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

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**Abstract** Most known species in the Physalacriaceae are saprotrophs that grow on decaying leaves and wood, and approximately 21 genera in the Physalacriaceae have been reported worldwide. During an ongoing survey of indigenous fungi in Korea, four specimens belonging to the Physalacriaceae 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 Physalacriaceae.

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

The family Physalacriaceae is classified in the order Agaricales (Agaricomycetes) and typified by the genus *Physalacria* Peck [1]. Approximately 21 Physalacriaceae genera have been reported worldwide. Fruit body shape in the Physalacriaceae is highly variable, ranging from agaricoid, cantharelloid, clavarioid, seccotioi and corticoid [2, 3]. Species in the Physalacriaceae 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 Physalacriaceae are saprobic, found on decaying leaves and wood, several species are parasitic [4]. Among the family Physalacriaceae, *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 Physalacriaceae using molecular analyses [9, 10]. The revision of the Physalacriaceae, 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. ellipsospora*) 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 Physalacriaceae have been reported in Korea: *Armillaria*, *Cylindrobasidium*, *Cytoptrama*, *Flammulina*, *Hymenopellis*, *Oudemansiella*, *Rhodotus*, *Strobilurus*, and *Xerula* [23]. On Ulleung Island, 10 species in seven genera of the family Physalacriaceae have been reported, which represents approximately one third of the recorded species and one...
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, Floocephala, 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 was constructed using the ITS dataset using MEGA with 1,000 bootstrap replicates. The outgroup species, Mycena aurantidiisca, 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 (≥ 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-reined 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. Pseudohiza swollen at ground line with long tapering base, 50–100 mm long. Pleurocystidia 89.0–131.1 × 22.7–31.7 μm, utriform to lageniform with broadly rounded apex, with clamp connection. Cheilocystidia 58.0–82.4 × 13.5–19.2 μm broadly fusiform, broadly cylindrical, with clamp connection. Basidia 74.2–79.8–84.3 × 16.9–18.2–19.9 μm, narrow clavate with 4 stigmata and basal clamp. Basidiospores 16.3–17.1–18.0 × 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 × 35–45 μm, while our specimen was 89.0–131.1 × 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 (Dörflert) R. H. Petersen, Nova Hedwigia, Beih. 137: 307 (2010) (Fig. 2B). Pileus 30–60 mm diameter broad, convex to planate, slightly umbonate, greyish yellow, pubescent with whitish hairs, context white. Lamellae close, free, white, with lamellulae. Stipe 70–90 × 3–8 mm, apex white, lower part brown to light brown, with whitish hairs. Pleurocystidia 91.9–97.0 × 17.8–20.9 μm, fusiform, capitate, abundant. Cheilocystidia 74.3–86.5 × 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.
Basidia 47.0–50.9–54.5 × 8.8–9.6–10.5 μm, clavate, with 4 sterigmata and clamp connection. Basidiospores 8.7–9.5–9.9 × 7.2–7.7–8.2 μm, Q = 1.16–1.23–1.29, ellipsoid, with a subacute or an obtusely rounded apex.

Specimens examined: Korea, Gyeongsangbuk-do, Ulleung-gun, Na-ri basin, on the dead wood of hardwood, 27 Sep 2016, M. S. Park, N. K. Kim, NIBRFG0000146699 (= SFC20160927-16, GenBank accession No. KY072829).

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 98–180 × 27–43 μm, while our specimen was 91.9–97.0 × 17.8–20.9 μm [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 lamellae. Stipe 70–90 × 7–10 mm, apex white, lower part brown to light brown, with whitish hairs, not annulus, with pseudohiza. Pleurocystidia 99.6–111.1 × 21.6–22.7 μm, subfusiform, slightly thick walled, with basal clamp connection. Cheilocystidia 116.5–126.7 × 17.6–21.1 μm, lageniform, digitate, and narrowly elongate-fusiform, with clamp connection. Basidia 55.3–63.9–74.9 × 14.6–15.7–17.2 μm, clavate with 4 sterigmata and basal clamp connection. Basidiospores 12.6–13.0–13.6 × 11.1–11.5–11.8 μm, Q = 1.06–1.15–1.21, 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 20–80 × 2–8 mm, while our specimen was 70–90 × 7–10 mm. Basidia size of type species was 45–60 × 12–15 μm [35], or 50–64 × 15–17 μm [11], while our specimen was 55.3–74.9 × 14.6–17.2 μm. 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 colllybioid, 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 sublignoniform 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 Oudemansella 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 Physalacriaceae, 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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REFERENCES

1. Peck CH. Fungi in wrong genera. Bull Torrey Bot Club 1882;9:1-4.
2. Henkel TW, Smith ME, Aime MC. Guyanagaster, a new wood-decaying sequestrate fungal genus related to Armillaria (Physalacriaceae, Agricales, Basidiomycota). Am J Bot 2010; 97:1474-84.
3. Moreau PA, Vila J, Aime MC, Antonin V, Horak E, Pérez-Buttrín JL, Richard F, Urban A, Wéti S, Vizzini A. Cibaomyces and Cyptotrama, two new genera for Europe, and an emendation of Rhizomarasmius (Basidiomycota, Physalacriaceae). Mycol Prog 2015;14:4.
4. Cannon PF, Kirk PM. Fungal families of the world. Wallingford: CABl; 2007.
5. Jing P, Zhao SJ, Lu MM, Cai Z, Pang J, Song LH. Multiple-fingerprint analysis for investigating quality control of Flammulina velutipes fruiting body polysaccharides. J Agric Food Chem 2014;62:12128-33.
6. Garraway MO, Huttermann A, Wargo PM. Ontogeny and physiology. In: Shaw CG, Kile GA, editors. Armillaria root disease. Agriculture handbook. No. 691. Washington, DC: U.S. Department of Agriculture, Forest Service; 1991. p. 21-47.
7. Corner EJ. Supplement to a monograph of Clavaria and allied genera. Beih Nova Hedwigia 1970;33:1-299.
8. Berthier J Les Physalacriaceae du globe (Hyménomycétales clavarioidés). Vaduz: Lubrecht & Cramer; 1985.
9. Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Catherine Aime M, Hofstetter V, Verduin SJ, Larsson E, Baroni TJ, et al. One hundred and seventeen clades of euagarcis. Mol Phylogenet Evol 2002;23:357-400.
10. Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Slot JC, Ammirati J, Baroni TJ, Bouger NL, et al. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 2006;98:982-95.
11. Petersen RH, Hughes KW. The Xerula/Oudemansella complex (Agricales). Nova Hedwigia Beih 2010;137:1-625.
12. Hao YJ, Qin J, Yang ZL. Cibaomyces, a new genus of Physalacriaceae from East Asia. Phytotaxa 2014;162:198-210.
13. Qin J, Hao YJ, Yang ZL, Li YC. Paraxerula ellipsospora, a new Asian species of Physalacriaceae. Mycol Prog 2014;13:639-47.
14. Moncalvo JM, Lutzoni FM, Rohnra SA, Johnson J, Vilgalys R. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 2000;49:278-305.
15. Matheny PB, Wang Z, Binder M, Curtiss JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati J, Schoch CL, Langer E, et al. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol Phylogenet Evol 2007;43:430-51.
16. Schoch CL, Seifert KA, Huhndorf S, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, et al. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 2013;22:5271-5.
19. Jargalmaa S, Park MS, Park JY, Fong JJ, Jang Y, Lim YW. Taxonomic study of the genus *Abundisporus* in Korea. Mycobiology 2015;43:225-30.

20. Park MS, Fong JJ, Lee H, Oh SY, Jung PE, Min YJ, Seok SJ, Lim YW. Delimitation of *Russula* subgenus *Amenula* in Korea using three molecular markers. Mycobiology 2013;41:191-201.

21. Cho HJ, Park MS, Lee H, Oh SY, Jang Y, Fong JJ, Lim YW. Four new species of *Amanita* in Inje County, Korea. Mycobiology 2015;43:408-14.

22. Kaburagi Y. Korean and Manchurian practical manual of forest. Korea Forest Experiment Station. Tokyo: Yokendo; 1940. p. 339-67.

23. Lee YS, Lim YW, Kim JJ, Yun HY, Kim C, Park JY. National list of species of Korea: Basidiomycota. Incheon: National Institute of Biological Resources; 2015.

24. Jung HS. Fungal flora of Ullung Island (I): on some corticioid fungi. Korean J Bot 1991;34:77-90.

25. Jung HS. Fungal flora of Ullung island (IV): on some agaric fungi. Korean J Mycol 1993;21:64-72.

26. Jung HS. Fungal flora of Ullung island (V): on additional agaric fungi. Korean J Mycol 1994;22:196-208.

27. Kim CS, Jo JW, Kwag YN, Sung GH, Lee SG, Kim SY, Shin CH, Han SK. Mushroom flora of Ulleung-gun and a newly recorded *Bovista* species in the Republic of Korea. Mycobiology 2015;43:239-57.

28. Rogers SO, Bendich AJ. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin SB, Schilperoort RA, editors. Plant molecular biology manual. Dordrecht: Springer; 1994. p. 183-90.

29. Garde M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113-8.

30. Lee H, Park MS, Jung PE, Fong JJ, Oh SY, Verbeken A, Lim YW. *Lactarius cucurbitoides* (Russulales, Basidiomycota), a new species from South Korea supported by molecular and morphological data. Phytotaxa 2015;205:168-76.

31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731-9.

32. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 2013;30:772-80.

33. Petersen RH, Nagasawa E. The genus *Xerula* in temperate East Asia. Rep Tottori Mycol Inst 2006;43:1-49.

34. Menolli N Jr, de Meijer AA, Capelari M. The genus *Pluteus* (Pluteaceae, Agaricales) from the state of Paraná, Brazil. Nova Hedwigia 2015;100:101-57.

35. Yang ZL. Further notes on the genus *Oudemansiella* from southwestern China. Mycotaxon 2000;74:357-66.