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Research Article

# Taxonomic Position and Species Identity of the Cultivated Yeongji '*Ganoderma lucidum*' in Korea

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**Abstract** *Ganoderma lucidum* has a long history of use as a traditional medicine in Asian countries. However, the taxonomy of *Ganoderma* species remains controversial, since they were initially classified on the basis of their morphological characteristics. Recently, it was proposed that *G. lucidum* from China be renamed as *G. sichuanense* or *G. lingzhi*. In the present study, phylogenetic analysis using the internal transcribed spacer region rDNA sequences of the *Ganoderma* species indicated that all strains of the Korean '*G. lucidum*' clustered into one group together with *G. sichuanense* and *G. lingzhi* from China. However, strains from Europe and North American, which were regarded as true *G. lucidum*, were positioned in a clearly different group. In addition, the average size of the basidiospores from the Korean cultivated Yeongji strains was similar to that of *G. lingzhi*. Based on these results, we propose that the Korean cultivated Yeongji strains of '*G. lucidum*' should be renamed as *G. lingzhi*.

**Keywords** *Ganoderma lingzhi*, *Ganoderma sichuanense*, ITS rDNA, Phylogeny, Taxonomy

*Ganoderma* has been used in traditional medicine in Korea, China, and Japan for thousands of years, and its use has spread to other regions of the world. It is known to prevent and treat immunological diseases and tumorigenesis, control blood glucose levels, modulate the immune system, have hepatoprotective and bacteriostatic effects [1, 2]. In addition, *Ganoderma* has been recognized as a potentially important source of lignin-degrading enzymes [3]. For these reasons, the cultivation of Korean *Ganoderma* is beneficial to both public health and industry.

The genus *Ganoderma* was initially classified on the basis of morphological characteristics [4, 5]. However, environmental factors, variability, interhybridization, and morphological propensity can lead to the inaccurate identification of *Ganoderma* species [6]. In the case of *Ganoderma lucidum*, the identification of species was often unclear, and the taxonomic segregation of East Asian and European *G. lucidum* has remained controversial [7, 8]. Based on analyses of nuclear rDNA gene regions, it was reported that the collections named *G. lucidum* in East Asia were not conspecific with European *G. lucidum* [9, 10]. Pegler and Yao [11] reported that, based on morphological examination, *G. lucidum* from East Asia and Europe are different. In addition, Hawksworth [12] proposed to retain the name *G. lucidum* for the Asian species and introduce a new name for the European species. A number of *Ganoderma* isolates have still been misidentified and misnamed [13]. Wang *et al.* [8] and Cao *et al.* [14] reported that, based on both morphological and molecular data, the identity of cultivated *G. lucidum* in China is conspecific with *G. sichuanense* and *G. lingzhi*. In the present study, we have investigated the taxonomic position of Korean *G. lucidum* by analyzing the internal transcribed spacer region (ITS) rDNA, and compared the results of our phylogenetic analysis with those obtained by the group from China [8].

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## MATERIALS AND METHODS

***Ganoderma* species and culture conditions.** The *Ganoderma* strains listed in Table 1 were obtained from the Korean Collection for Type Culture (KTCT, Jeongeup, Korea), the American Type Culture Collection (ATCC, Rockville, MD, USA), the Korean Agricultural Culture Collection (KACC, Suwon, Korea), the Mushroom Division

**Table 1.** *Ganoderma* strains used in the present study

| Species | Misidentified name | Collection ID | GenBank accession No. (ITS) | Origin | References |
|---|---|---|---|---|---|
| *G. lingzhi* | *G. lucidum* | ASI-7004 | JQ520167 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7013 | JQ520168 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7071 | JQ520169 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7074 | JQ520170 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7091 | JQ520171 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7094 | JQ520172 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | ASI-7135 | JQ520173 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | KU-4035 | JQ520207 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-0047 | JQ520174 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-0757 | JQ520175 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-0938 | JQ520176 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-3986 | JQ520177 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-4002 | JQ520178 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-4100 | JQ520179 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-4537 | KT717953 | Korea | This study |
| *G. lingzhi* | *G. lucidum* | IUM-4304 | JQ520183 | Bangladesh | This study |
| *G. lingzhi* | *G. lucidum* | KACC 42232 | KT717954 | Japan | This study |
| *G. lingzhi* | *G. lucidum* | KACC 51689 | KT717955 | Japan | This study |
| *G. lingzhi* | *G. lucidum* | KACC 51690 | KT717956 | Japan | This study |
| *G. lingzhi* | *G. sichuanense* | HMAS 251145 | JF915400 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 251146 | JF915401 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 251147 | JF915402 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 251148 | JF915403 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 62503 | JF915405 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 76566 | JF915406 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 99391 | JF915407 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 130128 | JF915404 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 130131 | JF915408 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 240175 | JF915393 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 240176 | JF915394 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 240177 | JF915395 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 240178 | JF915396 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 240187 | JF915397 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 250672 | JF915398 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 250677 | JF915399 | China | [8] |
| *G. lingzhi* | *G. sichuanense* | HMAS 60537 | JN197281 | China | [8, 14] |
| *G. lingzhi* | – | Wu 1006-38 (holotype) | JQ781858 | China | [14] |
| *G. lingzhi* | – | Cui 9164 | JQ781859 | China | [14] |
| *G. lingzhi* | – | Dai 12438 | JQ781861 | China | [14] |
| *G. lingzhi* | – | Cui 6982 | JQ781862 | China | [14] |
| *G. lucidum* | – | ATCC 46755 | JQ520185 | Canada | This study |
| *G. lucidum* | – | Gl-1-3 | JN588574 | France | [15] |
| *G. lucidum* | – | Glu1 | JN588575 | Italy | [15] |
| *G. lucidum* | – | Dai 11593 | JQ781852 | Finland | [14] |
| *G. lucidum* | – | Dai 2272 | JQ781851 | Sweden | [14] |
| *G. lucidum* | – | HMAS 86597 | AY884176 | UK | [8] |
| *G. meredithae* | – | ATCC 64492 | JQ520190 | USA | This study |
| *G. meredithae* | – | ASI-7140 | JQ520191 | Unknown | This study |
| *G. multipileum* | – | HMAS 242384 | JF915409 | China | [8] |
| *G. multipileum* | – | BCRC 37033 | EU021462 | Taiwan | [16] |
| *G. resinaceum* | – | IUM-3651 | JQ520204 | Czech | This study |
| *G. resinaceum* | – | HMAS 86599 | AY884177 | UK | [8] |
| *G. resinaceum* | – | CBS 152.27 | JQ520200 | UK | This study |
| *G. sichuanense* | – | HMAS 42798 (holotype) | JQ781877 | China | [14] |
| *G. sichuanense* | – | Cui 7691 | JQ781878 | China | [14] |
| *G. tropicum* | – | HMAS 263143 | JF915410 | China | [8] |
| *G. tropicum* | – | Wu 0407-2 | EU021458 | Taiwan | [16] |

**Table 1.** Continued

| Species | Misidentified name | Collection ID | GenBank accession No. (ITS) | Origin | References |
|---|---|---|---|---|---|
| *G. tsugae* | – | ATCC 64795 | JQ520215 | Canada | This study |
| *G. tsugae* | – | ASI-7064 | JQ520216 | USA | This study |
| *G. weberianum* | – | SUT H2 | AY569451 | Australia | [17] |
| *G. weberianum* | – | HMAS 97365 | JF915411 | China | [8] |
| *G. weberianum* | – | CBS 219.36 | JQ520219 | Philippines | This study |
| *Tomophagus colossus* | – | CGMCC 5.763 | ZQ081068 | Philippines | [8] |
| *Tomophagus colossus* | – | HCMC 10 | JN184396 | Vietnam | [18] |

ITS, internal transcribed spacer region; ASI, agricultural science institute.

of the Rural Development Administration (Eumseong, Korea), the Centraalbureau voor Schimmelcultures (CBS, Utrecht, Netherlands), Incheon University (Incheon, Korea), and Konkuk University (Seoul, Korea). *Ganoderma* species were cultured on potato dextrose broth (Difco, Detroit, MI, USA) and incubated at 30°C for 2 wk.

**Genomic DNA extraction and PCR amplification.** Cultured mycelia, filtered through 2 layers of MiraCloth (Calbiochem, La Jolla, CA, USA), were ground in liquid nitrogen, and genomic DNA was extracted using the cetyltrimethylammonium bromide method [19]. The ITS rDNA region was amplified using the primers ITS1 and ITS4 [20]. PCRs were performed using a premixed polymerase kit (Taq PreMix; TNT Research, Anyang, Korea) in a 20 μL reaction mixture containing 1 μL of DNA. Amplification of the ITS region was carried out using a thermal cycler (TaKaRa, Tokyo, Japan) at the following conditions: 5 min at 94°C for initial denaturation, followed by 30 cycles of 30 sec at 94°C for denaturation, 30 sec at 56°C for primer annealing, and 1 min at 72°C for extension, and 10 min at 72°C for a final extension. PCR products were detected by electrophoresis on 1.2% agarose gels in 0.5× Tris-acetate ethylenediaminetetraacetic acid buffer. Gels were stained with ethidium bromide and inspected visually under a UV transilluminator.

**Cloning and sequencing.** PCR products were ligated into the pGEM-T easy vector (Promega, Madison, WI, USA) according to the manufacturer's instructions. Ligation products were transformed into the *Escherichia coli* DH5α competent cell (RBC, Taiwan) by heat shock [21]. Plasmid DNAs were extracted using the DNA Hybrid-QTM Plasmid mini DNA Isolation Kit (GeneAll, Seoul, Korea). Recombinant clones were identified, and the presence of inserts was confirmed by *Eco*RI restriction enzyme digestion and sequencing (using the SP6 and T7 promoters; GenoTech, Daejeon, Korea).

**Sequence analysis.** Nucleotide sequences were deposited in the National Center for Biotechnology Information GenBank database (Table 1). The sequences of the ITS rDNA were aligned for phylogenetic analysis using the program BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The phylogenetic trees were constructed by using the MEGA5 program [22] and the neighbor-joining method [23]. Confidence levels for individual branches of the resulting tree were assessed using the bootstrap test [24] in which 1,000 replicate trees were generated from resampled data.

**Basidiospore observation.** The size of basidiospores from the Korean cultivated Yeongji strains was determined using a fluorescence microscope (Axio Observer A1; Carl Zeiss, Jena, Germany) and a 5% KOH solution as a mounting medium. At least 10 basidiospores of each mature specimen were measured. The size of each spore was calculated, and the mean value was used in the description.

## RESULTS AND DISCUSSION

The phylogenetic analysis of *Ganoderma* species in this study was generally consistent with the findings reported by the group from China [8, 14]. The 62 *Ganoderma* strains were divided into 7 groups, A to G (Fig. 1). This result was strongly supported by high bootstrap values ranging from 91% to 99%. In addition, *Ganoderma lucidum* were largely divided into two groups (groups A and G). Group A included all the Korean *G. lucidum* strains, as well as the *G. lucidum* strains from Bangladesh and Japan, and Chinese *G. sichuanense* and *G. lingzhi*. This group had a high bootstrap support value of 91%. *G. meredithae* (USA and unknown sources) in group B was closely related to *G. lucidum* in group A as evidenced by a high bootstrap value of 98%. *G. multipileum* (China and Taiwan) was grouped within group C and *G. tropicum* (China and Taiwan) within group D, with 96% and 99% bootstrap support, respectively. Group E includes three strains of *G. resinaceum* from the Czech Republic and UK (two strains), and was supported by very high bootstrap values of 99%. Strains of *G. weberianum* (Australia, China, and Philippines) and two other Chinese *G. sichuanense* strains (Cui 7691 and HMAS 86597; holotype) were grouped within group F with 99% bootstrap support. Groups E and F were closely related with bootstrap values of 96%.

It is worth noting that other *G. lucidum* strains from


**Fig. 1.** Phylogenetic relationships of 62 *Ganoderma* species based on their internal transcribed spacer region rDNA gene region sequences. This tree was obtained using the neighbor-joining method. Numbers at the branch nodes represent bootstrap values obtained from 1,000 replications (only values greater than 91% are shown). Two strains of *Tomophagus colossus* were used as the outgroup.

Europe (France, Finland, Italy, Sweden, and UK) and Canada could be clustered into group G with *G. tsugae* strains from North America (Canada and USA), supported by a very high bootstrap value of 99%. It was clearly separated from Korean *G. lucidum* (group A). The findings of the current study are consistent with those of Park *et al.* [25] who reported that *G. lucidum* strains from Europe and North America could be clustered into one group together with *G. tsugae* based on both analyses of the ITS rDNA gene and partial β-tubulin gene sequences. Interestingly, Korean *G. lucidum* strains could be clustered into A group together with *G. sichuanense* and *G. lingzhi* strains from China. This study produced results that corroborate the findings of previous work in this field.

Based on both morphological and molecular evidence, Wang *et al.* [8] reported that *G. lucidum* is incorrectly recorded in China, as well as in other countries, and suggested that the name '*G. lucidum*' as used for the Chinese species should be corrected as *G. sichuanense*. However, they did not obtain sequences from type specimens of *G. sichuanense* (the holotype; HMAS 42798). Liao *et al.* [26] reported that Korean *G. lucidum* (ASI-7004) could be clustered into one group together with Chinese *G. lucidum* and *G. lingzhi* based on the ITS2 sequences and RNA secondary structures.

Furthermore, Cao *et al.* [14] reported that *G. sichuanense* (included the holotype; HMAS 42798) was distantly phylogenetically related to *G. lingzhi* (included the holotype; Wu 1006-38), but it was closely related to *G. resinaceum*. With regard to the morphological characteristics, it has been reported that Chinese *G. lucidum* (*G. lingzhi*) has a yellow pore surface [27, 28], European *G. lucidum* has a white pore surface [29, 30], and Korean *G. lucidum* has pale brownish thread-like tissues in the middle of the context [31]. Cao *et al.* [14] found that the morphological characteristics of '*G. lucidum*' from East Asia were consistent with those of *G. lingzhi*. Thus, they concluded that *G. sichuanense* was a distinct species since they display obvious morphological differences from *G. lingzhi* and suggested that the name '*G. lucidum*' should be corrected as *G. lingzhi* not *G. sichuanense*.

*Ganoderma lucidum* (Curtis) P. Karst. was given its name by Petter Adolf Karsten in 1881 based on its morphology [32, 33]. It is commonly called "Yeongji" in Korea, "Lingzhi" in China, and "Reishi" or "Mannentake" in Japan [34]. Phylogenetic analyses placed *G. lucidum* from various regions of the world in different lineages [9, 13, 35-37]. However, the *Ganoderma* species from various countries including Africa, Oceania, America, Asia (China, Korea, and Japan), and Europe [8] have been incorrectly reported as *G. lucidum*. In addition, Moncalvo *et al.* [7, 9] classified *G. lucidum* collections from different regions (Asia and Europe) into different species based on ITS and partial nuclear large subunit ribosomal DNA sequences. This is similar to the results of our ITS nucleotide sequence analysis of *G. lucidum*. Likewise, Hong and Jung [35] reported that the *G. lucidum* from Korea and Japan were monophyletic, and were distinguished from the *G. lucidum* from Europe and North America based on sequence analysis of mitochondrial small-subunit ribosomal DNA.

As described above, the taxonomy of *G. lucidum* has been reported by many researchers. Nevertheless, the name *G. lucidum* has been misapplied to various species around the world. The results of this study also reveal that Korean *G. lucidum* was clustered into one group together with Chinese *G. sichuanense* and Chinese *G. lingzhi* (both formerly *G. lucidum*), and it was clearly separated from *G. lucidum* from Europe and North America. In addition, *G. sichuanense* were divided into two groups (groups A and F). *G. sichuanense* strains (Cui 7691 and HMAS 86597; holotype), including the holotype, could be clustered into group F together with *G. weberianum*, and it was closely related to *G. resinaceum*.

Cao *et al.* [14] also reported that the size of basidiospores of *G. lingzhi* [(9.85 ± 0.85) × (6.4 ± 0.6) μm] differs from that of *G. sichuanense* [(8.3 ± 0.9) × (5.8 ± 0.8) μm]. Interestingly, the average size of the basidiospores from the Korean cultivated Yeongji strains [(10.65 ± 0.65) × (6.6 ± 0.6) μm for ASI-7004, (10 ± 1) × (6.4 ± 0.3) μm for ASI-7071] were similar to that of *G. lingzhi* (Fig. 2).

Thus, the comparison of the ITS rDNA sequences and the estimation of the basidiospores size presented in this study confirm previous findings and contribute additional evidence that suggests the naming Korean cultivated Yeongji strains of '*G. lucidum*' should be renamed as *G. lingzhi*.

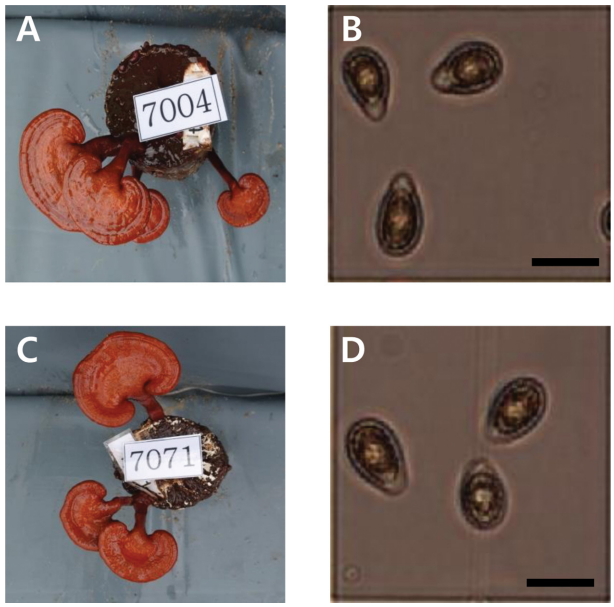


**Fig. 2.** The Korean cultivated Yeongji strains. A, Cultivated fruiting body of the Yeongji 1 strain (ASI-7004); B, Microscopic characteristics of basidiospores from the Yeongji 1 strain; C, Cultivated fruiting body of the Yeongji 2 strain (ASI-7071); D, Microscopic characteristics of basidiospores from the Yeongji 2 strain (scale bars: B, D = 10 μm).

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