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Research Article

Taxonomic Study of the Genus *Abundisporus* in Korea

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Abstract The polypore genus *Abundisporus* Ryvarden is characterized by resupinate to pileate fruitbodies with a purplish brown hymenophore, slightly thick-walled, pale yellowish and non-dextrinoid basidiospores, and causing white rot. A purple color hymenophore, an easily observable and striking character, was considered the main distinctive feature at the generic level within polypores. However, due to highly similar basidiocarp features, species identification within these purple polypores is particularly difficult. Three species of purple colored polypores have been reported in Korea (*Abundisporus fuscopurpureus*, *A. pubertatis*, and *Fomitopsis rosea*). Based on morphological re-examination, ecological information, and sequence analysis of the internal transcribed spacer, we showed that previous classification was incorrect and there is only one species (*A. pubertatis*) in Korea. We provide a detailed description of *A. pubertatis* in Korea, as well as a taxonomic key to distinguish wood rot fungi with a purple hymenophore.

Keywords *Abundisporus*, *Fomitopsis rosea*, Polypores, Purple color hymenenophore, Wood rot fungi

The polypore genus *Abundisporus* [1] is characterized by resupinate to pileate fruibodies with pale umber to deep purplish brown or greyish to amber brown context; ellipsoidal, slightly thick-walled, pale yellowish and non-dextrinoid basidiospores; dimitic hyphal systems with skeletal hyphae; and causing white rot [2]. This genus is mostly distributed in tropical and subtropical areas [1], but was recently found in temperate areas [3-5]. Eight *Abundisporus* species have been reported worldwide: *A. fuscopurpureus* (Pers.) Ryvarden, *A. mollissimus* Zhao, *A. pubertatis* (Lloyd) Parmasto, *A. quercicola* Y. C. Dai, *A. sclerosetosus* C. Decock and O. Laurence, *A. subflexibilis* (Berk. & Curtis) Parmasto (synonym of *A. roseoalbus*), *A. roseoalbus* (Jungh.) Ryvarden, and *A. violaceus* (Wakef.) Ryvarden [5]. Six of these species (*A. fuscopurpureus*, *A. mollissimus*, *A. pubertatis*, *A. quercicola*, *A. roseoalbus*, and *A. sclerosetosus*) are recorded in Asia [3-9].

Phylogenetic analysis based on the internal transcribed spacer (ITS) and the 28S nuclear ribosomal large subunit (LSU) showed that *Abundisporus* formed a monophyletic group [10-13]. This result was supported by a multigene phylogenetic analysis that included ITS, LSU, the small subunit mitochondrial rRNA gene (mtSSU), and the translation elongation factor 1-α gene (*tef1*) [5].

Before 2006, there were no records of *Abundisporus* in Korea. After detailed morphological and molecular analyses, two *Abundisporus* species were identified: *A. fuscopurpureus* [4] and *A. pubertatis* [8]. Both of these species were previously misidentified as *Fomitopsis rosea* [14], as they are superficially similar (e.g., have a purple hymenophore). In fact, these three species can be distinguished using microscopic features [1, 9, 15], DNA [5], or ecology [9, 16, 17]. In order to clarify the status of *Abundisporus* species in Korea, we investigated *Abundisporus* and *F. rosea* specimens collected across South Korea using microscopic characters, DNA sequence analyses of the ITS region, and ecology (habitat). Based on our findings, we address the status of *Abundisporus* in Korea and provide a taxonomic key to distinguish these species.

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MATERIALS AND METHODS

Samples and morphological analysis. Specimens originally identified as *A. fuscopurpureus*, *A. pubertatis*, *Abundisporus* sp., or *F. rosea* at the Seoul National University Fungus Collection (SFC) were used in this study. When available, information on host species was noted. First, we sorted specimens using macro- and micro-morphological observations [5, 6, 15]. Initially, three morphological features were observed for 45 specimens: the number of pores per mm, basidiospore shape, and basidiospore size. For observations of micro-morphological features for the representative specimens of *A. pubertatis*, slide preparations were made from dried tissue mounted in 3% KOH and viewed using a light microscope (Nikon 80i; Nikon, Tokyo, Japan). We specifically noted the shape and wall thickness of the basidiospores, as these are features that distinguish *Abundisporus* from *Fomitopsis—Abundisporus* basidiospores

Table 1. *Abundisporus pubertatis* used in this study

Original ID	Specimen No.	Substrate	Locality	Collection date
A. pubertatis	SFC20140626-27[a]	Broad-leaved tree	Guri-si, Gyeonggi-do, Korea	Jun 26, 2014
	SFC20140411-07	Broad-leaved tree	Jinan-gun, Jeollabuk-do, Korea	Apr 11, 2014
	SFC20140723-07	*Quercus* sp.	Jinan-gun, Jeollabuk-do, Korea	Jul 23, 2014
	SFC20140827-10	*Quercus* sp.	Jangsu-gun, Jeollabuk-do, Korea	Aug 27, 2014
	SFC20140921-17	Broad-leaved tree	Jinan-gun, Jeollabuk-do, Korea	Sep 21, 2014
A. fuscopurpureus	SFC20030927-10[a]	*Quercus* sp.	Hamyang-gun, Gyeongsangnam-do, Korea	Sep 27, 2003
	SFC20030921-11[a]	*Quercus* sp.	Hamyang-gun, Gyeongsangnam-do, Korea	Sep 21, 2003
	SFC20040205-02[a]	*Quercus* sp.	Jecheon-si, Chungcheongbuk-do, Korea	Feb 5, 2004
	SFC20050817-85[a]	Broad-leaved tree	Hadong-gun, Gyeongsangnam-do, Korea	Aug 17, 2005
	SFC20061013-24[a]	*Quercus mongolica*	Pyeongchang-gun, Gangwon-do, Korea	Oct 13, 2006
	SFC20110921-31[a]	*Quercus serrata*	Wonju-si, Gangwon-do, Korea	Sep 21, 2011
	SFC20140411-03[a]	Broad-leaved tree	Jinan-gun, Jeollabuk-do, Korea	Apr 11, 2014
	SFC20030614-06	Broad-leaved tree	Muju-gun, Jeollabuk-do, Korea	Jun 14, 2003
	SFC20030723-18	*Quercus* sp.	Muju-gun, Jeollabuk-do, Korea	Jul 23, 2003
	SFC20030921-60	Broad-leaved tree	Geochang-gun, Gyeongsangnam-do, Korea	Sep 21, 2003
	SFC20030927-05	*Quercus mongolica*	Hamyang-gun, Gyeongsangnam-do, Korea	Sep 27, 2003
	SFC20031016-05	Broad-leaved tree	Jangsu-gun, Jeollabuk-do, Korea	Oct 16, 2003
	SFC20031017-30	*Quercus* sp.	Jangsu-gun, Jeollabuk-do, Korea	Oct 17, 2003
	SFC20040317-04	*Juniperus rigida*	Yeongam-gun, Jeollanam-do, Korea	Mar 17, 2004
	SFC20040525-14	*Quercus* sp.	Hadong-gun, Gyeongsangnam-do, Korea	May 25, 2004
	SFC20040525-33	*Quercus* sp.	Hadong-gun, Gyeongsangnam-do, Korea	May 25, 2004
	SFC20040921-31	*Quercus serrata*	Hadong-gun, Gyeongsangnam-do, Korea	Sep 21, 2004
	SFC20040921-83	Broad-leaved tree	Hadong-gun, Gyeongsangnam-do, Korea	Sep 21, 2004
	SFC20040923-22	*Quercus serrata*	Hadong-gun, Gyeongsangnam-do, Korea	Sep 23, 2004
	SFC20050720-21	*Quercus mongolica*	Namwon-si, Jeollabuk-do, Korea	Jul 20, 2005
	SFC20051024-14	Broad-leaved tree	Hongcheon-gun, Gangwon-do, Korea	Oct 24, 2005
	SFC20060509-16	Broad-leaved tree	Gurye-gun, Jeollanam-do, Korea	May 9, 2006
	SFC20060525-19	Broad-leaved tree	Muju-gun, Jeollabuk-do, Korea	May 25, 2006
	SFC20060623-11	Broad-leaved tree	Hongcheon-gun, Gangwon-do, Korea	Jun 23, 2006
	SFC20060708-07	Broad-leaved tree	Muju-gun, Jeollabuk-do, Korea	Jul 8, 2006
	SFC20060809-08	*Quercus* sp.	Danyang-gun, Chungcheongbuk-do, Korea	Aug 9, 2006
	SFC20061013-06	*Quercus mongolica*	Pyeongchang-gun, Gangwon-do, Korea	Oct 13, 2006
	SFC20061128-30	*Quercus* sp.	Gapyeong-gun, Gyeonggi-do, Korea	Nov 28, 2006
Abundisporus sp.	SFC20041105-11[a]	*Quercus* sp.	Sinan-gun, Jeollanam-do, Korea	Nov 5, 2004
	SFC20140411-29	*Quercus* sp.	Jangsu-gun, Jeollabuk-do, Korea	Apr 11, 2014
Fomitopsis rosea	SFC20011114-35[a]	*Quercus* sp.	Gurye-gun, Jeollanam-do, Korea	Nov 14, 2001
	SFC20020515-12	*Quercus serrata*	Boeun-gun, Chungcheongbuk-do, Korea	May 15, 2002
	SFC20021011-22[a]	*Quercus* sp.	Boeun-gun, Chungcheongbuk-do, Korea	Oct 11, 2002
	SFC20111029-20[a]	*Quercus* sp.	Pyeongchang-gun, Gangwon-do, Korea	Oct 29, 2011
	SFC20111227-18[a]	*Quercus aliena*	Chuncheon-si, Gangwon-do, Korea	Dec 27, 2011
	SFC20120703-08[a]	*Quercus* sp.	Inje-gun, Gangwon-do, Korea	Jul 3, 2012
	SFC20010710-11	*Quercus* sp.	Gongju-si, Chungcheongnam-do, Korea	Jul 10, 2001
	SFC20021011-44	*Quercus* sp.	Boeun-gun, Chungcheongbuk-do, Korea	Oct 11, 2002
	SFC20021108-12[a]	*Quercus* sp.	Boeun-gun, Chungcheongbuk-do, Korea	Nov 8, 2002
	SFC20110506-08	Broad-leaved tree	Wonju-si, Gangwon-do, Korea	May 6, 2011

[a]Representative specimens for sequencing.

are ellipsoid in shape and have thick walls, while *Fomitopsis* basidiospores are cylindrical in shape and have thin walls [1, 15]. All specimens were identified and used in latter parts of this study (Table 1).

DNA extraction, amplification, and sequencing. Fifteen specimens were chosen for DNA sequencing (Table 1). Genomic DNA was extracted using a modified CTAB extraction protocol [16]. The ITS region was amplified using primers ITS1F and ITS4b [18, 19]. PCR reactions were performed on a thermal cycler (C1000TM; Bio-Rad, Richmond, CA, USA) using AccuPower PCR premix (Bioneer Co., Daejeon, Korea) following Park *et al.* [20]. PCR products were electrophoresed through a 0.8% agarose gel stained with EcoDye (SolGent Co., Daejeon, Korea) and purified using the Expin PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions. DNA sequencing was performed at Macrogen (Seoul, Korea) using an ABI3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis. Sequences were assembled and proofread using MEGA 6 [21] and aligned using the default settings of MAFFT v7 [22]. Alignments were checked by eye and ambiguous positions adjusted manually. We included available GenBank sequences of *Abundisporus* and *Fomitopsis*. *Bjerkandera adusta* (KJ704813) and *Phanerochaete chrysosporium* (KP689211) were used as outgroups following Binder *et al.* [10]. A maximum likelihood (ML) phylogenetic analysis was conducted with RAxML 8.0.2 [23] using the GTRGAMMA model of evolution and 1,000 bootstrap replicates.

RESULTS AND DISCUSSION

In Korea, all wood rot fungi with purple hymenophores were originally identified as *F. rosea* [14]. After detailed morphological and molecular studies, some specimens were identified as *A. fuscopurpureus* [4], *A. pubertatis* [8], or a new species *F. incarnatus* [24]. Although superficially similar, these species can be differentiated based on micromorphology, DNA, and ecology. In this study, we used these three data types to evaluate the status of *Abundisporus* in Korea.

The first step was to distinguish *Abundisporus* from *Fomitopsis*. These two genera can be differentiated based on rot type (*Abundisporus*, white rot; *Fomitopsis*, brown rot) [1, 17], but these data are seldom determined during field collection. For microscopic features differentiating *Abundisporus* and *Fomitopsis*, hyphal system, along with pore, basidia, and basidiospore sizes are slightly different, but measurements overlap in their ranges [6, 15]. Two clear microscopic characters that can be used to differentiate these two genera are basidiaspore shape and wall thickness; *Abundisporus* has ellipsoid basidiospores with thick walls, while *Fomitopsis* has cylindrical basidiospores with thin walls [5, 15, 24, 25]. Based on these two characters, we identified 45 specimens in SFC that are *Abundisporus* (Table 1).

The next step was to verify which *Abundisporus* species are present in Korea. All the specimens used in this study had similar morphology (Fig. 1); The hyphal systems were

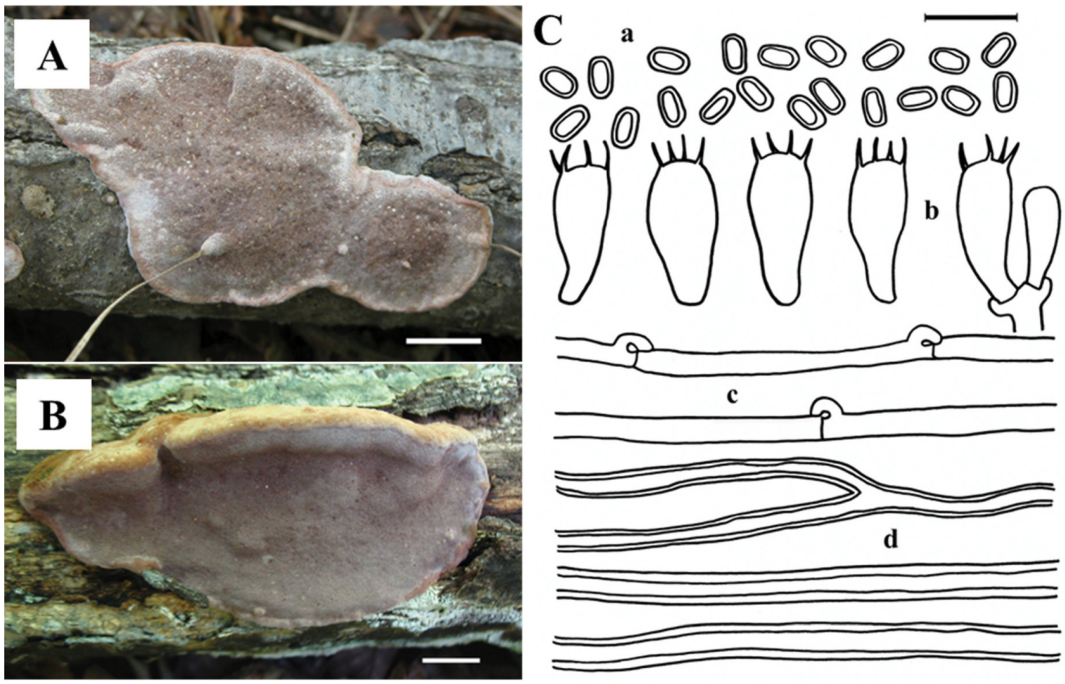

Fig. 1. *Abundisporus pubertatis*. A, Basidiocarp (SFC20030921-11); B, Basidiocarp (SFC20110921-31); C, Microscopic features (scale bars: A, B = 1 cm, C = 10 μm). a, basidiospores; b, basidia; c, generative hyphae; d, skeletal hyphae.

all dimitic, generative hyphae with clamp connections and skeletal hyphae. Depending on their growth condition, basidiocarps were either sessile or effused-reflexed. Hymenophores of old speciemens were dark brown or ocher, while fresh samples were purple or pinkish. Based on microscopic characters, *A. pubertatis* can be distinguished from *A. fuscopurpureus* by its larger pores (6~7 per mm vs. 7~9 per mm) and larger basidiospores (3.5~5.0 × 2.5~3.5 μm vs. 2.0~3.0 × 1.0~1.5 μm) [9]. For all 45 specimens, there was an average of 5 to 7 pores per mm and basidiospores were 4.5~5.0 × 2.4~3.0 μm. These features were more similar to *A. pubertatis*, despite some specimens originally being identified as *A. fuscopurpureus* or *F. rosea*.

ITS was sequenced for 15 specimens, selecting representatives specimens originally identified as *A. pubertatis*, *A. fuscopurpureus*, *Abundisporus* sp., or *F. rosea*. All sequences were approximately 600 bp in length. Sequences of SFC specimens were highly similar (similarity 99.6~100%). All the samples in Korea formed a monophyletic clade with *A. pubertatis* (ML bootstrap = 100%) (Fig. 2). Based on sequence similarity and ML analysis, all sequenced specimens were identified as *A. pubertatis*. Sequences were deposited in GenBank (accession numbers in Fig. 2).

Ecological data should not be the only data used for identification, but it may help. It is noted in the key to differentiating between the two *Abundisporus* species in East Asia [9] that *A. fuscopurpureus* is found in tropical to subtropical climates, while *A. pubertatis* is found in temperate climates. The climate in Korea is temperate, implying the existence of only *A. pubertatis* in Korea.

Data from micro-morphology, DNA, and ecology all support the existence of a single *Abundisporus* species in Korea—*A. pubertatis*. To check our results, we re-examined one of the specimens in the original record of *A. fuscopurpureus* in Korea (SFC20030927-10) [4]. Phylogenetic analysis of the ITS sequence clearly identified this species to be *A. pubertatis* (Fig. 2), and re-examination of the micro-morphology measurments also matched this specimen with *A. pubertatis*. Below we provide a detailed description of *A. pubertatis* in Korea, as well as a key to distinguish wood rot fungi with purple hymenophores.

Taxonomy.

Abundisporus pubertatis (Lloyd) Parmasto, Karstenia 40: 133 (2000).

Basidiocarps partly resupinate, perennial, vinaceous brown to fuscous, forming a thick pileus along upper edges; pileal surface narrow, smooth, often faintly sulcate, developing grey to dark brown crust; margin distinct, entire, obtuse, usually purple and pinkish when fresh; pore 5~7 per mm, angulate ellipsoid or round, pore surface pale purple to lilac grey; tubes 1~3 mm long; context vinaceous brown,

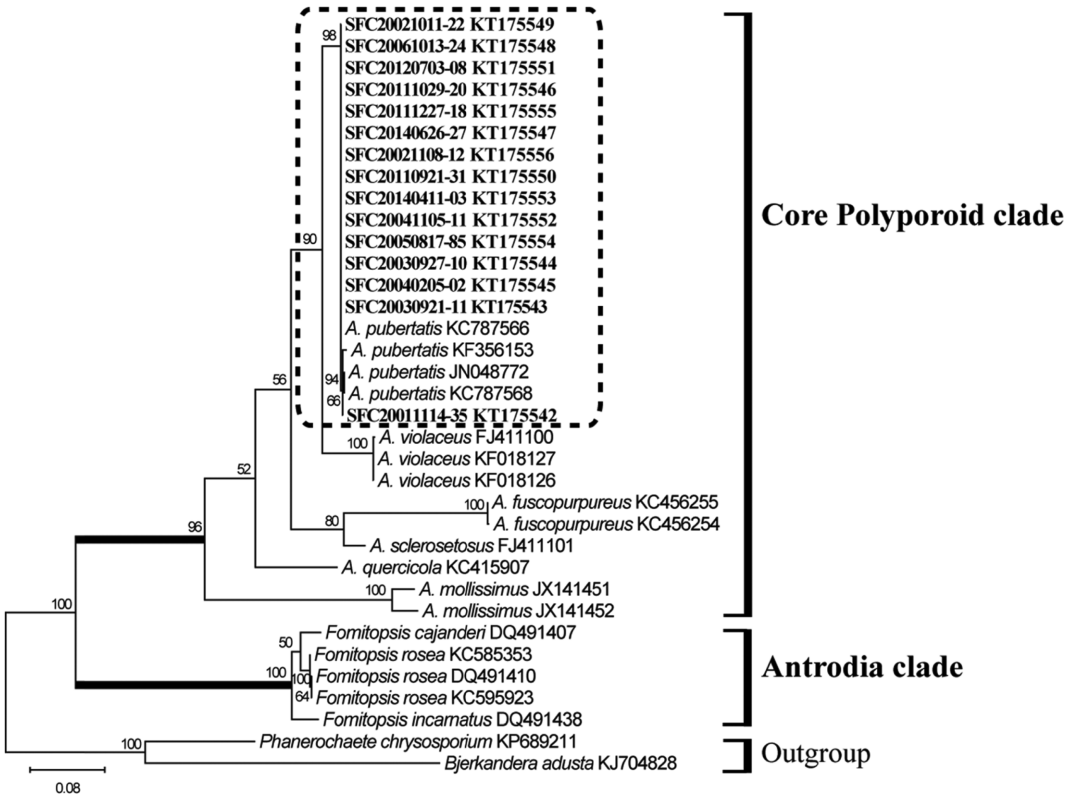

Fig. 2. Phylogenetic tree for *Abundisporus* and related species based on a maximum likelihood analysis of the internal transcribed spacer. Bootstrap scores of > 50 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site.

soft corky, becoming hard.

Hyphal system dimitic, generative hyphae septate with clamps, 2~3 μm wide; skeletal hyphae straight to moderately branched, 3~5 μm wide; basidia broadly clavate, 18.3~20.0 × 6.3~7.9 μm, 4 sterigmata; basidiospores pale brown, broadly ellipsoid, 4.5~5.0 × 2.4~3.0 μm.

Specimen examined: On a fallen trunk of *Quercus serrata*, Mt. Deogyu (SFC20030927-10).

Additional specimens examined: SFC20061013-24, SFC20120703-08, SFC20111029-20, SFC20111227-18, SFC20140626-27, SFC20021108-12, SFC20110921-31, SFC20050817-85, SFC20140411-03, SFC20041105-11, SFC20040205-02, SFC20030927-10, SFC20030921-11, SFC20011114-35, SFC20021011-22, SFC20150320-02.

Remarks: *Abundisporus pubertatis* is similar to *A. fuscopurpureus* and *F. rosea*, having a purple colored hymenophore. It can be distinguished from *F. rosea* by rot type and basidiospores with thick walls and ellipsoid shape. Size of pore, basidia, and basidiospore can be used to distinguish *A. pubertatis* from *A. fuscopurpureus*. Although smaller basidia size (10.0~13.0 × 3.0~4.5 μm) of *A. pubertatis* (KUC20080726-14) was proposed [8], we correct the basidia size as 18.3~20.0 × 6.3~7.9 μm through the re-examination of the originally recorded specimen (KUC20080726-14).

Taxonomic key to distinguish wood rot fungi with purple colored hymenophores (based on [5, 9, 15]).

1. Basidiospore thin wall and cylindrical shape, brown rot 2
1. Basidiospore thick wall and ellipsoid shape, white rot 4
   2. Pores 6~8 per mm ..... *F. incarnatus*
   2. Pores less than 5 per mm 3
3. Basidiospore curved cylindrical ..... *F. cajanderi*
3. Basidiospore non-curved cylindrical ..... *F. rosea*
   4. Pores 7~9 per mm, basidiospores 2.5~3.3 × 1.7~2.1 ..... *A. fuscopurpureus*
   4. Pores 5~7 per mm, basidiospores 4.5~5.0 × 2.4~3.0 ..... *A. pubertatis*

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