RESEARCH ARTICLE

Four unrecorded species of macrofungi from South Korea

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ABSTRACT

In 2022, our investigation focused on exploring the macrofungal diversity in South Korea. Through the examination of morphological characteristics and ITS sequences of the rDNA, we successfully identified four unrecorded species (*Laccaria striatula*, *Leotia atrovirens*, *Malvipezia emileia*, *Xerula strigosa*). Detailed morphological descriptions, accompanied by photographs and molecular phylogenetic analyses were performed.

Keywords: Macrofungi, Morphology, Phylogeny, Taxonomy

INTRODUCTION

Macrofungi play important roles in natural ecosystems, adopting saprophytic, parasitic, and symbiotic lifestyle. They encompass ascomycetes and basidiomycetes, which are characterized by conspicuous sporebearing structures that are easily observable [1]. While the worldwide compilation of macrofungi names by the Index fungorum exceeds 20,000, the recorded macrofungi species in Korea amount to approximately 2,100. Among these, 1,800 species were basidiomycetes fungi and 290 species were ascomycetes fungi [2].

In this study, we conducted taxonomic research on the diversity of wild macrofungi in the forests of South Korea. Four previously unrecorded macrofungal species were collected in 2022. Based on taxonomic identification (morphological features and DNA fungal barcodes), two basidiomycete fungi (*Laccaria striatula* and *Xerula strigosa*) and two ascomycete fungi (*Leotia atrovirens* and *Malvipezia emileia*) were confirmed. Among these, *Malvipezia* was discovered to be an unknown genus in South Korea.

MATERIALS AND METHODS

Four samples were collected during a mycological survey conducted in 2022 to investigate the mushroom diversity. In this study, these collections were examined for morphological identification based on their macroscopic and microscopic characteristics. The dried materials were mounted in distilled water



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under the terms of the Creative Commons Attribution Non-Commercial License (http: //creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. and 5% KOH using a Zeiss Axio Imager A1 microscope (Jena, Germany) and an Axiocam 503 color camera (Jena, Germany). The taxonomic classification of the studied taxa followed the guidelines provided by the Index Fungorum (http://www.indexfungorum.org). Dried specimens were archived at the herbarium of the National Institute of Biological Resources (NIBR), Incheon, South Korea.

For phylogenetic analysis, genomic DNA was extracted from the specimens using the ZR Genomic DNA Tissue MicroPrep kit (Zymo Research, USA). The ITS region was amplified using a universal primer pair (ITS1 and ITS4). PCR amplicons were purified with the QIAquick Purification Kit (Qiagen, Inc.) and sequenced by Macrogen sequencing service (Macrogen Inc., Seoul, Korea). For phylogenetic study, 61 sequences were download from GenBank (Table 1) [3-21]. The dataset was aligned using MAFFT v.7, with all other parameters set to default values. A Maximum Likelihood (ML) tree of the ITS sequences was constructed using RAxML-HPC2 on XSEDE (v. 8.2.4) [22] through the CIPRES Science Gateway [23]. The robustness of the individual branches was assessed by bootstrapping with 1,000 replicates.

Table 1. Specimens of species used in this study and their GenBank accession numbers.

Species name	Specimen No.	GenBank accession No. (ITS)	Reference
Geoscypha ampelina	15909	JF908554	[3]
Geoscypha ampelina	KH 00.011 (C)	AF491629	[4]
Laccaria acanthospora	HKAS45998	KU685719	[5]
Laccaria laccata	LLQ2	JX679363	-
Laccaria laccata	JMP0036	EU819477	[6]
Laccaria parva	ASIS21282	MG519526	[7]
Laccaria parva	SFC20120919-40	MG519525	[7]
Laccaria pseudomontana	pse1771	DQ149870	[8]
Laccaria pseudomontana	Cripps 1625	DQ149871	[8]
Laccaria striatula	CNV123	MT345281	[9]
Laccaria striatula	1475_PREMIX	OQ612526	-
Laccaria striatula	220922-10	OR168719	This study
Laccaria striatula	MQ17225-QFB29733	MN992607	-
Laccaria striatula	SAT1519306	KY777385	-
Laccaria striatula	136124799	OP749776	-
Laccaria striatula	3769	KM406967	-
Laccaria trullisata	WCG2072	KU685749	[5]
Laccaria trullisata	WCG2075	KM067894	[10]
Laccaria versiforma	SFC20120926-01	MG519556	[7]
Laccaria versiforma	ASIS20939	MG519557	[7]
Laccaria vinaceoavellanea	HKAS122366	ON794253	[11]
Laccaria vinaceoavellanea	HKAS122347	ON794247	[11]
Leotia atrovirens	(4)	OM972275	-
Leotia atrovirens	02V	AY144564	[12]
Leotia atrovirens	56690948	ON059220	-
Leotia atrovirens	JMP0048	EU819488	[6]
Leotia atrovirens	5NJ	AY144563	[12]
Leotia atrovirens	220922-05	OR168716	This study
Leotia atrovirens	HKAS122642	ON794411	[11]
Leotia lubrica	OTA:61895	JX178635	[13]
Leotia lubrica	06-Aug-2021	ON794306	[11]

Species name	Specimen No.	GenBank accession No. (ITS)	Reference
Leotia viscosa	14VT	AY144540	[12]
Leotia viscosa	1Chi	AY144537	[12]
Leotia viscosa	2MA	AY144538	[12]
Leotia viscosa	5VT	AY144539	[12]
Malvipezia emileia	220915-10	OR168717	This study
Malvipezia emileia	L 0833270	KJ728716	[14]
Malvipezia emileia	GM 03091006	KJ728717	[14]
Malvipezia emileia	L 0833260	KJ728715	[14]
Malvipezia howsei	6929	JF908528	[3]
Malvipezia howsei	PGJ 1742	KJ728714	[14]
Malvipezia invidula	14802	JF908545	[3]
Microglossum pratense	VK51	KJ513002	[15]
Mythicomyces corneipes	LE313648	OL739887	[16]
Peziza aurata	КН-97-133 (С)	AF491628	[4]
Peziza aurata	KH 00.023 (C)	AF491627	[4]
Peziza aurata	WTU-F-073122	MZ054370	-
Peziza depressa	HBAU15641	MW862407	-
Peziza proteana	PC 0084700	MN737812	[17]
Peziza proteana	OSC 100024	DQ491497	-
Strobilurus conigenoides	TENN52944	GQ892807	[18]
Xerula australis	RV95/413	AF321480	[19]
Xerula hispida	GMM4696	AF321486	[19]
Xerula longipes	LE1003 66b	HM005161	[18]
Xerula melanotricha	TFB12284	HM005163	[18]
Xerula melanotricha	KRAM F39960	HM005162	[18]
Xerula mundroola	PSC2263	FJ178106	[20]
Xerula pudens	179640	AF321492	[19]
Xerula pudens	179651	AF321493	[19]
Xerula strigosa	HKAS_48778_Liang162	KF530552	[21]
Xerula strigosa	HKAS_73323_Qin337	KF530553	[21]
Xerula strigosa	KP-06	LK932286	-
Xerula strigosa	HKAS_73908_Zhu513	KF530555	[21]
Xerula strigosa	HKAS_46276_WL398	KF530554	[21
Xerula strigosa	HKAS_73923_Zhu528	KF530556	[21
Xerula strigosa	220914-14	OR168718	This study

Table 1. Specimens of species used in this study and their GenBank accession numbers (continued).

RESULTS

As a result, the ITS sequences were subjected to an RaxML analysis, resulting in the resolution of the phylogenetic positions of four species (*Laccaria striatula, Leotia atrovirens, Malvipezia emileia, Xerula strigosa*) (Figs. 1-4). Each species formed well-supported clades in their respective phylogenetic trees. In *Laccaria* phylogeny (Fig. 1), the Korean collection grouped together with *L. striatula*. Within the *L. striatula* clade, this species exhibited three distinct groups, indicating the presence of a species complex, based on ITS sequences. Further studies are required to validate the phylogenetic position of *L. striatula*. Regarding the *Leotia* phylogeny (Fig. 2), the Korean specimen was clustered with *L. atrovirens* group. Based on the

phylogenetic analysis, the Korean collection was conclusively identified as *L. atrovirens*. In *Malvipezia* phylogeny (Fig. 3), the Korean collection clustered with *M. emileia* (KJ728715-KJ728717) with 100% BS values. Thus, the phylogenetic tree we constructed was supported by the position of *M. emileia*. In *Xerula* phylogeny (Fig. 4), *X. strigosa* clade was split into two groups. The Korean specimens clustered with *X. strigosa* from China (KF530556), with 100% BS values. Further studies are required to confirm this differentiation within *X. strigosa* clade.



Fig. 1. RAxML tree based on ITS sequences of *Laccaria striatula*. Sequences generated in this study are indicated in bold text.



Fig. 2. RAXML tree based on ITS sequences of *Leotia atrovirens*. Sequences generated in this study are indicated in **bold** text.



Fig. 3. RAxML tree based on ITS sequences of *Malvipezia emileia*. Sequences generated in this study are indicated in bold text.



- 0.01

Fig. 4. RAxML tree based on ITS sequences of Xerula strrgosa. Sequences generated in this study are indicated in bold text.

TAXONOMY

Laccaria striatula (Peck) Peck. Bull. N.Y. St. Mus. 157: 93 (1912) [1911] (Figs. 1 and 5)

Basionym. Clitocybe laccata var. striatula Peck 1897

Korean name: Julmunui-jolgakbeoseot (줄무늬졸각버섯); the epithet 'striatula' originates from the

Latin term for little stripes (Latin), referring to pattern of the pileus.

Description: Pileus: Diameter ranging from 15 to 40 mm., convex to flattened, with flesh pink to pinkish brown coloration, striated. Lamellae: Adnate, thick, orange-pinkish. Stipe: Colored in shades of orange, pinkish, or brown, finely fibrillose, 20–50 mm \times 2–4 mm. Basidia: Clavate-shaped, 4-spored, with dimensions of 53–68 \times 12–14.5 μ m. Basidiospores: Globose shape, echinulate with spines, measuring 8.0–12.0 \times 9.0–11.5 μ m; Q = 0.9–1.3; n = 20. Cheilocystidia rarely observed, filamentous, flexuous. Pileipellis a cutis, hyphae interwoven, cylindrical, bifurcating, clamped. Pileocystidia not observed.

Habitat: Typically found scattered on sand or moss in mixed forest (*Pinus koraiensis* and *Quercus mongolica*).

Specimen examined: Location: Gangwon-do, Jeongseon-gun, Korea, Coordinates: 37°9'12.3.53"N 128°54'24.59"E, alt. 1287 m. Collection date: Sep. 22, 2022. Specimen voucher: CKU20220922-10.

Remarks. *Laccaria striatula* is distinguished by its orange-pink to pinkish-brown basidiomata and globose spores with extensive echinulation. The Korean collection have morphologically similar characteristics from specimens of North America (shapes of basidiospores and pileus striations) [24]. This species is the most morphologically similar to *L. macrobasidia* with size of pileus, basidia, and basidiospore [25]. However, they can be differentiated by the size of stipe (20–50 mm \times 2–4 mm vs. 10–50 mm \times 0.5–1.0 mm). In Korea, this species is collected at high elevations (1287 m). Similarly, a collection of *L. striatula* from the USA (NCBI accession no. KY777385) was collected at a high elevation (1800 m). Therefore, this species may have grown at higher altitudes.



Fig. 5. A, B: Laccaria striatula. C, D: Basidiospores.

Leotia atrovirens Pers. Mycol. eur. (Erlanga) 1:202 (1822) (Figs. 2 and 6)

Korean name: jinnoksaek-dugeonbeoseot (진녹색두건버섯); the epithet "atrovirens" derives from the Latin term for dark green, referring to the color of the ascomata.

Description: Ascomata: Gelatinous, irregularly rounded or flattened, measuring 6-15 mm in width, with a yellowish to greenish hue, and appearing smooth or furrowed. Stipe: Typically 15-45 mm tall, pale green, and usually roughened. Asci: 8-spored, cylindrical, $120-168 \times 7.0-10 \mu m$, tapering towards a long base. Ascospores: Hyaline, ellipsoid, slightly curved, guttulate, measuring $18.0-24.0 \times 5.0-6.0 \mu m$; Q = 3.6-4.8; n = 20.

Habitat: Found in deciduous forests.

Specimen examined: Location: Gangwon-do, Jeongseon-gun, Korea, Coordinates: 37°09′02.49″N 128° 54′29.07″E, alt: 1316 m. Collection date: September 22, 2022. Specimen voucher: CKU20220922-05.

Remarks. According to a previous study [6], *L. atrovirens* may be *L. lubrica* parasitized by an asexual fungus, resulting in color change. However, phylogenetic analyses have shown that they are not monophyletic [26]. The constructed phylogenetic tree (Fig. 2) supports the results of this study.

Malvipezia emileia (Cooke) Van Vooren, Ascomycete.org 12(4):188 (2020) (Figs. 3 and 7)

Basionym: Peziza emileia Cooke 1879

Korean name: Naseon-damjasaekjubalbeoseot (나선담자색주발버섯); derived from the spiralshaped apothecia. The newly designated Korean genus name is Damjasaekjubalbeoseot (담자색주발버섯), derived from the characteristics of light purple apothecia.

Description: Apothecia: Cup or spiral shaped, with diameters ranging from 35 to 75 mm. The flesh was waxy, fragile, whitish, weakly brownish, and layered. Asci: Subcylindrical, hyaline, measuring 250–330 × 16.5–18 μ m, and containing eight-spores. Ascospores: Ellipsoid-shaped, measuring 16.0–23.0 × 9.0–12.0 μ m; Q = 1.7–2.5; n = 20; with warts in the form of irregular ridges. Paraphyses: Subcylindrical, 2.8–4.0 μ m wide at the base, 7–8 μ m at the apex, simple, and septate at the tip.

Habitat: Found on the ground of the forests.

Specimen examined: Location: Gangwon-do, Wonju-si, Korea. Coordinates: 37°21'47.75"N



Fig. 6. A: Leotia atrovirens. B: Ascospores. C: Ascus.

128°1'19.16"E, alt. 452 m. Collection date: Sep. 15, 2022. Specimen voucher: CKU20220915-10.

Remarks. There are four species have been recorded in the genus *Malvipezia (M. emileia, M. howsei, M. invidula, M. pauli*; IndexFungorum.org). Of those, *M. emileia* is morphologically similar to *M. howsei* with apothecia color (whitish or weakly brownish vs. brownish-violaceous with slight yellowish), spore size (*M. emileia* is slightly longer than *M. howsei*), and ornamentation (warts of *M. emileia* are slightly larger than *M. howsei*) [14]. This characteristic of *M. emileia* is similar to those observed in this study. However, *M. howsei* (Fig. 3). Therefore, more sequences are needed to understand the relationships among this taxon. The genus *Peziza* sensu lato is further split into four genera (*Peziza* sensu stricto, *Geoscypha, Malvipezia* and *Phylloscypha*), phylogenetically [27]. In Korea, the genus *Peziza* is also known as *Peziza* sensu lato. Therefore, a taxonomic study of *Peziza* sensu lato is needed based on the current research. In the present study, *Malvipezia* was recorded for the first time in South Korea.

Xerula strigosa Zhu L. Yang, L. Wang & G.M. Muell. 2008 (Figs. 4 and 8)

Korean name: Ganeunteol-Ppuribeoseot (가는털뿌리버섯); derived from the shape of lean stipe.

Description: Pileus: 25–45 mm in diameter, convex to plano-convex, light brown to dark brown. The surface is fibrillose or fine scaly, with an umbonate shape and broad depression over the disc. Lamellae: Cream to white, adnate to adnexed, edges entire. Stipe: $60–110 \times 3-4$ mm, yellowish-brown, centrally located, pubescent, cylindrical to subcylindrical in shape, tapering towards the base, firm. Basidia: 28.5– $35 \times 10.0-12.0 \mu$ m, 4–spored, clavate, hyaline. Clamp connections absent in all parts of basidiomata. Basidiospores: Ellipsoidal to broadly ellipsoidal, hyaline, pale yellow, measuring 11.0–15.0 × 10.5–12.5 μ m; Q = 1.0–1.4; n = 20. Pleurocystidia: 70–125 × 14–30 μ m, with a truncate apex, thick-walled mainly at the base. Pileipellis: hymeniform, 45–62 × 15–20 μ m, thick-walled.

Habitat: Found on litter and soil in coniferous forest.

Specimen examined: Location: Gangwon-do, Wonju-si, Korea. Coordinates: 37°25′12.12″N 128°3′13.53″E, alt. 322 m. Collection date: Sep. 14, 2022. Specimen voucher: CKU20220914-14.

Remarks. The description based on the Korean collection is in accordance with the holotype description [28]. *Xerula strigosa* has been documented in both China and Pakistan as well. Hence, considering its morphological features and molecular analysis, this represents the species' third recorded occurrence.



Fig. 7. A: Malvipezia emileia. B, C: Asci containing ascospores. D: Paraphyses.



Fig. 8. A, B: Xerula strigosa. C: Pleurocystidium. D: Pileipellis. E: Basidia. F: Basidiospores.

CONFLICT OF INTEREST

No potential conflict of interest is reported by the authors.

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