






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

Ji Seon Kim<sup>a</sup> , Wonjun Lee<sup>a</sup> , Changmu Kim<sup>b</sup>, Hanna Park<sup>a</sup> , Chang Sun Kim<sup>c</sup>  and Young Woon Lim<sup>a</sup> 

<sup>a</sup>School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul, Korea; <sup>b</sup>Species Diversity Research Division, National Institute of Biological Resources, Incheon, Korea; <sup>c</sup>Forest Biodiversity Division, Korea National Arboretum, Pocheon-si, Korea

### ABSTRACT

*Hydnum* is a genus of ectomycorrhizal fungi belonging to the Hydneaceae 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*; Hydneaceae; ectomycorrhiza; sequence-based identification


## 1. Introduction


The genus *Hydnum* L. belongs to the family Hydneaceae [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 Hydneaceae 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. aspratium*, *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](mailto:ywlim@snu.ac.kr)

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

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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 hydroid 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- $\alpha$  (*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 Lạc 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 proof-read 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	nrLSU	TEF1
<i>Hydnum albopallidum</i>	KA16-0626	Republic of Korea	Eorimok Valley, Haean-dong, Jeju-si, Jeju island	2016-07-18	OR211374	OR211385	
	KA16-1082	Republic of Korea	Eorimok Valley, Haean-dong, Jeju-si, Jeju island	2016-09-26	ON907799	ON907770	OR220049
<i>H. cremealbum</i>	SFC20181012-04	Republic of Korea	Hong island, Hongdo-ri, Heuksan-myeon, Sinan-gun, Jeollanam-do	2018-10-12	ON907782	ON907775	
<i>H. minus</i>	KA13-1475	Republic of Korea	Udu Mountain, Suwol-ri, Gajo-myeon, Geochang-gun, Gyeongsangnam-do	2013-10-17	ON907783	ON907767	
	KA16-1189	Republic of Korea	Sogwang-ri, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do	2016-10-05	ON907784	OR211387	
	KA17-0894	Republic of Korea	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, Gyeongsangbuk-do	2020-09-14	ON907787	OR211389	OR220056
	KA20-0770	Republic of Korea	Doota Mountain, Miro-myeon, Samcheok-si, Gangwon-do	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
<i>H. orientalbum</i>	NIBRFG0000122620	Republic of Korea	Daeam Mountain, Yanggu-gun, Gangwon-do	2012-09-27	OR211377	OR211393	
	NIBRFG0000109936	Republic of Korea	Hakgok-ri, Socho-myeon, Wonju-si, Gangwon-do	2009-08-15	OR211375	OR211391	
	SFC20150902-97	Republic of Korea	760, Jajangnamusup-gil, Inje-eup, Inje-gun, Gangwon-do	2015-09-02	OR211381	OR211399	OR220060
	NIBRFG0000113465	Republic of Korea	Unak Mountain, Hwahyeon-myeon, Pocheon-si, Gyeonggi-do	2009-09-18	OR211376	OR211392	
<i>H. pallidomarginatum</i>	KA16-0264	Republic of Korea	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, Jeollanam-do	2016-07-12	ON907791	ON907763	OR220047
	SFC20180705-81	Republic of Korea	Duryunsan, Samsan-myeon, Haenam-gun, Jeollanam-do	2018-07-05	ON907792	ON907764	OR220048
<i>H. pinicola</i>	KA13-1480	Republic of Korea	Udu Mountain, Suwol-ri, Gajo-myeon, Geochang-gun, Gyeongsangnam-do	2013-10-17	OR211373	OR211384	
	SFC20180928-18	Republic of Korea	Deokgye-ri, Yeongdeok-gun, Gyeongsangbuk-do	2018-09-28	OR211383	OR211401	OR220059
	SFC20170811-18	Russia	Ordzhonikidzevskiy Rayon, Khakassia	2017-08-11	OR211382	OR211400	OR220058
<i>H. paucispinum</i> sp. nov	NIBRFG0000503828	Vietnam	Đà Chais, Lạc Dương, Lâm Đồng	2018-06-16	OR211379	OR211395	
	NIBRFG0000507153	Vietnam	Xã Đà Nhịm, Lạc Dương, Lâm Đồng	2019-07-01	OR211380	OR211396	OR220063
	NIBRFG0000508573	Republic of Korea	Gaya Mountain, Gaya-myeon, Hapcheon-gun, Gyeongsangnam-do	2019-07-01	ON907801	ON907774	OR220057
<i>H. ventricosum</i>	NIBRFG0000502952	Russia	Sovetskaya Ulitsa, Tsukanovo, Primorskiy kray	2018-08-07	OR211378	OR211394	
	NIBRFG0000508910	Republic of Korea	Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do	2020-07-30	ON907795	OR211397	OR220050
	NIBRFG0000508951	Republic of Korea	Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do	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 Korea	Taebaek Mountain, Gohan-eup, Jeongseon-gun, Gangwon-do	2021-09-02	ON907798	ON907768	OR220051
	KA16-0826	Republic of Korea	415, Gwangneungsumogwon-ro, Soheul-eup, Pocheon-si, Gyeonggi-do	2016-07-28	ON907794	OR211386	
<i>Hydnum cf. tomaense</i>	KA20-0732	Republic of Korea	191, Geommasan-gil, Subi-myeon, Yeongyang-gun, Gyeongsangbuk-do,	2020-09-15	ON907793	ON907772	OR220061

**Table 2.** Summary of information about the published *Hydnum* sequences used in the phylogenetic analysis.

Subgenus	Section	Species	Voucher	Country	ITS	nrLSU		
<i>Alba</i>		<i>Hydnum albomagnum</i>	RAS231	United States	MH379943			
			AFTOL-ID 471	United States	DQ218305	AY700199		
				Wei 10247	China	MW980551	MW979537	
				Wei 10194	China	MW980550	MW979536	
		<i>H. cremeoalbum</i>	TUMH 40462	Japan	AB906674			
			HKAS92345	China	KU612619	KU612676		
				TUMH 60740	Japan	AB906678		
		<i>H. pinicola</i>	TUMH 64004	Japan	LC621813			
			TUMH 64003	Japan	LC621812			
				TU110688	Papua New Guinea	UDB013289		
<i>Alba s. lato</i>		<i>H. albidum</i>	RAS058	United States	MH379873			
			10640TJB	United States	MH379883			
				<i>H. alboaurantiacum</i>	TENN:073548	United States	MH379904	
					TFB12761	United States	MH379855	
		<i>H. alboluteum</i>	TUMH 63988	Japan	LC621802			
			TUMH 63983	Japan	LC621798			
				<i>H. flavidocanum</i>	Yuan 13900a	China	MW980560	MW979546
					Yuan 13903a	China	MW980559	MW979545
		<i>H. minus</i>	IFP 019483	China	MW980558	MW979544		
			IFP 019482	China	MW980557	MW979543		
			TUMH:60737	Japan	AB906675			
<i>H. orientalbidum</i>	TUFC 101371	Japan	LC377875					
	SuR20170915-3	Japan	LC377873					
		<i>H. tomaense</i>	TUMH:64086	Japan	LC621885			
			TUMH:64085	Japan	LC621884			
		<i>H. treui</i>	TU110403	Papua New Guinea	UDB013043			
		<i>H. zongolicense</i>	GO-2010-142a	Mexico	KC152121			
<i>Brevispina</i>		<i>H. brevispinum</i>	Wei 10258	China	MW980579	MW979560		
			Wei 10214	China	MW980578	MW979559		
			<i>H. tenuistipitum</i>	Wei 10410	China	MW980576	MW979557	
			Wei 10417	China	MW980577	MW979558		
<i>Hydnum</i>		<i>H. boreorepandum</i>	TUMH 64005	Japan	LC621814			
			H T. Niemela 1679	Finland	KX388658			
					H 6003711	Finland	KX388657	
		<i>H. olympicum</i>	SAT-10-208-05	United States	MT955159			
			H T Niskanen 09-134	United States	KX388661			
		<i>H. slovenicum</i>	LJU GIS 1340	Slovenia	AJ547884			
			LJU GIS 1338	Slovenia	AJ547870			
		<i>H. sphaericum</i>	IFP 019471	China	MW980565	MW979551		
			IFP 019470	China	MW980563	MW979549		
					IFP 019485	China	MW980564	MW979550
				<i>H. subolympicum</i>	DAOM744368	Canada	MH174257	
		<i>H. neorepandum</i>	H T. Niskanen 10-086	Canada	KX388660			
			H T. Niskanen 10-095	Canada	KX388659			
		<i>H. repando-orientale</i>	TUMH60743	Japan	AB906684			
			TUMH60745	Japan	AB906683			
				<i>H. repandum</i>	H 6003710	Finland	KX388650	
		<i>H. vegabundum</i>	CLO4985	United States	MH379909			
			10782TJB	United States	MH379949			
				<i>H. washingtonianum</i>	UBC F-32538	Canada	MF954990	
					strain 214	Canada	MH379846	
<i>Pallida</i>		<i>Hydnum sp.</i>	HKAS55410	China	KU612596			
			TUMH 63998	Japan	LC621808			
					TUMH 63997	Japan	LC621807	
		<i>H. flabellatum</i>	Yuan 14708	China	MW980575			
			MA-fungi 6457	Spain	AJ547879			
		<i>H. ibericum</i>	BIO:Fungi:12330	Spain	HE611086			
			Yuan 13928a	China	MW980566	MW979552		
		<i>H. pallidomarginatum</i>	Yuan 13940a	China	MW980567	MW979553		
			RAS180	United States	MH379918			
				<i>H. tottoriense</i>	TUMH 64091	Japan	LC621890	
			TUMH 64090	Japan	LC621889			
		<i>H. vesterholtii</i>	BIO:Fungi:12904	France	HE611087			
			BIO:Fungi:10429	Spain	HE611084			
<i>Rufescentia</i>	<i>Rufescentia</i>	<i>H. aerostatisporum</i>	Ex1399 BB 1456 PC0142475	United States	KY800344			
			IFP019484	China	MW980552	MW979538		
				<i>H. berkeleyanum</i>	H T. Niskanen 09-006	Canada	KX388681	
		<i>H. canadense</i>	RAS205	United States	MH379936			
			RAS246	United States	MH379944			
		<i>H. cuspidatum</i>	H T. Niskanen 12-036	Europe	KX388671			
			FD3281	Europe	KX086215	KX086217		
					0s5579	Germany	AY817138	
		<i>H. itachiharitake</i>	TUMH 64033	Japan	LC621830			
			TUMH 64031	Japan	LC621828			
		<i>H. melitosarx</i>	H T. Niskanen 11-056	United States	KX388683			
			K 176869	United Kingdom	KX388685			
<i>H. minospororufescens</i>	TUMH 64039	Japan	LC621835					
	TUMH 64041	Japan	LC621837					

(continued)

**Table 2.** Continued.

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

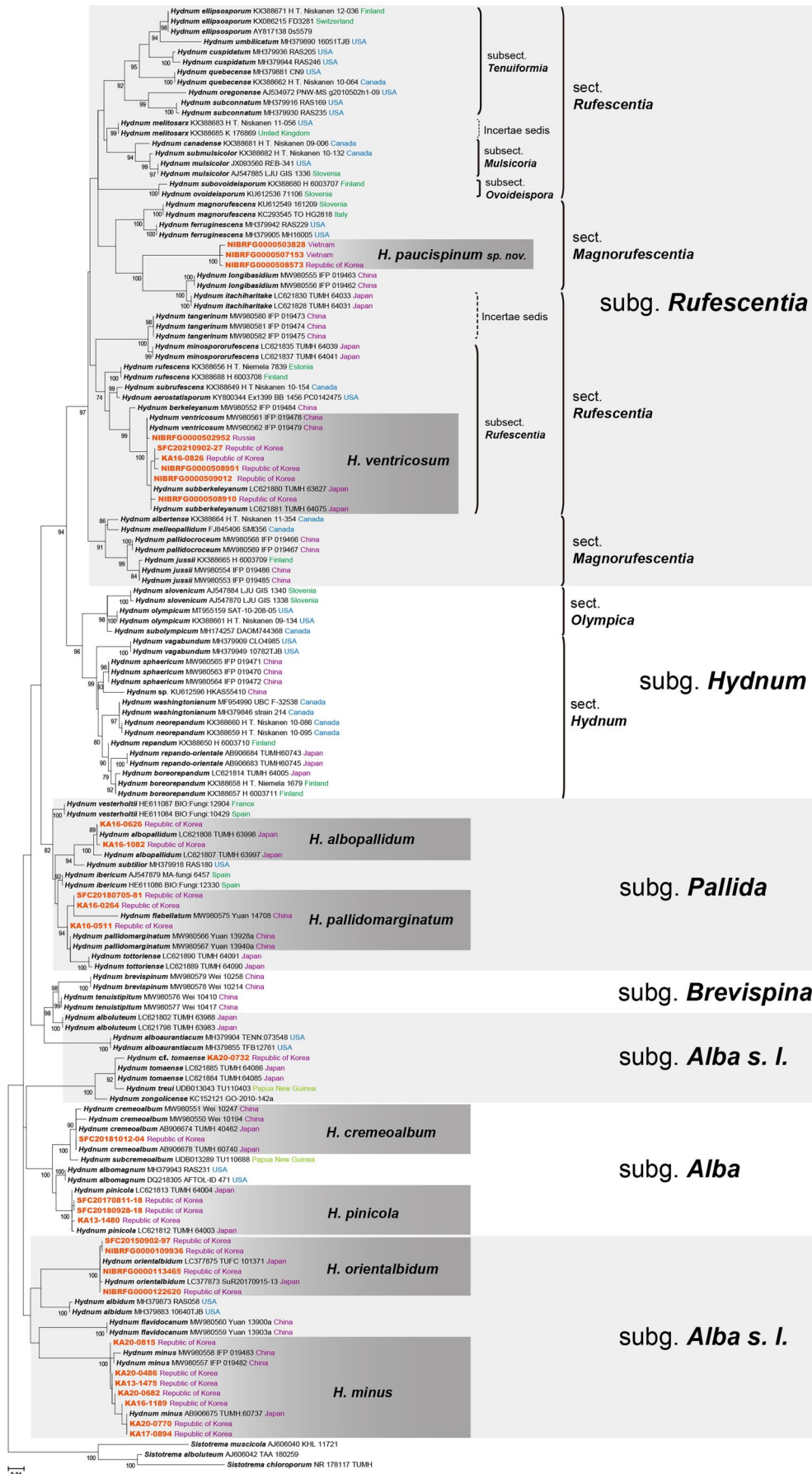
### 3. Results

#### 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.

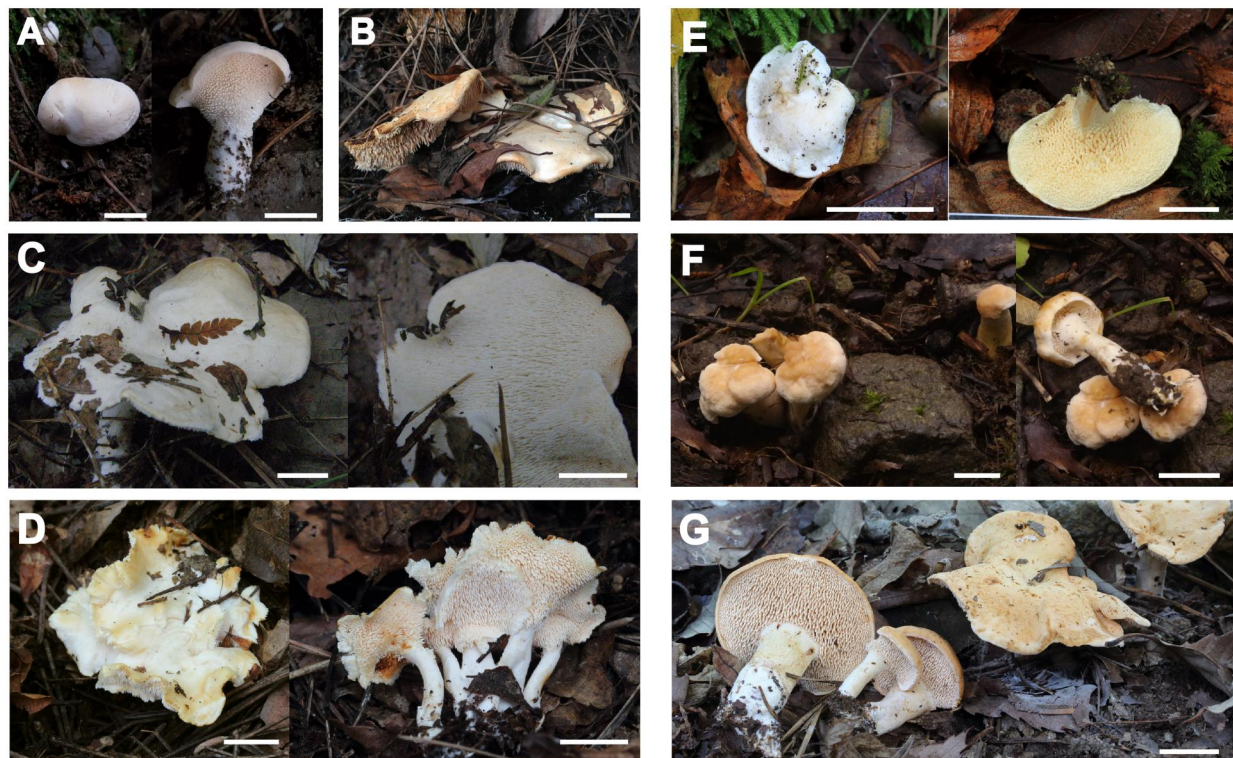
Thirty *Hydnum* specimens were classified into eight taxa in the phylogenetic tree. They formed distinct clades with their corresponding reference sequences supported by high bootstrap values (except for the new species candidate) in four subgenera: *Alba*, *Alba* s. l., *Pallida*, and *Rufescentia* (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. cremealbum* (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. orientalbum* (SFC20150902-97, NIBRFG0000109936, NIBRFG0000113465, and NIBRFG0000122620) and *H. minus* (KA13-1475,



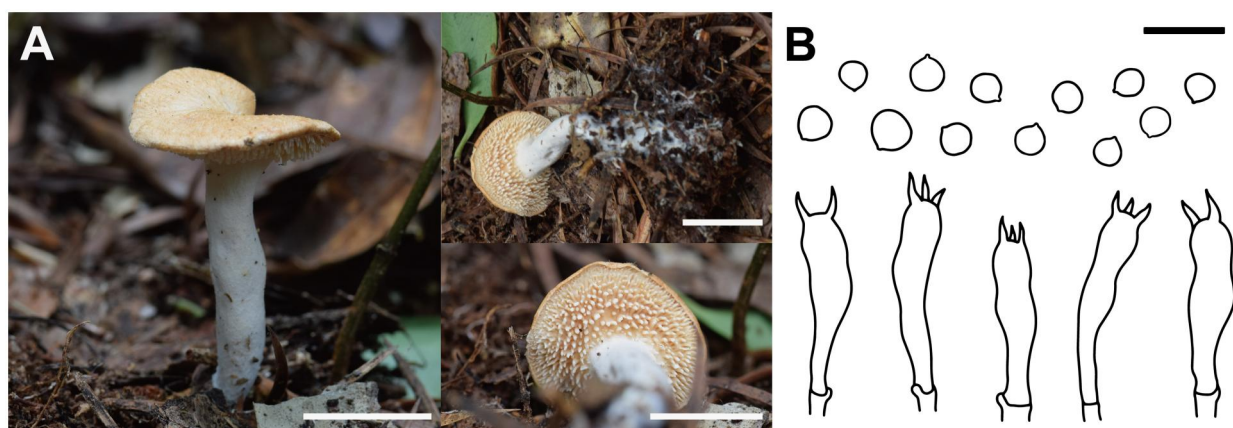
**Figure 1.** Phylogenetic tree based on Maximum Likelihood (RAxML) analysis of *Hydnium* based on sequence data of ITS. Bootstrap values exceeding 70% are indicated at the branch nodes. Newly generated sequences from this study are represented in orange.

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 *H. 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, NIBRFG0000509012, 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 *Magnorufescentia* 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/mm<sup>2</sup> crowded, concolorous with the pileus surface. **Stipe** cylindrical to subclavate, 16–18 × 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 × 5–7 μm, with a basal clamp, producing (4–)5 sterigmata. **Basidiospores** subglobose to ellipsoid, 4.5–6.5 × 3.2–5.7 μm,  $E = 1.07–1.43$ ,  $Q = 1.29$ , smooth, thin-walled, hyaline, sometimes with oil-drops, 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 × 3.5–5.5 μm 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 × 15–30 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/mm<sup>2</sup> crowded, concolorous with the pileus surface. **Stipe** cylindrical to subclavate, 26–36 × 9.5–12.5 mm, central or eccentric, solid; surface smooth, concolorous with the pileus surface. **Basidia** (narrowly) utriform, 28–37 × 5.3–7.8 μm, with a basal clamp, producing 2–6 sterigmata. **Basidiospores** subglobose, 4.2–5.7 × 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, Gyeongsangnam-do 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, Yeongdeok-gun, 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 orientalbum* 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, whitish; margin incurved to decurved, entire. **Hymenophore** shortly decurrent, 1–4 mm in length, 4–6 spines/mm<sup>2</sup> crowded, concolorous with the pileus surface. **Stipe** subclavate, 15–30 × 5.5–13 mm, central or eccentric, solid; surface smooth, concolorous with the pileus surface. **Basidia** urniform to utriform, 28–38.2 × 5.2–8 μm, with a basal clamp, producing 3–6 sterigmata. **Basidiospores** subglobose, 4.5–5.6 × 4.1–5.3 μm,  $E = 1.00–1.36$ ,  $Q = 1.15$ , smooth, hyaline, sometimes with oil-drops, non-amyloid.

**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, Hwahyeon-myeon, Pocheon-si, Gyeonggi-do, Republic of Korea, collected by Jae-Jin Kim, Yongil Kim, Yeongseon Jang; SFC20150902-97, Jajangnamusup-gil 760, Inje-eup, Inje-gun, Gangwon-do, Republic of Korea, September 2, 2015, collected by Young Woon Lim.

**Remark.** *Hydnum orientalbum* 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. orientalbum* 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 “*minus*”], 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/mm<sup>2</sup> crowded, more buff (6A2 to 7A3) than the pileus surface. **Stipe** cylindrical to obclavate, 15–20 × 2–4 mm, central or eccentric; surface smooth, concolorous with the pileus surface. **Basidia** subclavate to suburniform, constricted, 25.5–32.46 (35) × 4.8–6.7 μm, with a basal clamp, producing 4–6 sterigmata. **Basidiospores** subglobose to ellipsoid, 4–5.3 × 2.6–3.5 μm,  $E = 1.25$ –1.6,  $Q = 1.47$ , smooth, thin-walled, hyaline, with oil-drops, non-amyloid.

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

**Specimens examined.** KA13-1475, Udu Mountain, Suwol-ri, Gajo-myeon, Geochang-gun, Gyeongsangnam-do, 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, Gyeongsangbuk-do, 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, Jaesan-myeon, 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 × 2–5 mm in width; basidiospores 4.5–5.5 × 3–4.5 μm) [38]. The epithet “minus (minus)” 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/mm<sup>2</sup> 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 × 7–8 μm) [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/mm<sup>2</sup> crowded, white to orange white (5A2). **Stipe** cylindrical, 26–36 × 8.5–10 mm, central, solid;

surface smooth, concolorous with the hymenophore.

**Basidia** urniform to utriform,  $32\text{--}55 \times 5\text{--}12 \mu\text{m}$ , with a basal clamp, producing 2–4 sterigmata.

**Basidiospores** ellipsoid,  $7.4\text{--}10.1 \times 6.3\text{--}7.9 \mu\text{m}$ ,  $E = 1.27\text{--}1.38$ ,  $Q = 1.31$ , 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 spines/mm<sup>2</sup> crowded, concolorous with the stipe surface. **Stipe** cylindrical to subclavate,  $29\text{--}69 \times 7\text{--}19 \text{ mm}$ , central or eccentric, solid; surface smooth, concolorous with the pileus surface. **Basidia** clavate to narrowly urniform,  $33\text{--}51 \times 8.3\text{--}12 \mu\text{m}$ , with a basal clamp, producing 3–4 sterigmata. **Basidiospores** globose,  $6.8\text{--}8.6 \times 6.5\text{--}8.4 \mu\text{m}$ ,  $E = 1.01\text{--}1.05$ ,  $Q = 1.03$ , smooth, thin-walled, hyaline, sometimes with oil-drops, non-amyloid.

**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, Minkyong 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, Đa Chais, Lạc 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  $7.1\text{--}9.2 \times 6.4\text{--}8.6 \mu\text{m}$  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/mm<sup>2</sup> crowded, concolorous with the pileus surface. **Stipe** cylindrical,  $15\text{--}32 \times 2.3\text{--}7 \text{ mm}$ , 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,  $37\text{--}47.5 \times 8.1\text{--}11.4 \mu\text{m}$ , with a basal clamp, producing 2–4 sterigmata. **Basidiospores** subglobose,  $7.1\text{--}9.2 \times 6.4\text{--}8.6 \mu\text{m}$ ,  $E = 1.00\text{--}1.16$ ,  $Q = 1.06$ , smooth, thin-walled, 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 Đa 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 ( $15\text{--}32 \times 2.3\text{--}7 \text{ mm}$  vs.  $30\text{--}35 \times 10\text{--}15 \text{ mm}$ ), narrowly utriform basidia, and a slightly thinner pileipellis ( $4\text{--}7.5 \mu\text{m}$  vs.  $5\text{--}10 \mu\text{m}$ ) [19].

## 5. Taxonomic key of Korean *Hydnum* species

- |  |                             |
|--|-----------------------------|
| 1 Pileus white to pale yellow .....                                | 2                           |
| 1 Pileus pale orange to reddish orange (sometimes whitish) .....   | 5                           |
| 2 Spines less crowded ( $\leq 6$ spines/ $\text{mm}^2$ ) .....     | 3                           |
| 2 Spines crowded ( $\geq 6$ spines/ $\text{mm}^2$ ) .....          | <i>Hydnum minus</i>         |
| 3 The length of hymenophore $> 1$ mm .....                         | 4                           |
| 3 The length of hymenophore $< 1$ mm .....                         | <i>H. cremeoalbum</i>       |
| 4 Brownish discoloration of the stipe* .....                       | <i>H. orientialbidum</i>    |
| 4 Lack of discoloration of the stipe* .....                        | <i>H. pinicola</i>          |
| 5 Spines crowded ( $\geq 5$ spines/ $\text{mm}^2$ on average) .... | 6                           |
| 5 Spines less crowded ( $\leq 5$ spines/ $\text{mm}^2$ ) .....     | <i>H. paucispinum</i>       |
| 6 Q value of basidiospores $< 1.2$ .....                           | 7                           |
| 6 Q value of basidiospores $> 1.2$ .....                           | <i>H. pallidomarginatum</i> |
| 7 The length of hymenophore $> 1$ ....                             | <i>H. ventricosum</i>       |
| 7 The length of hymenophore $< 1$ .....                            | <i>H. albopallidum</i>      |
- \*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. subberkeleyanum*, 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](http://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  <http://orcid.org/0000-0003-1869-7347>  
 Wonjun Lee  <http://orcid.org/0000-0002-7227-0777>  
 Hanna Park  <http://orcid.org/0009-0000-9980-1665>  
 Chang Sun Kim  <http://orcid.org/0000-0001-6918-8863>  
 Young Woon Lim  <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.

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