Twelve Undescribed Species of Macrofungi from Korea

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ABSTRACT : A survey of the indigenous fungal resources of Korea was undertaken during 2014-2015. All specimens collected in this study were identified at the species level, based on their morphological characteristics and rDNA sequence data. Among them, 12 macrofungal species, viz. *Agaricus guizhouensis, Amanita orientifulva, Armillaria cepistipes, Crepidotus inhonestus, Daldinia childiae, Elmerina cladophora, Lycoperdon scabrum, Marasmius brunneoaurantiacus, Otidea bufonia, Pluteus hongoi, Pluteus variabilicolor, and Russula grisea have not been previously reported in Korea.*

KEYWORDS : Diversity, Macrofungi, Undescribed species

Introduction

Macrofungi usually called mushrooms are known to be one of the most important constituents of the forest ecosystem with forest trees. Most of them are in temperate regions and constitute a significant part of terrestrial ecosystems. Their edibility, poisonous nature, and medicinal value, have made them economically, biotechnologically, and medically important [1]. The study of fungal biodiversity has been carried out the world over and so far only 6.7% of 1.5 million species of fungi estimated in the world have been described [2], while 4,686 species of fungi are recorded in Korea by 2015 (National Institute of Biological Resources, https://species.nibr.go.kr). To secure, preserve, and manage the genetic biological resources in Korea, a research project entitled survey and discovery of Korean indigenous fungal species has been performed by aid of

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National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea since 2006.

Materials and Methods

In this study, the distribution of macrofungi at Chungnam, Chungbuk and Jeonnam Provinces, Korea was investigated by analyzing fungal specimens collected during from August 2014 to October 2015. 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 then brought to the laboratory and dried over mild heat for several days. Dried specimens were deposited in the NIBR.

Taxonomic classification of species and the associated nomenclature were assigned using the Index Fungorum database (http://www.index-fungorum.org). Measurements and drawings were made from slide preparations mounted in 3% KOH [3] using a BAM-102i light microscope (MRC Lab, Holon, Israel). Twenty randomly selected mature basidiospores and basidia from each specimen were measured. For molecular identification, total DNA was extracted from dried specimens using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The internal transcribed spacer (ITS) and partial nLSU rDNA regions were amplified using primers ITS5 [4] and LR3 [5], as described by Lee and Jung [6]. DNA sequencing was performed at the DNA Synthesis and Sequencing

234 Jin Sung Lee, Sun Young Choi, Changmu Kim and Hyang Bum Lee



Fig. 1. Basidiocarps and microscopic features of *Otidea bufonia* (A), *Daldinia childiae* (B), *Agaricus guizhouensis* (C), *Lycoperdon scabrum* (D), *Amanita orientifulva* (E), *Crepidotus inhonestus* (F), *Marasmius brunneoaurantiacus* (G), *Armillaria cepistipes* (H), *Pluteus hongoi* (I), *Pluteus variabilicolor* (J), *Russula grisea* (K), and *Elmerina cladophora* (L). a, asci; b, ascospores; c, paraphyses; d, basidia; e, basidiospores; f, pileipellis; g, generative hyphae; h, skeletal hyphae; i, cheilocystidia; j, pleurocystidia; k, caulocystidia (scale bar = 10 μm).

Facility, Macrogen (Seoul, Korea), using the aforementioned primers and an ABI 3730XL DNA Analyzer. The resulting nucleotide sequences were edited using jPHYDIT [7] and deposited in GenBank (accession nos. KX963782 ~KX963793). Specimens were initially identified on the basis of their macro- and microscopic features according to published descriptions (Fig. 1) [8-16].

Results and Discussion

Species identities were confirmed by comparison with GenBank reference sequences using BLASTn (Table 1) [17]. A Neighbor-joining (NJ) phylogenetic analysis was implemented in PAUP 4.0b10 [18] with a Jukes-Cantor correction. The robustness of inferred NJ topologies was

Species	Voucher No.	GenBank - accession No.	ITS rDNA		28S rDNA	
			The Closest	Maximum	The Closest	Maximum
			GenBank taxa	identity (%)	GenBank taxa	identity (%)
Otidea bufonia	JS150904-08	KX963782	Ectomycorrhizal fungus	99.8	Otidea bufonia	99.4
Daldinia childiae	JS150718-02	KX963783	Daldinia childiae	100	Daldinia cf. loculatoides	98.9
Agaricus guizhouensis	JS140903-06	KX963784	Agaricus guizhouensis	100	Agaricussp. GAL15446	100
Lycoperdon scabrum	JS150715-20	KX963785	Lycoperdon scabrum	98.7	Lycoperdon scabrum	99.5
Amanita orientifulva	JS150904-44	KX963786	Amanita fulva	93.1	Amanita orientifulva	99.6
Crepidotus inhonestus	JS150715-11	KX963787	Crepidotus sphaerosporus	97.5	Crepidotus inhonestus	99.6
Marasmius brunneoaurantiacus	JS150904-16-1	KX963788	Marasmius bambusiniformis	94.0	Marasmius brunneoaurantiacus	99.8
Armillaria cepistipes	JS140921-54	KX963789	Armillaria cepistipes	99.8	Armillaria ostoyae	99.7
Pluteus hongoi	JS150709-14	KX963790	Pluteus hongoi	99.8	Pluteus cervinus	99.7
Pluteus variabilicolor	JS150827-19	KX963791	Pluteus variabilicolor	100	Pluteus umbrosus	98.4
Russula grisea	JS140806-24	KX963792	Russula vesca	99.2	Russula grisea	100
Elmerina cladophora	JS140903-22-1	KX963793	Elmerina cladophora	99.8	Elmerina cladophora	99.8

Table 1. Closest GenBank matches of 12 undescribed species in this study

ITS, internal transcribed spacer.

tested with 1,000 bootstrap replicates (Fig. 2).

Using a combination of the morphological and phylogenetic analyses described above, all fungal taxa were enumerated and classified according to current taxonomies. Among these taxa, twelve species including *Agaricus guizhouensis*, *Amanita orientifulva*, *Armillaria cepistipes*, *Crepidotus inhonestus*, *Daldinia childiae*, *Elmerina cladophora*, *Lycoperdon scabrum*, *Marasmius brunneoaurantiacus*, *Otidea bufonia*, *Pluteus hongoi*, *Pluteus variabilicolor*, and *Russula grisea* have not been previously reported in Korea.

Taxonomy Ascomycota Whittaker Pezizomycetes O.E. Erikss. & Winka Pezizales J. Schröt. Pyronemataceae Corda 1. Otidea bufonia (Pers.) Boud., Histoire et Classification des Discomycètes d'Europe: 52 (1907) [19]

The cups are 3~4 cm tall and split on one side, with an irregularly wavy, dark brown inner surface and outer surface that is slightly whitish to ochre and smooth to furfuraceous. The flesh is thin and yellowish to light brown.

Asci are $85\sim100 \times 9\sim11 \mu m$ and eight-spored. Ascospores are $11\sim13 \times 7\sim8 \mu m$, elliptic-fusiform, smooth, and contain two oil drops. Paraphyses are slender and strongly curved at the tip.

Specimen examined: The specimens was collected on Mt. Taehak, Cheonan-si, Chungnam Province, Korea, from

the ground of a broad-leaved forest, 4th September 2015, JS150904-08 (GenBank accession no. KX963782).

Sordariomycetes O.E. Erikss. & Winka

Xylariales Nannf.

Xylariaceae Tul. & C. Tul.

2. *Daldinia childiae* J.D. Rogers & Y.M. Ju, *Mycotaxon* 72: 512 (1999) [20].

Stromata are irregular and spherical, depressed-spherical to turbinate, 0.9×1.1 cm in size, sessile, solitary, and smooth, with either inconspicuous or conspicuous perithecial mounds. The surface is brown or vinaceous, and later become black. If the stroma is cut vertically, it shows conspicuous concentric blackish and grayish growth zones.

Asci are at least 70 \times 7~8 µm. Ascospores are 6~7 \times 5~6 µm, dark brown to black, unicellular, and ellipsoidinequilateral, with narrowly rounded ends and a straight germ slit that is spore-length on the convex side.

Specimen examined: The specimen was collected on Mt. Weolbong, Asan-si, Chungnam Province, Korea, from the branch of a dead deciduous tree, 18th July 2015, JS 150718-02 (GenBank accession no. KX963783).

Basidiomycota R.T. Moore
Agaricomycetes Doweld
Agaricales Underw.
Agaricaceae Chevall
3. Agaricus guizhouensis Y. Gui, Zuo Y. Liu, K.D. Hyde,



Fig. 2. Neighbor-joining (NJ) tree inferred from the nLSU rDNA sequences of 12 macrofungal species newly recorded in Korea. *Mucor circinelloides* (JN315040) was used as an outgroup. Numbers before the slash are NJ bootstrap proportions, and those after the slash are MP bootstrap proportions.

Fungal Biology 119: 83 (2015) [21]

The pileus is $5.2 \sim 12$ cm in diameter, $0.5 \sim 0.8$ cm thick, convex to hemispherical, and applanate. The surface is smooth and white or covered with pastel yellow to greenish-grey appressed patches that are irregularly shaped. Lamellae are up to $0.4 \sim 0.6$ cm wide, free, crowded, and brown to brownish-black. The stipe is $9 \sim 15 \times 1 \sim 1.6$ cm, hollow in the center, and cylindrical, with many rhizomorphs. The surface is white and smooth or fibrillose. The context is white, turning yellowish-white or yellow, then brownish-red or pastel yellow, finally yellowish-green.

Basidia are $30 \sim 35 \times 7 \sim 10 \mu m$, clavate. Basidiospores $8 \sim 10 \times 4 \sim 5 \mu m$ and elongate-ellipsoid, with a prominent oblique apiculus, attenuated at the apex, smooth, thick-walled, and brown. Pileipellis are $60 \sim 70 \times 4 \sim 5 \mu m$, cylin-

drical and hyaline.

Specimen examined: The specimen was collected on Mt. Songni, Boeun-gun, Chungbuk Province, Korea, from leaf litter in a mixed forest, 3rd September 2014, JS140903-30-1 (GenBank accession no. KX963784).

4. *Lycoperdon scabrum* Willd., *Florae Berolinensis Prodromus*: 409 (1787) [22]

The fruiting body is pyriform, with a prominent pale cream base that is $26 \times 3 \sim 6$ cm and glabrous, with dark sepia spines on the upper surface that are $1\sim3$ mm long and that can be rubbed off. The stipe is white to pale cream, 15×20 mm, and more or less cylindrical, with rooting rhizomorphs at the base. The endoperidium is umber, smooth, and somewhat shiny. The gleba is a cottony or fibrous mass containing spores that olivaceous brown when mature.

Basidospores are $4 \sim 6 \times 4 \sim 5 \mu m$ and globose to subglobose, with a long pedicel.

Specimen examined: The specimen was collected on Mt. Bueong, Asan-si, Chungnam Province, Korea, from soil in a mixed forest, 15th July 2015, JS150715-20 (Gen-Bank accession no. KX963785).

Amanitaceae R. Heim ex Pouzar

5. Amanita orientifulva Zhu L. Yang, M. Weiss & Oberw., Mycologia 96(3): 643 (2004) [23]

The pileus is 4~9 cm in diameter, at first hemispherical, then convex to plano-convex and yellowish-brown to ochreous. The margin is tuberculate-striate, dirty white to cream-colored. Lamellae free, crowded, white to creamcolored. The stipe is cylindrical, 7~8 cm in length, tapering upward, and is dirty white to brownish, with squamules. The context is white and hollow in the center. The annulus is absent.

Generative hyphae are $6~7 \mu m$ in width, thin-walled, and hyaline. Basidia are $40~45 \times 13~15 \mu m$, clavate, with a 4-spored sterigmata and basal septa without clamps. Basidiospores are $10~12 \times 9~11 \mu m$, globose to subglobose, colorless, hyaline, thin-walled, and smooth, with a small apiculus.

Specimen examined: The specimen was collected on Mt. Taehak, Cheonan-si, Chungnam Province, Korea, from soil in a mixed forest, 4th September 2015, JS140806-20 (GenBank accession no. KX963786).

Inocybaceae J. Schröt.

6. Crepidotus inhonestus P. Karst., Meddn Soc. Fauna

Flora fenn. 13: 160 (1886) [24]

The pileus consists of multiple cap-like structures arising from one or that are gregarious, and is $1\sim2$ µm and laterally or centrally attached to the substrate without a stipe. The surface is smooth, dull, and white to creamyellow. The margin is acute and even. The fruit body is fleshy, becoming partially hollow at the core, and pale or brownish. The lower surface is smooth and shallowly wrinkled or has broad and poorly developed false gills.

The basidia are cylindrical and $20~25 \times 7~8 \mu$ m, with 4-spored sterigmata. Cystidia are not seen. Basidiospores are $6~7 \times 4~5 \mu$ m, smooth, and more or less elliptical.

Specimen examined: The specimen was collected on Mt. Weolbong, Asan-si, Chungnam Province, Korea, from the branch of a dead deciduous tree, 15th July 2015, JS 150715-11 (GenBank accession no. KX963787).

Marasmiaceae Roze ex Kühner

7. *Marasmius brunneoaurantiacus* Antonín & Buyck, Fungal Diversity 23: 24 (2006) [25].

The pileus is 6~7 mm in diameter, at first hemispherical-campanulate with an incurved margin, then broadly convex. The surface is smooth to slightly wrinkled, dull, and finely velutinous. The stipe is $1~10 \times 0.1~0.2$ cm, cylindrical, almost equal, smooth, and shiny, with an apex that is whitish, and red brown below. The lamellae are adnexed, broad, subdistant, and pale, nearly concolorous with the pileus.

The basidia are $25 \sim 30 \times 7 \sim 9 \mu m$ and clavate. Basidiospores are $7 \sim 8 \times 4 \sim 5 \mu m$, ellipsoid to oblong-ellipsoid, and thin-walled, without a germ pore. The cheilocystidia are $20 \sim 25 \times 9 \sim 11 \mu m$, and pleurocystidia are $50 \sim 95 \times 5 \sim 13.5 \mu m$ and fusoid-ventricose.

Specimen examined: The specimen was collected on Mt. Weolbong, Asan-si, Chungnam Province, Korea, from leaf litter in a mixed conifer-hardwood forest, 4th September 2015, JS150904-16-1 (GenBank accession no. KX 963788).

Physalacriaceae Bresinsky

8. Armillaria cepistipes Velen., Ceske Houby 2: 283 (1920) [26].

The pileus is $1\sim3$ cm in diameter, broadly convex to plano-convex, with a margin that is decurved and even to uplifted and wavy, with a reddish brown context that is very thin and concolorous with the surface. The lamellae are ascending, adnate to subdecurrent, and close to crowded. The stipe is $20\sim30 \times 4\sim10$ mm, central, and uniform below or sometimes gradually narrowing.

Basidia are $18 \sim 22 \times 6 \sim 7 \mu m$ and rather narrowly clavae and clamped. Cheilocystidia are $15 \sim 25 \times 4 \sim 6 \mu m$ and clavate. Caulocystidia are $40 \sim 60 \times 5 \sim 7 \mu m$, irregularly cylindrical to sinuous. Basidiospores are $4 \sim 6 \times 3 \sim 4 \mu m$, broadly ellipsoid to subglobose, smooth, and hyaline. Clamp connections are abundant in all tissues.

Specimen examined: The specimen was collected on Mt. Songni, Boeun-gun, Chungbuk Province, Korea, from buried wood remnants of a coniferous tree, 21st September 2014, JS140921-54 (GenBank accession no. KX963789).

Pluteaceae Kotl. & Pouzar

9. Pluteus hongoi Singer, Fieldiana Botany 21: 95 (1989) [27].

The pileus is 9 cm in diameter, hemispherical or campanulate when young, and expands to convex or planoconvex. The surface is smooth to innately radially fibrillose. The margin is smooth or slightly translucently striate. Lamellae are crowded, free, and ventricose. The stipe is 110×7 mm and cylindrical, with a slightly broad base. The surface is white, sometimes with a yellowish tint near the base, and is usually smooth.

Basidia are $30 \sim 35 \times 6 \sim 8 \ \mu\text{m}$ and clavate. Basidiospores are $5.5 \sim 6.5 \times 5 \sim 6 \ \mu\text{m}$ and ellipsoid or broadly ellipsoid. Pleurocystidia are metuloid, $18 \sim 20 \times 8 \sim 12 \ \mu\text{m}$, and fusiform. The pileipellis is $40 \sim 45 \times 7 \sim 10 \ \mu\text{m}$. It is solitary.

Specimen examined: The specimen was collected on Mt. Weolbong, Asan-si, Chungnam Province, Korea, from the humus layer of soil in a mixed forest, 14th July 2015, JS150714-14 (GenBank accession no. KX963790).

10. *Pluteus variabilicolor* Babos, *Annales Historico-Natureles Musei Nationalis Hungarici* 70: 93 (1978) [28].

The pileus is $5 \sim 7$ cm wide, smooth, and yellowishorange, with a darker central umbo, clearly striate or not striate at the margin, with well-defined squamules. Lamellae are free, quite crowded, ventricose, and yellowishorange. The stipe is $40 \sim 70 \times 412$ mm, cylindrical, slightly enlarged at the base, streaked-fibrillose over the entire length, and yellow, with reddish tinges at the base of mature specimens. The context is white-yellowish or yellowishorange under the pileus surface.

Basidia are $20 \sim 30 \times 5 \sim 7 \mu m$ and clavate. Pleurocystidia are lageniform and $60 \sim 70 \times 30 \sim 40 \mu m$. The pileipellis is a hymeniderm consisting of clavate, rounded terminal elements and cylindrical, elongated cells that are $70 \sim 90 \times 7 \sim$ $10 \mu m$. Caulocystidia are present over the whole length of the stipe, $45 \sim 50 \times 8 \sim 10 \mu$ m, cylindrical to claviform, and fusiform. Basidiospores are $5.5 \sim 7.0 \times 4.5 \sim 6.0 \mu$ m, broadly ellipsoid to subglobose, and thin-walled.

Specimen examined: The specimen was collected on Mt. Wolchul, Yeonggwang-eup, Jungnam Province, Korea, from a deciduous tree, 27th August 2015, JS150827-19 (GenBank accession no. KX963791).

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

Russulaceae Lotsy

11. Russula grisea Fr., Epicrisis Systematis Mycologici: 361 (1838) [29].

The pileus is $4{\sim}11$ cm wide and globose. The margin is fragile and whole to eroded, with warted striations. The surface is smooth and yellow-brown to light brown. Lamellae are adnate, closed, brittle, and white, frequently developing brown stains. The stipe is $3{\sim}5$ cm tall, $1{\sim}2$ cm thick, brittle, evenly white with brownish stains, especially at the base, and solid when young, but becoming nearly hollow at maturity. The veil is absent.

Basidia are $40 \sim 45 \times 6 \sim 7 \mu m$, narrowly clavate. Basidiospores are $7 \sim 8 \times 5 \sim 6 \mu m$, subglobose to elliptical, and warted. Pleurocystidia are $65 \sim 70 \times 8 \sim 10 \mu m$, cylindrical to fusoid,

Specimen examined: The specimen was collected on Mt. Mangyeong, Asan-si, Chungnam Province, Korea, from soil in a mixed conifer-hardwood forest, 6th August 2014, JS140806-24 (GenBank accession no. KX963792).

Tremellomycetes Doweld

Tremellales Fr.

Aporpiaceae Bondartsev & Bondartseva

12. Elmerina cladophora (Berk.) Bres., Annales Mycologici 10(5): 507 (1912) [30]

The basidiocarps are 2×3 cm, annual, and resupinate. Hymenophores are poroid, corky, cream, pinkish-buff, and curry yellow when dry. Pores are $4\sim5$ cm and circular, angular, or hexagonal. Dissepiments are whole and can be thin or thick.

The hyphal system is dimitic. Generative hyphae are $2\sim$ 3 µm in width, thin-walled, and hyaline. Skeletal hyphae are $3\sim5$ µm, are thick-walled and hyaline. Basidia are 20 $\sim25 \times 10\sim12$ µm, thin-walled, hyaline, vertically septate, and mostly clavate. Basidiospores are $9\sim12 \times 5\sim6$ µm, thin-walled, hyaline, allantoid to ellipsoid.

Specimen examined: The specimen was collected on Mt. Songni, Boeun-gun, Chungbuk Province, Korea, from the trunk of a dead *Quercus variabilis*, 3rd September

2014, JS140903-22-1 (GenBank accession no. KX963793).

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Twelve Undescribed Species of Macrofungi from Korea 239

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