

Ganoderma lingzhi (Polyporales, Basidiomycota): the scientific binomial for the widely cultivated medicinal fungus Lingzhi

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Abstract The traditional medicinal fungus, Lingzhi, has long been considered to be *Ganoderma lucidum*, but was recently described as *G. lingzhi* based on both morphological and molecular data. Months earlier than this description, the species was identified as *G. sichuanense* by another research group. A sequenced epitype for *G. sichuanense* was selected that, if accepted, meant that it became a synonym of, and an earlier name for, *G. lingzhi*. However, the holotype of *G. sichuanense* is well developed with distinct morphological characters, and its ITS sequence, the DNA barcode for fungal taxonomy, is available. The holotype of *G. sichuanense* does not possess the typical morphological characteristics of Lingzhi, and the designated epitype is not conspecific with the holotype from morphological, phylogenetic and ecological perspectives. Consequently, the designation of the epitype is considered to conflict with the regulations of the Code and should be rejected. As no other previously published species name

matches Lingzhi, the correct name for this fungus is *G. lingzhi*.

Keywords Epitypification · *Ganoderma sichuanense* · Nomenclature · Polypores

Introduction

Lingzhi, an economically important wood-decay polypore fungus, has been used in traditional Chinese medicine for more than 2000 years (Anonymous 1955). The successful production of Lingzhi fruitbodies in 1969 (Yu and Shen 2003) led to widespread cultivation in China and adjacent countries, stimulating scientific research on the identity and function of the medicinal metabolites. These metabolites include polysaccharides, triterpenes, lucidenic acids, adeno-

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sines, ergosterols, glucosamines and cerebrosides with medicinal functions in the prevention and treatment of diseases such as cancers, chronic bronchitis, diabetes mellitus, hyperlipidemia and hypertension, as well as improvement of immunity and anti-aging (Lin 2009). The products containing Lingzhi have an estimated annual value of US\$2.5 billion in global trade (Li et al. 2013). Besides the economic importance and health benefits, cultivation and utilization of Lingzhi is also considered an essential part of Chinese traditional culture.

Nomenclatural history of Lingzhi

The first possible name applied to Lingzhi (Mannen-take or Reishi in Japanese) in East Asia was *Boletus dimidiatus* Thunb. based on specimens collected on the Japanese mainland around Tokyo and Nagasaki (Thunberg 1784). Fries (1838) renamed this *Polyporus japonicus* Fr. Because this name is not sanctioned, it is illegitimate because there was no obstacle to recombining Thunberg's epithet *dimidiatus* into *Polyporus*, and Fries explicitly refers to Thunberg's name. Later, Yasuda (1913) suggested that *Fomes japonicus* (Fr.) Sacc. (= *P. japonicus*) recorded from Japan actually represents *F. lucidus* (Curtis) Sacc. [= *Polyporus lucidus* (Curtis) Fr.]. Imazeki (1939) made intensive studies of specimens of the *Ganoderma lucidum* (Curtis) P. Karst. complex collected in Japan, and concluded that specimens of "*G. lucidum* (Curtis) P. Karst." recorded after Yasuda (1913) included *G. lucidum* s.s., *G. neojaponicum* Imazeki and *G. curtisii* (Berk.) Murrill. Imazeki considered that Mannen-take represented *G. lucidum*, while specimens with almost black pilei and restricted to conifers were *G. neojaponicum*.

Likewise, for Chinese mycota, Patouillard (1907) identified Chinese Lingzhi collections from Guizhou Province as *Ganoderma lucidum* (Moncalvo and Ryvar den 1997). While *G. lucidum* was then broadly defined, more recently many species have been differentiated from the original concept. However, the name *G. lucidum* has been widely used for Lingzhi in later and even recent publications including studies of medicinal metabolites and functions (Kino et al. 1989; Bao et al. 2002; Sliva et al. 2002). Detailed morphological examinations (Pegler and Yao 1996) and phylogenetic analyses (Moncalvo et al. 1995; Smith and Sivasithamparam 2000; Hong and Jung 2004) have demonstrated that the application of the name *G. lucidum* for Lingzhi is no longer correct (Szedlay 2002; Wasser 2011). However, the nomenclatural consequences of these taxonomic results have been hotly debated.

Cao et al. (2012) sampled both natural and cultivated Lingzhi specimens from China and Japan as well as specimens of *Ganoderma* species resembling Lingzhi, including *G. curtisii* (Berk.) Murrill, *G. flexipes* Pat., *G. lucidum*, *G. multipileum* Ding Hou, *G. sichuanense* J.D. Zhao & X.Q.

Zhang and *G. tropicum* (Jungh.) Bres., and performed a phylogenetic analysis including a sequence from the holotype of *G. sichuanense*. They demonstrated that Lingzhi represented an independent lineage. Moreover, distinct morphological differences between *G. lingzhi* and these species were documented in Cao et al. (2012). Therefore, a new species, *Ganoderma lingzhi* Sheng H. Wu, Y. Cao & Y.C. Dai, was introduced for Lingzhi, a species with a wide natural distribution in temperate East Asia.

Months earlier than the description of *Ganoderma lingzhi*, Wang et al. (2012) proposed *Ganoderma sichuanense* as the correct name of Lingzhi. *Ganoderma sichuanense* was originally described based on two collections from Panzhuhua Iron and Steel (Group) Company Limited in Dukou City (former name of Panzhuhua City), southern Sichuan Province, southwestern China (Zhao et al. 1983). Wang et al. (2012) concluded that the two collections represented different species: the paratype (HMAS 43728, with basidiospore dimensions 8–8.5 × 6–6.5 μm) was identified as *Ganoderma weberianum* (Bres. & Henn. ex Sacc.) Steyaert, while the holotype (HMAS 42798) was considered to be conspecific with Lingzhi. However, Wang et al. (2012) could not obtain any DNA sequence from the holotype of *G. sichuanense*. Instead, for DNA analyses, they used four recently collected samples from Sichuan (HMAS 251145–251,148) that they considered to be morphologically similar, from an area close to but distinct from the type locality. These samples were considered by Wang et al. (2012) to be Lingzhi and to match *G. sichuanense*. To stabilize this concept, Yao et al. (2013) subsequently designated a sample recently collected from Huangqiao Village, Miyi County, Panzhuhua, Sichuan Province (HMAS 252081) and bearing an ITS sequence identical to that of Lingzhi, as the epitype of *G. sichuanense*. They justified this with the argument that DNA in the holotype of *G. sichuanense* had not been well preserved. This action, were it to be accepted, meant that *G. lingzhi* became a later synonym of *G. sichuanense*.

Evidence to support *Ganoderma lingzhi* as the correct scientific binomial for Lingzhi

As stated by Zhou et al. (2015), the epitypification of *G. sichuanense* does not comply with the International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Art. 9.8 defines an epitype as "an interpretative type" selected when the holotype or other original material "is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon".

The holotype of *G. sichuanense* is well developed with clear morphological characters and its ITS region, the DNA barcode for fungal taxonomy, was successfully sequenced by Cao et al. (2012). The failure of Wang et al. (2012) and Yao

et al. (2013) to amplify any sequence from the holotype was, in their statement, because of “the specimen’s condition”. However, a failed PCR can have more causes than damage of the DNA, so the interpretations of Yao et al. (2013) are vulnerable.

Furthermore, the epitype of *G. sichuanense* was inappropriately selected and could not be interpreted with certainty as conspecific with the holotype, because these collections differ in at least four aspects:

- a) According to phylogenetic analysis, the holotype (GenBank accession JQ781877, as sequenced by Cao et al. 2012) and the epitype (KC662402, as sequenced by Yao et al. 2013) occur in distinctly separated lineages (Yao et al. 2013). The two sequences are not identical and in our examination showed a difference of more than 8%.
- b) The basidiospore dimensions of the epitype [(9.0–)9.5–10.5(–11.0) × 6.5–8.0 μm] in Yao et al. (2013) are much larger than those of the holotype of *G. sichuanense* [(7–)7.4–9.2(–9.3) × (4.6–)5–6.6(–6.8)] in Cao et al. (2012; Table 2). Yao et al. (2013) stated that their measurements were from at least 30 basidiospores, and the quoted measurements from Cao et al. (2012) were also based on 30 basidiospores. Yao et al. (2013), following Wang et al. (2012), maintained that basidiospores from the holotype of *G. sichuanense* are 9–11.5 × 6.5–8 μm, and explained the discrepancy in basidiospore dimensions by stating that Cao et al. (2012) also included measurements from the paratype of *G. sichuanense* that had smaller basidiospores than the holotype. However, Cao et al. (2012) only borrowed the holotype, not the paratype, to study. Moreover, Cao et al. (2012)’s measurements of the holotype are consistent with the basidiospore dimensions (7.4–9.5 × 5–7 μm) mentioned in the original publication on *G. sichuanense* by Zhao et al. (1983), where the four illustrated basidiospores from the holotype are all shorter than 10 μm. Hence, there is no evidence of confusion of basidiospore size between the holotype and paratype.
- c) Macromorphologically, the holotype has a much darker pore surface than the epitype (see photographs in Cao et al. 2012; Yao et al. 2013).
- d) The ecological environment of the epitype is distinctly different from that of the holotype of *G. sichuanense*: 80 km distant in a straight line and, more importantly, at an altitude of nearly 1000 m above that of the holotype. The epitype from Miyi occurred in a humid and temperate forest at an altitude of 1933 m as mentioned by Yao et al. (2013), representing a temperate species like *Lingzhi*, while the locality of the holotype of *G. sichuanense* (Dukou) was found at an altitude of 985 m with dry and nearly tropical vegetation (indicated by mango cultivation on the basis of our observation in this area). Another

specimen of *G. sichuanense* (Cui 7691), with an identical ITS sequence to that of the holotype, was also collected from the subtropical to tropical zone in Guangdong (Cao et al. 2012). Yao et al. (2013) considered the sequence from the holotype “unusual” without further explanation. If this term was to indicate artifacts of DNA preparation, the 100% identity with the sequence from Cui 7691 is hardly explicable.

It is evident that the so-called epitype of *G. sichuanense* is different from the holotype of *G. sichuanense*, according to the above-mentioned phylogenetic, morphological and ecological perspectives. This means that the epitype represents *G. lingzhi*, but not *G. sichuanense*. The intention of Yao et al. (2013) in proposing the epitype actually serves to replace the holotype rather than add to it. Richter et al. (2015) agreed with Yao et al. (2013)’s opinion, but did not provide any evidence or new data to support this epitype.

Other potential synonyms

Like *Ganoderma sichuanense*, several other previously described Chinese species of *Ganoderma*, such as *G. atrum* J.D. Zhao, L.W. Hsu & X.Q. Zhang, *G. calidophilum* J.D. Zhao, L.W. Hsu & X.Q. Zhang, *G. chenghaiense* J.D. Zhao, *G. hainanense* J.D. Zhao, L.W. Hsu & X.Q. Zhang, *G. parviungulatum* J.D. Zhao & X.Q. Zhang and *G. simaoense* J.D. Zhao, are more or less morphologically similar to *G. lingzhi* (Zhao et al. 1979, 1986; Zhao 1987, 1989). However, Cao et al. (2012) clearly concluded these species are not conspecific with *G. lingzhi* by type studies. Moreover, in the absence of molecular sequences from the holotypes of these species, their species circumscriptions are not fully clear and it is not possible to use any of these names for *Lingzhi* (Ryvarden 1995).

Considering *Boletus dimidiatus* and *Polyporus japonicus*, the original descriptions and drawings suggest that they represent a member of the *G. lucidum* complex. The name *Ganoderma japonicum* (Fr.) Sawada (= *P. japonicus*) has often been applied to a Chinese species with a dark pileus and distribution in subtropical areas (e.g. Teng 1964). However, it is most likely a misapplication, because specimens of *G. japonicum* from China and Japan are not conspecific. The existing sequences of *G. japonicum* were obtained from Chinese specimens (e.g., Wang and Yao 2005). While they differ from those of *Lingzhi*, this is not a valid reason to conclude that *G. japonicum* is distinct from *Lingzhi*. However, the authentic material of *B. dimidiatus* and *P. japonicus* deposited at the Museum of Evolution Herbarium (UPS: BOT: F-118347) was not available for loan and exists as drawings provided by Thunberg (1784), and we

conclude that the rejection of the name *Boletus dimidiatus* is better in order to stabilize the scientific binomial for Lingzhi.

Conclusion

Ganoderma lingzhi is a temperate species widely distributed mainly in Fagaceae forests of East Asia. Using both morphological and phylogenetic evidence, this name unambiguously represents the economically important and widely cultivated Lingzhi species. In 2013, *G. lingzhi* was nominated as the fungus of the year in *Mycology*, an international journal issued by the Mycological Society of China (Yang and Feng 2013). Moreover, in addition to taxonomic mycologists (Hawksworth 2013; Li et al. 2015a, 2015b; Henniecke et al. 2016), the name *G. lingzhi* has also been accepted instead of *G. lucidum* by mycological chemists (Fatmawati et al. 2013; Gao et al. 2014; Li et al. 2014; Chen et al. 2015; Yan et al. 2015; Wang et al. 2016; Zhang et al. 2017), while to the best of our knowledge the name *G. sichuanense* has never been applied to these kinds of chemical studies.

Ganoderma sichuanense is a subtropical-tropical species from South China, and bears morphological features distinct from those of *G. lingzhi* (Cao et al. 2012), whereas the so-called epitype of *G. sichuanense* designated by Yao et al. (2013) represents *G. lingzhi* and is inadequate as an epitype. In order to stop the widespread misapplication of epitypification in fungi, Ariyawansa et al. (2014) proposed strict criteria for meaningful epitypification, such as multilocus-based phylogenetic analyses, which are not met in the epitypification of Yao et al. (2013). Wang et al. (2012) and Cao et al. (2012) presented different basidiospore measurements from the holotype of *G. sichuanense*, and the latter corresponds with the protologue. The single available DNA sequence from this specimen clusters in a clade comprising *G. weberianum* and *G. sichuanense*. The present inconsistent findings indicate that the species concept of *G. sichuanense* is ambiguous. There is urgent need to stabilize nomenclature for the economically important Lingzhi species. Currently, the only unambiguous name for the widely cultivated Lingzhi is *G. lingzhi*. A formal proposal to conserve *Ganoderma lingzhi* over *G. sichuanense* and *Boletus dimidiatus* will be prepared for solving the scientific binomial for the widely cultivated medicinal fungus Lingzhi thoroughly.

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