


## Seven Unrecorded Indigenous Fungi from Mudeungsan National Park in Korea

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### ABSTRACT

Fungi act as important decomposers in the forest environment. They recycle essential nutrients, promote plant growth through mycorrhizal relationships, and act as food for small animals. Samples of 265 indigenous fungal species were collected from Mudeungsan National Park in 2020. These species were identified based on morphological, molecular, and phylogenetic analyses using the internal transcribed spacer (ITS), nuclear large subunit rRNA (LSU), and RNA polymerase II second largest subunit (rpb2) regions. Subsequently, seven species were identified as unrecorded species in Korea: *Cordyceps cicadae*, *Dentocorticium bicolor*, *Hymenochaete nanospora*, *Physisporinus crataegi*, *Rigidoporus piceicola*, *Russula raoultii*, and *Scutellinia crinita*. This study reveals their detailed macro- and microscopic morphological characteristics with phylogenetic trees to report them as unrecorded species in Korea.

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## 1. Introduction

Fungi play important roles in forest environments. They act as wood decomposers and food for small animals. They also promote or inhibit growth of plants through mycorrhizal formation or parasitism, respectively. Studies have estimated that 2.2–3.8 million fungal species live on Earth [1]. However, about 10% of the fungal species, between 220,000 and 380,000, were assumed as macrofungal species in worldwide [1,2]. Meanwhile, only about 1900 macrofungal species have been recorded in Korea until 2013 [3], and currently, it is estimated that more than 2200 macrofungal species are identified in Korea [4]. This suggests that continuous research is required to discover new and unrecorded indigenous fungal species.

Mudeungsan National Park is located in Gwangju Metropolitan City and the province of Jeollanam-do in the western part of Korea. Mudeungsan was designated as a provincial park in 1972 and redesignated as a national park in 2012 owing to its importance in preserving biodiversity and natural resources in Korea. Flora and fauna in Mudeungsan National Park have been studied extensively to determine their diversity and composition [5–8].

However, studies surveying fungal diversity have not yet been conducted.

As a project to discover indigenous fungi in Korea, 246 fungal specimens were collected from Mudeungsan National Park in 2020. The surveys were conducted in the east region of Mudeungsan National Park. Among them, 200 specimens had previously been identified using DNA molecular and morphological analyses, whereas seven were identified as unrecorded in Korea: *Cordyceps cicadae*, *Dentocorticium bicolor*, *Hymenochaete nanospora*, *Physisporinus crataegi*, *Rigidoporus piceicola*, *Russula raoultii*, and *Scutellinia crinita*. Five species are wood-decaying fungi, except *C. cicadae*, which has parasitic form, and *Ru. raoultii*, which grows solitary on soil covered with woody debris. This study describes detailed macro- and micro-morphological characteristics of the species and provides phylogenetic trees to report them as newly recorded species in Korea.

## 2. Materials and methods

### 2.1. Sampling

Mudeungsan National Park (35°03'06"~35°12'59" N, 126°53'41"~127°05'01" E) is located across Buk-gu

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of Gwangju Metropolitan City, and Damyang-gun and Hwasun-gun of Jeollanam-do Province, Korea. Collection of fungal species in Mudeungsan National Park commenced from May to September 2020. Afterwards, they were dried at 60 °C for 72 h and stored in silica gel. The specimens were deposited in the Korea University Collection (KUC) and National Institute of Biological Resources (NIBR).

## 2.2. Molecular approach

Genomic DNA of the dried specimens was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The internal transcribed spacer (ITS) region was amplified with ITS5/ITS4 or ITS1F/ITS4 primer sets [9,10]. The nuclear large subunit rRNA (LSU) region was amplified with the primer sets, LR0R/LR5 or LR0R/LR7 [11,12]. For *Rigidoporus piceicola*, bRPB2-6F/bRPB2-7.1R primer set was used to amplify RNA polymerase II second largest subunit (rpb2) region [13]. The PCR products were purified using AccuPrep® PCR Purification Kit and AccuPrep® Gel Purification Kit (Bioneer) according to the manufacturer's instructions. DNA sequencing was performed by Cosmogenetech (Seoul, Korea), and each sequencing result was edited using SeqMan Lasergene package version 7.0.0 (DNASTar Inc., Madison, WI). Reference sequences were collected from the NCBI GenBank database ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)), and the edited sequences were compared with the reference sequences using BLAST. Each sequence was assembled and aligned using MEGA version 7 and MAFFT version 7.130 [14,15]. Phylogenetic analyses were performed using the maximum likelihood (ML) method, using RAxML with GTR+G model, and 1000 bootstrap replicates were used for tree inference [16]. All analyses were performed using CIPRES [17]. The obtained trees were edited using FigTree version 1.4.3. [18] and Adobe Illustrator CS6 (Adobe Systems, Inc., San Jose, CA). Bootstrap support values above 70% are shown in the tree. All newly generated

sequences of the seven species have been deposited in GenBank (Table 1).

## 2.3. Morphological observation

For morphological characteristics, microscopic features were observed using Olympus BX51 light microscope (Olympus, Tokyo, Japan), and pictures were taken using DP20 microscope camera (Olympus). The observations were performed using 5% KOH and Congo red solutions. The special color terms follow the Munsell Soil Color Book [19]. The following abbreviations are used: L = mean spore length, W = mean spore width, and Q = L/W ratio.

## 3. Results and discussion

Seven unrecorded species were identified using phylogenetic analyses of DNA sequences, particularly the ITS or LSU regions (Table 1). Six species were enough to be identified using ITS or LSU regions. However, *Ri. piceicola* needed multigene analysis (ITS, LSU, and rpb2) [20]. At the molecular level, each of the seven species was compared with its reference sequence and clearly identified with a high percentage of homology (Table 1). The identification results revealed the orders of the species: Polyporales (two spp.), Hymenochaetales (two spp.), Hypocreales (one sp.), Pezizales (one sp.), and Russulales (one sp.) (Table 1).

Seven species were clearly distinguished through ML method using ITS or LSU or rpb2 sequence datasets for phylogenetic analyses. According to the phylogenetic trees (Figures S1–S7, Supporting Information), *C. cicadae* formed monophyletic groups with high bootstrap values and its sister group was *C. tenuipes* (Figure S1). *D. bicolor* was clearly separated from other taxa with high bootstrap values (Figure S2). *H. nanospora* formed monophyletic groups with high bootstrap values and its sister group was *H. cana* (Figure S3). *P. crataegi* formed monophyletic groups with high bootstrap values and its sister group was *P. vitreus* (Figure S4). *Ri. piceicola* was not clearly separated from *Ri.*

**Table 1.** Molecular identification of seven unrecorded species in Korea.

Identity	Specimen ID	Order	Gen. Acc. No. <sup>f</sup>			Homology (%)	Accordance of bp	
			ITS	LSU	rpb2			
<i>Cordyceps cicadae</i>	KUC20200924-44	Hyp <sup>a</sup>	OK559813	–	–	KX017277	99	536/537 bp
<i>Dentocorticium bicolor</i>	KUC20200527-18	Pol <sup>b</sup>	OK559816	–	–	MF626355	100	394/394 bp
	KUC20200617-44		OK559817	–	–	MF626355	99	547/549 bp
<i>Hymenochaete nanospora</i>	KUC20200924-48	Hym <sup>c</sup>	OK559811	–	–	NR_160200	99	701/710 bp
<i>Physisporinus crataegi</i>	KUC20200903-15	Pol	OK559818	–	–	KY131845	99	544/547 bp
<i>Rigidoporus piceicola</i>	KUC20200924-53	Hym	OK559812	–	–	KT203301	99	528/535 bp
			–	ON171629	–	KT203322	98	873/889 bp
			–	–	ON204049	KT210376	99	595/598 bp
<i>Russula raoultii</i>	KUC20200616-18	Rus <sup>d</sup>	–	OK576271	–	KT933863	99	773/781 bp
<i>Scutellinia crinita</i>	KUC20200923-07	Pez <sup>e</sup>	OK559814	–	–	MN752431	99	540/543 bp

<sup>a</sup>Hyp: Hypocreales; <sup>b</sup>Pol: Polyporales; <sup>c</sup>Hym: Hymenochaetales; <sup>d</sup>Rus: Russulales; <sup>e</sup>Pez: Pezizales; <sup>f</sup>Gen. Acc. Num.: GenBank Accession Number; <sup>9</sup>Ref: reference.

*obducens*, *Ri. populinus*, and *Ri. subpopulinus* using ITS or LSU regions, so combined tree of ITS, LSU, and rpb2 regions is provided for accurate identification [20]. As a result, *Ri. piceicola* formed monophyletic groups with high bootstrap values (Figure S5). *Ru. raoultii* was clearly separated from the other taxa with high bootstrap values (Figure S6). *S. crinita* was clearly separated from the other taxa, but the clade was divided within the *S. crinita* group (Figure S7). Therefore, further research on the genus *Scutellinia* is required to determine why the clade was divided.

The genus *Cordyceps* Fr. is characterized by fusi-form to clavate fertile heads of the stroma and fili-form ascospores [21]. Additionally, this genus is widely used for medicinal applications, especially in Asia. Although 183 species are accepted as *Cordyceps* worldwide [22], only 46 species are listed in the NIBR database in Korea. Occurrence of *Cordyceps* is affected by conditions, such as humidity, temperature, elevation, and life cycle of its host species [21]. The host species are mostly insects, but other *Cordyceps* species, fungi *Elaphomyces*, spiders, nematodes, and plant tissues have also been reported as the hosts [23,24]. Many synonyms have been listed because of their sexual and asexual morphs [21]. Therefore, additional studies are required to correct sexual and asexual names and to reclassify hidden *Cordyceps* species.

The genus *Dentocorticium* (Parmasto) M.J. Larsen & Gilb was separated from the extinct genus *Laeticorticium* [25]. Currently, six species are listed in the genus, and five previously listed species have been reclassified as other genera [26–29]. *Dendrodontia* and *Fuscocerrena* have been identified as synonyms of *Dentocorticium* based on phylogenetic analysis [30]. These two genera have macroscopic features similar to *Dentocorticium* [30]; therefore, accurate observations of microscopic characteristics and molecular analyses are required to identify them correctly. *Dentocorticium* is characterized by effused basidiocarps, brown skeletal hyphae, and irregularly branched dendrohyphidia [30].

The genus *Hymenochaete* Lév is characterized by brown effused basidiocarps, hymenial setae, and small basidiospores [31,32]. More than 120 species have been reported globally [33], but only 12 species were reported in Korea. Phylogenetic analysis revealed that in addition to *Hymenochaete*, *H. tabacina* (Sowerby) Lév is closely related to the poroid genera [33,34]. Consequently, the new genus *Pseudochaete* was separated [33,34]. Therefore, molecular approaches are required because of the similar morphological characteristics of these two genera [33].

The genus *Physisporinus* P. Karst. has eight species worldwide and is new to Korea. *Physisporinus* species are characterized by soft basidiocarps, and lack of cystidia [20,35–37]. Phylogenetic analysis showed that *Physisporinus* and several species of *Rigidoporus* Murrill were closely related in the Polyporales clade [34,38–40]. However, it is difficult to distinguish between the two genera because of their similar morphological characteristics [20]. Therefore, accurate phylogenetic analysis is required to correctly identify the species in these genera [20].

The genus *Rigidoporus* Murrill has morphological characteristics similar to genera *Leucophellinus* Bondartsev & Singer, *Oxyporus* (Bourdot & Galzin) Donk, and *Physisporinus* P. Karst [35,37,41]. *Rigidoporus* is distinguished by ochraceous and corky basidiocarps, and hyphoid cystidia [20]. According to phylogenetic analyses, the four genera are closely related [34]. Several species of *Oxyporus* and *Rigidoporus* were classified in the order Hymenochaetales, but species of *Physisporinus* and some species of *Rigidoporus* were classified in the order Polyporales [34,38,39]. Therefore, additional research is required for accurate classification.

The genus *Russula* Pers. is the second largest genus in the class Agaricomycetes, with has 1331 recorded species; it is estimated that almost 3000 species exist globally [42,43]. However, only 82 species of this genus were reported in Korea. In some studies, *Russula* was studied using the LSU region, and it was proved that the LSU region is an effective tool for identifying this species [44,45]. *Russula* is characterized by a large, bright-colored pileus and spores with echinulate surfaces [46,47]. The species in this genus are difficult to distinguish through morphological characteristics because of their large population [48,49]. Since an accurate description of taxonomy is difficult, molecular and phylogenetic analyses are necessary [47].

The genus *Scutellinia* (Cooke) Lambotte is a cosmopolitan fungi which have been reported worldwide, usually in the Northern Hemisphere [50]. This genus is characterized by orange to red apothecia and blackish brown hairs around the margin of apothecia [51]. Although over 100 species are listed in the genus, only four species were reported in Korea [22]. Among the species, 10 species that occur in soil have globose to subglobose ascospores, and the others that occur on decayed wood have ellipsoid ascospores [50–54]. Phylogenetic analysis showed that the genus *Scutellinia* is well divided in the family Pyronemataceae [51].

This study identified several unrecorded species in Mudeungsan National Park. These records are important resources for determining the diversity and composition of fungal species in Korea.

The survey started in 2020; therefore, it is expected that many new and unrecorded species are yet to be discovered. Thus, additional surveys are required at different locations, altitudes, and seasons.

## 4. Taxonomy

### 4.1. *Cordyceps cicadae* (miq.) Masee, *annals of botany* 9: 38 (1895) [MB#311793]

#### 4.1.1. Asexual morphs

**Synnemata** erect, irregularly branched, clavate, with powdery and floccose apex due to the conidial mass, white (2.5Y, 9.5/1) to pale yellow (2.5Y, 8.5/2) mycelium, 30–50 mm in length and 0.5–2.5 mm in diam (Figures 1(A) and 2(A)). **Conidiophores** and **Phialides** were not observed. **Conidia** narrowly cylindrical with round apex, sometimes slightly curved, smooth, thin-walled, hyaline, containing one or two guttules,  $6.0\text{--}7.5 \times 2.0\text{--}3.5 \mu\text{m}$  [ $Q = 2.14\text{--}3.25$ ,  $Q = 2.7 \pm 0.5$ ] ( $n = 40$ ).

#### 4.1.2. Specimen examined

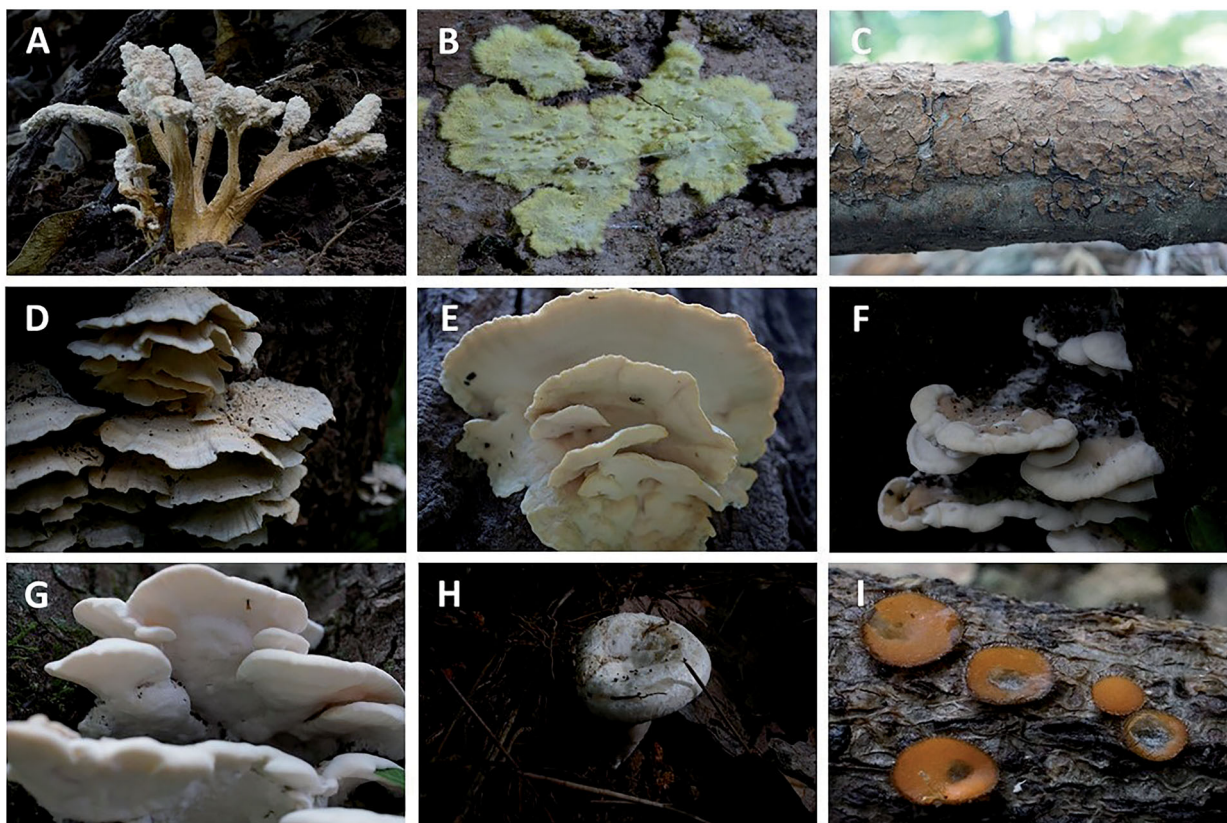
Korea. Gwangju Metropolitan City, Mudeungsan National Park,  $35^{\circ}07'51''$  N,  $126^{\circ}59'62''$  E, mixed hardwood forest, parasitic in cicada, 24 Sep 2020, S. L. Kwon, KUC20200924-44 (NIBRFG0000511371).

### 4.1.3. Remarks

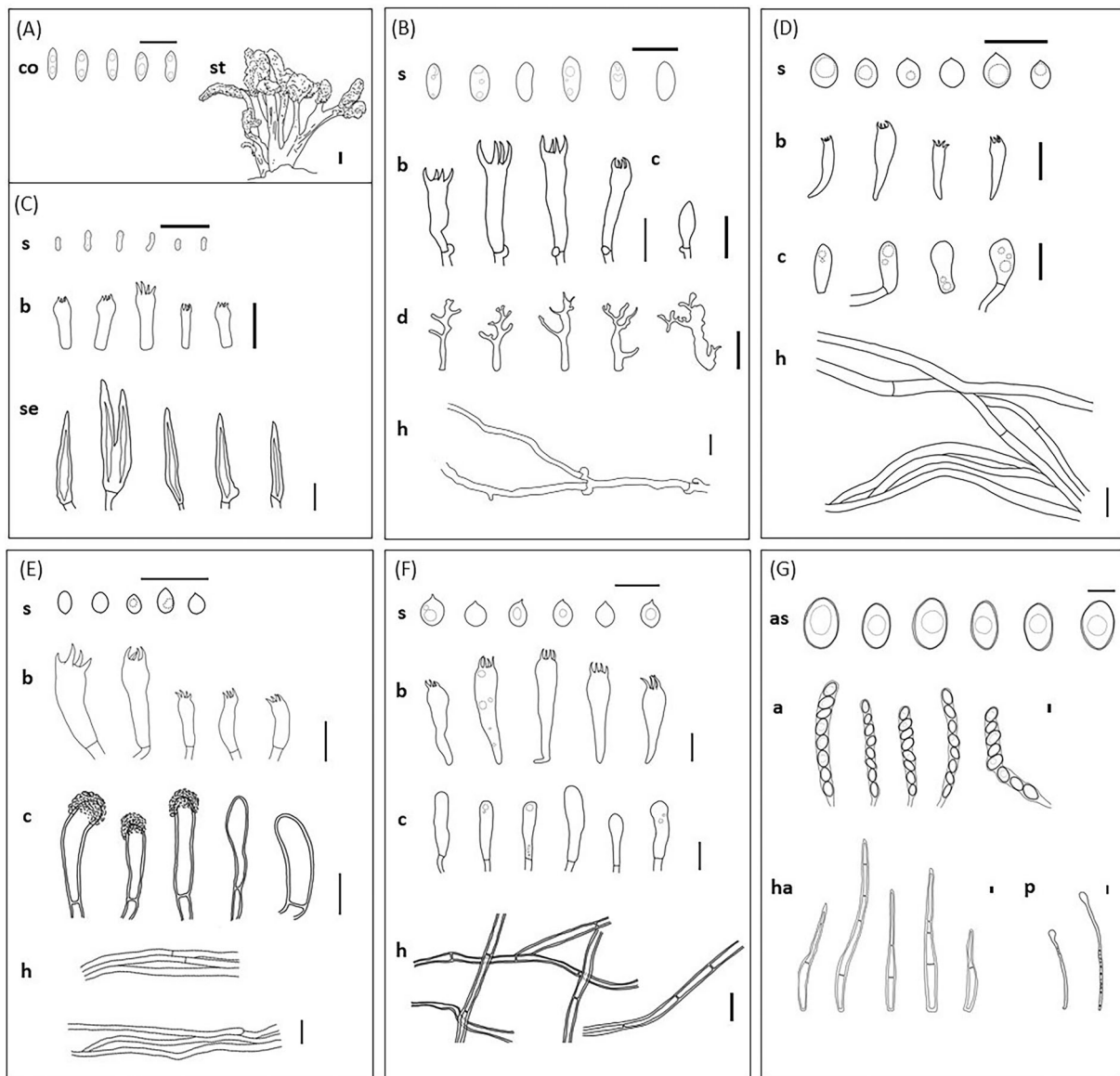
*Cordyceps cicadae* KUC20200924-44 matched well with *C. cicadae* (Miq.) Masee in the phylogenetic analysis. The size of the conidia was measured and found to be similar to the original description [55]. Further microscopic observations are required for accurate identification. According to phylogenetic analysis, *C. qingchengensis* is the most closely related species. The difference between *C. cicadae* and *C. qingchengensis* is the host species. The hosts of *C. cicadae* are cicada nymphs, while the host of *C. qingchengensis* has been identified to be the cocooned pupa of a large silk moth [21]. However, the asexual morph of *C. qingchengensis* is unknown; therefore, it was impossible to compare its morphological characteristics.

### 4.2. *Dentocorticium bicolor* (P.H.B. Talbot) Nakasone & S.H. He, *MycKeys* 32: 42 (2018) [MB#823073]

**Basidiocarp** annual, resupinate, loosely adnate, effused, little tough, easily separated from substrate when dried, up to 200–300  $\mu\text{m}$  thick (Figures 1(B) and 2(B)). **Hymenial surface** membranaceous, slightly odontoid, hydonoid, tuberculate because of hyphal pegs, margin abrupt, very pale yellow (2.5Y, 9.5/2) to



**Figure 1.** Fruiting bodies of the seven unrecorded species in Korea. (A) *Cordyceps cicadae*; (B) *Dentocorticium bicolor*; (C) *Hymenochaete nanospora*; (D and E) *Physisporinus crataegi*; (F and G) *Rigidoporus piceicola*; (H) *Russula raoultii*; (I) *Scutellinia crinita*.



**Figure 2.** Microscopic view of morphological characteristics of the seven unrecorded species in Korea. (A) *Cordyceps cicadae*; (B) *Dentocorticium bicolor*; (C) *Hymenochaete nanospora*; (D) *Physisporinus crataegi*; (E) *Rigidoporus piceicola*; (F) *Russula raoultii*; (G) *Scutellinia crinita*. (a: asci, as: ascospores, b: basidia, c: cystidia, co: conidia, d: dendrohyphidia, h: hyphae, ha: hair, p: paraphyses, s: basidiospores, se: setae, st: stroma; a scale bar = 10  $\mu$ m, for stroma, a scale bar = 1 cm).

yellow (5Y, 8/8) when fresh, pale yellow (5Y, 8/2) to yellow (5Y, 8/6) when dry. **Hyphal system** dimitic, generative hyphae located in subhymenium, moderately septate with clamp connections, branched, thin-walled, hyaline, 2.0–3.5  $\mu$ m in diam. Skeletal hyphae easily separated from generative hyphae, long, straight, aseptate with clamp connections, unbranched, thick-walled up to 1  $\mu$ m with thin-walled round apex, brownish yellow (10YR, 6/6), 2.7–4.1  $\mu$ m in diam. **Dendrohyphidia** arising from subhymenial hyphae, irregularly and strongly branched, tortuous and with bulges, thin-walled, hyaline, 1.0–2.5  $\mu$ m in diam. **Basidia** clavate, 4-spored, smooth, hyaline, containing one or two guttules (17–)18.5–21(–21.5)  $\times$  4.0–5.0  $\mu$ m ( $n=31$ ). **Basidiospores** ellipsoid to cylindrical, smooth, thin-walled, hyaline, containing over two guttules, 7.5–8.5  $\times$  3.0–4.5  $\mu$ m [ $Q=1.9-2.5$ ,  $Q=2.2 \pm 0.3$ ]

( $n=34$ ). **Cystidia** lacks, cylindrical to subclavate, smooth, thin-walled, hyaline, 17–27  $\times$  3.7–6.2  $\mu$ m ( $n=8$ ).

#### 4.2.1. Specimen examined

Korea. Gwangju Metropolitan City, Mudeungsan National Park, 35°08'80" N, 126°58'59" E, mixed hardwood forest, occurring on dead trees, 27 May 2020, S. L. Kwon, KUC20200527-18 (NIBRFG0000507813), 17 June 2020, S. L. Kwon, KUC20200617-44 (NIBRFG0000508874).

#### 4.2.2. Remarks

*Dentocorticium bicolor* KUC20200527-18 and KUC20200617-44 matched well with *D. bicolor* (P.H.B. Talbot) Hjortstam & Ryvarden in the phylogenetic analysis, with high bootstrap value.

The morphological characteristics of *D. bicolor* KUC20200527-18 and KUC20200617-44 are similar to the original description. According to the description of *D. bicolor* (Talbot) Hjortstam & Ryvarden, the size of the basidia was determined to be  $30\text{--}35 \times 6\text{--}7 \mu\text{m}$ . However, according to *Grandinia bicolor* (P.H.B. Talbot), a synonym of *D. bicolor*, the basidia size is smaller than that of *D. bicolor*, KUC20200527-18 and KUC20200617-44. In addition, the context color was reported as brown; however, brown-colored samples were not observed. Further studies are required to obtain more accurate results. Phylogenetic analysis shows that *D. sulphurellum* is the most closely related species. *D. sulphurellum* has white to yellow hymenial surface and basidiospores ( $7\text{--}9.5 \times 2.5\text{--}3 \mu\text{m}$ ), similar to *D. bicolor*. However, *D. sulphurellum* has only been reported in North America [30].

#### 4.3. *Hymenochaete nanospora* J.C. Léger, *cryptogamie mycologie* 4 (3): 235 (1983) [MB#108064]

**Basidiocarps** annual, resupinate, effused, adherent, margin thin, woody hard when dry, cortex present (Figures 1(C) and 2(C)). **Hymenial surface** crustaceous, smooth, densely cracked, light brown (7.5YR, 6/4) when fresh, brown (7.5YR, 4/4) when dry. **Hyphal system** monomitic, usually septate without clamp connections, occasionally branched, thin-walled, hyaline,  $2.1\text{--}3.3 \mu\text{m}$  in diam. **Setae** obclavate, fusiform, with acute apex, partition absent, smooth, thick-walled, reddish brown (7.5YR, 6/8),  $20\text{--}45 \times 4.0\text{--}5.0 \mu\text{m}$  ( $n = 30$ ). **Basidia** clavate, subcylindrical, 4-spored, smooth, hyaline ( $8.5\text{--}9.0\text{--}11.5\text{--}12.5$ )  $\times 2.0\text{--}3.5 \mu\text{m}$  ( $n = 48$ ). **Basidiospores** narrowly cylindrical to allantoid, smooth, thin-walled, hyaline, containing two guttules,  $2.5\text{--}3.5 \times 0.8\text{--}1.2 \mu\text{m}$  [ $Q = 2.92\text{--}3.125$ ,  $Q = 3.02 \pm 0.1$ ] ( $n = 23$ ). **Cystidia** absent.

##### 4.3.1. *Specimen examined*

Korea. Gwangju Metropolitan City, Mudeungsan National Park,  $35^{\circ}07'52''$  N,  $126^{\circ}59'62''$  E, mixed hardwood forest, occurring on hardwood branches, 24 Sep 2020, S. L. Kwon, KUC20200924-48 (NIBR FG0000511372).

##### 4.3.2. *Remarks*

*Hymenochaete nanospora* KUC20200924-48 was well-matched with *H. nanospora* J.C. Léger in the phylogenetic analysis. This species is morphologically characterized by small and narrow spores, and its width rarely exceeds  $1 \mu\text{m}$ . According to phylogenetic analysis, *H. cana* was identified as the most closely related species. *H. cana* has reddish brown

setae ( $20\text{--}40 \times 5.0\text{--}8.0 \mu\text{m}$ ) like *H. nanospora*. However, *H. cana* differs from *H. nanospora* in that it has mouse-gray to ash-gray basidiocarps, without a cortex, and larger basidiospores ( $2.8\text{--}3\text{--}3.4$ )  $\times (1.5\text{--})1.6\text{--}1.9\text{--}2 \mu\text{m}$  [56].

#### 4.4. *Physisporinus crataegi* F. Wu, Jia J. Chen & Y.C. Dai, *mycologia* 109 (5): 751 (2017) [MB#819191]

**Basidiocarps** annual, effused-reflexed, pileate, mostly imbricate, soft when fresh, fragile when dry (Figures 1(D,E) and 2(D)). **Pileus** elongated, up to 4 cm long, 8 cm wide, and 4.5 cm thick at the base. Pileus surface zonate, margin crenate, incurved when dry, pale orange yellow (10YR, 9.5/2) to very pale brown (10YR, 8.5/2). **Pores** angular to round, 6–7 per mm, dissepiments thin, lacerate. Pore surface white (2.5Y, 8/1) to yellow (2.5Y, 8/6) when fresh, becoming dark yellowish brown (18YR, 4/6) when dry. **Context** corky, azonate, 1.2 mm thick. **Tubes** concolorous with surface of the pores, tube layers distinct, each layer up to 5 mm long. **Hyphal system** monomitic, hyphae usually septate and occasionally with clamp connections, sometimes branched, thin-walled, hyaline,  $3.5\text{--}5.5 \mu\text{m}$  in diam. **Basidia** broadly clavate, 4-spored, smooth, hyaline ( $8.0\text{--}10.5\text{--}13.5 \times 4.5\text{--}5.5 \mu\text{m}$ ) ( $n = 17$ ). **Basidiospores** ellipsoid, smooth, thin-walled, hyaline, sometimes containing a guttule,  $4.5\text{--}5.0\text{--}5.5 \times 3.0\text{--}4.0 \mu\text{m}$  [ $Q = 1.25\text{--}1.5$ ,  $Q = 1.38 \pm 0.13$ ] ( $n = 44$ ). **Hymenial cystidia** absent. **Cystidioles** present, clavate, ovoid, fusoid, smooth, thin-walled, hyaline,  $10\text{--}14.5 \times 4.5\text{--}6.5 \mu\text{m}$  ( $n = 39$ ).

##### 4.4.1. *Specimen examined*

Korea. Gwangju Metropolitan City, Mudeungsan National Park,  $35^{\circ}08'59''$  N,  $126^{\circ}58'97''$  E, mixed hardwood forest, clustered or mesic in the lower part of a broad-leaved tree stump, 3 Sep 2020, S. L. Kwon, KUC20200903-15 (NIBRFG0000511374).

##### 4.4.2. *Remarks*

*Physisporinus crataegi* KUC20200903-15 is matched well with *P. crataegi* F. Wu, Jia. J. Chen & Y.C. Dai, Wu, Chen, Ji, Vlasak & Dai in the phylogenetic analysis. Besides the presence of various cystidioles observed on the new species, microscopic characteristics of *P. crataegi* KUC20200903-15 match the original description [20]. *P. crataegi* is characterized by effused-reflexed and soft basidiocarps, cystidia absent, and ellipsoid basidiospores, and grows on broad-leaved trees in temperate forests. The resupinate region of *P. crataegi* is similar to that of *P. vitreus* in having soft basidiocarps, but *P. vitreus* has larger basidiospores ( $5\text{--}6 \times 4\text{--}5 \mu\text{m}$ ) and pores (4–6 per mm) [20]. Phylogenetically, *P. cinereus* was

identified as the most closely related species (Núñez & Ryvarden) F. Wu *et al.* However, *P. cinereus* has fibrillose and grayish pileus, large pores (5–6 per mm), and large globose-shaped basidiospores, 5–6 µm in diam [57], whereas *P. crataegi* has glabrous and cream-colored pileus with distinct pellicle, smaller pores (6–8 per mm), and broadly ellipsoid to subglobose basidiospores (4.5–5.0(–5.5) × 3.0–4.0 µm). In addition, the basidiocarp of *P. crataegi* is fragile when dry, whereas that of *P. cinereus* is woody hard [57].

#### 4.5. *Rigidoporus piceicola* (B.K. Cui & Y.C. Dai) F. Wu, Jia J. Chen & Y.C. Dai, *mycologia* 109 (5): 761 (2017) [MB#819208]

**Basidiocarps** annual, effused-reflexed, pileate, mostly imbricate, soft when fresh, becoming woody hard when dry (Figures 1(F,G) and 2(E)). **Pileus** broadly attached, up to 5 cm wide, 4 cm long, and 3.5 cm thick at the base. Pileus surface very pale brown (10YR, 8/3) to reddish yellow (7.5YR, 8/6) when fresh, very pale yellow (2.5Y, 9.5/2) to margin yellow (2.5Y, 7/8) when dry, azonate, margin sinuate. **Pores** angular to round, 4–5 per mm, dissepiments thin, entire. Pore surface white (2.5Y, 8/1) to yellow (2.5Y, 8/6). Context pale brown (2.5Y, 8/2), corky, azonate, up to 5 mm thick. Tubes concolorous with pore surface, each layer up to 1.5 mm long, tube layers distinct. **Hyphal system** monomitic, hyphae usually septate and without clamp connections, occasionally branched, thin-walled, with round apex, hyaline, 2.5–4.0 µm in diam. **Basidia** clavate, 4-spored, smooth, hyaline, 11.5–13 × 3.5–4.0 µm ( $n = 30$ ). **Basidiospores** ellipsoid, smooth, thin-walled, hyaline, sometimes containing a guttule (2.5–)3.0–4.5 × 2.0–3.0(–3.5) µm [ $Q = (1.25–)1.3–1.5$ ,  $Q = 1.4 \pm 0.15$ ] ( $n = 38$ ). **Cystidia** arising from the trama, clavate, apically encrusted with coarse crystals, thick-walled, hyaline (19–)20.5–25.5(–28) × 4.0–5.0 µm ( $n = 42$ ).

##### 4.5.1. Specimen examined

Korea. Gwangju Metropolitan City, Mudeungsan National Park, 35°07'52" N, 126°59'62" E, mixed hardwood forest, clustered or mesic in the lower part of a broad-leaved tree stump, 24 Sep 2020, S. L. Kwon, KUC20200924-53 (NIBRFG0000511373).

##### 4.5.2. Remarks

*Rigidoporus piceicola* is characterized by thick-walled and apically encrusted cystidia with coarse crystals, and ellipsoid thin-walled basidiospores. The microscopic characteristics of *Ri. piceicola* KUC20200924-53 are similar to *Ri. piceicola* (B.K. Cui & Y.C. Dai) F. Wu, Jia J. Chen & Y.C. Dai (basionym: *Oxyporus*

*piceicola*) [20,58]. However, the basidiospore size of KUC20200924-53 is smaller than previously reported [58]. Although *Ri. piceicola* is parasitic only on gymnosperm trees mostly on the genus *Picea* in the original description, it was found on a broad-leaved tree stump in this study. Based on phylogenetic analysis, *Rigidoporus populinus* (Fr.) Donk is the most closely related species. *Ri. populinus* has clavate basidia and apically encrusted cystidia, like *Ri. piceicola*. However, the former could be distinguished by subglobose and thick-walled basidiospores, and shorter cystidia (10–15 × 4.0–5.0 µm) [59]. In addition, *Ri. subpopulinus* is similar with *Ri. piceicola* by having imbricate basidiocarps and ellipsoid basidiospores, but the former has shorter basidia (7.6–12 × 5–6 µm) [60]. Because of the structural similarities in *Rigidoporus*, it is highly recommended that the DNA-based identification with combined regions, ITS, LSU, and rpb2.

#### 4.6. *Russula raoultii* Qué., *comptes rendus de l'association française pour l'avancement des sciences* 14 (2): 449 (1886) [MB#208070]

**Pileus** largely depressed center, 20 mm in diam, soft when fresh, becoming woody hard when dry; Pileus surface smooth, white (2.5Y, 9.5/1) to cream (2.5Y, 9/2), pale yellow (2.5Y, 8/2) in the center; Margin entire, rounded-obtuse, incurved. Lamellae adnate, subclose to close, pale orange yellow (10YR, 9.5/2) to very pale brown (10YR, 8/4) (Figures 1(H) and 2(F)). **Stipe** cylindrical, soft-cottony, white (2.5Y, 9.5/1), 25 × 10 mm. **Hyphal system** monomitic, hyphae occasionally septate and without clamp connections, mostly branched, thin-walled, becoming narrower toward the end with round apex, hyaline, 1.8–4.3 µm in diam. **Basidia** clavate, 4-spored, smooth, hyaline, 30–36.5 × 8.0–8.5 µm ( $n = 25$ ). **Basidiospores** broadly ellipsoid, napiform, striate with variable ridges, thin-walled, hyaline, sometimes bearing a big guttule (5.0–)5.5–6.0(–7.0) × 4.0–5.0(–5.5) µm [ $Q = (1.2–)1.25–1.27(–1.38)$ ,  $Q = 1.26 \pm 0.1$ ] ( $n = 44$ ). **Cystidia** mainly fusiform, sometimes capitate, clavate, hyphoid, smooth, thin-walled, hyaline, 25–67 × 3.3–12 µm ( $n = 39$ ).

##### 4.6.1. Specimen examined

Korea. Gwangju Metropolitan City, Mudeungsan National Park, mixed hardwood forest, 35°08'26" N, 126°58'00" E, solitary on soil covered with woody debris, 16 Jun 2020, S. L. Kwon, KUC20200616-18 (NIBRFG0000511375).

##### 4.6.2. Remarks

*Russula raoultii* KUC20200616-18 was well-matched (99%) with *Ru. raoultii* Qué. in the phylogenetic

analysis using the LSU region. For an accurate analysis, additional regions must be identified. Microscopic characteristics of *Ru. raoultii* KUC20200616-18 matched the original description [61]. However, the basidiospore of KUC20200616-18 is smaller than the size described in the original description ( $7.0\text{--}8.0 \times 5.7\text{--}7.0 \mu\text{m}$ ).

#### 4.7. *Scutellinia crinita* (bull.) Lambotte, *Mémoires de la société royale des sciences de liège, sér. 2 14 (7): 299 (1887) [MB#433534]*

**Apothecia** sessile, scattered to gregarious, discoid, undulate; Disk round, 2.5–4 mm in diam; Margin distinct, covered by blackish brown (7.5YR, 2.5/2) hairs which are stretched inward and outward (Figures 1(I) and 2(G)). **Hymenial surface** smooth, ceraceous, orange (7.5YR, 7/8) when fresh, pale yellow (5Y, 8/4) when dry. **Marginal hair** acicular, stiff, with pointed apex, 10–12 septate, thick-walled, brown (7.5YR, 4/4) to dark brown (7.5YR, 3/2),  $1000\text{--}2300 \times 24\text{--}80 \mu\text{m}$ . Internal hair similar with marginal hair, but much shorter,  $< 300 \mu\text{m}$ . **Asci** cylindrical, with round apex, 8-spored, smooth, thin-walled, hyaline,  $98\text{--}140 \times 12.5\text{--}14 \mu\text{m}$  ( $n = 28$ ). **Ascospores** ellipsoid to cylindrical, pustule-cristate, uniseriate, thin-walled, hyaline, containing two guttules,  $17\text{--}19.5(-21) \times (9.0\text{--})10\text{--}13(-13.5) \mu\text{m}$  [ $Q = (1.5\text{--})1.6\text{--}1.7(-1.9)$ ,  $Q = 1.65 \pm 0.15$ ] ( $n = 40$ ).

##### 4.7.1. Specimen examined

Korea. Gwangju Metropolitan City, Mudeungsan National Park,  $35^{\circ}07'84''$  N,  $126^{\circ}59'26''$  E, mixed hardwood forest, occurring on rotten branches, 23 Sep 2020, S. L. Kwon, KUC20200923-07 (NIBRFG000511370).

##### 4.7.2. Remarks

*Scutellinia crinita* is similar to *S. scutellata* in terms of morphological characteristics, geographic distribution, and substrate [51]. *Scutellinia crinita* KUC20200923-07 has shorter asci and longer marginal hairs than *S. scutellata* [62]. Notably, *Scutellinia* species with globose to subglobose ascospores were found in soil. However, other *Scutellinia* species with ellipsoid ascospores have been found on decayed wood. This suggests that substrates may be crucial for understanding the diversity of the genus *Scutellinia* [50].

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