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Short Communication

Collection, Identification, and Culturing of *Phlebopus* sp. from Taiwan

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Abstract

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Mushrooms are rich in nutrients and popular delicacy. However, due to limitations in cultivation techniques, some mushrooms that are symbiotic with plants still rely on wild harvesting. Boletes are well-known edible wild mushrooms. In 2024, samples similar to bolete mushrooms were collected in Taichung and Yunlin, Taiwan. Both fruiting bodies were medium to large in size with a dark brown pileus surface and the porous structure of hymenium. Upon vertical sectioning of the pileus, the context was yellow sponge-like tissue. Two isolates, designated Ph-1 and Ph-2, were isolated from these samples. To further identify the two isolates, the internal transcribed spacer (ITS) region of rDNA was amplified by polymerase chain reaction (PCR) with the primers ITS1/ITS4 and sequenced. Phylogenetic analysis preliminarily identified both isolates as *Phlebopus* sp. Additionally, to investigate the optimal cultivation conditions of isolates, their colony lengths were measured on three different media including potato dextrose agar (PDA), potato rice bran (PR), and sawdust potato glucose (SPG) medium. The results showed that Ph-1 grew faster than Ph-2 on all three media, and both isolates exhibited the highest growth rate on the SPG medium. It indicated that sawdust effectively promotes the mycelial growth of *Phlebopus* sp. Bolete mushrooms are well-known for their unique flavor and fleshy texture. This study is the first report to identify the *Phlebopus* sp. isolated from Taiwan by combining phenotype and ITS sequence analysis. To establish commercial cultivation methods for bolete mushrooms in Taiwan, future research will focus on cultivating fruiting bodies and evaluating the feasibility of artificial cultivation.

Key words: Edible bolete, *Phlebopus* sp., Mycelium growth medium.

Mushrooms are rich in nutrients and highly favored by consumers. Due to limitations in cultivation techniques, some mushrooms that are symbiotic with plants still have to be obtained through wild harvesting. Among them, boletes are well-known edible wild mushrooms. They can be recognized by boletoid fruiting bodies, olive-brown spore print, ellipsoidal brown basidiospores, and numerous clamps on the hyphae (Putra *et al*. 2024). In 2024, two fruiting bodies with characteristics similar to bolete mushrooms were collected in Taiwan (Fig. 1). The mycelium-isolate Ph-1 (accession number: TARI-Ph1) was collected from Dongshi, Taichung, and the mycelium-isolate Ph-2 (accession number: TARI-Ph2) was from Douliu, Yunlin. Both fruiting bodies were found to be medium to large in size with a dark brown pileus surface. The data on the size of Ph-1 fruiting bodies was not obtained, so only the data for Ph-2 is presented. The height of the entire fruiting body was approximately 114.32 ± 14.21 mm. The length and width of the pileus were 106.43 ±

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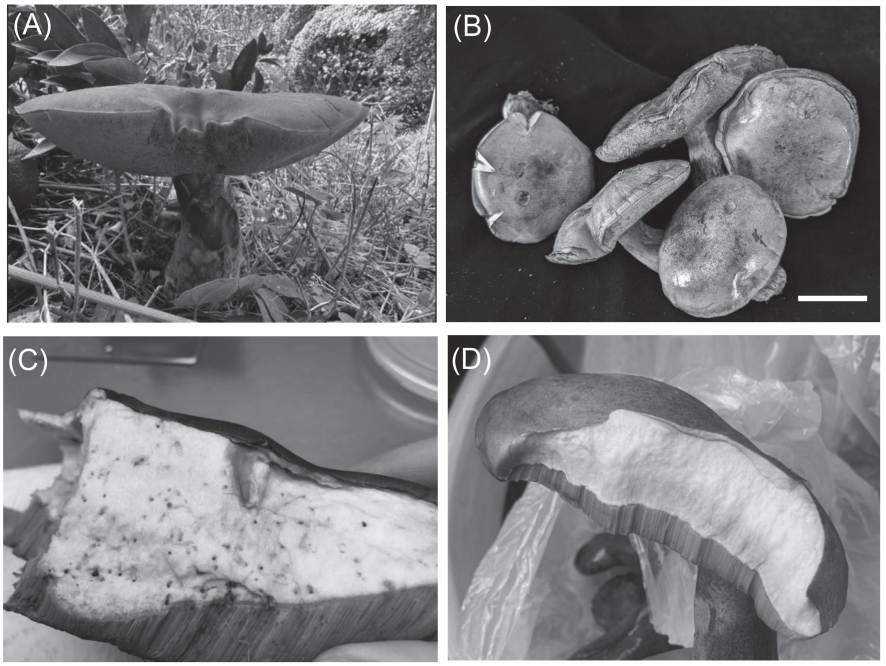

Fig. 1. The fruiting bodies of *Phlebopus* sp. samples. (A) Ph-1 and (B–D) Ph-2. Both fruiting bodies were medium to large in size with a dark brown surface. (C–D) Upon vertical sectioning, light yellow sponge-like tissues were observed. Scale bar = 50 mm. (The size of the Ph-1 fruiting body was not measured.)

9.25, and 86.3 ± 9.49 mm. Upon vertical sectioning of the pileus, light yellow sponge-like tissues were observed. The thickness of the pileus was 21.92 ± 3.28 mm, and the hymenium exhibited a porous structure. The morphology characteristics of the mushroom were similar to *Phlebopus* sp. However, due to the severe decay of the collected fruiting bodies, data on spore prints, basidia, and the number of basidiospores could not be obtained. To further identification, DNA was extracted by Maelstrom Switch 8 Automated DNA/RNA Purification Platform (Taiwan Advanced Nanotech Inc. (TANBead), Taoyuan, Taiwan), and the internal transcribed spacer (ITS) region was amplified by using the universal primers ITS1 and ITS4 (White *et al*. 1990; Blattner 1999). The ITS sequences were BLASTed against the National Center for Biotechnology Information (NCBI) blastn database. The ITS sequences of both isolates had identity with those of *Phlebopus* sp. (Ph-1: query coverage of 100%, identity of 96.62%, e-value of 0.0, NCBI number: MN962562; Ph-2: query coverage of 98%, identity of 85%, e-value of 0.0, NCBI number: MT272131). Six closely related species of Boletinellaceae family were used to construct the phylogenetic tree. ITS Sequences from other fungi were obtained from the NCBI database and aligned using ClustalW multiple alignments (Campi *et al*. 2023; Yang *et al*. 2023; Putra *et al*. 2024). Phylogenetic analysis based on ITS sequences was performed using Mega X (Kumar *et al*. 2018) by Maximum likelihood method (1,000 bootstraps) with Kimura 2-parameter and Gamma Distributed model (K2* + G model) (Fig. 2). Although, the Ph-1 (PQ049143); Ph-2 (PQ061505) can be grouped a complex with the isolates of *P. portentosus* and *P. spongiosus*, but we cannot accurately identify them. Therefore, based on their morphological characteristics, and ITS sequence analysis, the two isolates were preliminarily identified as *Phlebopus* sp. [NCBI number: Ph-1 (PQ049143); Ph-2 (PQ061505)]. Furthermore, the two isolates were cultured in

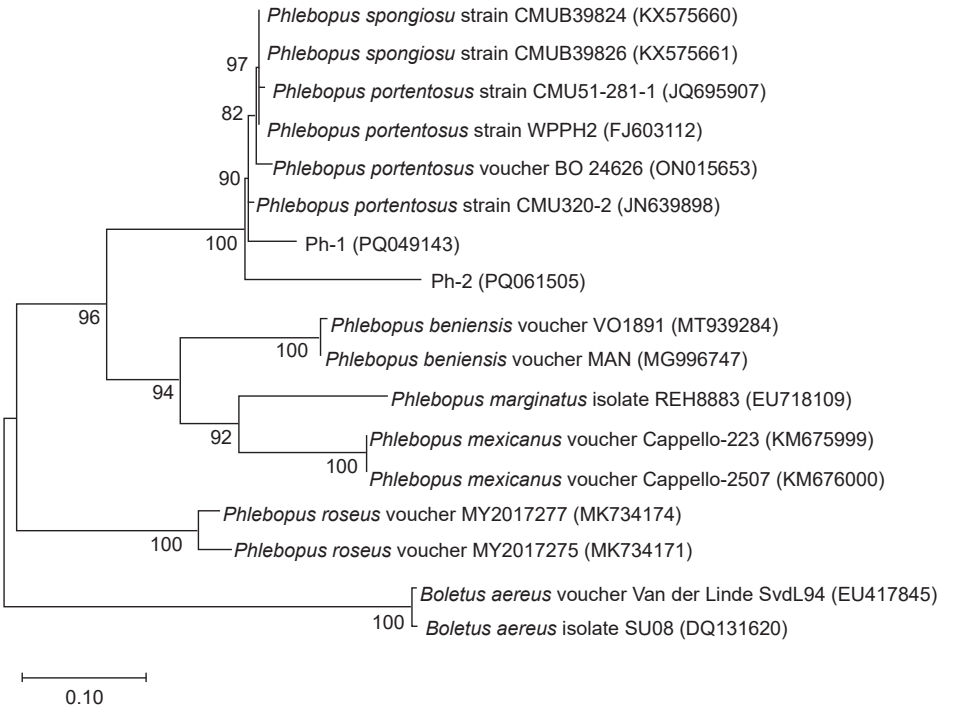

Fig. 2. Phylogenetic tree derived from maximum likelihood method with 1,000 bootstraps based on internal transcribed spacer (ITS) sequences of *Phlebopus* genera using ClustalW multiple alignments on MEGA X. *Boletus aereus* was used as outgroup. Bootstrap values above nodes represent the statistic support for clades. The scale indicates nucleotide substitution per site.

three different media at 28℃ to understand the suitable growth nutrients. The media used in this experiment were as follows: PDA (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), PR (PDA powder 39 g, agar 20 g, rice bran 2 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, $FeSO_4$ 1 g, dH_2O 1 L), and SPG (sawdust 100 g, potato 200 g, glucose 20.85 g, agar 20 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, $FeSO_4$ 1 g, dH_2O 1 L). The experiment was conducted in 2 independent trials, each containing 5 plates per isolate. Data (means ± standard error) with different letters are significantly different according to the least-significant difference test at $P < 0.05$ by using Statistical Analysis System (SAS) version 7.1. The results showed that regardless of the type of culture medium, the mycelium growth rate of Ph-1 was faster than that of Ph-2. Additionally, both isolates had the fastest mycelial growth on SPG after 14 d of cultivation (Table 1). Bolete mushrooms, belonging to the family Boletaceae, are a diverse group of fungi characterized by fleshy fruiting bodies and pores instead of gills on the underside of the cap. For example, *Phlebopus portentosus*, one of Boletaceae family, has been found in tropical and subtropical regions, including China and Northern Thailand. Due to its proteins, amino acids, and minerals, *P. portentosus* is considered a delicious wild edible mushroom (Zhang *et al*. 2017). This study is the first report to identify the *Phlebopus* sp. isolated from Taiwan by combining phenotype and ITS sequence analysis. And the results indicate that *Phlebopus* sp. cannot be identified only by ITS sequence analysis. To further identify the species, additional primers, such as RPB2 and EF1α, will need to be included for multilocus sequence analysis (MLSA) in the future. Moreover, previous studies have also reported that bolete mushrooms have symbiotic relationships with plants, and they can

Table 1. The mycelium growth rate of Ph-1 and Ph-2 on three different media.

	Ph-1[z]			Ph-2		
Day	PDA[y]	PR	SPG	PDA	PR	SPG
7 d	2.25 ± 0.05 a[x]	1.97 ± 0.03 b	1.98 ± 0.04 b	1.25 ± 0.05 d	1.41 ± 0.03 c	1.45 ± 0.07 c
10 d	2.89 ± 0.09 a	2.69 ± 0.07 b	2.82 ± 0.09 ab	2.02 ± 0.05 d	2.20 ± 0.04 c	2.19 ± 0.05 cd
14 d	3.55 ± 0.10 b	3.49 ± 0.05 b	4.26 ± 0.08 a	2.67 ± 0.04 d	2.98 ± 0.04 c	3.10 ± 0.11 c

[z] Ph-1/Ph-2: The colony radius was measured 7, 10, and 14 d after the start of mycelial growth.
[y] PDA: potato dextrose agar medium; PR: potato rice bran medium; SPG: sawdust potato glucose medium.
[x] Data (means ± standard error) with different letters are significantly different according to least significance difference (LSD) test at $P < 0.05$. Colony sizes of two isolates on three media on the same day were combined for analysis.

form ectomycorrhizal structures in the roots. Bolete mushrooms have broad hosts, including *Coffea arabica*, *Citrus grandis*, and *Pinus* sp. (Kumla *et al*. 2012, 2020). The complex symbiotic relationships make the artificial cultivation of boletes challenging. To simulate the natural growth environment of boletes, it is essential to understand the nutrition required for their growth. Kumla *et al*. (2020) found that the optimal growth conditions for the mycelium of *Phlebopus portentous* were 30℃ and pH 5.0. Additionally, they used various solid substrates for mycelium growth. Another study showed that the mixture of rice seed and sorghum grains with sawdust significantly promoted the mycelial growth of *P. portentosus* (Zhang *et al*. 2017). In this study, it was also found that the addition of sawdust in the medium could effectively increase the mycelial growth rate. Therefore, to establish commercial cultivation methods for bolete mushrooms in Taiwan, future research will focus on fruiting body cultivation and evaluating the feasibility of artificial cultivation.

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臺灣脈柄牛肝菌 (*Phlebopus* sp.) 之採集、鑑定與培養

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摘要

余祥萱、蔡承佑、呂昀陞。2025。臺灣脈柄牛肝菌 (*Phlebopus* sp.) 之採集、鑑定與培養。台灣農業研究 74(1):81–85。

菇類營養價值豐富，深受消費者喜愛。由於栽培技術之限制，部分與植物共生之菇類仍仰賴野外採集取得，牛肝菌便是其中著名可食用的野生菇類。2024 年分別於臺灣臺中市東勢區與雲林縣斗六市兩處，採集到外觀疑似牛肝菌之子實體樣本，經組織分離後暫稱為 Ph-1 與 Ph-2。兩樣本之菌傘外觀皆呈暗褐色，背面具有孔狀結構，而菌傘縱剖後，其內部構造呈淺黃色海綿狀組織。為進一步鑑定此兩菌株，本研究對其 rDNA 之內轉錄區間 (internal transcribed spacer; ITS) 進行增幅與序列分析，並由親緣分析結果初步鑑定此兩菌均為脈柄牛肝菌屬 (*Phlebopus* sp.)。此外為開發菌株之最適培養基，以供後續人工栽培參考，利用 3 種不同之營養培養基 (potato dextrose agar (PDA)、potato rice bran (PR) 及 sawdust potato glucose (SPG)) 進行菌絲生長速度測定。結果顯示 Ph-1 之菌絲於所有培養基上生長速度皆較 Ph-2 快，且兩菌株均於 SPG 培養基上具有最佳菌絲生長速度，顯見木屑添加可有效促進脈柄牛肝菌之菌絲生長。牛肝菌以其獨特的風味與肉質口感而聞名，本篇為首次結合子實體表型與 ITS 序列分析鑑定國內脈柄牛肝菌屬之研究，未來將持續進行相關栽培試驗，同時也將評估人工栽培之可行性，以建立臺灣牛肝菌之商業化栽培方法。

關鍵詞：可食用牛肝菌、脈柄牛肝菌屬、菌絲生長培養基。

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