

The Fungicolous Ascomycetes Genus *Hypomyces* in Korea

Minkyong Kim^{1,2}, Chorong Ahn¹ and Changmu Kim^{1,*}

¹Microorganism Resources Division, National Institute of Biological Resources, Incheon 22689, Korea

²Division of Life Sciences, Incheon National University, Incheon 22012, Korea

Abstract The genus *Hypomyces* contains fungi that grow on mushrooms, including agarics, boletes, and Aphyllophorales. While 53 *Hypomyces* species have been reported worldwide, only one was in Korea. In this study, two new Korean species were identified as *H. luteovirens* and *H. tubariicola* based on morphology and internal transcribed spacer sequencing.

Keywords Fungicolous fungi, *Hypomyces luteovirens*, *Hypomyces tubariicola*, New recorded

Hypomyces (class Sordariomycetes and order Hypocreales) is the largest genus of ascomycetes and consists exclusively of fungicolous fungi. Approximately 53 species in this genus have been identified worldwide [1, 2]. The type species of *Hypomyces* is *H. lactifluorum* (Schwein.) Tul. & C. Tul., which is characterized by forming brightly- or lightly-colored perithecia in the subiculum, thickening of the cylindrical asci at the apex, being fusiform and apiculate, and having 0–1 septa and a warted ascospore [3–6]. The most conspicuous characteristic of *Hypomyces* species is their basidiomycetous habitat, which includes agarics, boletes, and Aphyllophorales [4–6]. Few *Hypomyces* species have been found on discomycetes [3]. The range of hosts and morphological variation are regarded as the key characteristics defining this genus.

Hypomyces was first characterized by Fries [7] as a subgenus of *Hypocrea* Fr. and was later revised to a genus by Tulasne and Tulasne [8]. The first detailed taxonomic treatment of this group was performed by Arnold [9], who distinguished between *Hypomyces* and related genera.

Adopting the broader concepts, Rogerson and Samuels [3–6] subdivided the genus according to host group. Based on molecular phylogenetic analyses using large subunit rDNA, *Hypomyces* was determined to be polyphyletic and include five major clades [2, 10, 11]. More detailed studies using additional markers for genera, including internal transcribed spacer (ITS), *rpb1*, *rpb2*, and *tef* sequencing, have been performed to identify genera-specific characteristics [12–14]. Due to the high probability of successfully identifying a broad range of fungi [15] using ITS rDNA, it is considered a universal DNA barcode marker for fungi. To confirm the taxonomic status of the two previously unrecorded species discovered in this present study, phylogenetic analyses were performed on the ITS region.

Only one species in the genus *Hypomyces*, *H. chrysospermus*, has been previously reported in Korea, where it was isolated from tomato plant soil [16]. However, we were unable to examine this specimen owing to a lack of published information on the strain and specimen. Through a survey of indigenous fungal species, we discovered two *Hypomyces* species previously unidentified in Korea, *H. luteovirens* and *H. tubariicola*, and characterized them based on ITS phylogenetic analysis and morphology. Here, we provide the detailed morphological characteristics and phylogenetic relationships of these taxa.

H. luteovirens was found at Mt. Sohwangbyeong, Pyeongchang-gun, Gangwon-do, Korea on July 22, 2014, while *H. tubariicola* was found at Mt. Cheongnyang, Bonghwa-gun, Gyeongsangbuk-do, Korea on September 4, 2015. Following collection, the specimens were dried at 40°C and preserved in the herbarium at the National Institute of Biological Resources (NIBR) according to the specimen preservation protocol of NIBR. Each dried specimen was rehydrated using 3% KOH [17] and stained with 1% phloxine. The microstructure was observed using a Nikon ECLIPSE

Mycobiology 2017 September, 45(3): 209–212
<https://doi.org/10.5941/MYCO.2017.45.3.209>
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

*Corresponding author
E-mail: snubull@korea.kr

Received June 17, 2017
Revised July 4, 2017
Accepted July 19, 2017

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

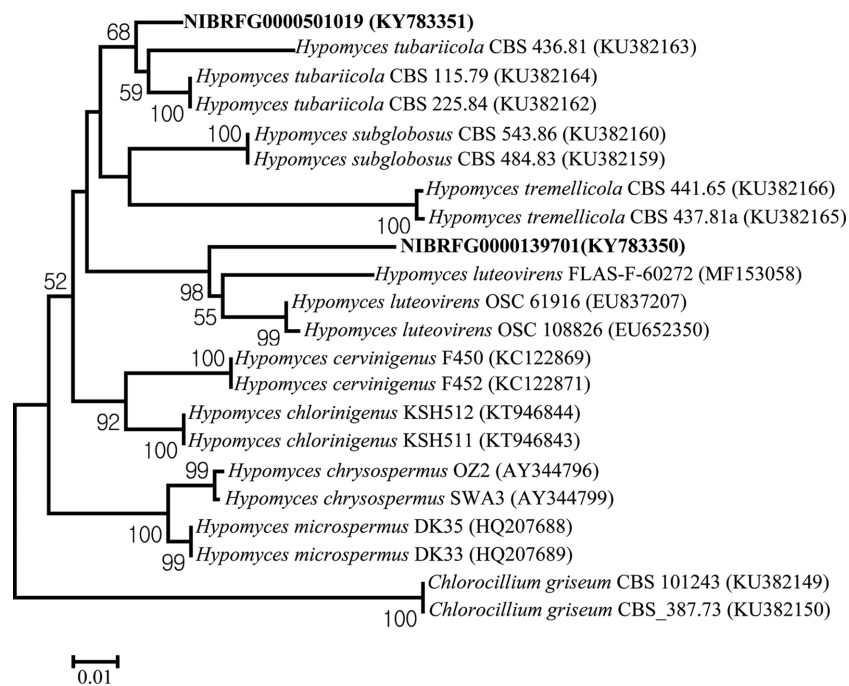


Fig. 1. Neighbor-joining tree of *Hypomyces* spp. based on the internal transcribed spacer rDNA sequences. Bootstrap values greater than 50% are indicated at the nodes. The sequences obtained in this study are shown in boldface. GenBank accession numbers are shown in parentheses. Scale bar indicates the number of substitutions per site for each branch.

80i light microscope (Tokyo, Japan). The lengths and widths of 20 randomly selected mature asci and ascospores were measured.

Genomic DNA was extracted using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The ITSs were amplified from a total of 1 μ L of genomic DNA using the primers ITS1 and ITS4 [18] and the following thermal cycling parameters: initial denaturation for 5 min at 94°C, 30 cycles of 1 min at 94°C, 30 sec at 55°C, and 1 min at 73°C, and a final elongation for 10 min at 72°C. The resulting PCR products were purified using the AccuPrep PCR Purification Kit (Bioneer) following the manufacturer's instructions. The amplicons were sequenced by Macrogen (Seoul, Korea) using the ABI prism 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The forward and reverse sequences were assembled using BioEdit ver. 7.2.5 [19].

Sequences were aligned using Clustal X ver. 2.1 [20] and the obtained nucleotide sequences were deposited in GenBank (accession Nos. KY783350 and KY783351). The sequences of closely related *Hypomyces* species were retrieved from GenBank and multiple alignments were performed using the default settings of MAFFT v7 [21]. Alignment positions that were ambiguous were manually edited. *Chlorocillium griseum* was used as outgroup because it has previously been shown to be in a sister group of *Hypomyces* [14]. Molecular phylogenetic analysis was conducted using the neighbor-joining method implemented in MEGA 6 [22] with the Kimura 2-parameter substitution model. Bootstrap analysis was performed with 1,000 replicates to evaluate

the robustness of the tree.

Phylogenetic analysis revealed one specimen (NIBRFG0000139701) clustered in a monophyletic group with the reference sequences of *H. luteovirens* (bootstrap support, 98%) (Fig. 1). The other specimen (NIBRFG0000501019) formed a monophyletic clade with the reference sequences of *H. tubariicola* (bootstrap support, 68%). The support value for this clade was relatively low, suggesting that *H. tubariicola* has high intraspecific variation in the ITS region of its rDNA.

H. chrysospermus Tul. & C. Tul. was reported in 1987 as the first *Hypomyces* species discovered in Korea [16]. However, only the species name was published. Therefore, *H. luteovirens* and *H. tubariicola* are the first *Hypomyces* species from Korea described.

Hypomyces luteovirens (Fr.) Tul. & C. Tul., Ann. Sci. Nat., Bot., Sér. 4 13: 12 (1860) (Fig. 2).

The subiculum went from white initially to yellowish/bright yellow to yellowish green/dark green to a final blackish green. It covered the surface of the deformed gills, stipe, lamellae, and pileus of its host, particularly Russulaceae species. *H. luteovirens* had subicular hyphae 3–5 μ m wide, and was septate and filamentous. This species was perithecia globose to subglobose, pyriform or flask-shaped, 415–477 \times 250–315 μ m, yellow when fresh, olivaceous to blackish when dry, usually darker than the surrounding subiculum, and immersed in crust except for the papilla. The papilla was truncate or obtuse. This species was KOH-. Asci filiform were long and cylindrical, were 165–186 \times 6–9 μ m, bore 8

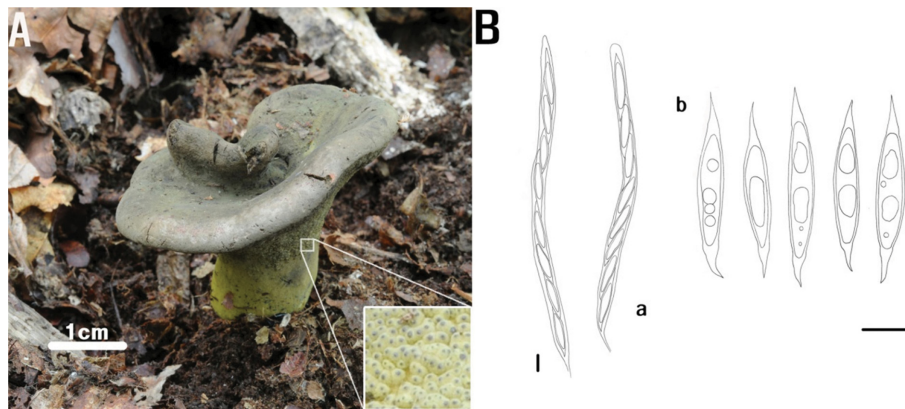


Fig. 2. Morphological features of *Hypomyces luteovirens*. A, Fruiting body; B, Microscopic features. a, ascus; b, ascospores (scale bars: B = 10 µm).

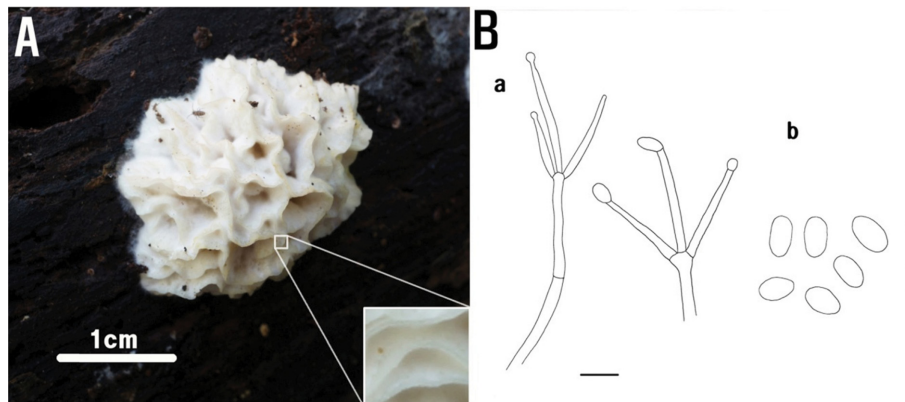


Fig. 3. Morphological features of *Hypomyces tubariicola*. A, Fruiting body; B, Microscopic features. a, conidiophore; b, conidia (scale bars: B = 10 µm).

spores, and had a thickened apex and pores when mature. Ascospores were fusiform to naviculate, 30–38 × 4.7–5.7 µm, one-celled, smooth to verrucose and apiculate, and hyaline. The apiculus were 5.0–8.3 µm long and straight to curved.

Specimens examined: Korea, Gangwon-do, Pyeongchang-gun, Mt. Sohwangbyeong, on *Russula* sp., 22 Jul 2014, C. Kim (NIBRFG0000139701).

Note: *Hypomyces luteovirens* was a conspicuous species with a distinct yellow or green color when on the host mushroom, with long unicellular ascospores and moniliform chains of cells present in the perithecial apex [6]. *H. chrysospermus* Tul. & C. Tul. partially resembles this present species. However, it differs from *H. luteovirens* based on its habitat being on boletes species and being golden yellow to reddish-brown in color. Moreover, these two species form two distant clades in the phylogenetic tree (Fig. 1).

***Hypomyces tubariicola* (W. Gams) Zare & W. Gams, Mycol. Prog. 15: 1014 (2016) (Fig. 3).**

The subiculum was white, effused, sometimes spread over the deformed host to nearby bark, and had subicular hyphae 2–3 µm wide. Conidiophores had whorls of 3–4 phialides, which usually arose from felty aerial hyphae, and were

erect and over 220 µm tall and 3–4 µm wide with smooth walls. Phialides branched off from conidiophores, were 22–45 µm long and gradually tapered from 1.7–2.7 µm to 0.8–1.7 µm. Conidia-forming globose heads were ovate, smooth-walled, 6–8.5 × 3.5–4.5 µm, moderately cyanophilic, and fell from phialides when mature.

Specimens examined: Korea, Gyeongsangbuk-do, Bonghwa-gun, Mt. Cheongnyang on *Crepidotus* sp., 4 Sep 2015, C. Kim (NIBRFG0000501019).

Note: *Hypomyces tubariicola* had a morphology similar to that of *H. tremellicola* (i.e. *Nectriopsis tremellicola*). However, the former differs from the latter in terms of rate of growth, colony pigmentation, conidial measurements, and the absence of teleomorphs *in vitro* [23]. Phylogenetic analysis based on ITS rDNA supports that these species form different clades (Fig. 1). This species was usually observed on *Tubaria* species and *Psathyrella* sp. [14], but in the present study it was found on *Crepidotus* sp.

ACKNOWLEDGEMENTS

This research was supported by the project on the survey and excavation of Korean indigenous species of the National

Institute of Biological Resources (NIBR 201701104) under the Ministry of Environment of the Republic of Korea.

REFERENCES

- Kirk PM, Cannon PF, Minter DW, Stalpers JA. Dictionary of the fungi. 10th ed. Wallingford: CABI; 2008.
- Põldmaa K, Larsson E, Kõljalg U. Phylogenetic relationships in *Hypomyces* and allied genera, with emphasis on species growing on wood-decaying homobasidiomycetes. *Can J Bot* 2000;77:1756-68.
- Rogerson CT, Samuels GJ. Species of *Hypomyces* and *Nectria* occurring on Discomycetes. *Mycologia* 1985;77:763-83.
- Rogerson CT, Samuels GJ. Boleticolous species of *Hypomyces*. *Mycologia* 1989;81:413-32.
- Rogerson CT, Samuels GJ. Polyporiculous species of *Hypomyces*. *Mycologia* 1993;85:231-72.
- Rogerson CT, Samuels GJ. Agaricolous species of *Hypomyces*. *Mycologia* 1994;86:839-66.
- Fries EM. Systema orbis vegetabilis. Primas lineas novae constructionis periclitatur Elias Fries. Pars I. Lund: Plantae Homonemae; 1825.
- Tulasne LR, Tulasne C. De quelques sphères fongicoles, à propos d'un mémoire de M. Antoine De Bary sur les Nyctalis. *Ann Sci Nat Bot* 1860;13:5-19.
- Arnold GR. Zur systematik der Hypomycetaceae. *Nova Hedwigia* 1971;21:529-35.
- Põldmaa K. Generic delimitation of the fungicolous Hypocreaceae. *Stud Mycol* 2000;45:83-94.
- Rehner SA, Samuels GJ. Molecular systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorphs. *Can J Bot* 1995;73:816-23.
- Põldmaa K. Tropical species of *Cladobotryum* and *Hypomyces* producing red pigments. *Stud Mycol* 2011;68:1-34.
- Tamm H, Põldmaa K. Diversity, host associations, and phylogeography of temperate aurofusarin-producing *Hypomyces/Cladobotryum* including causal agents of cobweb disease of cultivated mushrooms. *Fungal Biol* 2013;117:348-67.
- Zare R, Gams W. More white verticillium-like anamorphs with erect conidiophores. *Mycol Prog* 2016;15:993-1030.
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. Fungal Barcoding Consortium. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci U S A* 2012;109:6241-6.
- Lee SK, Suh JS, Kim YS, Park JK. Studies on phytotoxin in intensively cultivated upland crops. II. Population and identification of soil microorganisms in rhizosphere of upland crops. *J Korean Soc Soil Sci Fert* 1987;20:179-83.
- Largent DL, Thiers HD. How to identify mushrooms to genus II: field identification of genera. Eureka (CA): Mad River Press; 1977.
- White TJ, Bruns T, 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. San Diego (CA): Academic Press; 1990. p. 315-22.
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95-8.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876-82.
- Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772-80.
- Tamura K, Stecher K, Peterson D, Filipiński A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725-9.
- Gams W, Van Zaayen A. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. *Neth J Plant Pathol* 1982;88:57-78.