RESEARCH ARTICLE

Current Taxonomical Status of Korean Auricularia Species

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ABSTRACT

Auricularia (Auriculariaceae, Basidiomycota) is well-known jelly fungi, and most species are cultivated or utilized medicinally around the world. A total of 127 Auricularia specimens were collected during Korean mushroom forays by the Korea National Arboretum from 2008 to 2020. In this investigation, fungal barcode analysis and morphological examinations of 33 specimens were conducted and five Korean Auricularia species were identified. Of the species identified, A. americana, A. heimuer, and A. villosula were new to Korea, while A. auricula-judae which was previously identified in Korea, was not found. Herein, we discuss the Korean Auricularia species and provide a taxonomic key for their morphological identification.

Keywords: Auriculariaceae, Basidiomycota, Jelly fungi, Phylogeny, Taxonomy

INTRODUCTION

Auricularia species have ear-shaped fruiting bodies and are considered a wood-decaying fungus which commonly grows on wood. They are easily cultivated, and some species have high economic, nutritional, medicinal, and pharmacological values in Asian countries [1,2]. While the Index Fungorum (http://www. indexfungorum.org/) lists 177 species and variety names for Auricularia, only 10 to 15 identified species have been studied [3]. Auricularia species are well-known for their morphological characteristics and distribution, but species level identification can be difficult due to their similar colors, fruit bodies, and sizes. Auricularia mesenterica is a type species of Auricularia [4]. A recent study [5] showed that A. auricula-judae has been treated phylogenetically as a species complex. Some specimens considered to be A. auricula-judae were later identified as A. heimuer, A. minutissima, A. thailandica, and A. villosula [6,7].

Since 1978, A. hispida Iwade, A. mesenterica (Dicks.) Pers., and A. polytricha (Mont.) Sacc. (current name: A. nigricans (Sw.) Birkebak, Looney, and Sánchez-García) have been recorded in Korea [8,9]. Recently, two unrecorded species (Auricularia cornea and A. minutissima) were reported based on morphological and molecular phylogenetic analysis in Korea [10], but comprehensive phylogenic and



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License (http://creativecommons.org/licenses/bync/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. taxonomic studies for the identification of Auricularia in Korea have not yet been conducted. Based on DNA sequence data comparisons and morphological characteristics from 127 Auricularia specimens deposited in the Korea National Arboretum (KH), Korea five species of Auricularia were identified, of which A. americana, A. heimuer, and A. villosula were previously unidentified in Korea. Moreover, phylogenetic analysis demonstrated that A. auricula-judae, which had previously been identified in Korea, was not present in these samples.

MATERIALS AND METHODS

Fungal materials and morphological observations

A total of 127 *Auricularia* samples were collected from 2008 to 2020 from various urban parks, legally protected trees, and forests in Korea. Forest floors, leaf litter, twigs, fallen trunks, leaves, and soil were inspected for collections. Macro-morphological characteristics were examined using a dissecting microscope. For micro-morphological characterization, abhymenial hairs, basidiospores, and basidia were mounted in distilled water and 5% KOH and examined using a compound microscope (Olympus BX53, Tokyo, Japan) and photographed with a Jenoptik ProgRes C14 Plus Camera (Jenoptik Corporation, Jena, Germany). Microscopic parameters were measured and calculated using the ProgRes Capture Pro v.2.8.8. software (Jenoptik Corporation, Jena, Germany). Dried specimens were deposited in the herbarium of the Korea National Arboretum (KH).

DNA isolation, amplification, sequencing, and phylogenetic analysis

Genomic DNA from specimens was extracted using the DNeasy Plant Mini DNA Extraction Kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions. After dividing into morphology groups, a representative sample was selected from each group. Of the 127 specimens, 33 samples were selected as a representative sample and these samples were successfully extracted in this study. The extracted DNA was used for PCR amplification. The internal transcribed spacer (ITS) was amplified with primers ITS5 and ITS4 [11]. The PCR amplicons were purified using a QIAquick purification Kit (Qiagen Inc., Hilden, Germany), and then directly sequenced using an ABI Prism[®] 377 Automatic DNA Sequencer (Applied Biosystems, Foster City, CA, USA) with a BigDye Cycle Sequencing Kit Version 3.1 (Applied Biosystems). A BLASTN search against the NCBI database (http://www.ncbi.nlm.nih.gov) was carried out, and new sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/). Sequences of *Auricularia* used in the phylogenetic tree were downloaded from GenBank, and *Exidia grandulosa* (AF291279) was used as an outgroup. For phylogenetic analyses, the data sets were aligned in MAFFT version 7.475 [12], using the auto option. The DNA sequences generated in this study were assembled and edited using BioEdit v. 7.2.5 [13] and MEGA v. 7.0.26 [14]. A phylogenetic tree was constructed using RAXML in the CIPRES Science Gateway (https://www.phylo.org). The relative robustness of

the individual branches was estimated by bootstrapping using 1,000 replicates. The GenBank accession numbers of the ITS sequences used in this study are listed in Table 1.

Table 1. Korean Auricularia specimens used in this study with GenBank accession numbers.

Species	Locality	Collection date	Voucher specimen	GenBank No.
Auricularia americana	Halla Mt. Jeju	27-Sep-2016	KA16-1114 (as A. nigricans)	MW477754
A. comea	Gunsan, Jeollabuk-do	7-May-2012	KA12-0015 (as A. nigricans)	MW477738
	Ulleung, Gyeongsangbuk-do	28-Jun-2012	KA12-0404 (as A. nigricans)	MW477739
	Ulleung, Gyeongsangbuk-do	6-Sep-2012	KA12-1345 (as A. nigricans)	MW477741
	Chuja-do, Jeju	30-Aug-2013	KA13-0890 (as A. nigricans)	MW477742
	Ulleung, Gyeongsangbuk-do	4-Sep-2013	KA13-0937 (as A. nigricans)	MW477743
	Gwangneung forest, Gyeonggi-do	28-Jul-2014	KA14-0696 (as A. nigricans)	MW477746
	Shinan, Jeollanam-do	11-May-2016	KA16-0030 (as A. nigricans)	MW477748
	Shinan, Jeollanam-do	12-May-2016	KA16-0052 (as A. nigricans)	MW477750
	Shinan, Jeollanam-do	13-Jul-2016	KA16-0543 (as A. nigricans)	MW477752
	Shinan, Jeollanam-do	25-Aug-2016	KA16-0896 (as A. nigricans)	MW477753
	Busun	12-Jul-2017	KA17-0355 (as A. nigricans)	MW477761
	Gwangneung forest, Gyeonggi-do	28-Jun-2018	KA18-0192 (as A. nigricans)	MW477763
	Seocheon, Chungcheongnam-do	18-Jun-2019	KA19-0093 (as A. nigricans)	MW477764
	Jeju, Jeju-do	21-Jul-2020	KA20-0264 (as A. nigricans)	MW477770
A. heimuer	Soya-do, Incheon	7-Jul-2014	KA14-0423 (as A. auricula-judae)	MW477745
	Gangneung, Gangwon-do	24-Jun-2015	KA15-0078 (as A. auricula-judae)	MW477747
	Gwangneung forest, Gyeonggi-do	7-Jul-2016	KA16-0398 (as A. auricula-judae)	MW477751
	Uljin, Gyeongsangnam-do	3-Oct-2016	KA16-1174 (as A. auricula-judae)	MW477755
	Seongju, Gyeongsangbuk-do	11-May-2017	KA17-0017 (as A. auricula-judae)	MW477756
	Seongju, Gyeongsangbuk-do	27-Jun-2017	KA17-0237 (as A. auricula-judae)	MW477760
	Pocheon, Gyeonggi-do	18-May-2018	KA18-0050 (as A. auricula-judae)	MW477762
	Goseong, Gangwon-do	1-Jul-2020	KA20-0135 (as A. auricula-judae)	MW477768
	Goseong, Gangwon-do	2-Jul-2020	KA20-0158 (as A. auricula-judae)	MW477769
A. minutissima	Ulleung, Gyeongsangbuk-do	28-Jun-2012	KA12-0427 (as A. auricula-judae)	MW477740
	Suncheon, Jellanam-do	14-May-2014	KA14-0029 (as A. auricula-judae)	MW477744
	Shinan, Jeollanam-do	11-May-2016	KA16-0039 (as A. auricula-judae)	MW477749
	Haenam, Jeollanam-do	20-May-2020	KA20-0026 (as A. nigricans)	MW477766
A. villosula	Cheongdo, Gyeongsangbuk-do	27-Jun-2017	KA17-0221 (as A. auricula-judae)	MW477757
	Seongju, Gyeongsangbuk-do	27-Jun-2017	KA17-0233 (as A. auricula-judae)	MW477758
	Seongju, Gyeongsangbuk-do	27-Jun-2017	KA17-0234 (as A. auricula-judae)	MW477759
	Gangjin, Jeollabuk-do	3-Jun-2020	KA20-0064 (as A. nigricans)	MW477767

RESULTS

Phylogenetic analysis

A total of 33 ITS sequences were obtained from the *Auricularia* specimens in this study (Table 1). Three unrecorded species (*A. americana, A. heimuer* and *A. villosula*) were resolved by conducting RAxML analysis of the ITS sequences. Although there was sequence variation, they formed well-supported clades. A previous study [10] showed that phylogenetic position of *A. cornea* and *A. minutissima* with one sample, respectively. In this study, *A. cornea* and *A. minutissima* form a distinct clade with additional samples from Korea National Arboretum. *A. minutissma* formed a sister group to the *A. heimuer* in ML analysis.

A previous study demonstrated *A. auricula-judae* forming a species complex with ITS sequence data (not included Korean samples here) [5]. In this study, the Korean samples considered to be *A. auricula-judae* before were identified as *A. heimuer*, *A. minutissima*, and *A. villosula*. Therefore, the phylogenetic positions of those species were confirmed in this study.

TAXONOMY

Auricularia americana Parmasto & I. Parmasto ex Audet, Boulet & Sirard, in Boulet, Les Champignons des Arbres de l'Est de l'Amérique du Nord: 287. 2003. (Figs. 1 and 2)

Korean name: Jeonnamu-Mogi (전나무목이); derived from the Korean family name of host (Jeonnamu), 'Abies'

Description: Basidiocarps solitary or gregarious, sessile or stipitate, auriculate to cupulate, with undulate margin, hard-gelatinous to cartilaginous, 2-4 cm broad, 0.4-1 mm thick; upper surface velvety with folds, olive brown to light brown; lower surface smooth with folds, light brown. Medulla absent or indistinct. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 1.5-3 μ m, diam. Abhymenial hairs solitary or gregarious, cylindrical, flexuous, thick-walled with a narrow lumen, apically acute to obtuse, hyaline with pale brown pigmentation at the base, 45-153×7-11 μ m. Basidia narrowly clavate to cylindrical, transversely 3-septate, hyaline, 60-75×4-6 μ m. Basidiospores allantoid to reniform, thin-walled, smooth, hyaline, 11-14×4-5.5 μ m.

Notes: The *Abies balsamea* (L.) Mill. was recorded as a host of the holotype of *A. americana* (Index Fungorum; www.indexfungorum.org/). The Korean specimen (KA16-1114) was collected from *Abies koreana* E.H. Wilson on Jeju Island. According to a previous report [4], this species grows exclusively on gymnosperms. To date, this Korean collection has been preserved in KH.

Auricularia heimuer F. Wu, B.K. Cui & Y.C. Dai, in Wu, Yuan, Malysheva, Du & Dai, Phytotaxa 186: 248. (Figs. 1 and 3)

Korean name: Heung-Mogi (흑무이); as 'heimuer'is a black jelly fungi in Chinese; 'hei' means black and 'muer' means jelly fungi.

Description: Basidiocarps solitary or gregarious, sessile or stipitate, auriculate to cupulate, with undulate margin, hard-gelatinous to cartilaginous, 2-5 cm broad, 0.5-1 mm thick; upper surface velvety with folds, olive brown to light brown; lower surface smooth with folds, light brown. Medulla pale brown, 115-180 μ m, broad. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 2-4 μ m, diam. Abhymenial hairs are solitary to gregarious, cylindrical, flexuous, thick-walled with a narrow lumen, apically acute to obtuse, hyaline with pale brown pigmentation at the base, 45-140×4.5-7 μ m. Basidia narrowly clavate to cylindrical, transversely 3-septate, hyaline, 35-58×4.5-5.5 μ m. Basidiospores allantoid, reniform, thin-walled, hyaline, 9.5-14×5-6 μ m.

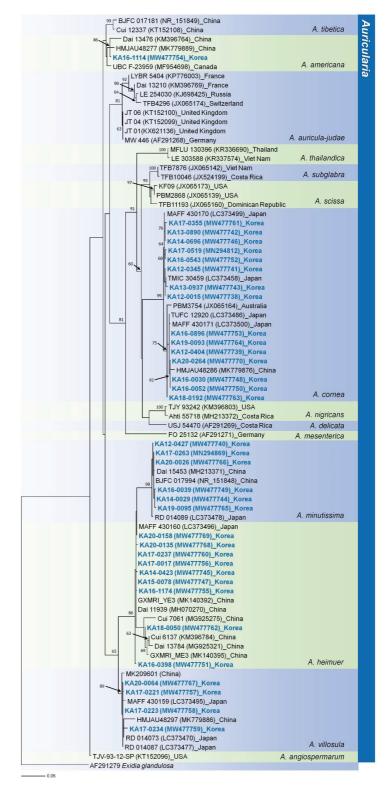


Fig. 1. Phylogenetic tree of Auricularia species based on RAxML analysis of the internal transcribed spacer (ITS) regions. The new sequences from the present study are shown in blue text. Bootstrap values higher than 60% are shown in the branches.

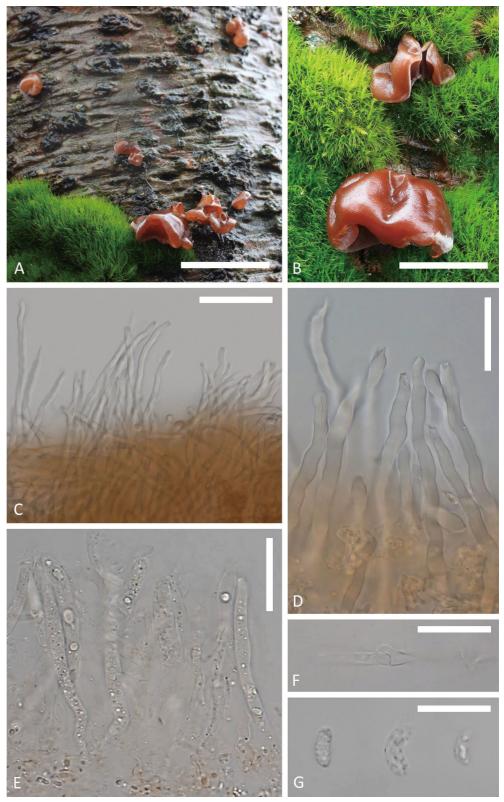


Fig. 2. Micro and macro morphological characteristics of *Auricularia americana* (KA16-1114). A, B: Basidiocarps; C, D: Abhymenial hairs; E: Basidioles and basidia; F: Clamp connenction; G: Basidiospores. Scale bars: A=5 cm; B=3 cm; C=40 µm; D-G=20 µm.

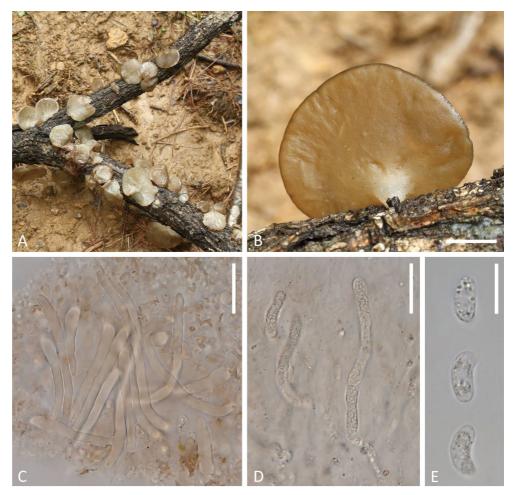


Fig. 3. Micro and macro morphological characteristics of *Auricularia heimuer* (KA20-0135). A, B: Basidiocarps; C: Abhymensial hairs; D: Basidioles and basidia; E: Basidiospores. Scal bars: B=3 cm; C-E=20 μm.

Notes: This species is the third most important cultivated edible mushroom worldwide [4,5]. Several studies of this species have recently been conducted using high-throughput sequencing technologies at the genomic level [15,16]. In Korea, specimens of *A. heimuer* were considered to be part of the *A. auricula-judae* complex. Morphological examination showed that *A. heimuer* produces smaller basidia (35-58 μ m long vs. 64-85 μ m long) and basidiospores (9.5-14 μ m long vs. 14.7-18.2 μ m long) than *A. auricula-judae* [4]. In addition, these two species are phylogenetically distant. Cultivated samples considered to be *A. auricula-judae* from Korea were morphologically and phylogenetically studied in this investigation. As a result of the phylogentic analysis, these samples were identified as *A. heimuer*, and *A. auricula-judae* was not identified.

Auricularia villosula Malysheva, Malysheva & Bulakh, Nov. sist. Niz. Rast. 48: 174. 2014. (Figs. 1 and 4) Korean name: Jjalpeunteol-Mogi (짧은털목이); derived from the species ephithet 'villosula', Latin meaning slightly hairy

Description: Basidiocarps solitary or gregarious, sessile or stipitate, auriculate to cupulate, with undulate margin, hard-gelatinous to cartilaginous, 1-7 cm broad, 1 mm thick; upper surface velvety with folds, brown to light brown; lower surface smooth with folds, light brown. Medulla absent or indistinct. Internal hyphae branched, septate, thin-walled, hyaline, with clamp connections, 2-4.5 μ m, diam. Abhymenial hairs solitary or gregarious, cylindrical, flexuous, thick-walled with a narrow lumen, apically acute to obtuse, hyaline with pale brown pigmentation at the base, 30-93×5.5-6 μ m. Basidia narrowly clavate to cylindrical, transversely 3-septate, hyaline, 52-62×4.5-6.5 μ m. Basidiospores allantoid to reniform, thin-walled, hyaline, 15-17×5.5-7 μ m.



Fig. 4. Micro and macro morphological characteristics of *Auricularia villosula* (KA17-0221). A, B: Basidiocarps; C: Abbhemenial hairs; D: Bsidioles and basidia; E: Basidiospores. Scale bars: B=3 cm; C-E=20 μm.

Notes: These specimens were also considered as *A. auricula-judae* complexes. Morphological comparisons showed that this species has shorter abhymenial hairs (30-93 μ m long vs. 100-150 μ m long) and basidia (52-62 μ m long vs. 64-85 μ m long) than *A. auricula-judae* [4]. Furthermore, phylogenetic analysis showed that *A. villosula* is distinctly different from *A. auricula-judae*.

Key to Auricularia species in Korea

Based on the collected specimens, we have provided a morphological distinguishing key for the five *Auricularia* species identified, as follows:

1. Abhymenial hairs <100 µm long·····2
1. Abhymenial hairs >100 µm long······3
2. Basidiospores usually >15 µm long·····A. minutissima
2. Basidiospores usually <15 µm long······A. villosula
3. Basidia >60 µm long·····A. heimuer
3. Basidia <60 µm long······4
4. Medulla present ····································
4. Medulla absent or indistinct ····································

DISCUSSION

Phylogenetic analysis confirmed five different species in this study. Of those, three species (*A. americana, A. heimuer* and *A. villosula*) were new to Korea, while *A. cornea* and *A. minutissima* have recently been described [10].

To date, six species of *Auricularia* have been recorded in Korea. *Auricularia auricula-judae* (Bull.) Quél. is a well-known fungus as judas ear mushroom in Auriculariales. In Korea, this species was recorded on Ulleung Island in 1959 for the first time. *A. auricula-judae* had previously been reported in Korea [17-20], but its taxonomical data had not been investigated. In Korea, phylogenetic studies of *A. auricula-judae* have never been conducted and it has mainly been identified based on its morphology. In this study, the Korean specimens considered to be *A. auricula-judae* based on their morphology were identified as three differnt species (*A. heimuer, A. minutissima,* and *A. villosula*) phylogenetically. Furthermore, the absence of *A. auricula-judae* in Korea was confirmed in our ITS analysis (Fig. 1). The geographical distribution of *A. auricula-judae has previously* been confined to Europe [4-6]. A phylogenetic tree confirmed the phylogenetic relationships of Korean *Auricularia* species for the first time.

The cultivated strains of *A. auricula-judae* from Korea were sequenced and analyzed phylogenetically (data not shown). As a result, the strains cultivated as products in the markets were identified as *A. heimuer*, which has a wide geographic distribution in East Asia. *Auricularia auricula-judae*. s. str. is not found in China, Japan, or the Russian Far East based on geological distribution [4], and this species is confined to European countries [4,7].

Auricularia hispida was introduced as a new species in Japan (Index Fungorum; https://www. indexfungorum.org/). This species was excluded in a recent Japanese taxonomical study [6], and there is no sequence data available for it in GenBank. In Korea, we could not find this species during the field surveys. *Auricularia mesenterica* is a type species of *Auricularia* and is considered a species complex [4]. *A. mesenterica* complex was split into three species (*A. mesenterica* s.str., *A. brasiliana*, and *A. orientalis*) based on phylogenetic analysis. *Auricularia mesenterica* s.str. is a European species, *A. orientalis* was found in China, and *A. brasiliana* was discovered in Brazil. We could not identify these species in Korea. Therefore, more samples are required to identify this species. *Auricularia polytricha* (Mont.) Sacc. was recorded in Korea, and the species is synonymized with *A. nigricans* (Sw.) Birkebak, Looney & Sánchez-García. According to a recent phylogenetic analysis [6], this species was recorded in the USA and Costa Rica, however it was not identified in this investigation. Therefore, additional collections are required to confirm its presence in Korea.

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