

# *Ganoderma multipileum*, the correct name for ‘*G. lucidum*’ in tropical Asia

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(Received April 28, 2008; Accepted April 17, 2009)

**ABSTRACT.** *Ganoderma lucidum* (generic type), originally described from England, has been reported worldwide. In the Orient, an economically important fungus commonly known as ‘ling-zhi’ or ‘chi-zhi’ for more than 2000 years, has also been named *G. lucidum*. However, the identity of the Oriental fungus has been questioned in recent years. Earlier molecular studies suggested that *G. lucidum* sensu stricto may be restricted to Europe and that ‘*G. lucidum*’ in Asia consists of at least two distinct species, one represented by material from mainland China and one by tropical Asian collections. This study attempts to clarify the identity of ‘*G. lucidum*’ in tropical Asia, with special emphasis on wild collections from Taiwan. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) of related species and strains were sequenced, and phylogenetic analyses were conducted. The results confirmed that *G. lucidum* is a name mistakenly applied to Oriental collections. The forgotten name *Ganoderma multipileum*, presented over half a century ago from Taiwan, was found in this study as the earliest valid name for ‘*G. lucidum*’ known from tropical Asia.

**Keywords:** Forgotten name; *Ganodermataceae*; Molecular phylogeny; Taxonomy.

## INTRODUCTION

*Ganoderma lucidum* (Curtis) P. Karst., the type of *Ganoderma* P. Karst., was originally described based on a specimen collected from Peckham, London, UK. Currently, this species has been reported worldwide, i.e. Europe (Ryvarden and Gilbertson, 1993), Asia (Núñez and Ryvarden, 2000; Zhao and Zhang, 2000), Oceania (McKenzie and Foggo, 1989), Africa (Ryvarden and Johansen, 1980), and America (Bazzalo and Wright, 1982; Gilbertson and Ryvarden, 1986). In the Orient, a fungus commonly known as ‘ling-zhi’ or ‘chi-zhi’ for more than 2000 years, has also been named *G. lucidum*. This fungus is widely grown on a commercial scale for its medicinal properties. However, the identity of this important fungus has been questioned in recent years. Pegler and Yao (1996) mentioned that the morphology of ‘*G. lucidum*’ in the Orient differs from that of *G. lucidum* found in Britain and throughout Europe in having basidiocarps of a more-slender stature. Moncalvo et al. (1995a, b) concluded that

collections of *G. lucidum* from Asia and Europe belong to different species based on the analysis of DNA sequences derived from the internal transcribed spacer (ITS) and partial nuclear large subunit ribosomal DNA (LSU nrDNA) regions. Furthermore, collections of Oriental ‘*G. lucidum*’ from mainland China and Taiwan were separated into two clades in the molecular analyses of Moncalvo et al. (1995a, b). This suggests that at least two species are included. The correct respective names for them have yet to be determined.

*Ganoderma lucidum* is a temperate species, so far known with certainty from Europe only. In Taiwan, however, the fungus identified by this name is common in lowland tropical and subtropical belts. To clarify the identity of ‘*G. lucidum*’ in tropical Asia, we performed a detailed investigation of *Ganoderma* species in Taiwan using both morphological and molecular characters, with special emphasis on the fungus called *G. lucidum*. The ITS region, a gene marker useful in separating related species and strains of *Ganoderma* (Moncalvo et al., 1995a; Smith and Sivasithamparam, 2000) was sequenced for the purpose of a molecular analysis. This study confirms that *G.*

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*lucidum* is a name mistakenly applied to Oriental collections. To our knowledge, *G. multipileum* Hou, presented over half a century ago (Hou, 1950), represents the earliest valid name published for '*G. lucidum*' in tropical Asia.

## MATERIALS AND METHODS

### Fungal materials and morphological observations

All studied specimens are deposited in the herbaria of the National Museum of Natural Science (TNM), Taichung, Taiwan, the National Taiwan Museum (TAIM), Taipei, Taiwan, the Forestry and Forest Products Research Institute (TF), Ibaraki, Japan, or in the National Museum of Nature and Science (TNS), Tokyo, Japan. Thin free-hand sections from basidiocarps were cut and mounted in 5% KOH to ensure rehydration, and in Melzer's reagent to test for amyloid or dextrinoid reactions. At least 20 basidiospores were measured for each mature specimen. In the descriptions, basidiospore sizes are given both with and without the myxosporium, but only those with the myxosporium were used for comparisons among species. Line drawings of the basidiospores and cuticle cells were made with the assistance of a camera lucida.

### DNA extraction, PCR amplification, DNA cloning and sequencing

The details of the molecular experiments, except for DNA cloning, were described in Wu et al. (2007). The primer pairs, ITS1/4 and ITS5/4 (White et al., 1990), were used in this study. For strains with intragenomic ITS heterogeneity, DNA cloning was performed using a  $\lambda$ T&A cloning vector and competent ECOSTM 9-5 cells (Yeastern Biotech, Taipei, Taiwan). A single positive colony was picked for PCR amplification and DNA sequencing.

### Sequence alignment and phylogenetic analysis

Sequences obtained from this study were compared with all *Ganoderma* sequences retrieved from GenBank and with those published by Smith and Sivasithamparam (2000, the sequences of which have not been deposited in GenBank and were retyped onto computer from the original publication) using Clustal X 1.83 (Thompson et al., 1997). After initial analyses of this broad sample, 29 sequences were chosen that include strains labeled '*G. lucidum*' and closely related taxa, representative lineages of *Ganoderma* species, and two outgroup taxa of *Amuroderma* Murrill and *Tomophagus* Murrill (Table 1). These selected sequences were realigned in Clustal X and adjusted by hand using BioEdit 7.0.4.1 (Hall, 1999). The optimized alignment (deposited in Treebase, accession no.: SN4381) was used for maximum-parsimony (MP) analysis in PAUP\* 4.0b10 (Swofford, 2002). The analytical parameter preferences are described in Wu et al. (2007). Bootstrap analysis (Felsenstein, 1985) was performed with 1000 replicates with random addition sequences to obtain estimates of the reliability of the nodes.

## RESULTS

### Phylogenetic analysis

Amplification of the ITS region yielded PCR products of approx. 650 bp long. The final alignment of the 29 sequences included 673 positions. After exclusion of the conserved 5.8S region and ambiguous sites at both ends, 413 sites were used for the MP analysis. Sixteen most parsimonious trees (281 steps, Consistency Index (CI) = 0.662, Retention Index (RI) = 0.773) were retained in this analysis. Of the 413 included sites, 271 were constant, and 46 were variable, but parsimoniously was uninformative, and 96 were parsimoniously informative.

The topologies of the 16 trees were generally identical. One of them is presented in Figure 2. In this tree, collections labeled *G. lucidum* were separated into three distinct clades, and *G. tropicum* from tropical Asia formed another separate clade. These four clades are labeled A, B, C, and D in Figure 2.

Clade A is composed of six strains originally determined as *G. lucidum*, with three from Taiwan, two from India, and one from the Philippines. Changes across the sequences consisted of none to three substitutions. This clade received 83% support in the bootstrap analysis. *Ganoderma steyaertanum*, a recently presented species based on collections from Indonesia and Australia (Smith and Sivasithamparam, 2003), served as the sister group of clade A, and they were grouped together with high bootstrap support (90%).

Clade B represents '*G. lucidum*' from mainland China and Japan, which was robustly supported in the bootstrap analysis (100%). There was no change or only a one-step change between the sequences of the strains. The relationship between the strains was not resolved.

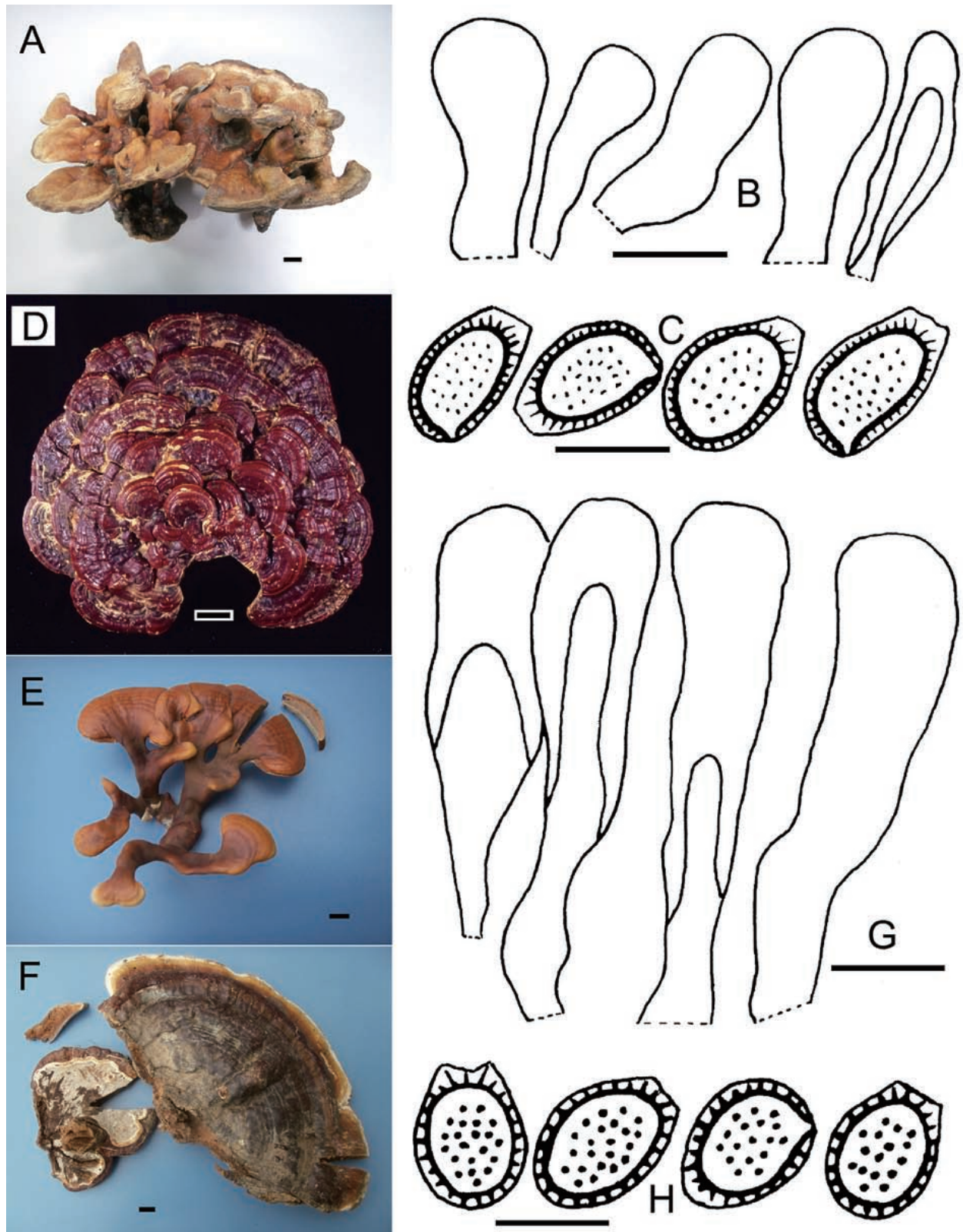
*Ganoderma tropicum* from Taiwan formed clade C. This clade was very strongly supported by the bootstrap analysis (100%). Changes among the sequences of the strains consisted of none to three steps. It was clearly separated from clade A, where '*G. lucidum*' from tropical Asia was seated.

Clade D, containing *G. lucidum* from Europe, the type locality, consisted of three strains respectively from the UK, France, and Norway. Changes among those sequences consisted of none to six steps. This clade was supported by a very high bootstrap value (99%). It was distantly related to the collections named *G. lucidum* in Asia.

### Taxonomical description

***Ganoderma multipileum*** Hou [as '*multipilea*'], Q. J. Taiwan Mus. 3: 101. 1950. Figure 1A-E

*Basidiocarp* annual, mostly stipitate, rarely sessile or only with a short base, corky. *Pileus* 1.5-9 × 2.8-16.5 cm, up to 2 cm thick, flabellate, reniform, dimidiate, sometimes with pilei growing from the lower pilei or growing together, up to 36 cm long and 54 cm wide in total; upper surface orange-yellow, orange-red to red-brown, weakly



**Figure 1.** Morphology of *Ganoderma* spp. A-E, *Ganoderma multipileum*. (A) Basidiocarp (holotype); (B) Sections of cutis (holotype); (C) Basidiospores (holotype); (D) Basidiocarp (TNM F0012903); (E) Basidiocarp (TNM F0020891). F-H. *Ganoderma tropicum* (Wu 0407-2). (F) Basidiocarp; (G) Sections of cutis; (H) Basidiospores. Bars = 1 cm in A, E, and F; = 5 cm in D; = 10 μm in B, C, G, and H.

**Table 1.** Taxa used in this study, along with their strain/specimen numbers, origins, and GenBank accession numbers.

Species <sup>a</sup>	Strain/Specimen no.	Origin	GenBank accession no. <sup>b</sup>
<i>Amauroderma rude</i> var. <i>intermedium</i>	JMM ASP.1	Taiwan	X78753&X78774
<i>Ganoderma adspersum</i>	CBS 351.74	Belgium	X78742&X78763
<i>G. australe</i>	UWA 108	Australia	AJ627590&AJ627591
<i>G. incrassatum</i>	DAR 73783	unknown	-
<i>G. lobatum</i>	CBS 222.48	USA	X78740&X78761
<b><i>G. lucidum</i></b>	BCRC 36123 = ATCC 32471	India	EU021459
<b><i>G. lucidum</i></b>	BCRC 37033	Nantou, Taiwan	EU021462
<b><i>G. lucidum</i></b>	BCRC 37043	Taitung, Taiwan	EU021460
<b><i>G. lucidum</i></b>	CWN 01740	Pingtung, Taiwan	EU021461
<i>G. lucidum</i>	JMM P93-1	Philippines	X78745&X78766
<i>G. lucidum</i>	ATCC 32472	India	X87351&X87361
<b><i>G. lucidum</i></b>	WD-565	Ibaraki, Japan	EU021455
<b><i>G. lucidum</i></b>	WD-2038	Ibaraki, Japan	EU021456
<i>G. lucidum</i>	ACCC 5.65	mainland China	X87354&X87364
<i>G. lucidum</i>	HMAS 60537	mainland China	Z37050&Z37074
<i>G. lucidum</i>	CBS 270.81	France	Z37049&Z37099
<i>G. lucidum</i>	RYV 33217	Norway	Z37096&Z37073
<i>G. lucidum</i>	CBS 176.30	UK	AF094511&AF044490
<i>G. philippii</i>	IMI 108700	Malaysia	AJ608714&AJ608715
<i>G. resinaceum</i>	CBS 194.76	Netherlands	X78737&X78758
<i>G. steyaertanum</i>	DAR 73779	Western Australia	-
<i>G. steyaertanum</i>	DAR 73780	Indonesia	-
<i>G. steyaertanum</i>	QFRI 8647.1	Queensland, Australia	-
<i>G. subamboinense</i> var. <i>laevisporum</i>	ATCC 52419	Argentina	X78736&X78757
<i>G. tropicum</i>	RSH 1111	Taiwan	Z37068&Z37088
<b><i>G. tropicum</i></b>	BCRC 37122	Nantou, Taiwan	EU021457
<b><i>G. tropicum</i></b>	Wu 0407-2	Nantou, Taiwan	EU021458
<i>G. weberianum</i>	CBS 219.36	Philippines	X78734&X78755
<i>Tomophagus (Ganoderma) colossus</i>	CBS 216.36	Philippines	Z37071&Z37091

<sup>a</sup>Taxa in bold indicate sequences from this study.

<sup>b</sup>Taxa without sequence accession nos. are those whose sequences published in Smith & Sivasithamparam (2000), but have not been submitted to GenBank.

to strongly laccate, conspicuously sulcate or not, rugose or not; margin obtuse or not, white to orange-yellow. *Pore surface* cream when young, becoming straw or pale brown with age; tubes up to 1.1 cm long, pale brown or brown; pores circular or subcircular, 6-8 per mm, 60-220  $\mu\text{m}$  in diam., dissepiments 25-110  $\mu\text{m}$  thick. *Stipe* when present, 1-9  $\times$  0.4-3 cm, flattened or subcylindrical, lateral, dorso-lateral or horizontally lateral, orange-yellow to red-brown, purple-black, strongly laccate or not. *Context* 0.1-1.8 cm thick, yellow-brown to dark brown, sometimes with melanoid substances, corky; generative hyphae 2-5  $\mu\text{m}$  in diam., colorless, thin-walled, with clamp-connexions; skeletal hyphae 4-7  $\mu\text{m}$  in diam., yellow-brown to red-brown in KOH, dextrinoid; binding hyphae of *bovista*-type, 1.2-2  $\mu\text{m}$  in diam., few, colorless, thick-

walled, much-branched. *Basidiospores* 8-13.5  $\times$  5.5-7.5  $\mu\text{m}$  (with myxosporium), 6.5-10.5  $\times$  4.5-6.5  $\mu\text{m}$  (without myxosporium), ovoid to ellipsoid, mostly truncate, brown, with a dark-brown eusporium bearing fine but slightly conspicuous echinulae, walls 0.5-0.8  $\mu\text{m}$  thick. *Cutis* composed of clavate cells, 15-60  $\times$  4-12.5  $\mu\text{m}$ , dextrinoid to slightly or strongly amyloid.

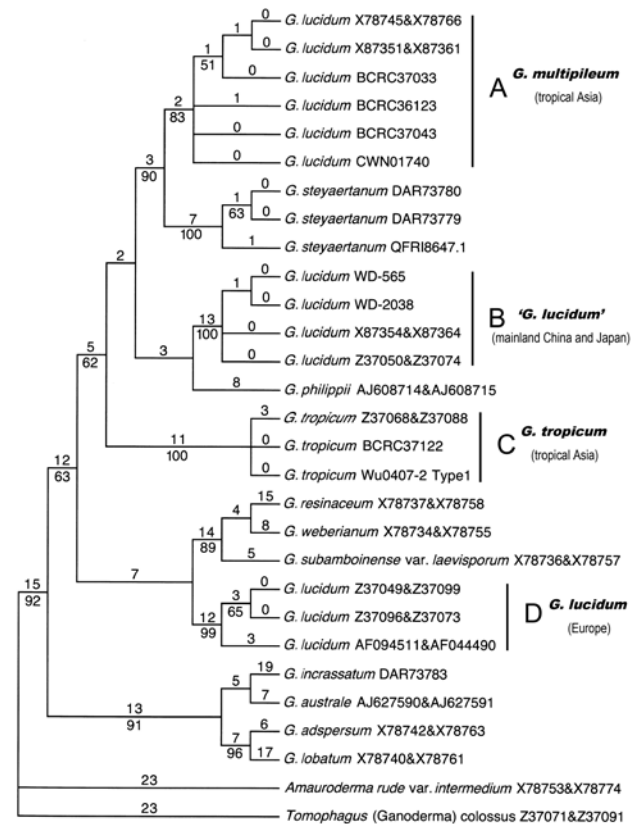
*Specimens examined.* **TAIWAN.** TAICHUNG: By Taichung Park, on stump, 7 Sep 1949, Y.F. Yu (TAIMF000001-holotype of *G. multipileum*); National Chungshing University, 24°07' N, 120°41' E, on trunk of *Delonix regia*, 5 Sep 2003, M.C. Fan and S.A. Liu, FL0309-1 (TNM F0015492); National Museum of Natural Science, 24°10' N, 120°39' E, elev. 100 m, on trunk base of *D. regia*, 23 Sep 1995, W.N. Chou, CWN 01269 (TNM

F0004355); Shalu, on rotten wood, 17 Nov 2005, H.J. Hou, *WAN 1077* (TNM F0019380); Tatushan, 24°06' N, 120°38' E, elev. 100 m, on wood, 7 Sep 1995, T.T. Yuan, *CWN 01246* (TNM F0004332); Veterans General Hospital, on dead trunk base, 9 Jun 2000, T.L. Li, *CWN 04670* (TNM F0014622); Veterans General Hospital, on dead trunk base of *Sterculia nobilis*, 11 Aug 2000, T.L. Li, *CWN 04782* (TNM F0014624). NANTOU: Fruiting body cultivated from BCRC 37033 in June 2006 in this study (TNM F0020892; BCRC 37033 = TARI 88-1-30, collected from Chungshingsintsun). CHIAYI: On *Acacia confusa*, Jul 1916, K. Sasaki, 201.962a-c (TNS-F-201962 with 4 basidiocarps, 201.962a-d; basidiocarp 201.962d redetermined as *G. tropicum* (Jungh.) Bres. in this study); Shuishang, Kuohsing, on stump of Leguminosae sp., Oct 1986, P.H. Fu (TNM F0012903). PINGTUNG: Paoli, 22°04' N, 120°45' E, elev. 160 m, on rotten trunk base of *A. confusa*, 6 Sep 1996, W.N. Chou, *CWN 01740* (TNM F0005258). **INDIA.** Fruiting body cultivated from BCRC 36123 in Jun 2006 in this study (TNM F0020891; BCRC 36123 = ATCC 32471 = FRI 55, collected from roots of *Acrocarpus fraxinifolius*).

## DISCUSSION

Collections identified as '*G. lucidum*' tested in this molecular analysis were separated into three clades: A, B, and D (Figure 2). The Asian collections assigned to *G. lucidum* (clades A and B) were distantly related to *G. lucidum* from Europe, the type locality (clade D). This suggests that *G. lucidum* is a name mistakenly applied to Asian collections. Furthermore, '*G. lucidum*' in Asia represents at least two distinct species, one from Taiwan, India and the Philippines, and another from mainland China and Japan. This study is consistent with the conclusions of Moncalvo et al. (1995a, b).

*Ganoderma multipileum*, a species originally known only based on the type specimen from lowland Taiwan, morphologically resembles Taiwanese '*G. lucidum*.' This species was originally described mainly based on three features: (1) two kinds of pilei, one from the stalk with some of the stipes and pilei growing together, and the other growing from the lower pilei; (2) a thin crust, composed of enlarged and bulbous ends of hyphae,  $16.5 \times 2.35\text{--}6.05 \mu\text{m}$ ; and (3) basidiospores  $8.2\text{--}9.4 \times 4.7 \mu\text{m}$ , ovoid, truncate, with numerous and minute echinulae. The former two characteristics are less useful for circumscription of *G. multipileum*. The feature of multiple-pileate basidiocarp was proven to be unreliable in a cultivation study of *G. multipileum* by Chang (1983). The presence of enlarged hyphal ends in the crust is not diagnostic because this feature is common to all laccate species of *Ganoderma*. Hence, only the feature of basidiospores is meaningful in identification. The holotype of *G. mutlipileum* shares all of the fundamental morphological characteristics with collections of '*G. lucidum*' from Taiwan, including the feature of basidiospores with fine echinulae. Their conspecific status was confirmed in this study.



**Figure 2.** One of the 16 most parsimonious trees derived from the ITS sequence data. The upper and lower numerals at the nodes denote the number of estimated substitutions and proportions of bootstrap replicates, respectively. Only bootstrap values  $\geq 50\%$  are shown.

According to Moncalvo and Ryvarden (1997), nearly ninety names of laccate *Ganoderma* have been proposed from Asian and Pacific regions. Over 90% of these names were excluded as an older name than *G. multipileum* for the same species, due to their later publications, or representing different species as referring to studies of types or authentic specimens by the present authors (unpublished) or by other researchers (e.g. Steyaert, 1972, 1980; Ryvarden, 1977, 1983, 1985, 1990). The remaining taxa lack modern descriptions or require neotypification (see Moncalvo and Ryvarden, 1997). So far, *G. multipileum* is the earliest valid name we can find for '*G. lucidum*' from tropical Asia, and is suggested herein as the correct name for this tropical fungus.

Chang and Chen (1986) stated that the culture from a specimen with multiple pilea, characteristic of *G. multipileum*, is compatible with that of the Taiwanese '*G. lucidum*' without multiple pilea; and these cultures were also compatible with two Indian isolates determined as *G. lucidum* (ATCC 32471 and ATCC 32472). Therefore, they concluded that *G. multipileum* is a synonym of *G. lucidum*. However, those two Indian isolates were grouped together with materials of *G. multipileum* (clade A), and clearly separated from European *G. lucidum* (clade D) in the ITS

phylogenetic tree (Figure 2). Further, the morphology of cultivated fruiting bodies from one of these two Indian strains, BCRC 36123 (= ATCC 32471) is very similar to that of *G. multipileum*, and the preference for Leguminosae plants of Indian '*G. lucidum*' (Sankaran et al., 2005) is also consistent with that of *G. multipileum*. In reality, those two isolates from India belongs to *G. multipileum*.

The material of '*G. lucidum*' from the Philippines (JMM P93.1) is identical to those of *G. multipileum* from Taiwan in ITS sequence except for one or two single-base substitutions, and embedded in the clade A where Taiwanese *G. multipileum* was nested (Figure 2). We re-determined this Philippine strain as *G. multipileum* owing to their high ITS similarity.

Some commercially grown strains of '*G. lucidum*' in the Orient, e.g. RSH RZ and RSH BLC, though not included in this study, were also re-determined as *G. multipileum* because they were grouped together with strains ATCC 32471 and JMM P93.1 in previous studies (Moncalvo et al., 1995a; Hseu et al., 1996).

Smith and Sivasithamparam (2003) described a new species, *G. steyaertanum* from Australian and Indonesian collections, which has been commonly mistaken for *G. lucidum*. They stated that a previous ITS sequence analysis by Smith and Sivasithamparam (2000) indicates the allopatric speciation of *G. steyaertanum* from a species from India and the Philippines, respectively, based on ATCC 32471 and JMM P93.1 (both determined as *G. multipileum* in this study). Furthermore, they concluded that the distribution of *G. steyaertanum* may not extend much further north of Indonesia. However, the morphology and hosts of *G. steyaertanum* (Smith and Sivasithamparam, 2003) are generally consistent with those of *G. multipileum*. The ITS sequences of these two species also exhibit high similarity. Although they formed two clusters in the phylogenetic tree (Figure 2), they were grouped together with strong bootstrap support (90%). The relationship between *G. steyaertanum* and *G. multipileum* merits further study.

'*Ganoderma lucidum*' from mainland China and Japan formed a distinct clade (clade B) separate from clade A where *G. multipileum* was nested (Figure 2). The morphology of '*Ganoderma lucidum*' from Japan examined in this study differs from *G. multipileum* by having moderately echinulate basidiospores and a paler context. Currently, the true identity of this fungus in clade B is still unknown.

*Ganoderma tropicum* is another species widely distributed in lowland tropical Asia. It resembles *G. multipileum* in having a similar habitat and morphology (Figure 1F, and G). *Ganoderma tropicum* differs from *G. multipileum* in having strongly echinulate basidiospores (Figure 1H). These two species were also separated from each other in the molecular analysis (clades A and C, Figure 2).

**Acknowledgments.** We are grateful to the curator of TAIM for arranging the study of the holotype of *G. multi-*

*pileum*, Dr. Tsuyoshi Hosoya (TNS) for the loan of a specimen of '*G. lucidum*' collected from Chiayi, Taiwan and Dr. Tsutomu Hattori (TFM) for arranging the loan of two specimens of '*G. lucidum*' collected from Ibaraki, Japan. We thank Ms. Shih-Yi Yu for her assistance in obtaining DNA sequence data for this study. This project was supported by the National Museum of Natural Science (ROC), Foundation of the National Museum of Natural Science (ROC), and a Postdoctoral Fellowship Grant of the National Science Council (ROC), no. NSC96-2816-B-178-001, to the senior author. The senior author also appreciates the support of a project grant (30270006) from the National Nature Science Foundation of China for her initial *Ganoderma* work in mainland China through Dr. Y.-J. Yao.

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## 重傘靈芝 (*Ganoderma multipileum*) 熱帶亞洲赤芝 “*G. lucidum*” 的正確學名

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*Ganoderma lucidum* (靈芝屬之模式種) 最早描述自英國，目前世界各地均有報導分布。兩千多年來在東方被稱為「靈芝」或「赤芝」的重要經濟真菌也被鑑定為 *G. lucidum*。然而，近年來這個東方赤芝真菌的種類鑑定受到質疑。早些年的分子研究可看出狹義 *G. lucidum* 可能僅局限於歐洲，而亞洲的“*G. lucidum*”至少包括兩個種：中國大陸種和熱帶亞洲種，均非 *G. lucidum*。本文旨在澄清熱帶亞洲赤芝的種類鑑別，且特別納入台灣的野生標本進行研究。定序相關種類和菌株的核糖體內轉錄間隔區 (ITS region)，運行系統發育分析。結果證實 *G. lucidum* 乃為東方赤芝真菌的錯誤命名。本研究並發現半世紀前發表於台灣而被遺忘的「重傘靈芝」(*G. multipileum*)，是熱帶亞洲地區赤芝所最早提出的有效名稱。

**關鍵詞：**被遺忘之名稱；靈芝科；分子系統學；分類學。