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

Seokyun Jang1, Yeongseon Jang2, Young Woon Lim1, Changmu Kim4, Byeong Jun Ahn2, Sung-Suk Lee2 and Jae-Jin Kim1,*

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
were obtained from Korea Mushroom Resource Bank (KMRB; Seoul, Korea). *Gymnopus* sp. NIBRFGR0000106907 (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]. Most of our specimens were clustered with the 6 known species: *G. confliuens* (Pers.) Antonin, *G. dryophilus* (Bull.) Murrill; *G. ioecephalus* (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. confliuens*, 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 parapolythetic in our phylogenetic tree. In one study on Korean *G. luxurians*, this strain was found to be monophylethic with other *G. luxurians* strains [8]. Analysis of additional DNA regions may be needed to identify such specimens exactly. *G. ioecephalus*, *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. polyphlylus* 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 ioecephalus* (Berk. & M. A. Curtis) Halling, Mycotaxon 63: 364 (1997) (Fig. 2).
Pileus 2–4 cm; convex with incurred 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 iocephaslus KUC20140804-02 (NIBRFG0000138670). B, Basidiospores; C, Basidia; D, Generative hyphae (scale bars: A = 1 cm, B–D = 10 µm).
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 × 5.5~7 μm. Cheilocystidia and pleurocystidia absent. Basidiospores smooth, inamyloid, lacrymoid to elliptical, (5~) 5.2~7.4 × 2.6~3.5 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 *G. iocephalus* are needed. In the phylogenetic tree (Fig. 1), *G. iocephalus* KUC20140804-02 is located in the monophyletic group of *G. iocephalus* with 100% posterior probabilities.

**Specimen examined:** Korea, Seoul, Bukhansan National Park, Mt. Dobong, 37°41’8’’ N, 127°2’10’’ E, on the leaf litter, 4 Aug 2014, Seokyoon Jang, KUC20140804-02 (NIBRFG0000138670, GenBank accession No. KX513745).

---

**Gymnopus polygrammus** (Mont.) J. L. Mata Mycotaxon 86: 313 (2003) (Fig. 3).

Pileus 1.5~2 cm; convex with incurved margin when young, becoming planoconvex; smooth, sometimes margin uplifted when aged; dark yellowish brown (10YR4/6) to very pale brown (10YR5/6) when fresh, becoming dark brown (10YR3/3) when dry; radially streaked; surface moist, but dries rapidly. Lamellae adnexed to free; subdistant, Stipe 3~4 cm long, 2~3 mm wide; central, terete, somewhat equal; fibrous, yellowish brown (10YR5/6). Partial veil absent. Odor not distinctive. Scattered to subcespitose on leaf litter.

Hyphal system monomitic, generative hyphae with clamps, thin or somewhat thick-walled, 2.5~4.5 μm diam. Basidia clavate with 4 sterigmata and basal clamp; 16.5~27.5 (~29) × 4~7 μm. Cheilocystidia and pleurocystidia absent. Basidiospores smooth, inamyloid, lacrymoid to elliptical, (5~) 5.2~7.4 × 2.6~3.5 μm.

**Note:** The fruit body of KUC20140804-01 is smaller than in the previous description of *G. polygrammus*, but microscopic characteristics are in good agreement except for the width of spores [18]. The small size of the fruit body was attributed to individual variation. Additional *G. polygrammus* specimens are needed to confirm this characteristic. In the phylogenetic tree (Fig. 1), *G. polygrammus* KUC20140804-01 is located in the monophyletic group of *G. polygrammus* with 100% posterior probabilities.

**Specimen examined:** Korea, Seoul, Bukhansan National Park, Mt. Dobong, 37°41’8’’ N, 127°2’10’’ E, on the leaf litter, 4 Aug 2014, Seokyoon Jang, KUC20140804-01 (NIBRFG0000138669, GenBank accession No. KX513746).

---

**Gymnopus subnudus** (Ellis ex Peck) Gilliam, Mycotaxon 4: 136 (1976) (Fig. 4).

Synonym: *Collybia subnuda* (Ellis ex Peck) Gilliam.

Pileus 2~4 cm; convex with incurved margin when young, becoming broadly convex; dry; very pale brown (10YR7/4) when dry; radially streaked; margin somewhat wrinkled and broadly lined; involute when dry. Lamellae adnate; distant to subdistant. Stipe 3~7 cm long, 3~5 mm wide; central, terete or compressed; somewhat equal; fibrous, very pale brown (10YR7/4) when dry. Partial veil absent. Odor not distinctive.

Hyphal system monomitic; generative hyphae with clamps, somewhat thick-walled, 2.0~5.0 μm diam. Basidia clavate with 4 sterigmata and basal clamp; 27~35 × 4.5~8 μm. Cheilocystidia variously shaped (often fusiform) with basal clamp, thin-walled, somewhat projecting, sometimes lobed, (25.5~) 27~34.5 (~37) × 4~5.5 μm. Pleurocystidia absent. Basidiospores smooth, lacrymoid to elliptical in front view.

---

*Fig. 3.* The basidiocarp (A) and microscopic features (B~D) of *Gymnopus polygrammus* KUC20140804-01 (NIBRFG0000138669). B, Basidiospores; C, Basidia; D, Generative hyphae (scale bars: A = 1 cm, B~D = 10 μm).
somewhat reniform in side view, (7.7~) 8.7~10.9 (~11.3) × 3.1~4.1 (~4.3) µm.

Specimen examined: Korea, Gyeonggi-do, Mt. Yongmun, 37°32’55” N, 127°34’21” E, on the leaf litter, 14 August 2015, Seokyoung 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, Seokyoung 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].

ACKNOWLEDGEMENTS

This research was supported by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea.

REFERENCES

1. Antonín V, Sedlák P, Tomšovský M. Taxonomy and phylogeny of European Gymnopus subsection Levipes (Basidiomycota, Omphalotaceae). Persoonia 2013;31:179-87.
2. Antonín V, Halling RE, Noordeloos ME. Generic concepts within the groups of Marasmius and Collybia sensu lato. Mycotaxon 1997;63:359-68.
3. Lee WS, Lim YW, Kim JI, Yun HY, Kim C, Park YJ. Korean Society of Mycology. National list of species of Korea: Basidiomycota. Incheon: National Institute of Biological Resources; 2015.
4. Antonín V, Ryoo R, Ka KH. Marasmioid and gymnopoid fungi of the Republic of Korea. 7. Gymnopus sect. Androsacei. Mycol Prog 2014;13:703-18.
5. White TJ, Bruns TD, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315-22.
6. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113-8.
7. Hong JH, Jang S, Heo YM, Min M, Lee H, Lee YM, Lee H, Kim JJ. Investigation of marine-derived fungal diversity and their exploitable biological activities. Mar Drugs 2015;13: 4137-55.
8. Jang S, Jang Y, Kim JJ. New records of two agarics: Galerina sideroides and Gymnopus luxurians in South Korea. Kor J Mycol 2015;43:88-91.
9. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 2013;30:772-80.
10. Maddison DR, Maddison WP. MacClade 4: analysis of phylogeny and character evolution. Version 4.08. Sunderland (MA): Sinauer Associates; 2005.
11. Nylander JA. MrModeltest v2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University; 2004.
12. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 2003;19:1572-4.
13. Largent DL, Johnson D, Watling R. How to identify mushrooms to genus, III. Microscopic features. Eureka (CA): Mad River Press; 1977.
14. Munsell Color. Munsell soil color charts with genuine Munsell.

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 µm).
color chips. Grand Rapids (MI): Munsell Color; 2009.
15. Park WH, Lee JH. New wild fungi of Korea. Seoul: Kyohaksa; 2011.
16. Seok SJ, Lim YW, Kim CM, Ka KH, Lee JS, Han SK, Kim SO, Hur JS, Hyun IH, Hong SG et al. List of List of mushrooms in Korea. Seoul: Korean Society of Mycology; 2013.
17. Roy Halling’s Collybia site [Internet]. New York: New York Botanical Garden; 2004[cited 2016 Apr 30]. Available from: http://www.nybg.org/bsci/res/col/vestiped. html.
18. Dennis RW. Some Agaricaceae of Trinidad and Venezuela. Leucosporae: Part 1. Trans Br Mycol Soc 1951;34:411-82.