

RESEARCH ARTICLE

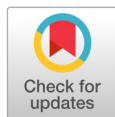
Six New Recorded Boletes from Mudeungsan National Park in Korea

Jae Young Park^{1,†}, Jin Sung Lee^{2,†}, Minkyong Kim³, Changmu Kim³, and Nam Kyu Kim^{4,*}¹Qmyco, Seoul 08793, Korea²Yurim Mushrooms, Asan 31582, Korea³Species Diversity Research Division, National Institute of Biological Resources, Incheon 22689, Korea⁴CatholicKwandong University, Gangneung 25601, Korea[†]These authors have contributed equally to this work.^{*}Corresponding author: namkyu0428@gmail.com

ABSTRACT

The indigenous fungi in Mudeungsan National Park were investigated between 2020 and 2022. All collected specimens were identified to the species level based on their morphological characteristics and rDNA sequences. Species belonging to the family Boletaceae were analyzed in depth. Six species, *Aureoboletus sinobadius*, *Hourangia densisquamata*, *H. nigropunctata*, *Tengioboletus glutinosus*, *Tylopilus himalayanus*, and *Xerocomus subparvus* were newly recorded as macromycota in Korea.

Keywords: Macrofungal flora, Mudeungsan National Park, Unrecorded species



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INTRODUCTION

Mudeungsan National Park is located in Gwangju Metropolitan City (Buk-gu and Dong-gu) and Jeollanam-do (Hwasun-gun and Damyang-gun), South Korea, and has an area of 75.425 km². The park provides habitats for several endangered species, *Lutra lutra*, *Pteromys volans aluco*, and *Lindera sericea*, etc and there have been several studies of plant and animal species in the park [1], but there has been little research regarding the fungal flora. The National Institute of Biological Resources conducted a fungal collection survey of Mudeungsan National Park from 2020 to 2022. About 351 mushroom specimens were obtained, among which species belonging to the Boletaceae Chevall. were analyzed in depth. The family Boletaceae is one of the most prominent and diverse basidiomycetes and primarily consists of ectomycorrhizal fungi, which play an essential ecological role in forest ecosystems. Members of the family are mainly tubulose with infrequent lamellate or loculate hymenophores and a fleshy appearance. Many mushrooms in the Boletaceae have attractive ornamentation on the cap, more ornamentation on the stalk, and various color changes in the fruiting body when bruised or cut [2]. The Boletaceae family is highly diverse, with about 50 genera and 800 species identified to date [3]. However, the numbers of genera and species are likely higher, as few studies have been in much of the tropics and subtropics. In Korea, 92 species in 22 genera of the Boletaceae have been reported to date [4]. New species and genera of Boletaceae

have been continuously described in recent years. To secure, preserve and manage the genetic biological resources of higher fungi in Korea, a research project was carried out in Mudeungsan National Park. This study of macrofungal flora in Mudeungsan National Park added six new recorded species belonging to this group in Korea.

MATERIALS AND METHODS

All specimens were initially identified based on macroscopic and microscopic features, according to published descriptions [5-13]. Measurements and drawings were obtained using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). Twenty randomly selected mature basidiospores and basidia were evaluated from each specimen. For molecular identification, total DNA was extracted from the dried specimens using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The nuclear large subunit rDNA D1-D2 domains (28S), specifically used for the molecular phylogenetic analysis of boletes, were amplified using the primer sets LR0R/LR5 or LR0R/LR7 [14,15]. DNA sequencing was performed at the Bioneer DNA Synthesis and Sequencing Facility (Daejeon, Korea) using the above primers. The nucleotide sequences were edited using MEGA 5 software [16] and deposited in GenBank. Species identification was confirmed by comparison with GenBank reference sequences using BLASTn. Neighbor-joining phylogenetic analysis was performed using MEGA 5 software with Kimura 2-parameter correction. The robustness of the inferred neighbor-joining topologies was tested using 1,000 bootstrap replicates. Using a combination of morphological and phylogenetic analyses, all fungal taxa were enumerated and classified according to the current taxonomies. Taxonomic classification of species and associated nomenclature were assigned using the MycoBank database (<http://www.mycobank.org/>).

RESULTS

This survey identified 351 species of macrofungi in Mudeungsan National Park, which were classified into 2 phyla, 5 classes, 14 orders, 51 families, and 115 genera. Among them, 22 species belonged to the Boletaceae family. Six of these boletoid species, *Aureoboletus sinobadius* Zhang & Li, 2019; *Hourangia densisquamata* Zeng, Wang & Liang, 2020; *H. nigropunctata* (Chiu) Zhu & Yang, 2015; *Tengioboletus glutinosus* Wu & Yang, 2016; *Tylopilus himalayanus* Chakr., Das, & Vizzini, 2018; and *Xerocomus subparvus* Zhu & Yang, 2016, were recorded for the first time in Korea. Here, we present photographs of fruiting bodies, drawings of microscopic features (Fig. 1), and descriptions and discussions of these species.

Ten nucleotide sequences of these six species have been deposited in GenBank (accession numbers: OQ804650-OQ804659). The sequences were compared with GenBank reference sequences (Table 1) and identified via nuclear large subunit rDNA sequence analysis (Fig. 2).

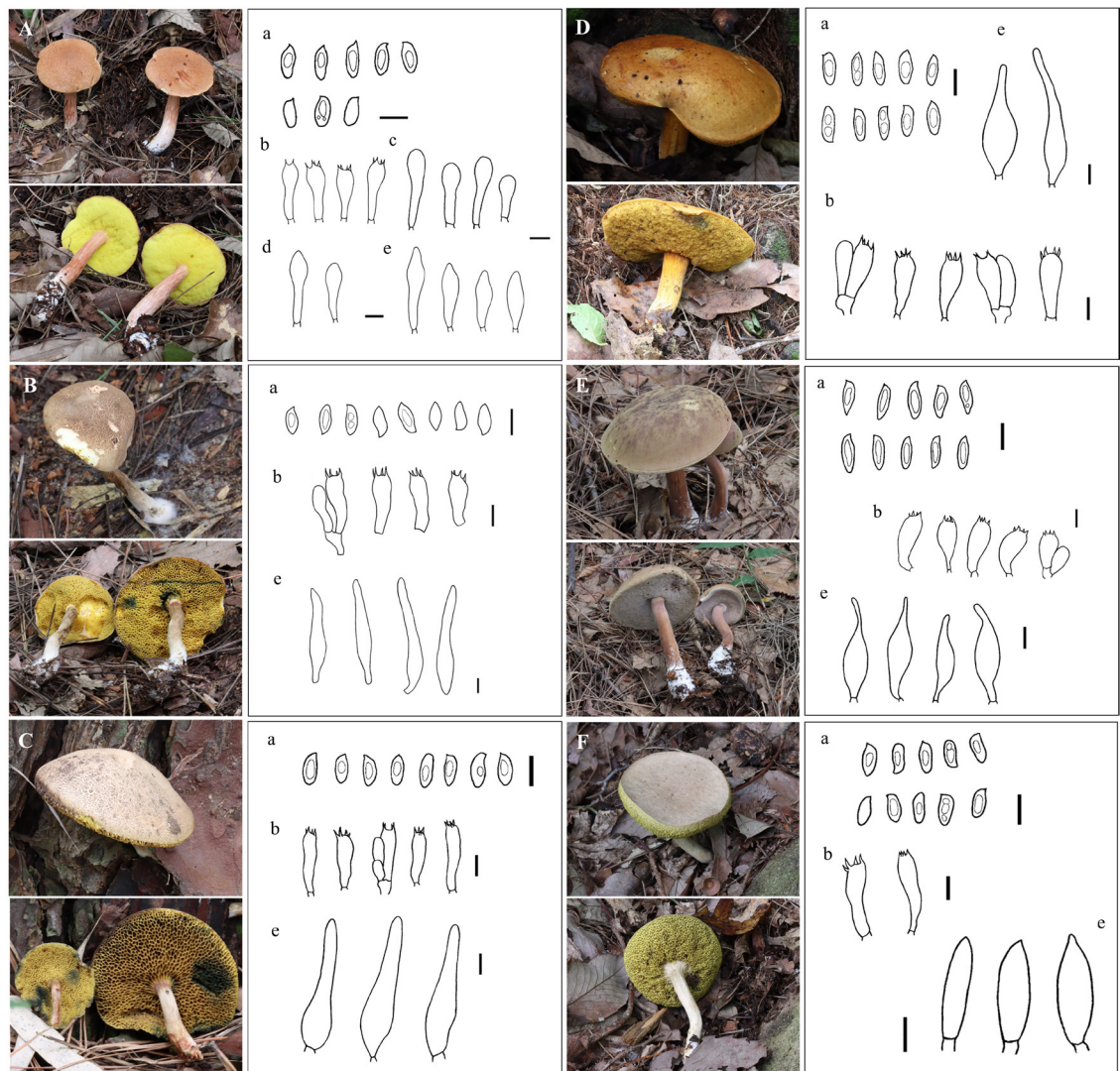


Fig. 1. Fruiting bodies and microscopic features of *Aureoboletus sinobadius* (A), *Hourangia densisquamata* (B), *Hourangia nigropunctata* (C), *Tengioboletus glutinosus* (D), *Tylopilus himalayanus* (E), and *Xerocomus subparvus* (F). a, basidiospores; b, basidia; c, basidioles; d, Cheilocystidia; e, Pleurocystidia. The scale bar is 10 μ m in the microscopic images.

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

Voucher No.	Genbank accession No.	28S rDNA	
		The closest GenBank Taxa	Identity (%)
NIBRFG0000510992	OQ804650	<i>Aureoboletus sinobadius</i>	99.74
NIBRFG0000513663	OQ804658	<i>Aureoboletus sinobadius</i>	100
NIBRFG0000513657	OQ804657	<i>Hourangia densisquamata</i>	100
NIBRFG0000511012	OQ804654	<i>Hourangia nigropunctata</i>	99.75
NIBRFG0000513834	OQ804659	<i>Tengioboletus glutinosus</i>	99.49
NIBRFG0000511001	OQ804651	<i>Tengioboletus glutinosus</i>	99.49
NIBRFG0000511023	OQ804655	<i>Tylopilus himalayanus</i>	100
NIBRFG0000511002	OQ804652	<i>Xerocomus subparvus</i>	100
NIBRFG0000511008	OQ804653	<i>Xerocomus subparvus</i>	100
NIBRFG0000513629	OQ804656	<i>Xerocomus subparvus</i>	100

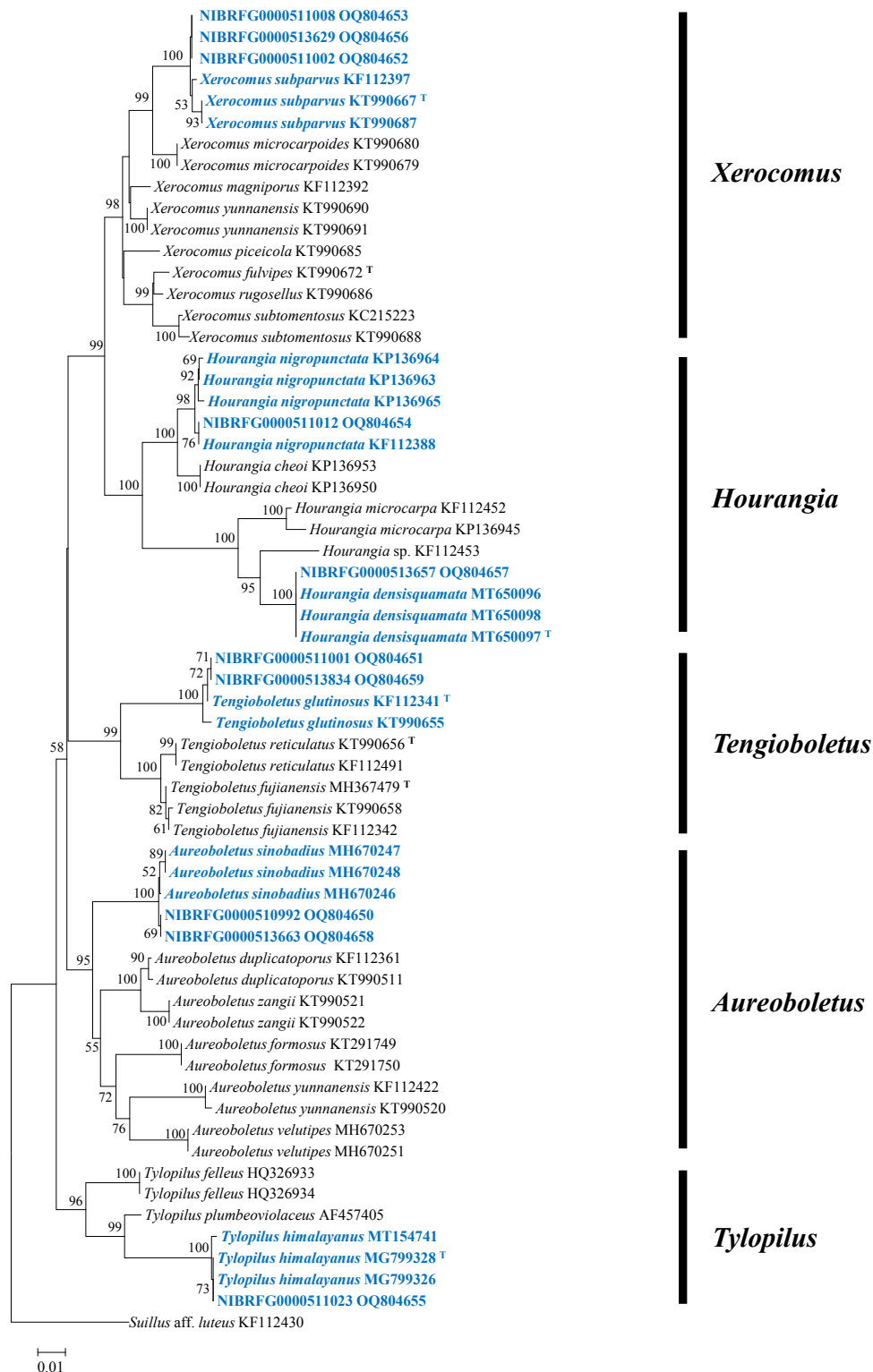


Fig. 2. Neighbor-joining tree of 6 unrecorded species constructed using Large subunit ribosomal ribonucleic acid (LSU rDNA). Bootstrap scores of >50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. The unrecorded species are marked with asterisks. "T" indicates the type specimens.

Two specimens (NIBRFG0000510992 and NIBRFG0000513663) formed a monophyletic clade with the reference sequence of *Aureoboletus sinobadius* (bootstrap=100%; sequence similarity=100%). One specimen (NIBRFG0000513657) formed a monophyletic clade with the reference sequence of *Hourangia densisquamata* (bootstrap value=100%; sequence similarity=100%). One specimen (NIBRFG0000511012) formed a monophyletic clade with the reference sequence, *Hourangia nigropunctata* (bootstrap value=100%; sequence similarity: 99.8-100%). Two specimens (NIBRFG0000511001 and NIBRFG0000513834) formed a monophyletic clade with the reference sequence of *Tengioboletus glutinosus* (bootstrap=100%; sequence similarity: 99.8-100%). One specimen (NIBRFG0000511023) formed a monophyletic clade with the reference sequences of *Tylopilus himalayanus* (bootstrap support=100%; sequence similarity: 99.8-100.0%). Three specimens (NIBRFG0000511002, NIBRFG0000511008, and NIBRFG0000513629) formed a monophyletic clade with the reference sequences of *Xerocomus subparvus* (bootstrap support=100%; sequence similarity: 99.8-100.0%) (Fig. 2).

TAXONOMY

Aureoboletus sinobadius Ming Zhang & T.H. Li, in Zhang, Li, Wang, Zeng & Deng, MycoKeys 61: 128 (2018)

Korean name. Bam-Saek-Sin-Geu-Mul-Beo-Seot, nom. nov. (밤색신그물버섯)

The Korean name refers to the colour of the basidiomata

Pileus 50-100 mm diam., hemispheric when young, convex to plane when old. **Upper-surface** is violet-brown when young, pastel red, brownish-red, reddish-brown to brownish-violet when old, and viscid, especially when young and wet, glabrous to minutely velvet-subtomentose, with a thin and slightly incurved margin. **Pore-surface** light yellow to greenish yellow. **Pores** 1-2 per mm, circular to angular. **Stipe** 40-80 × 5-10 mm, central, cylindrical, or clavate, enlarged downwards, pastel red, with basal mycelium white.

Basidia 25-35 × 9-12 µm, clavate, 4-sterigmata, partially 2-sterigmata, without a basal clamp. **Basidiospores** 10-13 × 4.5-5 µm, subfusiform, smooth. **Pleurocystidia** were 25-50 × 8-13 µm, fusiform, and thin-walled. **Cheilocystidia** 31-45 × 9-15 µm, clavate to subfusiform, thin-walled.

Specimen examined: Korea. Mudeungsan National Park, 20 Jul 2021, NIBRFG0000510992 (GenBank accession No. OQ804650), 8 Aug 2022, NIBRFG0000513663 (GenBank accession No. OQ804658)

Habitat: on soil in forests of broadleaf tree.

Remarks: *Aureoboletus sinobadius* is characterized by its pastel red, brownish-red, reddish-brown to brownish-violet upper-surface, light yellow pore surface in the pileus and white basal mycelium of the stipe.

***Hourangia densisquamata* N.K. Zeng, Yi Wang & Zhi Q. Liang, in Wang, Su, Jiang, Xue, Wu, Xie, Zhang, Liang & Zeng, Phytotaxa 472(2): 95 (2020)**

Korean name. Bi-Neul-Bol-Rok-Geu-Mul-Beo-Seot, nom. nov. (비늘볼록그물버섯)

The Korean name refers to the dense squamules on the pileus.

Pileus 35-55 mm diam., convex to applanate, marginally decurved. **Upper-surface** dry, densely covered with pale brown to brown. **Pore-surface** tubulate, yellow, rapidly changing blue. **Pores** angle, 0.5-1 per mm. **Stipe** 20-50×5-10 mm, central, sub-cylindrical, covered with brown squamules and basal white mycelium.

Basidia 17-35×6-10 µm, clavate, thin-walled, 4-sterigmata. **Basidiospores** 9-11×4-5 µm, subfusiform to ellipsoid, slightly thick-walled, smooth. **Pleurocystidia** 35-70×7-12 µm, subclavate or subfusiform, ventricose, thin-walled.

Specimen examined: Korea. Mudeungsan National Park, 8 Aug 2022, NIBRFG0000513657 (GenBank accession No. OQ804657)

Habitat: on soil in forests of broadleaf tree.

Remarks: *Hourangia densisquamata* is characterized by a pileus densely covered with pale brown to brown squamules, pore surface and context staining blue when bruised, a stipe covered with brown squamules, and a layer of white pruina.

***Hourangia nigropunctata* (W.F. Chiu) Xue T. Zhu & Zhu L. Yang, in Zhu, Wu, Zhao, Halling & Yang, Mycological Progress 14(no. 37): 7 (2015)**

Korean name. Geom-Eun-Jeom-Bol-Rok-Geu-Mul-Beo-Seot, nom. nov. (검은점볼록그물버섯)

The Korean name refers to the black spots scattered on the pileus when aged.

Pileus 30-70 mm diam., hemispherical when young, convex to plano-convex when old. **Upper-surface** densely covered by yellow-brown, red-brown, or dull brown squamules, which become rimose-diffracted to granular when aged. **Pore-surface** yellowish when young, becomes ochraceous when old, and stains blue when bruised. **Pores** compound, angular, 1 per mm. **Stipe** 20-80×5-12 mm, clavate, enlarged downwards, yellow-brown to brownish, basal mycelia dirty white.

Basidia 31-45×8-10 µm, clavate, 4-sterigmata. **Basidiospores** 7.5-9.8×3.5-4.6 µm, subfusiform, brownish yellow. **Pleurocystidia** 47-93×10-17 µm, clavate or ventricose, thin-walled.

Specimen examined: Korea. Mudeungsan National Park, 20 Jul 2021, NIBRFG0000511012 (GenBank accession No. OQ804654)

Habitat: on soil in mixed forests.

Remarks: *Hourangia nigropunctata* is characterized by a densely covered yellow-brown, red-brown, or dull brown squamous upper-surface in the pileus and large pores (1 per millimeter).

***Tengioboletus glutinosus* G. Wu & Zhu L. Yang, in Wu, Li, Zhu, Zhao, Han, Cui, Li, Xu & Yang, Fungal Diversity 81: 146 (2016)**

Korean name. A-Gyo-Ssi-Geu-Mul-Beo-Seot, nom. nov. (아교씨그물버섯)

The Korean name refers to the viscid to glutinous surface of the basidiomata.

Pileus 50-70 mm diam., convex to applanate. **Upper-surface** orange, deep orange to brownish orange, glabrous, and viscid to glutinous when wet. **Pore-surface** yellow to orange-yellow, staining blue when bruised. **Pores** stuffed when young, round when old, 1.5-3 per mm. **Stipe** 40-70 × 10-13 mm, central, sub-cylindrical, tapered downwards, concolorous with pileus, basal mycelium light yellow.

Basidia 25-36 × 8-13 µm, clavate, 4-sterigmata. **Basidiospores** 10-12 × 3.5-4.5 µm, subfusiform, smooth. **Pleurocystidia** 40-70 × 13-17 µm, fusoid-ventricose to clavate, subacute apex, thin-walled.

Specimen examined: Korea. Mudeungsan National Park, 20 Jul 2021, and NIBRFG0000511001 (GenBank accession No. OQ804651), Gayasan National Park, 5 Aug 2020, NIBRFG0000513834 (GenBank accession No. OQ804659)

Habitat: on soil in forests of broadleaf tree.

Remarks: *Tengioboletus glutinosus* is characterized by its pore-surface yellow to orange-yellow, staining blue when bruised, and light yellow basal mycelium on the stipe.

***Tylopilus himalayanus* D. Chakr., K. Das & Vizzini, in Chakraborty, Vizzini & Das, MycoKeys 33: 109 (2018)**

Korean name. Min-Ja-Ru-Sseun-Mat-Geu-Mul-Beo-Seot, nom. nov. (민자루쓴맛그물버섯)

The Korean name refers to the surface of stipe without any reticulum.

Pileus 70-110 mm diam., convex when young, plano-convex to applanate when old. **Upper-surface** dry, brownish-grey, dull red, reddish-grey to purplish-grey or greyish-magenta, paler greyish-yellow towards margin; margin entire. **Pore surface** greyish-white to pinkish-white; **Pores** angular, 2 per mm. **Stipe** 100-150 × 20-30 mm, central, cylindrical, or clavate, enlarged downwards, concolorous with pore surface at apex, brownish towards the base, with basal white mycelium.

Basidia 30-40 × 10-12 µm, clavate, with 4-sterigmata, without basal clamp, multiguttulate. **Basidiospores** 12.0-14.5 × 4.0-5.0 µm, elongated to fusiform, thin-walled, smooth. **Pleurocystidia** 38-60 × 8-12.5 µm, fusoid to ventricose.

Specimen examined: Korea. Mudeungsan National Park, 20 Jul 2021, NIBRFG0000511023 (GenBank accession No. OQ804655)

Habitat: on soil in forests of *Pinus*.

Remarks: *Tylopilus himalayanus* is characterized by reddish-grey or brownish-grey to purplish-grey or greyish-magenta pileus in the basidiomata and white basal mycelia of the stipe.

***Xerocomus subparvus* Xue T. Zhu & Zhu L. Yang, in Wu, Li, Zhu, Zhao, Han, Cui, Li, Xu & Yang, Fungal Diversity 81: 181 (2016)**

Korean name. Jak-Eun-San-Geu-Mul-Beo-Seot, nom. nov. (작은산그물버섯)

The Korean name refers to its similarity to the small-sized *Xerocomus parvus*.

Pileus 20-50 mm diam., convex to planate. **Upper-surface** pale yellow-brown, pale red-brown, pale brown, yellow-brown to brown, dry, tomentose. **Pore-surface** yellow, staining blue slowly when bruised. **Pores** angular, 1 per mm. **Stipe** 30-55 × 4-10 mm, sub-cylindrical, pale yellow-brown, upper part pale yellow, basal white mycelium.

Basidia 25-38 × 9-12 μm , clavate, 4-sterigmata. **Basidiospores** 9-10.5 × 3.5-4 μm , subfusiform, brownish yellow, smooth. **Pleurocystidia** 42-70 × 10-15 μm , clavate to ventricose, thin-walled, and colorless.

Specimen examined: Korea. Mudeungsan National Park, 20 Jul 2021, NIBRFG0000511002 (GenBank accession No. OQ804652), and NIBRFG0000511008 (GenBank accession No. OQ804653), 28 Jul 2022, and NIBRFG0000513629 (GenBank accession no. OQ804656)

Habitat: on soil in mixed forests.

Remarks: *Xerocomus subparvus* is characterized by its context and pore-surface that slowly and indistinctly staining blue when injured and large pores (1 per mm).

DISCUSSION

Boletaceae Chevall, 1826 [17] is one of the most prominent and diverse families among basidiomycetes, with more than 70 genera [18]. Most Boletaceae species are valuable to humans, forming essential mutualistic symbioses with trees [19,20]. The phylogeny and classification of the Boletaceae have long been inconsistent because the morphology of Boletaceae has convergent characteristics. However, advances in molecular biology have led to the use of genealogical concordance phylogenetic species recognition [21] to identify fungal species, which has contributed to a better understanding of the relationships within this family. In addition, the species diversity of this family has increased significantly over the past few decades [2,20,22,23]. In the present study, based on a combination of morphological characteristics and rDNA sequence analyses, we discovered six newly recorded boletoid species in Korea. Fungi have a limited basidiocarp-forming period, making their collection and observation difficult. To overcome this issue, a long-term collection survey over a long period within a specific site is necessary, and it is expected that more species diversity will be obtained through such selective and intensive research.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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