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Research Article

Re-evaluation of the Genus *Antrodia* (Polyporales, Basidiomycota) in Korea

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Abstract The wood decay fungi *Antrodia* P. Karst. play important ecological roles and have significant industrial and economic impacts as both wood degraders and sources of pharmaceutical and biotechnological products. Although each *Antrodia* species has distinct morphological characteristics, the misidentification rate is especially high due to their simple morphological characters. A combination of morphological and internal transcribed spacer region sequence analyses revealed that 27 of 89 specimens previously identified by morphology alone were correct, whereas 35 of these specimens were misidentified as other *Antrodia* species. We report here that seven *Antrodia* species exist in Korea (*A. albida*, *A. heteromorpha*, *A. malicola*, *A. serialis*, *A. sinuosa*, *A. sitchensis*, and *A. xantha*) and based on these specimens, we provide taxonomic descriptions of these species, except for *A. serialis*, which was only confirmed by isolate.

Keywords *Antrodia*, Biotechnological products, Fungal barcode, ITS, Wood decay fungus

Wood decay fungi of the genus *Antrodia* P. Karst (1880) are characterized by resupinate to effused-reflexed, mostly light-colored, and tough to hard basidiocarps and a white to pale cream pore surface. Microscopically, *Antrodia* display dimitic hyphal systems and smooth, cylindrical-ellipsoid and non-amyloid basidiospores [1]. *Trametes serpens* was chosen as a type species, which was subsequently amended as *Antrodia albida* (Fr.) Donk [2]. *Antrodia* has morphological characteristics that are similar to *Antrodiella* Ryvarden & Johans (1980) and *Diplomitoporus* Domański (1970), except rot type [1]. Recent studies have demonstrated a clear distinction in the phylogenetic position of the latter two genera from *Antrodia* [3, 4].

Because members of the genus *Antrodia* cause a brown rot that selectively degrades cellulose and hemicellulose, they influence forest structure and succession and carbon sequestration [5-9]. They also significantly weaken wood and wood products, which reduces their commercial value [5, 10]. However, some of them also have economic value as good sources of pharmaceutical and biotechnological products [11-13].

Approximately 50 *Antrodia* species have been described worldwide [14], and to date, eight species have been reported in Korea: *A. albida*, *A. crassa*, *A. heteromorpha*, *A. malicola*, *A. serialis*, *A. sinuosa*, *A. sitchensis*, and *A. xantha* [15, 16]. *A. sinuosa* was the first species reported in Korea as synonym *Poria vaporaria* [17] and later amended as *A. sinuosa* [18]. *A. albida*, which was first reported as *Trametes albida* in the 1950s [19], was also amended as a member of the genus *Antrodia* in 1992 by Jung [18]. In 1994, *Daedalea heteromorpha* was amended as *Antrodia heteromorpha*, and two new species, *A. crassa* and *A. serialis*, were reported [20]. Since then, three more *Antrodia* species have been described in Korea: *A. malicola* [21], *A. sitchensis* [16], and *A. xantha* [22]. Among them, only *A. sitchensis* was described based on nuclear large subunit rDNA region sequence data and morphological data [16].

With the recent advances in sequencing technology, DNA-based methods for molecular phylogeny and species identification have become faster, cheaper, less labor intensive, and easier to perform, even for non-experts. Fungal molecular phylogeny is based on the internal transcribed spacer (ITS) region, which is a commonly used molecular

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marker for fungal phylogenetics and has been formally proposed as the primary fungal barcode gene [23]. Because numerous ITS region sequences are available in public nucleotide sequence databases like GenBank, the ITS region can be used to correctly identify fungi and investigate their phylogenetic relationships.

Although species delimitation of fungi based on morphology is useful, the misidentification rate of wood decay fungi is especially high due to their simple morphological characters. In this study, we clarified the status of *Antrodia* species in Korea based on ITS sequence analyses and morphological characteristics.

MATERIALS AND METHODS

Samples and morphological analysis. Eighty-nine specimens kept in the Seoul National University Fungus Collection (SFC) and the Korea University Culture Collection (KUC) were analyzed in this study. They were originally identified as seven species; 81 specimens from SFC comprised six species (*A. albida*, *A. heteromorpha*, *A. malicola*, *A. serialis*, *A. sinuosa*, and *A. xantha*) and eight specimens from KUC comprised four species (*A. albida*, *A. heteromorpha*, *A. malicola*, and *A. sitchensis*). Measurements and drawings were made from slide preparations mounted in 3% KOH

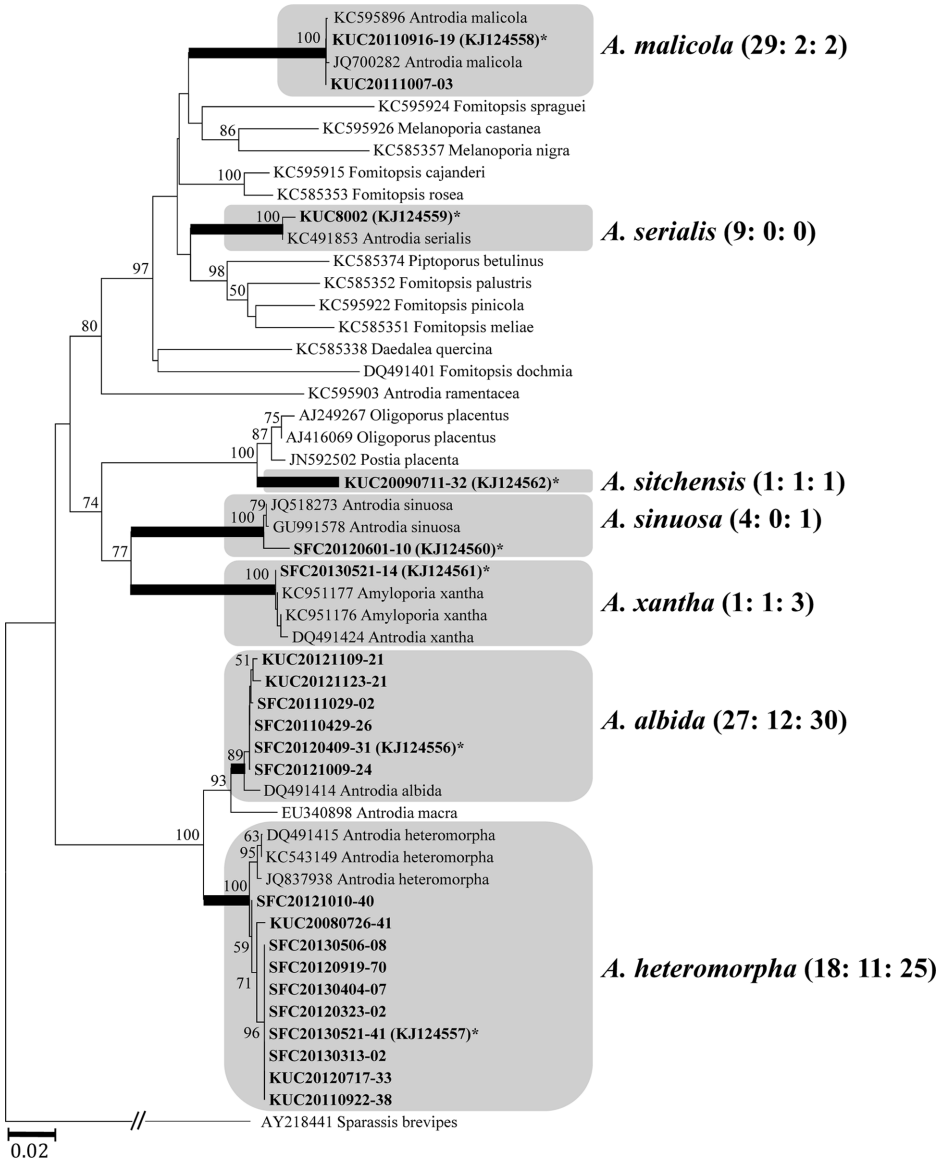

Fig. 1. Neighbor joining tree inferred from the internal transcribed spacer (ITS) sequences of seven Korean *Antrodia* species. Bootstrap value is presented on the line. The accession numbers of the representative specimens are marked with asterisks. The numbers shown in parentheses (A : B : C) indicate the number of specimens from the original morphological identification as stored in SFC (A), the number of correctly identified samples among herbarium specimens (B), the number of specimens after rearrangement according to the morphological and molecular analyses in this study (C). *A. serialis* (KUC8002) is the isolate obtained from wood products.

and 1% phloxine [24] by using a Nikon 80i light microscope (Nikon, Tokyo, Japan). More than 20 basidiospores were measured to ascertain average dimensions. The quotient (Q) is the ratio of variation between the mean spore length and the mean spore width of the studied specimens. Species identification via classical methodology was achieved by macro- and micro-morphological observations using taxonomic guides [1, 25, 26].

DNA extraction, PCR, sequencing, and analysis. Tissues from fresh basidiocarps and herbarium materials were placed in 2× CTAB buffer. Genomic DNA was extracted using a modified CTAB extraction protocol [27]. The ITS region was amplified by PCR using primers ITS5 and ITS4b [28, 29]. Each reaction was performed on a C1000 thermal cycler (Bio-Rad, Hercules, CA, USA) using AmpONE Taq premix (GeneAll Biotechnology, Seoul, Korea) in a final volume of 20 μL containing 10 pmol of each primer and 1 μL of DNA template. PCR amplification was performed as described by Park *et al.* [30]. The PCR products were electrophoresed in a 1% agarose gel, and then stained with loading STAR (Dyne Bio, Seoul, Korea) and purified using the Expin PCR Purification Kit (GeneAll Biotechnology) according to the manufacturer's instructions. DNA sequencing was performed by the DNA Synthesis and Sequencing Facility at Macrogen (Seoul, Korea) with an ABI3700 automated DNA sequencer.

Sequences were assembled, proofread, and edited using MEGA 5 [31]. Representative sequences were deposited in GenBank (accession Nos. are shown in Fig. 1). The sequences obtained in this study were compared to the reference sequences in GenBank using BLAST. Multiple alignments were performed using the default settings of MAFFT v7 [32]. DNA alignments were checked by eye, and ambiguously aligned positions were manually adjusted. The sequence of *Sparassis brevipes* Krombh. (AY218441) was used as an outgroup based on a previous study [33]. A neighbor joining tree was constructed with MEGA 5 [31] using the Kimura 2-parameter model [34]. Bootstrap analysis was performed with 1,000 replications for branch stability.

RESULTS AND DISCUSSION

Re-evaluation of *Antrodia* species in Korea. The specimens selected for this study were collected from different geographic locations in Korea and were initially identified by morphological methods as follows: *A. albida* (27 specimens), *A. heteromorpha* (18), *A. malicola* (29), *A. serialis* (9), *A. sinuosa* (4), *A. sitchensis* (1), and *A. xantha* (1) (Fig. 2). We determined their identity by combining morphological observations of the macro- and microscopic features with molecular analyses of their ITS sequences. The ITS sequencing and morphological characteristic analyses revealed that seven *Antrodia* species exist in Korea. Although *A. sitchensis* (KUC20090711-32) was closely related to *Oligoporus placentus* in the ITS tree, its dimitic hyphal system was distinguished from the monomitic hyphal system of *O. placentus* [1].

Since the *Antrodia* have been regarded as a heterogeneous

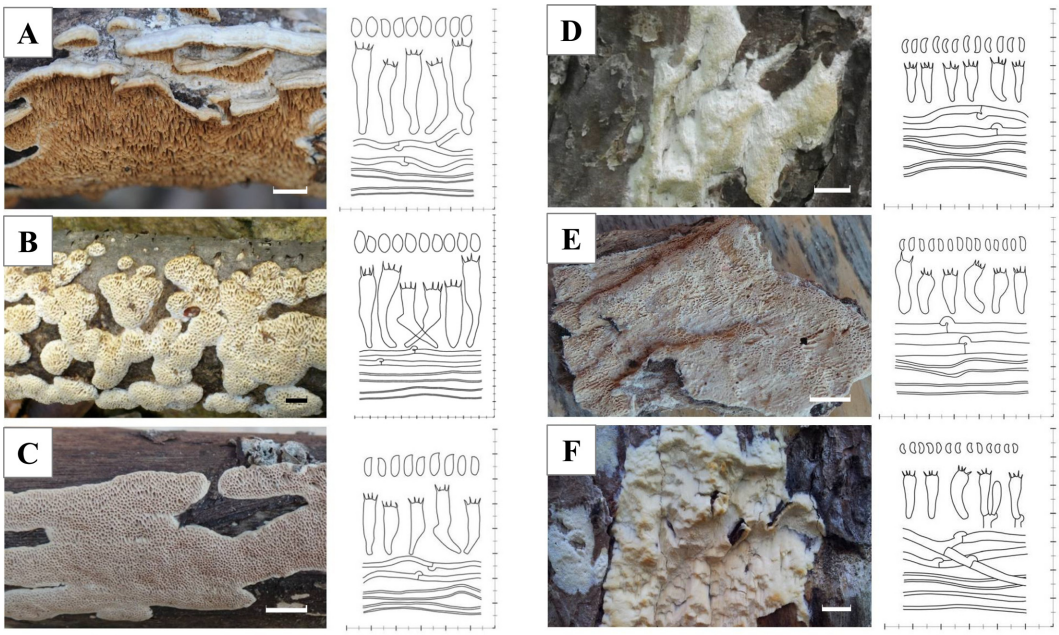

Fig. 2. Basidiocarps and microscopic features of *Antrodia albida* (A), *A. heteromorpha* (B), *A. malicola* (C), *A. sinuosa* (D), *A. sitchensis* (E), and *A. xantha* (F). The microscopic features, the basidiospores, basidia, generative hyphae with clamp connection, and skeletal hyphae are shown from the top. The scale bar is 5 mm in the basidiocarp images and 10 μm in the microscopic images.

and polyphyletic group, segregation of *Antrodia s. l.* into three genera (*Antrodia s. s.*, *Amyloporia* Singer, and *Fibroporia* Parmasto) has been proposed [14, 35-40]. *Fibroporia* has a rhizomorphic margin as a diagnostic characteristic [35], and its separation is well supported by molecular analysis [41]. *Amyloporia* is distinguished by amyloid skeletal hyphae [42], and it has a paraphyletic relationship [38]. Although *Fibroporia* have not been reported in Korea, three species that have been proposed for the genus *Amyloporia*, *A. sinuosa*, *A. sitchensis*, and *A. xantha* [33], are included in this study. We decided to leave these species in the genus *Antrodia* because of the paraphyletic relationship in the proposed genus [38].

In this study, we verified six *Antrodia* species among the 89 specimens. Twenty-seven of the specimens (approximately 30%) were correctly identified. Among the misidentified specimens, 35 were *Antrodia*; however, the species identification was incorrect. Twenty-seven specimens were identified as other genera such as *Trametopsis cervina, Perenniporia subacida,* and *Schizopora paradoxa*. Nine specimens that were initially identified as *A. serialis* were confirmed as other species, such as *A. albida*, *A. heteromorpha*, *A. sinuosa, Cinereomyces lindbladii*, *Perenniporia subacida*, *Schizopora paradoxa*, and two unidentified polypores. The specimen (SFC19910816-31) that was used for the original description of *A. serialis* in Korea [20] was lost from SFC. The existence of *A. serialis* in Korea was proved by an isolate that was obtained from playground wood products [43]. Among the four specimens of *A. sinuosa*, two were amended as *A. xantha* and *Trametes hirsuta,* and two were unidentified. One specimen (SFC20120601-10) originally recorded as *A. serialis*, was identified as *A. sinuosa*.

Antrodia crassa was only reported once in Korea at Mt. Sobaek National Park in 1994 [20]. However, the specimen was severely damaged by mold; therefore, we could not verify its identity. Although *A. crassa* has been known to be distributed in coniferous forests and to inhabit gymnosperms [1], according to the description, the Korean specimen was collected from a deciduous tree [20]. Therefore, further investigations in coniferous forests are required to confirm whether *A. crassa* exists in Korea or not. We report here that seven *Antrodia* species exist in Korea, and provide taxonomic descriptions based on the specimens, expect for *A. serialis*, which was only confirmed by one isolate.

Taxonomy.

***Antrodia albida* (Fr.) Donk**, Persoonia 4: 339 (1966) [2].

Basidiocarps annual, resupinate, effused to reflexed, imbricate growing on a vertical surface, up to 4.5 mm thick at the base, margin sharp; upper surface white to cream colored, smooth, first matted and adpressed velutinate, becoming glabrous in zones with age; context tough and corky, 0.3~0.5 mm thick at the base; hymenophore white to cream or yellowish in old specimens, pores round to angular, 1~2 per mm, radially elongate, sinuous and semilamellate on vertical substrates, slightly dentate, tube length, 2~4 mm at the base. Hyphal system dimitic; generative hyphae with clamps, moderately branched, 2.5~5 μm thick; skeletal hyphae dominating in context and the pileus surface, hyaline, thick-walled to solid, 2~3 μm thick. Basidia narrowly clavate, 34~45 × 6.2~9.2 μm. Basidiospores oblong elliptical, thin-walled, smooth, hyaline, 8.5~9.7 × 4~5.2 μm, Q = 1.83~2.42.

Specimens examined: KUC20121109-21, KUC20121123-21, SFC19990326-20, SFC19990326-31, SFC19990326-35, SFC19990326-36, SFC19990521-16, SFC20000922-01, SFC20021010-03, SFC20021011-21, SFC20021107-01, SFC20021123-02, SFC20030419-24, SFC20030827-01, SFC20030921-14, SFC20040512-04, SFC20040527-12, SFC20040923-42, SFC20050526-21, SFC20050609-48, SFC20110429-26, SFC20110519-32, SFC20111029-02, SFC20120409-02, SFC20120409-31, SFC20121009-24, SFC20130314-13, SFC20130315-33, SFC20130403-36, and SFC20130507-05.

Remarks: This species is commonly found on oak trees and is characterized by the white surface of the basidiocarp and a hymenophore with pores or a mixture of pores and lamellate. This taxon is often confused with *Trametopsis cervina*, which is separated from *A. albida* by the upper surface of the basidiocarp, which is hirsute to strigose and a pinkish buff to cinnamon in color, and short basidia (20~25 × 5~7 μm) [44].

***Antrodia heteromorpha* (Fr.) Donk**, Persoonia 4: 339 (1966) [2].

Basidiocarps annual, resupinate to semipileate growing on a sloped surface, tightly attached to the substrate; pileus protrudes 1.4 cm, margin distinctly bounded; upper surface creamy to beige colored, smooth; context soft or corky and thin; hymenophore cream or pale brown, pores round to angular, with lacerate pore mouths, 1~2 per mm, elongate, daedaleoid on vertical surface, tube length 3~8 mm. Hyphal system dimitic; generative hyphae with clamps, 2~3 μm thick; skeletal hyphae hyaline, thick-walled, 5~6 μm thick. Basidia clavate, 42~61 × 8.5~12.5 μm. Basidiospores oblong elliptical, thin-walled, smooth, hyaline, 10.8~14.8 × 5.7~7.4 μm, Q = 1.7~1.95.

Specimens examined: KUC20080726-41, KUC20110922-38, KUC20120717-33, SFC19900807-01, SFC19971008-07, SFC19980411-13, SFC19981217-24, SFC19991219-07, SFC19991219-19, SFC20030419-12, SFC20030613-30, SFC20030928-19, SFC20040525-35, SFC20040525-42, SFC20050609-05, SFC20120323-02, SFC20120508-06, SFC20120915-03, SFC20120919-70, SFC20121010-40, SFC20130313-02, SFC20130403-16, SFC20130404-07, SFC20130506-08, and SFC20130521-41.

Remarks: This species is characterized by a thick resupinate hymenophore with large lacerate pores tightly attached to the substrates, often found on oak trees and large basidiospores (10.8~14.8 × 5.7~7.4 μm).

***A. malicola* (Berk. & M. A. Curtis) Donk**, Persoonia 4: 339 (1966) [2].
Basidiocarps annual, resupinate, effused to reflexed, semipileate growing on a sloped surface projecting up to 1 cm on and up to 5 mm thick at the base, margin sharp; upper surface cream to buff colored, smooth; context corky, buff, 0.5~1 mm thick; hymenophore cream to buff, pores round, elongate on vertical surface, 2~3 per mm, tubes up to 4 mm long. Hyphal system dimitic; generative hyphae with clamps, 2~3 µm thick; skeletal hyphae hyaline, thick-walled, 2~4 µm thick. Basidia clavate, 25~31 × 5.7~6.8 µm. Basidiospores cylindrical, smooth, 7.8~9.2 × 3~4 µm, Q = 2.44~2.86.
Specimens examined: KUC20110916-19 and KUC20111007-03.
Remarks: Buff color basidiocarp, round pores, and short basidia can separate this species from *A. albida*, which has white basidiocarps and a pore surface with mixed round and lamellate pores.

***A. sinuosa* (Fr.) P. Karsten**, Medd. Soc. Fauna Flora Fenn. 6: 10 (1881) [45].
Basidiocarps annual, resupinate, becoming effused; margin cream color to light buff, distinctly bounded; hymenophore cream-colored, buffy brown when dried, pores angular, sinuous, 3~5 per mm, with thin dissepiments. Hyphal system dimitic; generating a hyaline hyphae, thin-walled, with clamps, 2.2~2.8 µm thick; skeletal hyphae hyaline, thick-walled, non-septate, 2.5~3 µm thick. Basidia clavate, 14~15 × 2.8~4.5 µm. Basidiospores cylindrical to allantoid, hyaline, smooth, 5.1~5.7 × 1.4~1.7 µm, Q = 3.05~4.12.
Specimen examined: SFC20120601-10.
Remarks: This species was detected only on a conifer tree. It may be confused with other morphologically similar species that grow on conifer trees, such as *A. xantha*, which has smaller pores and Basidiospores, or with *Schizopora flavipora*, which has white, sterile, and fimbriate margins and tramal hyphal ends with spherical swellings.

***Antrodia sitchensis* (D. V. Baxter) Gilb. & Ryv.**, Mycotaxon 22: 363 (1985) [46].
Basidiocarps resupinate, confluent; context thin, white to cream; hymenophore cream when fresh, light brown when old, pores round, elongated on vertical surface, 3~5 per mm. Hyphal system dimitic, generative hyphae with clamps, thin-walled and 3.8~4.5 µm thick, skeletal hyphae, predominant, thick-walled, 2.85~5.5 µm thick, drops of resinous substances commonly present in microscopic preparations. Basidia two to four sterigmata, clavate, 18~26 × 5.14~7.1 µm. Basidiospores cylindrical, smooth and thin walled, hyaline, 4.5~5.7 × 1.6~2.1 µm, Q = 2.44~2.86.
Specimen examined: KUC20090711-32.
Remarks: *A. sitchensis* may be confused with *A. sinuosa*; however, the former has larger basidia (18~26 × 5.14~7.1 µm) and does not crack at senescence.

***Antrodia xantha* (Fr.) Ryvarden**, Nor. J. Bot. 20: 8 (1973) [47].
Basidiocarps annual, resupinate, widely effused, narrow semipilei on vertical surfaces, adnate, up to 1 mm thick, soft when fresh, chalky when dry, margin narrow; context thin and white; hymenophore white when fresh, fading to light buff, pores round and 7~8 per mm. Hyphal system dimitic; generative hyphae clamped, thin-walled, 2~3 µm thick; skeletal hyphae abundant, thick-walled to semisolid, 3~7 µm thick. Basidia clavate, 17~20 × 4~5 µm. Basidiospores allantoid, hyaline, 3.4~4 × 1.2~1.7 µm, Q = 2.8~3.1.
Specimens examined: SFC20030614-03, SFC20040527-63, and SFC20130521-14.
Remarks: *Antrodia xantha* is characterized by its small pores (7~8/mm), cream-colored, thick, and resupinate basidiocarps, and host specificity on conifers. *A. sinuosa* and *A. sitchensis* are morphologically similar to *A. xantha*; however, pore size could be a distinguishing characteristic.

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