

Three New Recorded Species of the Physalaciaceae on Ulleung Island, Korea

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Abstract Most known species in the Physalaciaceae are saprotrophs that grow on decaying leaves and wood, and approximately 21 genera in the Physalaciaceae have been reported worldwide. During an ongoing survey of indigenous fungi in Korea, four specimens belonging to the Physalaciaceae were collected on Ulleung Island. These specimens were identified as three species based on morphological characteristics and molecular analysis of rDNA-internal transcribed spacer sequences. Three species in three genera were shown to be new records in Korea: *Hymenopellis orientalis*, *Paraxerula hongoi*, and *Ponticulomyces orientalis*. The latter two are the first records of these genera in Korea. In this study, we provide detailed morphological descriptions of these species and describe their phylogenetic position within the Physalaciaceae.

Keywords *Hymenopellis*, New records, *Paraxerula*, Physalaciaceae, *Ponticulomyces*, rDNA-ITS, Ulleung Island

The family Physalaciaceae is classified in the order Agaricales (Agaricomycetes) and typified by the genus *Physalacria* Peck [1]. Approximately 21 Physalaciaceae genera have been reported worldwide. Fruit body shape in the Physalaciaceae is highly variable, ranging from agaricoid, cantharelloid, clavarioid, secotioid, and corticoid [2, 3]. Species in the Physalaciaceae are characterized by a monomitic hyphal system with clamps, narrowly clavate basidia with two to four basidiospores, and smooth, thin walled, ellipsoidal, fusiform, cylindrical, or lacrimiform basidiospores [4]. While most species in the Physalaciaceae are saprobic, found on decaying leaves and wood, several species are parasitic [4]. Among the family Physalaciaceae, *Flammulina velutipes* is a valuable edible mushroom, while the genus *Armillaria*

contains species that are severe forest pathogens [5, 6].

The family was originally defined in 1970 [7] and revised in 1985 [8]. Monophyly was confirmed in the Physalaciaceae using molecular analyses [9, 10]. The revision of the Physalaciaceae, based on morphology as well as phylogenies using the internal transcribed spacer (ITS) and the large subunit of ribosomal DNA sequences (LSU), identified several new genera and the systematics of the *Oudemansiella/Xerula* complex were also drastically revised [11]. *Cibaomyces* was proposed as a new genus based on sequence analysis of two loci, ITS and LSU [12]. A new species in the genus *Paraxerula* (*Pa. ellipspora*) was confirmed at four loci, ITS, LSU, β-tubulin, and elongation factor 1-α [13]. Although several coding genes have been used in recent fungal phylogeny studies, the ITS region remains the most commonly used locus for fungal phylogenetics, and the accumulated DNA sequence information for the ITS region has greatly improved the accuracy of identification in the Fungi [14-17]. Previously, we showed the efficiency and accuracy of a two-step approach for fungal identification: phylogenetic analysis using ITS sequences followed by confirmation based on morphological observations [18-21].

Since the first reports of *Armillaria* and *Flammulina* species in 1940 [22], nine genera (including 19 species) in the Physalaciaceae have been reported in Korea: *Armillaria*, *Cylindrobasidium*, *Cyptotrama*, *Flammulina*, *Hymenopellis*, *Oudemansiella*, *Rhodotus*, *Strobilurus*, and *Xerula* [23]. On Ulleung Island, 10 species in seven genera of the family Physalaciaceae have been reported, which represents approximately one third of the recorded species and one

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half of the recorded genera of the Physalacriaceae in Korea [24–27]. During the 2016 Fungal Survey of Ulleung and Dokdo Islands, a part of the larger National Institute of Biological Resources initiative, three new records in the Physalacriaceae were discovered using morphological and ITS sequence analysis. Here, we provide detailed morphological characteristics and ITS phylogenetic analysis of these taxa.

MATERIALS AND METHODS

Specimens and morphological observation. Four specimens collected in Ulleung Island were re-examined using molecular analysis as well as macro- and microscopic characteristics for more accurate species classification. To observe microscopic characteristics, dried tissue from specimens was rehydrated in 3% (w/v) KOH and stained in 1% (w/v) phloxine and 1% congo red. Microscopy was performed using an Eclipse 80i light microscope (Nikon, Tokyo, Japan). We measured basidia (20 per specimen), cystidia (10 per specimen), and basidiospores (20 per specimen). Q refers to the length/width ratio of an individual basidiospore. The morphological features were characterized in detail.

DNA sequencing and phylogenetic reconstruction. DNA was extracted using a modified cetyltrimethylammonium bromide extraction protocol [28]. The ITS region was amplified with ITS1F and ITS4B [29] using previously described methods [30]. The amplicons were Sanger-sequenced in both forward and reverse directions using the PCR primers. Sequencing was performed by Macrogen (Seoul, Korea) using an automated DNA sequencer (ABI3700; Applied Biosystems, Foster City, CA, USA).

DNA sequences were proofread using MEGA ver. 5.0 [31] and aligned with *Armillaria*, *Dactylosporina*, *Flammulina*, *Floiocephala*, *Gutanagaster*, *Hymenopellis*, *Oudemansiella*, *Paraxerula*, *Physalacria*, *Ponticulomyces*, *Rhizomarasmius*, *Rhodotus*, *Strobilurus*, and *Xerula* ITS sequences downloaded from GenBank using MAFFT [32]. Alignments were checked by eye and ambiguous positions were adjusted manually. Neighbor-joining tree were constructed using the ITS dataset using MEGA with 1,000 bootstrap replicates. The outgroup species, *Mycena aurantiidisca*, was selected based on a previous study [12]. Intraspecific sequence similarity was calculated using MEGA.

RESULTS

Phylogenetic analyses. The four samples separated into two highly supported clades ($\geq 99\%$ bootstrap support) on the ITS-based phylogenetic tree (Fig. 1): the *Hymenopellis/Ponticulomyces* complex and the genus *Paraxerula*. In our analysis, the *Hymenopellis/Ponticulomyces* complex was comprised of nine species in two genera: *Hymenopellis* and *Ponticulomyces*. The *Paraxerula* clade was comprised of three species. Three of four specimens grouped within the complex,

two specimens (NIBRFG0000146698 and NIBRFG0000146696) were identified as *H. orientalis* (99% bootstrap support, 100%; 99.5–100% sequence similarity with reference sequences), and one (NIBRFG0000146697) was identified as *Ponticulomyces orientalis* (*Po. orientalis*) (100%; 98.9–100% sequence similarity and 100% bootstrap support with reference sequence). One specimen (NIBRFG0000146699) grouped within the *Paraxerula* clade, and was identified as *Pa. hongoi* (100%; 100% sequence similarity and bootstrap support with reference sequence) (Fig. 1).

Taxonomy.

***Hymenopellis orientalis* (R. H. Petersen & Nagas.) R. H. Petersen**, Nova Hedwigia, Beih. 137: 195 (2010) (Fig. 2A). Pileus 45–85 mm diameter, viscid, nearly plane with decurved margin, subacutely to shallowly umbonate at center, radially rugulose around umbo, usually reticulate-veined over margin; disc and umbo greyish yellow when young, yellowish white when mature. Lamellae close, subdecurrent, subventricose, white, with lamellulae. Stipe 100–110 mm, tapering upward from bottom, white at apex, greyish yellow or somewhat darker. Pseudorhiza swollen at ground line with long tapering base, 50–100 mm long. Pleurocystidia 89.0–131.1 \times 22.7–31.7 μm , utriform to lageniform with broadly rounded apex, with clamp connection. Cheilocystidia 58.0–82.4 \times 13.5–19.2 μm broadly fusiform, broadly cylindrical, with clamp connection. Basidia 74.2–79.8–84.3 \times 16.9–18.2–19.9 μm , narrow clavate with 4 sterigmata and basal clamp. Basidiospores 16.3–17.1–18.0 \times 10.9–11.8–12.6 μm , Q = 1.37–1.44–1.50, broadly ovate to broadly ellipsoid, smooth.

Specimens examined: Korea, Gyeongsangbuk-do, Ulleung-gun, Mt. Sunginbong, on soil, 15 Jun 2016, J. Y. Park, N. K. Kim, NIBRFG0000146696 (= SFC20160615-05, GenBank accession No. KY072826); Korea, Gyeongsangbuk-do, Ulleung-gun, Seokpo circumference tracking way, on soil, 26 Sep 2016, J. Y. Park, N. K. Kim, NIBRFG0000146698 (= SFC20160926-20, GenBank accession No. KY072828).

Remarks: Pleurocystidia of this specimen was slightly smaller than type species. Pleurocystidia size of type species was 104–146 \times 35–45 μm , while our specimen was 89.0–131.1 \times 22.7–31.7 μm [11]. *H. orientalis* was formerly identified as *H. radicata* because of their morphological similarity. These species can be distinguished by the developing state of the caulocystidia. The former species has well developed caulocystidia while those of the latter species are poorly developed [33].

***Paraxerula hongoi* (Dörfelt) R. H. Petersen**, Nova Hedwigia, Beih. 137: 307 (2010) (Fig. 2B).

Pileus 30–60 mm diameter broad, convex to applanate, slightly umbonate, greyish yellow, pubescent with whitish hairs, context white. Lamellae close, free, white, with lamellulae. Stipe 70–90 \times 3–8 mm, apex white, lower part brown to light brown, with whitish hairs. Pleurocystidia 91.9–97.0 \times 17.8–20.9 μm , fusiform, capitate, abundant. Cheilocystidia 74.3–86.5 \times 17.1–19.7 μm , similar to pleurocystidia, abundant.

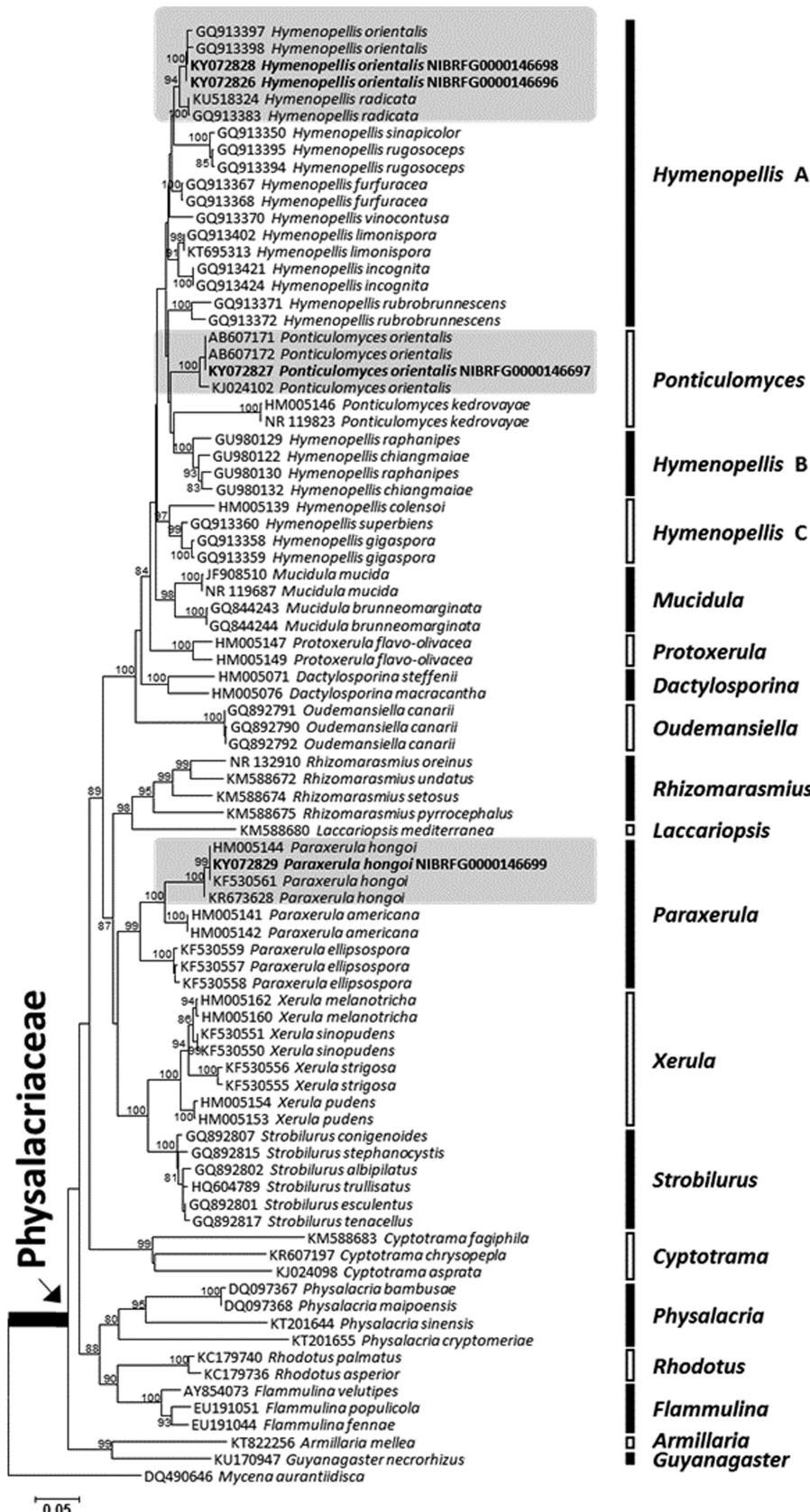


Fig. 1. Phylogenetic trees for Physalaciaceae species based on neighbor-joining analysis of the internal transcribed spacer. Bootstrap scores of >70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. Bold letters represent the species that were identified in this study.

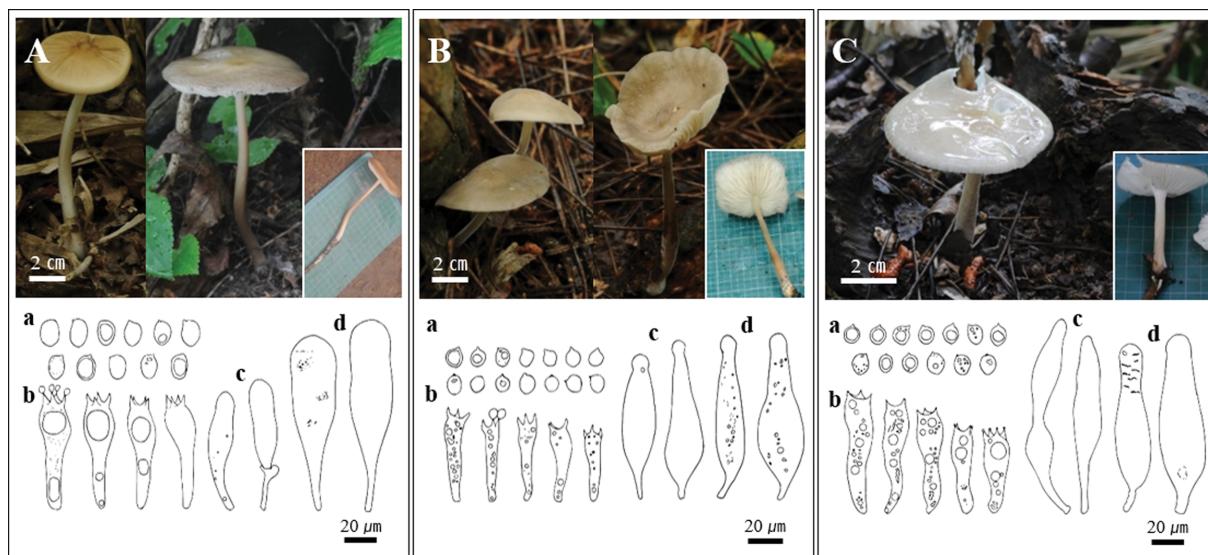


Fig. 2. Image and microscopic features of three new record species in Korea: *Hymenopellis orientalis* (A), *Paraxerula hongoi* (B), and *Ponticulomyces orientalis* (C). a, basidiospore; b, basidia; c, cheilocystidia; d, pleurocystidia.

Basidia $47.0\text{--}50.9\text{--}54.5 \times 8.8\text{--}9.6\text{--}10.5 \mu\text{m}$, clavate, with 4 sterigmata and clamp connection. Basidiospores $8.7\text{--}9.5\text{--}9.9 \times 7.2\text{--}7.7\text{--}8.2 \mu\text{m}$, $Q = 1.16\text{--}1.23\text{--}1.29$, ellipsoid, with a subacute or an obtusely rounded apex.

Specimens examined: Korea, Gyeongsangbuk-do, Ulleung-gun, Naesujeon, on leaf soil, 16 Jun 2016, J. Y. Park, Y. W. Lim, NIBRFG0000146697 (= SFC20160616-02, GenBank accession No. KY072827).

Remarks: Pileus size of this specimen is slightly larger than type species. In reference, pileus size of type species was 20–50 mm, while our specimen was 30–60 mm. Also, pleurocystidia of this specimen was slightly shorter and thicker than type species. Pleurocystidia size of type species was $98\text{--}180 \times 27\text{--}43 \mu\text{m}$, while our specimen was $91.9\text{--}97.0 \times 17.8\text{--}20.9 \mu\text{m}$ [11]. *P. hongoi* shares similar morphology with *Pluteus rimosellus* (*Pl. rimosellus*). However, they can be distinguished by lamella color (*Pa. hongoi* is white, *Pl. rimosellus* is pink), stipe color (*Pa. hongoi*, brown to light brown; *Pl. rimosellus*, white). Basidiospores, pleurocystidia and cheilocystidia of *Pa. hongoi* are larger than those of *Pl. rimosellus* [34].

***Ponticulomyces orientalis* (Zhu L. Yang) R. H. Petersen,** Nova Hedwigia, Beih. 137: 315 (2010) (Fig. 2C).

Pileus 50–60 mm diameter, plane, white, centre light brown, viscid when wet, context white, short translucent striations at margin. Lamellae close, white, subdecurrent, with lamellulae. Stipe $70\text{--}90 \times 7\text{--}10 \text{ mm}$, apex white, lower part brown to light brown, with whitish hairs, not annulus, with pseudorhiza. Pleurocystidia $99.6\text{--}111.1 \times 21.6\text{--}22.7 \mu\text{m}$, subfusiform, slightly thick walled, with basal clamp connection. Cheilocystidia $116.5\text{--}126.7 \times 17.6\text{--}21.1 \mu\text{m}$, lageniform, digitate, and narrowly elongate-fusiform, with clamp connection. Basidia $55.3\text{--}63.9\text{--}74.9 \times 14.6\text{--}15.7\text{--}17.2 \mu\text{m}$, clavate with 4 sterigmata and basal clamp connection.

Basidiospores $12.6\text{--}13.0\text{--}13.6 \times 11.1\text{--}11.5\text{--}11.8 \mu\text{m}$, $Q = 1.06\text{--}1.15\text{--}1.21$, subglobose to broadly ellipsoidal, smooth, nonamyloid.

Specimens examined: Korea, Gyeongsangbuk-do, Ulleung-gun, Naesujeon, on leaf soil, 16 Jun 2016, J. Y. Park, Y. W. Lim, NIBRFG0000146697 (= SFC20160616-02, GenBank accession No. KY072827).

Remarks: Stipe and basidia size of this specimen is slightly larger than type species. In reference, stipe size of type species was $20\text{--}80 \times 2\text{--}8 \text{ mm}$, while our specimen was $70\text{--}90 \times 7\text{--}10 \text{ mm}$. Basidia size of type species was $45\text{--}60 \times 12\text{--}15 \mu\text{m}$ [35], or $50\text{--}64 \times 15\text{--}17 \mu\text{m}$ [11], while our specimen was $55.3\text{--}74.9 \times 14.6\text{--}17.2 \mu\text{m}$. Other characteristics were similar to reference [11, 35]. *Po. orientalis* is often confused with *Oudemansiella mucida* but *Po. orientalis* can be distinguished from *O. mucida* due to its brown, slender, smooth stipe and solitary habitat while *O. mucida* is cespitose with corrugated stipes [35].

DISCUSSION

In this study, molecular analysis of ITS sequences followed by examination of morphological characters accurately identified three unrecorded species of the family Physalacriaceae in Korea. Using this two-step approach, we identified four specimens collected from Ulleung Island as members of the family Physalacriaceae: *H. orientalis*, *Po. orientalis*, and *Pa. hongoi*. Sequence identity of the specimens was greater than 98.9% with that of reference sequences, and species identifications were further supported by phylogenetic analysis. The genus *Ponticulomyces* clustered with the genus *Hymenopellis* in the phylogenetic analysis, which is consistent with a previous study [11]. We retained this taxonomic system because we focused on species identification (Fig. 1); however, further study is needed to resolve the phylogenetic relationships

of the *Hymenopellis* and *Ponticulomyces*.

The genus *Hymenopellis* is characterized by shallow convex to plane, moist, usually brown to olive-brown pileus, pseudorhiza swollen at ground line and slender to broadly cylindrical caulocystidia in stipe [11]. *H. orientalis* is often misidentified as *H. radicata* due to similar morphology [11]. However, the former can be identified from its well-developed caulocystidia [33]. The genus *Paraxerula* is characterized by collybioid, dry pileus with flexuous hairs, inflated pileocystidia, narrowly fusiform to fusiform pleurocystidia, and fruiting on deciduous trees [11, 13]. *Pa. hongoi* was reported in the list of mushroom flora of Ulleung Island without morphological description [27]. For this reason, we suggest *Pa. hongoi* as a new recorded species. This species can be misidentified as a member belonging to the genus *Pluteus* due to similar morphology and habitat, but it can be distinguished by the size of microscopic structures such as cystidia and color of lamellae and stipe (Fig. 2B) and distantly related phylogenetic relationship with *Pluteus* species [10, 34]. The genus *Ponticulomyces* is characterized by viscid or glutinous pileus, white to pallid pink lamellae, and ovate, ellipsoid or sublimoniform basidiospore [11]. Morphological characters of *Ponticulomyces orientalis* coincide with original description of the type species except slightly larger basidia size [11, 35]. *Po. orientalis* is often misidentified as *Oudemansiella mucida*. However, *O. mucida* is cespitose with corrugated stipes, while *Po. orientalis* is solitary and has brown, slender, smooth stipe [35].

In conclusion, from the results of 2016 Fungal Survey of Ulleung Islands, among 21 genera of the family Physalacriaceae, three species from three genera were confirmed as first records in Korea. *Pa. hongoi* and *Po. orientalis* were identified as the first recorded species in Korea. The genus *Hymenopellis* previously had only two recorded species in Korea: *H. radicata* and *H. raphanipes*. This study identified an additional Korean species record to this genus: *H. orientalis*. Through this survey, 3 unrecorded species from 2 unrecorded genera were found in Korean Phylsalacriaceae, and these results confirm that 12 species of 8 genera and 22 species of 11 genera were distributed in Ulleung Island and Korea, respectively.

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