Phyton-International Journal of Experimental Botany
DOI:10.32604/phyton.2020.09690
Article

Morphology, Phylogeny and Culture Characteristics of *Ganoderma gibbosum* Collected from Kunming, Yunnan Province, China

Thatsanee Luangharn[1,2,3,4], Samantha C. Karunarathna[1,3], Peter E. Mortimer[1,4,*], Kevin D. Hyde[3,5] and Jianchu Xu[1,3,4,*]

[1]Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China
[2]University of Chinese Academy of Sciences, Beijing, 100049, China
[3]East and Central Asia Regional Office, World Agroforestry Centre (ICRAF), Kunming, 650201, China
[4]Centre for Mountain Futures, Kunming Institute of Botany, Kunming, 650201, China
[5]Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand
*Corresponding Authors: Peter E. Mortimer. Email: peter@mail.kib.ac.cn; Jianchu Xu. Email: jxu@mail.kib.ac.cn
Received: 15 January 2020; Accepted: 04 March 2020

Abstract: *Ganoderma* is a genus of medicinally and economically important mushrooms in the family Ganodermataceae. *Ganoderma* species are popular medicinal mushrooms and their health benefits are well-documented. *Ganoderma* is a cosmopolitan genus that is widely distributed in both tropical and temperate regions. This genus is characterized by its unique laccate or non-laccate species with double-walled basidiospores. Here, we report on eight collections of *G. gibbosum* collected during surveys in Kunming, Yunnan Province, China. The specimens are described and illustrated based on macro- and micro-morphological characteristics. Total DNA of the eight *G. gibbosum* strains were extracted using the Biospin Fungal Extraction Kit following manufacturer protocol. Amplification of the Internal Transcribed Spacer (nrITS) region was carried out using ITS5/ITS4 primers and LROR/LR5 for the nuclear ribosomal large subunit 28S rDNA gene (LSU). Phylogenetic analysis with closely related species to *G. gibbosum* showed that all eight collections grouped with *G. gibbosum* with 100% bootstrap support. Phylogenetic similarity and morphological variations within the eight collections of *G. gibbosum* are discussed.

Keywords: Chinese medicine; medicinal mushroom; morphology; phylogeny

1 Introduction

Ganoderma P. Karst. was typified by Karsten in 1881 [1], with *Ganoderma lucidum* (Curtis) P. Karst as the type species [1,2]. This fungus was introduced by Curtis in 1781 [3] based on material from England [4]. Recently, the name of *G. lingzhi* (Lingzhi) has begun to be used instead of *G. lucidum* for those native to East Asia, and the true *G. lucidum* has been determined to be native to Europe [5]. The various species of *Ganoderma* are widely distributed in both tropical and temperate areas [6]. They grow saprophytically or parasitically and form a widespread group of white rot fungi on a wide variety of trees, fulfilling a vital ecological function as wood decomposers [7,8]. *Ganoderma* fungi are able to form conspicuous laccate

or non-laccate basidiocarps, bracket-like sporocarps with double walled basidiospores [9,10], while *G. gibbosum* forms non-laccate basidiocarps. *Ganoderma* has a long history of use in traditional medicine in East Asia. *Ganoderma* fungi are used to treat and remedy a wide range of pathological diseases [11,12]. *Ganoderma* are also used as ingredients in cosmetics [13], and are unique for their pharmaceutical properties and nutritional value [14,15].

Ganoderma species are genetically heterogeneous, and a wide range of genetic variation has resulted from outcrossing over multiple generations and between different geographical origins [16]. Different *Ganoderma* taxonomic characteristics have been identified by various authors [17,18,19], and fungal identifications were later carried out according to taxonomic keys [20]. Index Fungorum listed 455 *Ganoderma* records (http:/www.indexfungorum.org; accessed date: 10 January, 2020) and 400 records of taxa in MycoBank (http://www.mycobank.org; accessed date: 10 January, 2020), while 80 species have been reported as synonyms [21].

Ganoderma species are found worldwide, displaying significant variation in the morphological features of each species. Specimens of *Ganoderma* have been recorded across Africa [22,23], Australia [9,24], Europe [25,26], North America [27], South Africa [28], and Asia [18,19], including China [29]. *Ganoderma gibbosum* is not the only fungal species shown to be different from any known taxon within China. *Ganoderma hainanense* [30], *G. japonicum* [31], *G. leucocontextum* [32], *G. lucidum* [33], and *G. mutabile* [6] have also been proposed.

Southwest China contains some of the highest concentrations of fungal biodiversity in the world, and Yunnan Province in particular has the varied topography, environmental conditions, and variety of habitats for a diverse range of fungi. An estimated 853 macrofungi species belonging to 172 genera worldwide in Yunnan [34], and different taxa within the genus *Ganoderma* have been discovered [35]. Although there have been numerous collections of *Ganoderma* specimens from Yunnan Province, including *G. lucidum* [36], *G. tropicum* [33], *G. lingzhi* [32,36], and *G. applanatum* [8], there have been no reports detailing the macro–micro morphological characteristics of *G. gibbosum* from Yunnan to date. Previous Chinese specimens of *G. gibbosum* were collected in Guizhou, Hainan, and Jiangxi provinces [37]. Furthermore, past studies of *G. gibbosum* have focused on the phylogenetic features of this species and have not provided detail on macro- and micro-morphological data [8,9]. Thus, we aim to provide a more conclusive description of cosmopolitan *G. gibbosum* by analyzing various specimens from Yunnan Province, providing detailed macro- and micro-morphological descriptions along with phylogenetic analyses.

2 Materials and Methods

2.1 Sample Collection and Isolation of Mycelial Cultures

Eight specimens of *G. gibbosum* were collected from Yunnan Province, China from August to December in 2016 and 2017. Documentation based on macro-morphological characteristics such as color, host, fruiting growing stages (young, mature, and old), and perennial strains were noted in the field. The specimens were hot air dried at 40°C for 48 hours until they were completely dehydrated, and then were kept in ziplock plastic bags containing dehydrated silica gel as a desiccant to control humidity. All dried herbarium specimens were described based on macro–micro morphological characteristics.

Pure cultures of all *G. gibbosum* were isolated under aseptic conditions (Stamets 2000) by transferring sections of internal tissues from basidiocarps onto potato dextrose agar (PDA), and incubated at 30°C for 10 days [38]. After the agar surface was fully covered with the fungal mycelium, the stock pure culture was deposited in the culture collection of the Kunming Institute of Botany culture collection (KUMCC). The cultures were maintained at 4°C for further study.

2.2 Macro–Micro Morphological Examination

Macro-morphological characteristics were described based on fresh material which was photographed in the field prior to collection. Morphological descriptions mainly followed the methods described previously by Lodge et al. [39], while colors were recorded following Ridgeway [40]. Macroscopic characteristics were determined according to the methodology described by Largent [41]. Microscopic characteristics were observed using free-hand sections; basidiospores were rehydrated and observed by mounting in a solution of 3%–5% potassium hydroxide (KOH), a drop of 1%–3% Congo red, and 3%–5% Melzer's reagent for highlighting all tissues [42]. Microphotography was carried out with magnification of up to 100x using a Nikon ECLIPSE Ni (Nikon, Tokyo, Japan) compound microscope, and specimens were photographed with a Canon EOS 600D (Tokyo, Japan) digital camera fitted to the microscope. Measurements were taken using the Tarosoft® Image Framework program v.0.9.0.7. Measurement of the size and shape of basidiospores followed [$Q = L/W$], indicating Q variation in quotient of spore length and width of individual spores; L = mean length; W = mean width was calculated considering the mean value of the lengths and widths, with a minimum of 50 basidiospores from each basidioma [43]. Fungal identification was performed according to taxonomic keys [20].

2.3 DNA Extraction, PCR Amplification, and DNA Sequencing

DNA was extracted from the internal tissue of dried specimens using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). The internal transcribed spacer (ITS) regions of nuclear rDNA were amplified by a polymerase chain reaction (PCR) with primers ITS5 and ITS4; primer pairs of LROR/LR5 were used to amplify the partial sequence of the large subunit (LSU). For PCR amplification, the 25 µl PCR reaction mixture contained 12.5 µl of 10 mM 2Mix, 10 µM ITS5 1 µl, 10 µM ITS4 1 µl, 40–50 ng/µl DNA 1 µl, and 9.5 µl of sterile ddH_2O. The PCR thermal cycles profile used the following modifications: 94°C for 5 min for the initial denaturation step, followed by 35 cycles of 94°C for 30 sec, a step of 55°C for 30 sec, and a final extension step of 70°C for 10 min. The purified PCR products were directly sequenced. ITS5/ITS4 and LROR/LR5 were used to sequence both strands of the DNA molecules [44]. The sequencing of PCR products was carried out by Sangon Biotech Co., Shanghai, China. The nuclear ribosomal Internal Transcribed Spacer region (nrITS) and nuclear ribosomal large subunit (nrLSU) of the mushroom were amplified and the sequence was deposited in GenBank to obtain the accession number. The Faces of Fungi database number was obtained as detailed in Jayasiri et al. [45].

2.4 Phylogenetic Analyses

Sequences were obtained from GenBank with our eight *G. gibbosum* sequences were used for the analyses (Tab. 1). Sequences were aligned and manually adjusted in Bioedit v. 7.0.9 [46] and Clustal X [47]. Sequences of *Amauroderma calcitum* (FLOR:50931) were used as outgroup taxa in the phylogenetic analyses. Gaps were set as missing data. A phylogenetic tree was performed by using PAUP* 4.0b10 [48]. Clade results from parsimony analyses were assessed by Maximum parsimony analyses (MP) performed with PAUP v. 4.0b10 [48]. Maximum likelihood analyses (ML) was performed with the CIPRES web portal [49] and using RAxML-HPC2 on XSEDE (v. 8.2.8) [50], including 1,000 bootstrap replicates random sequence additions. The best fitting substitution model was determined in MrModeltest 2.3 [51], for Bayesian inference posterior probabilities (PP). The Bayesian inference posterior probabilities (PP) distribution [52] was estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 [53]. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100 generations, thus 10,000 trees were obtained. Burn-in phases were determined by traces analysis in Tracer version 1.6 [54]. Based on the tracer analysis, the first 2,000 trees representing 20% of burn-in phase of the analyses were discarded, while the remaining 8,000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree [55].

Table 1: Taxa information used in nuc-ITS and nuc-LSU rDNA analysis with their voucher, GenBank accession numbers and locality used in this study

Fungal species	Voucher	GenBank accession no.		Locality
		ITS	LSU	
Ganoderma applanatum	Wei 5787a	KF495001	KF495011	China
G. applanatum	Dai 8924	KU219987	KU220014	China
G. applanatum	SFC20141001-24	KY364255	–	Korea
G. applanatum	SFC20141001-25	KY364256	–	Korea
G. applanatum	SFC20141012-02	KY364257	–	Korea
G. australe	HUEFS:DHCR417	MF436676	MF436673	Brazil
G. australe	HUEFS:DHCR411	MF436675	MF436672	Brazil
G. australe	CTRA1	KU569531	KU570929	Brazil
G. australe	CTRA2	KU569532	KU570930	South America
G. australe	CTRA3	KU569533	KU570931	South America
G. australe	CTRA4	KU569534	KU570932	South America
G. australe	CTRA12	KU569541	KU570940	South America
G. australe	CTRA11	KU569540	KU570939	South America
G. australe	CTRA10	KU569539	KU570938	South America
G. adspersum	FGA1	AM269771	AM269829	Italy
G. adspersum	SFC20150918-07	KY364248	–	Korea
G. adspersum	SFC20141001-16	KY364251	–	Korea
G. adspersum	SFC20140701-31	KY364253	–	Korea
G. adspersum	SFC20160115-20	KY364254	–	Korea
G. austroafricanum	CMW41454	KM507324	KM507325	South America
G. chalceum	URM80457	JX310812	JX310826	Brazil
G. destructans	CBS 139793	NR132919	NG058157	South Africa
G. destructans	CMW43670	KR183856	KR183860	South Africa
G. destructans	CMW43671	KR183857	KR183861	South Africa
G. destructans	CMW43672	KR183858	KR183862	South Africa
G. enigmaticum	CBS 139792	NR_132918	NG_058156	South Africa
G. enigmaticum	Ghana2/938397	KR014265	KR014266	Ghana
G. enigmaticum	Ghana1a/938398	KR150678	KR150679	South America
G. enigmaticum	CMW43669	KR183855	KR183859	South America
G. gibbosum	UB2	KU569557	KU570955	South America
G. gibbosum	SPC2	KU569547	KU570946	South America
G. gibbosum	SPC6	KU569550	KU570948	South America
G. gibbosum	SPC7	KU569551	KU570949	South America

Table 1 (continued).

Fungal species	Voucher	GenBank accession no.		Locality
		ITS	LSU	
G. gibbosum	IBT3	KU569543	KU570942	South America
G. gibbosum	CTRA6	KU569535	KU570934	South America
G. gibbosum	CTRA8	KU569537	KU570936	South America
G. gibbosum	CC23	KU569529	KU570927	South America
G. gibbosum	AS5.624 type3	AY593856	–	China
G. gibbosum	AS5.624 type4	AY593857	–	China
G. gibbosum	SFC20150630-23	AY593858	–	Korea
G. gibbosum	SFC20150812-36	AY593859	–	Korea
G. gibbosum	SFC20150918-08	AY593860	–	Korea
G. gibbosum	SFC20140702-12	AY593861	–	Korea
G. gibbosum	KUMCC 17-0003	MH035681	MH553157	Kunming, China
G. gibbosum	KUMCC 17-0005	MH035682	MH553158	Kunming, China
G. gibbosum	KUMCC 17-0008	MH035683	MH553159	Kunming, China
G. gibbosum	KUMCC 17-0009	MH035684	MH553160	Kunming, China
G. gibbosum	KUMCC 17-0010	MH035685	MH553161	Kunming, China
G. gibbosum	KUMCC 17-0013	MH035686	MH553162	Kunming, China
G. gibbosum	KUMCC 17-0014	MH035687	MH553163	Kunming, China
G. gibbosum	KUMCC 18-0007	MH035688	MH553164	Kunming, China
G. lucidum	IUM00298	–	DQ208410	Korea
G. lucidum	IUM01122	–	DQ208411	Korea
G. lucidum	C-1	–	DQ208412	Korea
G. multiplicatum	URM 83346	JX310823	JX310837	Brazil
G. multiplicatum	CWN 04670	KJ143913	–	Taiwan
G. orbiforme	URM 83332	JX310813	JX310827	Brazil
G. orbiforme	URM 83334	JX310814	JX310828	Brazil
G. orbiforme	URM 83335	JX310815	JX310829	Brazil
G. parvulum	URM 83339	JX310817	JX310831	Brazil
G. parvulum	URM 83340	JX310818	JX310832	Brazil
G. resinaceum	URM 83400	JX310824	JX310838	Brazil
Amauroderma calcitum	FLOR:50931	KR816528	KU315207	Brazil

The phylogenetic trees were printed with FigTree v. 1.4.0 [56], edited using Microsoft Office PowerPoint 2010 and exported to Adobe Illustrator CS v.3. The phylogram of Maximum likelihood and Maximum parsimony bootstrap values equal to or greater than 70%, with Bayesian Posterior Probabilities (PP) equal or greater than 0.95, are shown above the branches presented in Fig. 1. The sequences generated in this study were submitted to GenBank (Tab. 1).

3 Results

3.1 Phylogenetic Analyses

The final partial sequences of the internal transcribed spacer (ITS) and of large subunit (LSU) alignment were used to resolve the phylogenetic placement of *G. gibbosum* (Fig. 1). The alignment dataset is comprised of 62 ingroup taxa, which represent 13 *Ganoderma* species with 1 outgroup taxa (*Amauroderma calcitum* FLOR:50931). The maximum parsimony dataset consists of 1,506 characters, of which 1,302 were constant, 145 variable characters parsimony-informative, and 59 characters parsimony-uninformative. The phylogenetic analysis showed that our eight Ganoderma grouped in Asia *G. gibbosum* clade, with 73% ML and 70% MP bootstrap support.

3.2 Basidiocarp Morphology and Isolation for Mycelial Cultures

3.2.1 Macro-Morphological Characteristic Description

High variation of *G. gibbosum* was mostly observed on macro-morphological characteristics, those of eight samples were typical basidiocarps in annual (Figs. 3a, 3b, 4a, 4b, 5a, 5b, and 8a, 8b) and some was perennial; pileus was 4–24 cm with applanate to applanate with umbonate pileus; stipe varied from sessile by usually distinctly the attached base (Figs. 3a, 3b–8b, and 10a) to short stipe attached nearly to central pileus (Fig. 9a); pileus surface was smooth when young (Figs. 6a, 6b), with some radial furrows, and cracked when older (Fig. 9a), and some was thickness of several layers (Figs. 7a, 7b), consistency is tough and hard when mature; pore usually pale orange (5A3), leathery when broken, and light in weight on drying.

3.2.2 Micro-Morphology Characteristic Description

Microscopic structures of eight *G. gibbosum* are shown in Figs. 3–10. The dimensions of basidiospores, hyphae, pore, and tube layers of eight *G. gibbosum* collections are shown in Tab. 2, and their basidiospore characteristics are presented in Tab. 3. Microscopically, basidiospores were characterized by being broadly ellipsoid and oblong ellipsoid, with some subglobose to globose distinctly tapering at the distal end, truncated, with double wall (ganodermoid), thick-walled inner endosporium; 4–8 × 6–12 µm, reddish brown; *Context* some distinctive of hymenial with sword-like apices hyphae (Figs. 3q, 3r, 4q, 4r, 5d–5g) and dendroid of the Bovista-type (Figs. 4s, 8p–8r, 9l–9m, 9r, and 10p), brown (6E8) to dark brown (8F4-5). *Hyphal system* hyaline, thin to thick walled with septa hyphae, composed of generative hyphae, skeletal hyphae and tri-dimitic hyphal with clamps connections.

3.3 Characteristics of Mycelial Cultures

Eight *G. gibbosum* produced white mycelium on PDA medium after incubation for 3–4 days. The fungal culture was fully colonized after an incubation period of 9–12 days, and a range of colors was present after an incubation period of 16–20 days. All cultures were photographed during each different incubation period. For instance, *G. gibbosum* KUMCC17-0003 was isolated from internal tissue and incubated for 7 days, and it showed white (5A1) with radius light yellow (4A4) zone from colony center, and its active mycelium (margin) thinner than the center (Figs. 2a, 2b); *G. gibbosum* KUMCC17-0005 was incubated for 24 days, and it produced velvety mycelial and smooth surface (Fig. 2c) with light yellow (4A4) when observed under the culture petri dish (Fig. 2d); *G. gibbosum* KUMCC17-0008 could observed white scattered cotton mycelium (Fig. 2e) and slightly produce pale orange (5A3) to brown (6D8) at center to the margin (Fig. 2f) after incubated for 16 days; *G. gibbosum* KUMCC17-0009 was photograph when incubation for 28 days, it could observed white radial furrows on agar surface (Fig. 2g), while under petri dish occurred light yellow (4A4) with brown (6D8) at the center of culture colony (Fig. 2h). *Ganoderma gibbosum* KUMCC17-0010 is also produced white with few cottony mycelium after incubated for 16 days, it slightly circles furrows (Fig. 2i), brown (6D8) to pale orange (5A3) at the center to the margin after incubated for 24 days (Fig. 2j). *Ganoderma gibbosum* KUMCC17-0013 produced white to brownish

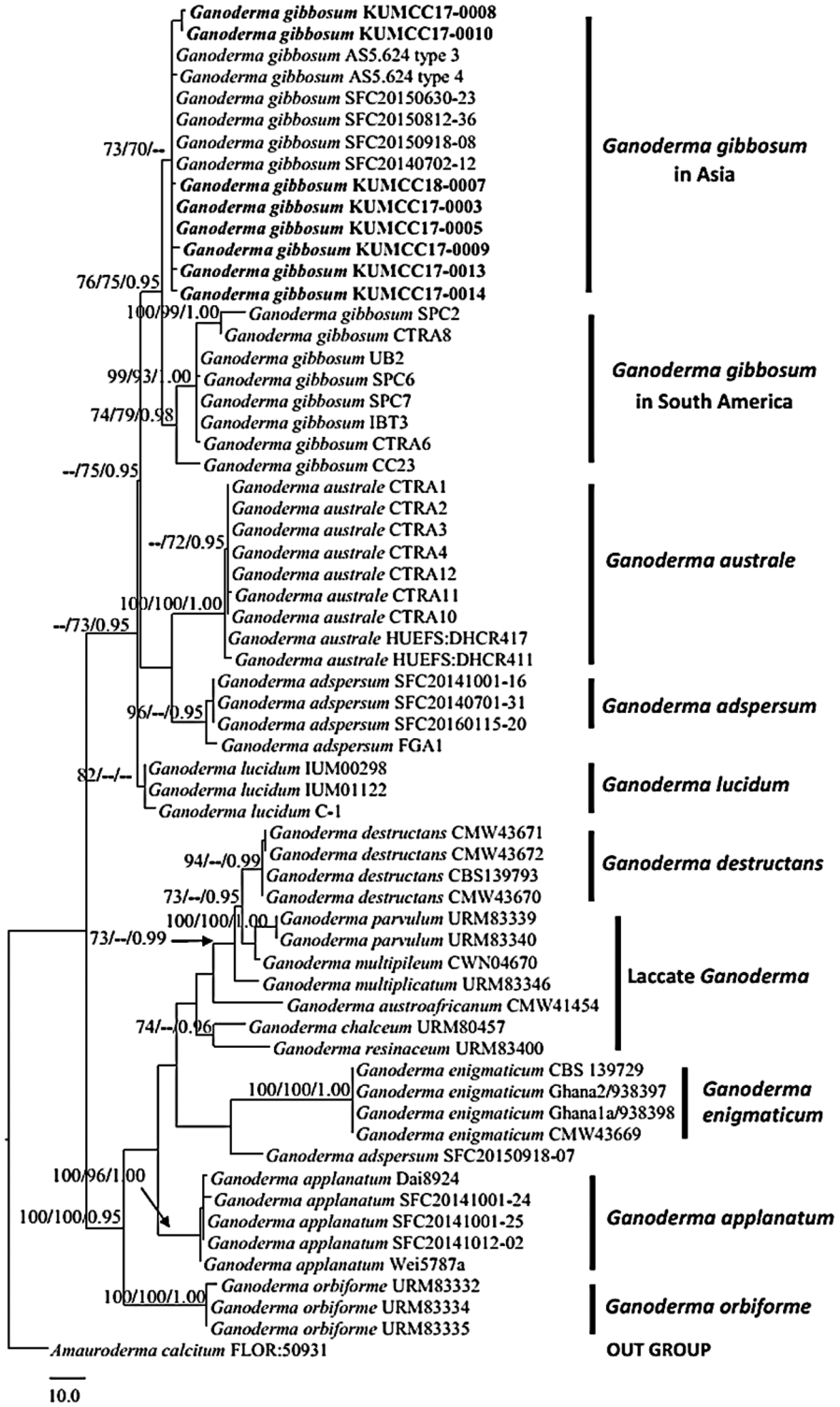

Figure 1: Phylogenetic tree showing the phylogenetic position of *G. gibbosum* specimens collected in Yunnan Province, China in comparison with available LSU and ITS rDNA sequence data of *Ganoderma* in GenBank. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 70% and Bayesian posterior probabilities greater than 0.95 are indicated above the nodes as MLBS/MPBS/PP. The data was analyzed with random addition sequence and unweighted parsimony, and gaps were treated as missing data. The tree is rooted with *Amauroderma calcitum* FLOR:50931. *Ganoderma gibbosum* collected in this study are indicated in black bold

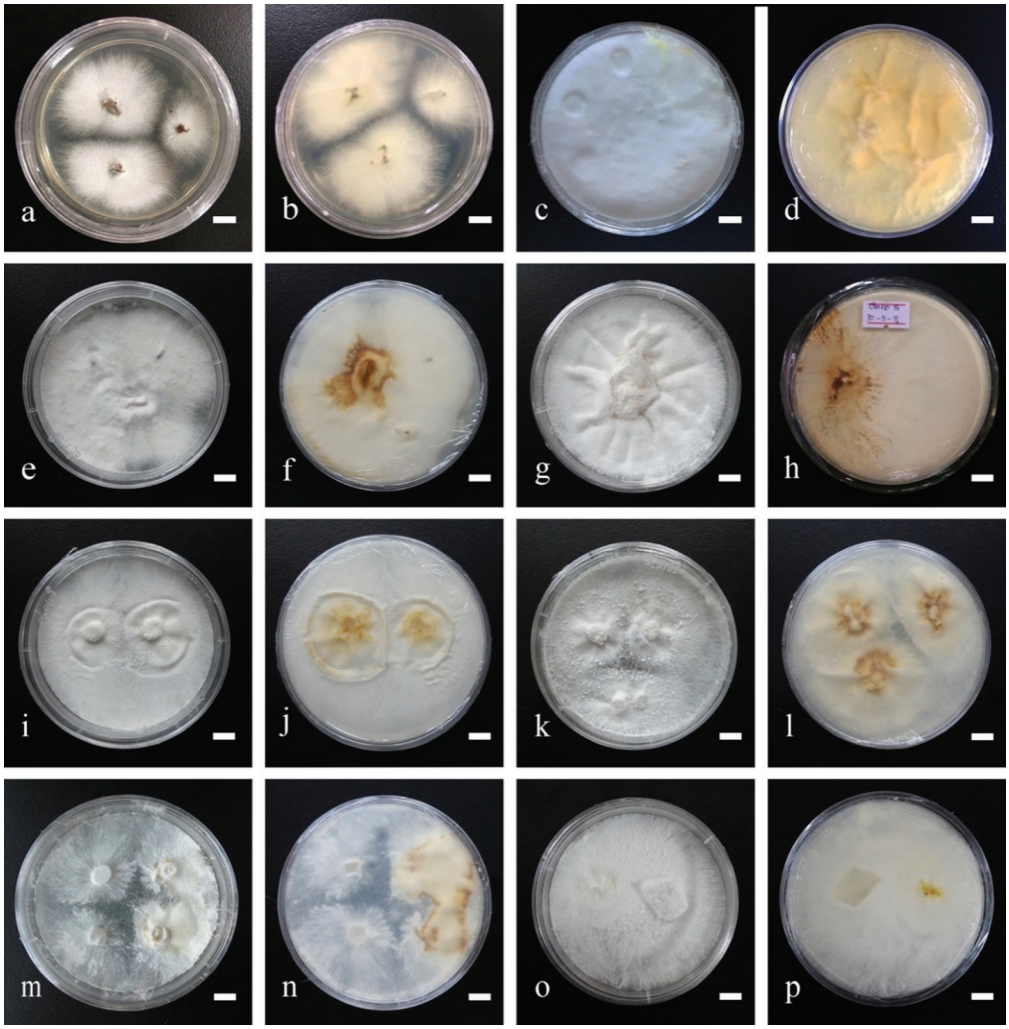

Figure 2: Morphology of *Ganoderma gibbosum* cultures which were incubated at 25°C for 7–28 days on Potato Dextrose Agar (PDA). a–b) strain KUMCC 17-0003, c–d) KUMCC 17-0005, e–f) strain KUMCC 17-0008, g–h) strain KUMCC 17-0009, i–j) strain KUMCC 17-0010, k–l) strain KUMCC 17-0013, m–n) strain KUMCC 17-0014, o–p) strain KUMCC 18-0017. Scale bars: a–p = 1 cm

yellow (5C7-5C8), tightly scattered cottony on agar surface after incubation for 21 days (Figs. 2k, 2l), while white soft mycelium and some tight velvety light yellow (4A5) has occurred at the center of strain KUMCC17-0014 after incubated for 14 days (Figs. 2m, 2n), and white with few cottony mycelia is also could be observed in *G. gibbosum* KUMCC18-0017 after incubated for 14 days (Figs. 2o, 2p), it changes to yellowish white (4A2) mycelial after incubated for 18 days and slightly unshaped furrows on agar surface after incubated for 22 days (data not show). Those of eight strains, which of 5 strains including; strain KUMCC17-0008, KUMCC17-0009, KUMCC17-0010, KUMCC17-0013, and KUMCC18-0007 were distinctive brittle when plugged, while the culture strain KUMCC17-0005 was sticky.

3.4 Taxonomy Study

Basidiocarps; annual or perennial, with non-laccate, 2–15 cm wide, 4–24 cm long, 1–6.5 cm thick. *Pileus* orbicular to subflabellate or subdimidiate. *Pileus shape* convex, umbonate to uneven or ungulate,

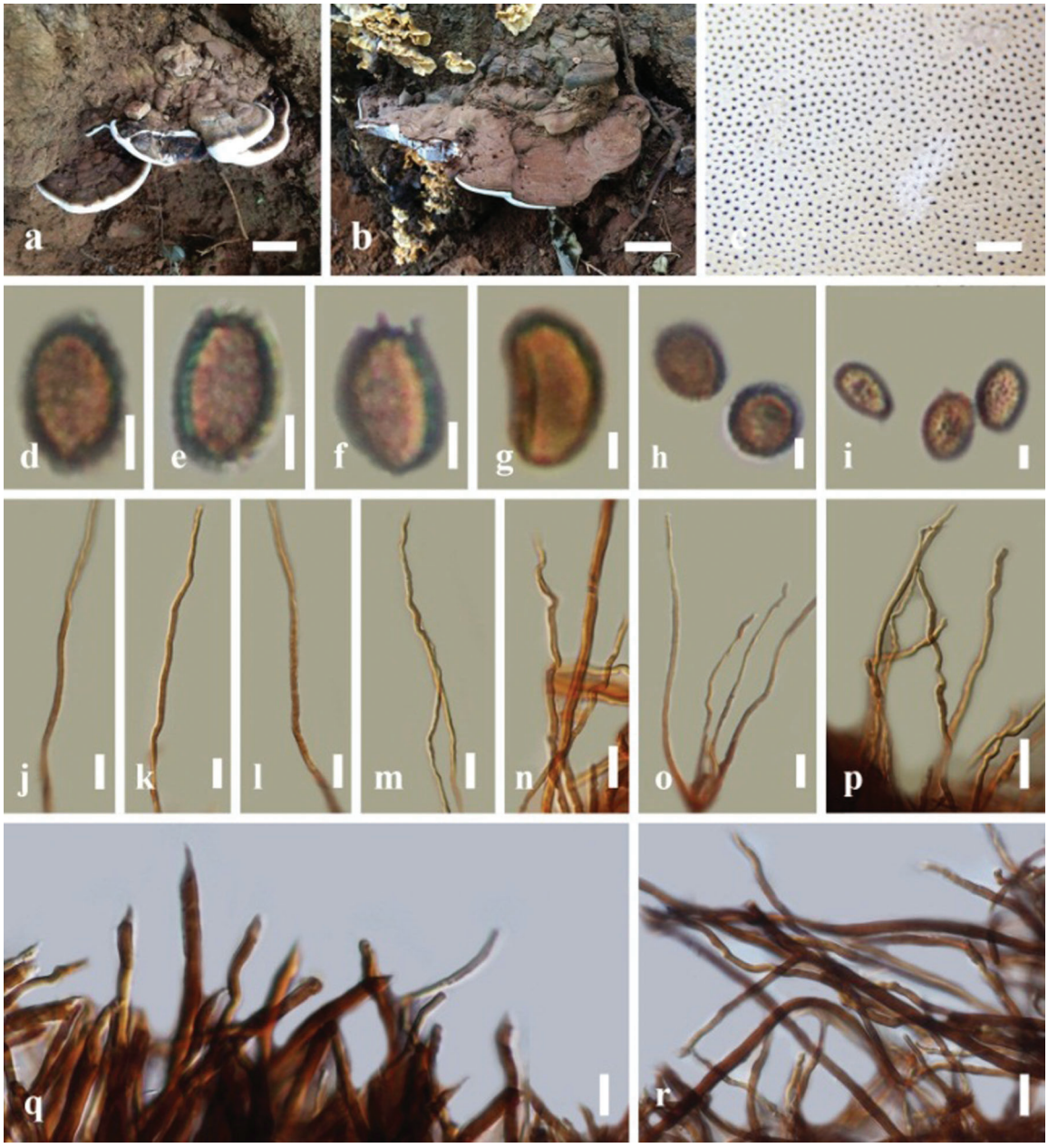

Figure 3: *Ganoderma gibbosum* strain KUMCC17-0003. a) young basidiocarp morphology characteristics, b) mature basidiocarp morphology, c) pore characteristic, d–i) basidiospore in Congo red, j–m) generative hyphae of context in KOH, n) skeletal hyphae of context in KOH, o–p) hyphae and clamp connections of context in KOH, q–r) hyphae of tubes in KOH. Scale bars: a–b) = 2 cm, c) = 1000 µm, d–i) = 3 µm, j–p) = 20 µm, q–r) = 10 µm

some was round and plump when young (Figs. 6a, 6b), somewhat imbricate or overlapping shelves (Figs. 3a, 3b, 4a, 4b, 8a, 8b), with broadly attached, imbricate, when seen from above flabelliform (fan shape), although can be thicker and mostly thicker at base slightly soft at margin when mature, and some cracked crust when old. *Pileus surface* smooth, soft, usually silky and slippery when young, woody when mature to older, most of the mature specimens are furrowed on the surface with sulcate, tuberculate to undulating, somewhat

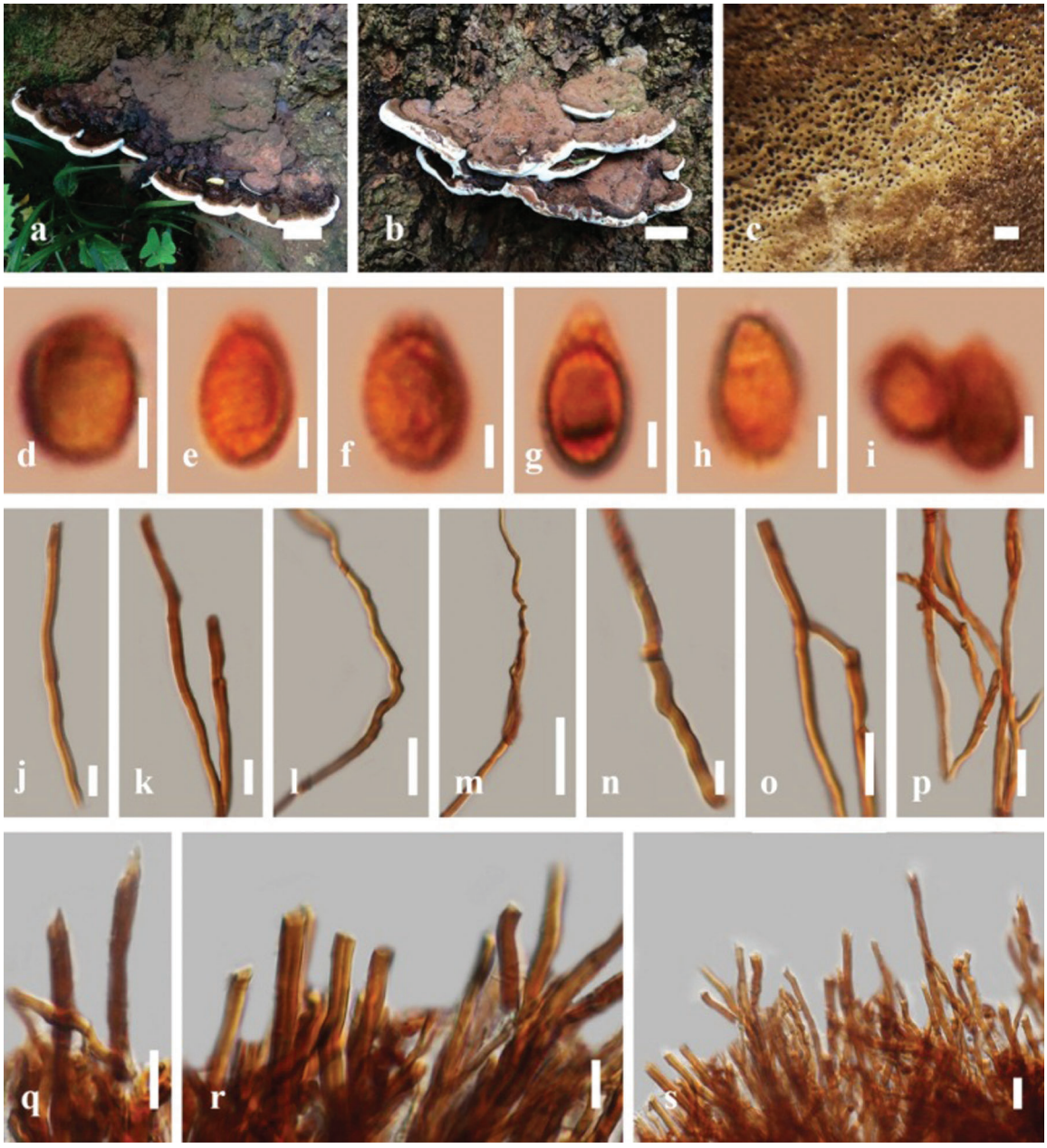

Figure 4: *Ganoderma gibbosum* strain KUMCC17-0005. a) perennial basidiocarp morphology characteristics, b) mature basidiocarp morphology, c) pore characteristic, d–i) basidiospore in Congo red, j–l) skeletal hyphae of context in KOH, m–p) hyphae and clamp connections of context in KOH, q–s) hyphae of tubes in Melzer's reagent. Scale bars: a) = 3 cm, b) = 2 cm, c) =1000 µm, d–i) = 3 µm, j–p) = 20 µm, q–s) = 10 µm

spathulate to uneven, incised, compact and hard, covered with a crust, non-shiny texture (dull), slightly dull and faded when mature to old, and lined or cracked crust when older, the thickness of pileus appeared as a thin layer (less than 0.5 cm) and several layers (upper than 3.5 cm). *Pileus color* greyish (1C3) at base and slightly yellow grey (3B2), and pale yellow (1A5) to pale orange (6A3) at center, and white (6A1) at margin in young basidiocarps, and usually the color changes to reddish brown (8E6) upon touch, when young. Dark

Figure 5: *Ganoderma gibbosum* strain KUMCC17-0008. a–b) basidiocarp morphology characteristics, c) generative hyphae of context in KOH, d) skeletal hyphae of context in KOH, e) hyphal and clamp connections of context in KOH, f) binding hyphae and clamp connections of context in KOH, g) trimitic hyphal system of context in KOH. Scale bars: a–b) = 1 cm, c–g) = 10 µm

brown (6F7), brownish orange (6C5), or greyish orange (6B3) towards the center of maturity basidiocarps; often greyish brown (11D4) to violet brown (11F8) close to pileus margin when young, and become dull red (11C3), reddish brown (8F6), greyish red (8C4), and greyish orange (5B3) when old. *Pore* angular, 4–7 per mm in fresh. *Tube layer* 0.2–1.8 cm long, wall thick 40–230 µm when dried (Figs. 3c, 4c, 7c, 8c, and 10b) and non-presented when young. *Pore color* white (11A1) to pale orange (5A3), fertile undersurface when mature, immediate color change to dark brown (6F8) or reddish brown (8D8) when cut, scratched or bruised, and discolored when touched, with a slippery surface when fresh (Fig. 9b).

Basidiospores ellipsoid to broadly ellipsoid, sub-globose to globose, and some oblong-ellipsoid, (5.1–) 5.3–7.0 (–7.2) × (8.0–) 7.9–10.8 (–11.0), overlaid by hyaline, inner wall echinulate brown, light brown (6D4) to brown (6E8) in 5% KOH. *Context* up to 2.5 cm thick, brown (6E7) to dark brown (6F8), mostly dark brown near the tube layers; Bovista-type ligative hyphae, hymenial with sword-like apices in the context; *hyphal system* hyaline, thin to thick walled with simple septa, composed of narrow and sparingly branched, thin wall of generative hyphae with hyaline, skeletal hyphae thicker wall 2.2–7.9 µm wide, binding hyphal, and tri-dimitic hyphal, with clamps connections. *Basidia* not seen.

Margin wavy, blunt edged, and slippery, thinner than the base and softer than the center, often white (8A1) to orange white (6A2) when young, violet brown (11F8) and brownish red (10F6) when mature. *Stipe* almost sessile (without stipe) with broadly attached or short stipe when present or centrally stipitate, attachment of stipe to pileus varied from lateral to nearly central (Fig. 9a), and mostly found on trunks of many broad-leafed trees. *Habitat* solitary on dead trunks, or decaying stumps, and occasionally occurring

Figure 6: *Ganoderma gibbosum* strain KUMCC17-0009. a–b) young basidiocarp morphology characteristics, c–d) generative hyphae of context in KOH, e–f) skeletal hyphae of context in KOH, g–h) hyphae and clamp connections of context in KOH. Scale bars: a–b) = 1 cm, c–h) = 20 µm

on standing trees of *Albizia mollis* (Wall.) Boiv., *Machilus yunnanensis* Lecomte., and *Neocinnamomum delavayi* (Lec.) H. Liou.

Specimens examined China, Yunnan Province, Kunming Botanical Garden, Chinese Academy of Sciences, 25°07'58'N, 102°44'39'E, on August to December, 2016–2017.

4 Discussion

This study is the first comprehensive report of the morphological characteristics and molecular analyses within the species of *G. gibbosum* collected from Yunnan Province, China. Phylogenetic analyses, based on LSU and ITS, showed 10 clades of *Ganoderma*, of which two clades belong to *G. gibbosum*. One is the Asian *G. gibbosum* clade, and the other is *G. gibbosum* from South America; both clades coincide with their geographic distributions. Our *G. gibbosum* collections formed a clade with Asia *G. gibbosum* collections originating from China and Korea, with 73% ML and 70% MP statistical supports; however, in a previous phylogenetic analysis Korean *Ganoderma* was identified as *Ganoderma* cf. *gibbosum* [57]. *Ganoderma gibbosum* collections from South America formed a sister clade to our collections, with 74% ML, 79% MP and 0.98 PP statistical supports, and both *G. gibbosum* clades of Asia and South America are clustered together with 76% ML, 75% MP, and 0.95 PP statistical supports.

Ganoderma gibbosum was first described in Australia [58]. It was considered to be a subspecies of *G. applanatum* [59], while *G. applanatum* was the earlier name of *G. australe* [59,60]. *Ganoderma australe* and *G. gibbosum* were renamed *G. incrassatum* based on their monophyletic origin [24], since it had been well recognized that *G. lipsiense* was synonymous with *G. applanatum* [2]. This study shows that *G. gibbosum* is

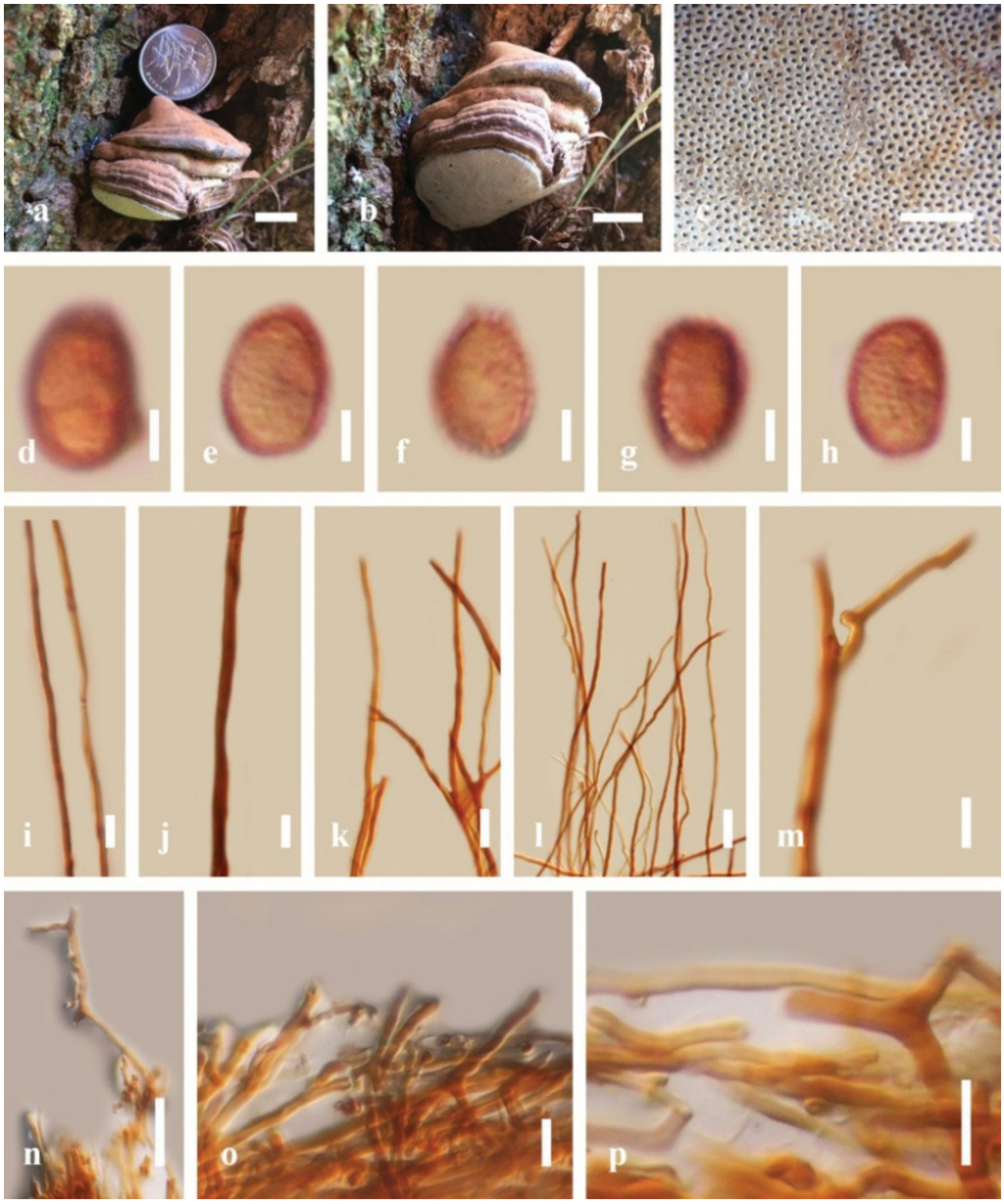

Figure 7: *Ganoderma gibbosum* strain KUMCC17-0010. a–b) Basidiocarp morphology characteristics, c) pore characteristic, d–h) basidiospore in Congo red, i) generative hyphae of context in KOH, j. skeletal hyphae of context in KOH, l–m) binding hyphae and clamp connections of context in KOH, n–p) binding hyphae of tubes in Melzer's reagent. Scale bars: a–b = 1 cm, c = 1000 µm, d–h = 3 µm, i–p = 20 µm

closely related with *G. australe* (75% MP and 0.95 PP). Our results are similar to those of Kaliyaperumal et al. [61], who considered *G. gibbosum* to be a sister clade to *G. australe*. Here, we did not include holotype descriptions and sequences, as the current taxa sampling lacks available data with respect to taxonomy and sequence evidence. Moreover, a few strains have been reported from Australia and North America [24,61]. However, *G. gibbosum* is still the verified species worldwide [62].

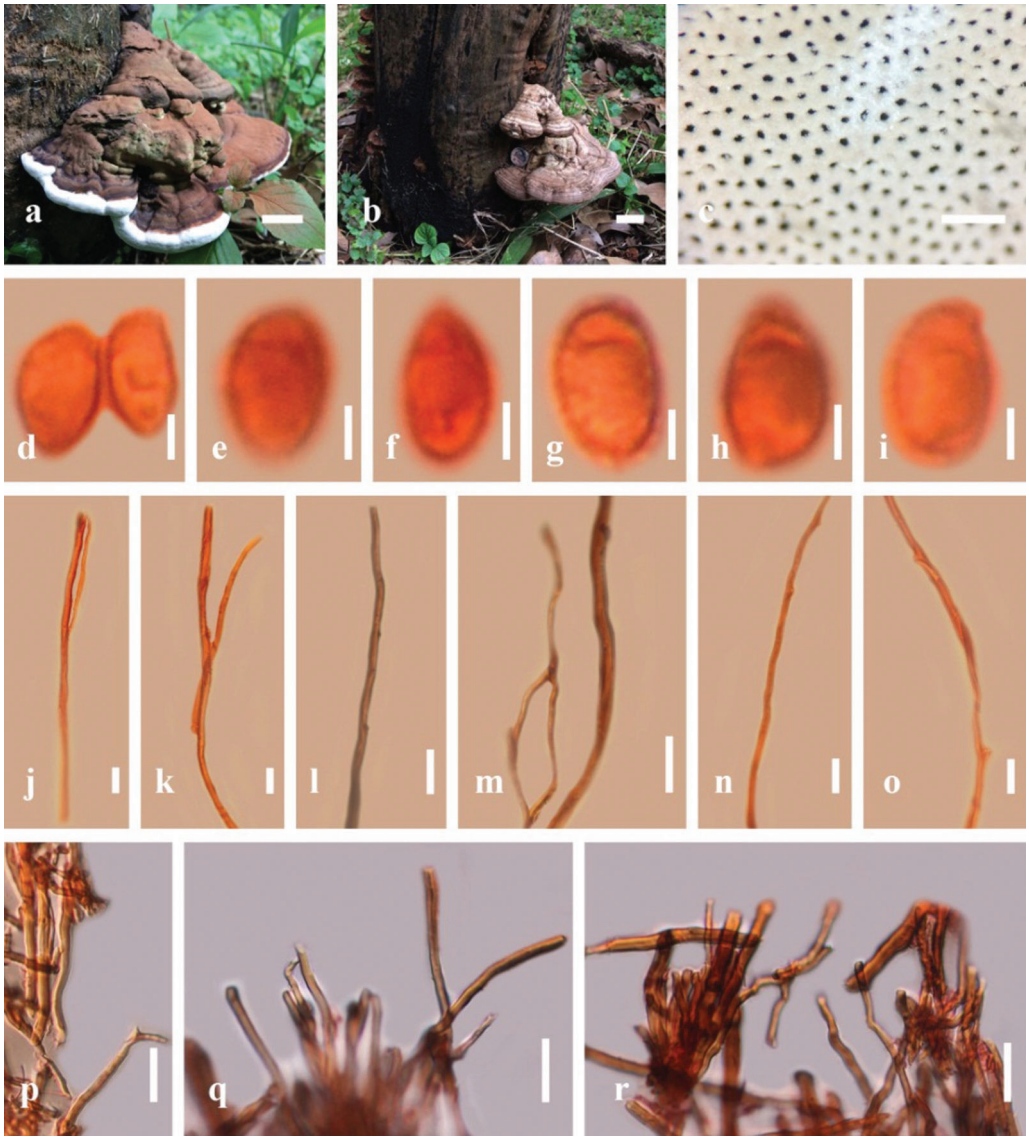

Figure 8: *Ganoderma gibbosum* strain KUMCC17-0013. a–b) perennial basidiocarp morphology characteristics, c) pore characteristic, d–i) basidiospore in Congo red, j–k) generative hyphae of context in Congo red, l–m) skeletal hyphae in Congo red, n–o) hyphae and clamp connections of context in Congo red, p–r) binding hyphae of tubes in KOH. Scale bars: a–b) = 2 cm, c) = 1000 µm, d–i) = 3 µm, j–r) = 20 µm

Our *G. gibbosum* has shared macro-morphology with the Hainan strain, as they both show sessile and annual crust basidiocarps [63]. The Guangzhou strain is sub-flabellate to sub-dimidiate [37], while the Korean strain is short stiped [57]. Regarding stipe attachment, the Korean *Ganoderma* and our collections share identical features in common, including broad attachment or short stipe with host, and pores between 4–6 to 4–7 per mm. In addition, we also illustrate differences in macro-morphological characteristics among the eight non-laccate *Ganoderma*. Lodge [39] proposed that geographical area, host range, weather and environmental parameters often influence fungal morphological characteristics. Although our *G. gibbosum* were collected from a limited geographical area, their tree hosts and substrates

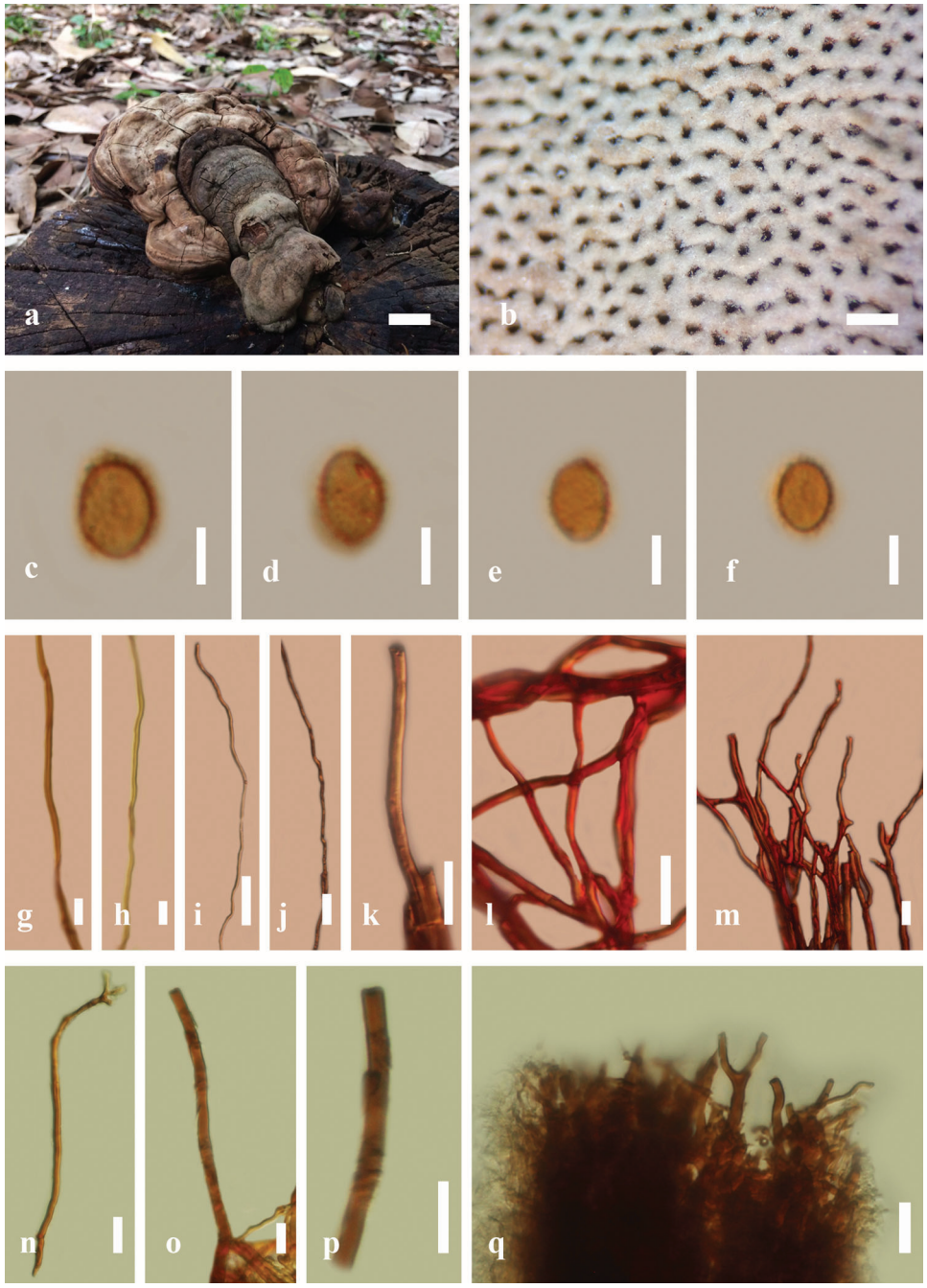

Figure 9: *Ganoderma gibbosum* strain KUMCC17-0014. a) mature basidiocarp morphology characteristics, b) pore characteristic, c–f) basidiospore in Congo red, g–l) hyphae of context in Congo red, m) hyphae and clamp connections of context in Congo red, n–q) hyphae of tubes in Melzer's reagent. Scale bars: a) = 2 cm, b) = 500 µm, c–g) = 3 µm, h–r) = 20 µm

are different. For example, perennial and mature basidiocarps of *G. gibbosum* KUMCC17-0003 are associated with living *Castanopsis* spp.; however, perennial and mature *G. gibbosum* KUMCC17-0005, perennial and young *G. gibbosum* KUMCC17-0009, and old basidiocarps of *G. gibbosum* KUMCC17-0010

Table 2: Dimensions of morphological characteristics of basidiospores, hyphae, pores and tubes layer of *Ganoderma gibbosum* in this study

Fungal specimen	Basidiospores (μm)	Hyphae (μm)	Pores (per mm)	Tubes layer
Ganoderma gibbosum KUMCC17-0003	(4.7–) 4.8–7.2 (–7.4) × (7.2–) 7.4–10.7 (–10.9)	2.8–7.4	4–7	0.4–1.7 cm long, 50–164 μm wall thick
G. gibbosum KUMCC17-0005	(5.1–) 5.2–6.6 (–6.8) × (6.9–) 6.11–11.10 (–11.9)	2.3–7.6	4–7	0.4–1.5 cm long, 60–175 μm wall thick
G. gibbosum KUMCC17-0008	Not observed	2.2–7.3	Not observed	0.3 × 0.8 cm long, 50–130 μm wall thick
G. gibbosum KUMCC17-0009	Not observed	2.4–7.6	Not observed	0.2 × 0.6 cm long, 40–132 μm wall thick
G. gibbosum KUMCC17-0010	(5.7–) 5.9–7.0 (–7.2) × (8.8–) 8.9–10.2 (–10.4)	3.2–7.9	4–6	0.2 × 1.1 cm long, 50–215 μm wall thick
G. gibbosum KUMCC17-0013	(5.3–) 5.5–6.8 (–7.0) × (8.7–) 8.10–10.4 (–10.6)	3.1–7.9	4–5	0.3 × 1.8 cm long, 80–230 μm wall thick
G. gibbosum KUMCC17-0014	(5.1–) 5.4–7.4 (–7.5) × (8.8–) 8.9–11.3 (–11.5)	2.1–7.7	4–7	0.2 × 1.2 cm long, 60–176 μm wall thick
G. gibbosum KUMCC18-0007	(4.6–) 4.8–7.0 (–7.2) × (7.8–) 8.0–11.3 (–11.4)	3.1–7.1	4–6	0.3 × 0.8 cm long, 50–160 μm wall thick

Table 3: Basidiospore characteristics of the *Ganoderma gibbosum* specimens analyzed in this study

Fungal specimen	Basidiospore characteristics
Ganoderma gibbosum KUMCC17-0003	Subglobose to globose, some broadly ellipsoid, and some oblong ellipsoid
G. gibbosum KUMCC17-0005	Subglobose to globose, and some broadly ellipsoid
G. gibbosum KUMCC17-0008	Not observed
G. gibbosum KUMCC17-0009	Not observed
G. gibbosum KUMCC17-0010	Subglobose and broadly ellipsoid
G. gibbosum KUMCC17-0013	Subglobose, some broadly ellipsoid, and some oblong ellipsoid
G. gibbosum KUMCC17-0014	Subglobose and broadly ellipsoid
G. gibbosum KUMCC18-0007	Oblong ellipsoid and some subglobose

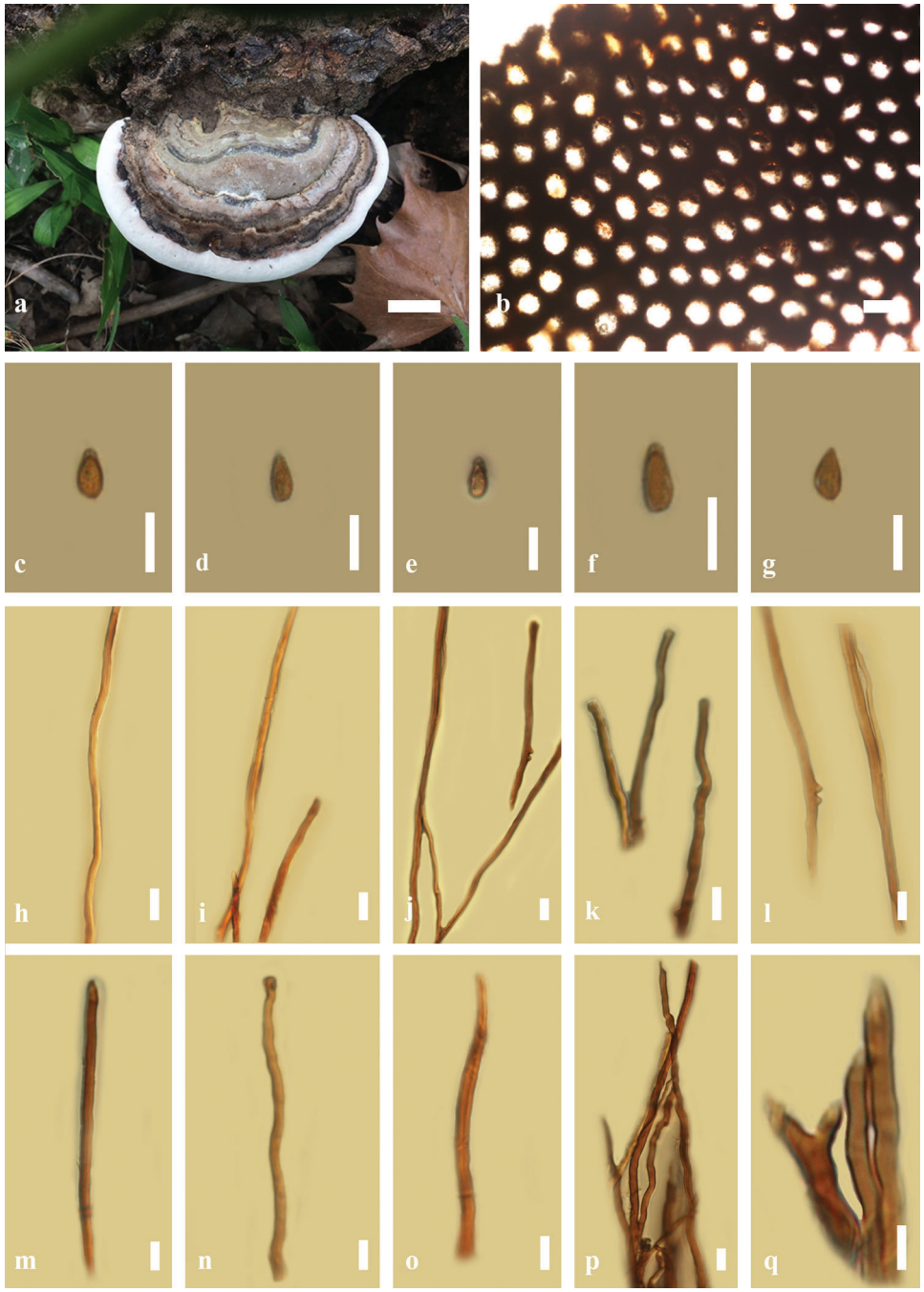

Figure 10: *Ganoderma gibbosum* strain KUMCC18-0007. a) basidiocarp morphology characteristics, b) pore characteristic, c–g) basidiospore in Congo red, h) generative hyphae of context in KOH, i) = skeletal hyphae of context in KOH, j–l) hyphae and clamp connections of context in KOH, m–q) hyphae of tubes in Melzer's reagent. Scale bars: a) = 2 cm, b = 100 µm, c–g) = 10 µm, h–j) = 15 µm, k–p) = 10 µm, q) = 5 µm

are associated with living *Albizia mollis* (Wall.) Boiv.; perennial *G. gibbosum* KUMCC17-008 is associated with living *Machilus yunnanensis* (Lec.); perennial and mature basidiocarps of *G. gibbosum* KUMCC17-0013 and old *G. gibbosum* KUMCC17-0014 were growing on dry stump of an unknown tree species, and perennial

G. gibbosum KUMCC18-0007 was growing at the base of *Quercus glaucoides* (Schottky). Thus, we propose that *G. gibbosum* can grow on different host tree species, as mentioned above. However, *G. steyaertanum*, *G. mastoporum* (= *G. orbiforme*) and *G. philippii* are associated with other tree species, such as *Acacia mangium* [64]; *G. carocalcareus* is associated with *Anthocleista nobilis* [22]. In addition, weather is also considered very important [65]. For example, *G. gibbosum* KUMCC17-0008 was collected after heavy rain, thus its basidiocarps were dark in color and laccate-like when recorded in the field, while *G. gibbosum* KUMCC17-0013 and KUMCC17-0014 were collected when the weather was sunny so basidiocarps were pale in color. Our study is in line with Imazeki [66], which claims that *Ganoderma* is distributed in tropical areas, as well as several other studies that reported the same [67]. Kim et al. [68] reported that Korean *Ganoderma* is usually collected from tropical areas. Thus, we concur with the previous studies that the geographical area, host, substrate, and weather-related factors could affect fungal physiology even within species [69].

Based on our intensive microscopic observations, some *G. gibbosum* collections are within the range of the type species [37], and *G. gibbosum* KUMCC17-0010 and KUMCC17-0013 have double-walled ellipsoid basidiospores, while *G. gibbosum* KUMCC18-0004 has mostly distinctive double-walled oblong-ellipsoid basidiospores. Hapuarachchi et al. [63] has described a Hainan strain of *G. gibbosum* which has ellipsoid to elongate basidiospores. The basidiospore size range of our *G. gibbosum* collections is (5.1–)5.3–7.0(–7.2) × (8.0–)7.9–10.8(–11.0), larger than the Korean collections (4.9–)5.6–6.0(–6.5) × (7.7–)8.5–9.2(–9.4) µm [57] as well as the Hainan collections (4.3–)6.9–9.2(–10.5) × (3.8–)4.6–5.7(–6.1) µm [63]. However, *G. gibbosum* from South America lack detailed macro-micro description.

Ganoderma gibbosum were isolated into PDA media and after 7–28 days of incubation at 25°C, different culture morphological characteristics were observed. *Ganoderma gibbosum* KUMCC17-0008 (Figs. 2e, 2f) culture was identical to KUMCC17-0013, having tightly scattered cottony mycelia after 21 days of incubation. *Ganoderma gibbosum* KUMCC17-0013 culture was similar to KUMCC17-0008 culture after 15–16 days of incubation. Garibova et al. [70] reported that culture conditions affect *Ganoderma* mycelia morphological characteristics. The shape of the original cultures; i.e., internal tissue, circles or irregular shape of culture when plugged, also affect the development of cultures. Moreover, mycelial density also depends on the number of active mycelia plugged on the agar media.

This study clearly shows the morphological variations and phylogenetic affinity among *G. gibbsosum* collected from a limited geographical area in Yunnan, China. Wang et al. [71] have also reported that there are high morphological variations within Chinese *Ganoderma*. *Ganoderma* species from different geographic areas have also showed separate lineages in phylogenetic analyses [72,73]. However, high variability in macro-morphological characteristics such as color, shape and size mean that it is often difficult to identify *G. gibbosum* by morphological characteristics alone. So to evaluate the variations in *G. gibbosum* or any *Ganoderma* species, important taxonomic characteristics should be carefully observed in collection and linked with phylogenetic analysis. In the future, worldwide collections of *G. gibbosum* are needed to evaluate taxonomic and phylogenetic variations within the species.

Acknowledgement: We appreciate the kind support given by University of Chinese Academy of Sciences, Beijing 100049, China; Key Laboratory for Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China; Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China; Centre for Mountain Ecosystem Studies (CMES), Kunming, Yunnan, China. We thank the Germplasm Bank of Wild Species, Kunming Institute of Botany, Kunming 650201, Yunnan, China for enabling our molecular laboratory work. The authors also would like to thank William Julian for contributions to the English editing.

Funding Statement: Peter E. Mortimer thanks the National Sciences Foundation, China, NSFC-TRF, Grant No. 41761144055; National Science Foundation, China (NSFC), Grant No. 41771063; Chinese Academy of

Sciences (CAS), Grant No. 2017CASSEABRIZD003 and Grant No. Y4ZK111B01. Samantha C. Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (Grant No. 2018PC0006) and the National Science Foundation of China (NSFC, project code 31851110759).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Karsten, P. A. (1881). Enumeratio boletinearum et polyporearum fenicarum systemate nova dispositarum. *Revue de Mycologie, 3,* 16–23.
2. Moncalvo, J. M., Ryvarden, L. (1881). A nomenclatural study of the ganodermataceae donk. *Synopsis Fungorum, 11,* 1–114.
3. Curtis, W. (1781). *Flora Londinensis: or plates and descriptions of such plants as grow wild in the environs of London*. London: Biodiversity Heritage Library.
4. Fries, E. M. (1821). *Systema mycologicum, sistens fungorum ordines, genera et species, huc usque cognitas, quas ad normam methodi naturalis determinavit, disposuit atque descripsit Elias Fries*. Lundæ, ex officina Berlingiana, Sweden.
5. Cao, Y., Wu, S. H., Dai, Y. C. (2012). Species clarification of the prize medicinal *Ganoderma* mushroom Lingzhi. *Fungal Diversity, 56(1),* 49–62. DOI 10.1007/s13225-012-0178-5.
6. Cao, Y., Yuan, H. S. (2013). *Ganoderma mutabile* sp. nov. from southwestern China based on morphological and molecular data. *Mycological Progress, 12(1),* 121–126. DOI 10.1007/s11557-012-0819-9.
7. Chang, S. T., Miles, P. G. (2004). *Mushroom cultivation, nutritional value, medicinal effect, and environmental impact*. Boca Raton, Florida: CRC Press.
8. Moncalvo, J. M., Buchanan, P. K. (2008). Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycological Research, 112(4),* 425–436. DOI 10.1016/j.mycres.2007.12.001.
9. Smith, B. J., Sivasithamparam, K. (2000). Internal transcribed spacer ribosomal DNA sequence of five species of *Ganoderma* from Australia. *Mycological Research, 104(8),* 943–951. DOI 10.1017/S0953756200002458.
10. Li, Y. B., Liu, R. M., Zhong, J. J. (2013). A new ganoderic acid from *Ganoderma lucidum* mycelia and its stability. *Fitoterapia, 84,* 115–122. DOI 10.1016/j.fitote.2012.11.008.
11. De Silva, D. D., Rapior, S., Hyde, K. D., Bahkali, A. H. (2012). Medicinal mushrooms in prevention and control of diabetes mellitus. *Fungal Diversity, 56(1),* 1–29. DOI 10.1007/s13225-012-0187-4.
12. Hapuarachchi, K. K., Wen, T. C., Jeewon, R., Wu, X. L., Kang, J. C. et al. (2016). Mycosphere Essays 7. *Ganoderma lucidum*—are the beneficial anticancer properties substantiated? *Mycosphere, 7(3),* 305–332. DOI 10.5943/mycosphere/7/3/6.
13. Hyde, K. D., Bahkali, A. H., Moslem, M. A. (2010). Fungi-an unusual source for cosmetics. *Fungal Diversity, 43 (1),* 1–9. DOI 10.1007/s13225-010-0043-3.
14. De Silva, D. D., Rapior, S., Sudarman, E., Stadler, M., Xu, J. et al. (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity, 62(1),* 1–40. DOI 10.1007/s13225-013-0265-2.
15. Wachtel-Galor, S., Yuen, J., Buswell, J. A., Benzie, B. (2011). *Ganoderma lucidum (Lingzhi or Reishi): a medicinal mushroom*. Boca Raton: CRC Press, Taylor & Francis.
16. Miller, R. N. G., Holderness, M., Bridge, P. D., Chung, G. F., Zakaria, M. H. (1999). Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology, 48(5),* 595–603. DOI 10.1046/j.1365-3059.1999.00390.x.
17. Loyd, A. L., Richter, B. S., Jusino, M. A., Truong, C., Smith, M. et al. (2018). Identifying the mushroom of immortality: assessing the *Ganoderma* species composition in commercial Reishi products. *Frontiers in Microbiology, 9,* 1–14. DOI 10.3389/fmicb.2018.01557.

18. Hapuarachchi, K. K., Karunarathna, S. C., McKenzie, E. H. C., Wu, X. L., Kakumyan, P. et al. (2019). High phenotypic plasticity of *Ganoderma sinense* (Ganodermataceae, Polyporales) in China. *Asian Journal of Mycology, 2(1),* 1–47. DOI 10.5943/ajom/2/1/1.
19. Luangharn, T., Karunarathna, S. C., Mortimer, P. E., Hyde, K. D., Xu, J. C. (2019). Additions to the knowledge of *Ganoderma* in Thailand: *Ganoderma casuarinicola*, a new record; and *Ganoderma thailandicum* sp. nov. *MycoKeys, 59(4),* 47–65. DOI 10.3897/mycokeys.59.36823.
20. Zhao, J. D., Xu, L. W., Zhang, X. Q. (1983). Taxonomic studies on the family Ganodermataceae of China II. *Acta Mycologica Sinica, 2,* 159–167.
21. Kirk, P. M., Cannon, P. F., Minter, D. W., Stalpers, J. (2008). *Dictionary of the fungi*. 10th. Wallingford: CABI publishing.
22. Douanla-Meli, C., Langerm, E. (2009). *Ganoderma carocalcareus* sp. nov., with crumbly-friable context parasite to saprobe on *Anthocleista nobilis* and its phylogenetic relationship in G. resinaceum group. *Mycological Progress, 8(2),* 145–155. DOI 10.1007/s11557-009-0586-4.
23. Kinge, T. R., Mih, A. M. (2010). *Ganoderma ryvardense* sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon. *Mycosphere, 2,* 179–188.
24. Smith, B. J., Sivasithamparam, K. (2003). Morphological studies of *Ganoderma* (Ganodermataceae) from the Australasian and Pacific region. *Australian Systematic Botany, 16(4),* 487–503. DOI 10.1071/SB02001.
25. Steyaert, R. L. (1972). Species of *Ganoderma* and related genera mainly of the Bogor and Leiden Herbaria. *Persoonia, 7,* 55–118.
26. Ryvarden, L., Gilbertson, R. L. (1993). European polypores 1. *Synopsis Fungorum, 6,* 1–387.
27. Gilbertson, R. L., Ryvarden, L. (1986). *North American Polypores*, *1,* Oslo: Fungiflora,
28. Tchoumi, J. M. T., Coetzee, M. P. A., Rajchenberg, M., Wingfield, M. J., Roux, J. (2018). Three *Ganoderma* species, including *Ganoderma dunense* sp. nov., associated with dying *Acacia Cyclops* trees in South Africa. *Australasian Plant Pathology, 47(4),* 431–447. DOI 10.1007/s13313-018-0575-7.
29. Xing, J. H., Sun, Y. F., Han, Y. L., Cui, B. K., Dai, Y. C. (2018). Morphological and molecular identification of two new *Ganoderma* species on *Casuarina equisetifolia* from China. *MycoKeys, 34,* 93–108. DOI 10.3897/mycokeys.34.22593.
30. Zhao, J. D., Xu, L. W., Zhang, X. Q. (1979). Taxonomic studies on the subfamily Ganodermataceae of China III. *Acta Mycologica Sinica, 19,* 265–279.
31. Wang, D. M., Yao, Y. J. (2005). Intrastrain internal transcribed spacer heterogeneity in *Ganoderma* species. *Canadian Journal of Microbiology, 51(2),* 113–121. DOI 10.1139/w04-118.
32. Li, T. H., Hu, H. P., Deng, W. Q., Wu, S. H., Wang, D. M. et al. (2015). *Ganoderma leucocontextum*, a new member of the G. lucidum complex from southwestern China. *Mycoscience, 56(1),* 81–85. DOI 10.1016/j.myc.2014.03.005.
33. Zhou, L. W., Cao, Y., Wu, S. H., Vlasak, J., Li, W. D. et al. (2015). Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. *Phytochemistry, 114,* 7–15. DOI 10.1016/j.phytochem.2014.09.023.
34. Liu, P. G., Wan, X. H., Yu, F. Q., Chen, J., Tian, X. F. et al. (2009). Fungous kingdom: Yunnan of China and her Ectomycorrhizal macrofungi species diversity. *Acta Botanica Yunnanica, 16,* 15–20.
35. Teng, S. C. (1934). Notes on Polyporaceae from China. *Sinensia, 5,* 198–200.
36. Yang, Z., Feng, B. (2013). What is the Chinese Lingzhi?—a taxonomic mini-review. *Mycology, 4,* 1–4.
37. Chang, S. T., Buswell, J., Chiu, S. W. (1993). *The macrofungus flora of China's Guangdong Province*. Hong Kong: Chinese University Press.
38. Luangharn, T., Karunarathna, S. C., Khan, S., Xu, J. C., Mortimer, P. E. et al. (2017). Antibacterial activity, optimal culture conditions and cultivation of the medicinal *Ganoderma australe*, new to Thailand. *Mycosphere, 8(8),* 1108–1123. DOI 10.5943/mycosphere/8/8/11.

39. Lodge, D. J., Ammirati, J., O'Dell, T. E., Mueller, G. M. (2004). Collecting and describing macrofungi: inventory and monitoring methods. In: Mueller, G. M., Bills, G., Foster, M. S. (Eds.), *Biodiversity of Fungi: Inventory and Monitoring Methods*. pp. 128–158. Elsevier Academic Press, San Diego, CA.
40. Ridgeway, R. (1912). *Color standards and color nomenclature*. Ridgeway: United State.
41. Largent, D. L. (1986). *How to identify mushrooms to genus*. Eureka, CA : Mad River Press.
42. Kreisel, H., Schauer, F. (1987). *Methoden des mykologischen Laboratoriums veb*. Jena: Gustav. Fischer Verlag.
43. Miettinen, O., Larsson, K. H. (2006). *Trechispora elongata* species nova from North Europe. *Mycotaxon, 96,* 193–198.
44. White, T. J., Bruns, T., Lee, S., Taylor, J. W. (1990). *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. New York: Academic Press.
45. Jayasiri, S. C., Hyde, K. D., Ariyawansa, H. A., Bhat, J., Buyck, B. et al. (2015). The faces of fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity, 74(1),* 3–18. DOI 10.1007/s13225-015-0351-8.
46. Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41,* 95–98.
47. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25(24),* 4876–4882. DOI 10.1093/nar/25.24.4876.
48. Swofford, D. L. (2002). *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0 beta version*. Sunderland, MA: Sinauer Associates.
49. Miller, M. A., Pfeiffer, W., Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, Los Angeles.*
50. Stamatakis, A. (2006). RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics, 22(21),* 2688–2690. DOI 10.1093/bioinformatics/btl446.
51. Nylander, J. A. A. (2004). *MrModeltest v.2 Program distributed by the author.* Sweden: Uppsala University.
52. Zhaxybayeva, O., Gogarten, J. P. (2002). Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics, 3(1),* 1–4. DOI 10.1186/1471-2164-3-4.
53. Huelsenbeck, J. P., Ronquist, F. (2001). MRBAYES: bayesian inference of phylogenetic trees. *Bioinformatics, 17 (8),* 754–755. DOI 10.1093/bioinformatics/17.8.754.
54. Rambaut, A., Suchard, M. A., Xie, D., Drummond, A. J. (2019), *Tracer v. 16.,* http://tree.bio.ed.ac.uk/software/tracer/.
55. Larget, B., Simon, D. L. (1999). Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution, 16(6),* 750–759. DOI 10.1093/oxfordjournals.molbev.a026160.
56. Rambaut, A., Drummond, A. (2008). *FigTree: tree figure drawing tool*. 12 edition, Institute of Evolutionary Biology, University of Edinburgh, United Kingdom.
57. Jargalmaa, S., Eimes, J. A., Park, M. S., Park, J. Y., Oh, S. Y. et al. (2017). Taxonomic evaluation of selected *Ganoderma* species and database sequence validation. *PeerJ, 5,* 1–16. DOI 10.7717/peerj.3596.
58. Saccardo, P. A., Polyporeae, Hydneae, Thelephoreae (1888). Sylloge Hymenomycetum, Clavarieae. *Tremellineae Sylloge Fungorum, II,* 156.
59. Zhao, J. D. (1989). The Ganodermataceae in China. *Bibliotheca Mycologica, 132,* 1–176.
60. Hseu, R. S. (1990). *An identification system for cultures of Ganoderma species, (Ph.D. Thesis)*. National Taiwan University, Taiwan.
61. Kaliyaperumal, M., Kalaichelvan, P. T. (2007). *Ganoderma austral* from southern India. *Microbiological Research, 163(3),* 286–292. DOI 10.1016/j.micres.2007.01.003.
62. Dai, Y. C., Cui, B. K., Yuan, H. S., He, S. H., Wei, Y. L. et al. (2011). Wood-inhabiting fungi in southern China. 4. Polypores from Hainan Province. *Annales Botanici Fennici, 48(3),* 219–231. DOI 10.5735/085.048.0302.

63. Hapuarachchi, K. K., Karunarathna, S. C., Raspé, O., De Silva, K. H. W. L., Thawthong, A. et al. (2018). High diversity of *Ganoderma* and *Amauroderma* (Ganodermataceae, Polyporales) in Hainan Island. *China Mycosphere, 9(5),* 931–982. DOI 10.5943/mycosphere/9/5/1.
64. Glen, M., Bougher, N. L., Francis, A. A., Nigg, S. Q., Lee, S. S. et al. (2009). *Ganoderma* and *Amauroderma* species associated with root-rot disease of *Acacia mangium* plantation trees in Indonesia and Malaysia. *Australasian Plant Pathology, 38(4),* 345–356. DOI 10.1071/AP09008.
65. Castellano, M. A., Trappe, J. M., Luoma, D. L. (2004). *Sequestrate fungi*. London: Elsevier Academic Press.
66. Imazeki, R. (1939). Studies on *Ganoderma* of Nippon. *Bulletin of the National Science Museum Tokyo, 1,* 29–52.
67. Ayissi, B. M. K., Mossebo, D. C. (2014). Some noteworthy taxonomic variations in the complex wood decayer *Ganoderma resinaceum* (Basidiomycota) with reference to collections from tropical Africa. *Kew Bulletin, 69,* 1–14.
68. Kim, C. S., Jo, J. W., Kwag, Y. N., Sung, G. H., Lee, S. et al. (2015). Mushroom flora of Ulleung-gun and a newly recorded Bovista species in the republic of Korea. *Mycobiology, 43(3),* 239–257. DOI 10.5941/MYCO.2015.43.3.239.
69. Pilotti, C. A., Sanderson, F. R., Aitken, A. B., Armstrong, W. (2004). Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. *Mycopathologia, 158(2),* 251–265. DOI 10.1023/B:MYCO.0000041833.41085.6f.
70. Garibova, L. V., Antimonova, A. V., Zavyalova, L. A., Krasnopolskaya, L. M. (2003). Growth and morphological features of *Ganoderma lucidum* mycelium depending on culture conditions. *Mikologiya I Fitopatologiya, 37(3),* 14–19.
71. Wang, X. C., Xi, R. J., Wang, D. M., Yao, Y. J. (2012). The species identity of the widely cultivated *Ganoderma*, G. lucidum (Lingzhi) in China. *PLoS One, 7(7),* 1–12.
72. Moncalvo, J. M., Wang, H. F., Hseu, R. S. (1995). Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences: comparison with traditional taxonomic characters. *Mycological Research, 99(12),* 1489–1499. DOI 10.1016/S0953-7562(09)80798-3.
73. Gottlieb, A. M., Ferrer, E., Wright, J. E. (2000). rDNA analyses as an aid to the taxonomy of species of *Ganoderma*. *Mycological Research, 104(9),* 1033–1045. DOI 10.1017/S095375620000304X.