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New Records of Two Agarics: *Galerina sideroides* and *Gymnopus luxurians* in South Korea

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ABSTRACT : Studies on the diversity of indigenous fungi were conducted in the central districts of South Korea from 2008 to 2013. During the studies, potentially unrecorded agarics were collected. All specimens collected in this study were examined morphologically and phylogenetic analysis was also performed. They were identified as *Galerina sideroides* and *Gymnopus luxurians*. These fungi have never been reported in South Korea. We report them here with detailed descriptions and figures.

KEYWORDS : Agaricomycetes, ITS, Phylogeny, Taxonomy

Introduction

Biodiversity has decreased with massive extinctions since the appearance of human. Climate change has also accelerated the loss of biodiversity [1]. In line with the effectuation of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity [2], knowledge of biodiversity is becoming much more important.

Fungi have major roles as decomposers and symbiotes in ecosystems. Fungi have also been useful biological resources [3]. However, the known diversity of fungi is only a small part of the actual fungal diversity. In Korea, there are more unrecorded fungi than recorded fungi [4]. Therefore, diversity studies on indigenous fungi have been conducted [5, 6].

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During the studies, agarics were collected in the central districts of South Korea from 2008 to 2013. Observation of phenotypic characteristics and molecular analysis were performed for identification of the collected specimens. Among them, two species; *Galerina sideroides* and *Gymnopus luxurians* were recorded for the first time in the Korean fungous flora. In this paper, the detailed description and figures for two species are provided with a phylogenetic tree for each fungus.

Materials and Methods

Morphological observation

Measurements and drawings were made from slide preparations mounted in Melzer's reagent using an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan) [7]. More than 30 measurements from different positions were made to ascertain the average dimensions of each characteristic. In the case of basidiospores, 5% of the measurements were excluded from each end of the range, and are shown in parenthesis. Munsell color [8] was followed as the color standard. The voucher specimens were deposited at the Herbarium of the National Institute of Biological Resources, Korea (KB).

Molecular analysis

Genomic DNAs were extracted from the two dry specimens using an Accuprep Genomic DNA extraction kit (Bioneer, Daejeon, Korea). In addition, *Galerina sideroides* CBS 162.46 was received and its genomic DNA was ext-

racted according to the described method. Internal transcribed spacer (ITS) region [9, 10] was analyzed by performing polymerase chain reaction (PCR) according to the previously described method [11]. For each species under analysis, closely related sequences were downloaded from GenBank. The obtained sequences were proofread and aligned with the selected reference sequences from GenBank using MAFFT 7.130 [12] and modified manually using MacClade 4.08 [13]. Neighbor joining trees were created using PAUP 4.0b10 [14]. The Kimura 2-parameter model was applied [15]. One thousand replications of bootstrap analysis were performed for branch stability.

Results and Discussion

Taxonomy

Galerina sideroides (Bull.) Kühner, Encyclop. Mycol. 7: 215 (1935) (Fig. 1)

Synonym: *Galerula stylifera* G.F. Atk., P. Amer. Philos. Soc. 57: 365. 1918

Pileus 1~2 cm diam, convex, appearing viscid, at first brown (7.5YR5/8) to brownish yellow (10YR7/8), becoming brown (7.5YR5/8) when dry, margin faintly striate. Lamellae adnate, subclose pale brown (10YR8/4), becoming brown (7.5YR6/6) when dry; spore print brown. Stipe 1~2 cm long, 0.1~0.3 cm wide, central, terete, longitudinally striate, very pail brown (10YR8/2), becoming brown (7.5YR6/6) when dry. Partial veil fibrous. Hyphal system monomitic; generative hyphae with clamps, thin-walled or somewhat thick-walled, 2.5~4 μm diam. Basidia clavate; with 4-sterigmata and basal clamp; 18.5~25 × 6~7 μm. Cheilocystidia tibiiform, with basal clamp, thin-walled, slightly projecting, 23.5~28.5 × 5.5~6 μm. Basidiospores smooth, ellipsoid, inamyloid, (5.1~)5.5~7(7.2) × 3.5~4.5 (~4.9) μm.

Specimen examined: Korea, Gangwon-do, Mt. Odae, 37°44'30" N, 128°35'03" E, on the branch of *Abies holophylla*, 2 Oct 2013, Seokyoon Jang, KUC20131001-02 (KB, NIBRFG0000132751; GenBank accession No. KM45 3735).

Note: In South Korea, four species of *Galerina*; *G. calyptrata* P.D. Orton, *G. fasciculata* Hongo, *G. helvoliceps* (Berk. & M.A. Curtis) Singer, and *G. vittiformis* (Fr.) Singer have been reported [4]. This species is easily recognized by its smaller fruit body and basidiospores compared to the other Korean *Galerina* species [16-18]. The obtained ITS sequence of *Galerina sideroides* KUC20131 001-02 was 400 bp. It was placed in the monophyletic clade with the reference sequences of *G. sideroides* (Fig. 2A). Hence, we report KUC20131001-02 as *G. sideroides*.

Gymnopus luxurians (Peck) Murrill, N. Amer. Flora 9: 362. 1916. (Fig. 3)

Pileus 2.5~6.5 cm diam, convex when young, later plano-convex or plane, convex when dry, margin at first incurved, even, smooth, later decurved, radially streaked; when young reddish brown (5YR5/3) or reddish grey (5YR5/2) overall, becoming brown (10YR4/4) when dry. Lamellae shallowly adnexed to sinuate, close; very pale brown (10YR9/2) colored at first, becoming brown (7.5

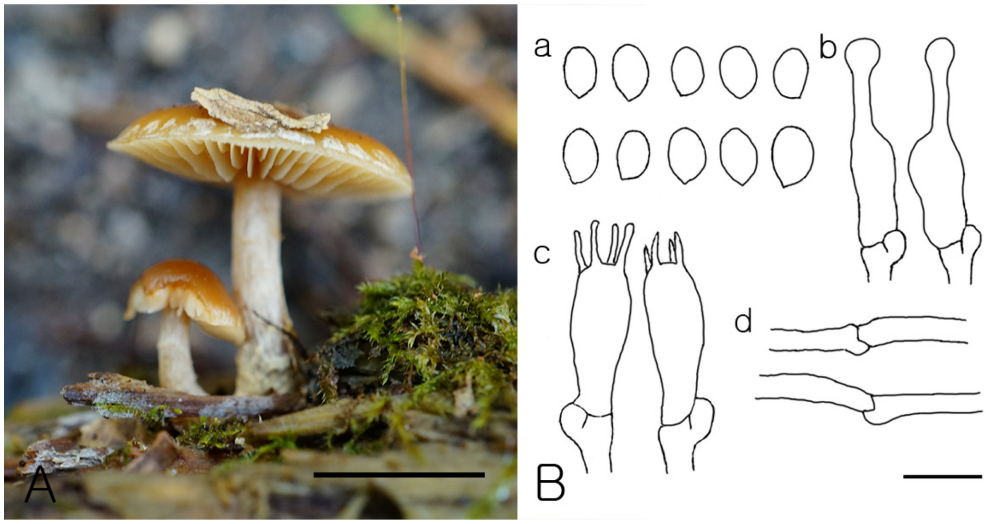

Fig. 1. *Galerina sideroides*. A, Basidiocarps; B, Microscopic features; a, basidiospores; b, cheliocystidia; c, basidia; d, generative hyphae (scale bars: A = 1 cm , B = 10 μm).

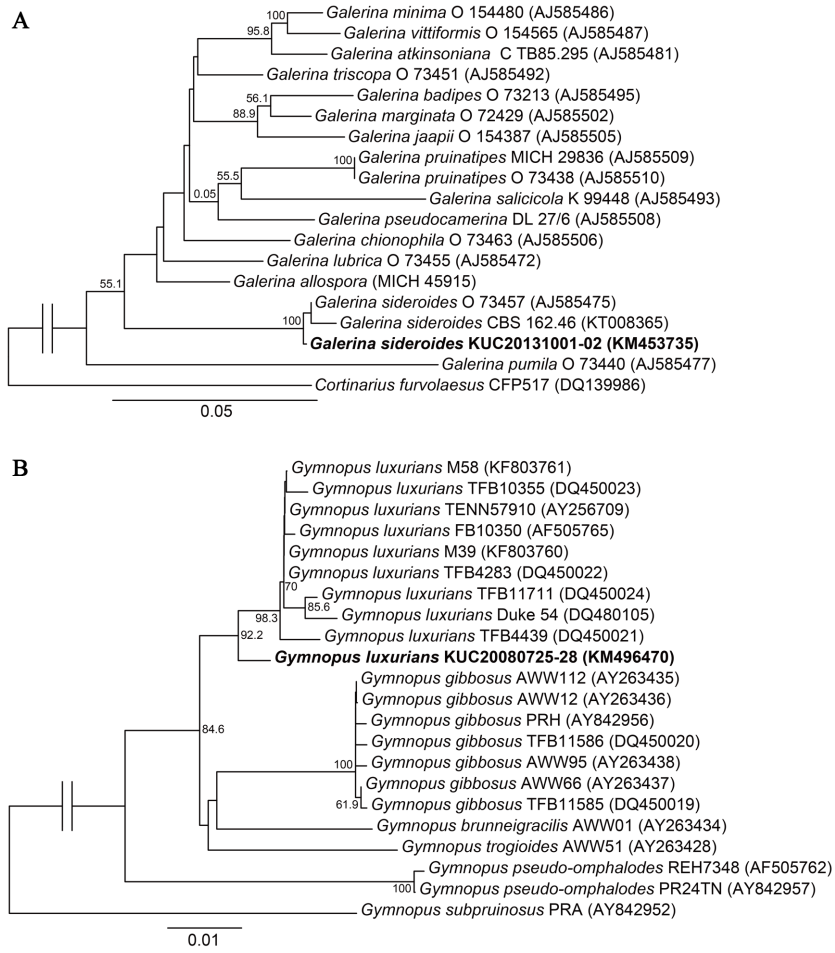

Fig. 2. Neighbor joining trees of internal transcribed spacer region sequences of the examined agarics; *Galerina sideroides and Gymnopus luxurians*. A, The dataset was created from 19 taxa and 393 characters; B, The dataset was created from 22 taxa and 664 characters. Bootstrap values less than 50 were not shown. Specimens examined in this study are in bold. GenBank accession numbers are in parentheses.

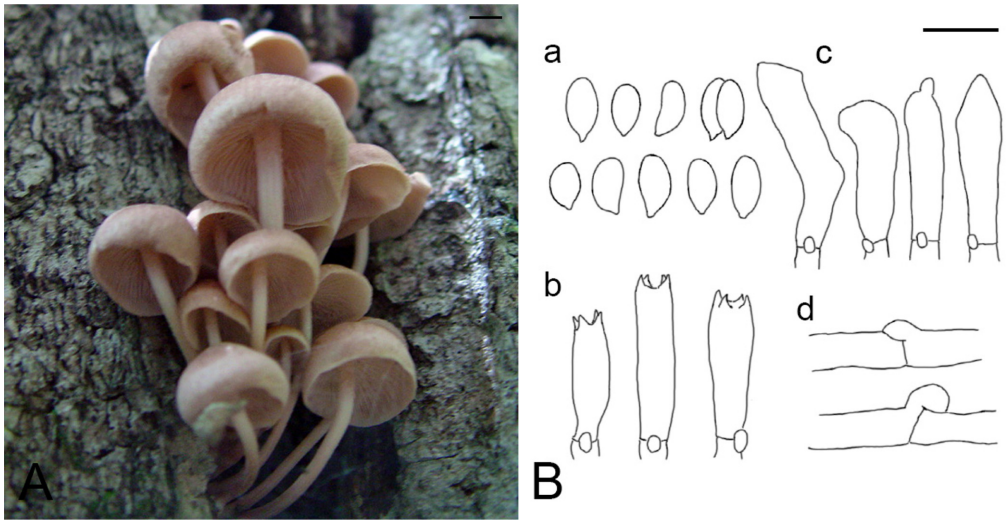

Fig. 3. *Gymnopus luxurians*. A, Basidiocarps; B, microscopic features; a, basidiospores; b, basidia; c, cheliocystidia; d, generative hyphae (scale bars: A = 1 cm, B = 10 μm).

YR5/8) when dry. Stipe 3~8 × 0.3~0.6 cm, central, terete or compressed, longitudinally striate, fibrous, hollow, caespitose, pale brown (10YR8/2) at first, becoming brown (10YR4/4) when dry. Partial veil absent. Hyphal system monomitic; generative hyphae with clamp, 2~6.5 μm diam. Basidia clavate; with 4-sterigmata and basal clamp; 18.5~26.5 × 5~7 μm. Cheilocystidia with basal clamp, abundant; versiform, often irregularly clavate or fusoid, ventricose; hyaline, thin-walled, 15.5~24 × 4~7 μm. Basidiospores more or less ellipsoid, thin-walled, smooth, inamyloid, (5.5~)5.9~8.8(~9) × (3~)3.2~4.5(~5.1) μm.

Specimen examined: Korea, Chungcheongbuk-do, Mt. Joryeong, 36°46'12" N, 128°02'24" E, on hardwood, 10 Jul 2008, Jae-Jin Kim, KUC20080710-14 (KB, NIBRFG0000107194); Seoul, Seongbuk-gu, 37°35'26" N, 127°01'26" E, 10 Jul 2008, Jae-Jin Kim, KUC20080725-28 (KB, NIBRFG0000107805; GenBank accession no. KM496470).

Note: In Korea, four species of *Gymnopus*; *Gymnopus confluens* (Pers.) Antonín, Halling & Noordel, *G. dryophilus* (Bull.) Murrill, *G. erythropus* (Pers.) Antonín, Halling & Noordel, and *G. peronatus* (Bolton) Gray have been reported [4]. The characteristics of our specimens were in agreement with the previous description of *Gymnopus luxurians* [19]. *G. luxurians* is characterized by its larger size of basidiocarp compared with other Korean *Gymnopus* spp., brown and convex pileus, close lamellae, and caespitose stripe. In our phylogenetic tree (Fig. 2B), *G. luxurians* KUC20080725-18 was placed in the monophyletic group of *G. luxurians* with a high bootstrap value (92.2).

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