

The First Report of Two Species of *Polyporus* (Polyporaceae, Basidiomycota) from South Korea

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Based on morphological examination, two species of *Polyporus*, *P. dictyopus*, and *P. tuberaster*, were identified, which constitutes the first record of these species in South Korea. To confirm their affinity within the genus *Polyporus*, the phylogenetic relationships of *Polyporus* and allied genera were established from nuclear large subunit ribosomal DNA (nLSU rDNA) sequences, and a morphological diagnostic key is presented to clarify the Korean species of *Polyporus*.

Keywords: phylogeny, *Polyporus*, taxonomy, wood-rotting fungi

The fungal genus *Polyporus* Adans.:Fr. is an assemblage of morphologically heterogeneous white-rot, lignicolous basidiomycetes. It is characterized by the formation of stipitate basidiocarps, a dimitic hyphal system, and causing white rot (Donk, 1964; Gilbertson and Ryvarden, 1987). Most species of *Polyporus* grow on dead wood but several species are found on other substrates such as grass or bamboo roots (Ryvarden and Gilbertson, 1994; Núñez and Ryvarden, 1995; Sotome *et al.*, 2007). Some *Polyporus* species, e.g., *P. tuberaster* and *P. umbellatus*, are known as edible and medicinal fungi (Imazeki and Hongo, 1989; Park and Lee, 1999).

The name *Polyporus* has been used since 1729 (Micheli, 1729). Adanson (1763) validated the name *Polyporus* because Micheli's publication predated the International Code of Botanical Nomenclature (ICBN) and has no nomenclatural status (McNeill *et al.*, 2006). The first use of the name by Fries is as "*Polyporus* Mich. Restit. Fr." (1815), and he applied the name to almost all polypores (1821). As all names used by Fries in *Systema Mycologicum* have been conserved (McNeill *et al.*, 2006), Núñez and Ryvarden (1995) suggested that the name *Polyporus* Adans.:Fr. be cited.

The typification of *Polyporus* has been disputed. The following lectotypifications have been proposed: i) *P. ulmi* (Murrill, 1903), ii) *P. brumalis* (Clements and Shear, 1931; Krüger and Gargas, 2004), iii) *P. tuberaster* (Donk, 1933), and iv) *P. squamosus* (Corner, 1984). Although *P. squamosus* has been used as a type species in Korea, we followed Donk's selection for this study because most subsequent authors have followed Donk's selection of *P. tuberaster* (Ryvarden, 1991; Núñez and Ryvarden, 1995; Silveria and Wright, 2005; Sotome *et al.*, 2008).

The latest monographic treatment contained only 32 species of *Polyporus* s. l. (Núñez and Ryvarden, 1995). Without given ranks, they are divided into six subgroups by their particular basidiocarp morphology and consistency as follows:

Polyporus s. str., *Admirabilis*, *Dendropolyporus*, *Melanopus*, *Polyporellus*, and *Favolus*. Subsequently, more species have been added or resurrected (Dai, 1996, 1999; Buchanan and Ryvarden, 1998; Popoff and Wright, 1998; Hattori, 2000; Thorn, 2000). To date, 1,740 *Polyporus* species have been proposed in the Index Fungorum (<http://www.indexfungorum.org>).

In Korea, *Polyporus* has been reported since the late 20th century by mycologists including Lee *et al.* (1959), Lee and Jung (1972), Lim and Kim (1972), Lee (1975, 1988), Jung (1975, 1992, 1994), and Lee (1990). A total of 11 species of Korean *Polyporus* have been recorded (Lee and Jung, 2005): *P. alveolaris* (DC.) Bondartsev & Singer, *P. arcularius* (Batsch) Fr., *P. badius* Jungh. (= *P. picipes* Fr.), *P. brumalis* (Pers.) Fr., *P. grammacephalus* Berk., *P. leptcephalus* (Jacq.) Fr. (= *P. elegans* Fr.), *P. melanopus* (Pers.) Fr., *P. semiclausus* Berk., *P. squamosus* (Huds.) Fr., *P. umbellatus* (Pers.) Fr., and *P. varius* (Pers.) Fr.. Nowadays, *P. leptcephalus* is treated as a synonym of *P. varius* (Núñez and Ryvarden, 1995; Mycobank: <http://www.mycobank.org>). Therefore, ten species of *Polyporus* have been recorded in Korea to date.

The national biological inventory organized by the National Institute of Biological Resources (NIBR, www.nibr.go.kr) is currently underway, and has yielded numerous collections of fungi from within Korea. While investigating fungal specimens of *Polyporus* deposited at NIBR, we identified five recorded species, *P. alveolaris*, *P. arcularius*, *P. brumalis*, *P. squamosus*, and *P. varius*. In addition, we found two unreported *Polyporus* species for Korea. To confirm their affinity within the genus *Polyporus*, phylogenetic analysis was carried out based on the sequence data of nuclear large subunit ribosomal DNA (nLSU rDNA) regions. Here we present a detailed description of the two new species for Korea and a key for the Korean *Polyporus*.

Materials and Methods

Microscopic observation

The seven *Polyporus* species examined in this study are listed in Table 1.

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Table 1. Specimens used in this study

Species	Voucher no.	Habitat	Locality	GenBank accession no.
<i>Polyporus alveolaris</i>	SFC040525-29	-	Mt. Jiri, Korea	HM003895
<i>P. arcularius</i>	HK01490	-	Seongnam-si, Korea	HM003896
<i>P. brumalis</i>	F20090219LJS19	<i>Abies holophylla</i>	Mt. Odae, Korea	HM003897
	F20090219LYW38	-	Mt. Odae, Korea	HM003898
<i>P. dictyopus</i>	SFC070618-06	hardwood	Mt. Sobaek, Korea	HM003899
	SFC070915-26	hardwood	Mt. Seorak, Korea	HM003900
<i>P. squamosus</i>	NIBRFG0000102475	-	Mt. Gyeryong, Korea	HM003901
<i>P. tuberaster</i>	F20090619LJS52	on soil	Mt. Seorak, Korea	HM003902
	F20080606LYW28	<i>Actinidia arguta</i>	Mt. Odae, Korea	HM003903
<i>P. varius</i>	SGS060818-04	-	Mt. Gyeryong, Korea	HM003904

Macroscopic and microscopic characteristics were based on voucher specimens deposited at the National Biological Resources Center (KB). Measurements and drawings were made from slide preparations mounted in 3% KOH (Largent *et al.*, 1977) using an Olympus BX51 light microscope. A total of 20 basidiospores and basidia were measured for the specimens. A key to *Polyporus* species found in Korea was constructed. The descriptions of three Korean species not examined in this study (*P. badius*, *P. melanopus*, and *P. umbellatus*) were given in previous studies (Jung, 1975; Lee, 1988; Jung, 1992). *Polyporus grammacephalus* and *P. semiclausus* were excluded from this study because no descriptions of these species were found in any references, even though some authors list them (Lim and Kim, 1972; Jung, 1975; Lee, 1975; Lee and Jung, 2005).

Molecular analyses

Dried fungal materials were ground using a freeze-crusher (SK200; Tokken, Japan), and genomic DNAs were extracted using the Plant DNA Mini-kit (QIAGEN, Germany) according to the manufacturer's instructions. The nLSU rDNA region was amplified using primers LR0R (Rehner and Samuels, 1994) and LR5 (Vilgalys and Hester, 1990). PCR was performed using Quick PCR Premix (Bioneer, Korea). PCR proceeded as follows: 5 min initial denaturation at 94°C, 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 52°C, and 1 min extension at 72°C, and 10 min final extension at 72°C. Amplified products were purified using a PCR purification kit (Bioneer). DNA sequencing was performed using the aforementioned primers and an ABI 3730XL (Macrogen, Korea). The nucleotide sequences determined in this study have been deposited in GenBank. Accession numbers are presented in Table 1. Sequences were proofread, edited, and aligned using the jPHYDIT program (Jeon *et*

al., 2005). Phylogenetic trees were inferred from sequence alignment using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods implemented in PAUP 4.0b10 (Swofford, 2002). Sequences were examined using equal weighting. In the NJ analyses, rates for variable sites were assumed equal and no sites were assumed invariable. Data matrices were corrected using Jukes-Cantor correction. The robustness of inferred NJ topologies was tested by 1,000 bootstrap replicates. MP analyses were conducted with a heuristic search method with tree bisection reconnection (TBR) branch swapping and MAXTREES set to autoincrease. A bootstrap analysis was performed with 1,000 replicates, ten random taxon addition sequences, MAXTREES set to autoincrease, and TBR branching (Felsenstein, 1985). Likelihood settings were parameterized using the best-fit model (GTR+I+G) using AIC in Modeltest 3.7 (Posada and Crandall, 1998). ML analyses were conducted with a heuristic search method with "asis" addition sequence and TBR branch swapping. Branch support was assessed using bootstrapping with simple taxon addition, with 100 replicates, and five trees held at each step (Salamin *et al.*, 2003). The trees were rooted with the sequence of *Lenzites betulinus* (AB368073), which is a sister group to the genus *Polyporus* s. lat (Sotome *et al.*, 2008).

Results and Discussion

In this study, we found two species that have not previously been reported in Korea. The first *Polyporus* species (SFC070618-06, SFC070915-26) has a similar morphology to *P. badius* and *P. varius*, which have particularly short blackish stipes, but has different microscopic characters. The clamped generative hyphae were differentiated from those of *P. badius*,

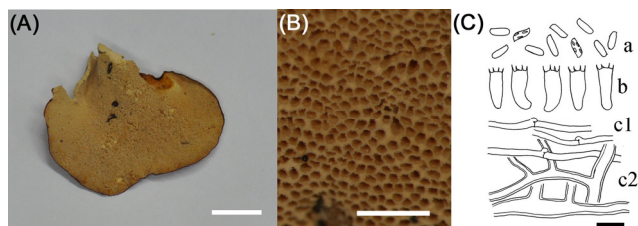


Fig. 1. Basidiocarps (A), Hymenophores (B) and microscopic features (C) of *Polyporus dictyopus*. a, basidiospores. b, basidia. c1, generative hyphae. c2, skeletal hyphae. Scale bars=1 cm in (A), 1 mm in (B) and 10 µm in (C).

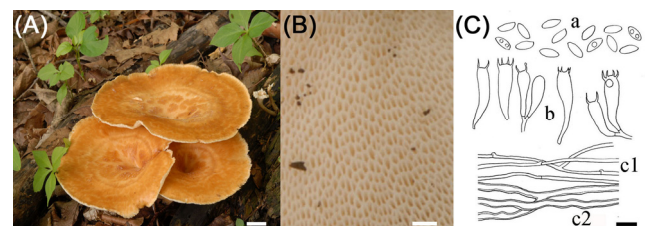
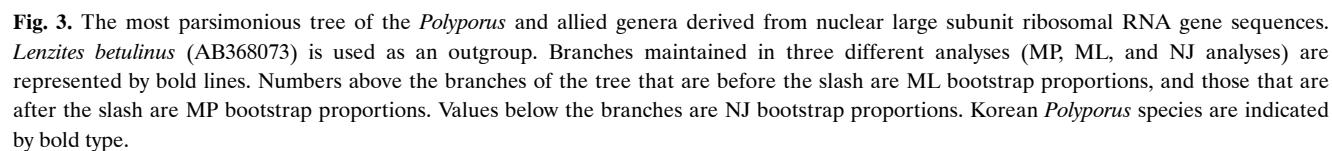


Fig. 2. Basidiocarps (A), Hymenophores (B) and microscopic features (C) of *Polyporus tuberaster*. a, basidiospores. b, basidia. c1, generative hyphae. c2, skeletal hyphae. Scale bars=1 cm in (A), 5 mm in (B) and 10 µm in (C).

characterized by thicker and more robust basidiocarps, rounded and agglutinated scales, and fruiting on dead standing or fallen trees as well as on living hardwoods (Gilbertson and Ryvarden, 1987). However, these specimens differed from *P. squamosus* by large pileus (up to 80-150 mm wide), pores (2-4 per mm), and large cylindrical basidiospores ($10-11 \times 6-7 \mu\text{m}$), finely fimbriate and raised scales (Fig. 2), terrestrial habitat, and occurring on buried wood or fallen leaves, which are characteristics similar to previous descriptions of *P. tuberaster* (Jacq. ex Pers.) Fr. (Núñez and Ryvarden, 1995). Because of their similar basidiocarp morphology, *P. tuberaster* may have been misidentified in the past as *P. squamosus*, which has been



treated as a type species in Korea.

To confirm the affinity of the Korean *Polyporus* species within the genus *Polyporus* s. l., ML, MP, and NJ analyses were carried out based on nLSU rDNA sequences of 37 polyporoid taxa, which showed that *Polyporus* has a close relationship with *Datronia* and *Lentinus* (Fig. 3). The aligned dataset of 37 rDNA sequences was composed of 619 nucleotide sites, after excluding ambiguously aligned sites. Among the 619 sites, 485 sites were constant, 47 sites were variable but non-informative, and 87 sites were parsimony-informative. The nLSU rDNA sequences of the two specimens of Korean *P. dictyopus* (SFC070618-06 and SFC070915-26) showed 99.7% similarity (two out of 741 positions showed nucleotide differences) to a Japanese specimen (AB368087), and those of Korean *P. tuberaster* (F20090619LJS52 and F20080606LYW28) showed 100% similarity (739 positions showed no nucleotide difference) to the Japanese specimen (AB368104) according to a BLAST search of GenBank.

ML analysis based on sequences of the nLSU rDNA yielded the best-resolved tree depicted in Fig. 3. The three ML trees show that *P. dictyopus* and *P. tuberaster* form distinct clades with 99% and 98% confidence level in the nLSU rDNA tree, respectively. These independent lineages had 100% bootstrap support in the MP (tree length=550, CI=0.355, RI=0.605) and NJ analyses.

The phylogenetic analysis showed that *Polyporus* is a polyphyletic genus, as previously suggested by some authors (Ko and Jung, 2002; Sotome *et al.*, 2008). The ML tree was recovered in the MP and NJ analyses. The members of *Polyporellus* sensu Núñez and Ryvarden (1995) with some *Lentinus* species were transferred recently to *Polyporus* (Krüger and Gargas, 2004), and are more closely related to *Microporus*, *Hexagonia*, *Daedaleopsis*, *Ganoderma*, *Perenniporia*, and *Cryptoporus* than other *Polyporus* species (Fig. 3). *Polyporus dictyopus* and *P. tuberaster* belong to another closely related clade. Considering results of this study and those of Sotome *et al.* (2008), *Polyporus* and its allied genera should be reorganized into several genera after careful morphological examination and phylogenetic analysis.

Including the two new Korean species, a total of 12 species of *Polyporus* are included in the Korean biological inventory. To our knowledge, however, no detailed morphological descriptions of two species, *P. grammacephalus* and *P. semiclausus*, have been reported for Korea. Therefore, we compared macro- and microscopic features of ten Korean *Polyporus* species (Table 2) and constructed a diagnostic key for them (see below). The descriptions of three *Polyporus* species, *P. brumalis*, *P. melanopus*, and *P. umbellatus*, which were not found in this study, were obtained from previously reported data (Jung, 1992, 1994).

Taxonomy

1. *Polyporus dictyopus* Mont., *Annls Sci. Nat., Bot., sér. 2* 3: 349 (1835) (Fig. 1).

Synonymy

Atroporus diabolicus (Berk.) Ryvarden, *Norw. J Bot.* 20: 2 (1973); *Atroporus infernalis* (Berk.) Ryvarden, *Norw. J Bot.* 20: 2 (1973); *Melanopus dictyopus* (Mont.) Pat., *Essai Tax. Hyménomyc.* (Lons-le-Saunier): 80 (1900)

Basidiocarps annual, growing solitary, pileate with a short lateral stipe, 3.5 cm wide, 5.0 mm thick, flabelliform, spatulate to semicircular; upper surface dark ochraceous, rusty to reddish-brown, finely tomentose to glabrous; margin acute; stipe lateral, very short, blackish; hymenophore decurrent on the stipe, ochraceous to umber; pores round to angular, 6-8 per mm. **Hyphal system** dimitic; generative hyphae with clamps, hyaline, thin-walled, 2.2-2.8 μm wide; binding hyphae 2.5-7.5 μm wide. **Basidia** clavate with narrow base, 12-17 $\times$ 4.0-5.8 μm . **Basidiospores** elliptical, hyaline, smooth and thin walled, 6.8-7.9 $\times$ 2.2-2.8 μm .

Specimens examined

KOREA: Gangwon Province, Mt. Seorak, on hardwood, 15 Sep 2007, Hack Sung Jung (070915-26); Chungbuk Province, Mt. Sobaek, *Ibid*, 24 Sep 2006, *Ibid* (070618-06).

Table 2. Morphological characteristics of ten Korean *Polyporus* species

Species	Basidiocarp.	Pore	Stipe	Basida	Basidiospores
<i>P. alveolaris</i>	circular to dimidiate up to 7 cm wide	1-2/mm	centally	20-22 $\times$ 7-8 μm	9.5-10 $\times$ 3.5-4 μm
<i>P. arcularius</i>	circular up to 2.5 cm wide	1-2/mm	centally,	25-35 $\times$ 5-6 μm	6-8 $\times$ 2-3 μm
<i>P. badius</i> (Lee, 1988) ^a	circular to flabelliform up to 15 cm wide	5-7/mm	laterally to centrally	ND ^b	6-7 $\times$ 2-2.5 μm
<i>P. brumalis</i>	surface bronze, hairy up to 6 cm wide	3-5/mm	centrally	17-22 $\times$ 4-5.5 μm	6-8.5 $\times$ 2-3 μm
<i>P. dictyopus</i>	spatulate up to 3.5 cm wide	6-8/mm	laterally	12-17 $\times$ 4-5.8 μm	6.8-7.9 $\times$ 2.2-2.8 μm
<i>P. melanopus</i> (Jung, 1992) ^a	circular up to 5 cm, wide	6-7/mm	centrally	20-25 $\times$ 6-7 μm	6-8 $\times$ 3 μm
<i>P. squamosus</i>	circular up to 10 cm wide	1-2 mm	centrally	42-55 $\times$ 10-12 μm	12-15 $\times$ 4.5-5.5 μm
<i>P. tuberaster</i>	circular up to 8 cm wide	2-4/mm	centrally	25-35 $\times$ 6-10 μm	10-11 $\times$ 6-7 μm
<i>P. umbellatus</i> (Lee, 1988) ^a	with several pilei up to 30 cm wide	2-4/mm	branched	ND	7-10 $\times$ 3-4 μm
<i>P. varius</i>	flabelliform wide up to 4 cm	4-5/mm	laterally to centrally	20-25 $\times$ 6-7.5 μm	7-10 $\times$ 2.8-3.5 μm

^a The characteristics of the species are just referred to previous studies

^b ND, There is no descriptions

Notes

Basidiocarps of *Polyporus dictyopus* are usually easy to recognize because of the rusty to reddish-brown pileus, blackish stipe, and small-sized pores (6-8 per mm). There is wide variation in pore and spore size. Korean species have similar basidiocarps to those from America, which have smaller spores (6-7 × 2.5 µm) than those from Africa (7-8 × 2.5 µm on average) (Núñez and Ryvarden, 1995).

2. *Polyporus tuberaster* (Jacq. ex Pers.) Fr., Observ. Mycol. (Havniae) 1: 121 (1815) (Fig. 2).

Synonymy

Boletus tuberaster Jacq., Collnea bot. 5: pls 8 & 9 (1796); *Melanopus lentus* (Berk.) Bourdot & Galzin, Bull. Soc. Mycol. Fr. 41: 109 (1925); *Melanopus squamosus* var. *lentus* (Berk.) Bourdot & Galzin, Bull. Trimest. Soc. Mycol. Fr. 41: 109 (1925); *Polyporellus tuberaster* (Jacq. ex Pers.) Pilát, Beih. Bot. Zbl., Abt. 2 56: 18 (1936)

Basidiocarps annual, solitary or imbricate, stipitate, 8.0 cm wide, 5-15-mm thick, fleshy, reniform or flabelliform; upper surface whitish, ochraceous to pale yellowish-brown, covered with small tan to dark brown agglutinated scales especially towards the margin; stipe central, very short; hymenophore decurrent on the stipe, whitish, soon cream to straw-colored, darker when dry; pores irregular and angular, large, 2-4 per mm. **Hyphal system** dimitic; generative hyphae with clamps, hyaline, thin-walled, 2-3 µm wide; binding hyphae 4-6 µm wide. **Basidia** clavate with narrow base, 25-35×6-10 µm. **Basidiospores** cylindric, smooth, thin-walled, oil drops, 10-11×6-7 µm.

Specimens examined

KOREA: Gangwon Province, Mt. Seorak, on soil, 19 Jun 2009, Jin Sung Lee and Young Woon Lim (F20090619LJS52); *Ibid*, on decayed hardwood, 30 Aug 2006, Yang Sup Kim (HK01477); *Ibid*, Mt. Odae, on dead branch of *Actinidia arguta*, 6 Jun 2008, Young Woon Lim (F20080606LYW28); Jeonnam Province, Mt. Wolchul, decayed hardwood, 30 Sep 2006, Yang Sup Kim (HK01917); Jeonbuk Province, Mt. Deoggyu, *Ibid*, 7 Jul 2007, Jun Tae Jeon (HK00649); *Ibid*, *Ibid*, 6 Jul 2007, *Ibid* (HK00485).

Notes

The species is the type species of the genus *Polyporus* and can be confused with *P. squamosus*, which also has scales on the pileus, although they are not agglutinated. *P. squamosus* normally has thicker and more robust basidiocarps. *Polyporus tuberaster* grows on the ground or on well-decayed wood and fallen leaves (Jahn, 1980; Gilbertson and Ryvarden, 1987).

Key to Korean *Polyporus* species

1. Basidiocarp with several pilei from a common base *P. umbellatus* (Pers.) Fr.
1. Basidiocarp with a single pileus 2
 2. Basidiospores longer than 9 µm 3
 2. Basidiospores shorter than 9 µm 6
3. Stipe lateral, pileus 0.3-0.7 cm thick *P. alveolaris* (DC.) Bondartsev & Singer
3. Stipe central, pileus 0.7-2.0 cm thick 4

4. Stipe blackish or dark brown *P. varius* (Pers.) Fr.
4. Stipe not black, with lighter color 5
5. Squamules usually agglutinated, in dead standing trees *P. squamosus* (Huds.) Fr.
5. Squamules finely fimbriate and raised, on ground or fallen leaves *P. tuberaster* (Jacq. ex Pers.) Fr.
6. Pores 1-2 per mm *P. arcularius* (Batsch) Fr.
6. Pores 3-8 per mm 7
7. Pileus bronze to purplish brown, with dark hairs, pores 3-4 per mm *P. brumalis* (Pers.) Fr.
7. Pileus pale buff to dark brown, glabrous, pores 5-8 per mm 8
- 8 Hyphae simple-septate *P. badius* Jungh.
- 8 Hyphae with clamps 9
9. Fruiting on dead wood *P. dictyopus* Mont.
9. Fruiting on the ground, usually from buried, rotten roots *P. melanopus* (Pers.) Fr.

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