Fungal Diversity (2012) 56:49–62
DOI 10.1007/s13225-012-0178-5

Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi"

Yun Cao · Sheng-Hua Wu · Yu-Cheng Dai

Received: 11 May 2012 / Accepted: 22 May 2012 / Published online: 10 June 2012

Abstract "Lingzhi" is a mushroom that has been renowned in China for more than 2,000 years because of its claimed medicinal properties plus its symbolic fortune. "Lingzhi" has high economic value mostly as a dietary supplement in the modern market especially in East Asia, and its medicinal functions have become a hot study topic. For over a century, the highly prized medicinal fungus, known as "Lingzhi" in East Asia, has been assigned to *Ganoderma lucidum*, a species originally described from Europe. Molecular studies in recent years have revealed that the commercially cultivated '*G. lucidum*' ("Lingzhi") in East Asia is a different species from the true *G. lucidum*. The present study aims to clarify the species identity of "Lingzhi" based on morphological studies and analysis of rDNA nuc-ITS sequences, and additional gene fragments of mt-SSU, RPB1, RPB2, and TEF1-α of "Lingzhi" were provided. All *Ganoderma* species that mostly resemble "Lingzhi" in phylogeny and /or morphology were included for analysis. We propose a new species *G. lingzhi* for "Lingzhi", which has an East Asia distribution. The most striking characteristics which differentiate *G. lingzhi* from *G. lucidum* are the presence of melanoid bands in the context, a yellow pore surface and thick dissepiments (80–120 μm) at maturity. *G. curtisii* is most closely related to *G. lingzhi* in phylogeny and is from North America. *Ganoderma flexipes*, *G. multipileum, G. sichuanense*, *G. tropicum* and '*G. tsugae*', are also closely related with *G. lingzhi* and are reported from China. These species are compared and discussed. '*Ganoderma tsuage*' reported from China is determined as conspecific with *G. lucidum*, hence the distribution of *G. lucidum* extends from Europe to northeastern China.

Keywords Ganodermataceae · *Ganoderma lingzhi* · *G. lucidum* · Medicinal fungus · Phylogeny · Taxonomy

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Introduction

The Chinese word "Lingzhi" translates as 'the polypore genus *Ganoderma* P. Karst.' in a broad sense, and in a narrow sense it represents the highly prized medicinal *Ganoderma* species distributed in East Asia. In this paper, we treat "Lingzhi" in its narrow sense: the widely medicinally applied species. "Lingzhi", also known as "Chi-zhi" or "Rui-zhi" in China, is one of the world's most important medicinal fungi. In the Orient, it is viewed as 'herb of spiritual potency' or 'mushroom of immortality', and symbolizes sanctity, success, goodness and longevity (Gao and Zhou 2003; Wasser 2005; Lin 2009; De Silva et al. 2012). The earliest report about Lingzhi's medicinal value appeared in the oldest Chinese medicinal monograph *Shennong's*

Compendium of Material Medica about 100 B.C. The knowledge of "Lingzhi" was also noted and renewed in the subsequent medicinal literatures including the famous Chinese *Compendium of Materia Medica* complied by Shi-Zhen Li in 1590 during the Ming Dynasty (Yu and Shen 2003).

Modern medicinal studies indicate that "Lingzhi" is effective in preventing or relieving various human diseases (Lin 2007; Dai et al 2009). Its medicinal properties include anti-aging, lowering blood pressure, improving immunity, and preventing and treating various cancers, chronic bronchitis, diabetes mellitus, gastric ulcers, hepatitis, hyperlipidemia, hypertension, neurasthenia and thrombosis (Lin 2007; Dai et al. 2009; Aly et al. 2011; De Silva et al. 2012). The fungus is also used in various cosmetics (Hyde et al. 2010). The medicinal effects of "Lingzhi" come from its metabolites including polysaccharides, triterpenes, lucidenic acids, LZ-8 protein, adenosine, ergosterol, glucosamine, cerebrosides *et al.* (Jong and Birmingham 1992; Gao and Zhou 2003; Lin 2009). Currently, "Lingzhi" is among the most sort after medicinal mushrooms in the world market. Various "Lingzhi" products made from the cultivated fruiting bodies have been commercialized as dietary supplements worldwide and especially in Asian countries; its estimated annual global turnover is approximately US$2.16 billion (Lai et al. 2004; Wachtel-Galor et al. 2004).

Ganoderma lucidum (Curtis) P. Karst., the generic type, was originally reported from Peckham, London, UK (Moncalvo and Ryvarden 1997). Unfortunately, the holotype of *G. lucidum* was lost and attempts to seek a neotype from the type locality also failed (Steyaert 1972; Moncalvo and Ryvarden 1997). At present, this taxon has been reported to have a worldwide distribution based on gross similarity of morphological features, e.g., Europe (Steyaert 1972; Ryvarden and Gilbertson 1993), Asia (Hongo and Izawa 1994; Núñez and Ryvarden 2000; Zhao and Zhang 2000), America (Bazzalo and Wright 1982; Gilbertson and Ryvarden 1986), Oceania (Quanten 1997), and Africa (Ryvarden and Johansen 1980).

Patouillard (1907) first reported *G. lucidum* from China, based on collections from Guizhou Province. Later, the Chinese mycologist SC Teng (Teng 1934) reported more collections of *G. lucidum* from different regions of China. In recent decades, *G. lucidum* has been reported from China by other Chinese mycologists, e.g. Tai (1979), Zhao and Zhang (2000), Wu and Dai (2005) and Dai (2012).

A cluster of "Lingzhi" specimens were collected from Laoshan, in Shandong Province, China in 1969 and determined as '*G. lucidum*' by the mycologists of Institute of Microbiology, Chinese Academy of Sciences (Beijing). With these specimens as mother strains, the first successful cultivation of fruiting bodies of "Lingzhi" was performed in the same year (Yu and Shen 2003). Since then, the cultivation of "Lingzhi" has been popularized in China and its adjacent countries, and *G. lucidum* has always been adopted as the scientific binomial for the commercially cultivated "Lingzhi".

Liu (1974) compiled a monograph of Chinese medicinal fungi, and he assigned *G. lucidum* to "Lingzhi" in his book. Since then, *G. lucidum* was accepted as the scientific binomial of "Lingzhi" in many reports on the Chinese edible and medicinal mushrooms (Ying et al. 1987; Mao 1998; Dai et al. 2009). Presently, *G. lucidum* has been widely used for naming the commercialized "Lingzhi" products in the world market of mushroom industry (Lai et al. 2004). Meanwhile, the oriental "Lingzhi" materials used for biochemical, medicinal and pharmaceutical studies were also labelled *G. lucidum* (Kino et al. 1989; Min et al. 2000; Bao et al. 2002; Hu et al. 2002; Sliva et al. 2002; Zhang et al. 2002).

Studies of Moncalvo et al. (1994, 1995) indicated that the collections of '*G. lucidum*' reported from different regions of the world (Europe, America, South and East Asia) do not represent a single species, based on analysis of sequence data from the internal transcribed spacers of nuclear rDNA (nuc-ITS) and the divergent domain D2 of the large ribosomal subunit gene of nuclear rDNA (D2 of nuc-LSU). Smith and Sivasithamparam (2000) further detected that the materials of '*G. lucidum*' from Australia cluster with '*G. lucidum*' from tropical Asia and they differ from European *G. lucidum* through nuc-ITS analysis. Hong and Jung (2004) reached a similar conclusion with Moncalvo et al. (1994, 1995) in their study of *Ganoderma* phylogeny based on the nearly complete mitochondrial small-subunit ribosomal DNA sequences (mt-SSU).

Smith and Sivasithamparam (2003) proposed a new species, *G. steyaertanum* B.J. Smith & K. Sivasithamparam, to replace the mistakenly named *G. lucidum* in Australia and Indonesia. Wang et al. (2009b) showed that the '*G. lucidum*' distributed in tropical Asia is *G. multipileum* Ding Hou, which is neither conspecific with the true *G. lucidum* distributed in Europe, nor conspecific with the real "Lingzhi" distributed in East Asia.

Their phylogenetic analyses (Moncalvo et al. 1994, 1995; Smith and Sivasithamparam 2000; Hong and Jung 2004; Wang et al. 2009b) also clearly indicated that the '*G. lucidum*' from East Asia represents a different species from the true *G. lucidum*. From the morphological perspective, Pegler and Yao (1996) noticed that "Lingzhi" bears a more slender basidiocarp as compared to *G. lucidum* from Europe. In recent years, Szedlay (2002), Wasser et al. (2006) and Wasser (2011) also considered that *G. lucidum* was mistakenly applied to this famous medicinal fungus "Lingzhi". Obviously, the clear recognition of the scientific binomial to represent the "Lingzhi" species remains unknown before our study. To determine the correct identity of the "Lingzhi" species, we performed a detailed

morphological and phylogenetic study of nine *Ganoderma* species that mostly resemble "Lingzhi".

Materials and methods

To search the most similar species with "Lingzhi", we have referred to numerous literatures published during 1902–2011 with special emphasis on those with recent descriptions from the type or authentic specimens (Steyaert 1972, 1980; Ryvarden and Johansen 1980; Ryvarden 1981, 1983, 1985, 2004; Gilbertson and Ryvarden 1986; Ryvarden and Gilbertson 1993; Hattori and Ryvarden 1994; Moncalvo and Ryvarden 1997; Gottlieb and Wright 1999; Núñez and Ryvarden 2000; Smith and Sivasithamparam 2003; Wang and Wu 2008; Welti and Courtecuisse 2010). In particular, we also studied the type specimens of *Ganoderma* species described from China that share similar morphological features with "Lingzhi". We also consulted the studies of Moncalvo et al. (1994) and Wang et al. (2009b) to select the species for the phylogenetic analysis. Molecular data can be used to infer relationships amongst groups of morphologically similar basidiomycetes (Ge et al. 2010; Zhao et al. 2010; Yang 2011; He and Dai 2012; Zhao et al. 2012).

Samples for the analysis included wild collections, commercially cultivated fruiting bodies or strains, and sequences derived from GenBank (Table 1). The commercially cultivated materials '*G. lucidum*' were obtained from the major cultivation bases in ten provinces of China. Most wild specimens were collected by the authors from 15 provinces of China over the last decade. All specimens used in this study are mainly deposited at the herbaria of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Shenyang and Beijing Forestry University (BJFC), Beijing. Some were from the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS), Beijing, Herbarium of the National Museum of Natural Science (TNM), Taichung, and Botanical Museum, University of Helsinki (H), Helsinki.

The microscopic procedure used in this study follows Dai (2010) with some minor amendments. Sections were examined at magnification up to×1000 under a Nikon Eclipse E 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. As the turgid vesicular appendix at distal end of basidiospores in *Ganoderma* are collapsed at maturity, the size of spores was measured with the turgid vesicular appendix excluded and included respectively (Niemelä and Miettinen 2008). In presenting the variation in the size of spores, 5 % of the measurements were excluded from each end of the range, and are given in parentheses. The following abbreviations used in the text include: IKI=Melzer's reagent, IKI–=negative in Melzer's reagent, KOH=5 % potassium hydroxide, CB=Cotton Blue, CB+= cyanophilous, L=mean spore length (arithmetic average of all spores), W=mean spore width (arithmetic average of all spores), Q=variation in the L/W ratios between the specimens studied, n=number of spores measured from given number of specimens. Special colour terms are from Anonymous (1969) and Petersen (1996).

The nuc-ITS sequences were amplified for "Lingzhi" and its related species for phylogenetic analysis. The mt-SSU sequences were also obtained for the Chinese '*G. tsuage*' to study its relationship with the true *G. lucidum*. In addition, the gene fragments of mt-SSU, RPB1, RPB2, and TEF1-α were amplified for "Lingzhi". Total genomic DNA was extracted from dried specimens or living cultures using the Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instructions with slight modifications. The primer pairs, ITS5/ITS4 (White et al. 1990) or ITS1F/ITS4B (Gardes and Bruns 1993) were used to amplify the nuc-ITS region. For some specimens deposited in herbarium for more than 10 years, three new *Ganoderma*-specific primers, G-ITS-F1 (ACC CTG TCG CTG AGA ACT TGA), G-ITS-R1 (AGC ACT GGT AGT CCG TGT CA) and G-ITS-R2 (TTG AGA GCG CAT CAC AAA GC), were designed to select for *Ganoderma* specimens rather than possible internal inhabitats. Combined with the published primers for nuc-ITS amplification, the primer pairs G-ITS-F1/G-ITS-R2 and G-ITS-F1/ITS4B were selected for successful amplification. The primers for amplifying the mt-SSU gene fragment were BMS05/BMS173 (Hong and Jung 2004). The RPB1 gene fragment was amplified using the primer pairs RPB1-2.2f/RPB1-Cr (Matheny et al. 2002; Binder et al. 2010). The RPB2 gene fragment was amplified with the primer pair fRPB2-5F/bRPB2-7R2 (Liu et al. 1999; Matheny et al. 2007). The TEF1-α gene fragment was amplified using the primer pair EF1-983F/EF1-2218R (Rehner and Buckley 2005). The obtained genomic DNA was diluted 1–10 times if necessary. PCR reactions were performed in 50 μL reaction mixtures containing 25 μL of 2×Phire® Plant PCR buffer, 1μL Phire® Hot Start II DNA Polymerase, 5 μL of each PCR primer (10 μM), 2–5 μl dilution protocol and total volume was adjusted to 50 μL with sterile deionized water. The following PCR procedure for Phire® Plant Direct PCR Kit was: initial denaturation for 5 min at 98 °C, followed by 39 cycles at 98 °C for 5 s, annealing temperature for 5 s and 72 °C for 20 s, and a final extension of 72 °C for 2 min. PCR amplification was performed at the following annealing temperatures: 59 °C (ITS5/ITS4), 60 °C (ITS1F/ITS4B), 69.5 °C (G-ITS-F1/ITS4B), 66.5 °C (G-ITS-F1/ G-ITS-R2), 55 °C(BMS05/BMS173), 50 °C (RPB1-2.2f/RPB1-Cr), 55 °C (fRPB2-5F/bRPB2-7R2) and 59 °C (EF1-983 F/EF1-2218R). The successful PCR products were sent to Beijing Genomics Institute, China for purification and

Table 1 Species used in nuc-ITS rDNA analysis, along with their specimen/strain numbers, locality and GenBank accession numbers

Accepted names	Names from specimens/GenBank	Specimen/ strain numbers	Locality	GenBank No.	Reference
*Amauroderma rude*var. *intermedium*	—	JMM ASP.1	Chinese Taiwan	X78753&X78774	Moncalvo et al. 1994
Ganoderma curtisii	*G. curtisii*	CBS 100131	North Carolina, USA	JQ781848	This study
G. curtisii	*G. curtisii*	CBS 100132	North Carolina, USA	JQ781849	This study
Ganoderma flexipes	*G. flexipes*	Wei 5491	Hainan, China	JQ781850	This study
G. flexipes	*G. flexipes*	Wei 5494	Hainan, China	JN383979	Cao and Yuan 2012
Ganoderma lucidum	*G. lucidum*	Dai 2272	Sweden	JQ781851	This study
G. lucidum	*G. lucidum*	RYV 33217	Norway	Z37096&Z37073	Moncalvo et al. 1994
G. lucidum	*G. lucidum*	CBS 270.81	France	Z37049&Z37099	Moncalvo et al. 1994
G. lucidum	*G. lucidum*	CBS 176.30	UK	AF094511&AF044490	Park et al. (unpublished)
G. lucidum	*G. lucidum*	Dai 11593	Finland	JQ781852	This study
G. lucidum	*Ganoderma tsugae*	Dai 3937	Jilin, China	JQ781853	This study
G. lucidum	*G. tsugae*	Yuan 5649	Jilin, China	JQ781854	This study
G. lucidum	*G. tsugae*	Zhang 0981	northern China	X78748&X78769	Moncalvo et al. 1994
Ganoderma lingzhi	*G. tsugae*	RSH J2*	Japan	X78746&X78767	Moncalvo et al. 1994
G. lingzhi	*G. tsugae*	RSH 1109	Japan	X78747&X78768	Moncalvo et al. 1994
G. lingzhi	*G. tsugae*	RSH BLC*	Chinese Taiwan	Z37097&Z37078	Moncalvo et al. 1994
G. lingzhi	*G. lucidum*	HMAS 60537	China	Z37050&Z37074	Moncalvo et al. 1994
G. lingzhi	*G. lucidum*	ACCC 5.65	China	X87354&X87364	Hseu et al. 1996
G. lingzhi	*G. lucidum*	WD-565	Japan	EU021455	Wang et al. 2009b
G. lingzhi	*G. lucidum*	WD-2038	Japan	EU021456	Wang et al. 2009b
G. lingzhi	*G. lucidum*	Dai 12573*	Liaoning, China	JQ781855	This study
G. lingzhi	*G. lucidum*	Cui 4018	Jiangsu, China	JQ781856	This study
G. lingzhi	*G. lucidum*	Cui 10165	Zhejiang, China	JQ781857	This study
G. lingzhi	*G. lucidum*	Wu 1006-38 (holotype)	Hubei, China	JQ781858	This study
G. lingzhi	*G. lucidum*	Cui 9164	Shandong, China	JQ781859	This study
G. lingzhi	*G. lucidum*	Dai 10631	Anhui, China	JQ781860	This study
G. lingzhi	*G. lucidum*	Dai 12438*	Henan, China	JQ781861	This study
G. lingzhi	*G. lucidum*	Cui 6982	Tianjin, China	JQ781862	This study
G. lingzhi	*G. lucidum*	Li 245	Henan, China	JQ781863	This study
G. lingzhi	*G. lucidum*	Dai 12479*	Anhui, China	JQ781864	This study
G. lingzhi	*G. lucidum*	IFP 01021*	Hubei, China	JQ781865	This study
G. lingzhi	*G. lucidum*	Dai 12443*	Guangdong, China	JQ781866	This study
G. lingzhi	*G. lucidum*	Dai 12374	Yunan, China	JQ781867	This study
G. lingzhi	*G. lucidum*	Dai 3583	Hunan, China	JQ781868	This study
G. lingzhi	*G. lucidum*	Dai 12441*	Zhejiang, China	JQ781869	This study
G. lingzhi	*G. lucidum*	Dai 12426*	Sichuan, China	JQ781870	This study
G. lingzhi	*G. lucidum*	Dai 12425*	Shandong, China	JQ781871	This study
G. lingzhi	*G. lucidum*	Dai 12447*	Fujian, China	JQ781872	This study
G. lingzhi	*G. lucidum*	Dai 12449*	Jiangsu, China	JQ781873	This study
Ganoderma multipileum	*G. lucidum*	JMM P93-1	Philippines	X78745&X78766	Moncalvo et al. 1994
G. multipileum	*G. lucidum*	BCRC 36123	India	EU021459	Wang et al. 2009b
G. multipileum	*G. lucidum*	BCRC 37043	Chinese Taiwan	EU021460	Wang et al. 2009b
G. multipileum	*G. lucidum*	Dai 9521	Hainan, China	JQ781874	This study
Ganoderma oregonense	*G. oregonense*	CBS 265.88	Oregon, USA	JQ781875	This study
G. oregonense	*G. oregonense*	CBS 266.88	Washington, USA	JQ781876	This study
G. resinaceum	*G. resinaceum*	CBS 194.76	Netherlands	X78737&X78758	Moncalvo et al. 1994

Table 1 (continued)

Accepted names	Names from specimens/GenBank	Specimen/ strain numbers	Locality	GenBank No.	Reference
G. resinaceum	*G. resinaceum*	CBS 152.27	UK	Z37062&Z37085	Moncalvo et al. 1994
Ganoderma sichuanense	*G. sichuanense*	HMAS 42798 (holotype)	Sichuan, China,	JQ781877	This study
G. sichuanense	*G. sichuanense*	Cui 7691	Guangdong, China	JQ781878	This study
Ganoderma tropicum	*G. tropicum*	Dai 9724	Hainan, China	JQ781879	This study
G. tropicum	*G. tropicum*	Yuan 3490	Yunnan, China	JQ781880	This study
G. tropicum	*G. tropicum*	BCRC 37122	Chinese Taiwan	EU021457	Wang et al. 2009b
Tomophagus colossus	—	CBS 216.36	Unknown	Z37071&Z37091	Moncalvo et al. 1994

* indicates the specimens or strains were artificially cultivated.

sequencing with the same primers used in PCR procedure and/or the additional newly designed primers in this study. G-Intron F1 (GGA ACC TAA GGA AGA CTA TTA C), G-Intron R1 (TCA GGG ATG TTA GTT TCT ACA) and G-Intron R2 (GGG TAT CTA ACC GTG GAA TCA GA) were newly designed for sequencing the intron of mt-SSU region. Two additional primers G-RPB2-F1 (CAT CGA GTT CTT GGA GGA GTG G) and G-RPB2-R1 (CGG AAT GAT GCT GGC ACA GAC A) were for RPB2. G-TEF1-F1 (GGT GAG TTC GAG GCT GGT ATC T) and G-TEF1-R1 (CGG GTA CTC GTT GTA AGA CTC) were for TEF1-α.

The newly-generated sequences were assembled and modified manually according to the chromatograms in ContigExpress (Vector NTI Suite 6.0, InforMax Inc.) and then submitted to GenBank. The phylogenetic analysis included nuc-ITS sequences from our lab work and GenBank (Table 1). *Amauroderma rude* var. *intermedium* J.S. Furtado and *Tomophagus colossus* (Fr.) Murrill were selected as outgroup (Moncalvo et al. 1994; Wang et al. 2009b). All sequences were aligned with Clustal X 2.0 (Larkin et al. 2007) using default settings and further optimized manually using BioEdit 7.0.5.3 (Hall 1999) to allow maximum alignment and minimize gaps. The alignment was deposited at TreeBASE (Accession No: 12548).

Phylogenetic analysis was performed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian algorithm (BA). The parameters were set as follows: 1) MP: the analysis was performed in PAUP* 4.0b10 (Swofford 2002). Heuristic search with TBR branch swapping was implemented with 1000 replicates of random-addition sequence. All characters were equally weighted and gaps were treated as missing data. MAXTREES was set to auto-increase. Bootstrap analysis was carried out with 1,000 replicates using the heuristic search (Felsenstein 1985). Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all parsimony trees. 2) ML: The analysis was conducted in PhyML3.0 (Guindon and Gascuel 2003). The best-fit model was selected by jModelTest (Posada 2008) according to Corrected Akaike Information Criterion (AICc). Bootstrap analysis was performed with 100 replicates. 3) BA: The analysis was run in MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit model of nucleotide evolution was selected by Hierarchical Likelihood Ratio Tests (hLRT) in MrModeltest 2.3 (Nylander 2004). Markov Chain Monte Carlo (MCMC) algorithm (Larget and Simon 1999) was conducted to calculate Bayesian posterior probabilities. Four Markov chains were run for 3,000,000 generations with the trees sampled every 100th generation. The average standard deviation of split frequencies was restricted to be below 0.01. The option of burnin was set to discard 10 % trees (Hall 2004).

Results

Phylogeny

The nuc-ITS dataset contains 53 sequences representing 12 taxa. Amplification of the nuc-ITS region yielded approximately 650 bp fragments. After deleting the ambiguous sites at both ends and 5.8S region in the alignment, 409 characters remained for phylogenetic analysis.

For MP analysis, 286 characters are constant, 33 characters are variable, but parsimony-uninformative, and 90 characters are parsimony-informative. MP analysis yielded 2992 most parsimonious trees (CI=0.769608, RI=0.928463, RC =0.714552, HI =0.230392), and one of them is shown in Fig. 3. For ML analysis: the best-fit model was HKY+G selected by AICc in jModelTest. For BA analysis, the best-fit model was HKY+G selected in MrModeltest 2.3. Four simultaneous Monte Carlo chains were run for 3,000,000 generations, and the average standard deviation of split

frequencies was 0.006618. As the trees generated by ML and BA analyses shared nearly identical topology with that of MP analysis, MP bootstrap, ML bootstrap and Bayesian Posterior Probabilities were respectively labelled near the nodes in the same tree (Fig. 1).

In the phylogenetic tree, the samples named *G. lucidum* separated into three distinct clades. They were respectively listed as Clade I, Clade II and Clade III in Fig. 1.

Clade I includes 22 wild and cultivated samples named *G. lucidum,* as well as three samples named *G. tsugae,* all from East Asia. These taxa form a monophyletic lineage with strong support (MP/ML/BA=95/97/1.00), and are distantly related to *G. lucidum* from Europe in Clade III. Samples of *G. curtisii* (Berk.) Murrill from North America form a well-supported clade (MP/ML/BA=84/92/0.95) and serves as the sister clade to Clade I.

Clade II comprises four samples named '*G. lucidum*' from tropical Asia, and represents a separate lineage of '*G. lucidum*' from Asia and has high bootstrap support value and posterior probability value (MP/ML/BA=97/94/1.00). All four samples in this clade represent *G. multipileum* (Wang et al. 2009b).

Clade III contains five samples of *G. lucidum* from Europe as well as three samples labelled '*G. tsugae*' from northern China. This clade has quite low support (ML/Bayes=71/0.73), but forms a robust group with high support (MP/ML/BA=100/99/1.00) with its sister clade containg *G. oregonense* Murrill from America.

In addition to *G. multipileum* and '*G. tsugae*' mentioned above, another three similar species, *G. flexipes, G. sichuanense* and *G. tropicum,* form the well-supported clades (*G. flexipes*, MP/ML/BA=100/100/1.00; *G. sichuanense* MP/ML/BA=100/100/1.00; *G. tropicum* MP/ML/BA=100/98/1.00; Fig. 1). They are not closely related to '*G. lucidum*' from East Asia in phylogeny.

Taxonomy

Ganoderma lingzhi Sheng H. Wu, Y. Cao & Y.C. Dai, **sp. nov.** (Figs. 2, 3)

MycoBank no.: MB 564240

CHINA. Hubei Province, Wuhan, Jiufeng National Forest Park, alt. 90 m, on the ground in forest of *Quercus*, 29 June 2010, *Wu 1006–38* (holotype in TNM, isotype in BJFC, IFP).

Etymology lingzhi (Lat.): referring to the Chinese name "Lingzhi", a highly prized *Ganoderma* fungus in Chinese folklore.

Fruitbody Basidiocarps annual, stipitate, corky and without odor, but bitter when fresh, becoming hard corky to woody hard when dry. Pilei variable, from semicircle, shell-like, reniform to circular, occasionally lobed, corky, projecting up to 16 cm, 17 cm broad and 2.7 cm thick. Pileal surface weakly laccate when juvenile, strongly laccate with age, cinnamon-buff to clay-buff when juvenile, orange-brown to reddish brown with age, often with more or less concentric zones especially near the margin and fine furrows in the middle; margin acute to obtuse, sometimes with slight waves, color variable, orange-brown, yellowish brown, cinnamon-buff to buff when juvenile, becoming orange-brown to reddish brown with age. Stipe flattened or subcylindrical, lateral, dorso-lateral, horizontally lateral or eccentric, orange-yellow to yellowish brown when juvenile, becoming reddish brown to vinaceous brown with age, up to 22 cm long and 3.5 cm thick. Pore surface white when juvenile, turning sulphur yellow at maturity, turning brown to dark brown when bruised, usually straw-colored when dry; pores circular or angular, mostly entire, (4–)5–6(–7) per mm, (40–)80–140(–160) μm in diam; dissepiments (60–)80–120(–140) μm. Context not completely homogeneous in color, the upper part buff, the lower part clay-buff, corky, without concentric growth zones, up to 0.5 cm thick at base, often with 1–2 black melanoid bands in the mature basidiocarps. Tubes clay-buff, woody hard, not stratified, up to 2.2 cm long.

Hyphal structure Hyphal system trimitic; generative hyphae bearing clamp connections, hyaline, thin-walled, infrequent; skeletal hyphae dominant, thick-walled to subsolid, frequently dichotomous branched; binding hyphae thick-walled with a narrow lumen to subsolid; all the hyphae IKI–, CB+; tissues darkening in KOH.

Context Generative hyphae not observed; skeletal hyphae dominant, pale brown to brown, thick-walled to subsolid, frequently branched, interwoven, 3–4.5 μm in diam; binding hyphae abundant, brownish, thick-walled with a narrow lumen to subsolid, flexuous, interwoven, 1–2.1 μm in diam.

Tubes Generative hyphae hyaline, thin-walled, moderately branched and clamped, 1.3–2.8 μm in diam; skeletal hyphae dominant, pale brown to distinctly brown, thick-walled with a medium or narrow lumen to subsolid, frequently branched, strongly interwoven, 2–4 μm in diam; binding hyphae brownish, thick-walled to almost solid, frequently branched, flexuous, interwoven, 1–1.8 μm in diam; basidia barrel-shaped with 4 sterigmata and a basal clamp connection, 14–18×6–10 μm; basidioles in shape similar to basidia, but slightly smaller.

Cutis Composed of a vertical and closely-packed palisade of cells; cells clavate, brown, thick-walled to subsolid, occasionally with blunt outgrowths or protuberances in the apical or lateral parts, moderately to strongly amyloid at maturity, 30–50×5–10 μm.

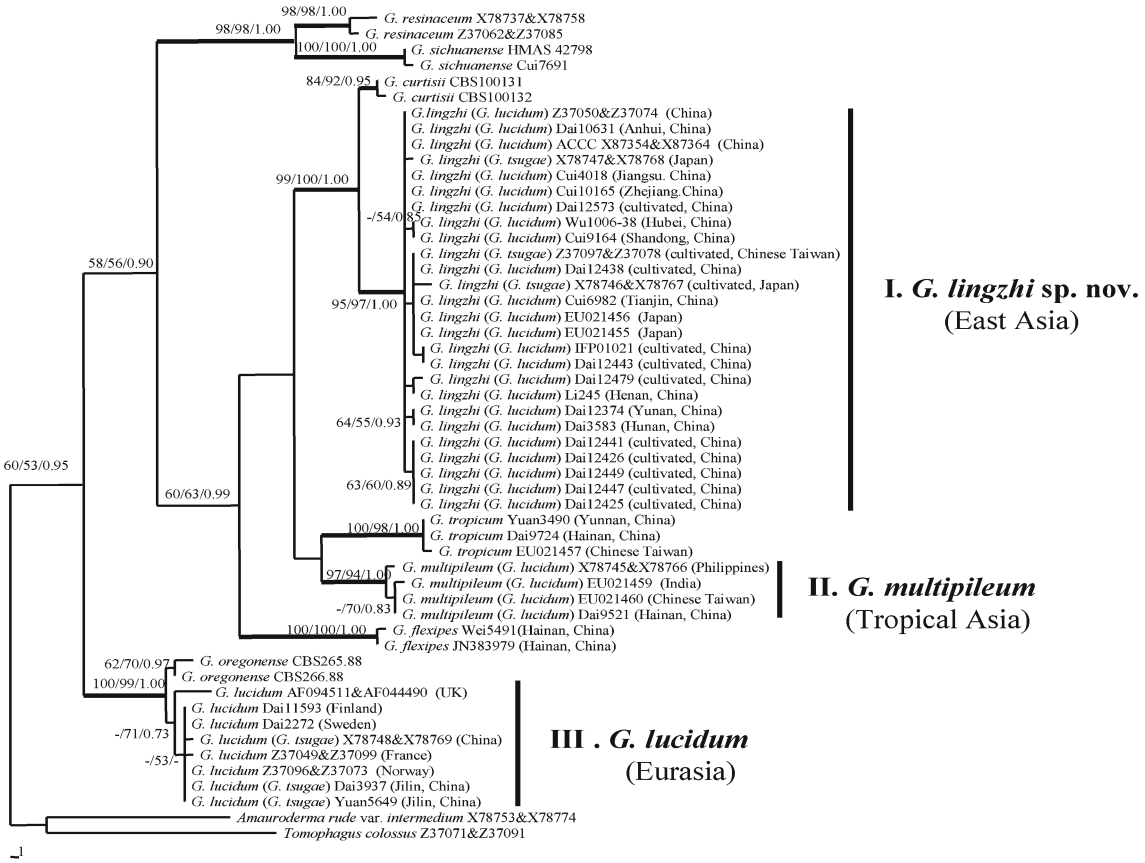

Fig. 1 One of the most parsimonious trees illustrating the phylogeny of *Ganoderma lingzhi* and related species within the genus based on nuc-ITS sequences. Clade stabilities were calculated from MP (≥50 %), ML (≥50 %) and BA (≥0.70). Branches that got strong support from all three analyses (MP, ML and BA) are in bold

Spores Basidiospores ellipsoid, truncate at maturity, yellowish brown, IKI–, CB+, double-walled, exospore smooth, endospore with moderate to coarse echinulae, sometimes even to short ridges up to 1.2 μm long, (8–)9–10.7(–12)×(5.2–)5.8–7(–7.5) μm, L=9.74 μm, W=6.38 μm, Q=1.45–1.59 (n=300/10; the exospore included and the turgid vesicular appendix excluded); (9.2–)9.5–11.2(–13)×(5.2–)5.6–7(–7.8) μm, L=10.27 μm, W=6.28 μm, Q =1.54–1.74 (n=90/6; the exospore included and the turgid vesicular appendix included); (6.4–)7–8.2(–9)×(4.5–)4.9–5.7(–6.2) μm, L=7.58 μm, W=5.19 μm, Q=1.38–1.56 (n=300/10; the exospore excluded).

Additional specimens (paratypes in IFP or mentioned by herbarium code) *studied.* **China.** Anhui Province, Hefei, on dead tree of *Quercus*, 25 September 2008, *Dai 10631*. Hubei Province, Wuhan, Maanshan National Forest Park, on the ground in forest of *Quercus*, 30 June 2010, *Wu 1006-75* (TNM), *Wu 1006-76* (TNM). Hunan Province, Changsha, Yuelushan, on root of *Castanopsis*, 14. July.2011 *Dai 12458*; on rotten angiosperm stump, 5 July 2002, *Dai 3583*; Yizhang County, Mangshan Nature Reserve, on stump of *Castanea*, 26 June 2007, *Dai 8172*. Jiangsu Province, Nanjing, Zijinshan, on angiosperm stump, 22 August 2006, *Cui 4018*. Jiangxi Province, Jiujiang County, South Lake Park, on angiosperm stump 10 October 2008, *Cui 6109*; Fenyi County, Dagangshan Nature Reserve, on angiosperm wood, 18 September 2008, *Dai 10457*; on living tree of *Quercus*, 22 September 2009, *Cui 7848 & 7849*. Shandong Province, Taian, Taishan Nature Reserve, on fallen trunk of *Quercus*, 2 August 2010, *Cui 9166*; on rotten wood of *Quercus*, 2 August 2010, *Cui 9164*; Yantai, Kunyushan Nature Reserve, on stump of *Quercus*, 23 August 2010, *Dai 11701*. Sichuan Province, Dujiangyan County, Qingchenshan, on angiosperm stump, 13 September 2010, *Cui 9223*; Mianyang, Longshan, on stump of *Cyclobalanopsis*, 10 October 2009, *Dai 11958*. Tianjin, Ji County, Panshan, on root of *Quercus*, 1 August 2009, *Cui 6982*. Yunnan Province, Tengchong County, Gaoligongshan Nature Reserve, on angiosperm stump, 24 October 2009, *Cui 8032*; Puer County, Laiyanghe Nature Reserve, on root of *Castanea*, 9 June 2011, *Dai 12374*. Zhejiang Province,

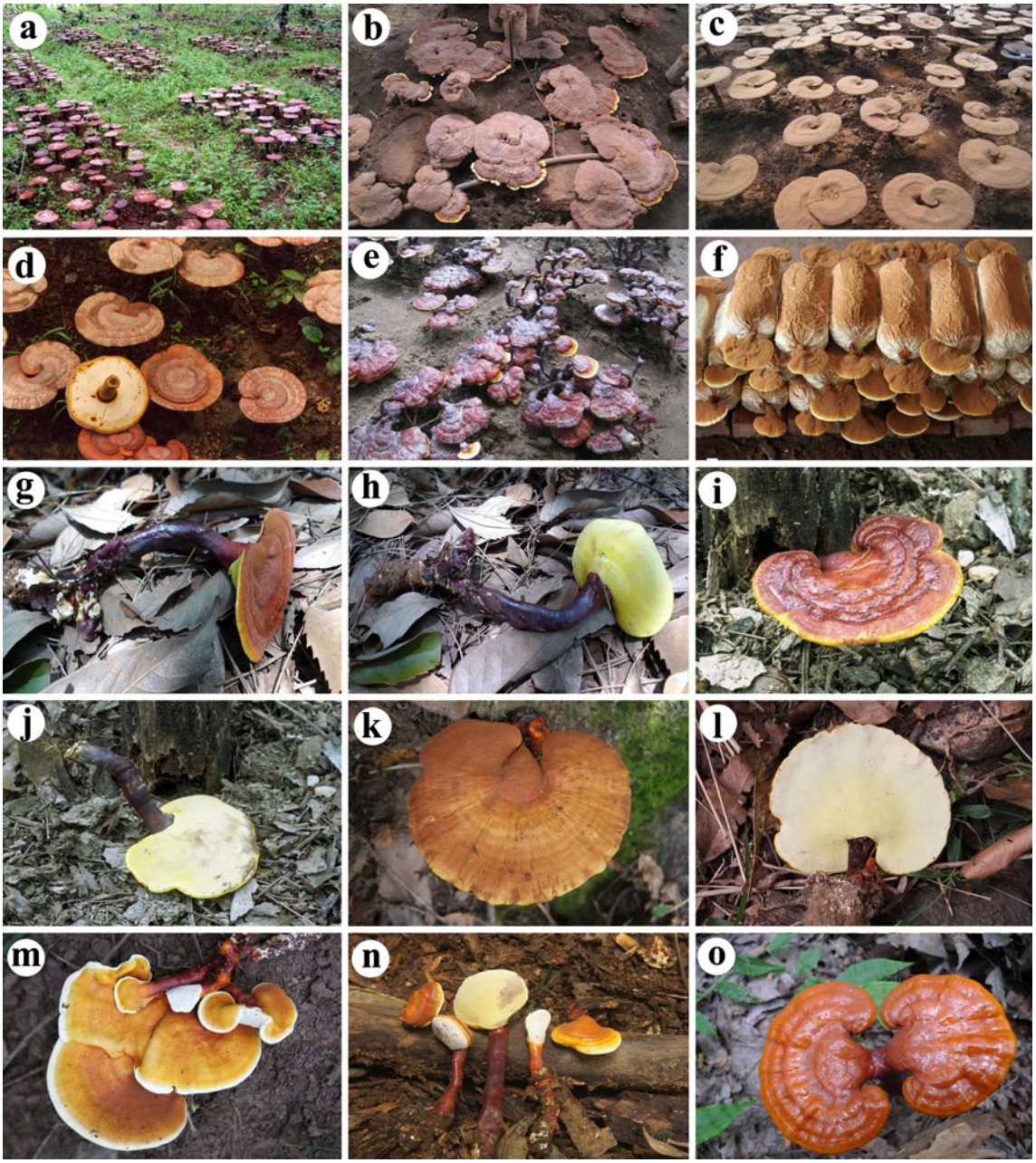

Fig. 2 Basidiocarps of *Ganoderma lingzhi*. **a–f**. Cultivated fruting bodies of *Ganoderma lingzhi* **g–o**. Wild fruting bodies of *Ganoderma lingzhi* (**g–h**. holotype; **i–o**. paratypes)

Yongjia County, Longwan Lake Forest Park, on angiosperm stump, 21 August 2011, *Cui 10165*.

Molecular data In addition to nuc-ITS sequences (Table 1) for phylogenetic analysis, another four gene fragments of mt-SSU, RPB1, RPB2, and TEF1-α are supplied in GenBank for further comparison with the other related species (mt-SSU:Cui9166, Shandong, JX029987; Dai12479, cultivated, Anhui, JX029988; Wu 1006-38, Hubei, JX029989; Dai12574 cultivated, Liaoning, JX029990. RPB1: Cui9166, Shandong, JX029982; Dai12479, cultivated, Anhui, JX029983; Wu 1006-38, Hubei, JX029984; Dai12574, cultivated, Liaoning, JX029985. RPB2: Cui9166, Shandong, JX029978; Dai12479, cultivated, Anhui, JX029979; Wu 1006-38, Hubei, JX029980; Dai12574, cultivated, Liaoning, JX029981. TEF1-α: Cui9166, Shandong, JX029974; Dai12479, cultivated, Anhui, JX029975; Wu 1006-38, Hubei, JX029976; Dai12574, cultivated, Liaoning, JX029977). Although the above mentioned gene sequences are available for *G. lingzhi*, it is impossible to carry out multigene analysis for phylogeny, because these genes are not available for other species of the genus.

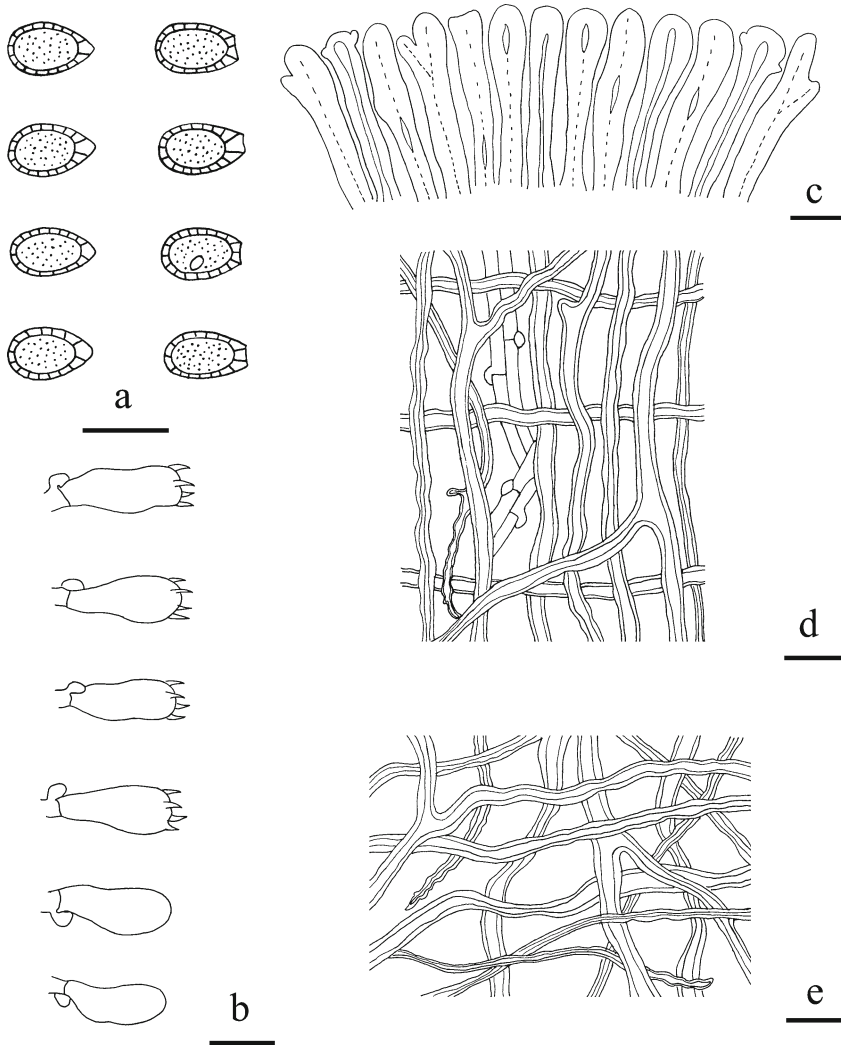

Fig. 3 Microscopic structures of *Ganoderma lingzhi* **a**. basidiospores; **b**. basidia and basidioles; **c**. apical cells from the cuticle; **d**. hyphae from trama. **e**. hyphae from context (scale bars: 10 μm)

Discussion

Analysis of nuc-ITS sequences shows that the strains of wild and cultivated *G. lingzhi* from East Asia (Fig. 1) are nested in a strongly-supported Clade I (MP/ML/BA=98/98/1.00). Clade I is clearly separated from Clade III in phylogeny, where the European *G. lucidum* is nested (Fig. 1). Therefore, the newly introduced species, *G. lingzhi*, which is widely distributed and cultivated in East Asia, is not conspecific with *G. lucidum* from Europe.

Ganoderma lingzhi was long assigned to *G. lucidum* as both species have a reddish brown pileal surface, similar-sized basidiospores and mostly regular clavate cuticle cells. However, several morphological features separate *G. lingzhi* from the European *G. lucidum*. In mature basidiocarps, *G. lingzhi* bears 1–2 black melanoid bands in the context while *G. lucidum* lacks this structure. *G. lingzhi* normally has a sulphur-yellow to straw-colored pore surface and thick dissepiments (80–120 μm) at maturity. In contrast, *G. lucidum* has a white pore surface and thin dissepiments (40–80 μm). The cuticle cells of *G. lucidum* are also usually longer (47–70 μm) than that of *G. lingzhi*.

In addition to the identification of our materials of *G. lingzhi* and *G. lucidum*, we also conducted morphological studies for the species similar to *G. lucidum*. Zhao and Zhang (2000) and Wu and Dai (2005) also have mentioned that '*G. lucidum*' (*G. lingzhi*) from China has yellow pore surface, while the European *G. lucidum* always has a white pore surface (Ryvarden and Gilbertson 1993; Ryvarden 1994). Kim et al. (2001) also found that the specimens labelled *G. lucidum* from Korea have pale brownish thread-like tissues in the centre of context (i.e., melanoid bands). These descriptions of '*G. lucidum*' from East Asia are consistent with our observation of *G. lingzhi*.

After studying type specimens of *Ganoderma* species from China that share similar morphological features with *G. lingzhi*, we found that they can be divided into 3 categories: 1) *Ganoderma* species with concentric growth zones in

the context: *G. chenghaiense* J.D. Zhao and *G. simaoense* J.D. Zhao. *G. chenghaiense* has been resolved as *G. multipileum* by Wang et al. (2009a). Based on our study, *G. simaoense* is a tropically-distributed species due to its concentric growth zones in the context, which can separate this species from *G. lingzhi* clearly. 2) *Ganoderma* species with small pilei and slender stipes: *G. atrum* J.D. Zhao *et al*., *G. calidophilum* J.D. Zhao *et al*., *G. hainanense* J.D. Zhao *et al*. and *G. parviungulatum* J.D. Zhao & X.Q. Zhang. They were all reported from Hainan Province of China, and they are conspecific with *G. flexipes* Pat. (Data to be published in another paper). 3) *G. sichuanense* is an accepted species and its morphological differences from *G. lingzhi* are discussed below.

Among the Chinese *Ganoderma* species, *G. flexipes* Pat., *G. multipileum* Ding Hou, *G. sichuanense* J.D. Zhao & X.Q. Zhang, *G. tropicum* (Jungh.) Bres. and '*G. tsugae*' Murrill are the mostly similar species to *G. lingzhi* because they share a reddish brown pileal surface, similar basidiospores and cuticle cells (Fig. 4). However, the morphological characteristics can distinguish *G. lingzhi* from these five species through careful observation. *G. flexipes* is separated from *G. lingzhi* by its mostly small pileus, slender and long stipe, and yellow brown to dark brown context when mature. *G. multipileum* is distinguished from *G. lingzhi* by its distinct concentric growth zones in context at maturity, and finely echinulate basidiospores. *Ganoderma lingzhi* is often associated with Fagaceae (e.g., *Castanea*, *Cyclobalanopsis* and *Quercus*) and is distributed in temperate to subtropical East Asia, while *G. multipileum* inhabits Fabaceae trunks (e.g., *Acacia*, *Delonix*) and is distributed in tropical and subtropical Asia (Wang et al. 2009b). *Ganoderma sichuanense* differs from *G. lingzhi* in its sessile basidiocarps and smaller basidiospores (7.4–9.2× 5–6.6 μm). *Ganoderma tropicum* differs from *G. lingzhi* by its mostly sessile basidiocarps, dark brown context, concentric growth zones in the context and mostly irregular cuticle cells. Similar to *G. multipileum*, *G. tropicum* also inhabits Fabaceae trees. The Chinese '*G. tsugae*' is separated from *G. lingzhi* by the absence of melanoid bands in the context, white pore surface and thin dissepiments (20–60 μm) when mature. The feature differences among *G. lingzhi* and some other similar species are listed in Table 2.

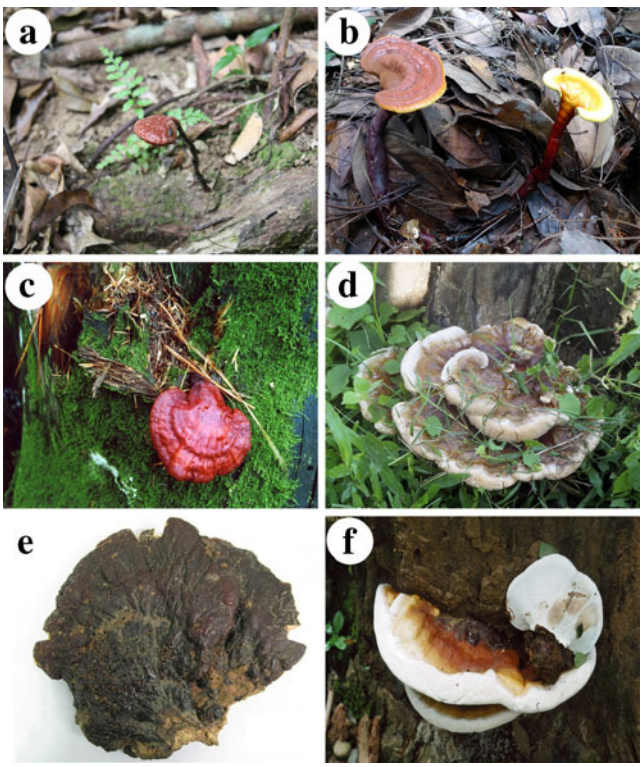

Fig. 4 Basidiocarps of *Ganoderma lingzhi* and its most similar species in China. **a**. *Ganoderma flexipes*; **b**. *Ganoderma lingzhi* (holotype); **c**. *Ganoderma lucidum* ('*Ganoderma tsugae*'). **d**. *Ganoderma multipileum*; **e**. *Ganoderma sichuanense* (holotype); **f**. *Ganoderma tropicum*

Ganoderma curtisii, a species originally described from North America (Moncalvo and Ryvarden 1997), was found to be the most closely phylogenetically related species to *G. lingzhi* (MP/ML/BA=99/100/1.00, Fig. 3). Two strains of *G. curtisii* (CBS 100131, 100132) from the type locality were cultivated to obtain their fruiting bodies for comparison. Fruiting bodies of *G. curtisii* resemble *G. lingzhi* when juvenile as they share a yellow to buff pileal surface and white pore surface. At maturity, *G. curtisii* still bears yellowish brown to olivaceous buff pileal surface with a thin and easily scaled off soft crust, while *G. lingzhi* often bears a reddish brown pileal surface with a hard and non-breakable crust. In addition, *G. curtisii* usually has a white pore surface and thin dissepiments (40–80 μm) when mature. In contrast, *G. lingzhi* has a yellow pore surface when mature and its dissepiments are thick (80–120 μm). Microscopically, *G. curtisii* has more inflated, loosely-arranged cuticle cells with wider apical parts ((6–)8–12(–14) μm, W=9.98 μm), while *G. lingzhi* often has more slender, closely-packed cuticle cells with narrower apical parts ((5–)6–9(–10) μm, W=7.34).

Wang and Yao (2009) reported that *G. sichuanense* can represent '*G. lucidum*' in China. Our analysis indicates that *G. sichuanense* is distantly related to *G. lingzhi*, but it is closely phylogenetically related to *G. resinaceum* (Fig. 1). Coupled with the obvious morphological differences from *G. lingzhi* (Fig. 4, Table 2), we confirm that *G. sichuanense* is a distinct species.

The nuc-ITS sequences obtained from four samples of '*G. lucidum*' (ACCC 5.65 and HMAS 60537 from China; WD–565 and WD–2038 from Japan) in previous studies (Moncalvo et al. 1994, 1995; Wang et al. 2009b) are also

Table 2 Morphological characters of *Ganoderma lingzhi* and its most similar species in China

Species	Stipe	Melanoid bands in mature fruiting body	Context color	Concentric growth zones in context	Dissepiments (µm)	Cuticle cells	Basidiospores (µm)	Pore color (at maturity)
Ganoderma flexipes	present	present	not completely homogeneous; yellow brown to dark brown	absent	thick; 80–120 (–140)	mostly regular; clavate	(8.5–)9–10.3(–11)×(5–) 5.3–7	white to pale sulphur yellow
Ganoderma lingzhi	present	present	not completely homogeneous; light buff, buff, clay buff to snuff buff	absent	thick; (60–)80–120(–140)	mostly regular; clavate	(8–)9–10.7(–12)×(5.2–) 5.8–7(–7.5)	pale yellow, sulphur yellow to straw-colored
Ganoderma lucidum (='*G. tsugae*' from China)	present	absent	not completely homogeneous; white, cream to pale clay brown	absent	thin; 20–60(–80)	mostly regular; clavate	(8.8–)9–10.7(–11)×(5.3–) 5.7–6.6(–7)	white
Ganoderma multipileum	present, rarely absent	present	not completely homogeneous; clay buff to fulvous	present	thick; 60–120 (–160)	mostly regular; clavate	(8–)8.8–10.4(–11.3)×(5–) 5.6–6.9(–7.2)	cream to straw-colored
Ganoderma sichuanense	absent	present	not completely homogeneous; buff to pale clay buff	absent	thick; (80–)100–140(–200)	mostly regular; clavate	(7–)7.4–9.2(–9.3)×(4.6–) 5–6.6(–6.8)	buff yellow
Ganoderma tropicum	absent, sometimes present	present	homogeneous; fulvous	present	thick; (60–)80–140(–160)	mostly irregular; clavate, often with blunt outgrowths or protuberances	(8.3–)8.8–10.7(–11.2)× (5–)5.4–6.3(–6.8)	cream to pale straw-colored

included in this study. They are also nested in Clade I (Fig. 1), which is *G. lingzhi*.

The mt-SSU sequences of '*G. lucidum*' (IFO 31863 (KCTC 6729) from Japan; IMSNU 30042, KCTC 6366, KCTC 6530b, KCTC 6531 and KCTC 6532 from Korea) in the study of Hong and Jung (2004) share very high similarity (>99 %) with that of *G. lingzhi* in our study. These Japanese and Korean '*G. lucidum*' are determined as *G. lingzhi* in this study. Accordingly, *G. lingzhi* has a distribution in China, Japan and Korea.

Moncalvo et al. (1995) concluded that an Asian species was mistaken for *G. tsugae* from his phylogenetic study of the cultivated *Ganoderma* species labelled *G. tsugae* (RSH J2, RSH 1109, RSH BLC) from East Asia. These sequences were also included in our analysis and these strains are nested in the well-supported Clade I (Fig. 3). Consequently, they are determined in this study as *G. lingzhi*.

We investigated 35 specimens or strains of cultivated "Lingzhi" from 10 representative production sites in China. In spite of the highly variable basidiocarps of the different "Lingzhi" varieties, the analysis of nuc-ITS sequences shows that they share high similarity (>99 %) and clustered in a single clade with a robust support. This indicates that the strains of "Lingzhi" widely cultivated in China represent a single species (i.e., *G. lingzhi*).

The strain labelled *G. tsugae* (Zhang 0981) from studies of Moncalvo et al. (1994, 1995) and two specimens named '*G. tsugae*' (Dai 3937, Yuan 5649) collected on *Larix* from north-eastern China were also analyzed. The results showed that these samples are nested in Clade III and cluster with *G. lucidum* from Europe. Similar results were mentioned by Moncalvo et al. (1994; 1995). The Chinese '*G. tsugae*' (Yuan 5649, nuc-ITS:JQ781854, mt-SSU:JX029986) also shares

Table 3 The studied *Ganoderma* species from China in this paper

	Species Names
Accepted species	*G. flexipes* Pat.
	G. lingzhi Sheng H. Wu, Y. Cao & Y.C. Dai
	G. lucidum (Curtis) P. Karst.
	G. multipileum Ding Hou
	G. sichuanense J.D. Zhao & X.Q. Zhang
	G. tropicum (Jungh.) Bres.
Unaccepted species	*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
	G. simaoense J.D. Zhao
	G. tsugae Murrill

high similarity (>99 %) in nuc-ITS and mt-SSU sequences with *G. lucidum* from UK (CBS 176.30, nuc-ITS: AF094511&AF044490, mt-SSU:AF248324&AF248325). In addition, the Chinese '*G. tsugae*' has stipitate basidiocarps, a reddish brown pileus, a white pore surface and thin dissepiments (20–60(–80) μm) when mature, but lacks melanoid bands in the context. All of these macro-morphological characters fit that of *G. lucidum* from Europe with such micromorphological features as regular cuticle cells and similar basidiospores. Thus, the '*G. tsugae*' samples from conifers in northeastern China are identified as *G. lucidum* in this study. Studies of Moncalvo et al. (1995) indicated that *G. lucidum* sensu stricto was distributed in northern and southern Europe, and probably extended to China. Based on more molecular and morphological evidences from more samples, our study confirmed that the distribution of *G. lucidum* includes northeastern China. Geographic distribution of *G. lucidum* resembles the Europe (-Siberia)-Northeast Asia (-Southwestern China) distribution pattern of *Heterobasidion* Bref. and *Chroogomphus* (Singer) O.K. Mill (Dai et al. 2003; Li et al. 2009).

Ganoderma species have been widely cultivated in Asia and consumed worldwide, among which most commercial materials are named as *G. lucidum*. In addition to the Asian origin, some commercial strains are from America, e.g. Fungi Perfecti '*G. lucdium*', which was also determined as another different species from the true *G. lucidum* (Smith and Sivasithamparam 2000). As misidentification of *Ganoderma* strains may hinder strategies for drung discovery (Wasser et al. 2006) and create complications for publications, patents and products (Wasser 2011), the correct identification of commercial and research-oriented *Ganoderma* strains especially those labelled *G. lucidum* is obviously important. The species identity of the '*G. lucidum*' collections outside Eurasia should be further studied. The species of *Ganoderma* studied and accepted in this study from China can be found in Table 3.

Specimens of related Ganoderma species studied ***G. curtissi***. **USA**. North Carolina, Durham, Duke gardens, on the base of living tree of *Quercus*, November 1996, *JM 96/80* (*CBS 100131*); Durham, yard Alabama street, on the base of living hardwood tree, 6 December 1996, *JM 96/81*(*CBS 100132*). ***G. flexipes***. **China**. Fujian Province, Wuyishan County, Tianyoufeng, on the ground of mixed forest, 26 August 2006, *Cui 4122*. Hainan Province, Baoting County, Diaoluoshan Nature Reserve, on the ground of angiosperm forest, 30 June 2010, *Wei 5491 & 5494*; Ledong County, Jianfengling Nature Reserve, on the root of dead angiosperm, 25 June 2010, *Wei 5200*; on the ground of forest, 15 November 2007, *Yang 407*. ***G. lucidum***. **Finland**. Espoo, Westend, Varsasaari, on the stump of *Picea*, 1 August 2005, *Stella* (H). Helsinki, on the dead tree of *Quercus* 4 September 2004, *Dai 5857*. Vantaa, 18 October 2009, *Dai 11593*. Varsinais-Suomi, on ground, *Salo 11212* (H). **Romania**. Transsilvania Distr., on *Quercus*, 16 September 1955, *Silaghi* (H). **Sweden**. Halland, Västergotland, on rotten wood of *Corylus*, 22 August 1996, *Dai 2272*. Skane, Brunby, on *Fagus*, 29 September 1985, *Nordin* (H). **UK**. England, Windsor Great Park, X 2003, *HMAS 86598* (HMAS). ***G. lucidum*** **('*G. tsugae*')**. **China**. Jilin Province, Antu County, Changbaishan Nature Reserve, on fallen gymnosperm trunk, 6 September 1993, *Dai 1115*; on the stump of *Pinus*, 21 September 2002, *Dai 3935*; on the fallen trunk of *Larix*, 21 September 2002, *Dai 3937*, 12 December 2007, *Dai 9028*, 10 August 2011, *Yuan 5649*. ***G. multipileum***. **China**. Hainan Province, Haikou, on angiosperm stump, 21 May 2008, *Yuan 4146*; on the stump of *Acacia*, 23 May 2008, *Dai 9523 & 9524*; on the stump of *Hevea*, 4 June 2008, *Dai 10042*. ***G. sichuanense***. **China**. Guangdong Province, Guangzhou, South China Botanical Garden, on angiosperm stump, 19 September 2009, *Cui 7691*. Sichuan Province, Panzhihua, on angiosperm stump, 1976, *HMAS 42798* (holotype, HMAS). ***G. tropicum***. **China**. Guangxi Autonomous Region, Chongzuo County, Nonggang Nature Reserve, on dead angiosperm root, 7 July 2007, *Zhou 269*. Hainan Province, Baoting County, Tropical Botanical Garden, on angiosperm stump, 27 May 2008, *Dai 9724*. Yunnan Province, Xishuangbanna, Menglun Green Stone Forest Park, on angiosperm stump, 4 August 2005, *Dai 6721*; Menglun Tropical Botanical Garden, on living tree of *Cycas*, 10 September 2005, *Yuan 2300*; on the living angiosperm, 12 September 2007, *Yuan 3490*.

Acknowledgements We thank Drs. T.Z. Wei and X.C. Wang (HMAS, China), Prof. L. Ryvarden and Dr. K.-H. Larsson (O, Norway) for loans of specimens. We are grateful to Prof. S. P. Wasser (UOH, Israel) for offering some important literatures and to CBS for offering some *Ganoderma* cutltures. We are indebted to Dr. D.M. Wang (GIM, China) and Dr. J.M. Moncalvo (ROM, Canada) for offering suggestions during this study. We appreciate Dr. H.S. Yuan (IFP, China) for improving the drawings, and Dr. S.H. He (BJFC, China) for offering some *Ganoderma* images. Y.C. Dai and S.H. Wu are deeply indebted to Dr. T. Niemelä (H, Finland) for his friendly support of their studies at Botanical Museum of the University of Helsinki. We are grateful to Prof. K.D. Hyde (Chiang Rai, Thailand) for improving the manuscript. The research was financed by the National Natural Science Foundation of China (Project Nos. 30910103907, 31070022).

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