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First report of four unrecorded macrofungal species in two national parks of South Korea

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During a mycological field survey conducted from 2023, 4 specimens of mushrooms belonging to different species were collected from national parks in South Korea. Based on molecular phylogenetic analyses and morphological examinations, four species (*Entoloma cornicolor*, *Homophron spadiceum*, *Pluteus chrysophlebius*, and *Xeromphalina enigmatica*) were reported for the first time in South Korea. This study presents morphological descriptions, color photographs, and phylogenetic analyses of these species.

Keywords: morphology, mushrooms, phylogeny, taxonomy

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Introduction

Macrofungi are a group of fungi with large, easily observable, spore-bearing structures (Niego *et al*., 2023). Approximately 14,000 species of this genus are globally distributed (Lu *et al*., 2020). They were classified under the phylum Ascomycota Caval.-Sm. and Basidiomycota R.T. Moore, based on how sexual spores are formed, according to Kinge *et al*. (2020). Ascomycota include cup, flask, and truffle-like fungi. Most mushrooms, jelly fungi, Gasteromycetes, and boletes belong to Basidiomycota. Macrofungi are an integral part of forest ecosystems and provide various ecosystem services, including innovative biotechnological, medicinal, and ecological applications (Hyde *et al*., 2019).

According to the Korean National Park Service, South Korea has 22 national parks. In this study, four basidiomycete fungi were identified in Juwangsan National Park (Gyeongsangbuk-do) and Taebaeksan National Park (Gangwon-do) of Korea. Based on their morphology and phylogeny, these species were identified as *Entoloma cornicolor* O.V. Morozova, Reschke & Noordel, *Homophron spadiceum* (P. Kumm.) Örstadius and E. Larss., *Pluteus chrysophlebius* (Berk. & M.A. Curtis) and *Xeromphalina enigmatica* K.W. Hughes & R.H. Petersen, respectively. Detailed descriptions, photographs, and phylogenetic analyses were provided here.

Materials and Methods

Sampling and morphological examination

In 2023, Wild mushroom samples were collected to assess mycological diversity in two national parks in South

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Korea, namely Juwangsan National Park and Taebaeksan National Park. The species were identified based on their macroscopic and microscopic characteristics. The dried collected samples were then mounted in distilled water and 5% KOH and observed using a Zeiss Axio Imager A1 microscope equipped with an Axiocam 503 color camera (Zeiss, Germany). The systematics of the taxa in this study correspond to those of the Index Fungorum (http://www.indexfungorum.org). The dried specimens used in this study were maintained at the National Institute of Biological Resources in Incheon, Korea.

DNA extraction, polymerase chain reaction (PCR), and phylogenetic analyses

Genomic DNA was extracted from the specimens using a TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (Takara, Japan). The internal transcribed spacer (ITS) region of rDNA was amplified using ITS1 and ITS4 primers. PCR amplicons were purified and sequenced using the Macrogen sequencing service (Seoul, Korea). The dataset was aligned using MAFFT version 7 (Katoh and Standley, 2013), and all other parameters were set to their default values. A phylogenetic tree was constructed using RAxML-HPC2 on XSEDE (v. 8.2.4) in the CIPRES Science Gateway (https://www.phylo.org). The relative robustness of the individual branches was estimated using ML bootstrap support values. GenBank accession numbers were obtained from the National Center for Biotechnology Information (NCBI), and the sequences generated in this study were assigned to NCBI (accession numbers PQ187058−PQ187061).

Results

Phylogenetic analysis

Phylogenetic tree analyses of the ITS sequences revealed the presence of four different species, namely *En-*

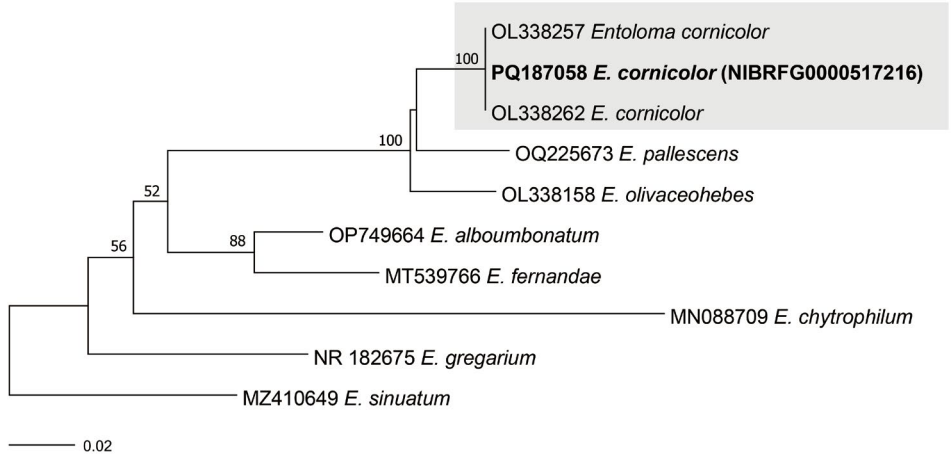


Fig. 1. RAxML phylogenetic tree based on ITS sequences of *Entoloma cornicolor*: Sequences generated in this study are indicated in bold text.

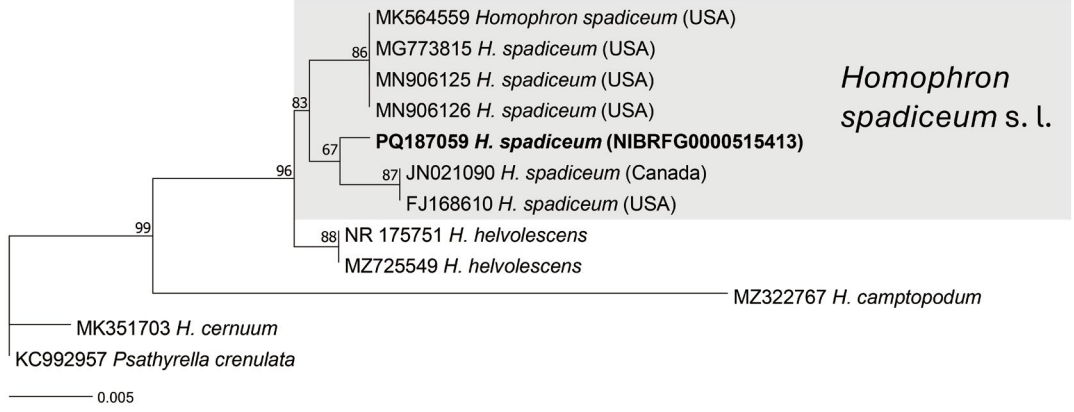


Fig. 2. RAxML phylogenetic tree based on ITS sequences of *Homophron spadiceum*: Sequences generated in this study are indicated in bold text.

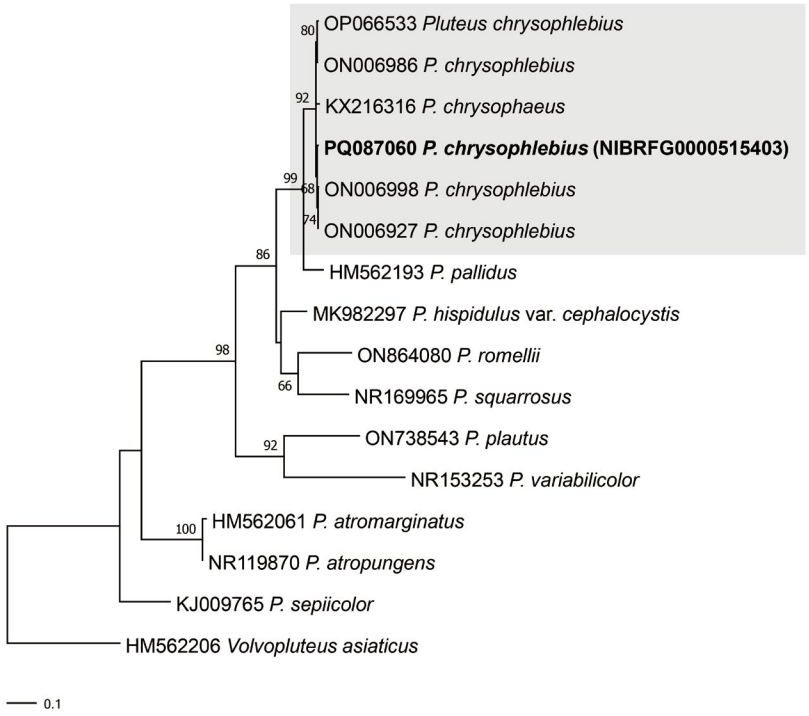


Fig. 3. RAxML phylogenetic tree based on ITS sequences of *Pluteus chrysophlebius*: Sequences generated in this study are indicated in bold text.

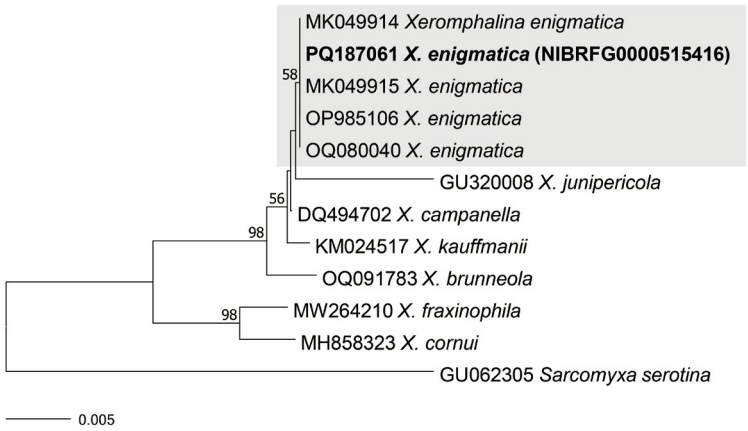


Fig. 4. RAxML phylogenetic tree based on ITS sequences of *Xeromphalina enigmatica*: Sequences generated in this study are indicated in bold text.

toloma cornicolor, *Homophron spadiceum*, *Pluteus chrysophlebius*, and *Xeromphalina enigmatica* (Figs. 1–4). The Korean collection of *E. cornicolor* clustered with *E. cornicolor*, with a bootstrap (BS) value of 100%. The Korean collection of *H. spadiceum* was found to belong to *H. spadiceum sensu lato* (Eberhardt *et al*., 2022). Although *H. spadiceum* is a type species of the genus, it was not possible to determine its species limits phylogenetically. Therefore, in this study, the Korean collection was identified as *H. spadiceum*. The Korean collection of *P. chrysophlebius* clustered within the *P. chrysophlebius* clade. This result is phylogenetically consistent with that of a previous study (Qi *et al*., 2024). In this study (Qi *et al*., 2024), the phylogenetic position of *P. chrysophlebius* was studied based on the ITS + TEF1-a sequence data, and this clade was described as the *P. chrysophlebius* clade. Therefore, the Korean collection was identified as *P. chrysophlebius*. Finally, the Korean collection of *X. enigmatica* clusters with the other sequences of *X. enigmatica*.



Fig. 5. Morphological characters of *Entoloma cornicolor*: A, B, CKU20230731-02; C, basidium; D, basidiospores. Scale bars: 3 cm (A, B); 10 μm (C, D).

Taxonomy

1. *Entoloma cornicolor* O.V. Morozova, Reschke & Noordel., in Reschke, Morozova, Dima, Cooper, Corriol, Biketova, Piepenbring & Noordeloos, Persoonia 49: 158 (2022) (Figs. 1, 5)

Diagnostic characters. Pileus 15−30 mm in diameter, conic, broadly conic to hemispherical with small umbo when young, convex and papillulate when old; upper surface yellowish-brown or grayish brown when young, pale ochraceous, yellowish beige, with paler margin, darker toward center when old, smooth, glabrous; context thin, concolorous with upper surface or paler. **Lamellae** adnate, ventricose, moderately distant, whitish when young and pinkish when old. **Stipe** 40−60 × 1.5−3.0 mm, cylindrical, slightly tapering upward, yellowish-brown, smooth, polished, basal mycelium white, tomentose. **Basidiospores** 7.6−9.7 × 5.6−7.0 μm, heterodiametrical, with 5−7 angles. **Basidia** 28.5−35.5 × 10.5−12.5 μm, clavate, thin-wall, with 4-sterigmata.

Habitat. Solitary or small groups on litter and soil in mixed forests.

Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°12′24.97″N 128°54′58.60″ E, 31 vii 2023, NK Kim, NIBRFG0000517216.

Remarks. This species is characterized by its relatively small mycenoid basidiocarps, with deeply translucently striated, beige to yellowish-brown pileus with a contrasting dark center, and polished, small, heterodiametrical basidiospores (Reschke *et al*., 2022). Their morphology closely resembled that of the Korean collection.

2. *Homophron spadiceum* (P. Kumm.) Örstadius & E. Larss., in Örstadius, Ryberg & Larsson, Mycol. Progr. 14(no. 25): 35 (2015) (Figs. 2, 6)

Diagnostic characters. Pileus 25–50 mm, convex, with inflexed margin when young, plane when old; upper surface pale pinkish when young, pale reddish-brown with dark brown center when old, covered with minute hairs at margin; context flesh colored or pink. **Lamellae** adnate, very crowded, and pinkish to pale brown when young, and brown with red tinges when old. **Stipe** 30–80 × 5–11 mm, cylindrical, equal to or slightly tapering upward, white when young, pale brown when old, flocculose at apex. **Basidiospores** 6.7–10.5 × 4.2–5.5 µm, ovoid, oblong, subcylindrical, rarely amygdaloid, thin-walled. **Basidia** 16–35 × 6.5–8.0 µm, thin-wall, with 4-sterigmata. **Pleurocystidia** 30–85 × 10–30 µm, fusiform to conical, and abundant.
Habitat. Small groups on soil in mixed forests.
Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°5′35.1″N 128°56′8.96″E, 4 vii 2023, NK Kim, NIBRFG0000515413.
Remarks. The phylogenetic tree analyzed in this study showed that this species formed an *H. spadiceum* s.l. clade consisting of three subclades (Fig. 6). In addition, this species appears to have no restricted geographical distribution. The phylogenetic results were supported by previous molecular results (Eberhardt *et al*., 2022).

3. *Pluteus chrysophlebius* (Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 5: 678 (1887) (Figs. 3, 7)

Diagnostic characters. Pileus 15–25 mm, bell-shaped to broadly conic when young, broadly convex to flat when old; upper surface bright yellow when young, dull yellow or brownish-yellow when old, veined, margin finely lined. **Lamellae** free, close, or crowded, whitish when young, pinkish when old. **Stipe** 20–45 × 1–3 mm, cylindrical, equal or slightly tapering upward, pale yellow, fragile, bald, basal mycelia white. **Basidiospores** 5.1–6.9 × 4.5–6.1 µm, subglobose to ellipsoid, smooth; hyaline, inamyloid. **Basidia** 20–30 × 7.5–11 µm, fusiform to clavate, thin-walled, 4-sterigmata. **Pleurocystidia** 35–60 × 15–20 µm, widely lageniform, thick-walled, and hyaline.
Habitat. Solitary on litter and soil in mixed forests.
Material examined. KOREA, Juwangsan National Park, Cheongsong-gun, Gyeongsangbuk-do, 36°24′1.78″N 129° 10′43.8″E, 20 vi 2023, NK Kim, NIBRFG0000515403.
Remarks. As shown in a previous study (Justo *et al*., 2011), the sequences of *P. chrysophlebius* were grouped into three distinct subclades. According to Malysheva *et al*. (2016), *Pluteus* sect. *Celluloderma* can recognize ten taxa including three species complexes, namely *P. chrysophlebius*, *P. phlebophorus*, and *P. cinereofuscus*. Therefore, *P. chrysophlebius* is recognized as a species complex, and further studies are required for the taxonomic identification of this species.



Fig. 6. Morphological characters of *Homophron spadiceum*: A, CKU20230704-01; B, basidium; C, cystidia; D, basidiospores. Scale bars: 3 cm (A); 10 µm (B–D).

4. *Xeromphalina enigmatica* K.W. Hughes & R.H. Petersen, in Aldrovandi, Johnson, O'Meara, Petersen & Hughes, Mycologia 107(6): 1280 (2015) (Figs. 4, 8)

Diagnostic characters. Pileus 7–15 mm in diameter, convex to plano-convex with a depressed center; upper surface orange-brown to yellow-brown, with stria margin. **Lamellae** decurrent, distant, bright yellow to orange, with

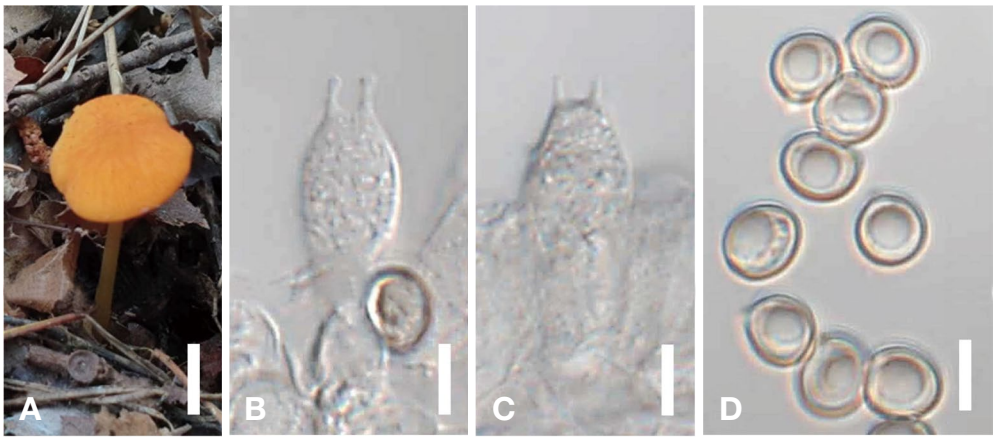


Fig. 7. Morphological characters of *Pluteus chrysophlebius*: A, CKU20230620-12; B, C, basidia; D, basidiospores. Scale bars: 1 cm (A); 5 μm (B–D).

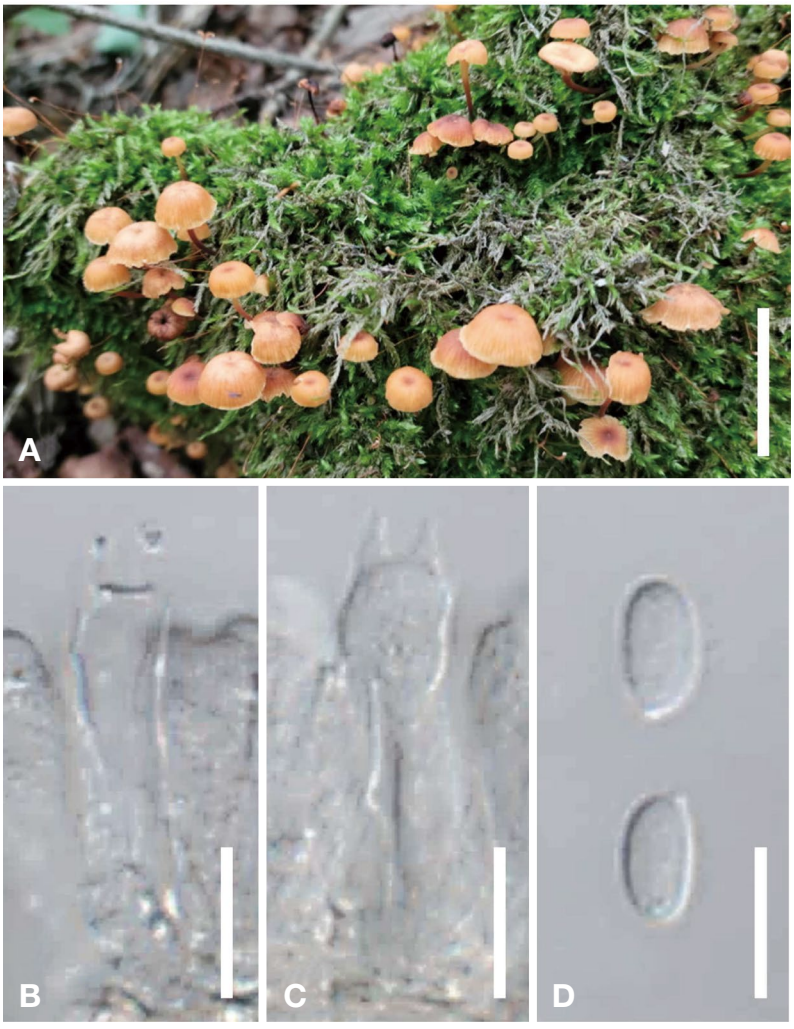


Fig. 8. Morphological characters of *Xeromphalina enigmatica*: A, CKU20230704-08; B, C, Basidia; D, basidiospores. Scale bars: 2 cm (A); 10 μm (B–D).

intervenose. **Stipe** 10–40 × 0.5–2.0 mm, cylindrical, equal to tapering downward, curved, yellowish above, reddish-brown to dark brown with hairs, smooth. **Basidiospores** 5.2–8.2 × 3.1–4.2 µm, ellipsoid, smooth, thin-walled, hyaline. **Basidia** 19–32 × 3.9–7.5 µm, clavate, hyaline, smooth, with 4-sterigmata. **Pleurocystidia** 32–62 × 5.0–14 µm, clavate to cylindrical, hyaline, thin-walled.

Habitat. Gregarious on moss in mixed forests.

Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°5′ 32.08″N 128°55′57.76″E, 4 vii 2023, NK Kim, NIBRFG0000515416.

Remarks. In a previous phylogenetic analysis based on ITS, 28S, and *RPB2* sequences, this species comprised several clades (Aldrovandi *et al*., 2015). The phylogeny in this study showed that the Korean collection is clustered with a well-supported monophyletic clade (Fig. 8). Therefore, the Korean collection was identified as *X. enigmatica*.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowlegements

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