pallida		H. albopallidum	TUMH 63998	Japan	LC621808	
			TUMH 63997	Japan	LC621807	
		H. flabellatum	Yuan 14/08	China	MW980575	
		H. IDericum	BIO:Eupgi:12330	Spain	AJ54/8/9 HE611086	
		H pallidomarainatum	Yuan 13928a	China	MW980566	MW979552
		n. panaomarginatam	Yuan 13940a	China	MW980567	MW979553
		H. subtilior	RAS180	United States	MH379918	
		H. tottoriense	TUMH 64091	Japan	LC621890	
			TUMH 64090	Japan	LC621889	
		H. vesterholtii	BIO:Fungi:12904	France	HE611087	
Defenset	Deferrentia		BIO:Fungi:10429	Spain	HE611084	
Rufescentia	Rufescentia	H. aerostatisporum	EX1399 BB 1456 PC0142475	China	KY800344	MM/070529
		H. canadense	H T Niskanen 09-006	Canada	KX388681	10100 97 9550
		H. cusnidatum	RAS205	United States	MH379936	
		··· ··· <i>p</i> ·····	RAS246	United States	MH379944	
		H. ellipsosporum	H T. Niskanen 12-036	Europe	KX388671	
			FD3281	Europe	KX086215	KX086217
			0s5579	Germany	AY817138	
		H. itachiharitake	TUMH 64033	Japan	LC621830	
		H molitocan	IUMH 64031	Japan	LC621828	
		n. mentosarx	п 1. INISKAITETI 11-050 К 176869	United Kingdom	KX388682	
		H. minospororufescens	TUMH 64039	Japan	LC621835	
			TUMH 64041	Japan	LC621837	

Table 2. Summary of informati	on about the published	Hydnum sequences	used in the phylogenetic analysi	is.
-------------------------------	------------------------	------------------	----------------------------------	-----

(continued)

Subgenus	Section	Species	Voucher	Country	ITS	nrLSU
		H. mulsicolor	REB-341	United States	JX093560	
			LJU GIS 1336	Europe	AJ547885	
		H. oregonense	PNW-MS g2010502h1-09	United States	AJ534972	
		H. ovoidesporum	71106	Slovenia	KU612536	
		H. quebecense	CN9	United States	MJ379881	
			H T. Niskanen 10-064	Canada	KX388662	
		H. rufescens	H T. Niemela 7838	Europe	KX388656	
			H 6003708	Europe	KX388688	
		H. subberkeleyanum	TUMH63627	Japan	LC621880	
			TUMH64075	Japan	LC621881	
		H. subconnatum	RAS169	United States	MH379916	
			RAS235	United States	MH379930	
		H. submulsicolor	H T. Niskanen 10-132	Canada	KX388682	
		H. subovoideisporum	H 6003707	Europe	KX388680	
		H. subrufescens	H T. Niskanen 10-154	Canada	KX388649	
		H. tangerinum	IFP 019473	China	MW980580	MW979561
		5	IFP 019474	China	MW980581	MW979562
			IFP 019475	China	MW980582	MW979563
		H. umbilicatum	16051TJB	United States	MH379890	
		H. ventricosum	IFP 019478	China	MW980561	MW979547
			IFP019479	China	MW980562	MW979548
	Magnorufescentia	H. albertense	H T. Niskanen 11-354	Canada	KX388664	
		H. ferruginescens	RAS228	United States	MH379942	
		-	MH16005	United States	MH379905	
		H. jussii	H 6003709	Finland	KX388665	
			IFP 019486	China	MW980554	MW979540
			IFP 019485	China	MW980553	MW979539
		H. longibasidium	IFP 019463	China	MW980555	MW979542
			IFP 019462	China	MW980556	MW979541
		H. magnorufescens	161209	Portugal	KU621549	KU612669
			TO HG2818	Europe	KC293545	
		H. melleopallidum	SMI356	Canada	FJ845406	
		H. pallidocroceum	IFP 019466	China	MW980568	MW979554
			IFP 019467	China	MW980569	MW979555
Outgroup		Sistotrema muscicola	KHL 11721	Finland	AJ606040	AJ606040
		Sistotrema alboluteum	TAA 180259	Estonia	AJ606042	
		Sistotrema chloroporum	TUMH 64399	Japan	NR178117	
		Sistotrema confluens	FCUG298	United States	DQ267125	AY647214
			PV174	Czechia	AY463466	AY586712

aligned using MAFFT v7 [36] and the alignments were manually edited using Geneious (https:// www.geneious.com). For ITS phylogeny, three Sistotrema sequences were selected as the outgroup [19]. For Maximum Likelihood (ML) analysis utilizing the combined ITS and nrLSU sequence datasets, only specimens that possessed both ITS and nrLSU sequences were included in the analysis. The sequences of two Sistotrema specimens possessing both ITS and nrLSU sequences were selected as outgroups. The final alignment was generated as a concatemer by manually merging the aligned sequences of two regions (ITS and nrLSU). Maximum Likelihood analysis was performed using RAxML-HPC v8 and a GTR+GAMMA model with 1,000 bootstrap [37].

3. Results

Table 2 Continued

3.1. Phylogenetic analysis

We generated 76 sequences from 30 specimens, comprising 30 ITS, 28 nrLSU, and 18 *TEF1* sequences. Since many reference sequences of

Hydnum species were available only for the ITS region, we conducted ML analysis using only the sequence of the ITS region. In total, 144 ITS sequences, including three sequences of *Sistotrema* as an outgroup, were used. In the phylogenetic tree, *Hydnum* was divided into six subgenera, consistent with previous studies [6,19], and each subgenus was supported by a high bootstrap value, except for the subgenus *Alba* s. l.

Thirty Hydnum specimens were classified into eight taxa in the phylogenetic tree. They formed distinct clades with their corresponding reference sequences supported by high bootstrap values (except for the new species candidate) in four subgenera: Alba, Alba s. l., Pallida, and Rufescentia (Figure 1). Seven of the taxa were identified as described species and one was identified as a new species candidate. In the subgenus Alba, two species were identified with four specimens: H. cremeoalbum (SFC20181012-04) and H. pinicola (KA13-1480, SFC20170811-18, and SFC20180928-18). In the subgenus Alba s. l., 11 specimens were assigned to two species, H. orientalbidum (SFC20150902-97, NIBRFG0000109936, NIBRFG0000113465, and NIBRFG0000122620) and H. minus (KA13-1475,



Figure 1. Phylogenetic tree based on Maximum Likelihood (RAxML) analysis of *Hydnum* based on sequence data of ITS. Bootstrap values exceeding 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in orange.

KA16-1189, KA17-0894, KA20-0486, KA20-0682, KA20-0770, and KA20-0815), whereas one specimen (KA20-0732) was not identified at the species level. In subgenus Pallida, three specimens (KA16-0264, KA16-0511, and SFC20180705-81) were identified as Н. pallidomarginatum and two specimens (KA16-0626 and KA16-1082) were identified as H. albopallidum. Within the subgenus Rufescentia, six specimens (KA16-0826, NIBRFG0000502952, NIBRFG0000508910, NIBRFG0000508951, NIBRFG 0000509012, and SFC20210902-27) were identified as H. ventricosum. The sequences of three other specimens (NIBRFG0000503828, NIBRFG0000507153, and NIBRFG0000508573) did not match any of the described *Hydnum* species and formed a distinct monophyletic clade in the section *Magnorufesentia* in the subgenus *Rufescentia*, suggesting that they represent a new species candidate. To confirm the robust phylogenetic placement of these species, a phylogenetic analysis was conducted for species with both ITS and nrLSU sequences. All species identified in this study were supported by high bootstrap values. The morphological characteristics of eight *Hydnum* species are depicted in Figures 2 and 3, while their corresponding Korean names are suggested in Supporting Information Table S1.



Figure 2. Pictures of basidiomata of seven *Hydnum* species from East Eurasia. (A) *Hydnum cremeoalbum* (SFC20181012-04); (B) *H. pinicola* (KA13-1480); (C) *H. orientalbidum* (NIBRFG0000113465); (D) *H. minus* (KA17-0894); (E) *H. albopallidum* (KA16-1082); (F) *H. pallidomarginatum* (SFC20180705-81); and (G) *H. ventricosum* (KA16-0826). Scale bars: 1 cm.



Figure 3. Basidiomata and microscopic characteristics of *Hydnum paucispinum* sp. nov. (A) Basidiomata of holotype (NIBRFG0000503828); (B) Microscopic characteristics (basidiospores and basidia) of holotype (NIBRFG0000503828). Scale bars: 1 cm for (A), 20 μm for (B).

4. Taxonomy

4.1. Hydnum subgenus Alba

4.1.1. Hydnum cremeoalbum Liimat. & Niskanen, 2018 (Figure 2A)

Description. Pileus 20-30 mm in diameter, expanding to plane, often umbilicate, entire to irregular in outline; surface dull, dry, smooth, and glabrous, not zonate, whitish; margin incurved to straight, undulating, entire to obscurely lobed. Hymenophore slightly decurrent, 0.4-0.6 mm in length, 3-7 spines/ mm² crowded, concolorous with the pileus surface. Stipe cylindrical to subclavate, $16-18 \times 7.5-9$ mm, central or eccentric, solid; surface smooth, whitish, or concolorous with the pileus surface. Basidia narrowly clavate to narrowly urniform, $35-49 \times 5 7 \,\mu\text{m}$, with a basal clamp, producing (4–)5 sterigmata. Basidiospores subglobose to ellipsoid, E = 1.07 - 1.43, $4.5-6.5 \times 3.2-5.7 \,\mu\text{m}$, Q = 1.29, smooth, thin-walled, hyaline, sometimes with oildrops, non-amyloid.

Habitat and distribution. Solitary, in a mixed forest of dominant with *P. densiflora*.

Specimens examined. SFC20181012-04, Hongdo-ri, Heuksan-myeon, Sinan-gun, Jeollanam-do, Republic of Korea, October 12, 2018, collected by Jae Young Park.

Remark. *Hydnum cremeoalbum* is characterized by the whitish pileus, cylindrical to subclavate spine, and $5-7 \times 3.5-5.5 \,\mu\text{m}$ sized, ellipsoid basidiospores [38]. The characteristics of the specimens used in this study mostly fell within the range of those described in the original description [38]. The only difference observed was that the basidiomata size of the Korean *Hydnum cremeoalbum* was smaller than that in the original description (pileus, 30–70 mm; stipe, $25-35 \times 15-30 \,\text{mm}$) [38].

4.1.2. Hydnum pinicola R. Sugaw. & N. Endo, 2022 (Figure 2B)

Description. Pileus 50–75 mm in diameter, plane, depressed; surface dull, dry, smooth, and not zonate, whitish to orange white (5A2); margin entire, incurved to straight. Hymenophore shortly decurrent, 2–4.5 mm in length, 4–6 spines/mm² crowded, concolorous with the pileus surface. Stipe cylindrical to subclavate, $26-36 \times 9.5-12.5$ mm, central or eccentric, solid; surface smooth, concolorous with the pileus surface. Basidia (narrowly) utriform, 28– $37 \times 5.3-7.8$ µm, with a basal clamp, producing 2–6 sterigmata. Basidiospores subglobose, $4.2-5.7 \times 3.2-5$ µm, E=1.05-1.33, Q=1.17, smooth, hyaline, sometimes with oil-drops, non-amyloid.

Habitat and distribution. Solitary or gregarious, in a mixed forest of dominant with *P. densiflora*.

Specimens examined. KA13-1480, Udu Mountain, Suwol-ri, Gajo-myeon, Geochang-gun, Gyeongsangnamdo Republic of Korea, July 17, 2013, collected by Sang Kuk Han; SFC20170811-18, Ordzhonikidzevskiy Rayon, Khakassia, Russia, August 11, 2017, collected by Young Woon Lim; SFC20180928-18, Deokgye-ri, Yeongdeokgun, Gyeongsangbuk-do, Republic of Korea, collected by Young Woon Lim, Ki Hyeong Park, Abel.

Remark. Most features were consistent with the original description of *Hydnum pinicola* in Japan [34]. Despite the geographical distance, the specimen (SFC20170811-18) collected in Central Siberia shared the same habitat and DNA sequence as those collected in East Asia.

4.2. Hydnum subgenus Alba s. l

4.2.1. Hydnum orientalbidum *R. Sugaw. & N. Endo, 2022 (Figure 2C)*

Description. Pileus 11-45 mm in diameter, expanding to pulvinate, often plane, entire to irregular in outline; surface dull, dry, smooth, not zonate, whitmargin incurved to decurved, ish; entire. Hymenophore shortly decurrent, 1-4 mm in length, 4-6 spines/mm² crowded, concolorous with the pileus surface. Stipe subclavate, $15-30 \times 5.5-13$ mm, central or eccentric, solid; surface smooth, concolorous with the pileus surface. Basidia urniform to utriform, $28-38.2 \times 5.2-8 \,\mu\text{m}$, with a basal clamp, producing 3-6 sterigmata. Basidiospores subglobose, $4.5-5.6 \times 4.1-5.3 \,\mu\text{m}$, E = 1.00-1.36, Q = 1.15, smooth, hyaline, sometimes with oil-drops, nonamyloid.

Habitat and distribution. Solitary to gregarious, in a mixed forest.

Specimens examined. NIBRFG0000109936, Hakgok-ri, Socho-myeon, Wonju-si, Gangwon-do, Republic of Korea, 15 August 2009, collected by Young Woon Lim; NIBRFG0000113465, Unak Mountain, Hwahyeonmyeon, Pocheon-si, Gyeonggi-do, Republic of Korea, collected by Jae-Jin Kim, Yongil Kim, Yeongseon Jang; SFC20150902-97, Jajangnamusup-gil 760, Inje-eup, Injegun, Gangwon-do, Republic of Korea, September 2, 2015, collected by Young Woon Lim.

Remark. *Hydnum orientalbidum* has white basidiomata that resemble those of *H. albidum*. While the latter species has been reported in North America, the former has been confirmed to occur only in Asia and exhibits distinct differences in terms of its DNA sequence, as mentioned in previous study [34]. The Korean *H. orientalbidum* specimen matched the original morphological, geographical, and ecological characteristics with the original description. However, the basidiomata of the studied specimens are generally small, particularly in the pileus and stipes [34].

4.2.2. Hydnum minus Yanaga & N. Maek., [as "minum"], 2015 (Figure 2D)

Description. Pileus 16-38 mm in diameter, expanding to plane or sometimes concave, depressed to umbilicate, irregular in outline; surface dull, glabrous, slightly zonate in center, whitish to yellowish white (4A2) to pale orange (5A3); margin curved, becoming undulate in age, entire to obscurely lobed. Hymenophore spinose, decurrent; spines conical to aciculate, 0.78–1.7 mm in length, 6–11 spines/mm² crowded, more buff (6A2 to 7A3) than the pileus surface. Stipe cylindrical to obclavate, $15-20 \times 2-4$ mm, central or eccentric; surface smooth, concolorous with the pileus surface. Basidia subclavate to suburniform, constricted, 25.5–32.46 (35) \times 4.8–6.7 µm, with a basal clamp, producing 4-6 sterigmata. Basidiospores subglobose to ellipsoid, $4-5.3 \times 2.6-3.5 \ \mu m$, E = 1.25-1.6, Q = 1.47, smooth, thin-walled, hyaline, with oildrops, non-amyloid.

Habitat and distribution. Solitary to gregarious, in a mixed forest.

Specimens examined. KA13-1475, Udu Mountain, Suwol-ri, Gajo-myeon, Geochang-gun, Gyeongsangnamdo, Republic of Korea, October 17, 2013, collected by Sang Kuk Han; KA16-1189, Sogwang-ri, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, Republic of Korea, October 5, 2016, collected by Sang Kuk Han; KA17-0894, Sogwang-ri, Seo-myeon, Ulleung-gun, Gyeongsangbukdo, September 14, 2017, collected by Jong Won Jo, Yeongnam Gwak, Juyeong Park, Huisu Jeong; KA20-0486, Dongmyeon-ri, Jaesan-myeon, Bonghwa-gun, Gyeongsangbuk-do, Republic of Korea, August 13, 2020, collected by Jong Won Jo, Hyun Lee, Hyeongso Kim, Sangyeong Park; KA20-0682, Dongmyeon-ri, Jaesanmyeon, Bonghwa-gun, Gyeongsangbuk-do, Republic of Korea, September 14, 2020, collected by Jong Won Jo, Hyeongso Kim, Sangyeong Park; KA20-0770, Doota Mountain, Miro-myeon, Samcheok-si, Gangwon-do, Republic of Korea, September 16, 2020, collected by Jong Won Jo, Hyeongso Kim, Sangyeong Park; KA20-0815, 1610, Guryongnyeong-ro, Seo-myeon, Yangyang-gun, Gangwon-do, Republic of Korea, September 17, 2020, collected by Jong Won Jo, Hyeongso Kim, Sangyeong Park.

Remark. All specimens were collected in the Republic of Korea, and the Korean *Hydnum minus*

has a wider pileus diameter range, wider and longer stipe, and slightly smaller basidiospores when compared with the reference (pileus 10–25 mm in diameter; stipe $8-15 \times 2-5$ mm in width; basidiospores $4.5-5.5 \times 3-4.5 \mu$ m) [38]. The epithet "minus (minum)" was originated from the small basidiomata of the holotype, but the Korean *Hydnum minus* has a greater pileus width range compared to that of the original description. Furthermore, the phylogenetic tree revealed intraspecific sequence variation in *Hydnum minus* (Figure 1), which was within the acceptable range for this species (less than 0.05%).

4.3. Hydnum subgenus Pallida

4.3.1. Hydnum albopallidum R. Sugaw. & N. Endo, 2022 (Figure 2E)

Description. Pileus 8–30 mm in diameter, expanding to plane, pulvinate; surface dull, glabrous, slightly zonate in center, whitish to orange white (5A2); margin curved, entire. **Hymenophore** spinose, decurrent; spines 0.5–0.8 mm in length, 6–8 spines/mm² crowded, concolorous with the pileus surface. **Stipe** cylindrical, 8–15 × 3–7.3 mm, central or slightly eccentric; surface smooth, concolorous with the pileus surface. **Basidia** urniform to (narrowly) utriform, sometimes clavate, 37.2–53.8 (56) × 8.5–11.4 µm, with a basal clamp, producing 3–4 sterigmata. **Basidiospores** subglobose, 7.1–8.7 × 6.2–8 µm, E = 1.03-1.29, Q = 1.11, smooth, thin-walled, hyaline, with oil-drops, non-amyloid.

Habitat and distribution. Solitary, in a mixed forest, nearby the bryophyte.

Specimens examined. KA16-0626, Eorimok Valley, Haean-dong, Jeju-si, Jeju Island, Republic of Korea, July 18, 2016, collected by Sang Kuk Han; KA16-1082, Eorimok Valley, Haean-dong, Jeju-si, Jeju Island, Republic of Korea, September 26, 2016, collected by Sang Kuk Han.

Remark. Overall, the characteristics of these specimens matched the original description; however, their basidiospores were slightly smaller than those of the original description $(8-9.5 \times 7-8 \,\mu\text{m})$ [34].

4.3.2. Hydnum pallidomarginatum T. Cao & H. S. Yuan., 2021 (Figure 2F)

Description. Pileus 10–42.9 mm in diameter, plane, depressed in center; surface dull, dry, smooth, and not zonate, orange white (5A2) to light orange (5A4); margin entire, incurved to straight, whitish. **Hymenophore** subdecurrent, 2–5 mm in length, 4–7 spines/mm² crowded, white to orange white (5A2). **Stipe** cylindrical, $26-36 \times 8.5-10$ mm, central, solid;

surface smooth, concolorous with the hymenophore. **Basidia** urniform to utriform, $32-55 \times 5-12 \mu m$, with a basal clamp, producing 2–4 sterigmata. **Basidiospores** ellipsoid, 7.4–10.1 × 6.3–7.9 μm , E = 1.27-1.38, Q = 1.31, smooth, hyaline, sometimes with oil-drops, non-amyloid.

Habitat and distribution. Solitary to gregarious, in a mixed forest.

Specimens examined. KA16-0264, Bakji island, 209, Bakjido-gil, Anjwa-myeon, Sinan-gun, Jeollanam-do, Republic of Korea, June 28, 2016, collected by Sang Kuk Han; KA16-0511, Bakji island, 209, Bakjido-gil, Anjwa-myeon, Sinan-gun, Jeollanam-do, Republic of Korea, July 12, 2016, collected by Sang Kuk Han; SFC20180928-18, Duryunsan, Samsan-myeon, Haenam-gun, Jeollanam-do, Republic of Korea, July 05, 2018, collected by Hae Jin Cho, Ki Hyeong Park, Dohye Kim.

Remark. Korean basidiomata have prominent features present in the original description, including a brighter pileus margin, an orange whitish to light orange pileus, and broadly ellipsoid basidiospores [19]. However, the Korean *H. pallidomarginatum* has a larger pileus when comparing to the original description (pileus 20–35 mm in diameter) [19]. In the phylogenetic tree, *H. pallidomarginatum* exists in the same clade as *H. flabellatum*, which can be distinguished based on the scabrous texture of the pileus and longer stipe [19]. The morphological differences were subtle and did not show significant distinctions, suggesting that further research is needed to establish the criteria for the delimitation between these two species.

4.3.3. Hydnum ventricosum *Cao & H.S. Yuan, 2021 (Figure 2G)*

Description. Pileus 20-55 mm in diameter, convex to plane, occasionally depressed in the center; surface dull, dry, smooth, and glabrous, not zonate, pale ocher, occasionally orangish ocher with whitish at the margin; margin entire. Hymenophore spinose, adnate, decurrent; spines conical to aciculate, -1.2-5.4 mm in length, $2-7 \text{ spines/mm}^2$ crowded, concolorous with the stipe surface. Stipe cylindrical to subclavate, $29-69 \times 7-19$ mm, central or eccentric, solid; surface smooth, concolorous with the pileus surface. Basidia clavate to narrowly urniform, $33-51 \times 8.3-12 \,\mu\text{m}$, with a basal clamp, producing 3–4 sterigmata. Basidiospores globose, 6.8–8.6 \times $6.5-8.4 \,\mu\text{m}, E = 1.01-1.05, Q = 1.03$, smooth, thinwalled, hyaline, sometimes with oil-drops, nonamyloid.

Habitat and distribution. Solitary or in groups, occasionally gregarious, in a pile of leaves of mixed deciduous forest.

Specimens examined. KA16-0826, 415, Gwangneungsumogwon-ro, Soheul-eup, Pocheon-si, Gyeonggi-do, Republic of Korea, July 28, 2016, collected by Sang Kuk Han; NIBRFG0000509012, Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do, Republic of Korea, September 10, 2020, collected by Changmu Kim, Minkyeong Kim, Chorong Ahn; SFC20210902-27, Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do, Republic of Korea, September 2, 2021, collected by Young Woon Lim, Ki Hyeong Park, Shinnam Yoo, Yoonhee Cho.

Remark. *Hydnum ventricosum* is characterized by an orangish pileus, fusiform to subcylindrical basidia, and subglobose basidiospores [19]. Korean *H. ventricosum* has a relatively larger pileus than original description (up to 35 mm) [19].

4.3.4. Hydnum paucispinum *JS Kim, C Kim, and YW Lim, sp. nov. (Figure 3) MycoBank no.: MB#849344* **Holotype**. NIBRFG0000503828, Da Chais, Lac Dương, Lâm Đồng, Vietnam, June 16, 2018, collected by Changmu Kim, Jae Young Park, Jin Sung Lee, in mixed forest dominant with *P. densiflora*. GenBank: ITS = OR211379; nrLSU = OR211395; *TEF1* = OR220062.

Etymology. *Paucispinum*, refers to the relatively less crowded aculeate hymenophore (paucus means fewer, and spinum refers from aculeate hymenophore)

Diagnosis. *Hydnum paucispinum* is characterized by whitish to light orange pileus, a non-decurrent hymenophore, cylindrical stipe, and $7.1-9.2 \times 6.4-8.6 \,\mu\text{m}$ sized, subglobose basidiospores. Ecologically, this species occurs in solitary or gregarious forms in Pinaceae forests.

Pileus 15–35 mm in diameter, convex to plane, irregularly round, depressed in the center; surface dry, smooth, glabrous, zonate; pale orange (5A4) when dry, light orange (5A5 to 6A5) when wet; margin entire, arched when young. **Hymenophore** spinose, non-decurrent; spines conical, aculeate, 1.1–3 mm in length, 3–5 spines/mm² crowded, concolorous with the pileus surface. **Stipe** cylindrical, 15–32 \times 2.3–7 mm, central or slightly eccentric, solid; surface smooth, whitish.

Tramal hyphae of the spines $3-5.5 \,\mu\text{m}$ in diameter, smooth, thin-walled. Pileipellis an indefinite trichodermium, composed of hyphae similar to those of the tramal hyphae of the spine, $4-7.5 \,\mu\text{m}$ in

diameter. **Basidia** narrowly utriform, $37-47.5 \times 8.1-11.4 \,\mu\text{m}$, with a basal clamp, producing 2-4 sterigmata. **Basidiospores** subglobose, $7.1-9.2 \times 6.4-8.6 \,\mu\text{m}$, E = 1.00-1.16, Q = 1.06, smooth, thinwalled, hyaline, sometimes with oil-drops, non-amyloid.

Habitat and distribution. Solitary or gregarious, in a mixed forest dominant with *P. densiflora*.

Additional specimens examined. NIBRFG0000507153, Xã Đạ Nhim, Lạc Dương, Lâm Đồng, Vietnam, July 1, 2019, collected by Changmu Kim, Jin Sung Lee, Jae Young Park, Nam Kyu Kim; NIBRFG0000508573, Gaya Mountain, Gaya-myeon, Hapcheon-gun, Gyeongsangnam-do, Republic of Korea, July 1, 2019, collected by Changmu Kim.

Remark. The morphological features of *Hydnum* paucispinum match the representative features of the subgenus *Rufescentia* [6]. This species belongs to the *Magnorufescentia* section within this subgenus based on the Q value and morphology of basidiospores, which are characteristics that differentiate the two sections in this subgenus [6]. *Hydnum paucispinum* differs from *H. ventricosum* in several aspects, including having shorter spines (1.1–3 mm vs. 1–5 mm long), a relatively sparse density of hymenophore (1–2 spines per mm vs. 2–4 spines per mm), a shorter and slender stipe (15–32 × 2.3–7 mm vs. 30–35 × 10–15 mm), narrowly utriform basidia, and a slightly thinner pileipellis (4–7.5 µm vs. 5–10 µm) [19].

5. Taxonomic key of Korean Hydnum species

1 Pileus white to pale yellow 2
1 Pileus pale orange to reddish orange (sometimes
whitish)
2 Spines less crowded (≤ 6 spines/mm ²)
2 Spines crowded (≥ 6 spines/mm ²) Hydnum minus
3 The length of hymenophore $> 1 \text{ mm}$
3 The length of hymenophore < 1 mm
4 Brownish discoloration of the stipe [*]
4 Lack of discoloration of the stipe* H. pinicola
5 Spines crowded (\geq 5 spines/mm ² on average) 6
5 Spines less crowded (\leq 5 spines/mm ²)
6 Q value of basidiospores < 1.2
6 Q value of basidiospores > 1.2
7 The length of hymenophore> 1 \dots H. ventricosum
7 The length of hymenophore $< 1 \dots H$. albopallidum
*This characteristic was not described in this study
but was derived from the reference paper [9].

5. Discussion

An integrative study combining morphological and molecular analyses confirmed the presence of eight *Hydnum* species in the Republic of Korea. Previously, all the specimens deposited in the three Korean fungaria were identified as *Hydnum repandum*. However, *Hydnum repandum*, the type species of *Hydnum* reported in Europe [6,39], seems absent from the Republic of Korea. The eight *Hydnum* species identified have aculeate hymenophores, which may lead to confusion with *H. repandum*. Nevertheless, as demonstrated in the taxonomic key of this study, closer examination revealed distinct morphological differences. Additionally, the sequences of these eight species were distinct from *H. repandum*.

Based on the results of phylogenetic and morphological analyses, seven of the eight Hydnum species were identified as described species. In the phylogenetic analysis, all species formed well-resolved clusters with the reference sequences of each species, supported by high bootstrap values, and no significant differences were observed when compared to the original descriptions. However, certain species exhibited variations in the size of their basidiomata compared with the original descriptions. These differences may be attributed to variations in the growth of basidiomata in different climatic zones, or to the limited number of specimens. Therefore, to generalize the characteristics of the Korean Hydnum, further examination using a larger number of specimens is necessary. In addition, further research on interspecies delimitation is required for some species. For example, H. ventricosum formed a monophyletic clade with H. subberkelyanum, showing no genetic variation in the ITS region between them. Their morphological characteristics were almost identical except for the width of the stipipellis [19,34]. Therefore, H. subberkeleyanum should be synonymized with H. ventricosum, as the latter species was proposed earlier.

Consistent with previous studies, taxonomic investigations of fungal specimens collected in Asia differed considerably from those conducted in Western regions. In the case of Hydnum, an ectomycorrhizal fungus, the species distribution may vary depending on the distribution of the host plants [40,41]. The disjunct distributions of certain endemic species have been reported in Hydnum [17]. Due to this reason, numerous new species have been proposed in recent taxonomic studies in Asia. The seven described species identified in the Republic of Korea have been recorded exclusively in Asia and not on any other continents [6,17,19,38]. Additionally, the new species proposed in this study, Hydnum paucispinum, was found in the Republic of Korea and the high mountains of Vietnam, and its molecular distinctiveness

from other described species was clearly shown. Our results indicate that despite the distance between East Asia, the mountainous areas of Vietnam, and central Siberia, their *Hydnum* species share similar ecological traits. These findings support those of previous studies highlighting the differences between Asian ectomycorrhizal fungi and those in western regions, which are attributed to variations in continents and ecological environments [42–44]. Therefore, continued research on the fungal taxonomy in the eastern region provides an opportunity to further explore the diversity of fungi, which has only been partially revealed thus far [45].

In conclusion, the present study confirmed the presence of eight *Hydnum* species in the Republic of Korea using SBI and morphological analyses. None of these species correspond *to Hydnum repandum*. Given the similarity in morphology among certain *Hydnum* species, careful observation of microscopic features and SBI processes are essential to prevent misidentification. The findings of this study will enhance accurate identification of *Hydnum* species in the Republic of Korea by providing validated sequence data and comprehensive morphological characteristics.

Acknowledgment

We would like to thank Editage (www.editage.co.kr) for English language editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Korea National Arboretum under Grant [KNA1-1-25, 19-2]; National Institute of Biological Resources under Grant [NIBR202102107, NIBR202304104]; and the Institute for Peace and Unification Studies (IPUS) at Seoul National University under the project of "Laying the Groundwork for Unification and Peace."

ORCID

Ji Seon Kim (b) http://orcid.org/0000-0003-1869-7347 Wonjun Lee (b) http://orcid.org/0000-0002-7227-0777 Hanna Park (b) http://orcid.org/0009-0000-9980-1665 Chang Sun Kim (b) http://orcid.org/0000-0001-6918-8863 Young Woon Lim (b) http://orcid.org/0000-0003-2864-3449

Data availability statement

The data generated in this study are all included in the article, and the sequence data have been deposited in GenBank.

References

- Miller LW. The genera of Hydnaceae. Mycologia. 1933;25(4):286–302. doi: 10.1080/00275514.1933. 12020669.
- [2] Moncalvo JM, Nilsson RH, Koster B, et al. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia. 2006;98(6):937–948. doi: 10.3852/mycologia.98.6.937.
- [3] Petersen RH. The typification of *Hydnum* L. ex Fries. Taxon. 1973;22(1):99–104. doi: 10.2307/ 1218039.
- [4] Donk M. The generic names proposed for Hymenomycetes. V, "Hydnaceae" (continuation). Taxon. 1956;5(5):95–115. doi: 10.2307/1216240.
- [5] Maas Geesteranus R. Hydnaceous fungi of the Eastern old world. Vehr K Ned Akad Wet. 1971; II.60:1–176.
- [6] Niskanen T, Liimatainen K, Nuytinck J, et al. Identifying and naming the currently known diversity of the genus *Hydnum*, with an emphasis on European and North American taxa. Mycologia. 2018;110(5):890–918. doi: 10.1080/00275514.2018. 1477004.
- [7] Diamandis S, Perlerou C. The mycoflora of the chestnut ecosystems in Greece. For Snow and Landscape Res. 2001;76(3):499–504.
- [8] Raidl S, Agerer R. Studien an ektomykorrhizen. XLII: ontogenie der rhizomorphen von Laccaria amethystina, Hydnum rufescens und Sarcodon imbricatus. Nova Hedwigia. 1992;55(3-4):279-307.
- [9] Sugawara R, Sotome K, Maekawa N, et al. Mycorrhizal synthesis, morpho-anatomical characterization of mycorrhizae, and evaluation of mycorrhiza-forming ability of *Hydnum albidum*– like species using monokaryotic and dikaryotic cultures. Mycorrhiza. 2021;31(3):349–359. doi: 10. 1007/s00572-021-01024-7.
- [10] Harrison KA. New or little known North American stipitate *Hydnums*. Can J Bot. 1964; 42(9):1205–1233. doi: 10.1139/b64-116.
- [11] Maas Geesteranus RA. Notes on hydnums. Persoonia-Molecular Phylogeny and Evolution of Fungi. 1960;1(3):341–384.
- [12] Murrill WA. Additions to Florida fungi-V. Vol. 67(4), In: Bulletin of the Torrey Botanical Club; New York (NY): Torrey Botanical Society; 1940. p. 275–281. doi: 10.2307/2481174.
- [13] Grigoriev IV, Cullen D, Goodwin SB, et al. Fueling the future with fungal genomics. Mycology. 2011;2(3):192–209.
- [14] Hibbett DS, Pine EM, Langer E, et al. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc Natl Acad Sci USA. 1997;94(22):12002–12006. doi: 10.1073/pnas. 94.22.12002.
- [15] Baird RE. Type studies of North American and other related taxa-of stipitate *Hydnums*. In: *Bibliotheca Mycologica*; 1986. ISBN 978-3-443-59004-8. [AQ]
- [16] Larsson KH, Svantesson S, Miscevic D, et al. Reassessment of the generic limits for *Hydnellum* and *Sarcodon* (Thelephorales, Basidiomycota). MycoKeys. 2019;54:31–47. doi: 10.3897/mycokeys. 54.35386.

- [17] Feng B, Wang X-H, Ratkowsky D, et al. Multilocus phylogenetic analyses reveal unexpected abundant diversity and significant disjunct distribution pattern of the hedgehog mushrooms (*Hydnum* L.). Sci Rep. 2016;6(1):25586. doi: 10. 1038/srep25586.
- [18] Swenie RA, Baroni TJ, Matheny PB. Six new species and reports of *Hydnum* (Cantharellales) from Eastern North America. MycoKeys. 2018;42(42): 35–72. doi: 10.3897/mycokeys.42.27369.
- [19] Cao T, Hu Y-P, Yu J-R, et al. A phylogenetic overview of the *Hydnaceae* (Cantharellales, Basidiomycota) with new taxa from China. Stud Mycol. 2021;99(1):100121-100121.
- [20] Huhtinen S, Ruotsalainen J. Variability of *Hydnum rufescens* in Finland: three taxa hidden under one name-and appearance? Karstenia. 2006;46(1):17-24. doi: 10.29203/ka.2006.412.
- [21] Ostrow H, Beenken L. Hydnum ellipsosporum spec. nov. (Basidiomycetes, Cantharellales)-ein doppelgänger von Hydnum rufescens Fr. Zeitschrift Fuer Mykologie. 2004;70(2):137-156.
- [22] Vizzini A, Picillo B, Ercole E, et al. Detecting the variability of *Hydnum ovoideisporum* (Agaricomycetes, Cantharellales) on the basis of Italian collections, and *H. magnorufescens* sp. nov. Mycosphere. 2012;4(1):32–44. doi: 10.5943/mycosphere/4/1/2.
- [23] NIBR. National Institute of Biological Resources, 2022; [cited 2022 Mar 23]. Available from: https:// kbr.go.kr/
- [24] Kaburagi Y. Korea forest experiment station. Korean and Manchurian practical manual of forest. Tokyo: Yokendo; 1940. p. 339–367.
- [25] Cho HJ, Park MS, Lee H, et al. A systematic revision of the ectomycorrhizal genus *Laccaria* from Korea. Mycologia. 2018;110(5):948–961.
- [26] Lee H, Wissitrassameewong K, Park MS, et al. Taxonomic revision of the genus *Lactarius* (Russulales, Basidiomycota) in Korea. Fungal Divers. 2019;95(1):275–335. doi: 10.1007/s13225-019-00425-6.
- [27] Wisitrassameewong K, Looney BP, Le HT, et al. Lactarius subgenus Russularia (Basidiomycota, Russulales): novel Asian species, worldwide phylogeny and evolutionary relationships. Fungal Biol. 2016;120(12):1554–1581. doi: 10.1016/j.funbio. 2016.08.004.
- [28] Kornerup A, Wanscher J. Methuen handbook of colour. London: Eyre Methuen Ltd; 1978. ISBN 978-0413334008.
- [29] Collins TJ. ImageJ for microscopy. Biotechniques. 2007;43(1 Suppl):25–30. doi: 10.2144/000112517.
- [30] Snell WH, Dick EA. A glossary of mycology. Cambridge (MA): Harvard University Press; 1971. ISBN 0674-35451-6.
- [31] Largent DL, Stuntz DE. How to identify mushrooms to genus. 1973. ISBN: 978-0916422004.
- [32] Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol. 1993;2(2):113–118. doi: 10.1111/j.1365-294x.1993. tb00005.x.

- [33] White TJ, Bruns TD, Lee SB, et al. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315–322.
- [34] Sugawara R, Maekawa N, Sotome K, et al. Systematic revision of *Hydnum* species in Japan. Mycologia. 2022;114(2):413–452. doi: 10.1080/ 00275514.2021.2024407.
- [35] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870– 1874. doi: 10.1093/molbev/msw054.
- [36] Katoh K, Misawa K, Kuma K, et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30(14):3059–3066. doi: 10.1093/ nar/gkf436.
- [37] Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006; 22(21):2688–2690. doi: 10.1093/bioinformatics/ btl446.
- [38] Yanaga K, Sotome K, Ushijima S, et al. *Hydnum* species producing whitish basidiomata in Japan. Mycoscience. 2015;56(4):434–442. doi: 10.1016/j. myc.2015.01.001.
- [39] Linnaeus C. Species plantarum, exhibentes plantas rite cognitas ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas, Impensis GC Nauk; 1797. doi: 10.5962/ bhl.title.37656.
- [40] van der Linde S, Suz LM, Orme CDL, et al. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature. 2018;558(7709): 243–248. doi: 10.1038/s41586-018-0189-9.
- [41] Yang H, Zang Y, Yuan Y, et al. Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata. BMC Evol Biol. 2012;12(1):50. doi: 10. 1186/1471-2148-12-50.
- [42] Cho HJ, Lee H, Park MS, et al. Two new species of *Laccaria* (Agaricales, Basidiomycota) from Korea. Mycobiology. 2020;48(4):288–295.
- [43] Wilson AW, May TW, Mueller GM. Biogeography of the ectomycorrhizal mushroom genus *Laccaria*. In: Tedersoo L, editor. Biogeography of mycorrhizal symbiosis. ecological studies, Vol. 230. Cham: Springer; 2017. doi: 10.1007/978-3-319-56363-3_13.
- [44] Wu BW, Gao C, Chen L, et al. Host phylogeny is a major determinant of Fagaceae-associated ectomycorrhizal fungal community assembly at a regional scale. Front Microbiol. 2018;9:2409. doi: 10.3389/fmicb.2018.02409.
- [45] Phukhamsakda C, Nilsson RH, Bhunjun CS, et al. The numbers of fungi: contributions from traditional taxonomic studies and challenges of metabarcoding. Fungal Diver. 2022;114(1):327–386. doi: 10.1007/s13225-022-00502-3.