RESEARCH ARTICLE

# Four Unrecorded Macrofungi from Mudeungsan National Park in Korea

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ABSTRACT

Macrofungi are major decomposers in terrestrial ecosystems, acting as nutrient cyclers, wood decomposers, and plant symbionts. Despite their beneficial effects, the diversity of macrofungi is not as well understood as that of the flora and fauna. Mudeungsan National Park is one of the biggest National Parks in Southwestern Korea. We conducted surveys in Mudeungsan National Park from 2021 to 2023 to identify indigenous macrofungi. The collected specimens were identified based on morphological characteristics and molecular and phylogenetic analyses using the internal transcribed spacer (ITS), nuclear large subunit ribosomal DNA (nLSU), and translation elongation factor 1 gene (*tef1*) regions. Four species were identified as unrecorded in Korea: *Anomoloma luteoalbum*, *Fibrodontia alba*, *Russula pallidula*, and *Schizocorticium parvisporum*. In this study, we provide detailed morphological figures and phylogenetic trees to support the four unrecorded species in Mudeungsan National Park.

Keywords: Basidiomycota, Indigenous fungi, Phylogeny, Taxonomy
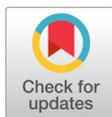
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INTRODUCTION

Macrofungi, such as ectomycorrhizal, saprobic, parasitic, and wood-decaying fungi, are major players in terrestrial ecosystems. They contribute to nutrient cycling, wood decomposition, and plant growth through mycorrhizal interactions [1–4]. In addition, several macrofungi provide economic benefits owing to their medicinal and nutritional values [5]. Technological advancements in DNA-based analyses estimate that 11.7–13.2 million fungal species exist on Earth [6]. However, macrofungal species have been recently estimated to be 220,000–380,000 [7], following the result of 2.2–3.8 million fungal species in 2017 [8]. According to Hawksworth (2001), 10% of the fungal species are macrofungi [9]. Thus, the current estimate should be updated to 1.2–1.3 million species following Hawksworth's calculations [9]. In Korea, 5,054 fungal species have been recorded until 2023, of which 2,188 have been identified as macrofungal species [10]. This indicates that macrofungal diversity in Korea is still limited compared to global diversity.

Mudeungsan National Park is located across Hwasun-gun and Gwangju Metropolitan City in Jeollanam-do province, Korea. Mudeungsan National Park was first established as a provincial park in 1972 and later upgraded to a National Park in 2012. Moreover, it was registered as a UNESCO Global Geopark in 2018, recognizing its biological and geological value. Several studies have identified the diversity of flora and fauna in Mudeungsan National Park [11–14], but data on macrofungal diversity are still limited [15].

Previous research in Mudeungsan National Park showed the possibility of high macrofungal diversity and argued for the need for further research [15]. This survey was conducted as part of a project by the National Institute of Biological Resources to gain a better understanding of macrofungal diversity in Mudeungsan National Park. In the present study, we identified four previously unrecorded species based on morphological and molecular analyses. This study provides micro- and macro-morphological descriptions of each species, along with phylogenetic trees, and reports them as new to Korea.

MATERIALS AND METHODS

Specimen collection

Specimens were collected from Mudeungsan National Park ($35°03'06''$–$35°12'59''$ N, $126°53'41''$–$127°05'01''$ E) between 2021 and 2023. After collection, the samples were dried at 55℃ for 72 h and stored in paper bags containing silica gel to avoid contamination. The specimens were subsequently deposited at the Korea University Culture Collection (KUC) and National Institute of Biological Resources (NIBR).

PCR amplification and sequencing

Genomic DNA was isolated from each specimen using the AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) and subjected to PCR amplification. The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using primer sets ITS1F/ITS4 and ITS5/ITS4 [16,17]. For nuclear large subunit ribosomal DNA (nLSU), the LR0R/LR5 primer set was used [18,19], and the translation elongation factor 1 (*tef1*) gene region was amplified using EF1-983F/EF1-1567R primers [20]. PCR products were purified using the AccuPrep® PCR Purification Kit (Bioneer), following the protocol provided by the manufacturer. The sequencing was performed by Bioneer and Cosmogenetech (Seoul, Korea). The obtained sequences were assembled, proofread, and edited using Geneious Prime 2023.2.1. (https://www.geneious.com). The sequences obtained in this study were submitted to GenBank and are listed in Table 1.

Table 1. List of studied specimens with GenBank accession numbers of ITS, nLSU, and *tef1* sequences.

Species name	Specimen voucher	Order	GenBank accession number		
			ITS	nLSU	*tef1*
Anomoloma luteoalbum	KUC20210714-74	Amy	PQ619322	PQ619355	–
	KUC20230317-01	Amy	PQ620178	–	–
Fibrodontia alba	KUC20210930-17	Tre	PQ619331	PQ619362	–
Russula pallidula	KUC20220715-36	Rus	PQ619349	PQ619379	–
Schizocorticium parvisporum	KUC20220414-13	Hym	–	PQ619380	PQ661210
	KUC20220414-22	Hym	PQ619350	PQ619381	PQ661211

ITS: internal transcribed spacer; nLSU: nuclear large subunit ribosomal DNA; *tef1*: translation elongation factor 1 gene; Amy: Amylocortiales; Tre: Trechisporales; Rus: Russulales; Hym: Hymenochaetales.

Phylogenetic analyses

For the phylogenetic analysis, reference sequences of related species were retrieved from the NCBI GenBank database [21]. Sequence alignment was performed using MAFFT v. 7.490 [22], with manual adjustments for ambiguous regions. Concatenated sequences were prepared using Geneious Prime 2023.2.1. A phylogenetic tree was constructed using the maximum likelihood (ML) method in the CIPRES web portal [23]. ML analysis was performed using RAxML-HPC2 in XSEDE v.8.2.12, applying the GTR+G model and 1,000 bootstrap replicates [24]. Phylogenetic trees were edited using FigTree v. 1.4.4 [25] and Adobe Illustrator CS6 v. 25.2.0 (Adobe Systems Inc., San Jose, CA, USA).

Morphological observation

Macroscopic images of the specimens were captured using a Sony α 6500 camera (Sony, Tokyo, Japan). Micromorphological characteristics were observed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan), and pictures were taken using a DP20 microscope camera (Olympus). Tissues from each specimen were mounted in 5% KOH for microscopic observation. Color terminology adhered to the standards outlined in the Munsell Soil Color Book [26]. The abbreviations used are as follows: L = average spore length, W = average spore width, and Q = L/W ratio.

RESULTS AND DISCUSSION

This study discovered four previously unrecorded species through multi-marker phylogenetic analyses (Figs. 1–4). An additional *tef1* region was used for *Schizocorticium parvisporum* owing to the uncertainty of species identification using ITS+nLSU data [27]. The four previously unrecorded species belong to the orders Amylocorticiales, Hymenochaetales, Russulales, and Trechisporales (Table 1).

According to the phylogenetic analyses, all unrecorded species showed high sequence similarity to their reference sequences and formed distinct clades with high bootstrap values (Figs. 1–4). Two genera were new to Korea: *Fibrodontia* Parmasto and *Schizocorticium* Sheng H. Wu. *Schizocorticium* was previously classified as *Skvortzoviella* Jia Yu, Xue W. Wang, S.L. Liu, and L.W. Zhou, and is currently synonymized as *Schizocorticium*. Additionally, three species—*Fibrodontia alba*, *Russula pallidula*, and *Schizocorticium parvisporum*—are new to temperate regions above 30° latitude, and were previously reported in tropical to subtropical areas, such as Southern China and Taiwan [27–29].
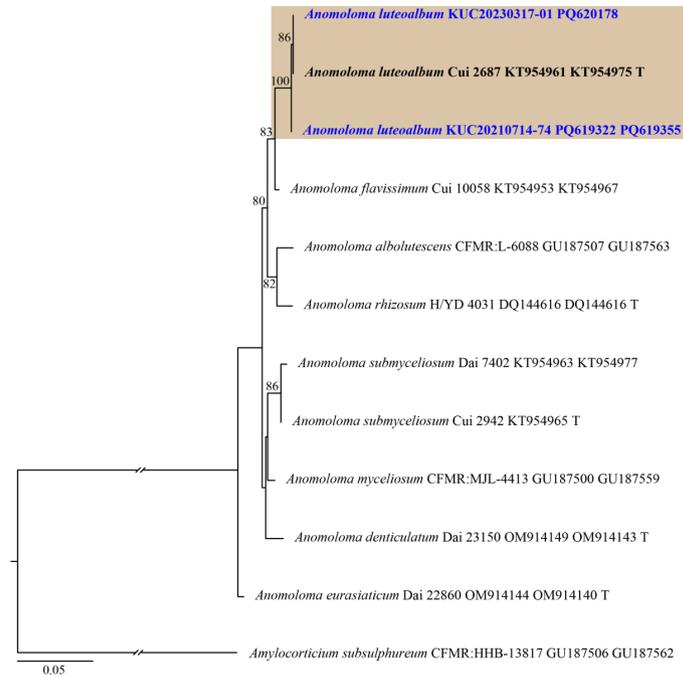
Fig. 1. ML tree based on the ITS and nLSU concatenated sequence datasets of the genus *Anomoloma*. The node numbers indicate the bootstrap support values above 70%. Newly generated sequences in this study are shown in blue and bold. *Amylocorticium subsulphureum* (CFMR:HHB-13817) was used as an outgroup. Type specimens are indicated with "T". ML: maximum likelihood; ITS: internal transcribed spacer; nLSU: nuclear large subunit ribosomal DNA.
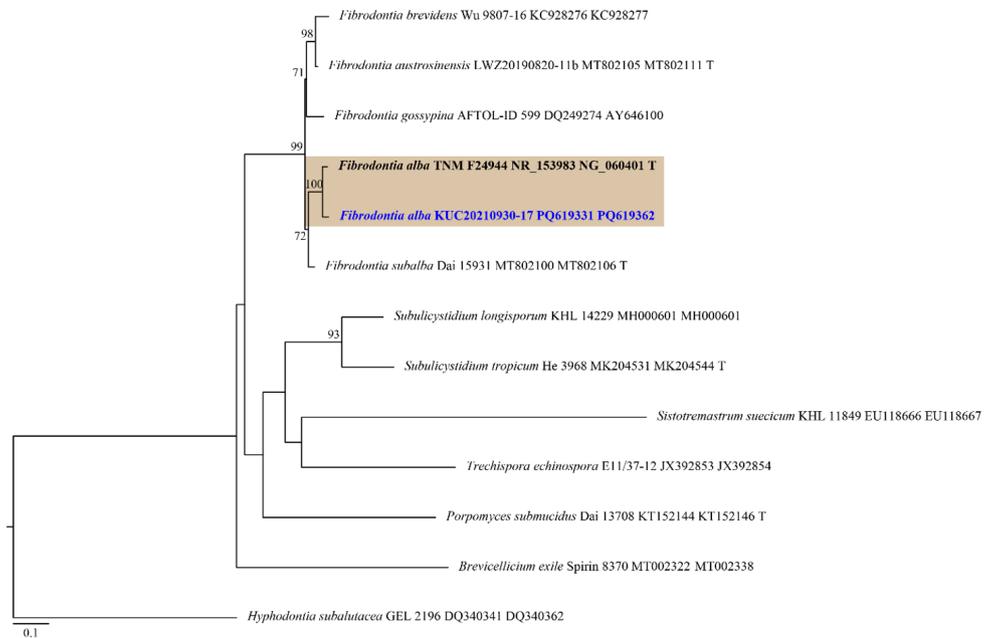

Fig. 2. ML tree based on the ITS and nLSU concatenated sequence datasets of the genus *Fibrodontia* and allied genera. The node numbers indicate the bootstrap support values above 70%. Newly generated sequence in this study is shown in blue and bold. *Hyphodontia subalutacea* (GEL 2196) was used as an outgroup. Type specimens are indicated with "T". ML: maximum likelihood; ITS: internal transcribed spacer; nLSU: nuclear large subunit ribosomal DNA.
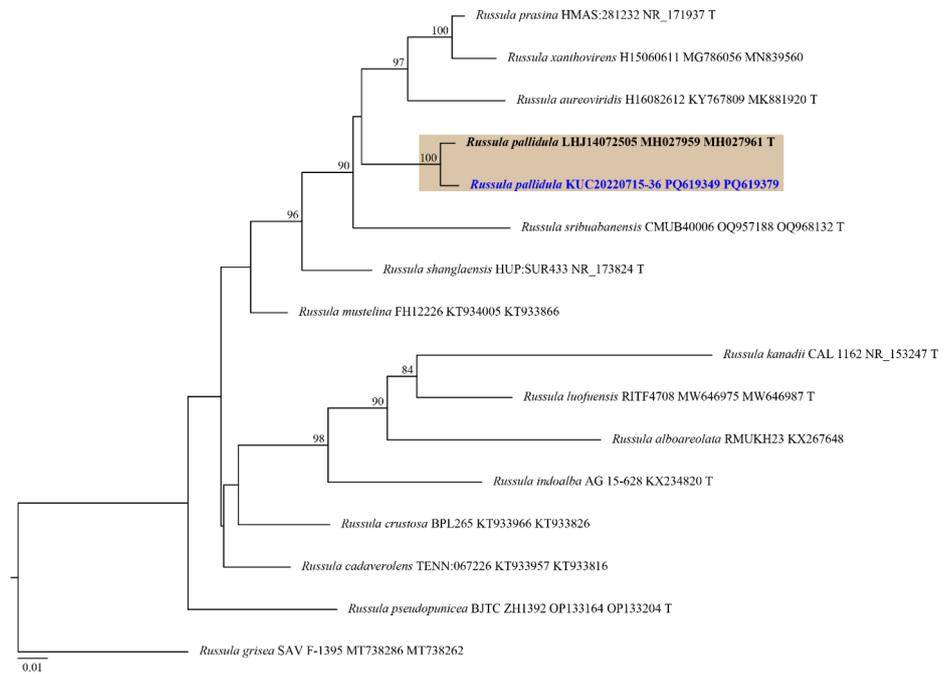
Fig. 3. ML tree based on the ITS and nLSU concatenated sequence datasets of the genus *Russula*. The node numbers indicate the bootstrap support values above 70%. Newly generated sequence in this study is shown in blue and bold. *Russula grisea* (SAV F-1395) was used as an outgroup. Type specimens are indicated with "T". ML: maximum likelihood; ITS: internal transcribed spacer; nLSU: nuclear large subunit ribosomal DNA.
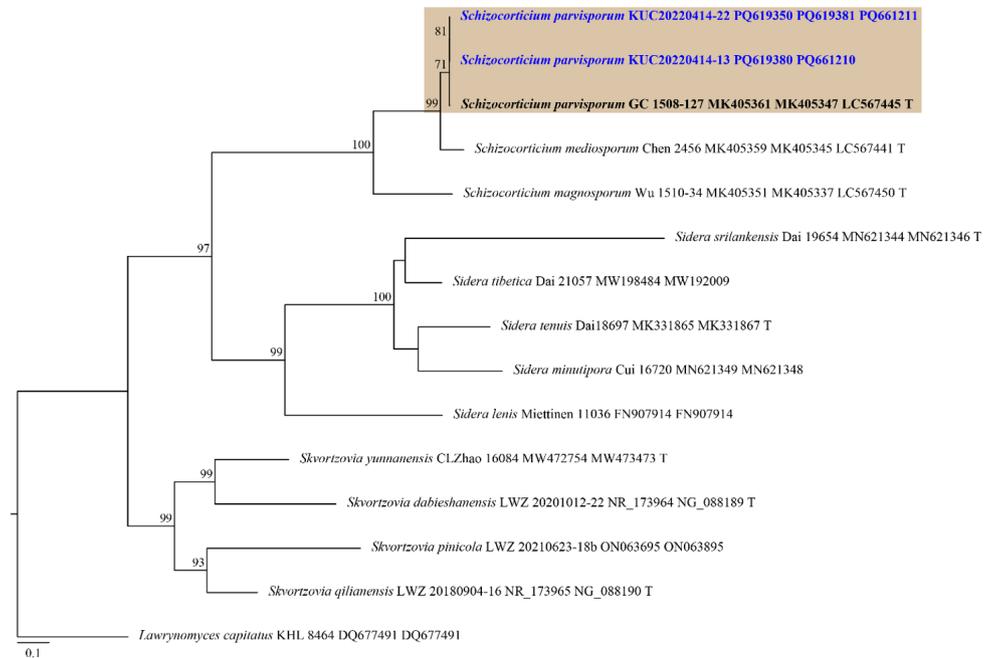

Fig. 4. ML tree based on the ITS, nLSU and *tef1* concatenated sequence datasets of the genus *Schizocorticium* and allied genera. The node numbers indicate the bootstrap support values above 70%. Newly generated sequences in this study are shown in blue and bold. *Lawrynomyces capitatus* (KHL 8464) was used as an outgroup. Type specimens are indicated with "T". ML: maximum likelihood; ITS: internal transcribed spacer; nLSU: nuclear large subunit ribosomal DNA; *tef1*: translation elongation factor 1 gene.

The finding that several macrofungi previously reported in tropical to subtropical areas are now found in temperate regions suggests that climate change affects macrofungal distribution. Because the life cycle of macrofungi is closely associated with trees, they are likely to be affected by changes in tree distribution due to climate change. Several studies have shown that macrofungal composition and diversity are affected by the composition and diversity of trees [30–32]. In addition, a recent study revealed that future climate and tree distribution changes affect the biodiversity of macrofungi [33]. Mudeungsan National Park is located in the Southern region of Korea. It is expected to enter a subtropical climate more quickly and to be more strongly impacted by climate change than other National Parks in Northern areas [34,35]. Therefore, further research is required to identify the long-term macrofungal diversity and discover new or unrecorded species in Mudeungsan National Park.

TAXONOMY

Anomoloma luteoalbum J. Song & B.K. Cui, Mycological Progress 15 (1/11): 5 (2016) (Fig. 5)
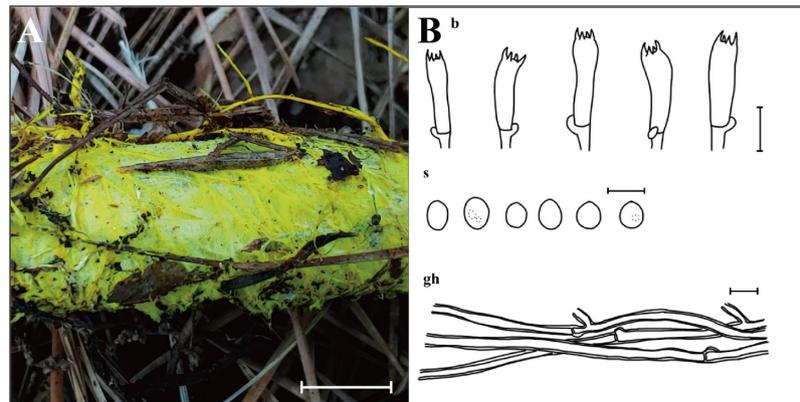

Fig. 5. Morphological characteristics of *Anomoloma luteoalbum* KUC20210714-74 and KUC20230317-01. A: Basidiome; B: Microscopic structures, b: basidia; gh: generative hyphae; s: basidiospores (Scale bars: A = 1 cm, B = 10 μm, 5 μm for s).

MycoBank number: MB 821239

Basidiome resupinate, effused, soft when fresh, corky when dried. Margin irregular, abundant yellow (2.5Y, 8/8) rhizomorphs grow out from margin. Pores angular, 5–6 per mm, surface bright yellow (2.5Y, 8/8). Tubes soft, corky, concolorous with pore surface, up to 0.3 mm long. Subiculum yellow (2.5Y, 8/8) to pale brown (2.5Y, 8/4) when dry, up to 0.7 mm thick. Basidia with 4–spored, cylindrical to subclavate, 16.1–23.8 $\times$ (3.8–)4.1–7.3 μm, smooth, hyaline, thin-walled, with a basal clamp connection. Basidiospores broadly ellipsoid to ellipsoid, (2.5–)2.9–4.2 $\times$ 2.0–3.2(–3.4) μm, L = 3.3 μm, W = 2.6 μm, Q = 1.08–1.60, smooth, hyaline, thin-walled, a few bearing droplets. Cystidia absent. Hyphal system monomitic, generative

hyphae septate, branched, with clamp connections, thick-walled, hyaline, 2.6–4.8 μm in diam.

Specimens examined: Korea. Jeollanam-do, Gwangju Metropolitan City, Mudeungsan National Park, 35°06′21″N, 126°59′53″E, 14 Jul 2021, M. Cho, S. L. Kwon & Y. Yoo, KUC20210714-74 (=NIBRFG0000510827); Korea. Jeollanam-do, Gwangju Metropolitan City, Mudeungsan National Park, 35°15′26″N, 126°99′04″E, 17 Mar 2023, M. Cho, S. L. Kwon, S. H. Lee & D. Y. Kwon, KUC20230317-01 (=NIBRFG0000514708).

Habitat: Occurs on dead trees in mixed hardwood forest.

Notes: *Anomoloma luteoalbum* is characterized by angular pores, abundant yellow rhizomorphs, absence of cystidia, and ellipsoid basidiospores. The basidiome of KUC20210714-14 appeared more yellowish, with thicker walls of basidia and basidioles compared to the type specimen (Cui 2687) [36]. The resupinate form of *A. luteoalbum* resembles *A. rhizosum*, though the latter has larger basidiospores (4.1–5.3 × 3–4 μm) [37]. In the phylogenetic tree, *A. flavissimum* is closely related to *A. luteoalbum*. Both species share features, such as yellow rhizomorphs and bright chrome or sulfur-yellow pore surfaces [38]. However, *A. flavissimum* can be distinguished by its larger basidiospores (3–4 × 2–3 μm) and the presence of cystidia [38]. Initially published as *A. luteoalba* [36], it was later revised to *A. luteoalbum* [39].

Fibrodontia alba Yurchenko & Sheng H. Wu, Mycoscience 55 (5): 339 (2014) (Fig. 6)
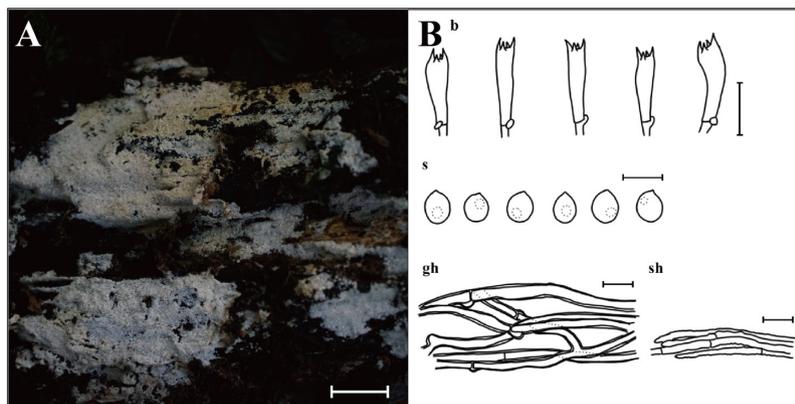

Fig. 6. Morphological characteristics of *Fibrodontia alba* KUC20210930-17. A: Basidiome; B: Microscopic structures, b: basidia; gh: generative hyphae; s: basidiospores; sh: skeletal hyphae (Scale bars: A = 1 cm, B = 10 μm, 5 μm for s).

MycoBank number: MB 564320

Basidiome effused, odontoid, soft when fresh, fragile when dry, covered with abundant aculei, 6–10 per mm, up to 0.4 mm long. Hymenial surface white (7.5YR, 9.5/1) when fresh, pale yellow (2.5Y, 8.5/2) when dry. Margin fimbriate or abrupt. Basidia with 4–spored, cylindrical to subclavate, 7.6–13.4 × 2.3–4.6

µm, smooth, hyaline, with a basal clamp connection. Basidiospores subglobose to ellipsoid, (2.8–)2.9–3.9 $\times$ 2.2–3.3 µm, L = 3.3 µm, W = 2.7 µm, Q = 1.03–1.38, smooth, hyaline, thin-walled, bearing a droplet, with acute apex. Cystidia absent. Hyphal system pseudodimitic, generative hyphae septate, branched, with clamp connections, thick-walled, hyaline, 2.3–3.1 µm in diam. Skeletal-like hyphae septate, unbranched, thin- or thick-walled up to 1 µm with round apex, encrusted, hyaline, 1.7–2.6 µm in diam.

Specimen examined: Korea. Jeollanam-do, Gwangju Metropolitan City, Mudeungsan National Park, 35°05′90″N, 126°59′28″E, 30 Sep 2021, M. Cho, S. L. Kwon & Y. Yoo, KUC20210930-17 (=NIBRFG0000518401).

Habitat: Occurs on conifer tree stump in mixed hardwood forest.

Notes: *Fibrodontia alba* is characterized by an odontoid basidiome covered with numerous slender aculei and skeletal-like hyphae encrusted with crystals. The morphology of our specimen closely resembles the type specimen (TNM F24944), though observed basidia were shorter than those in the original description (11–19 $\times$ 3–3.5(–4.5) µm) [28]. Phylogenetic analysis indicates that *F. subalba* is most closely related to *F. alba*. *Fibrodontia subalba* shares an odontoid hymenophore and a white hymenial surface with *F. alba* [40] but differs in having a dimitic hyphal system and larger basidiospores ((3.5–)3.7–4.4(–4.6) $\times$ (2.7–)2.8–3.4(–3.5) µm) [40]. Interestingly, although *F. alba* was found on a conifer stump in this survey, it was reported on broad-leaved trees in the original description [28]. This species was previously reported only in central Taiwan [28]. This is the first report of *F. alba* in a temperate region and the first record of the genus *Fibrodontia* in this country.

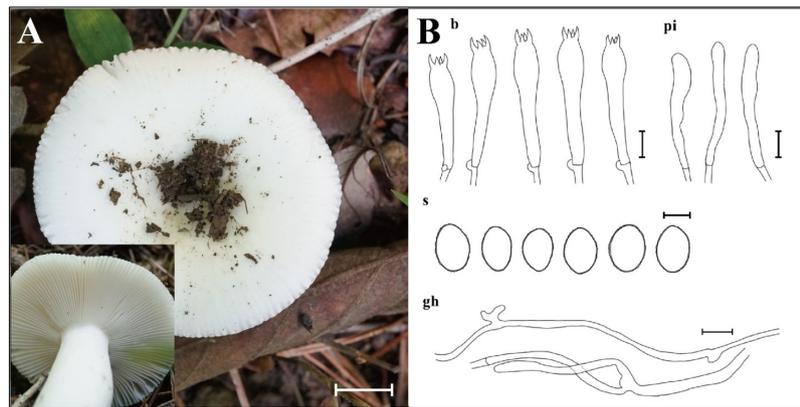## *Russula pallidula* Bin Chen & J.F. Liang, Sydowia 71: 2 (2019) (Fig. 7)

Fig. 7. Morphological characteristics of *Russula pallidula* KUC20220715-36. A: Basidiome; B: Microscopic structures, b: basidia; gh: generative hyphae; pi: pileocystidia; s: basidiospores (Scale bars: A = 1 cm, B = 10 µm, 5 µm for s).

MycoBank number: MB 825075

Pileus smooth, convex to plane, center depressed, white (10YR, 9.5/1), 40–50 mm in diam. Lamellae adnate, subfree, subcrowded to crowded, white (10YR, 9.5/1). Stipe central, equal, smooth, concolorous

with pileus, 45 × 12 mm. Basidia with 4–spored, clavate to subclavate, 47.1–53.4 × 9.7–11.9 μm, smooth, hyaline, thin-walled. Basidiospores broadly ellipsoid to ellipsoid, asperulate, 7.5–9.8 × 5.8–7.3 μm, L = 8.7 μm, W = 6.5 μm, Q = 1.18–1.50, olive brown (2.5Y, 4/3). Pileocystidia narrowly cylindrical, 26.2–41.2 × 4.2–7.1 μm, smooth, hyaline, thin-walled. Hyphal system monomitic, generative hyphae septate, branched, thin-walled, hyaline, 2.5–3.5 μm in diam.

Specimen examined: Korea. Jeollanam-do, Hwasun-gun, Mudeungsan National Park, 35°10′29″N, 126°98′38″E, 15 Jul 2022, M. Cho, S. L. Kwon & S. H. Lee, KUC20220715-36 (=NIBRFG0000518398).

Habitat: Solitary in the mixed hardwood forest.

Notes: *Russula pallidula* is characterized by white pileus and ellipsoid, asperculate basidiospores. It is classified under *Russula* subsect. *Virescentinae*. The morphological features of KUC20220715-36 generally align with those of the type specimen (RITF2613) [29], though cheilocystidia and pleurocystidia were rarely observed in the collected specimen. Morphologically and phylogenetically, *R. sribuabanensis* is closely related to *R. pallidula*. However, *R. sribuabanensis* is distinguishable by its larger pileus (2.5–10.5 cm) and subglobose basidiospores (6.2–8.2 × 5.6–7.2 μm, Q = 1.0–1.2) [41]. Previously reported from Southern China (Guangdong, Yunnan, and Zhejiang) [29], and this is the first report of *R. pallidula* in the temperate region.

Schizocorticium parvisporum Sheng H. Wu & C.L. Wei, Mycological Progress 20 (6): 777 (2021) (Fig. 8)
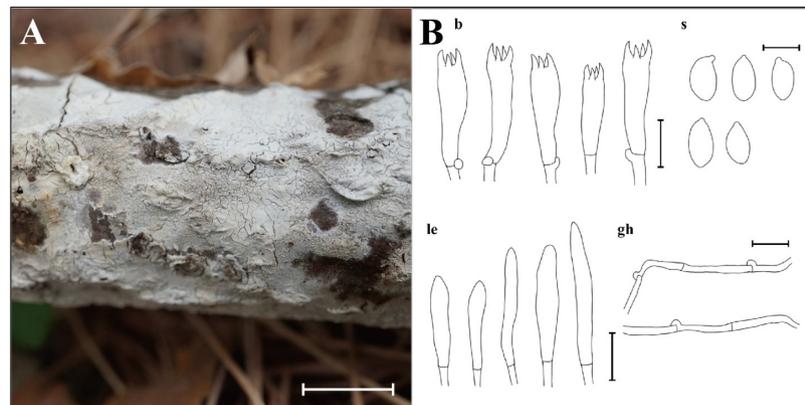

Fig. 8. Morphological characteristics of *Schizocorticium parvisporum* KUC20220414-13 and KUC20220414-22. A: Basidiome; B: Microscopic structures, b: basidia; gh: generative hyphae; le: leptocystidia; s: basidiospores (Scale bars: A = 1 cm, B = 10 μm, 5 μm for s).

MycoBank number: MB 830193

Basidiome adnate, widely effused, resupinate, membranous, with cracks. Hymenial surface smooth or sometimes irregular, white (7.5YR, 9.5/1) when fresh, pale yellow (2.5Y, 8.5/2) when dried. Hyphal system monomitic, generative hyphae septate, with clamp connections, thin-walled, hyaline, 2.1–3.3 μm in diam.

Basidia with 4–spored, subclavate to cylindrical, 16.2–25.0 × 5.2–7.0 μm, smooth, hyaline, thin-walled, with a basal clamp connection. Basidiospores narrowly ellipsoid to cylindrical, 6.1–6.8 × 3.1–4.0 μm, L = 6.3 μm, W = 3.6 μm, Q = 1.6–1.9, smooth, hyaline, thin-walled, with acute apex. Cystidia absent. Hyphidia tubular, narrowly cylindrical, smooth, thin-walled, hyaline, 15.8–32.9 × 2.2–3.9 μm.

Specimens examined: Korea. Jeollanam-do, Hwasun-gun, Mudeungsan National Park, 14 Apr 2022, 35°10′35″N, 126°98′81″E, M. Cho, S. L. Kwon & S. H. Lee, KUC20220414-13 (=NIBRFG0000513227); Korea. Jeollanam-do, Hwasun-gun, Mudeungsan National Park, 35°10′33″N, 126°98′79″E, 14 Apr 2022, M. Cho, S. L. Kwon & S. H. Lee, KUC20220414-22 (=NIBRFG0000513233).

Habitat: Occurs on the dead angiosperm branches in the hardwood forest.

Notes: *Schizocorticium parvisporum* is characterized by effused and cracked basidiome, and the presence of hyphidia. It is typically found in angiosperm branches or trunks [27]. The studied specimens share a similar morphology with the type specimen (GC 1508-127), although numerous branched hyphidia were observed in the type specimen [27]. Morphological similarities are common among *Schizocorticium* species [27], but they can be differentiated by basidiospore size: *S. magnosporum* has the largest basidiospores (9.8–11.5 × 4.2–5.5 μm), followed by *S. mediosporum* (8–9.8 × 3.8–4.8 μm), and *S. parvisporum* with the smallest [27]. Previous research has emphasized the necessity of *tef1* region analysis for precise identification of *Schizocorticium* species [27]. For instance, in the phylogenetic tree, *S. mediosporum* is the closest to *S. parvisporum*, with only 18 bp differences in the ITS and nLSU combined dataset. This limited variation caused an unclear separation when using these two regions alone. However, in the *tef1* region, the difference increased significantly to 70 bp, thereby facilitating better resolution. Therefore, combined phylogenetic analyses using the ITS+nLSU+*tef1* datasets are essential for the accurate identification of *Schizocorticium* species. Previously known from subtropical Taiwan and Yunnan, China [27], this is the first report of *S. parvisporum* in the temperate region.

CONFLICT OF INTEREST

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

ACKNOWLEDGEMENTS

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