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Identification of three wood decay fungi in Yeoninsan Provincial Park, Korea

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Though several wood decay fungi have been reported in the world-wide, only about 600 wood decay fungi have been reported in Korea to date. Thus, the objective of this study was to secure resources for the wood decay fungi in Korea. We investigated wood decay fungi in Yeoninsan Provincial Park, Korea, and the collected specimens were identified based on ITS sequence analysis. Two species were unrecorded species in Korea: *Postia hirsuta* (Polyporales, Basidiomycota) and *Hyphodontia reticulata* (Hymenochaetales, Basidiomycota). Another species was previously reported without detailed description: *Ceriporia alachuana* (Polyporales, Basidiomycota). Here, we provided additional detailed microscopic features and phylogenetic analysis of these species.

Keywords: Basidiomycota, ITS, morphology, phylogeny, taxonomy

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Introduction

Wood decay fungi play a significant role in forest ecosystems through wood decomposition, such as soil humus formation and nutrient recycling (Gilbertson 1984; Bader *et al*., 1995; Misra *et al*., 2014). They use various enzymes to obtain carbon sources and other nutrients from moist wood. The enzymes have been used in many industries such as pharmaceuticals and bioremediation (Buswell *et al*., 1987; Wasser, 2002; Asgher *et al*., 2008; Karigar and Rao, 2011; Lee *et al*., 2015a). Specifically, the lignin-olytic fungi can degrade environmental pollutants; such as polychlorinated biphenyl (PCB), dichloro-diphenyl-trichloroethane (DDT), dioxins, and polycyclic aromatic hydrocarbons (PAHs) by using lignin peroxidase, laccase, and manganese peroxidases (Eriksson *et al*., 1990; Joshi and Gold, 1993; Reddy, 1995).

Traditionally, macro-fungi were classified by morphological characters (Johnson and Watling, 1977). Molecular methods have been applied for fungal research, and the internal transcribed spacer (ITS) region is used for fungal barcode marker (Schoch *et al*., 2012). Although the ITS show low species resolution for some fungi (Hong *et al*., 2015), it is suitable for most wood decay fungi (Jang *et al*., 2016b). In Korea, 5,056 fungal species have been reported (National Biodiversity Center, 2017). Among them, approximately 600 species are wood decay fungi (Lee *et al*., 2015b; Kim *et al*., 2016). Many of these species were identified based only on morphological characters, taxonomic studies have used molecular markers to identify species.

In order to describe indigenous basidiomycetes in Korea, we surveyed fungal diversity in Yeoninsan Provincial Park (Gapyeong-gun, Gyounggi-do, Korea) from 2015 to 2016 (Jang *et al*., 2016a). In a previous study, 79 fungal species were identified based on the ITS and nuclear large subunit ribosomal DNA region (LSU) sequence analysis and several wood decay fungi were confirmed as new species without morphological description (Jang *et al*., 2016a). During the process of updating the floral list of Yeoninsan Provincial Park, we found that *Hyphodontia niemelaei* (KUC20160721B-26) was misidentified. In this study, we correct this misidentification and provide detailed morphological descriptions. We also describe

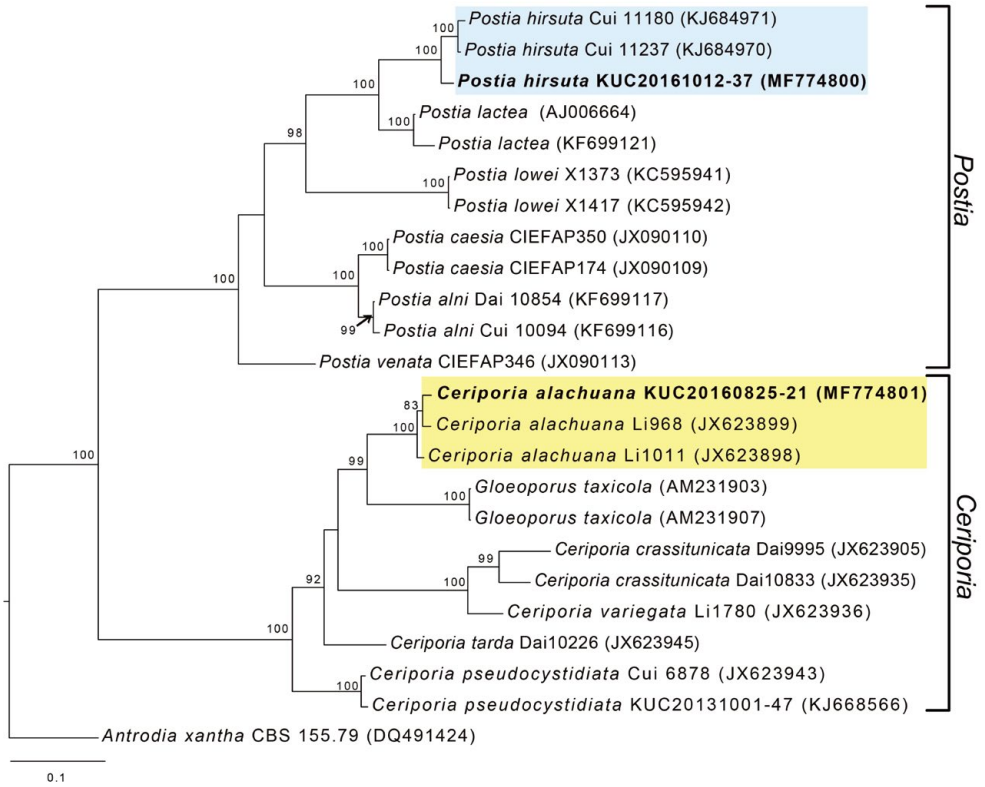

Fig. 1. The phylogenetic tree of *Ceriporia alachuana* (KUC20160825-21), *Postia hirsuta* (KUC20161012-37) and related species based on internal transcribed spacer region sequences. The dataset was created from 24 taxa and 740 characters. The specimen examined in this study is boldfaced. The posterior probability values ≥70 are shown above branch. The scale bar indicates nucleotide substitutions per position.

previously unrecorded wood decay fungi in Korea.

Materials and Methods

Strains

We reassessed wood decay fungi collected from Mt. Yeonin in 2016 using ITS sequence analysis. *Hyphodontia niemelaei* (KUC20160721B-26) was shown to be to *H. reticulata*, which is confirmed as new species in Korea. Although *Ceriporia alachuana* (KUC20160825-21) was also an unrecorded species, no morphological description was previously provided. The other wood decay fungus, *Postia* specimen (KUC20161012-37), was determined as an unrecorded species. Here, we confirmed the identification of three specimens by morphological examination and molecular phylogenetic analysis.

Morphological examination

For the accurate identification, detailed morphological features were investigated. Macro- and microscopic features were noted from those specimens of unrecorded species. The colors of fruiting bodies were indicated with Munsell colors (2009). Measurements and drawings for microscopic features were performed from slide preparations mounted in Melzer's reagent under an Olympus BX51 light microscope (Tokyo, Japan) (Johnson and Watling, 1977) at either 400× or 1000× magnification.

At least 30 basidiospores and 20 basidia (if possible) were measured per specimen.

The following abbreviations in the paper were used: L = mean spore length, W = mean spore width, n = number of spores from given number of specimens, Q = variation in the L/W ratios. In case of basidiospores, 5% of the measurements were rejected from each end of the range and are given in parentheses.

Molecular analysis

The AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) was used for genomic DNA extraction from the dry specimens. PCRs were conducted using the primers ITS1F and LR3 for the internal transcribed spacer (ITS) region amplification (White *et al*., 1990). The determined sequences were proofread using MAFFT

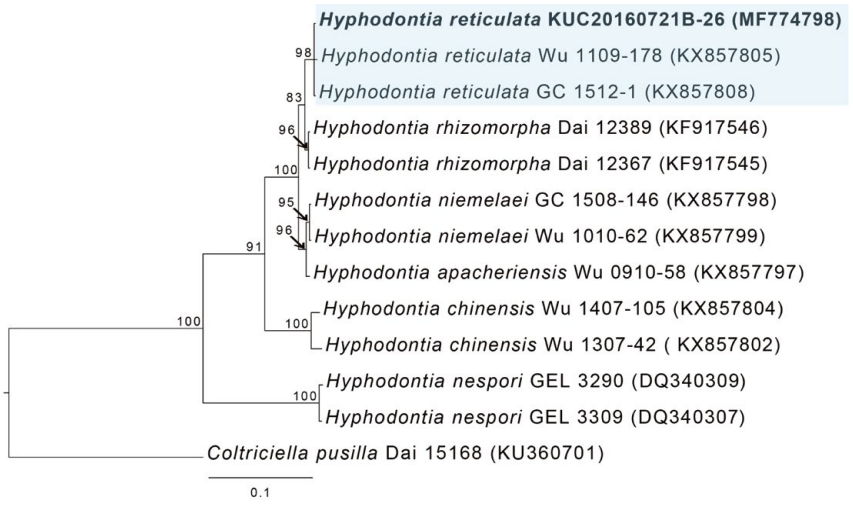

Fig. 2. The phylogenetic tree of *Hyphodontia reticulata* (KUC20160721B-26) and related species based on internal transcribed spacer region sequences. The dataset was created from 13 taxa and 799 characters. The specimen examined in this study is boldfaced. The posterior probability values ≥70 are shown above branch. The scale bar indicates nucleotide substitutions per position.

7.130 (Katoh and Standley, 2013). To analyze each species, closely related sequences were downloaded from GenBank. These were aligned using MAFFT 7.130 (Katoh and Standley, 2013) and modified manually using MacClade 4.08 software (Maddison and Maddison, 2005). Datasets were tested by MrModeltest 2.3 using the Akaike information criterion (AIC) criteria with default options (Nylander, 2004). The GTR + I + G model for the Polyporales dataset, and the GTR + G for the Hymenochaetales were chosen for the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003).

Results

Phylogenetic analysis

The molecular phylogenies were constructed by Bayesian analysis using the ITS sequence datasets. The two phylogenetic trees were constructed using 24 taxa for KUC20160825-21 and KUC20161012-37 (Polyporales) and 13 taxa for KUC20160721B-26 (Figs. 1 and 2). KUC20160825-21 clustered with the *Ceriporia alachuana* and was placed in monophyletic clade with 100% posterior probabilities (PP) (Fig. 1). KUC20160721B-26 was in a clade with *Hyphodontia reticulata* with 97% PP (Fig. 2). *H. rhizomorpha H. niemelaei*, and *H. apacheriensis* are morphologically and phylogenetically similar to *H. reticulata* (Fig. 2). KUC20161012-37 clustered with *Postia hirsuta* (Fig. 1) and monophyletic relationship is well supported (100% PP). The allied species was *P. lactea* (AJ006664).

Taxonomy

***Ceriporia alachuana* (Murrill) Hallenb**, Iranian Journal of Plant Pathology 15(1-4): 14 (1979) (Fig. 3)
Korean name: 연노랑그물구멍버섯

Basidiocarp annual, resupinate, pore surface pale yellow (2.5Y8/2, 2.5Y8.5/2) to light yellowish brown (2.5Y5/6), pores angular 3-4 per mm. margin sterile, white (7.5YR9.5/1), soft-floccose, paler than the pore surface. Hyphal system monomitic, hyphae thin- to thick-walled, simple-septate, 2.5-5.0 (-5.4) μm. Basidia clavate with 4 sterigmata, simple septate at the bases, 12.0-17.0 (-17.5) × 3.0-4.5 μm. Cystidia or other sterile hymenial elements absent. Basidiospores narrowly ellipsoid, ovoid, hyaline, smooth, 3.7-4.6 (-4.8) × 1.8-2.5 (-2.7) μm, L = 4.05 μm, W = 2.18 μm, Q = 1.86 (n = 30/1).
Habitat: On hardwood
Distribution: Iran, Nepal, and Thailand, rare in Europe, and widespread in the Southern United States (Ryvarden, 1993); and Republic of Korea.
Note: The microscopic characteristics of *Ceriporia alachuana* KUC20160825-21 accord with Ryvarden (1993). It is known as a white rot fungus. The basidiospores are recognized with shorter length or more width than other *Ceriporia* species in Korea (Jung, 1994; Lim *et al*., 2000; Lee *et al*., 2002; Jang *et al*., 2012; Jang *et al*., 2016b). *C. reticulata* has similar macro-morphological characters with *C. alachuana*, but it is distinguished from spore size (6.0-8.0 × 2.0-3.0 μm) (Jung, 1994).
Specimen examined: Korea, Gyeonggi, Yeoninsan Provincial Park, Mt. Yeonin, 37°53′55″ N, 127°24′51″ E, 25 Aug 2016, on hard wood, Seokyoon Jang,

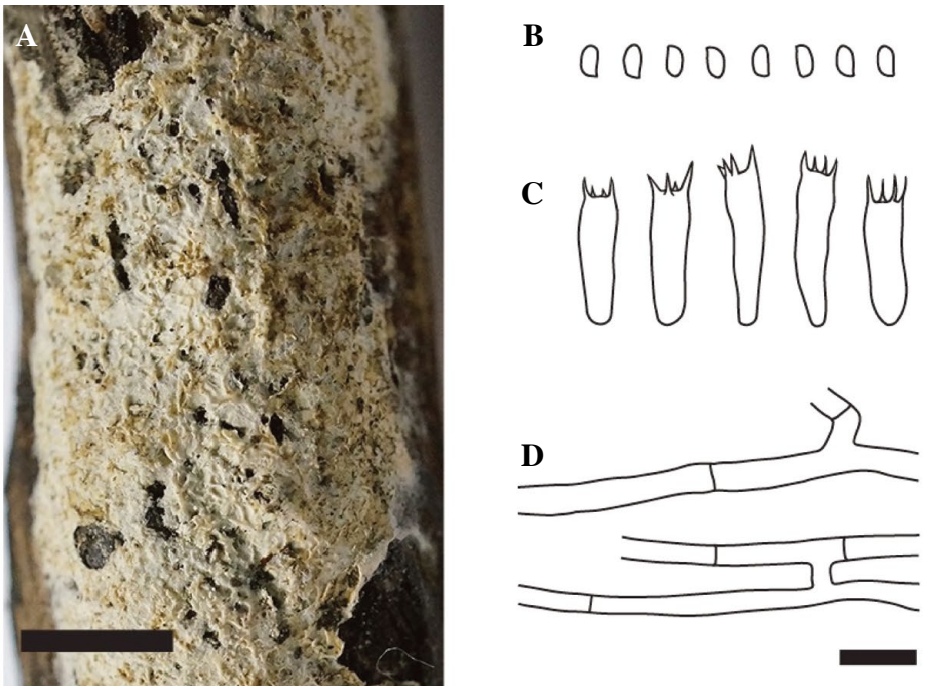

Fig. 3. The basidiocarp (A) and microscopic features (B-D) of *Ceriporia alachuana* KUC20160825-21. B, Basidiospores; C, Basidia; D, hyphae (scale bars: A = 1 cm, B-D = 10 μm).

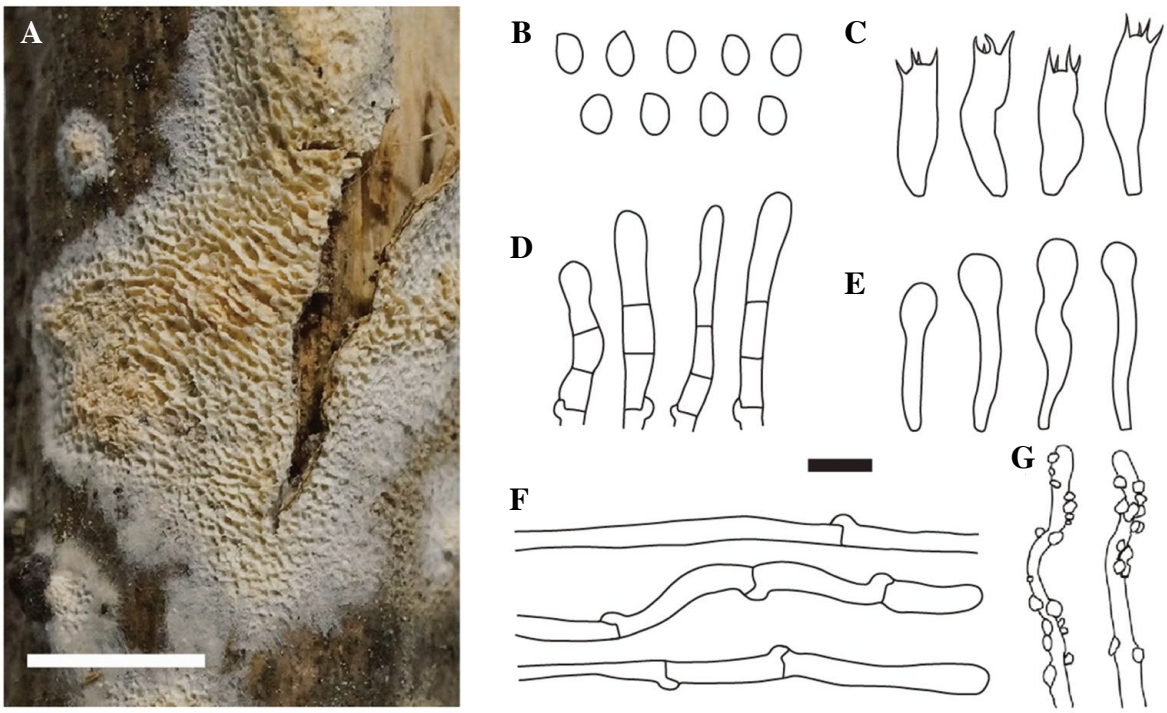

Fig. 4. The basidiocarp (A) and microscopic features (B-F) of *Hyphodontia reticulata* KUC20160721B-26. B, Basidiospores; C, Basidia; D, Septated cystidia; E, Capitate cystidia; F, hyphae; G, Encrusted hyphae part of a dissepiment edge (scale bars: A = 0.5 cm, B-F = 10 μm).

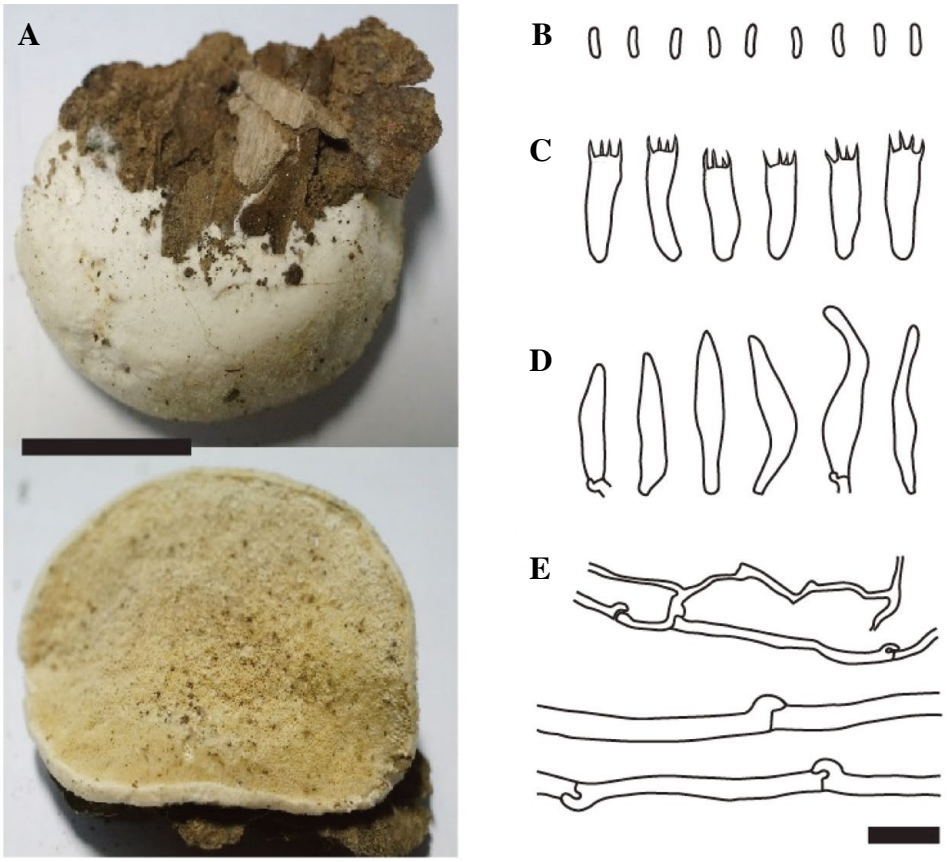

Fig. 5. The basidiocarp (A) and microscopic features (B-D) of *Postia hirsuta* KUC20161012-37. B, Basidiospores; C, Basidia; D, Fusoid cystidioles; E, hyphae (scale bars: A = 0.5 cm, B-D = 10 μm).

KUC20160825-21 (KB, NIBRFG0000499641; Genbank accession no. MF774801).

Hyphodontia reticulata C.C. Chen & Sheng H. Wu, Mycological Progress 16 (5): 558 (2017) (Fig. 4)
Korean name: 그물돌기고약버섯

Basidiocarp annual, resupinate, loosely adnate, effused, pore surface yellow (2.5Y8/6), pores angular 3-4 per mm; margin white (2.5Y9.5/1). Hyphal system monomitic, smooth, thin- to slightly thick-walled, nodose-septate, sometimes encrusted, 2.0-3.0 μm. Basidia narrowly clavate with 4 sterigmata and basal clamp, sometimes slightly constricted, 15.0-20.0 × 4.0-5.5 μm. Capitate cystidia with basal clamp, smooth, thin-walled, originating from the subhymenial or subicular hyphae, 20-37 × (2.0-)2.5-4.0. Basidiospores broadly ellipsoid, smooth, thin-walled, 4.9-6.1 × 3.4-3.9 μm, L = 5.36 μm, W = 3.66 μm, Q = 1.47 (n = 30/1).
Habitat: On hardwood
Distribution: Taiwan, Japan (Chen *et al*., 2017); and Republic of Korea.
Note: The capitate cystidia width of *Hyphodontia reticulata* (KUC20160721B-26) is narrower than that of *H. reticulata* in the other study (5-6.5 μm), but microscopic characteristics are comparable (Chen *et al*., 2017). *H. reticulata* is morphologically similar to *H. apacheriensis*, *H. niemelaei*, and *H. rhizomorpha* (Chen *et al*., 2017). Especially, *H. niemelaei* has similar basidiospores, but it is distinguished from small capitate cystidia (20-30 × 2.5-3.0 μm).
Specimen examined: Korea, Gyeonggi, Yeoninsan Provincial Park, Mt. Yeonin, 37°53′55″ N, 127°24′51″ E, 21 Jul 2016, on hard wood, Seokyoon Jang, KUC2016 0721B-26 (Genbank accession no. MF774798).

Postia hirsuta L.L. Shen & B.K. Cui, Cryptog. Mycol. 35(2): 202 (2014) (Fig. 5).
Korean name: 털손등버섯

Pileate basidiocarps annual, solitary, effused-reflexed, soft corky, white (2.5Y9.5/1) to very pale yellow (2.5Y9/2). Pore surface very pale yellow (2.5Y9/2) to yellow (10YR8/6). Pores round to angular 3-4 per mm; Hyphal system monomitic, generative hyphae with clamp connection, (2.6-)3.0-4.5 (-4.9) μm. Basidia clav-

ate with 4 sterigmata and basal clamp, (12.5-)13.5-16.5 (-17.0) × (2.5-)3.0-4.0 (-4.5) μm. Cystidioles fusoid, 16.7-25.4 × 3.0-4.6 μm. Basidiospores allantoid to cylindrical, smooth, thin-walled, 4.1-4.7 (-4.9) × (0.9-)1.0-1.2 μm, L = 4.41 μm, W = 1.1 μm, Q = 4.04 (n = 30/1).

Habitat: On hardwood

Distribution: China (Shen and Cui, 2014), and Republic of Korea.

Note: The genus *Postia* is known as brown rot fungi. The basidia size is slightly smaller than those of *P. hirsuta* in the other study (14.5-18 × 5-7.5 μm) (Shen and Cui, 2014), but other microscopic characters are comparable. Morphologically, *P. hirsuta* is similar to *P. lactea*, *P. amurensis*, *P. lowei*, *P. gloeocystidiata*, *P. cana*, and *P. caesia* in their pileate basidiocarps and pore surface (Shen and Cui, 2014). However, they can be distinguished by their hyphae or basidiospores size.

Specimen examined: Korea, Gyeonggi, Yeoninsan Provincial Park, Mt. Yeonin, 37°53′55″ N, 127°24′51″ E, 05 Aug 2016, on hard wood, Seokyoon Jang, KUC20161012-37 (KB, NIBRFG0000499758; Genbank accession no. MF774800).

Discussion

We identified three species in Korea based on both morphological features and molecular DNA sequence analysis. Three species were identified as *Ceriporia alachuana* KUC20160825-21 and *Postia hirsuta* KUC20161012-37 in Polyporales, and *Hyphodontia reticulata* KUC20160721B-26 in Hymenochaetales. Polyporales and Hymenochaetales are dominant orders of wood decay fungi world-wide (Kim *et al*., 2016).

KUC20160825-21 and KUC20160721B-26 were previously reported as an unrecorded species by Jang et al (2016a), but this study did not provide detailed descriptions. In the previous report by Jang *et al*. (2016a), the KUC20160721B-26 was identified as *H. niemelaei*. However, through phylogenetic analysis, KUC20160721B-26 was re-identified as *H. reticulata* (Fig. 2). *Hyphodontia niemelaei* has similar morphological features and is allied species with *H. reticulata*. Therefore, it is difficult to identify accurately. *Hyphodontia reticulata* was also reported as an unrecorded species in Korea, which recorded as a new species in 2017 by Chen et al (2017). The genus *Hyphodontia* J. Erikss is one of the largest genera of wood decay fungi (Hjortstam *et al*., 2007; Hjortstam and Ryvarden, 2009). *Hyphodontia* species are widely distributed and are saprophytic on wood in diverse forest ecosystems (Xiong *et al*., 2010; Yurchenko *et al*., 2013). In Korea, only eleven species of *Hyphodontia* species have been reported, even though there are 126 species recorded worldwide (Lee *et al*., 2015b; Yurchenko and Wu, 2016).

Ceriporia Donk is a genus that is widely distributed from subtropic to subpolar regions (Dai, 2012). Approximately 70 species of *Ceriporia* have been reported (Spirin *et al*., 2016). Among them, only five species were reported in South Korea (Jung, 1994; Lim *et al*., 2000; Lee *et al*., 2002; Jang *et al*., 2012; Jang *et al*., 2016b).

Postia Fr. is a large and globally distributed genus, and it consists of brown rot fungi mostly grown on conifer wood (Shen and Cui, 2014). Over 69 species of the Genus *Postia* Fr. have been reported in Mycobank (http://www.Mycobank.org/) and Index Fungorum (http://www.indexfungorum.org/) as current name, but only seven species have been reported in South Korea (Lee *et al*., 2015b).

According to the Wensberg *et al*. (2003), wood decay fungi have the ability to degrade recalcitrant molecules that aid degradation of xenobiotics through biological metabolism. Such practical use can be found in the use of *Ceriporia alachuana* (Murrill) Hallenb (Kondo *et al*., 2003), which has been used in degrading dioxins. Further, fungi have been used in various industrial products such as clinical drugs, industrial enzymes (pulp and paper), biomimetics and pharmaceutical purposes (Margulis and Chapman, 2009). Additionally, some *Postia* species economically important. In China, *Postia guttulata* (Peck) Jülich and *P. lactea* (Fr.) P. Karst have been used in medicine. In the near future, these three unrecorded wood decay fungi in Korea can be studied for practical use.

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