

RESEARCH NOTE

Six New Recorded Species of Macrofungi on Gayasan National Park in Korea

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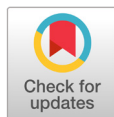
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ABSTRACT

The fungi play important roles in maintaining the balance and homeostasis of natural ecosystems. We investigated fungal specimens indigenous to the Gayasan National Park located in the deep inlands of southeastern Korea from 2017-2020. Six fungal species in the Korean macromycota—*Chiuva olivaceoreticulata*, *Entoloma conchatum*, *Galerina sulciceps*, *Hebeloma radicosoides*, *Spongiporus gloeoporus*, and *Tricholoma sinoacerbum*—were identified based on morphological characteristics and rDNA sequences. The six fungal species were newly revealed on the Korean Peninsula, and it supports that the continuous investigation is the best way to realize the mycosis.

Keywords: Gayasan National Park, Macrofungal flora, Unrecorded species



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Gayasan National Park is located in the deep inlands of southeastern Korea and covers an area of 77 km². It borders on Hapcheon-gun and Geochang-gun of Gyeongsangnam-do, and Seongju-gun of Gyeongsangbuk-do. The National Institute of Biological Resources surveyed the Gayasan National Park 31 times over 4 years from 2017 to 2020 and collected about 1,000 mushroom specimens. Initially, we referred to published descriptions to identify each specimen on the basis of its macroscopic and microscopic features [1-10]. Measurements and drawings were made using a Nikon eclipse 80i microscope (Nikon, Tokyo, Japan). Moreover, from each specimen 20 basidiospores and basidia were randomly evaluated (Fig. 1). To identify the specimens at the molecular level, total DNA was extracted from dried specimens using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). Subsequently, DNA was amplified and sequenced at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea), using primers specific for the internal transcribed spacers (ITS) ITS1F and ITS4B [11]. The sequenced DNA were edited using the MEGA 5 software [12] and deposited at GenBank (accession numbers: MZ854164-MZ854166, Z854348-MZ854353). Identities of the fungal species were confirmed by comparing their

sequences with GenBank reference sequences using BLASTn (Table 1). A neighbor-joining phylogenetic tree was constructed using the MEGA 5 software with Jukes–Cantor correction. Additionally, the robustness of inferred neighbor-joining topologies was tested with 1,000 bootstrap replicates (Fig. 2). Subsequently, all fungal species were enumerated and taxonomically classified by morphological and phylogenetic analyses. The Mycobank database (<http://www.mycobank.org/>) was used to classify the species into taxa and assign them a nomenclature.

Table 1. Closest GenBank matches of 6 undescribed species in this study.

Species	Voucher No.	Gene	Genbank accession No.	The closest GenBank Taxa	Similarity (%)
<i>Chiuva olivaceoreticulata</i>	NIBRFG0000508552	LSU rDNA	MZ854164	<i>Chiuva olivaceoreticulata</i>	100.0
	NIBRFG0000508549	LSU rDNA	MZ854165	<i>Chiuva olivaceoreticulata</i>	99.8
	NIBRFG0000508514	LSU rDNA	MZ854166	<i>Chiuva olivaceoreticulata</i>	99.8
<i>Entoloma conchatum</i>	NIBRFG0000508463	ITS rDNA	MZ870348	<i>Entoloma conchatum</i>	100.0
<i>Galerina sulciceps</i>	NIBRFG0000507914	ITS rDNA	MZ870349	<i>Galerina sulciceps</i>	100.0
<i>Hebeloma radicosoides</i>	NIBRFG0000508444	ITS rDNA	MZ870350	<i>Hebeloma radicosoides</i>	100.0
<i>Spongiporus gloeoporu</i>	NIBRFG0000509343	ITS rDNA	MZ870351	<i>Spongiporus gloeoporu</i>	100.0
<i>Tricholoma sinoacercbum</i>	NIBRFG0000508511	ITS rDNA	MZ870352	<i>Tricholoma sinoacercbum</i>	100.0
	NIBRFG0000508519	ITS rDNA	MZ870353	<i>Tricholoma sinoacercbum</i>	100.0

LSU, large subunit ; ITS, internal transcribed spacers.



Fig. 1. Fruiting bodies and microscopic features of (A) *Chiuva olivaceoreticulata*, (B) *Entoloma conchatum*, (C) *Galerina sulciceps*, (D) *Hebeloma radicosoides*, (E) *Spongiporus gloeoporus*, and (F) *Tricholoma sinoacercbum*. Scale bar: 1 cm. a, basidiospores; b, basidia and basidioles; c, cystidia; d, gelatinous hyphae. Scale bar: 10 µm.

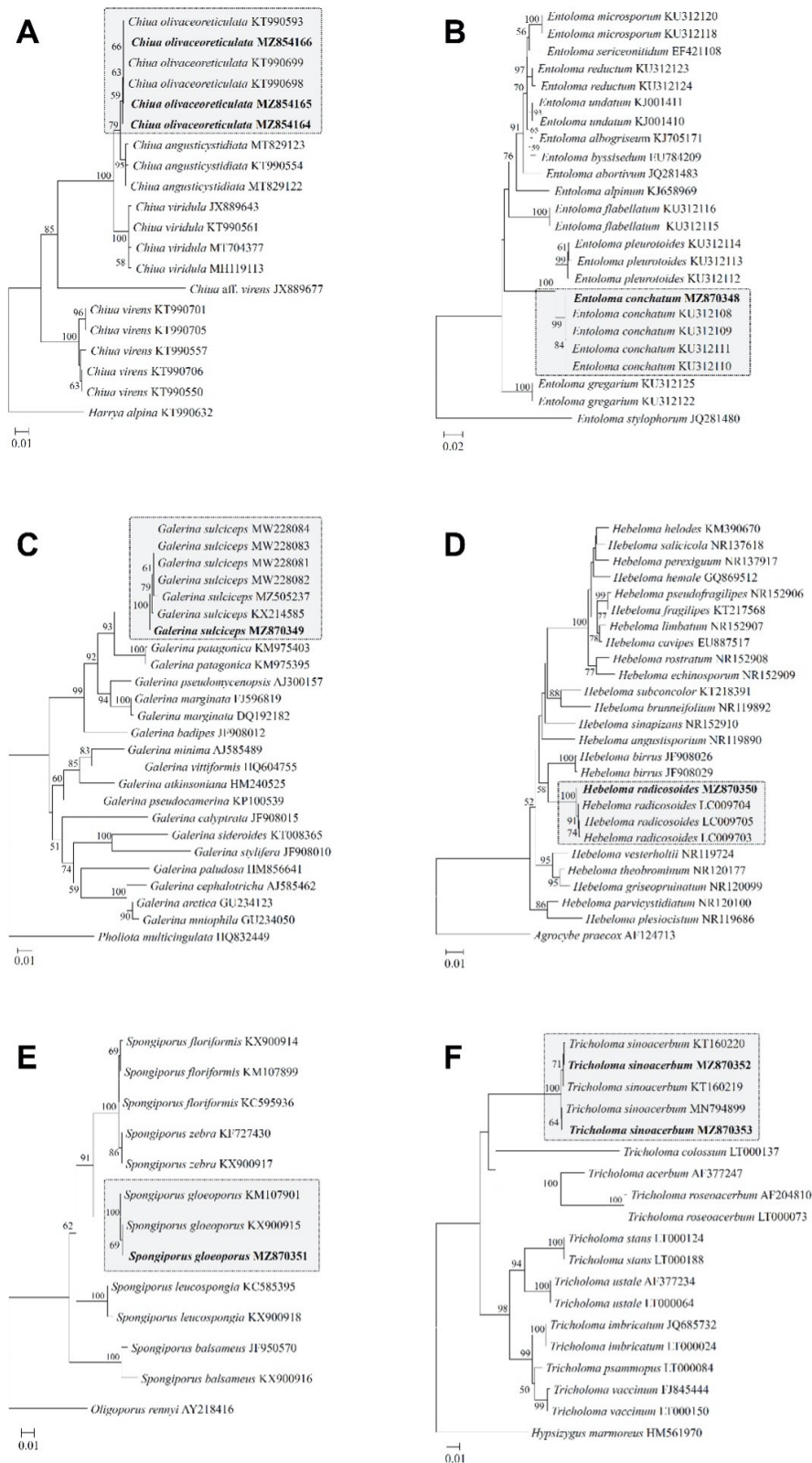


Fig. 2. Neighbor-joining tree of 6 unrecorded species constructed using (A) LSU rDNA and (B-F) internal transcribed spacer sequences. Bootstrap scores of >50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. Samples from the present study are indicated in bold. A, *Chiuia olivaceoreticulata*; B, *Entoloma conchatum*; C, *Galerina sulciiceps*; D, *Hebeloma radicosoides*; E, *Spongiporus gloeoporus*; F, *Tricholoma sinoacerbum*.

We collected 267 macrofungal species from the Gayasan National Park and classified them into 2 phyla, 5 classes, 19 orders, 59 families, and 146 genera. Among them, 112 species belonged to the order Agaricales; 17 species, including *Amanita caesareoides*, were the most diverse species belonging to the genus *Amanita*. In addition, our paper reports six species that have never been reported in Korea: *Chiuva olivaceoreticulata*, *Entoloma conchatum*, *Galerina sulciceps*, *Hebeloma radicosoides*, *Spongiporus gloeoporus*, and *Tricholoma sinoacervum*. The boletoid specimen was identified using the nuclear large subunit (nLSU) rDNA sequence analysis, while other specimens were identified using the ITS sequence analysis (Fig. 2). Three specimens (NIBRFG0000508514, NIBRFG0000508549, and NIBRFG0000508552) formed a monophyletic clade with *Chiuva olivaceorediculata* (bootstrap=59%; sequence similarity: 99.8-100%). Moreover, they showed 99.2-99.4% sequence similarity with *C. angusticystidiata* and 98.1-98.2% with *C. viridula* (Fig. 2A). Whereas, NIBRFG0000508463 formed a monophyletic clade with *E. conchatum* (bootstrap=99%; sequence similarity: 100%) and had 97.2-97.7% sequence similarity with *E. pleurotoides* (Fig. 2B). The specimen NIBRFG0000507914 formed a monophyletic clade with *G. sulciceps* (bootstrap=100%; sequence similarity: 99.8-100%) and had a 95.7% sequence similarity with *G. patagonica* (Fig. 2C). On the other hand, NIBRFG0000508444 formed a monophyletic clade with *H. radicosoides* (bootstrap=100%; sequence similarity: 99.8-100%) and exhibited 96.7-96.9% sequence similarity with *H. birrus* (Fig. 2D). Furthermore, NIBRFG0000509343 formed a monophyletic clade with *S. gloeoporus* (bootstrap support=100%; sequence similarity, 99.8-100.0%). It exhibited 95.4-96.0% sequence similarity with *S. floriformis* and 95.6% sequence similarity with *S. zebra* (Fig. 2E). Additionally, the two specimens NIBRFG0000508511 and NIBRFG0000508519 formed a monophyletic clade with *T. sinoacervum* (bootstrap support=100%; sequence similarity: 99.8-100.0%; Fig. 2F).

Lee et al. (2020) reported 17 unrecorded species such as *Crepidotus crocophyllus* from Gayasan National Park [13]. Although there is a lack of research in Gayasan National Park, it is thought that intensive research has increased the possibility to find new species. Fungi have a very limited basidiocarp forming period, making it difficult to collect and observe. The best way to overcome this is to conduct a collection survey over a long period of time for the specified research place. Among the collected specimens, there are still some that have caused confusion in identification, and if identification is completed, additional discovery of new or unrecorded species is possible.

Taxonomy

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Boletales E.-J. Gilbert

Boletaceae Chevall.

1. *Chiuva olivaceoreticulata* Yan C. Li & Zhu L. Yang, Fungal Diversity 81: 78 (2016)

(Jin-No-Ran-Gue-Mul-Beo-Seot, 진노란그물버섯)

Pileus hemispherical when young, becoming plane when mature, 40-85 mm diam. **Upper surface** dark green to yellowish green, paler towards margin, with fibrillose to tomentose. **Pore surface** depressed around apex of stipe, white when young, pinkish with age. **Pores** angular, 2-3 per mm, without discoloration when bruised. **Stipe** subcylindrical to narrow clavate, 50-80 × 15-25 mm, reddish pink in the upper part, yellow to bright yellow downward.

Basidia clavate, 30-45 × 8-10 μm, with 4-sterigmata. **Basidiospores** subfusiform, 10.5-12.5 × 4-5.5 μm, colorless, smooth. **Pleuro and cheilo-cystidia** cylindrical to subcylindrical, 30-45 × 4-7 μm, colorless, smooth.

Remarks: *Chiuia* is the genus first reported in Korea from this study. *C. olivaceoreticulata* is characterised by dark green to yellowish green pileus and white to pinkish pore surface.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 20 Aug 2020, NIBRFG0000508552 (GenBank accession no. MZ854164), NIBRFG0000508549 (GenBank accession no. MZ854165), NIBRFG0000508514 (GenBank accession no. MZ854166).

Agaricales Underw.

Entolomatacea Kotl. & Pouzar

2. *Entoloma conchatum* Xiao L. He & E. Horak, MycoKeys 61: 5 (2019)

(So-Ra-Oe-Dae-Beo-Seot, 소라외대버섯)

Pileus conchate in ventral view, broadly convex in side view, 7-15 mm diam. **Upper surface** white when young, becoming orange-white when mature, with tomentose. **Lamellae** adnexed, subdistant, white at first, becoming pinkish when mature. **Context** white, fine. Stipe absent or sometimes slightly developed, white fibrils, base with white mycelium.

Basidia subclavate, 30-35 × 10-12 μm, 4-sterigmata. **Basidiospores** polygonal (5-6 angled), 8-10.5 × 6-8 μm, with apiculus.

Remarks: *E. conchatum* is characterised by small size pileus, pinkish lamellae, polygonal (5-6 angled) basidiospores.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 6 Aug 2020, NIBRFG0000508463 (GenBank accession no. MZ870348).

Hymenogastraceae Vittad

3. *Galerina sulciceps* (Berk.) Boedijn, Sydowia 5 (3-6): 224 (1951)

(Jeok-Gal-Saek-E-Mil-Jong-Beo-Seot, 적갈색에밀종버섯)

Pileus convex at first, becoming slightly depressed in the center when mature, 20-30 mm diam. **Upper surface** dark rusty to dark vinaceous brown, smooth, subgelatinous. **Lamellae** distant, broadly adnate to slightly decurrent, concolorous with upper surface, with lamellulae. **Stipe** subcylindrical, 10-25 × 2-3 mm, concolorous with pileus or darker, solid.

Basidia cylindrical to slightly club-shaped, $32-44 \times 5-6 \mu\text{m}$ with 4-sterigmata. **Basidiospores** ellipsoid to almond-shaped, $7.0-9.5 \times 4.5-5.5 \mu\text{m}$, yellowish brown.

Remarks: *G. sulciceps* is characterised by dark rusty to dark vinaceous brown, subgelatinous pileus, ellipsoid to almond-shape basidiospores.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 8 Jul 2020, NIBRNIBRFG0000507914 (GenBank accession no. MZ870349).

Strophariaceae Singer & A.H. Sm.

4. *Hebeloma radicosoides* Sagara, Hongo & Y. Murak., *Mycol. Res.* 104(8): 1017 (2000)

(Ppu-Ri- Ja-Gal-Beo-Seot-Bu-Chi, 뿌리자갈버섯부치)

Pileus hemispherical with incurved margin when young, becoming broadly convex or plane with obtusely umbonate when mature 50-70 mm diam. **Upper surface** pale yellow, light yellow, with brown scales, viscid to glutinous when wet. **Lamellae** adnexed, crowded, whitish when young, becoming light brown when mature. **Stipe** slightly tapering toward apex with swollen base and pseudorhiza, 60-100 \times 10-15 mm, white, squamulose, solid to stuffed. **Annulus** membranous, brown, movable.

Basidia clavate to subclavate, $20-25 \times 5-8 \mu\text{m}$, with 4-sterigmata. **Basidiospores** subamygdaliform, $8.5-11.0 \times 4.5-5.5 \mu\text{m}$, finely punctate.

Remarks: *H. radicosoides* is characterised by pale yellow, light yellow, with brown scales pileus, swollen base with pseudorhiza stipe, movable annulus.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 9 Jul 2020, NIBRFG0000508444 (GenBank accession no. MZ870350).

Polyporales Gäum.

Dacrybolaceae Jülich

5. *Spongiporus gloeoporus* (L.L. Shen, B.K. Cui & Y.C. Dai) B.K. Cui, L.L. Shen & Y.C. Dai, *Persoonia* 42: 122 (2018)

(Kkeun-Jeok-Hae-Myeon-Gu-Meong-Beo-Seot, 끈적해면구멍버섯)

Basidiocarp annual, sessile. **Pileus** flabelliform, 50 \times 80 mm and 10 mm thick at base. **Upper surface** velutinate, sinuous, white colored when young, becoming cream to greyish when wet, margin concolorous with pileal surface. **Pore surface** white when fresh, becoming buff when dry, pores angular, 3-4 per mm, glue pores when dry. **Context** white to cream, up to 5 mm thick, corky. **Tubes** concolorous with pore surface, up to 2 mm long.

Hyphal system monomitic; generative hyphae with clamp connections, hyaline, 3-5 μm in diam. **Basidia** clavate, $12-18 \times 4-5 \mu\text{m}$, with 4-sterigmata. **Basidiospores** ellipsoid, $3.5-4.5 \times 2-2.5 \mu\text{m}$, hyaline, thin-walled, smooth.

Remarks: *Spongiporus* is the genus first reported in Korea from this study. *S. gloeoporus* is characterized by flabelliform and white to cream color pileus, glue pores when dry.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 8 Jul 2020, NIBRFG0000509343 (GenBank accession no. MZ870351).

Agaricales Underw.

Tricholomataceae R. Heim ex Pouzar

6. *Tricholoma sinoacervum* T.H. Li, Iqbal Hosen & Ting Li, Mycoscience 57 (4): 234 (2016) (Gub-Eun-Sseun-Song-I, 굽은쓴송이)

Pileus hemispherical to convex when young, becoming plano-convex, with slightly incurved margin, 50-110 mm diam. **Upper surface** cream-colored to pale yellow at margin and brownish buff at center, smooth, slightly viscid when wet. **Context** white, solid, 5-10 mm thick. **Lamellae** adnate, crowded, white to yellowish white. **Stipe** cylindrical to slightly tapering toward apex, slightly curve, 70-120×12-18 mm, dull white with whitish mycelium at base.

Basidia clavate to narrowly clavate, 18-23×5-7 μm, hyaline, thin-walled, with 4-sterigmata. **Basidiospores** globose to subglobose, 3.0-5.5×3.5-4.0 μm, thin-walled, pale yellowish, smooth, with apiculus.

Remarks: *T. sinoacervum* is characterized by cream-colored to pale yellow pileus, basidiospores globose to subglobose.

Specimen examined: Korea. Gyeongsangnam-do Hapcheon-gun, Gayasan National Park, 20 Aug 2020, NIBRFG0000508511 (GenBank accession no. MZ870352), NIBRFG0000508519 (GenBank accession no. MZ870353).

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