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RESEARCH NOTE

Eight Previously Unreported Species of Macrofungi from Korea

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Abstract

This study evaluated macrofungal specimens collected from the Gangwon, Jeonnam, and Chungnam provinces of Korea in 2015~2016. All specimens were identified at the species level using a combination of morphological characteristics and rDNA sequence data. Among them, eight macrofungal species, *Coprinopsis romagnesiana*, *Daldinia loculata*, *Gymnopus biformis*, *Gymnopus montagnei*, *Gymnopus sepiiconicus*, *Hebeloma danicum*, *Pholiota limonella*, and *Russula brevipes*, are new records for macromycota in Korea.

Keywords: Diversity, Taxonomy, Unrecorded macrofungal species

Fungi decompose organic matter and comprise a major proportion of the microbial biomass. Because of global climate change and the well-known role of fungi in biogeochemical cycles [1, 2], investigations of fungal diversity have become increasingly important. To secure, preserve, and manage genetic biological resources from Korea, a research project entitled “Survey and Discovery of Korean Indigenous Fungal Species” has been underway since 2006 with support from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment, Republic of Korea. In this study, the distribution of macrofungi in the Gangwon, Jeonnam, and Chungnam provinces of Korea was analyzed using fungal specimens collected in 2015~2016. Each specimen was photographed and details regarding the collection site, habitat, host, substrates, and fruiting bodies of each specimen were recorded prior to collection. Specimens were brought to the laboratory and dried by mild heating for several days. Dried specimens were deposited at the NIBR.

Specimens were initially identified based on their macroscopic and microscopic features according to published descriptions [3-11]. Taxonomic classification of species and associated nomenclature were assigned using the Mycobank database (http://www.mycobank.org/). Measurements and drawings were made from slide preparations mounted

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in 3% KOH [12] using a BAM-102i light microscope (MRC Lab, Holon, Israel). Twenty randomly selected mature basidiospores and basidia from each specimen were evaluated (Fig. 1). For molecular identification, total DNA was extracted from dried specimens using

Table 1. Closest GenBank matches of eight undescribed species in this study

Species	Voucher no.	GenBank accession no.	ITS rDNA		28S rDNA	
			The Closest GenBank taxa	Maximum identity (%)	The Closest GenBank taxa	Maximum identity (%)
Daldinia loculata	JS160901-49	MG407674	*Xylaria* sp.	99.1	*Daldinia loculata*	99.6
Gymnopus biformis	JS160901-64	MG407676	*Gymnopus biformis*	98.0	*Gymnopus biformis*	99.3
Gymnopus montagnei	JS160901-60	MG407677	*Gymnopus iocephalus*	98.2	*Gymnopus montagnei*	99.5
Gymnopus sepiiconicus	JS160903-08	MG407678	ND	ND	*Gymnopus sepiiconicus*	99.8
Coprinopsis romagnesiana	JS161006-01	MG407679	*Coprinopsis atramentaria*	98.7	*Coprinopsis romagnesiana*	99.8
Hebeloma danicum	JS150827-21	MG407680	*Hebeloma danicum*	99.8	*Hebeloma* sp.	99.6
Pholiota limonella	JS161006-27	MG407680	*Pholiota squarrosa-adiposa*	99.4	*Pholiota limonella*	99.8
Russula brevipes	JS160927-01	MG407682	*Russula brevipes*	99.4	*Russula* aff. *brevipes*	99.3

ITS, internal transcribed spacer; ND, no data in GenBank.

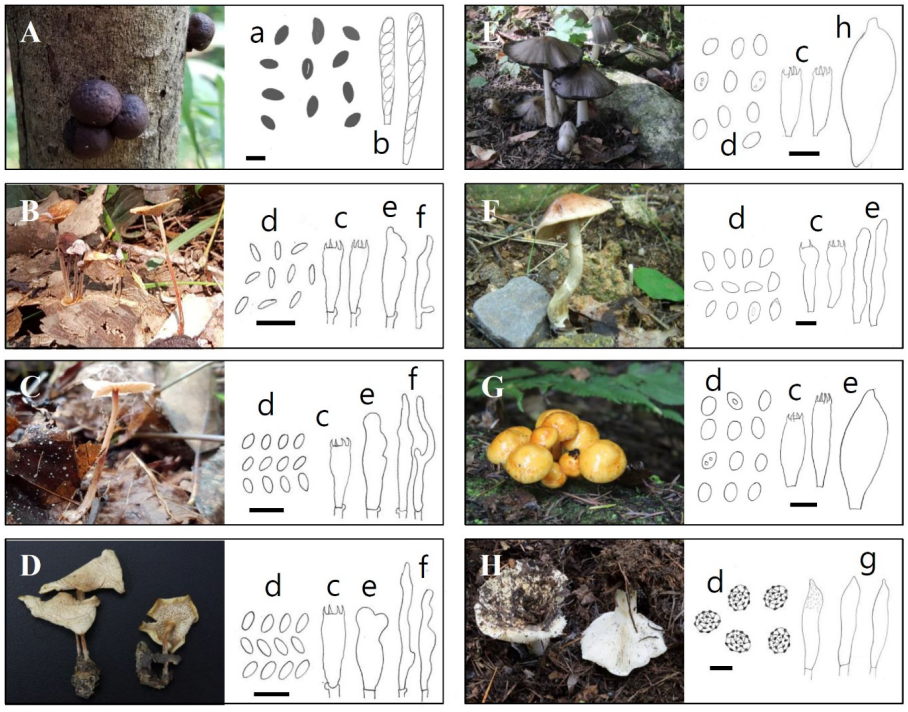

Fig. 1. Fruiting bodies and microscopic features of *Daldinia loculata* (A), *Gymnopus biformis* (B), *Gymnopus montagnei* (C), *Gymnopus sepiiconicus* (D), *Coprinopsis romagnesiana* (E), *Hebeloma danicum* (F), *Pholiota limonella* (G), *Russula brevipes* (H). a, asci; b, ascospores; c, basidia; d, basidiospores; e, cheilocystidia; f, caulocystidia; g, pleurocystidia; h, hymenial cystidia (Scale bars = 10 μm).

an AccuPrep Genomic DNA Extraction kit (Bioneer, Daejeon, Korea). Internal transcribed spacer (ITS) and partial nuclear large subumit (LSU) rDNA regions were amplified using primers specific for ITS5 [13] and LR3 [14] as described by Lee and Jung [15]. DNA sequencing was performed at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea) using the primers mentioned above and an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Resulting nucleotide sequences were edited using MEGA7 software [16] and deposited at GenBank (accession numbers: MG407674~MG407682). Species identities were confirmed by comparison with GenBank reference sequences using BLAST [17] (Table 1). A neighbor-joining (NJ) phylogenetic analysis was constructed in PAUP 4.0b10 [18] with Jukes-Cantor correction. The robustness of inferred NJ topologies was tested with 1,000 bootstrap replicates (Fig. 2). Using a combination of morphological and phylogenetic analyses described above, all fungal taxa were enumerated and classified according to current taxonomies (Figs. 1 and 2). Among these taxa, eight species (*Coprinopsis romagnesiana*, *Daldinia loculata*, *Gymnopus biformis*, *Gymnopus montagnei*, *Gymnopus sepiiconicus*, *Hebeloma danicum*, *Pholiota limonella*, and *Russula brevipes*) have not been previously reported in Korea.

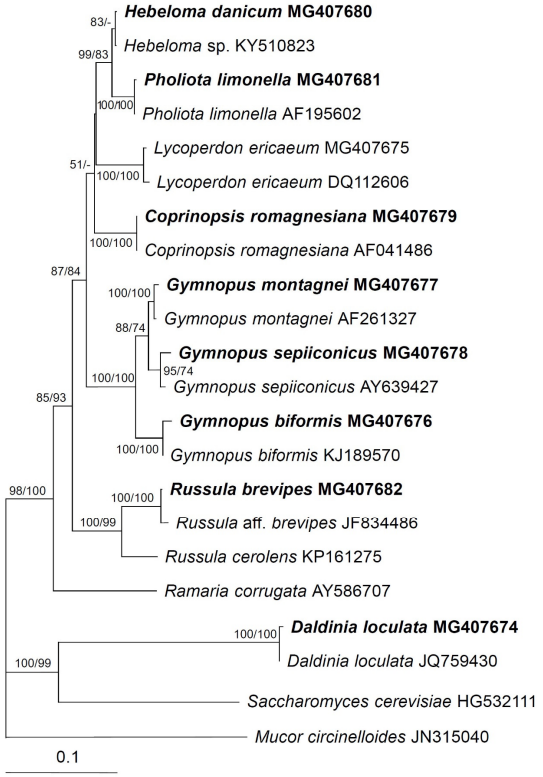

Fig. 2. Neighbor-joining (NJ) tree inferred from nuclear lgarge subunit rDNA sequences of eight macrofungal species newly recorded in Korea. *Mucor circinelloides* (JN315040) was used as an outgroup. Numbers before the slash are NJ bootstrap proportions. Those after the slash are maximum parsimony bootstrap proportions.

Taxonomy

Ascomycota Whittaker

Sordariomycetes O.E. Erikss. & Winka

Xylariales Nannf.

Xylariaceae Tul. & C. Tul.

***Daldinia loculata* (Lév.) Sacc.**, Sylloge Fungorum 1:394 (1882)

Stromata irregularly spherical, depressed-spherical to turbinate, 7 × 10 mm, sessile, solitary to gregariously. Surface smooth to undulating-tuberculate, brown or vinaceous, later black. Conspicuous concentric blackish and grayish growth zones in stroma vertical cut. Flesh tough and leathery when fresh, brittle when old. Asci at least 60~90 × 7~10 μm. Ascospores 13~14 × 6~7.5 μm, dark brown to black, unicellular, ellipsoid-inequilateral, with narrowly rounded ends and a straight germ slit.

Specimen examined: The specimen (JS160901-49, GenBank accession no. MG407674) was collected from the bark of *Betula* on September 1, 2016 at Seongdong-ri, Bukbang-myeon, Hongcheon-gun, Gangwon Province, Republic of Korea.

Remarks: *Daldinia loculata* resembles *D. concentrica* which has a large spore in the mature form. *D. loculata* species frequently occurs on *Betula*.

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Agaricales Underw.

Omphalotaceae Bresinsky

***Gymnopus biformis* (Peck) Halling**, Mycotaxon 63:363 (1997)

Pileus 1.5~2 cm in diameter, broadly convex to plano convex, shallowly sunken, sometimes with a small central bump, cinnamon brown context very thin. Flesh whitish, very thin, pliant. Surface furrowed. Lamellae whitish, ascending, adnate to subdecurrent, close to crowded. Stipe 3~5 × 0.3 cm, central, equal below or seldom gradually narrowed downward.

Basidiospores 5~6 × 2~2.3 μm, elongate-ellipsoid, smooth, hyaline. Basidia 19~23 × 4.5~6 μm, clavate, 4-spored. Pleurocystidia absent. Cheilocystidia abundant cylindrical, 20~40 μm in length. Caulocystidia 30~35 × 4~6 μm, cylindrical, clavate slightly irregular. Clamp connections abundant in all tissues.

Specimen examined: The specimen (JS160901-64, GenBank accession no. MG407676) was collected from soil in a mixed forest on September 1, 2016 at Seongdong-ri, Bukbang-myeon, Hongcheon-gun, Gangwon Province, Republic of Korea.

Remarks: *Gymnopus subnudus* is similar to *G. biformis* in pileus color and texture but differs by widely spaced lamellae, and grows on sticks, leaves, and humus. *G. biformis* typically grows directly in soil.

***Gymnopus montagnei* (Berk.) Redhead**, Index Fungorum 148:1 (2014)

Pileus thin fleshed, 3 cm wide, broadly convex to plane, with broad central bump, cinnamon to reddish brown. Surface concolorous. Lamellae white to pinkish buff, attached or almost free, close to crowded. Flesh whitish, very thin, pliant. Stipe 6 × 0.4 cm, pale pinkish-cinnamon, central, equal below, hollow.

Basidiospores 4.6~5.0 × 3.4~3.8 μm, elongate-ellipsoid, smooth, hyaline, thin-walled. Basidia 19 × 4 μm. Pleurocystidia absent. Cheilocystidia 15~25 × 4~5 μm, irregular, with projections or coralloid. Caulocystidia 20~30 × 3~5 μm, cylindrical, clavate, subfusoid, regular or slightly irregular.

Specimen examined: The specimen (JS160901-60, GenBank accession no. MG407677) was collected from organic compost in a mixed forest on September 1, 2016 at Seongdong-ri, Bukbang-myeon, Hongcheon-gun, Gangwon Province, Republic of Korea.

Remarks: *Gymnopus montagnei* is known as a lignicolous species which typically produces numerous pileus, mostly on branches and sticks of dead deciduous trees.

***Gymnopus sepiiconicus* (Corner) A.W. Wilson**, Desjardin & E. Horak, Sydowia 56 (1):163 (2004)

Pileus 1.5~2 cm in diameter, broadly convex to plano convex, margin decurved, straight to uplifted and wavy, reddish brown context very thin, concolorous with surface. Lamellae ascending, adnate to subdecurrent, close to crowded. Stipe 3~5 × 0.3 cm, central, equal below or seldom gradually narrowed downward.

Basidiospores 5.5~6 × 3.5~3.8 μm, elongate-ellipsoid, smooth, hyaline. Basidia 19~22 × 6~7 μm. Cheilocystidia 19~35 × 4~6 μm, irregular, with projections or coralloid. Caulocystidia 22~40 × 5~7 μm, cylindrical, clavate, subfusoid, regular or slightly irregular.

Specimen examined: The specimen (JS160903-08, GenBank accession no. MG407678) was collected from old fallen and partially decayed leaves in a mixed forest on September 3, 2016 at Mt. Manggyeongsan, Baebang-eup, Asan-si, Chungnam Province, Republic of Korea.

Remarks: *Gymnopus dryophilus* is similar to *G. sepiiconicus* in pileus color and size but differs by hairy stipe. *G. sepiiconicus* has smooth stipe and is scattered in fallen decayed leaves.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

***Coprinopsis romagnesiana* (Singer) Redhead**, Vilgalys & Moncalvo, Taxon 50 (1):230 (2001)

Pileus 8~10 cm across, oval when young, expanding to bell-shaped or broadly convex, whitish to purplish-brown, with orangish brown scales. Lamellae attached or free, white at first, becoming purplish black, eventually deliquescing, crowded. Stipe 5~10 × 0.8~1.5 cm long, relatively equal, white, hollow, sometimes with a slightly rimmed basal bulb.

Basidiospores 9.2~10 × 6.2~7 μm, elliptical, smooth. Basidia 30 × 8 μm, 4-spored, surrounded by brachybasidia. Hymenial cystidia cylindric to utriform, up to about 75 μm long. Clamp connections present.

Specimen examined: The specimen (JS161006-01, GenBank accession no. MG407679) was collected from organic compost in a mixed forest on October 1, 2016 at Mt. Odaesan, Pyeongchang-gun, Gangwon Province, Republic of Korea.

Remarks: *Coprinopsis romagnesiana* is closely related to C. atramentaria. However, *C. romagnesiana* is decorated with orangish brown scales on the pileus and stipe.

Strophariaceae Singer & A.H. Sm.

***Hebeloma danicum* Gröger**, Zeitschrift für Mykologie 53:53 (1987)

Pileus 4 cm broad, conic with margin slightly incurved, later expanding and becoming convex or plane, smooth. Surface viscid to glutinous, reddish brown to buff at pileus center, becoming pale buff to cream toward margin. Flesh 1.5~2 cm thick at the disc, white or yellowish. Lamellae adnate or sinuate, very crowded, 0.3~0.5 cm broad, whitish when young, becoming light brown. Stipe 6 cm long above ground level, 0.7~0.9 cm thick at apex, equal or slightly attenuated upward from thickened and long-rooting, yellowish white to pale yellow, pruinose-squamulose at apex, with often recurved brownish scales, solid to stuffed. Veil absent.

Basidiospores white or brownish, 9~10 × 4.5~5.5 μm, elliptical, finely punctate (tuberculate). Basidia 28~30 × 8~12 μm, 4-spored. Cheilocystidia 50 × 10 μm, numerous, clavate, hyaline, thin-walled.

Specimen examined: The specimen (JS150827-21, GenBank accession no. MG407680) was collected from sandy or gravelly soil in a mixed forest on August 27, 2015 at Mt. Wolchulsan, Yeonggwang-gun, Jeonnam Province, Republic of Korea.

Remarks: *Hebeloma danicum* is easily identified by its reddish brown to buff pileus, ellipsoidal punctate spores, and long solid stipe.

***Pholiota limonella* (Peck) Sacc.**, Sylloge Fungorum 5:753 (1887)

Pileus 3~13 cm, convex, becoming broadly convex, broadly bell-shaped. Surface sticky or slimy, orange-yellow to orange colored, covered with brownish to reddish brown scales that rapidly scattered and often gelatinized. Lamellae attached to stem, close, whitish to yellowish when young, becoming rusty brown, initially covered by a whitish to yellowish partial veil. Stipe 3~4 cm long, up to 0.6 cm thick, silky near apex, with reddish brown to brownish scales that may be scattered or densely packed. Base sometimes covered with whitish mycelium.

Basidiospores 7~8.5 × 4~4.5 μm, smooth, relatively elliptical. Basidia clavate to clavate-mucronate, up to 25 × 13 μm. Cheilocystidia variously shaped, up to 45 × 16 μm.

Clamp connections present.

Specimen examined: The specimen (JS161006-27, GenBank accession no. MG407681) was collected from mossy soil in a conifer forest on October 6, 2016 at Mt. Manggyeongsan, Mt. Odaesan, Pyeongchang-gun, Gangwon Province, Republic of Korea.

Remarks: *Pholiota limonella* is very closely related to *P. aurivella* and *P. adiposa* species. However, *P. limonella* is distinguished based on their spores. The size of *P. limomella* (7~8.5 × 4~4.5 μm) is larger than those of *P. aurivella* (6.0~7.5 × 4.0~4.5 μm) and *P. adiposa* (5~6.5 × 3.5~4 μm).

Russulaceae Lotsy

***Russula brevipes* Peck**, Annual Report on the New York State Museum of Natural History 43:66, t. 2:5－8 (1890)

Pileus 7~10 cm broad, convex with a central depression and an inrolled margin when young, later broadly convex with a central depression and eventually shallowly vase-shaped, margin remaining somewhat inrolled or straightening, initially white to whitish or creamy, becoming brownish to orangish. Lamellae adnate, closed, brittle, and white, frequently developing creamy. Stipe 3~5 cm tall, 1~2 cm thick, brittle, equal white with brownish or bluish stains, particularly at the base, solid when young, becoming nearly hollow at maturity, Veil absent.

Basidiospore 10~12 × 10~11 μm, subglobose to subovoid to subellipsoid, warted. Ornamentation typically approximately 0.5 μm high. Pleurocystidia 46~54 × 10 μm, fusiform, cylindric, or subclavate.

Specimen examined: The specimen (JS160927-01, GenBank accession no. MG407682) was collected from soil in a mixed forest on September 1, 2016 at Nocheon-ri, Dong-myeon, Hongcheon-gun, Gangwon Province, Republic of Korea.

Remarks: *Russula brevipes* is most frequently found in mixed woods with sandy soil and easily identified by its shape, large dimension, and white coloration.

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