RESEARCH ARTICLE

First Report of *Cantharellus* Species, *C. hainanensis*, in Korea

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ABSTRACT

As part of a study on the diversity of edible *Cantharellus* species in South Korea, molecular and morphological identifications were performed on specimens collected at a local market in Goesan-gun, Chungcheongbuk-do. Based on morphological characteristics and sequence information of the transcription elongation factor 1-alpha gene, the specimen was verified as *Cantharellus hainanensis*. This study presents the first record of *C. hainanensis* in South Korea and its distribution outside of China.

Keywords: Chanterelle, Ectomycorrhiza, Macrofungi, Mushroom, Unreported species

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INTRODUCTION

The genus *Cantharellus* is among the most highly prized genera of wild, edible mushrooms worldwide. Because of their distinctive apricot-like aroma and excellent flavor, *Cantharellus* spp. have been used as popular culinary ingredients in various countries, and their commercial value has been estimated at billions of USD [1–3]. Recent pharmacological and biochemical studies have improved their market potential by elucidating their bioactive properties. For instance, extracts of *C. cibarius* have exhibited diverse functional activities, including antimicrobial [4], antioxidant [5,6], anti-aging [7], analgesic [8], and anticancer [9–11]. Similarly, extracts of *C. applanatus* have demonstrated anti-bacterial and anticancer activities [12]. A recent report added its ability to inhibit tyrosinase activity and suppress the expression of melanin synthesis-related proteins, highlighting its potential application in cosmeceutical formulations, particularly skin-whitening products [12].

In Korea, *Cantharellus* spp. are commonly called "*Kwe-Koh-Ri* (oriole)", "*Sahl-Goo* (apricot)", or "*Wee-Got* (cucumber flower)" mushrooms [13,14]. Several documents record that *C. cibarius*, *C. cinnabarinus*, *C. ferruginascens*, *C. friesii*, and *C. tarbenensis* are native to Korea, but the reliability of earlier taxonomic studies is limited by a reliance on morphological identification and weak genetic data. Since the late 2010s, collaborative efforts between the National Institute of Forest Science and Czech mycologists have resulted in the molecular verification of several additional taxa, namely *C. albovenosus*, *C. applanatus* (previously identified as *C. anzutake*), *C. citrinus*, *C. curvatus*, *C. hongneungensis*, *C. koreanus*, and *C.*

subvaginatus [15–18]. Despite these advancements, species diversity within Korean *Cantharellus* remains under-documented when compared to neighboring China, where molecular analyses have confirmed 28 distinct species [19].

To address this gap, the present study evaluates the species diversity and improves the taxonomic resolution of wild *Cantharellus*. First, bulk chantarelles collected from a local market were subjected to morphological examination and molecular identification. We identified *C. hainanensis*, a species previously collected only from Hainan Island and Henan Province in China. We will continue to explore native *Cantharellus* taxa, study their conservation, and develop commercial cultivation methods.

MATERIALS AND METHODS

Specimen and morphology

Fresh wild chanterelles collected from Goesan-gun, Chungcheongbuk-do, were purchased in July 2024. The specimen was dried at 45℃ for 48 hours and deposited in the herbarium of the National Institute of Forest Science. Macroscopic characteristics of fresh basidiocarps were studied by examining dried materials mounted in 3% KOH using a TCS SPE confocal microscope (Leica Microsystems, Wetzlar, Germany) at 630 x magnification.

PCR amplification and sequencing

Genomic DNA was isolated from fresh basidiocarps or fruiting body tissue using a DNeasy Plant Pro kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. The nuclear ribosomal large subunit (nucLSU) and internal transcribed spacer (ITS) regions were amplified using the primer pairs LR0R/LR5 and ITS1F/ITS4, respectively [20–22]. Partial regions of transcription elongation factor 1-alpha (*TEF-1α*) and the second largest subunit of RNA polymerase II (*RPB2*) were amplified using the primer pairs *tef-1*Fcanth/*tef-1*Rcanth and *RPB2*-5FCanth/*RPB2*-7cRCanth, respectively [23]. The PCR products were sequenced by Macrogen Inc. (Seoul, Korea) using an ABI 3730xl DNA Analyzer (Applied Biosystems, CA, USA). The obtained sequences were deposited in GenBank (accession numbers: PV776357, PV777658, PV817755, and PV817756).

Phylogenetic analysis

Phylogenetic relationships were inferred using the maximum likelihood method in MEGA 12 [24]. Multiple sequences were aligned by Multiple Sequence Comparison by Log-Expectation (MUSCLE) using the sequences of *TEF-1α* of 55 taxa of 31 *Cantharellus* species. The Tamura-Nei model was used for substitution. The robustness of the inferred phylogeny was assessed using bootstrap analysis with 1,000 replicates. The final tree was presented and annotated using MEGA 12.

RESULTS

The most likely tree inferred from the alignment of *TEF1-α* sequences of 55 taxa of *Cantharellus* species placed the specimen NIFoS20240725-01 within a monophyletic clade with the reference sequences of *C. hainanensis*, with substantial bootstrap support of 99% (Fig. 1).

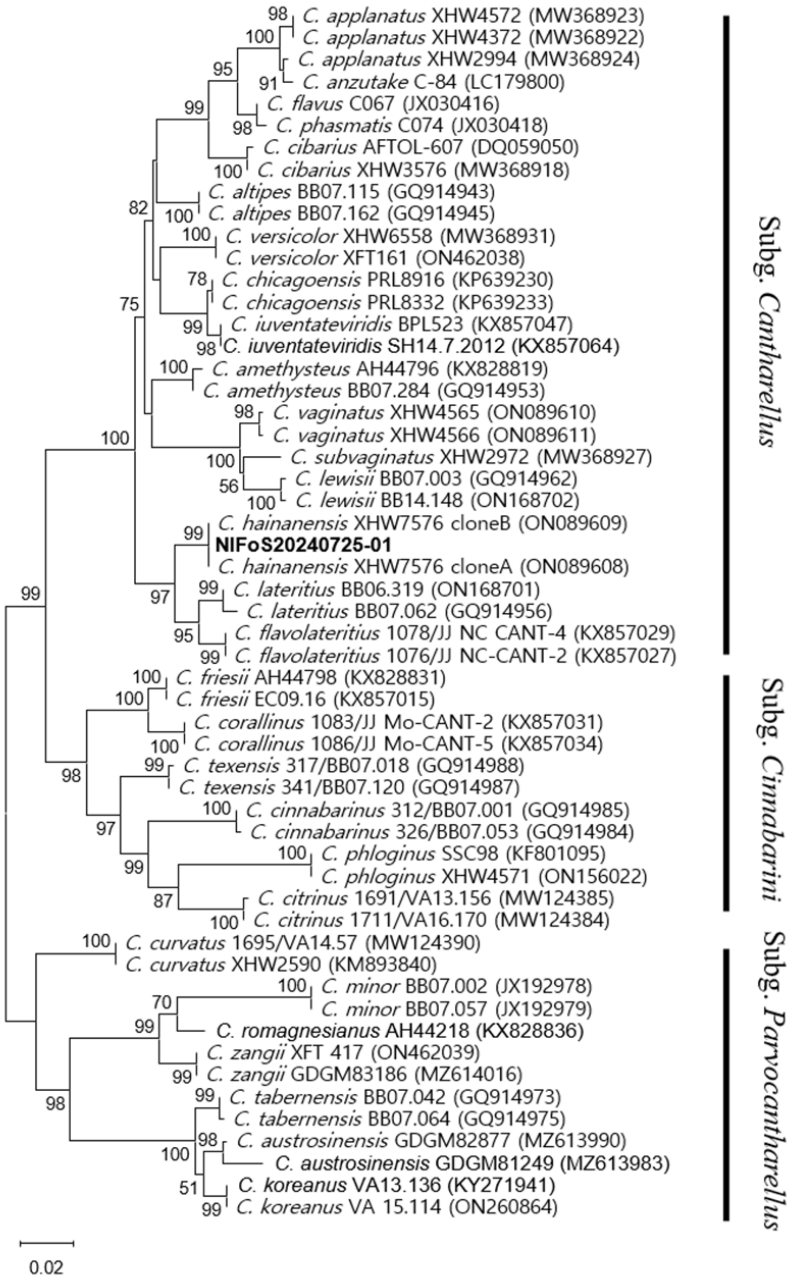

Fig. 1. The maximum-likelihood tree (-lnL = 3,672.08) constructed using transcription elongation factor 1-alpha gene sequences of *Cantharellus* species. Numbers on the nodes indicate bootstrap support (> 70%). The sequence obtained in this study is highlighted in bold.

Taxonomy

Cantharellus hainanensis N. K. Zeng, Zhi Q. Liang & S Jiang, Mycoscience 58(6): 439 (2017) (Figs. 2 and 3)

Position in classification: Hydnaceae, Cantharellales, Agaricomycetes, and Basidiomycota.

Pileus 3.8-5.1 cm broad, yellow to yellowish-brown, infundibuliform; surface dry and dull; margin incurved and irregularly undulating; no change in color when injured. **Hymenophore** decurrent, variable development, usually smooth, weakly veined, sometimes with distinct veins, cream to yellowish-white. **Stipe** 3.8-4.5 × 1.0–1.6 cm, central, sub-cylindrical; yellowish white to yellowish brown. **Smells** weakly fruity. **Spore print** not obtained.

Basidiospores 5.3-6.8-7.8 × 4.3-5.2-6.4 µm, Q (length/width) = 1.11-1.33-1.65 (n = 53), smooth, oval, ellipsoid, thin-walled, hyaline in KOH. **Basidia** clavate to subcylindrical, (4-)5-spored, and hyaline in KOH. **Pileipellis** 3.3-9.2 µm wide, cylindrical or subclavate, infrequently branched, hyaline to colorless in KOH. **Clamp connections** present in all the tissues.

Edibility: Edible

Distribution: South Korea; China

Specimen examined: Cheongcheon-myeon, Goesan-gun, Chungcheongbuk-do, Republic of Korea; July 25, 2024 (NIFoS20240725-01).

Notes:

Phylogenetically, *C. hainanensis* is related to *C. applanatus* and *C. subvaginatus* among the previously recorded Korean species. However, *C. hainanensis* is differentiated by its infundibuliform basidiomata and decurrent hymenophores with smooth veins.

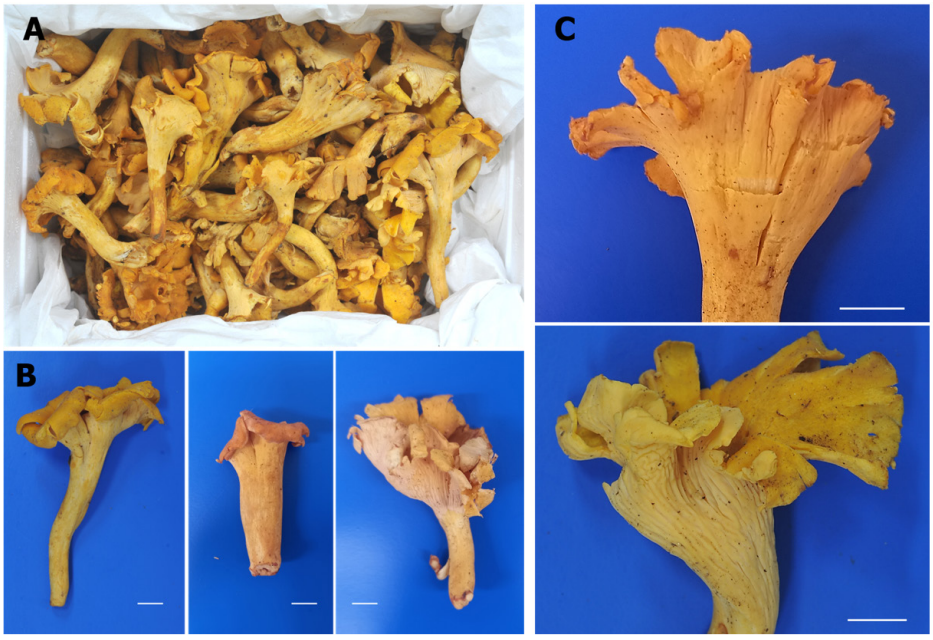

Fig. 2. Basidiomata of *Cantharellus hainanensis* (NIFoS20240725-01) collected at a local market in Goesan-gun, Chungcheongbuk-do. Bars = 1 cm.

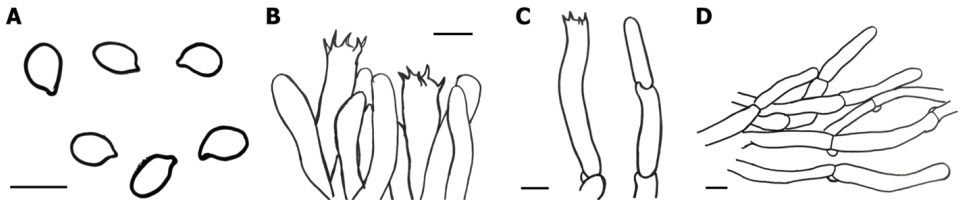

Fig. 3. Microscopic features of *Cantharellus hainanensis* (NIFoS20240725-01). A, Basidiospores; B and C, Basidia; D, Pileipellis. Bars = 10 μm.

DISCUSSION

Cantharellus spp. are widely consumed as food ingredients in Korea, and are distributed as fresh mushrooms in local and online markets. Based primarily on morphological characteristics, more than ten species, including *C. cibarius*, are presumed to be native to Korea [13,14]. Due to the inherent difficulty in distinguishing species within the genus, identification "in the pre-molecular era" relied predominantly on general attributes such as size and color [19]. Consequently, as in other Asian countries, the taxonomic identification of these fungi may have significant flaws. In support of this, molecular identification conducted in Korea during the 2010s by the National Institute of Forest Science and Czech mycologists revealed six novel species [15–18]; previously recorded species were not found, indicating the urgent need for accurate species identification.

The ITS region is commonly used for fungal identification owing to its high copy number, which facilitates amplification. However, in *Cantharellus* spp., the ITS region exceeds 1 kb and exhibits substantial heterogeneity (e.g., the *C. applanatus* strain KUN-HKAS109695 harbors a 131 bp deletion), complicating molecular identification. Recent comprehensive analyses of Asian *Cantharellus* spp. incorporating both morphological and genetic data has led to significantly advanced species identification [19]. To our knowledge, eight species, *C. hainanensis*, *C. albovenosus*, *C. applanatus*, *C. citrinus*, *C. curvatus*, *C. hongneungensis*, *C. koreanus*, and *C. subvaginatus*, have been molecularly verified in Korea [15–18]. This number is considerably lower than previous estimates based on morphological classification, and starkly contrasts with the 28 species reported in China, highlighting the necessity for further taxonomic studies on Korean *Cantharellus* species.

As ectomycorrhizal basidiomycetes, *Cantharellus* spp. relies on symbiotic associations with host plants for energy acquisition, which makes artificial cultivation more challenging than for saprotrophic fungi. However, Korean researchers have successfully cultivated the ectomycorrhizal basidiomycete *Tricholoma matsutake* [25–28], inducing mycorrhizal formation in the host plant *Pinus densiflora* in the forest. Subsequently, they facilitated the spread of mycorrhizae to neighboring hosts, leading to the production of *T. matsutake* fruiting bodies. This method was scientifically verified using molecular marker microsatellite or simple sequence repeat markers, demonstrating that fruiting bodies produced at the pine tree transplant site indeed originated from the same site where the *T. matsutake*-infected pine trees were made [28]. Moreover, continuous fruiting was observed for over eight years. Reports indicate that artificial fruiting of certain *Cantharellus* spp. has also been achieved under laboratory conditions [29,30]. Using the recently

obtained pure isolates of *C. hainanensis* and *C. subvaginatus*, we will further develop cultivation methods for *Cantharellus* spp.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest for this study.

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