2015 Global diversity of the Ganoderma lucidum complex

Data · May 2015

CITATIONS
0

READS
1,510

7 authors, including:

Wei Li
Ocean University of China
53 PUBLICATIONS **357** CITATIONS
SEE PROFILE

Yun Cao
Nanjing Institute of Environmental Sciences
35 PUBLICATIONS **528** CITATIONS
SEE PROFILE

Sheng-Hua Wu
National Museum of Natural Science
162 PUBLICATIONS **2,429** CITATIONS
SEE PROFILE

Josef Vlasák
Biology Centre CAS
125 PUBLICATIONS **1,759** CITATIONS
SEE PROFILE

Phytochemistry 114 (2015) 7–15

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny

Li-Wei Zhou [a,1], Yun Cao [a,1], Sheng-Hua Wu [b], Josef Vlasák [c], De-Wei Li [d], Meng-Jie Li [a], Yu-Cheng Dai [a,*]

[a] State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, China
[b] Department of Botany, National Museum of Natural Science, Taichung 404, Taiwan, China
[c] Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic
[d] The Connecticut Agricultural Experiment Station Valley Laboratory, 153 Cook Hill Road, Windsor, CT 06095, USA

ARTICLE INFO

Article history:
Available online 25 October 2014

Keywords:
Taxonomy
Polypore
ITS
tef1α
rpb1
rpb2

ABSTRACT

Species of the *Ganoderma lucidum* complex are used in many types of health products. However, the taxonomy of this complex has long been chaotic, thus limiting its uses. In the present study, 32 collections of the complex from Asia, Europe and North America were analyzed from both morphological and molecular phylogenetic perspectives. The combined dataset, including an outgroup, comprised 33 ITS, 24 *tef1α*, 24 *rpb1* and 21 *rpb2* sequences, of which 19 ITS, 20 *tef1α*, 20 *rpb1* and 17 *rpb2* sequences were newly generated. A total of 13 species of the complex were recovered in the multilocus phylogeny. These 13 species were not strongly supported as a single monophyletic lineage, and were further grouped into three lineages that cannot be defined by their geographic distributions. Clade A comprised *Ganoderma curtisii*, *Ganoderma flexipes*, *Ganoderma lingzhi*, *Ganoderma multipileum*, *Ganoderma resinaceum*, *Ganoderma sessile*, *Ganoderma sichuanense* and *Ganoderma tropicum*, Clade B comprised *G. lucidum*, *Ganoderma oregonense* and *Ganoderma tsugae*, and Clade C comprised *Ganoderma boninense* and *Ganoderma zonatum*. A dichotomous key to the 13 species is provided, and their key morphological characters from context, pores, cuticle cells and basidiospores are presented in a table. The taxonomic positions of these species are briefly discussed. Noteworthy, the epitypification of *G. sichuanense* is rejected.

1. Introduction

Ganoderma P. Karst. 1881, erected by Karsten (1881), is an old polypore genus typified by *Ganoderma lucidum* (Curtis) P. Karst. 1881. The generic type was originally reported from the UK (Moncalvo and Ryvarden, 1997), and later was considered to have a worldwide distribution (Gilbertson and Ryvarden, 1986; Núñez and Ryvarden, 2000; Quanten, 1997; Ryvarden and Gilbertson, 1993; Ryvarden and Johansen, 1980). Several species morphologically similar to *G. lucidum* have also been described from all over the world; these include *Ganoderma multipileum* Ding Hou 1950 (Hou, 1950), *Ganoderma sichuanense* J.D. Zhao & X.Q. Zhang 1983 (Zhao et al., 1983) and *Ganoderma lingzhi* Sheng H. Wu et al. 2012 (Cao et al., 2012) from China, *Ganoderma resinaceum* Boud. 1889 (Patouillard, 1889) from Europe, and *Ganoderma oregonense* Murrill 1908, *Ganoderma sessile* Murrill 1902, *Ganoderma tsugae* Murrill 1902 and *Ganoderma zonatum* Murrill 1902 (Murrill, 1902, 1908) from the USA. These species are accepted as members of the *Glucidum. complex, which is mainly characterized within the genus by laccate pilei.*

Ganoderma has long been regarded as one of the most important medicinal fungi worldwide (Paterson, 2006), and laccate species of *Ganoderma*, i.e., *G. lucidum* complex, have been used as medicinal fungi in traditional Chinese medicine for over two millennia (Anon., 1955). Nowadays, many health products with *Ganoderma* as an ingredient are available, especially in East Asia and the USA for having supposedly anti-cancer, anti-aging, and anti-microbial/viral functions, among others (Paterson, 2006). However, the species concepts in the *G. lucidum* complex lack consensus in morphology, and taxonomy of this fungal species complex is thus problematic. This limits both further research on these fungi and their medical usefulness. For example, the widely used medicinal species in biochemical and pharmaceutical studies has been assumed to be *G. lucidum*, but evidence has emerged that this medicinal species is, in fact, a different species (Moncalvo et al., 1995) and was recently described as *G. lingzhi* (Cao et al., 2012).

The taxonomy of the *G. lucidum* complex has long been subject to debate, and diverse opinions have been voiced regarding the

* Corresponding author. Tel./fax: +86 24 83970348.
E-mail address: yuchengd@yahoo.com (Y.-C. Dai).
[1] The two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.phytochem.2014.09.023
0031-9422/

validity of its members. *G. sessile* was treated as a synonym of *G. resinaceum* by Haddow (1931), while Overholts (1953) considered that *G. lucidum* should be the true name of specimens classified as *G. sessile*. *G. lucidum* has also been considered with the prior name over its later synonym *G. tsugae* (Haddow, 1931; Steyaert, 1977). However, based on mating test evidence, Nobles (1965) pointed out that the specimens classified as *G. lucidum* in the USA actually represented *G. sessile*.

In East Africa, Ryvarden and Johansen (1980) parsimoniously treated all names of the *G. lucidum* complex as the "*G. lucidum* group", because of the lack of a morphological taxonomic solution to the problem of this complex.

With the aid of molecular phylogeny, Wang et al. (2009) divided Asian specimens classified as *G. lucidum* into two clades; both were separated from European *G. lucidum*. One clade, composed of tropical collections, represented *G. multipileum*, while the other clade was unknown (Wang et al., 2009). Wang et al. (2012) recognized the unknown clade as *G. sichuanense*. However, a parallel paper by Cao et al. (2012) found that the holotype of *G. sichuanense* was not conspecific with the unknown clade, and proposed the unknown clade as a new species *G. lingzhi*, which also is the most widely cultivated species in China. Meanwhile, the distribution of genuine *G. lucidum* in China was also confirmed (Cao et al., 2012; Yang and Feng, 2013).

After several decades of debate, the taxonomy of the *G. lucidum* complex remains problematic. Most previous studies focused on the species in a specific region or continent (Cao et al., 2012; Wang et al., 2012), or described a phylogeny with low resolution to certain clades (Moncalvo et al., 1995; Yang and Feng, 2013). Moreover, a robust phylogeny including species originally described from the USA is critically needed, because most of these species are old and never referred to in any phylogenetic analysis. In this study, the phylogeny of this complex, on the basis of four genetic loci, was examined with an inclusive taxon sampling covering species from Asia, Europe and North America to clarify the phylogenetic relationships within this complex.

2. Results and discussion

2.1. Molecular phylogeny

In this study, 32 collections of the *G. lucidum* complex from Asia, Europe and North America were critically examined with respect to morphology, and then investigated with molecular phylogenetic approaches.

A total of 76 sequences were generated for this study, comprising 19 ITS, 20 *tef1α*, 20 *rpb1* and 17 *rpb2* sequences (Table 1). The concatenated dataset resulted in an alignment with 3478 characters, of which 2631 are constant, 165 parsimony-uninformative and 682 parsimony-informative. The maximum likelihood (ML) searches stopped after 200 bootstrap (BS) replicates, while the maximum parsimony (MP) analysis generated eight equally most-parsimonious trees of 1407 steps (CI = 0.736, RI = 0.885). Because the ML and MP analyses produced nearly congruent topologies, only the topology derived from ML is presented, along with BS values from both analyses when simultaneously above 50%.

In the current phylogeny, based on a combination of four loci (Fig. 1), the 32 collections of the *G. lucidum* complex formed 13 highly supported terminal clades representing 13 species. These 13 species are *Ganoderma boninense* Pat. 1889, *Ganoderma curtisii*

Table 1
Information on species used in phylogenetic analysis.

Species	Voucher	Origin	GenBank accession numbers[a]			
			ITS	*tef1α*	*rpb1*	*rpb2*
G. boninense	WD 2028 (FFPRI)	Japan	**KJ143905**	**KJ143924**	**KJ143944**	**KJ143964**
G. boninense	WD 2085 (FFPRI)	Japan	**KJ143906**	**KJ143925**	**KJ143945**	**KJ143965**
G. curtisii	CBS 100131	NC, USA	JQ781848	**KJ143926**	**KJ143946**	**KJ143966**
G. curtisii	CBS 100132	NC, USA	JQ781849	**KJ143927**	**KJ143947**	**KJ143967**
G. flexipes	Wei 5491 (IFP)	Hainan, China	JQ781850	–	–	**KJ143968**
G. flexipes	Wei 5494 (IFP)	Hainan, China	JN383979	–	–	–
G. lingzhi	Cui 9166 (BJFC)	Shandong, China	**KJ143907**	JX029974	JX029982	JX029978
G. lingzhi	Dai 12479 (IFP)	Anhui, China	JQ781864	JX029975	JX029983	JX029979
G. lingzhi	Dai 12574 (IFP)	Liaoning, China	**KJ143908**	JX029977	JX029985	JX029981
G. lingzhi	Wu 1006-38 (Holotype, TNM)	Hubei, China	JQ781858	JX029976	JX029984	JX029980
G. lucidum	Rivoire 4195	France	**KJ143909**	–	**KJ143948**	KJ143969
G. lucidum	Cui 9207 (BJFC)	Yunnan, China	**KJ143910**	**KJ143928**	**KJ143949**	KJ143970
G. lucidum	K 175217	UK	**KJ143911**	**KJ143929**	**KJ143950**	KJ143971
G. lucidum	MT 26/10 (BRNM)	Czech Republic	**KJ143912**	**KJ143930**	**KJ143951**	–
G. multipileum	CWN 04670 (TNM)	Taiwan, China	**KJ143913**	**KJ143931**	**KJ143952**	KJ143972
G. multipileum	Dai 9447 (IFP)	Hainan, China	**KJ143914**	**KJ143932**	**KJ143953**	KJ143973
G. oregonense	CBS 265.88	OR, USA	JQ781875	**KJ143933**	**KJ143954**	KJ143974
G. oregonense	CBS 266.88	WA, USA	JQ781876	–	**KJ143955**	KJ143975
G. resinaceum	CBS 194.76	Netherlands	**KJ143916**	**KJ143934**	**KJ143956**	–
G. resinaceum	Rivoire 4150	France	**KJ143915**	–	**KJ143957**	–
G. sessile	LDW 20121017 (IFP)	CT, USA	**KJ143917**	**KJ143935**	–	–
G. sessile	JV 1209/9	AZ, USA	**KF605629**	**KJ143936**	**KJ143958**	–
G. sessile	JV 1209/27	AZ, USA	**KF605630**	**KJ143937**	**KJ143959**	KJ143976
G. sessile	NY 00985711	NY, USA	**KJ143918**	–	–	–
G. sichuanense	Cui 7691 (BJFC)	Guangdong, China	JQ781878	–	–	–
G. sichuanense	HMAS 42798 (Holotype)	Sichuan, China	JQ781877	–	–	–
G. tropicum	Dai 9724 (IFP)	Hainan, China	JQ781879	–	–	–
G. tropicum	Yuan 3490 (IFP)	Yunnan, China	JQ781880	**KJ143938**	–	–
G. tsugae	Dai 12751b (BJFC)	CT, USA	**KJ143919**	**KJ143939**	**KJ143960**	KJ143977
G. tsugae	Dai 12760 (BJFC)	CT, USA	**KJ143920**	**KJ143940**	**KJ143961**	KJ143978
G. zonatum	FL-02 (TNM)	FL, USA	**KJ143921**	**KJ143941**	**KJ143962**	KJ143979
G. zonatum	FL-03 (TNM)	FL, USA	**KJ143922**	**KJ143942**	–	KJ143980
Outgroup						
Tomophagus colossus	TC-02 (TNM)	Vietnam	**KJ143923**	**KJ143943**	**KJ143963**	–

[a] The accession numbers in bold-face indicate the newly generated sequences for this study.

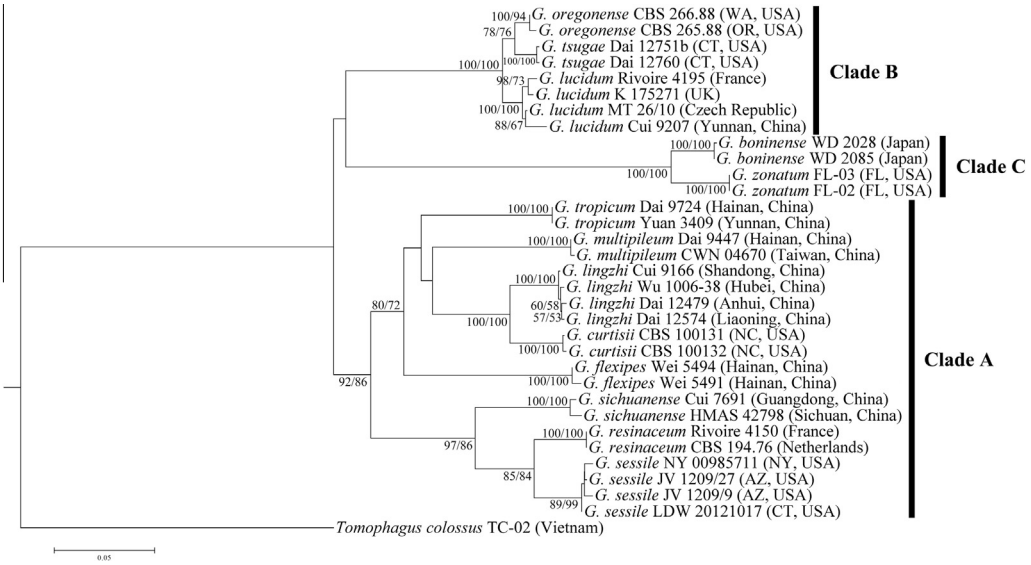

Fig. 1. Phylogeny of the *G. lucidum* complex based on the data from a combination of ITS, *tef1α*, *rpb1* and *rpb2*. Topology is derived from the maximum likelihood analysis, while bootstrap values were obtained from maximum likelihood (before slash) and maximum parsimony analysis (after slash) when both were above 50%.

(Berk.) Murrill 1908, *Ganoderma flexipes* Pat. 1907, *G. lingzhi*, *G. lucidum*, *G. multipileum*, *G. oregonense*, *G. resinaceum*, *G. sessile*, *G. sichuanense*, *Ganoderma tropicum* (Jungh.) Bres. 1910, *G. tsugae* and *G. zonatum*. Their representatives are mostly collected around type localities, if not type specimens. A dichotomous key to the 13 species is provided below, and their morphological characters are presented in Table 2. The photographs of their basidiomes can be seen in Figs. 2–14. The monophyly of these 13 species in a single lineage did not receive reliable statistical support (BS values from ML and MP analyses below 50%), which is similar to the ITS-based phylogeny presented by Cao et al. (2012). In Wang et al. (2012) and Yang and Feng (2013), species of this complex were well resolved based on ITS, IGS and *rpb2* sequences; however taxon sampling in these two studies was small in size and biased due to the lack of species from North America.

In the presented phylogeny, the 13 species of the *G. lucidum* complex were clustered into three clades, each with high support and not coinciding with their geographic distributions (Fig. 1). Therefore, evolutionary histories for the species in this complex have not been simply constraint with only morphology or biogeographic distribution, and the hypothesis of a single origin of laccate species within *Ganoderma* is not supported. More data from other laccate and non-laccate species are needed to settle the position of the *G. lucidum* complex within *Ganoderma*.

2.2. Species in Clade A

Three species from China, *G. lingzhi*, *G. multipileum* and *G. sichuanense*, were clustered together in Clade A. They had all been misidentified as *G. lucidum* previously. Wang et al. (2009) proposed that the so-called *G. lucidum* collections from tropical China should be classified as *G. multipileum*. Cao et al. (2012) further stated that *G. multipileum* is also found in other tropical Asian countries, such as India and the Philippines.

The validity of *G. lingzhi* and *G. sichuanense* has been hotly debated. Cao et al. (2012) obtained the ITS sequence from the holotype of *G. sichuanense* and found that it was different from the most widely cultivated *Ganoderma* species, Ling-Zhi, in China. The latter was described as a new species *G. lingzhi* by Cao et al. (2012). Wang et al. (2012), however, failed to amplify any sequence from the holotype of *G. sichuanense*. Instead, they treated specimens recently collected from the type province as representatives of *G. sichuanense*, based on morphological characters, and considered these collections conspecific with the most widely cultivated *Ganoderma* species (Wang et al., 2012). Later, Yao et al. (2013) selected a specimen recently collected from the type province of *G. sichuanense* as an epitype and treated *G. lingzhi* as a synonym of *G. sichuanense*. This epitypification can be considered redundant as the holotype is fertile and its ITS sequence is available; i.e., the holotype is "not demonstrably ambiguous" as required by the Code to merit any epitypification. Furthermore, the ITS sequence of the proposed epitype was different from that of the holotype (Yao et al., 2013), which suggests that the holotype and a paratype could have been switched during loans, and that explanation for their results does not appear to have been tested. Geographically, the locality of the proposed epitype, Huangqiao Village, Miyi County, Panzhihua City, Sichuan Province, at an altitude of 1933 m (Yao et al., 2013) is about 80 km away from that of the holotype, Panzhihua Iron and Steel (Group) Company Limited, Dukou City (former name of Panzhihua City), Sichuan Province, at an altitude of 985 m, so the two collections grow in different ecological environments. Because of widespread cultivation of *G. lingzhi*, the new collections from the type province of *G. sichuanense* are very likely *G. lingzhi* rather than *G. sichuanense*. Given the above, we reject the epitype of *G. sichuanense* by Yao et al. (2013), and consider both *G. lingzhi* and *G. sichuanense* to be independent and taxonomically valid species.

The other two Asian species recovered in Clade A were *G. flexipes* and *G. tropicum*, originally from Vietnam and Indonesia, respectively. Evidence has been presented in previous phylogenetic studies that these are independent species (Cao et al., 2012; Wang et al., 2012), widely found across subtropical and tropical Asia (Moncalvo and Ryvarden, 1997). Cao et al. (2012) proposed that *Ganoderma atrum* J.D. Zhao et al. 1979, *Ganoderma calidophilum* J.D. Zhao et al. 1979, *Ganoderma hainanense* J.D. Zhao et al. 1979 and *Ganoderma parviungulatum* J.D. Zhao & X.Q. Zhang 1986 all described from Hainan Province, China by Prof. Ji-Ding Zhao and his colleagues were synonyms of *G. flexipes*, although the data to support this were not presented.

The other three species in Clade A were *G. curtisii*, *G. resinaceum* and *G. sessile*. The ITS-based phylogeny presented by Cao et al. (2012) clustered the former two species together with the five Asian species mentioned above, but with weak support. The current phylogeny based on four loci confirmed the result of Cao et al. (2012) with high statistical support (Fig. 1).

Table 2
Morphological characters of the species of the *G. lucidum* complex.

Species	Context[a]			Pore[b]		Cuticle cell[c]	Basidiospore	
	Color	Concentric growth zones	Melanoid bands in mature fruiting body	Color (at maturity)	Size (per mm)	Shape	Shape	Size (μm)
G. boninense	Nearly homogeneous, dark clay-buff	Absent	Usually present	Straw-yellow	4–5	Often irregular, clavate or cylindrical, often with blunt outgrowths or protuberances	Oblong-ellipsoid to ellipsoid, finely echinulate	(8–)8.7–12.8(–13.5) × (4.2–)4.7–6(–6.3)
G. curtisii	Not completely homogeneous, light buff to clay-buff	Absent	Present	Yellowish	4–6	Mostly regular, clavate	Ellipsoid, moderately coarsely echinulate	(9–)9.2–11(–11.2) × (5.8–)6–6.8(–7)
G. flexipes	Not completely homogeneous, yellow brown to dark brown	Absent	Present	White to pale sulfur yellow	4–6	Mostly regular, clavate	Ellipsoid, moderately coarsely echinulate	(8.5–)9–10.3(–11) × (5–)5.3–7
G. lingzhi	Not completely homogeneous, light buff, buff, clay-buff to snuff-buff	Absent	Present	Pale yellow, sulfur yellow to straw-colored	5–6	Mostly regular, clavate	Ellipsoid, moderately coarsely echinulate, sometimes with short ridges	(8–)9–10.7(–12) × (5.2–)5.8–7(–7.5)
G. lucidum	Not completely homogeneous, cream or pinkish buff to clay buff	Occasionally present	Absent	White	4–5	Mostly regular, clavate	Ellipsoid to broadly pear-shaped, coarsely echinulate, but more commonly with sinuous ridges	(8.8–)9.7–12.2(–13.2) × (6–)6.3–8(–8.5)
G. multipileum	Not completely homogeneous, clay-buff to fulvous	Present	Present	Cream to straw-colored	4–6	Mostly regular, clavate	Ellipsoid, finely and thickly echinulate	(8–)8.8–10.5(–11.3) × (5–)5.5–7(–7.2)
G. oregonense	Not completely homogeneous, cream to pinkish-buff	Absent	Absent	White	3–4	Mostly regular, clavate	Ellipsoid, coarsely echinulate	11–12.5(–12.8) × (6.8–)7–8
G. resinaceum	Not completely homogeneous, straw-yellow or buff to clay buff	Present	Absent	White	3–4	Mostly regular, clavate	Ellipsoid to ovoid, finely to moderately coarsely echinulate	(8.8–)9–11.7(–12) × (6–)6–7.5(–8)
G. sessile	Not completely homogeneous, pinkish-buff to clay-buff	Mostly present	Sometimes present	Straw-yellow	4–6	Mostly regular, clavate	Ellipsoid, slightly to moderate-coarsely echinulate	(9–)9.2–12(–12.5) × (5.3–)6–7.8(–8.2)
G. sichuanense	Not completely homogeneous, buff to pale clay-buff	Absent	Present	Buff yellow	5–6	Mostly regular, clavate	Ovoid to ellipsoid, finely to moderate-coarsely echinulate	(7–)7.5–9.2(–9.3) × (4.5–)5–6.5(–6.8)
G. tropicum	Homogeneous, fulvous	Present	Present	Cream to pale straw-colored	4–6	Mostly irregular, clavate, often with blunt outgrowths or protuberances	Ellipsoid to broadly ellipsoid, coarsely echinulate	(8.3–)8.8–10.7(–11.2) × (5–)5.5–6.3(–6.8)
G. tsugae	Not completely homogeneous, white to pinkish-buff or clay-buff	Absent	Absent	White	4–5	Mostly regular, clavate	Ellipsoid, moderately coarsely echinulate	(8.8–)9–10.8(–11) × (5.3–)5.8–6.8(–7)
G. zonatum	Nearly completely homogeneous, umber	Present	Absent	Cream	4–5	Mostly irregular, clavate or cylindrical, with protuberances or slight branches	Oblong-ellipsoid, finely echinulate	(9.8–)10–12(–12.8) × (5–)5.3–6.3(–6.7)

[a] Means the sterile part of fruiting body between tubes and the pileus surface.
[b] Means the mouth of a tube that bears fertile tissue of fruiting body.
[c] Means the tissue of the outer layer in the pileus of fruiting body.

Fig. 2. Basidiome of *G. boninense* in situ.

Fig. 3. Basidiomes of *G. curtisii* (specimen JV 1209/13) in situ.

Fig. 4. Basidiome of *G. flexipes* (specimen Dai 12018 in IFP) in situ.

Fig. 5. Basidiomes of *G. lingzhi* (specimen Dai 8172 in IFP) in situ.

Fig. 6. Basidiomes of *G. lucidum* (specimen Dai 2272 in IFP) in situ.

Fig. 7. Basidiome of *G. multipileum* (specimen Dai 9521 in IFP) in situ.

G. sessile was described from New York, USA by Murrill, but was later treated as synonyms of the European species *G. lucidum* and *G. resinaceum* (Haddow, 1931; Overholts, 1953). Nobles (1965) considered that the American specimens classified as *G. lucidum* were actually *G. sessile*. The phylogeny here confirmed that the specimens of *G. sessile* from the type locality and other USA states were different from European *G. lucidum* and *G. resinaceum*.

2.3. Species in Clade B

G. lucidum, the generic type, and two species of North American origin, *G. oregonense* and *G. tsugae*, were clustered together as Clade B.

Fig. 8. Basidiome of *G. oregonense* (specimen JV 0709/51) in situ.

Fig. 9. Basidiomes of *G. resinaceum* (specimen JV 8208/23) in situ.

Fig. 10. Basidiomes of *G. sessile* (specimen JV 1209/27) in situ.

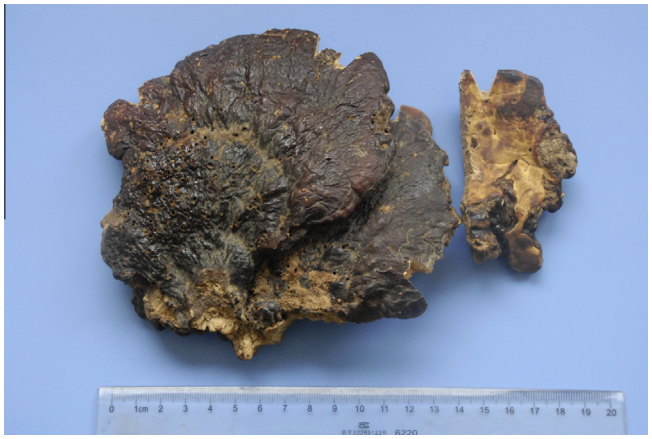

Fig. 11. Basidiomes of *G. sichuanense* (specimen HMAS 42798, holotype).

Fig. 12. Basidiomes of *G. tropicum* (specimen Dai 6721 in IFP) in situ.

Fig. 13. Basidiomes of *G. tsugae* (specimen Dai 12760 in BJFC) in situ.

Cao et al. (2012) considered *G. lucidum* and *G. oregonense* as two separate lineages, but without strong support. The four locus-based phylogeny here strongly supported each species as an independent lineage (Fig. 1).

Cao et al. (2012) and Wang et al. (2012) confirmed that the genuine *G. lucidum* had a distribution in northern China, and then Yang and Feng (2013) extended its range to southwestern China. The current phylogeny also suggested a specimen (Cui 9207) from southwestern China as *G. lucidum*.

Fig. 14. Basidiomes of *G. zonatum* (specimen JV 0904/116) in situ.

An ITS-based phylogeny indicated that *G. oregonense* and *Ganoderma carnosum* Pat. 1889, a species with a European origin, may be conspecific in topology (unpublished data). *G. carnosum* has priority over *G. oregonense*; however, we could not treat the latter as a synonym of the former until reliable phylogeny is available.

G. tsugae has been treated as a synonym of *G. lucidum* (Haddow, 1931; Steyaert, 1977). Again, the phylogeny here supported *G. tsugae* as an independent species distinct from *G. lucidum*. *G. tsugae* grows exclusively on conifers, especially on *Tsuga* and *Abies*, while *G. lucidum* inhabits mostly angiosperm trees.

2.4. Species in Clade C

Clade C comprised two subtropical species *G. boninense* and *G. zonatum*. Of these, Moncalvo et al. (1995) proposed that *G. boninense* might be the correct name of the American specimens labeled as *G. lucidum*. The phylogeny herein, however, clearly distinguished *G. boninense*, this being represented by collections from the type locality, i.e., the Bonin Islands, from *G. sessile* and *G. tsugae*, both of which have been wrongly considered to be the American *G. lucidum* (Haddow, 1931; Overholts, 1953; Steyaert, 1977).

Gottlieb et al. (2000) provided evidence that *G. sessile* and *G. zonatum* were conspecific, based on ITS phylogeny, and considered their differences in morphology to be a result of divergent evolution. However, the ITS sequences used by Gottlieb et al. (2000) were from Argentinean specimens, which may have been misidentified. In the phylogeny used here, *G. sessile* and *G. zonatum* were represented by specimens from their type localities, New York and Florida, USA, respectively, and were clearly shown to be two independent species (Fig. 1). With respect to their ecology, *G. sessile* is a temperate species on hardwoods (dicots), while *G. zonatum* grows on subtropical palms (monocots).

A dichotomous key to 13 species in the *G. lucidum* complex

1.	Grown mostly on gymnosperms	2
1.	Grown mostly on angiosperms	3
2.	Basidiospores larger, 11–12.5 × 7–8 μm	*G. oregonense*
2.	Basidiospores smaller, 9–10.8 × 5.8–6.8 μm	*G. tsugae*
3.	Distributed in America	4
3.	Distributed in Asia and/or Europe	6
4.	Basidiospores-oblong ellipsoid, finely echinulate	*G. zonatum*
4.	Basidiospores ellipsoid, moderately coarsely echinulate	5
5.	Pileal surface yellowish brown, concentric growth zones absent in context	*G. curtisii*
5.	Pileal surface reddish brown, concentric growth zones present in context	*G. sessile*
6.	Cuticle cells irregular, mostly with outgrowths or protuberances	7
6.	Cuticle cells regular, occasionally with outgrowths or protuberances	8
7.	Basidiospores ellipsoid to broadly ellipsoid, coarsely echinulate	*G. tropicum*
7.	Basidiospores oblong-ellipsoid to ellipsoid, finely echinulate	*G. boninense*
8.	Basidiospores mostly with distinct sinuous ridges	*G. lucidum*
8.	Basidiospores rarely with distinct sinuous ridges	9
9.	Distributed in tropical regions	10
9.	Distributed in temperate regions	11
10.	Basidiome with a long, slender stipe, concentric growth zones absent in context	*G. flexipes*
10.	Basidiome sessile or with a short stipe, concentric growth zones present in context	*G. multipileum*
11.	Pore surface white when mature, concentric growth zones present in context	*G. resinaceum*
11.	Pore surface buff yellow, sulfur yellow or straw-colored when mature, concentric growth zones absent in context	12
12.	Basidiome sessile, basidiospores mostly ovoid, cuticle cells inflated, loosely-arranged	*G. sichuanense*
12.	Basidiome stipitate, basidiospores ellipsoid, cuticle cells constrictive, tightly-arranged	*G. lingzhi*

3. Conclusions

The study here considers 32 collections belonging to the *G. lucidum* complex from Asia, Europe and North America, in terms of their morphology and phylogeny as derived from analysis of four loci (ITS, *tef1α*, *rpb1* and *rpb2*). Of these 13 well-delineated species, *G. boninense*, *G. curtisii*, *G. flexipes*, *G. lingzhi*, *G. lucidum*, *G. multipileum*, *G. oregonense*, *G. resinaceum*, *G. sessile*, *G. sichuanense*, *G. tropicum*, *G. tsugae* and *G. zonatum*, were recognized. All 13 species are morphologically similar but, with respect to phylogeny, all form at least three lineages that were not mirrored in their geographic distributions.

4. Experimental

4.1. Studied collections

The studied specimens and cultures studied are deposited in the collections of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP), the Institute of Microbiology, Beijing Forestry University (BJFC), the New York Botanical Garden (NY), the Royal Botanical Garden, Kew (K), the Forest Products Research Institute (FFPRI), the CBS-KNAW Fungal Biodiversity Centre (CBS) and the Moravian Museum in Brno (BRNM), as well as in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS), the Herbarium of the National Museum of Natural Science (TNM), the private collection of Bernard Rivoire (Rivoire) and the private herbarium of Josef Vlasák (JV). The collections studied in this study from Rivoire and JV are also deposited in IFP.

4.2. Morphology

The microscopic procedure follows Cao et al. (2012). Specimen sections prepared in Cotton Blue were examined at magnification up to 1000× under a Nikon Eclipse 80i microscope and phase contrast illumination. The size of basidiospores was measured with the inclusion of exospore and the exclusion of the turgid vesicular appendix. In presenting the basidiospore size variation, 5% of the

measurements were excluded from each end of the range, and are given in parentheses. Special color terms follow Anon. (1969) and Petersen (1996).

4.3. Molecular sequencing

The molecular sequencing procedure follows Cao et al. (2012). The sequences of ITS, *tef1α*, *rpb1* and *rpb2* from the *G. lucidum* complex were amplified and sequenced for phylogenetic analysis. The primer pairs ITS5/ITS4 (White et al., 1990) and ITS1F/ITS4B (Gardes and Bruns, 1993) were usually used to amplify the ITS region. To avoid possible disturbance from internal inhabitants in dried specimens, the primer pairs G-ITS-F1/G-ITS-R2 and G-ITS-F1/ITS4B (Cao et al., 2012) were also adopted in this study. The *tef1α*, *rpb1* and *rpb2* gene fragments were amplified using the primer pairs EF1-983F/EF1-2218R (Rehner and Buckley, 2005), RPB1-2.2f/RPB1-Cr (Binder et al., 2010; Matheny et al., 2002) and fRPB2-5F/bRPB2-7R2 (Liu et al., 1999; Matheny et al., 2007), respectively. Total genomic DNA was extracted from dried specimens or living cultures using the Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland). The obtained genomic DNA was diluted up to 10 times, if necessary. PCR was performed in a reaction mixture containing 25 μl of 2× Phire® Plant PCR buffer, 1 μl of Phire® Hot Start II DNA Polymerase, 5 μl of each PCR primer (10 μM), and 2–5 μl of diluted genomic DNA. The total volume was adjusted to 50 μl with sterile deionized H_2O. The PCR procedure was as follow: 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. The annealing temperatures for various primer pairs were as follows: 59 °C (ITS5/ITS4), 60 °C (ITS1F/ITS4B), 66.5 °C (G-ITS-F1/G-ITS-R2), 69.5 °C (G-ITS-F1/ITS4B), 59 °C (EF1-983F/EF1-2218R), 50 °C (RPB1-2.2f/RPB1-Cr) and 55 °C (fRPB2-5F/bRPB2-7R2). The successful PCR products were purified and sequenced in Beijing Genomics Institute, China with the primers used in Cao et al. (2012).

The newly generated sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>; Table 1).

4.4. Phylogenetic analysis

Besides the newly generated sequences, other related sequences were obtained from GenBank and included in the subsequent phylogenetic analysis (Table 1). *Tomophagus colossus* (Fr.) Murrill 1905 was selected as an outgroup (Cao et al., 2012). Four loci were combined and then aligned using MAFFT 7.110 (Katoh and Toh, 2008; Katoh et al., 2002) with the Q-INI-I option. The combinability of these four loci was evaluated by an incongruence length difference test (Farris et al., 1994) implemented in PAUP* 4.0b10 (Swofford, 2002). This test, using a heuristic search and 1000 homogeneity replicates, gave a *P* value of 1.000, i.e., much greater than 0.01; this means there is no discrepancy among the four loci in reconstructing phylogenetic trees.

raxmlGUI 1.2 (Silvestro and Michalak, 2012; Stamatakis, 2006) with GTR+I+G model and the auto FC option (Pattengale et al., 2010) for BS replicates was used to conduct ML analysis. MP analysis was performed using PAUP* 4.0b10 (Swofford, 2002) with heuristic searches and 1000 BS replicates, and with all characters equally weighted, gaps treated as missing, the starting tree obtained via stepwise addition, tree-bisection-reconnection branch swapping, the steepest descent option not in effect, and the MULTREES option selected.

Acknowledgments

Dr. Tsutomu Hattori (FFPRI, Japan) is thanked for providing the photograph of *G. boninense* in situ. The curators of BJFC, NY, K, FFPRI, CBS, BRNM and HMAS, and Mr. Bernard Rivoire (Lyon, France) are thanked for loans of *Ganoderma* specimens and cultures. The research was financed by National Natural Science Foundation of China (Project No. 30910103907).

References

Anon., 1955. Shen Nong materia medica, 102–200 A.D (E. Han). Reprinted. People's Hygiene Press, Beijing.
Anon., on. 1969. Flora of British fungi. Colour identification chart. Her Majesty's Stationery Office, London.
Binder, M., Larsson, K.H., Matheny, P.B., Hibbett, D.S., 2010. Amylocorticiales ord. nov. and Jaapiales ord. nov.: early-diverging clades of Agaricomycetidae were dominated by corticioid forms. Mycologia 102, 865–880.
Cao, Y., Wu, S.H., Dai, Y.C., 2012. Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". Fungal Divers. 56, 49–62.
Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.
Gilbertson, R.L., Ryvarden, L., 1986. North American Polypores 1. Fungiflora, Oslo.
Gottlieb, A.M., Ferrer, E., Wright, J.E., 2000. RDNA analyses as an aid to the taxonomy of species of *Ganoderma*. Mycol. Res. 104, 1033–1045.
Haddow, W.R., 1931. Studies in *Ganoderma*. J. Arnold Arbor. 12, 25–46.
Hou, D., 1950. A new species of *Ganoderma* from Taiwan. Quat. J. Taiwan Mus. 3, 101–105.
Karsten, P.A., 1881. Enumeralio boletinearum et polyporearum fennicarum, systemate novo dispositarum. Rev. Mycol. 3, 16–19.
Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinf. 9, 286–298.
Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.
Liu, Y.L., Whelen, S., Hall, B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. 16, 1799–1808.
Matheny, P.B., Liu, Y.J., Ammirati, J.F., Hall, B.D., 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). Am. J. Bot. 89, 688–698.
Matheny, P.B., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Nilsson, R.H., Hughes, K.W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., Langer, E., Langer, G., McLaughlin, D.J., Wilson, A.W., Frøslev, T., Ge, Z.W., Kerrigan, R.W., Slot, J.C., Yang, Z.L., Baroni, T.J., Fischer, M., Hosaka, K., Matsuura, K., Seidl, M.T., Vauras, J., Hibbett, D.S., 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol. Phylogenet. Evol. 43, 430–451.
Moncalvo, J.M., Ryvarden, L., 1997. A nomenclatural study of the Ganodermataceae Donk. Synop. Fung. 11, 1–114.
Moncalvo, J.M., Wang, H.H., Hseu, R.S., 1995. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. Mycol. Res. 99, 1489–1499.
Murrill, W.A., 1902. The Polyporaceae of North America. I. The genus *Ganoderma*. Bull. Torrey Bot. Club 29, 599–608.
Murrill, W.A., 1908. Polyporaceae (*pars*). N. Am. Flora 9, 73–131.
Nobles, M.K., 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Can. J. Bot. 43, 1097–1139.
Núñez, M., Ryvarden, L., 2000. East Asian polypores 1. Ganodermataceae and Hymenochaetaceae. Synop. Fung. 13, 1–168.
Overholts, L.O., 1953. The Polyporaceae of the United States, Alaska and Canada. University of Michigan Press, Ann Arbor.
Paterson, R.R.M., 2006. *Ganoderma* – a therapeutic fungal biofactory. Phytochemistry 67, 1985–2001.
Patouillard, N.T., 1889. Le genre *Ganoderma*. Bull. Soc. Mycol. France 5, 64–80.
Pattengale, N.D., Alipour, M., Bininda-Emonds, O.R.P., Moret, B.M.E., Stamatakis, A., 2010. How many bootstrap replicates are necessary? J. Comput. Biol. 17, 337–354.
Petersen, J.H., 1996. Farvekort. The Danish Mycological Society's Colour-Chart. Foreningen til Svampekundskabens Fremme, Greve.
Quanten, E., 1997. The polypores (Polyporaceae s.l.) of Papua New Guinea. Opera Bot. Belgica 11, 1–352.
Rehner, S.A., Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-*α* sequences: evidence for cryptic diversification and links to *Cordyceps teleomorphs*. Mycologia 97, 84–98.
Ryvarden, L., Gilbertson, R.L., 1993. European polypores 1. Synop. Fung. 6, 1–387.
Ryvarden, L., Johansen, I., 1980. A Preliminary Polypore Flora of East Africa. Fungiflora, Oslo.
Silvestro, D., Michalak, I., 2012. RaxmlGUI: a graphical front end for RAxML. Org. Divers. Evol. 12, 335–337.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Steyaert, R.L., 1977. Basidiospores of two *Ganoderma* species and others of two related genera under the scanning electron microscope. Kew Bull. 31, 437–442.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods). Sinauer, Sunderland.
Wang, D.M., Wu, S.H., Su, C.H., Peng, J.T., Shih, Y.H., Chen, L.C., 2009. *Ganoderma multipileum*, the correct name for '*G. lucidum*' in tropical Asia. Bot. Stud. 50, 451–458.
Wang, X.C., Xi, R.J., Li, Y., Wang, D.M., Yao, Y.J., 2012. The species identity of the widely cultivated *Ganoderma*, '*G. lucidum*' (Ling-zhi), in China. PLoS ONE 7, e40857.
White, T.J., Bruns, T.D., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols, A Guide to Methods and Applications. Academic Press, San Diego, pp. 315–322.
Yang, Z.L., Feng, B., 2013. What is the Chinese "Lingzhi"? – a taxonomic mini-review. Mycology 4, 1–4.
Yao, Y.J., Wang, X.C., Wang, B., 2013. Epitypification of *Ganoderma sichuanense* J.D. Zhao & X.Q. Zhang (Ganodermataceae). Taxon 62, 1025–1031.
Zhao, J.D., Xu, L.W., Zhang, X.Q., 1983. Taxonomic studies on the family Ganodermataceae of China II. Acta Mycol. Sin. 2, 159–167.