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Eight Previously Unreported Species of Fungi Identified in Mt. Manggyeong, Korea

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ABSTRACT : A total of 118 fungal specimens were collected from Mt. Manggyeong, Chungnam province, Korea between July and October 2014. All specimens were identified to the species level using a combination of morphological characteristics and DNA sequencing. Out of 118 specimens, eight species, *Amanita manginiana*, *A. pilosella*, *Calvatia holothurioides*, *Cantharellus tabernensis*, *Dicephalospora rufocornea*, *Gymnopus menehune*, *Marasmius brunneospermus*, and *Russula cerolens*, have not been previously reported in Korea.

KEYWORDS : Macrofungal flora, Mt. Manggyeong, Unrecorded species

Mt. Manggyeong (600 m) is located in the southern part of Asan-si, Chungnam province, Korea, between Mt. Taehak (455 m) and Mt. Gwangdeok (699 m), which together form the administrative border between Cheonan-si and Asan-si. The valleys surrounding Mt. Manggyeong serve as the source of the Pungseocheon river, a minor tributary of the Gokgyocheon river, which together empty into Lake Sapgyo-ho. The mountain is densely forested, with conditions adequate for supporting fungal growth; however, until recently no fungal studies have been performed in this region.

To fully characterize the distribution of fungi on this mountain, regular surveys were conducted between July and October 2014. Each specimen was photographed, and details regarding the collecting site, habitat, host, substrates, and fruiting bodies of each specimen were recorded prior to collection. All collected materials were then brought to the laboratory, and dried over mild heat for several days. Dried specimens were deposited in the National Institute of Biological Resources.

Specimens were initially identified on the basis of their macro- and microscopic features according to published descriptions [1-8]. Taxonomic classification of species and the associated nomenclature were assigned using the Index Fungorum (http://www.indexfungorum.org). Measurements and drawings were made from slide preparations mounted in 3% KOH [9] using an Olympus BX51 light microscope. Size measurements were made using 20 randomly selected mature basidiospores and basidia from each specimen (Fig. 1). For molecular identification, total DNA was extracted from dried specimens using an Accu Prep genomic DNA extraction kit (Bioneer, Korea). The ITS and partial nLSU rDNA regions were amplified using primers ITS5 [10] and LR3 [11], as described by Lee and Jung [12]. DNA sequencing was performed using an ABI 3730XL sequencer (Macrogen, Seoul, Korea). The resulting nucleotide sequences were proofread and edited using the jPHYDIT program [13] and deposited in GenBank (accession Nos. KP161275~KP161282). Species identification was confirmed by comparison with GenBank reference sequences using BLAST [14]. Phylogenetic trees were inferred from sequence alignment using neighbor-joining (NJ) and maximum parsimony (MP) methods implemented in PAUP 4.0b10 (Swofford 2002). In the NJ analyses,

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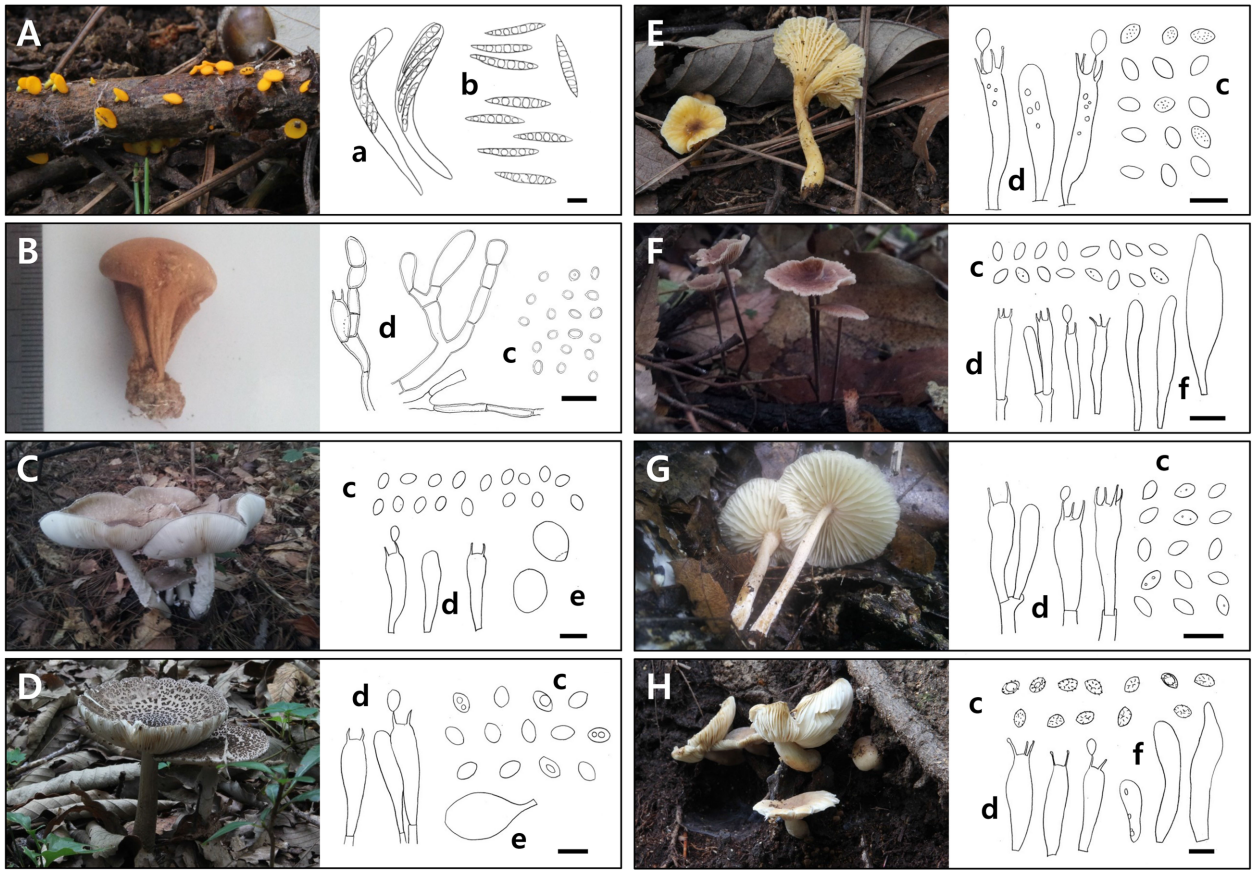

Fig. 1. Basidiocarps and microscopic features of *Dicephalospora rufocornea* (A), *Calvatia holothurioides* (B), *Amanita manginiana* (C), *A. pilosella* (D), *Cantharellus tabernensis* (E), *Marasmius brunneospermus* (F), *Gymnopus menehune* (G) and *Russula cerolens* (H). a, ascus; b, ascospores; c, basidiospores; d, basidia; e, marginal cells; f, cystidia. The scale bar is 10 μm in microscopic images.

rates for variable sites were assumed equal and no sites were assumed invariable. Data matrices were corrected using Jukes-Cantor correction. The robustness of inferred NJ topologies was tested by 1000 bootstrap replicates. MP analyses were conducted with a heuristic search method with tree bisection reconnection (TBR) branch swapping, MAXTREES set to autoincrease. A bootstrap analysis was performed with 1000 replicates, with ten random taxon addition sequences (Fig. 2).

Using the combination of morphological and phylogenetic analyses described above, 118 fungal taxa were enumerated and classified according to current taxonomies. Together, these taxa represented 29 unique families, consisting of 89 species in 51 genera. Among these samples, eight species, *Amanita manginiana*, *A. pilosella*, *Calvatia holothurioides*, *Cantharellus tabernensis*, *Dicephalospora rufocornea*, *Gymnopus menehune*, *Marasmius brunneospermus*, and *Russula cerolens*, have not been previously reported in Korea.

Taxonomy

Ascomycota Whittaker

Sclerotiniaceae Whetzel

1. *Dicephalospora rufocornea* (Berk. & Broome) Spooner, *Biblthca Mycol.* 116: 272 (1987) [16].

Stroma black, substratal. Apothecia scattered or gregarious, discoid 3-5 mm wide, disc flat to convex, yellow when flesh, becoming orange-red to dark red when dried. Asci 8-spored, subcylindrical, 100-140 × 10-13 μm. Ascospores long fusiform, 31-38 × 3-4 μm, hyaline, usually with 7-12 drops. Paraphyses filiform, 1-1.5 μm at base, slightly enlarged to 2-4 μm at tips.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected from the branch of a dead deciduous tree, 13 August 2014, JS140813-06 (GenBank accession no. KP161277).

Basidiomycota R.T. Moore

Agaricaceae Chevall

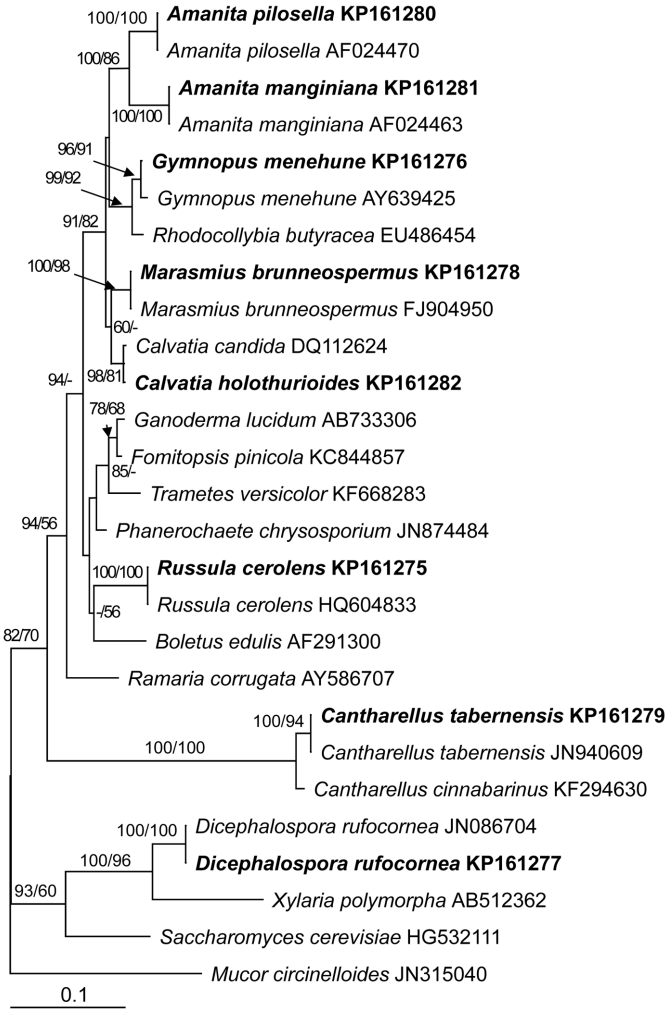

Fig. 2. Neighbor joining tree inferred from the nLSU rDNA sequences of eight species newly recorded in Korea. *Mucor circinelloides* (JN315040) was used as outgroup. Numbers before the slash are NJ bootstrap proportions, and those after the slash are MP bootstrap proportions.

2. *Calvatia holothurioides* Rebriev, Mikol. Fitopatol. 47(1): 21 (2014) [17].

Fruiting body pyriform, turbinate to broadly excipuliforme, 3.5 cm high and 2 cm diam. Exoperidium 2-layered, thin, easily detachable from the gleba, fragile, tomentosum, white later olive-brown. Gleba cottony, light yellow. Basidiospores ellipsoid to oblong-ovoid, hyaline, 3-4 × 2.5-3.5 μm, Capillitium 2-4 μm diam, thin-walled, branching, subhyaline to light brown, formed by fragile threads, septate.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected from soil in a meadow, 26 July 2014, JS140728-08 (GenBank accession no. KP161282).

Amanitaceae R. Heim ex Pouzar

3. *Amanita manginiana* (Har. & Pat.) E.-J. Gilbert, in Bresadola, Iconogr. Mycol. 27(Suppl. 1): 78 (1941) [18].

Pileus 7-8 cm wide, chestnut brown, darker in the center, convex then applanate. Flesh white to brownish. Lamellae adnate, white, broad. Stipe 5-8 cm, cylindric, stuffed, white, becoming orangish-brown. Annulus membranous, white, skirt-like. Volva membranous, limbate. Basidiospores 7-8 × 6 μm, subglobose. Basidia clavate 23-30 × 7 μm, probably lacking clamps. Marginal cells subglobose, 15-13 μm.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on soil in mixed conifer-hardwood forest, 6 August 2014, JS140806-20 (GenBank accession no. KP161281).

4. *Amanita pilosella* Corner & Bas, Persoonia 2(3): 267 (1962) [19].

Pileus 8-11 cm wide, grayish brow, darker in the center,

convex then applanate, smooth to faintly striate margin. Flesh thin cream colored. Lamellae crowded, white or grayish-white. Stipe 8-11 cm, cylindrical, stuffed, white, becoming orangish-brown. Volva membranous, limbate. Basidiospores 7-8 × 6 μm, broadly ellipsoid to ellipsoid. Basidia clavate, 35-40 × 8-10 μm, probably lacking clamps. Marginal cells subglobose, clavate, 37-15 μm.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on soil in mixed conifer-hardwood forest, 13 August 2014, JS140813-08 (GenBank accession no. KP161280).

Cantharellaceae J. Schröt.

5. *Cantharellus tabernensis* Feib. & Cibula, in Feibelman, Bennett & Cibula, Mycologia 88(2): 299 (1996) [20].

Pileus consisting of multiple cap-like structures arising from one or more single or conglomerated stem structures 1.5-5 cm long, up to 1 cm thick, fairly slender, tapering downward, brownish or yellow-brown. Fleshy becoming partially hollow at the core, pale or brownish. Lower surface smooth, shallowly wrinkled, or with broad and poorly developed false gills. Basidiospores 6-7 × 4-5 μm, smooth, more or less elliptical. Basidia cylindrical, 20-26 × 6-8 μm. Cystidia not seen.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on soil in conifer forest, 13 August 2014, JS140813-07 (GenBank accession no. KP16 1279).

Marasmiaceae Roze ex Kühner

6. *Marasmius brunneospermus* Har. Takah., Mycoscience 40(6): 477 (1999) [21].

Pileus 2-5 cm in diam, at first hemispherical-campanulate with incurved margin, then broadly convex. Surface smooth to slightly wrinkled, dull, finely velutinous. Stipe 6-7 cm, cylindrical, almost equal, smooth, shiny, apex whitish, red brown below. Lamellae adnexed broad, subdistant paler concolorous with the pileus. Basidiospore 5-6 × 2-3 μm, ellipsoid to oblong-ellipsoid, thin-walled, without germ pore. Basidia clavate, 23-25 × 3-5 μm. Cystidia fusoid-ventricose, 35-45 × 5-13 μm.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on leaf litter in mixed conifer-hardwood forest, 8 August 2014, JS140808-13 (GenBank accession no. KP161278).

Omphalotaceae Bresinsky

7. *Gymnopus menehune* Desjardin, Halling & Hemmes, Mycologia 91(1): 173 (1999) [22].

Pileus 2.5-3 cm diam., broadly convex to plano convex, margin decurved, straight to uplifted and wavy; reddish brown context very thin, concolorous with the surface. Lamellae ascending, adnate to subdecurrent, close to crowded. Stipe 3-4 cm, central, equal below or seldom gradually narrowed downward.

Basidiospores 7.5-9 × 3-4 μm, elongate-ellipsoid, smooth, hyaline. Basidia clavate, 19-24 × 6-7 μm.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on soil in mixed conifer-hardwood forest, 6 August 2014, JS140806-10 (GenBank accession no. KP161276).

Russulaceae Lotsy

8. *Russula cerolens* Shaffer, Mycologia 64(5): 1036 (1972) [23].

Pileus 4-9 cm broad, globose; margin fragile, entire to eroded, with warted striations; surface smooth, yellow-brown to light brown, Lamellae adnate, closed, brittle, and white, frequently developing brown stains. Stipe 3-5 cm tall, 1.5-2 cm thick, brittle, equal white with brownish stains, especially at the base; solid when young, becoming nearly hollow at maturity.

Basidiospore 7-8 × 5-6 μm, subglobose to elliptical, warted. Basidia clavate, 36-42 × 8-12 μm. Cystidia clavate 30-55 × 8-14 μm.

Specimen examined: Chungnam Province, Mt. Manggyeong, Korea, collected on soil in mixed conifer-hardwood forest, 13 August 2014, JS140808-13 (GenBank accession no. KP161275).

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