

The First Report of *Antrodia sitchensis* (Polyporaceae, Basidiomycota) in Korea

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An unrecorded *Antrodia* species was collected in South Korea and based on morphological characteristics, the species was identified as *Antrodia sitchensis*. To confirm its affinity within the polypores, the phylogenetic relationships of *A. sitchensis* and allied species were established using large subunit rDNA sequences.

KEYWORDS : *Antrodia*, Large subunit rDNA, Phylogeny, Taxonomy

Antrodia Karsten is a cosmopolitan genus with the characteristics of resupinate to effused-reflexed basidiocarp, dimitic hyphal system with clamped generative hyphae, white or pale context, oblong to ellipsoid, hyaline, thin-walled, smooth and non-amyloid spores, and brown rot [1]. They selectively degrade cellulose and hemicelluloses from wood and significantly weaken its structural properties. Some *Antrodia* species have been reported to cause decay in wood products from playground [2] as well as in building materials [3]. In ecosystems, brown rot residues increase the water holding capacity and cation exchange capacity of soils. Such soils also have a favorable acid pH as well as temperature [1]. In Korea, a total of seven *Antrodia* species has been reported to date [4]: *Antrodia albida* (Fries) Donk, *A. crassa* (P. Karsten) Ryvardeen, *A. heteromorpha* (Fries) Donk, *A. malicola* (Berkeley & M. A. Curtis) Donk, *A. serialis* (Fries) Donk, *A. sinuosa* (Fries) P. Karsten, and *A. xantha* (Fries) Ryvardeen.

During our studies on the diversity of indigenous fungi,

an *Antrodia* species (KUC20090711-32) was collected from Gyeonggi Province in Korea on July 11, 2009. It had a similar morphology to *A. xantha*, but was slightly different. In order to identify this fungus at the specific level, macro- and microscopic features of the basidiocarp were observed and final identification was complemented based on sequence analysis of large subunit (LSU) rDNA. Measurements and drawings were made from slide preparations mounted in 3% KOH [5] using an Olympus BX51 light microscope (Olympus, Tokyo, Japan). More than 20 measurements were made to ascertain the average dimensions of each characteristic. The voucher specimen was deposited at the National Biological Resources Center (KB). A key to *Antrodia* species found in Korea was constructed. The six *Antrodia* species that were examined in this study are listed in Table 1 and the descriptions of two species (*A. crassa* and *A. serialis*) were described in a previous study [6].

To carry out sequence analysis, DNA was extracted

Table 1. Specimens used in this study

Species	Voucher No.	Habitat	Locality
<i>Antrodia albida</i>	SFC030921-14	Hardwood	Mt. Deogyu, Korea
<i>A. heteromorpha</i>	SFC031017-10	<i>Quercus mongolica</i>	Mt. Deogyu, Korea
<i>A. malicola</i>	SFC990320-33	<i>Quercus</i> sp.	Mungyeong, Korea
	SFC991003-23	<i>Quercus</i> sp.	Mungyeong, Korea
<i>A. sinuosa</i>	SFC030921-20	Conifers	Mt. Deogyu, Korea
<i>A. sitchensis</i>	KUC20090711-32	Conifers	Mt. Cheonma, Korea
<i>A. xantha</i>	SFC031101-09	<i>Pinus densiflora</i>	Mt. Deogyu, Korea

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from the specimen using Accuprep genomic DNA extraction kit (Bioneer, Daejeon, Korea) and LSU rDNA region was amplified using the primers LR0R/LR3 [7]. PCR reactions were performed using Accupower PCR premix kit (Bioneer). The reaction conditions were the same as those described by Jang *et al.* [8]. The sequences obtained in this study were matched with the reference sequences in

GenBank using a BLAST search. They were aligned initially with MUSCLE ver. 3.8.31 [9] and optimized manually with MacClade ver. 4.08 [10]. For analysis, maximum parsimony (MP) as well as neighbor joining (NJ) tree with the options of 10 random addition sequences was established using PAUP* 4.0b10 [11]. Bootstrap values were determined to support individual branches (1,000 replicates of MP

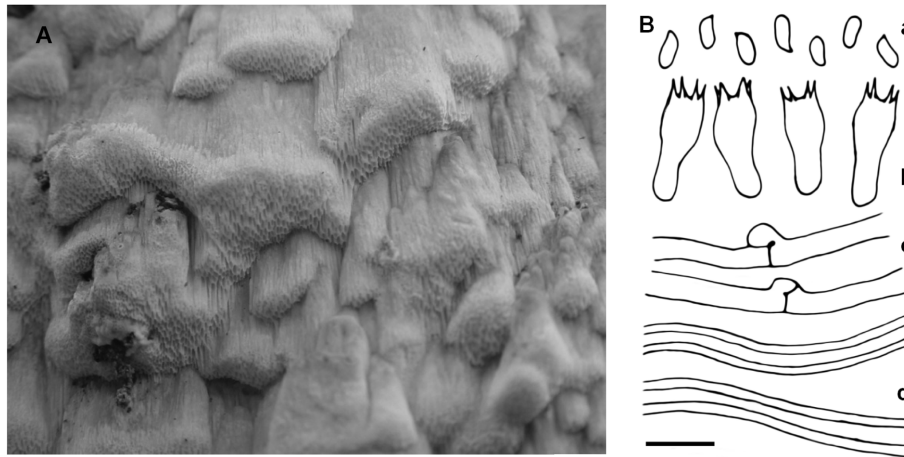


Fig. 1. (A) Basidiocarp and (B) microscopic features of *Antrodia sitchensis*. a, basidiospores; b, basidia; c, generative hyphae; d, skeletal hyphae (scale bar = 10 μ m).

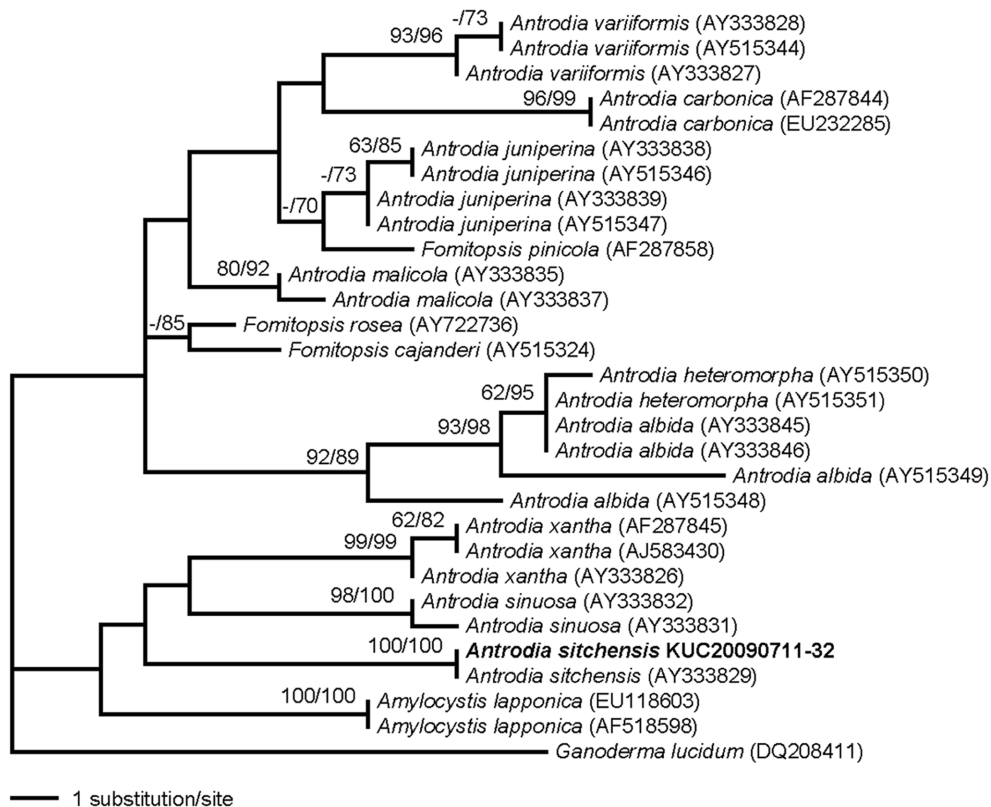


Fig. 2. Maximum parsimonious tree of *Antrodia sitchensis* and its allied species. Bootstrap values greater than 50 are shown above branches (maximum parsimony bootstrap proportions/neighbor joining bootstrap proportions). The specimen found in this study is in bold font.

and NJ bootstrap).

The specimen (KUC20090711-32) was found to belong to the genus *Antrodia* based on its resupinate basidiocarp and whitish to cream poroid hymenophore and to the species *Antrodia sitchensis* by the shape and size of its basidia and basidiospores. This species was first reported in America as *Poria sitchensis* by Baxter, D. V. in 1937. Later, it was transferred to *Antrodia* by Gilbertson and Ryvar den [12]. *A. sitchensis* has been reported in Europe and North America as well as in Asia, particularly in China [13]. According to Jung [6], Lee *et al.* [14], and Gilbertson and Ryvar den [1], the macroscopic features of *A. sitchensis* were similar to *A. xantha*, but the microscopic features of *Antrodia* species found in Korea were clearly different from each other. *A. sitchensis* is distinguished by cylindrical and large spores ($4.5\text{--}6 \times 2\text{--}2.5 \mu\text{m}$) when compared to allantoid and the small spores of *A. xantha* ($2.8\text{--}3.5 \times 1.5 \mu\text{m}$) (Fig. 1). Basidiocarp of *A. xantha* also cracks into squares when old [14]. *A. sitchensis* (KUC20090711-32) is mostly in agreement with the description of Gilbertson and Ryvar den [1] but has smaller basidia when compared to the American specimens.

The rDNA region sequence (527 bp) was deposited in GenBank under accession number of JF510484. It matched to *A. sitchensis* (AY333829) with 100% similarity. The MP and NJ analyses showed that *A. sitchensis* was clearly separated from *A. xantha* (Fig. 2). However, the phylogenetic placement of this species still needs to be resolved before making on definitive conclusions.

Taxonomy

Antrodia sitchensis (D. V. Baxter) Gilb. & Ryvar den, Mycotaxon 22: 363 (1985).

Synonymy. *Amyloporia sitchensis* (D. V. Baxter) Vampola & Pouzar, Česká Mykol. 46: 213 (1993); *Poria sitchensis* D. V. Baxter, Pap. Mich. Acad. Sci. 23: 293 (1938).

Basidiocarps: Resupinate, confluent, corky when fresh, brittle when dry, pore surface cream when fresh, light brown in old specimens, pores round, elongated on vertical surface, 3~6 per mm, whitish to cream, context thin and cream. **Hyphal system:** Dimitic, generative hyphae with clamps, thin-walled and $2.5\text{--}3.5 \mu\text{m}$ wide, skeletal hyphae, predominant, thick-walled, $2.5\text{--}5.5 \mu\text{m}$ wide, drops of resinous substances commonly present in microscopic preparations. **Basidia:** 4 sterigmate, clavate, $13\text{--}17 \times 5\text{--}7 \mu\text{m}$. **Basidiospores:** Cylindrical, slightly bent, smooth and thin walled, $4.5\text{--}6 \times 2\text{--}2.5 \mu\text{m}$, hyaline.

Distribution. Asia, Europe, and North America.

Specimen examined. Korea, Gyeonggi Province, Mt. Cheonma, on the trunk of coniferous deadwood. July 11,

2009, Yeongseon Jang (KUC20090711-32: N $37^{\circ}40'52''$, E $127^{\circ}16'30''$).

Remarks. *Antrodia sitchensis* may be confused with *A. xantha*, but the former has larger basidiospores and does not crack when old.

Key to Korean species of genus *Antrodia*

1. Host is coniferous tree 2
1. Host is deciduous tree or both
 - (coniferous and deciduous)..... 6
 2. Pore number per 1 mm is less than 3 *A. sinuosa*
 2. Pore number per 1 mm is more than 3 3
3. Spores longer than $7 \mu\text{m}$ *A. serialis*
3. Spores shorter than $7 \mu\text{m}$ 4
 4. Pore surface pale sordid brown *A. crassa*
 4. Pore surface cream to white 5
5. Spores $4.5\text{--}6 \times 2\text{--}2.5 \mu\text{m}$ *A. sitchensis*
5. Spores $2.8\text{--}3.5 \times 1\text{--}1.5 \mu\text{m}$ *A. xantha*
 6. Spore longer than $10 \mu\text{m}$ 7
 6. Spore shorter than $10 \mu\text{m}$ *A. malicola*
7. Pore number per 1 mm is less than 2 ... *A. heteromorpha*
7. Pore number per 1 mm is 2 to 3 *A. albida*

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