

Phylogenetic Identification of Korean *Gymnopus* spp. and the First Report of 3 Species: *G. iocephalus*, *G. polygrammus*, and *G. subnudus*

Seokyeon Jang¹, Yeongseon Jang², Young Woon Lim³, Changmu Kim⁴, Byoung Jun Ahn², Sung-Suk Lee² and Jae-Jin Kim^{1,*}

¹Division of Environmental Science & Ecological Engineering, College of Life Science & Biotechnology, Korea University, Seoul 02841, Korea

²Division of Wood Chemistry & Microbiology, National Institute of Forest Science, Seoul 02455, Korea

³School of Biological Sciences, Seoul National University, Seoul 08826, Korea

⁴Microorganism Resources Division, National Institute of Biological Resources, Incheon 22689, Korea

Abstract *Gymnopus* is a cosmopolitan genus of agaric fungi and consists of ~300 species. In Korea, *Gymnopus* represents common saprobic mushrooms, and 12 species have been reported in Korea. Several *Gymnopus* specimens were collected in Korea between 2008 and 2015. To identify them exactly, phylogenetic analysis was performed by means of the internal transcribed spacer region of ribosomal-DNA sequences from the collected *Gymnopus* specimens. Among them, *G. iocephalus*, *G. polygrammus*, and *G. subnudus* have not been reported in Korea. A phylogenetic tree and images are provided.

Keywords Agaricomycetes, ITS, Phylogeny, Taxonomy

Gymnopus (Pers.) Roussel (*Omphalotaceae*, *Agaricales*) is a large fungal genus, which consists of ~300 species and is distributed all over the world. It is characterized by a collybioid mushroom; convex to applanate or slightly concave pileus; free, emarginate or adnate and crowded to fairly distant lamellae; central stipe; white spore print; nonamyloid or nondextrinoid hyphae with clamp; ellipsoid to oblong, thin-walled, hyaline, nonamyloid basidiospores; often present cheilocystidia; and usually absent pleurocystidia [1]. The morphological characteristics of *Gymnopus* are similar to those of other genera: *Marasmius* (*Marasmiaceae*, *Agaricales*) Fr. and *Collybia* (Fr.) Staude (*Tricholomataceae*, *Agaricales*), except for its pileipellis: *Gymnopus* is distinguished from

other collybioid mushrooms by the pileipellis containing a frequently encrusted pigment and a cutis or trichoderm made of projected hyphae [2]. These genera can be easily discriminated by molecular biological assays as well because they are classified into different families.

Gymnopus represents common saprobic fungi on leaf litter and dead wood, and ~12 species have been reported in Korea [3, 4]. Nonetheless, we presume the diversity of Korean *Gymnopus* to be much greater than the reported species because 12 known species is a small number as compared to the high worldwide diversity. To study the diversity of indigenous *Gymnopus* spp., the specimens were collected in Korea. Phylogenetic analysis was performed to identify them. Among them, *G. iocephalus* (= *Collybia iocephala*), *G. polygrammus* (= *Collybia polygramma*), and *G. subnudus* (= *Collybia subnuda*) have not been reported in Korea. Thus, morphological examination of the macro- and microscopic characteristics was conducted, and we report the 3 *Gymnopus* species as new in Korea, with detailed descriptions.

MATERIALS AND METHODS

Materials studied. A total of 14 specimens were collected (Table 1). Among them, the specimens that are named Korea University Collection (KUC) were collected and preserved by the Environmental Biotechnology Laboratory (Korea University, Seoul, Korea). Five *G. subnudus* specimens

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***Corresponding author**

E-mail: jae-jinkim@korea.ac.kr

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Table 1. Specimens analyzed in this study

Fungal name	Collection ID	Accession No.	Date of collection	Location
<i>Gymnopus confluens</i>	KUC20140820A-21	KX513743	20 Aug 2014	Gangwon-do, Odaesan National Park
<i>G. dryophilus</i>	KUC20140627-37	KX513744	27 Jun 2014	Chungcheonbuk-do, Songnisan National Park
<i>G. iocephalus</i>	KUC20140804-02	KX513745	4 Aug 2014	Seoul, Bukhansan National Park, Mt. Dobong
<i>G. luxurians</i>	KUC20080725-28	KM496469	25 Jul 2008	Seoul, Seongbuk-gu, Korea Univ.
<i>G. polygrammus</i>	KUC20140804-01	KX513746	4 Aug 2014	Seoul, Bukhansan National Park, Mt. Dobong
<i>G. subnudus</i>	KUC20150814-11	KX513747	14 Aug 2015	Gyeonggi-do, Yangpyeong-gun, Mt. Yongmun
	KUC20150911-19	KX513748	11 Sep 2015	Gyeonggi-do, Gwacheon-si, Seoul Grand Park
	KMRB 15081230	KX513749	12 Aug 2015	Seoul, Jongno-gu, Jongmyo
	KMRB 15082001	KX513750	20 Aug 2015	Gyeonggi-do, Uiwnag-si, Mt. Cheonggye
	KMRB 15082059	KX513751	20 Aug 2015	Gyeonggi-do, Uiwnag-si, Mt. Cheonggye
	KMRB 15090201	KX513752	2 Sep 2015	Gyeongsangbuk-do, Ulleung-gun, Nari Basin
	KMRB 15090317	KX513753	3 Sep 2015	Gyeongsangbuk-do, Ulleung-gun, Nari Basin
<i>Gymnopus</i> sp.	KUC20080914-03	KX513754	14 Sep 2008	Gyeongsangbuk-do, Gyeongju-si, Mt. Seonam
	KUC20150819-52	KX513755	19 Aug 2015	Seoul, Seodaemun-gu, Mt. An

were obtained from Korea Mushroom Resource Bank (KMRB; Seoul, Korea). *Gymnopus* sp. NIBRFG0000106907 (KUC20080914-03) was obtained from the National Institute of Biological Resources (NIBR, Incheon, Korea).

Molecular analysis. Genomic DNA samples were extracted from dry specimens with the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). PCR amplification of the internal transcribed spacer (ITS) region was performed according to the previously described method [5-7]. To analyze each species, closely related sequences of *Gymnopus* spp. and reference sequences from other studies were retrieved from GenBank [1, 4, 8]. The determined sequences were proofread and aligned using MAFFT 7.130 [9] and modified manually in the MacClade 4.08 software [10]. Datasets were tested by MrModeltest 2.3 using the Akaike information criterion (AIC) criteria with default options [11]. The GTR + I + G model was chosen for the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 [12]. A phylogenetic tree was constructed by a method described elsewhere [7].

Morphological examination. Measurements and drawings were made for the unrecorded species from slide preparations mounted in Melzer's reagent under an Olympus BX51 light microscope (Tokyo, Japan) [13]. More than 30 measurements at different positions were made to ascertain the average dimensions of each part. In case of basidiospores, 5% of the measurements were excluded from each end of the range and are given in parentheses. Munsell colors were used as color standards [14]. Voucher specimens were deposited in the Herbarium of NIBR.

RESULTS AND DISCUSSION

Phylogenetic analysis. A total of 61 taxa were used to construct the phylogenetic tree (Fig. 1). Despite the differences among the target taxa, several parts of our phylogenetic tree were found to be similar to trees from

other studies [1, 4, 8]. Most of our specimens were clustered with the 6 known species: *G. confluens* (Pers.) Antonín, Halling & Noordel.; *G. dryophilus* (Bull.) Murrill; *G. iocephalus* (Berk. & M. A. Curtis) Halling; *G. luxurians* (Peck) Murrill; *G. polygrammus* (Mont.) J. L. Mata; and *G. subnudus* (Ellis ex Peck) Gilliam. The specimen KUC20140820A-21 was identified as *G. confluens*, which is the most common *Gymnopus* species in Korea and a well-known edible and medicinal mushroom [15, 16]. KUC20140627-37 ended up in a clade of *G. dryophilus*. It is also a common Korean *Gymnopus* species, known as an edible and medicinal mushroom [15, 16]. *G. luxurians* KUC20080725-28 was previously reported in Korea [8] but is paraphyletic in our phylogenetic tree. In one study on Korean *G. luxurians*, this strain was found to be monophyletic with other *G. luxurians* strains [8]. Analysis of additional DNA regions may be needed to identify such specimens exactly. *G. iocephalus*, *G. polygrammus*, and *G. subnudus* have not been reported in Korea. Thus, additional examination of morphological features of these specimens was carried out.

Two specimens—KUC20080914-03 and KUC20150819-52—are in their own clade without any reference sequences. They were identified at the genus level: *Gymnopus* sp. They are located in a clade of *G. polyphyllus* with long branches and match *G. barbipes* KJ416266 according to a BLAST search but show low similarity (KUC20150819-52: 552/591, 93%). We presume that they are new species candidates, and further research is needed.

Taxonomy.

Gymnopus iocephalus (Berk. & M. A. Curtis) Halling, Mycotaxon 63: 364 (1997) (Fig. 2).

Pileus 2~4 cm; convex with incurved margin when young, becoming planoconvex; sometimes margin uplifted when aged; yellowish brown (10YR5/6) to very pale brown (10YR8/2) when fresh, becoming very pale brown (10YR7/4) when dry, radially streaked; surface moist, but dries rapidly; margin somewhat wrinkled. Lamellae adnate to adnexed; close. Stipe 4~8 cm long, 3~4 mm wide; central, terete,

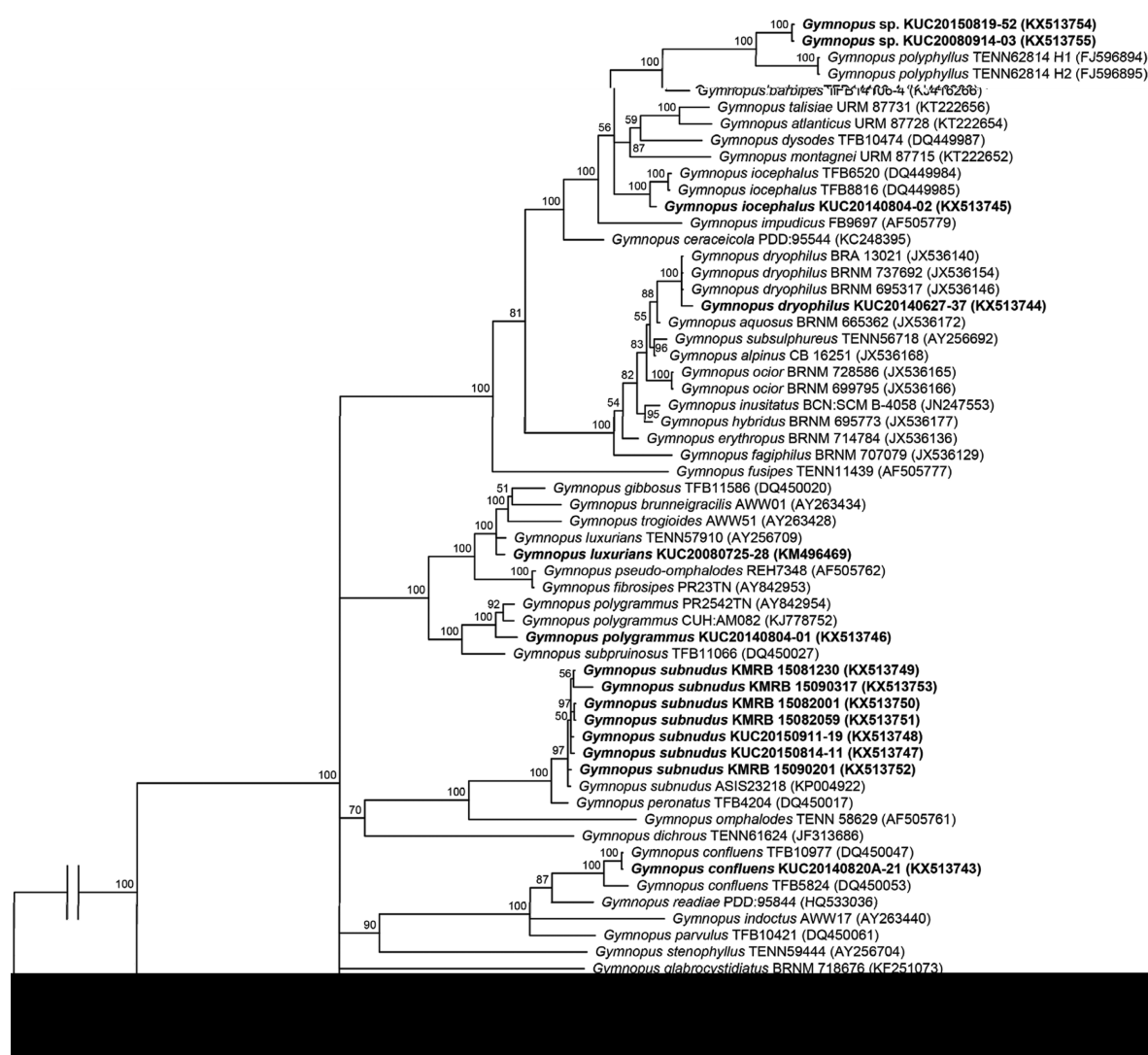


Fig. 1. The phylogenetic tree of *Gymnopus* spp. based on internal transcribed spacer region sequences. The dataset was created from 61 taxa and 1,032 characters. The specimens examined in this study are boldfaced. GenBank accession numbers are shown in parentheses. The numbers above branches indicate posterior probabilities. The scale bar indicates nucleotide substitutions per position.



Fig. 2. The basidiocarp (A) and microscopic features (B~D) of *Gymnopus iocephalus* KUC20140804-02 (NIBRFG0000138670). B, Basidiospores; C, Basidia; D, Generative hyphae (scale bars: A = 1 cm, B~D = 10 μ m).

somewhat equal; fibrous, light brownish grey (10YR6/2). Partial veil absent. Odor not distinctive. Cespitose on leaf litter.

Hyphal system monomitic, generative hyphae with clamps, somewhat thick-walled, 2.5~5.0 μm diam. Basidia clavate with 4 sterigmata and basal clamp; 18.5~29 $\times$ 5.5~7 μm . Cheilocystidia and pleurocystidia absent. Basidiospores smooth, inamyloid, lacrymoid to elliptical, (6.1~) 6.4~9.1 (~9.7) $\times$ (3.2~) 3.7~5.1 (~5.4) μm .

Note: The fruit body of KUC20140804-02 is somewhat larger than the previously described fruit body of *G. iocephalus*, but microscopic characteristics are a match [17]. Although an odor was reported in a previous description, our specimen has no distinctive odor. The odor of fresh Korean *G. iocephalus* is uncertain because we examined a dried specimen. To verify these data, additional specimens of Korean

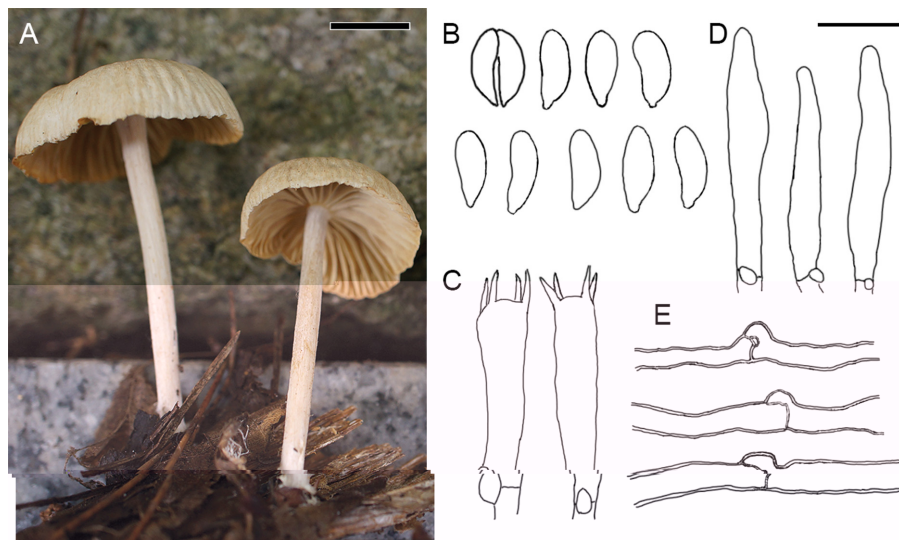


Fig. 4. The basidiocarp (A) and microscopic features (B-E) of *Gymnopus subnudus* KUC20150814-11 (NIBRFG0000141859). B, Basidiospores; C, Basidia; D, Cheilocystidia; E, Generative hyphae (scale bars: A = 1 cm, B-E = 10 μ m).

somewhat reniform in side view, (7.7~) 8.7~10.9 (~11.3) $\times$ 3.1~4.1 (~4.3) μ m.

Specimen examined: Korea, Gyeonggi-do, Mt. Yongmun, 37°32'55" N, 127°34'21" E, on the leaf litter, 14 August 2015, Seokyeon Jang, KUC20150814-11 (NIBRFG0000141859, GenBank accession No. KX513747); Korea, Gyeonggi-do, Gwacheon-si, Seoul Grand Park. 37°25'18" N, 127°1'29" E, on leaf litter, 11 Sep 2015, Seokyeon Jang, KUC20150911-19 (NIBRFG0000141933, GenBank accession No. KX513748).

Note: The characteristics of our specimens are consistent with the previous description of *Gymnopus subnudus* except the shape of basidiospores [17]. *G. subnudus* is characterized by its somewhat longer basidiospores than other Korean *Gymnopus* spp. In the phylogenetic tree (Fig. 1), the sequences of our specimens were placed in the monophyletic group of *G. subnudus* with 97% of posterior probabilities. Its sister-group species was *G. peronatus*, and it is corresponded with the previous study [4].

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