MYCOBIOLOGY
2024, VOL. 52, NO. 1, 58–67
https://doi.org/10.1080/12298093.2024.2306012

RESEARCH ARTICLE

OPEN ACCESS Check for updates

A New Species of *Ganoderma* (Ganodermataceae, Polyporales) from Southern China and Optimum Condition for Mycelia Production

Qiu-Lu Wei[a], Hai-Fu Zheng[b], Yuan-Yuan Shao[c], Usman Rasheed[a], Jian-Tian Lin[a], Fu-Chang Huang[a] and Bin Liu[a]

[a]Institute of Applied Microbiology, College of Agriculture, Guangxi University, Nanning, China; [b]Guangxi Forest Inventory & Planning Institute, Nanning, China; [c]Key Laboratory of Beibu Gulf Environment Change and Resources Utilization, Ministry of Education, Nanning Normal University, Nanning, China

ABSTRACT

The present study sought to propose *Ganoderma guixiense* sp. nov. as a new species based on phenotypic and genotypic evidence. Phylogenetic analyses were carried out based on the internal transcribed spacer (ITS), the large subunit of nuclear ribosomal RNA gene (nLSU), and the second subunit of RNA polymerase II (RPB2) sequence data. *G. guixiense* has been characterized by pileate basidiomata, long stipe, in addition to reddish-black zonate pileal surface. Basidiospores are broadly ellipsoid with one end tapering at maturity, and measuring 9–12.8 × 6.5–9.3 µm. Basidia are oval to subglobose. This study marks the first exploration of the biological characteristics of *G. guixiense*. The result indicated that the optimal medium of mycelial growth was observed on malt extract agar (MEA) and yeast extract peptone dextrose agar (YPD) while the optimal temperature was found to be 25–30 °C with pH range of 6–7.

ARTICLE HISTORY
Received 20 July 2023
Revised 30 October 2023
Accepted 11 January 2024

KEYWORDS
Basidiomycota; phylogeny; taxonomy; biological characteristics

1. Introduction

Ganoderma P. Karst was initially established by Karsten (1) and it was included in Polyporaceae. Up until the 1948s, Ganodermataceae was proposed by Donk (2). Meanwhile, *Ganoderma* with most rich species was included into the family. Up to now, there are 313 records from the genus in Index Fungorum (http://www.indexfungorum.org/; accessed date: July 15 2023) and 479 records in MycoBank (http://www.mycobank.org/; accessed date: July 15 2023). *Ganoderma* is characterized by distinctive features, double-walled, columnar or coronal ornamented endospore on the walls, and truncated colored apically basidiospores (3). Although it is a cosmopolitan genus, its macromorphology varies greatly in different environments, whereas their micromorphological characters remain the same. Therefore, there are nearly half of the species belonging to this genus are treated as synonyms (4,5). This scenario made taxonomic characterization of this genus more challenging.

As early as in 1994, Ryvarden and Gilbertson (6) found that changes in morphological characteristics of *Ganoderma* were difficult to define by traditional morphological taxonomy through studying the color, shape, and size of fruiting bodies, microcosms of basidia, host morphology, and mycelium arrangement while studying the composition of 53 *Ganoderma* collected specimens. With the development of molecular biology, species identification has become more accurate thereby filling the gaps left by morphological studies. Moncalvo et al. (7) studied the role of ITS and nLSU D2 region sequence for the classification of *Ganoderma* and provided the evidence that nLSU sequence was suitable for the identification of different genera (7), while ITS was suitable for the identification of inter-species within *Ganoderma*. Liu et al. (8) studied phylogenetic relationship of phylum of ascomycota family and showed that a slowly evolving protein-coding gene such as RPB2 was conductive for diagnosing phylogenetic relationships among fungi. Therefore, in our study, three genes of ITS, nLSU, and RPB2 were used to study the phylogeny of *Ganoderma* (8).

At present, *Ganoderma* is widely distributed in the tropics and temperate areas (4). However, the diversity of *Ganoderma* species is also abundant in China and more than 40 species have previously been reported (5,9–16). Guangxi is rich in *Ganoderma* diversity, accounting for about 30% of the total *Ganoderma* diversity in the country (17). Two new species namely *G. bubalinomarginatum* and *G. guangxiense* were collected from Guangxi, China and reported in 2022.

CONTACT Fu-Chang Huang hfch5685@126.com; Bin Liu liubin@gxu.edu.cn

However, there are still numerous resources of *Ganoderma* to be explored in Guangxi, China.

In this study, a new species of *Ganoderma* from southern China has been described and illustrated based on morphological identification and phylogenetic analysis, meanwhile conditions for the mycelium growth were optimized.

2. Materials and methods

2.1. Sample collection and culture obtain

Three specimens were collected from White-headed Langur Nature Reserve, Chongzuo, China, which is located at a subtropical humid monsoon area. One specimen was preserved in silicone; the other were dried and stored in the Herbarium of Edible Fungi Research Institute of Guangxi University. Tissues samples of the specimen were aseptically isolated by sterilized forceps and scalpel, from the cap or stipe and transferred onto potato dextrose agar (PDA). Agar plates were incubated at 25 °C under dark conditions to attain pure culture which were preserved for further studies (18).

2.2. Morphological studies

Color descriptions followed the method by Ridgeway (19). Microscopic characteristics were obtained by cutting, separating, and sectioning dry specimens. Observation was carried out by a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan). Small pieces of tissue from each part of the specimens were cut using a blade according to the content to be observed. Samples were placed on a slide to which 5% KOH, or 1–3% Congo Red, or Melzer's reagent was added for highlighting all tissues. Basidiospores, basidia, hyphal system, size, color, and shape were observed, recorded and photographed. Measurements were taken using Spot 32 software.

The following abbreviations are used: IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = Cyanophilous (15). The equation to measure basidiospore size is given as (a) b–c (d), where "a" represents the minimum, "d" represents the maximum, "b" and "c" covers a minimum of 90% of the values, *L* = mean length, *W* = mean width, Qm = length–width ratio (10,20).

2.3. DNA extraction, PCR amplification, and sequencing

The dry specimens were pulverized with Automatic sample rapid grinding instrument JXFSTPRP-24L and DNA was extracted using Omega kit. The genes ITS, nLSU, and RPB2 were amplified by polymerase chain reaction (PCR) technique. The primers ITS1/ITS4 were used to amplify the internal transcribed spacer (ITS) rDNA region. Primers LR0R/LR5 were used for PCR amplification of the ribosomal large subunit 28S rDNA gene (nLSU), TRPB2-5f/TRPB2-7cR for the second largest subunit region of some RNA polymerases (RPB2) (8,21,22). PCR reactions (25 µL) contained mixture: The PCR reaction mixture (25 µL) contained 12.5 µL Taq DNA polymerase 1 µL forward primer, 1 µL reverse primer, 1 µL DNA template, and 9.5 µL double distilled water. The PCR program was as follows: initial denaturation of 94 °C for 5 min, followed by 30 cycles at 94 °C for 40 s, 56 °C for 40 s, and 72 °C for 1 min, as well as extension at 72 °C for 10 min and keeping warm at 4 °C. Amplified PCR products were verified by 1% agarose gel electrophoresis stained with ethidium bromide in 1 × TAE. The PCR products were directly purified and sequenced by Beijing Genomics Institute (BGI, Shenzhen, China). The sequences were deposited at GenBank to obtain the accession number.

2.4. Phylogenetic analysis

Sequences of samples were obtained from sequencing companies and standard BLAST searches were performed on GenBank to determine the primary identity of fungal isolates. In addition to the sequences generated in this study, other reference sequences were selected from GenBank for phylogenetic analyses (Table 1). Among them, 35 sequences of ITS, six sequences of nLSU, and 22 sequences of RPB2 were downloaded from GenBank. *Amauroderma rugosum* Cui 9011 was treated as outgroup. The sample sequences were first edited by ContigExpress, and then multi-sequence alignment was performed with all the downloaded sequences by MEGA X software to optimize the alignment (23). Model prediction was performed using Model in Mega followed by maximum parsimony (MP) analysis using PAUP V.4.0 B10 (24). The best-fit evolutionary model of alignment was determined by JMODELTEST 2.3 (25). BI algorithm was conducted using MRBAYES V.3.2.2 (26). The number of generations was set at 2,000,000 with trees being sampled every 1000 generations (a total of 2000 trees), the resulting in an average standard deviation of split frequencies below 0.01 (27).

Phylogenetic trees and datasets were shown in FIGTREE V. 1.4.3 (28), edited using TREEGRAPH

Table 1. Species, specimens, geographic origin, and GenBank accession numbers of sequences treated in the present study.

Species	Voucher/strain	Origin	ITS	nLSU	RPB2	Reference
Amauroderma rugosum	Cui9011	Liaoning, China	KJ531664	–	–	(36)
Ganoderma adspersum	GACP15061220	Thailand	MK345425	–	MK371437	(30)
G. adspersum	SFC20141001-22	Korea	KY364252	–	KY393271	(37)
G. australe	K(M)120828	UK	AY884183	–	–	(38)
G. guixiense	**GXU3457 (holotype)**	**Guangxi, China**	**OQ788244**	**OQ788236**	**PP187389**	**This study**
G. guixiense	**GXU3709**	**Guangxi, China**	**OR271986**	**OR287443**	**–**	**This study**
G. guixiense	**GXU4317**	**Guangxi, China**	**OQ788243**	**OQ788235**	**–**	**This study**
G. curtisii	CBS 100131	NC, USA	JQ781848	–	KJ143966	(39)
G. curtisii	CBS 100132	NC, USA	JQ781849	–	KJ143967	(39)
G. dianzhongense	L4331	Yunnan, China	MW750237	–	MZ467043	(10)
G. dianzhongense	L4737	Yunnan, China	MW750238	–	MW839000	(10)
G. dianzhongense	L4759	Yunnan, China	MW750239	–	MW839001	(10)
G. dianzhongense	L4969	Yunnan, China	MW750240	–	MZ467044	(10)
G. gibbosum	MN14091109	Thailand	MK345435	–	–	(30)
G. gibbosum	SPC2	Colombia	KU569547	KU570946	–	(40)
G. gibbosum	UB1	Colombia	KU569556	KU570954	–	(40)
G. heohnelianum	Cui13982	Guangxi, China	MG279178	–	MG367515	(41)
G. heohnelianum	Dai11995	Yunnan, China	KU219988	–	MG367497	(41)
G. lingzhi	Cui 9166	China	KJ143907	–	JX029978	(42)
G. lingzhi	Dai12574	Liaoning, China	KJ143908	–	JX029981	(42)
G. lucidum	K175217	UK	KJ143911	–	KJ143971	(39)
G. lucidum	MT26/10	Czech Republic	KJ143912	–	–	(39)
G. multipileum	CWN04670	Taiwan, China	KJ143913	–	KJ143972	(39)
G. multipileum	Dai 9447	Hainan, China	KJ143914	–	KJ143973	(39)
G. multiplicatum	URM83346	Brazil	JX310823	JX310837	–	(40)
G. neojaponicum	FFPRI WD-1285	Tokyo, Japan	MN957784	–	–	(14)
G. neojaponicum	FFPRI WD-1532	Chiba, Japan	MN957785	–	–	(14)
G. pfeifferi	JV 0511/11	Unknown	KF605660	–	–	GenBank
G. pfeifferi	K(M)120818	UK	AY884185	–	–	GenBank
G. resinaceum	HMAS86599	UK	AY884177	–	JF915435	(43)
G. resinaceum	HSBU200830	Iran	KT343303	–	–	GenBank
G. sessile	111TX	USA	MG654306	–	MG754866	(44)
G. sessile	113FL	USA	MG654307	–	MG754867	(44)
G. shanxiense	BJTCFM423 (type)	Shanxi, China	MK764268	–	MK783940	(12)
G. shanxiense	Dai18921	China	MZ354909	MZ355044	MZ345740	(5)
G. sichuanense	HMAS42798 (type)	China	JQ781877	–	–	(42)
G. weberianum	CBS:128581	Taiwan	MK603805	MH876427	MK611971	(45)
G. weberianum	CBS:219.36	Philippines	MK603804	MH867289	MK611972	(45)

V.2.4.0 and MICROSOFT OFFICE POWERPOIT 2010 (29). The MP bootstrap values, equal to or greater than 50%, and the Bayesian posterior probability (BPP) equal to or greater than 0.95, are indicated above each node (Figure 1).

2.5. Optimal conditions for mycelial growth

Optimal medium was screened according to the method described by Luangharn et al. (30) with some modifications. Briefly, six different agar media were chosen to screen the optimal medium for the new strain: Czapek's agar (CZA), PDA, yeast extract peptone dextrose agar (YPD), yeast malt extract agar (YMA), malt extract agar (MEA), and martin broth modified (MBM). All Petri dishes containing culture medium dishes were incubated at the value of pH 6, 28 °C in the dark. The optimal culture medium was used to screen the optimal pH value and temperature. pH of the optimized media was adjusted to 4.5, 5, 6, 7, 8, 9, and 10, with 1 mol/L HCl or 1 mol/L NaOH at 28 °C in the dark. The growth of the new strain was evaluated in darkness at 15 °C, 20 °C, 25 °C, 28 °C, 30 °C and 35 °C at the value of pH 6. All inoculations were 9 mm in diameter and placed in the center of the plate (18).

The diameter of colonies (mm) was measured from the second day to the sixth day; average vertical and horizontal lengths of colonies were calculated. Characteristics of colonies were recorded according to Qian et al. (31), as follows: mycelial densities were very rare (+), rare (++), moderate (+++), somewhat abundant (++++), and abundant (+++++). The optimal conditions, growth rates and hyphal densities were tested in five replications.

Statistical program SPSS was employed to analyze the data inferred from the experiments (Softonic International SA, Barcelona, Spain). All means were compared using Tukey's test ($p < .05$ and $p < .01$) and then mean separation was performed using post hoc test (30). Results were expressed in one-way analysis of variance (ANOVA).

3. Results

3.1. Phylogenetic analysis

The datasets consisted of ITS, nLSU, and RPB2 genes and contained 1577 characters in total, ITS

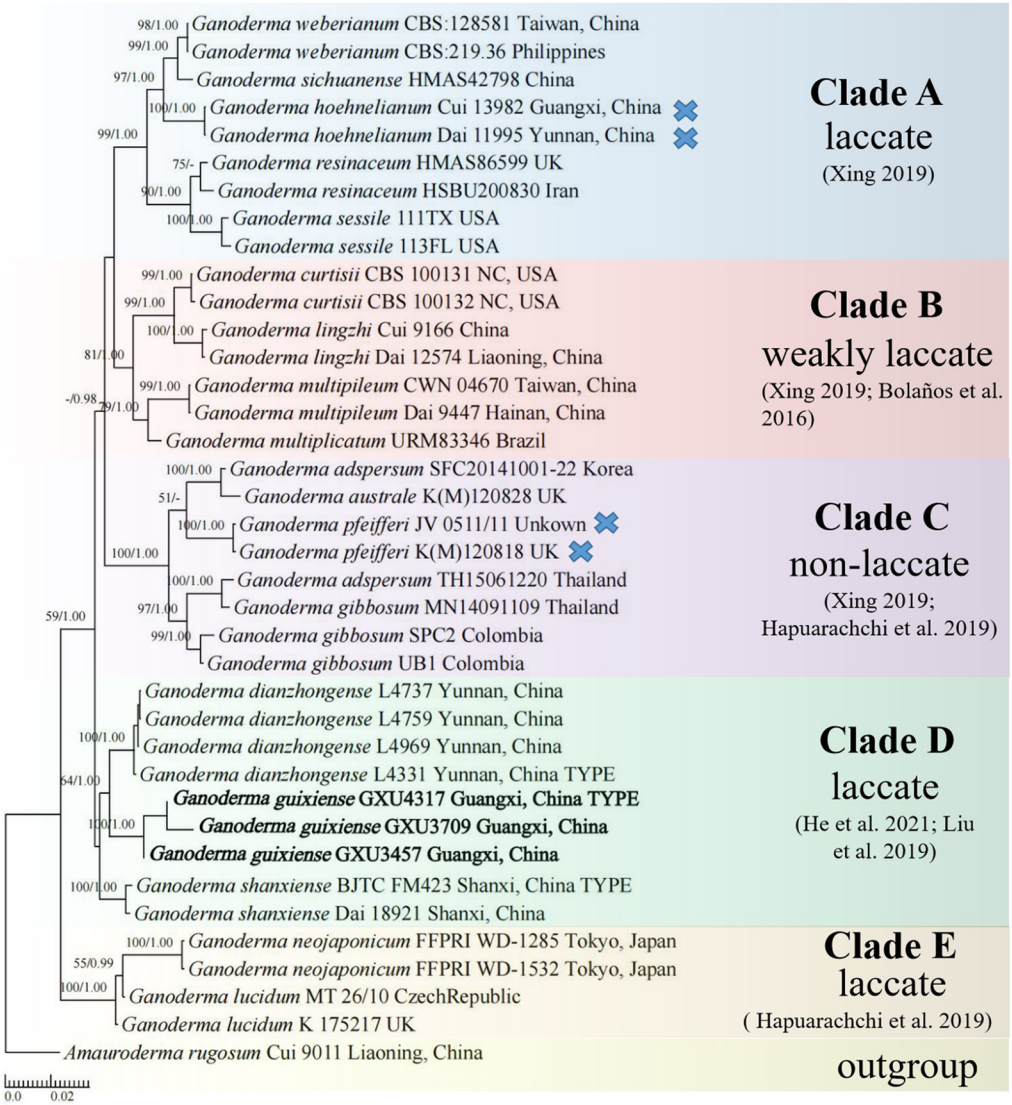

Figure 1. Phylogenetic tree (Bayesian tree) obtained from the DNA sequence data of LSU, ITS, and RPB2 datasets. Bootstrap values from maximum parsimony (MP, left) greater than 50% and Bayesian posterior probabilities (BPPs, right) greater than 0.95 are indicated above the nodes as MPBS/PP. The tree is rooted with *Amauroderma rugosum* Cui 9011. Newly recorded species are indicated in bold. Clades of phylogenetic tree with different colors indicated that those species had laccate or not, and references were noted below. The X-mark indicated that the species didn't possess this characteristic.

(1–549 bp), nLSU (550–1230 bp) in addition to RPB2 (1231–1577 bp). Phylogenetic analyses included 38 taxa with *Amauroderma rugosum* Cui 9011 as outgroup. The best model for the three genes combination datasets was found to be TrNef + I + G, lset nst = 6, Rates = gamma, prset statefreqpr = dirichlet (1,1,1,1), –In*L* = 4834.6710. The base rate was as follows: AC = 1.0000, AG = 4.3406, AT = 1.0000, CG = 1.0000, CT = 7.5139, GT = 1.0000, gamma distribution shape: α = 0.6160. The average standard deviation of split frequencies of Bayesian's analysis remained 0.001320. Phylogenetic analysis showed that trees from MP and BI had the same topological structures, and it only showed the trees built by the Bayesian inference. Bootstrap support values with MP greater than 50%, and BPPs greater than 0.95 are given above the nodes (Figure 1).

The phylogenetic tree showed that the new species was closely related to *G. shanxiense* and *G. dianzhongense*, and clustered together with the latter with a high bootstrap support (MP–BS = 64%, BPP = 1.00, Figure 1).

4. Taxonomy

4.1. *Ganoderma guixiense* Q.L. Wei & H.F. Zheng & F.C. Huang & Bin Liu, sp. nov.

MycoBank: MB851810.

Figures 2,3.

Diagnosis. G. guixiense is characterized by its greatly different basidiomata and pileus, reddish to reddish-black pileal surface, broadly ellipsoid with one end tapering basidiospores, clavate to cylindrical with granules at the apex pileipellis cells.

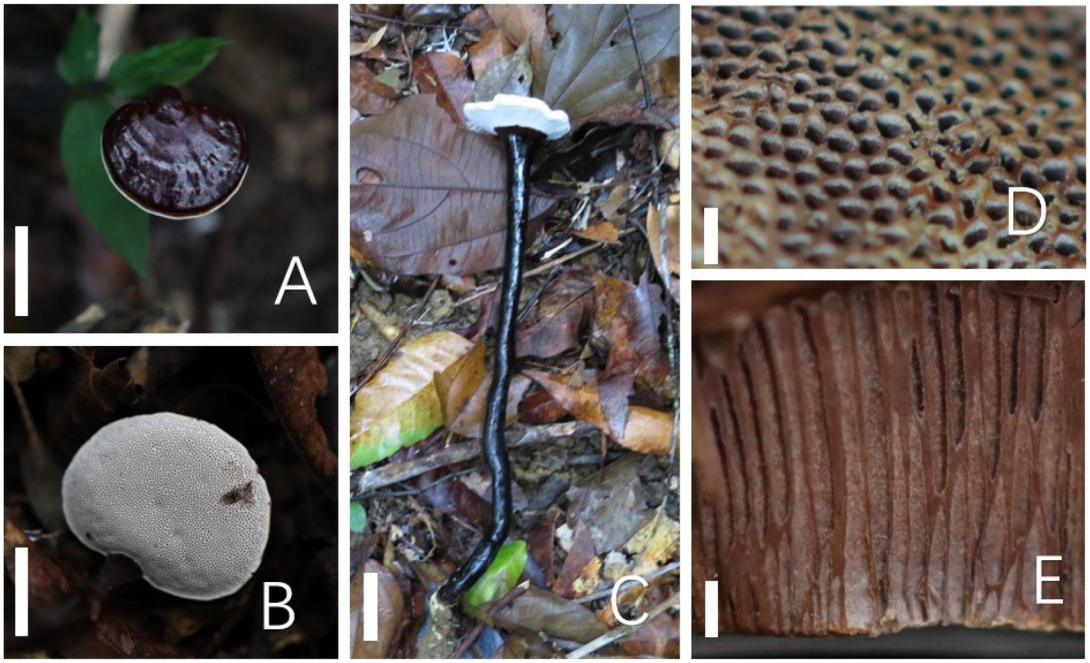

Figure 2. Basidiomata of *Ganoderma guixiense*. (A–C) Basidiomata; (D–E) pore characteristics. Scale bars: (A, B) 1 cm; (C) 4 cm; (D, E) 1 mm. Photos by: Hai-Fu Zheng, Jian-Tian Lin, and Qiu-Lu Wei.

Etymology. The epithet "guixi" refers to the place where it was collected.

Ecology and distribution. Known from White-headed Langur Nature Reserve, Chongzuo City, and Guangxi Zhuang Autonomous Region. GXU3457 (Holotype), GXU4317, GXU3709.

Description. Basidiomata annual, pileate, stipitate, corky to hard corky. *Pileus* solitary, glossy, spathulate to suborbicular, up to 0.8–3.5 × 0.8–4.3 cm, 0.3–0.7 cm thick at the base, strongly laccate, occasionally appears shallow sulcate on upper surface, shallow zonate, reddish to reddish black when fresh, turning slightly dark when dry. *Margin* slightly obtuse, cream when juvenile, becoming reddish black at maturity, concolorous with the pileus, entire. *Stipe* up to 6.5–28 cm long, cylindrical, mesopodal to lateral, strongly laccate, concolorous with the pilei, reddish black, fibrous to woody. Parts of the stipe buried in the soil are light yellow. *Context* up to 0.2 cm thick, composed of coarse loose fibrils, light brown to dark brown, without black melanoid line. *Tube* 0.2–0.6 cm long, hard corky to woody, pale brown, concolorous with the context or little lighter than that of context, non-stratified. *Pore* 3–4 per mm, circular or angular; dissepiments thin, entire. *Pore surface* white when fresh, turning brown to dark brown when scratched or bruised, turning yellowish white when dry. *Sterile margin* distinct, 0.4 cm wide, milky white when fresh, turning dark brown when dry.

Hyphal system trimitic: Generative hyphae from tubes 0.6–2.3 µm diameter, colorless, thin-walled, with clamp connections; skeletal hyphae from tubes 2.1–6.4 µm diameter, subthick-walled, non-septate, with few branches, yellowish to golden-yellow; binding hyphae from tubes 0.9–2.6 µm diameter, thin-walled, frequently branched, interwoven, colorless to yellowish, septate; generative hyphae from context colorless, thin-walled, with clamp connections, 2.2–3.3 µm diameter; skeletal hyphae from context yellowish, thick-walled, non-septate, arboriform with few branches, 3–6.1 µm diameter; binding hyphae from context thin-walled, colorless to yellowish, branches, septate, 0.9–2.0 µm diameter all the hyphae IKI–, CB+; tissues darkening in KOH. *Pileipellis* cells (16.4–)18.3–29.4(–35.1) × (5.7–)6.8–11.6(–14.3) µm, L = 23.16 µm, W = 8.9 µm, Qm = 2.6 (n = 40), clavate to cylindrical, thick-walled, with granulations in the apex, golden-yellow to yellowish-brown, moderately amyloid at maturity, forming a regular palisade. *Basidiospores* mostly melon seed shaped when juvenile, broadly ellipsoid and usually with one end tapering at maturity, as well as with apical germ pore, untruncated, yellowish to medium brown, IKI–, CB+, double-walled, exospore wall smooth, endospore wall with coarse interwall pillars, (6.8–)7.0–9.5(–9.7) × (5.2–)5.4–7.2(–7.5) µm, L = 8.08 µm, W = 6.48 µm, Qm = 1.25 (n = 40) (excluding myxosporium); (8.9–)9–12.8(–13.0) × (6.0–)6.5–9.3(–9.6) µm, L = 11.14 µm, W = 8.38 µm, Qm = 1.33 (n = 40) (including myxosporium). *Basidia* oval to subglobose, with two to four sterigmata, (11.8–)12.4–23.2(–26.2) × (9.5–)10.7–17.8(–17.9) µm, L = 16.31 µm, W = 13.62 µm, Qm = 1.2 (n = 50) (Figures 2,3).

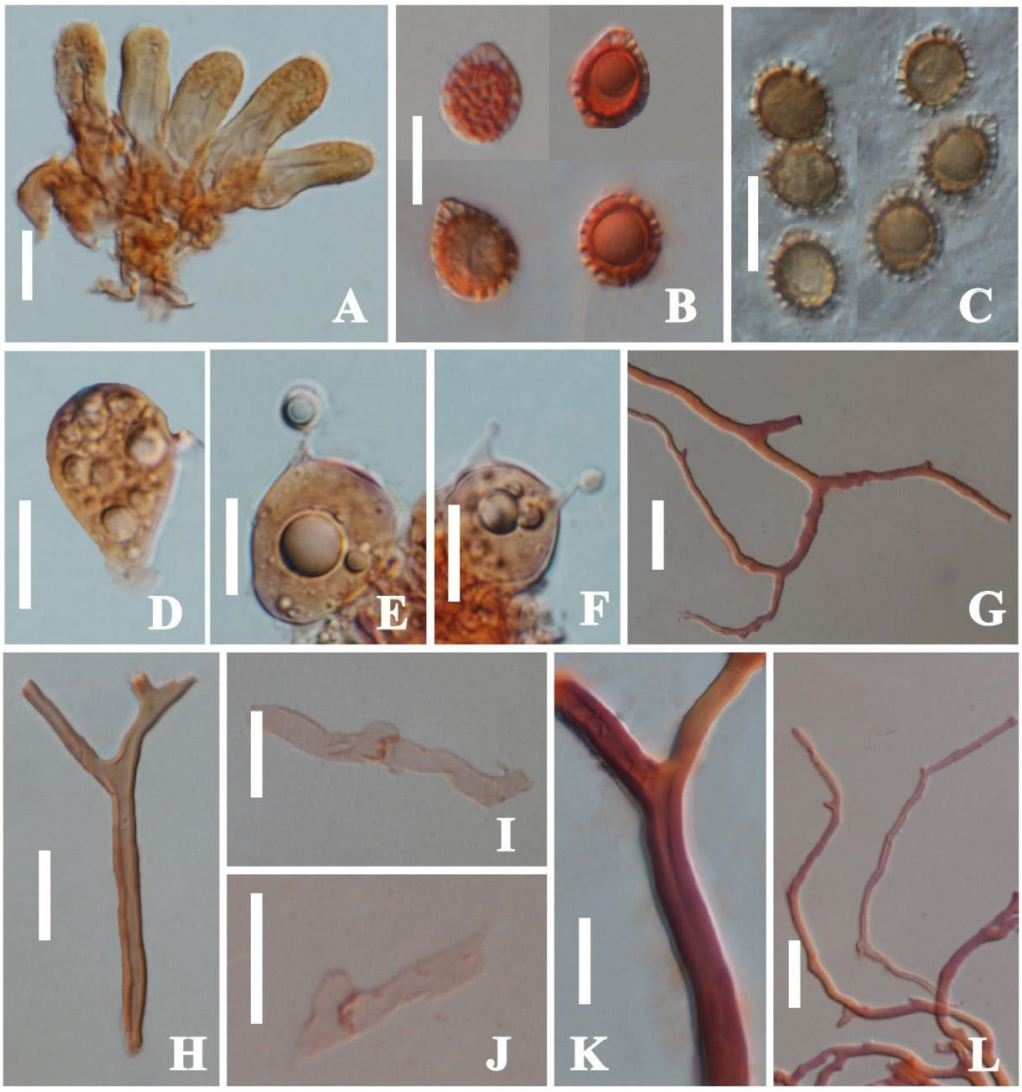

Figure 3. *Ganoderma guixiense* (A) sections of pileipellis; (B, C) Basidiospores; (D) Basidioles. (E, F) Basidia; (G) binding hyphae from tube; (H) skeletal hyphae from tube; (I) generative hyphae from tube (J) generative hyphae from context; (K) skeletal hyphae from context; (L) binding hyphae from context. Scale bars: (A–L) 10 µm. Photos by: Hai-Fu Zheng and Qiu-Lu Wei.

4.2. Screening optimal conditions for mycelial growth

After six days of incubation at 28 °C on six different Agar medium, the result showed that the mycelial growth rate and colony diameter were superior on PDA where average growth rate 7.08 ± 0.46 mm/day was recorded, surpassing the others media, followed by YPD and MEA, while CZA did not prove to be suitable for the growth of *G. guixiense* (Table 2, Figure 4).

4.3. Optimal pH conditions for mycelial growth

PDA and YPD medium were selected to optimize media pH. Overall, there little difference was observed in the growth rate of mycelium and colony diameter. However, the mycelial growth rate in YPD medium was faster than in PDA. The pH range of 5–7 was the most suitable for the mycelial growth *G. guixiense*. In PDA medium, mycelia grew the fastest at pH 6, 4.55 ± 0.86 mm/day, followed by pH 5 and pH 7, with growth rate of 3.46 ± 0.77 mm/day and 3.59 ± 0.37 mm/day, respectively, while no growth was observed at pH 9. However, in YPD medium, mycelia grew vigorously at pH 5, 5.22 ± 0.33 mm/day, followed by pH 7. Mycelia could not grow when pH was raised to 9. Therefore, it can be concluded that *G. guixiense* requires slightly acidic pH conditions for vigorous mycelial growth. Mycelia growth was better in slightly acidic YPD medium than PDA medium (Tables 3 and 4).

4.4. Optimal temperature conditions for mycelial growth

After six days of incubation at different temperatures in PDA and YPD medium, it was found that the most suitable temperature for the enhanced growth

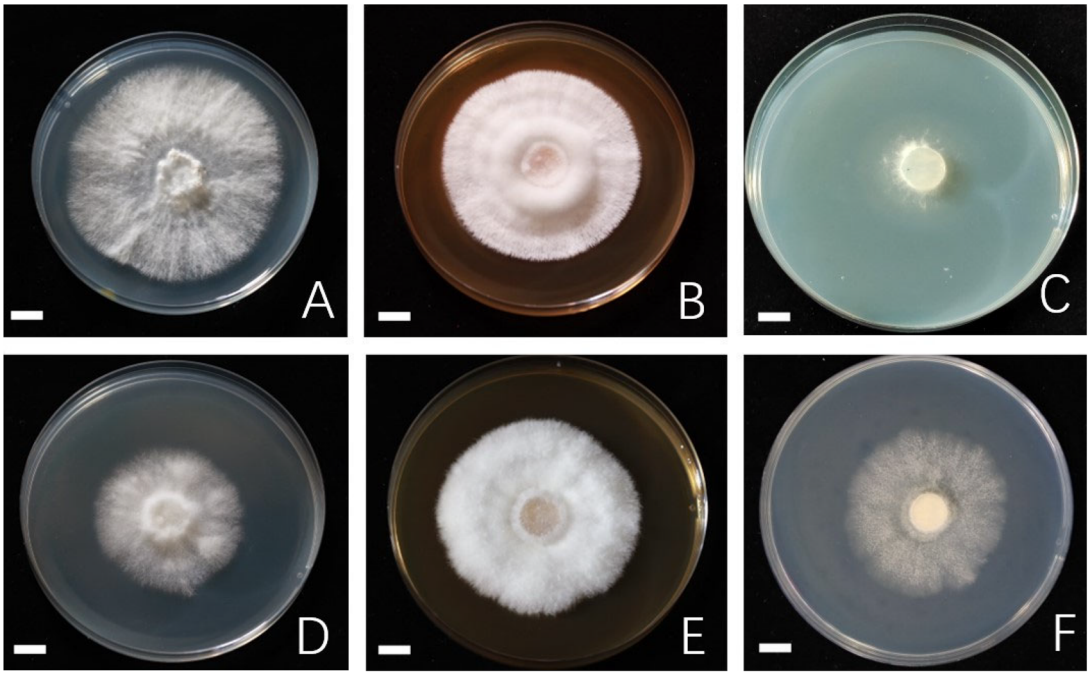

Figure 4. Mycelium growth characteristics of *Ganoderma guixiense* GXU4317 mycelial cultures were incubated at 28 °C for six days on different agar media. (A) Potato dextrose agar (PDA); (B) yeast extract peptone dextrose agar (YPD); (C) Czapek's agar (CZA); (D) Martin broth modified (MBM); (E) Malt extract agar (MEA); (F) beef extract peptone medium (YMA). Scale bars: 1 cm. Photos by: Qiu-Lu Wei.

Table 2. Effect of different medium on mycelial growth.

Agar media	Growth rate (mm/day)	Colony diameter (mm)	Mycelial density
PDA	7.08 ± 0.46^{Aa}	37.6 ± 0.98^{Aa}	++++
YPD	4.79 ± 0.23^{Bb}	30.89 ± 2.26^{Bb}	+++++
YMA	3.09 ± 0.2^{Cc}	22.52 ± 0.94^{Dd}	++
MEA	4.77 ± 0.07^{Bb}	27.28 ± 0.99^{Cc}	+++++
MBM	2.83 ± 0.05^{CcDd}	20.38 ± 0.64^{Ee}	+++
CZA	2.56 ± 0.1^{Dd}	17.73 ± 0.24^{Ff}	+

Lowercase letters indicate the significance level of .05, uppercase letters indicate the significance level of .01, and there is no significant difference between values of the same letters.

Table 3. Effects of different pH conditions on mycelial growth in PDA medium.

pH value	Growth rate (mm/day)	Colony diameter (mm)	Mycelial density
4.5	–	–	–
5	3.46 ± 0.77^{Ab}	21.75 ± 5.21^{Aa}	+++
6	4.55 ± 0.86^{Aa}	23.49 ± 4.26^{Aa}	+++
7	3.59 ± 0.37^{Ab}	21.87 ± 3.93^{Aa}	++
8	3.45 ± 0.23^{Ab}	18.09 ± 3.58^{Ab}	+++
9	–	–	–
10	–	–	–

Lowercase letters indicate the significance level of .05, uppercase letters indicate the significance level of .01, and there is no significant difference between values of the same letters.

rate of *G. guixiense* mycelia was in the range of 25–30 °C. On PDA medium, the growth rate of *G. guixiense* mycelia was greater than 5 mm/day, and increased along with temperature from 15 °C to 30 °C, while the mycelial growth was the slowest at 35 °C. Whereas it was maximum at 25 °C, at the rate of 5.72 ± 0.84 mm/day, followed by 30 °C and 28 °C.

Table 4. Effect of different pH conditions on mycelial growth in YPD medium.

pH value	Growth rate (mm/day)	Colony diameter (mm)	Mycelial density
4.5	4.02 ± 0.26^{Cc}	23.25 ± 0.64^{Bb}	++++
5	5.22 ± 0.33^{Aa}	29.23 ± 4.93^{Aa}	+++
6	4.23 ± 0.16^{BCc}	20.55 ± 0.69^{Bb}	+++
7	4.73 ± 0.34^{ABb}	22.33 ± 2.51^{Bb}	++++
8	4.07 ± 0.3^{Cc}	19.86 ± 2.11^{Bb}	+++++
9	–	–	–
10	–	–	–

Lowercase letters indicate the significance level of .05, uppercase letters indicate the significance level of .01, and there is no significant difference between values of the same letters.

In the case of YPD medium, the fastest mycelial growth rate of *G. guixiense* was at 28 °C, 5.00 ± 1.16 mm/day, followed by 30 °C and 25 °C. Overall, it indicated that the growing rate of mycelium was greatly different at different temperature on PDA. Furthermore, result indicated the variability in optimal growth temperature of *G. guixiense* on different culture media (Tables 5 and 6).

5. Discussion

In the phylogenetic inferences, the phylogenetic tree can be divided into five clades which reflect the morphological and molecular systematical characteristics of *G. guixiense* and related species. Species in clade I have laccate pileal surface in addition to *G. hoehnelianum*, clade II have weakly laccate pileal surface, while clade III have non-laccate pileal

Table 5. Effect of different temperatures on mycelial growth on PDA medium.

Temperature	Growth rate (mm/day)	Colony diameter (mm)	Mycelial density
15 °C	–	–	–
20 °C	1.88 ± 0.26^{Cc}	15.45 ± 0.65^{Dd}	+++
25 °C	5.72 ± 0.84^{Aa}	36.65 ± 3.88^{Aa}	+++++
28 °C	5.56 ± 0.31^{Aa}	30.43 ± 1.89^{Bbc}	+++++
30 °C	5.68 ± 0.05^{Aa}	35.66 ± 0.57^{AaBb}	+++++
35 °C	3.22 ± 0.55^{Bb}	25.47 ± 3.24^{Cc}	++

Lowercase letters indicate the significance level of .05, uppercase letters indicate the significance level of .01, and there is no significant difference between values of the same letters.

Table 6. Effect of different temperatures on mycelial growth on YPD medium.

Temperature	Growth rate (mm/day)	Colony diameter (mm)	Mycelial density
15 °C	–	–	–
20 °C	2.59 ± 0.16^{Cc}	22.78 ± 1.59^{BCbc}	++++
25 °C	3.76 ± 0.59^{BCb}	27.86 ± 2.35^{ABab}	+++++
28 °C	5.00 ± 1.16^{Aa}	30.94 ± 8.96^{Aba}	+++++
30 °C	4.78 ± 0.52^{Aab}	31.31 ± 2.37^{Aa}	+++++
35 °C	2.60 ± 0.30^{Cc}	18.49 ± 1.31^{Cc}	+++

Lowercase letters indicate the significance level of .05, uppercase letters indicate the significance level of .01, and there is no significant difference between values of the same letters.

surface besides *G. pfeifferi* and clade IV have laccate pileal surface. The study showed the same evolutionary trend as Xing (32) and according to phylogenetic tree of this study proposed a novel evolutionary trend from laccate, weakly laccate to non-laccate species.

In the phylogenetic tree, *G. guixiense* clustered in the same clade with *G. dianzhongense* and *G. shanxiense* (Figure 1). Morphologically, *G. guixiense*, *G. dianzhongense* in addition to *G. shanxiense* all have a mesenchymal stem with a cover of nearly circular to kidney-shaped, broadly ellipsoidal spores. *G. dianzhongense* and *G. shanxiense* are distinct from *G. guixiense* because pileus is oxblood red to violet brown and red to red-brown, respectively. Both of the pileipellis cells have no granules at the apex, basidiospores are greater than that of *G. guixiense* in addition to smaller pore (10,12). Although the color of *G. dianzhongense* pileus is more similar to the new species, it is purple cast, and concentrically zonate or azonate more evidently.

In this study, the effects of different medium, pH and temperature on the growth rates of *G. guixiense* mycelial were explored. PDA, MEA, and YPD were determined to be the best medium for the growth of *G. guixiense* mycelial. It is show that glucose as carbon source is conducive to hyphal growth density from PDA, YPD, MBM, and MEA medium. As well as it is concluded that sucrose is not suitable for the mycelium growth of *G. guixiense* from CZA medium. As for nitrogen source, peptone contributes to the mycelium growth of *G. guixiense*, but has little effect on mycelium densily from CZA and YMA. This result is consistent with those reported (30,33). From the results of *G. guixiense* incubated in different media at different pH value, the range of pH adaptation of the mycelia is also different along with changing of medium conditions. PDA and YPD medium were employed to evaluate the optimal pH value of mycelial growth. It was concluded that mycelia grew well in the range of slightly acidic pH conditions. These findings are consistent with other study on biological characteristics of *Ganoderma* (34,35). Derived from the results of *G. guixiense* grown in YPD and PDA medium at different temperatures, it is indicated that there was no difference in the optimal temperature of *G. guixiense* between two media.

In conclusion, morphological characteristics and phylogenetic analysis were used to confirm *G. guixiense* as a new species. The optimal conditions for mycelial growth of *G. guixiense* were studied. PDA and YPD medium were found to be the most favorable for mycelial growth under pH = 5–7. The optimum temperature is 25–30 °C.

6. Identification key to *G. guixiense* sp. nov. and their closest relatives in the combined phylogeny

1. Pileal surface has no laccate 2
2. Pileal surface is strongly laccate or weakly laccate 5
3. Basidiospores ≥9 µm in length in average 3
4. Basidiospores <9 µm in length in average *G. gibbosum*
5. Context with black melanoid line.....4
6. Context without black melanoid line.....*G. adspersum*
7. Basidiospores are subglobular ..... *G. australe*
8. Basidiospores are ellipsoid .....*G. hoehnelianum*
9. Basidiomata is sessile or usually with a distinctly contracted base.....6
10. Basidiomata is stipite 10
11. Pore <5 per mm .....*G. pfeifferi*
12. Pore ≥5 per mm 7
13. Pileipellis cell ≥60 µm in length in mini value.....*G. weberianum*
14. Pileipellis cell <60 µm in length in mini value..8
15. Distributed in South America or North America 9
16. Distributed in Asia .....*G. multipileum*
17. Basidiospores are ellipsoid, not obviously truncated.....*G. multiplicatum*
18. Basidiospores are ellipsoid, truncated when mature.....*G. resinaceum*
19. Context with black melanoid line..... 12
20. Context without black melanoid line..... 15

21. Basidiospores endospore ornamentation is sparse and almost smooth..... *G. sichuanense*
22. Basidiospores endospore ornamentation is densely..... 13
23. Pileal surface is reddish black to brownish black and pore surface is grayish brown .....*G. neojaponicum*
24. Pileal surface is yellow to duff and pore surface is white when juvenile..... 14
25. Pore surface is white and thin dissepiments (40–80 µm) when mature .....*G. curtisii*
26. Pore surface is yellow and thick dissepiment (80–120 µm) when mature ..... *G. lingzhi*
27. First discovered in Europe, Basidiospores are ellipsoid, 7.7–11.5 × 5.2–8.4 µm .....*G. lucidum*
28. First discovered in China, Basidiospores are broadly ellipsoid..... 14
29. Basidiospores >8 µm in width in average.....*G. shanxiense*
30. Basidiospores <8 µm in width in average..... 15
31. Pileal surface red to reddish black, pileipellis cells present granulations in the apex, 16.4–35.1 × 5.7–14.3 µm .....***G. guixiense***
32. Pileal surface oxblood red to violet brown, pileipellis cells without granulations in the apex, 20–45 × 5.5–7.5 µm .....*G. dianzhongense*

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by National Natural Science Foundation of China [No. 31960006]. and GuangXi Forestry Research [2022ZC] No.54.

ORCID

Qiu-Lu Wei http://orcid.org/0009-0001-1725-1506
Hai-Fu Zheng http://orcid.org/0009-0007-7304-461X
Yuan-Yuan Shao http://orcid.org/0009-0002-0808-7983
Usman Rasheed http://orcid.org/0000-0002-6001-299X
Jian-Tian Lin http://orcid.org/0009-0000-0538-4427
Fu-Chang Huang http://orcid.org/0000-0002-2648-9751
Bin Liu http://orcid.org/0000-0001-9345-4674

References

[1] Karsten PA. Enumeratio Boletinearum et Polyporearum Fennicarum, systemate novo dispositarum. Rev Mycol Toulouse. 1881;3:16–19.
[2] Donk MA. Notes on Malesian fungi I. Bull Jard Bot Buitenzorg Sér. 1948;317:473–482.
[3] Moncalvo JM, Ryvarden L. A nomenclatural study of the Ganodermataceae Donk. Synop Fung. 1997;11:1–114.
[4] Cao Y, Yuan HS. *Ganoderma mutabile* sp. nov. from southwestern China based on morphological and molecular data. Mycol Progress. 2013;12(1):121–126. doi: 10.1007/s11557-012-0819-9.
[5] Sun YF, Xing JH, He XL, et al. Species diversity, systematic revision and molecular phylogeny of Ganodermataceae (Polyporales, Basidiomycota) with an emphasis on Chinese collections. Stud Mycol. 2022;101(1):287–415. doi: 10.3114/sim.2022.101.05.
[6] Ryvarden L, Gilbertson RL. European polypores. Synop Fung. 1994;6:1–743.
[7] Moncalvo JM, Wang HH, Hseu RS. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia. 1995;87(2):223–238. doi: 10.2307/3760908.
[8] Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol. 1999;16(12):1799–1808. doi: 10.1093/oxfordjournals.molbev.a026092.
[9] Hapuarachchi KK, Karunarathna SC, Raspé O, et al. High diversity of *Ganoderma* and *Amauroderma* (Ganodermataceae, Polyporales) in Hainan Island, China. Mycosphere. 2018;9(5):931–982. doi: 10.5943/mycosphere/9/5/1.
[10] He J, Luo ZL, Tang SM, et al. Phylogenetic analyses and morphological characters reveal two new species of *Ganoderma* from Yunnan province, China. Mycokeys. 2021;84:141–162. doi: 10.3897/mycokeys.84.69449.
[11] Li TH, Hu HP, Deng WQ, et al. *Ganoderma leucocontextum*, a new member of the *G. lucidum* complex from southwestern China. Mycoscience. 2015;56(1):81–85. doi: 10.1016/j.myc.2014.03.005.
[12] Liu H, Guo LJ, Li SL, et al. *Ganoderma shanxiense*, a new species from Northern China based on morphological and molecular evidence. Phytotaxa. 2019;406(2):129–136. doi: 10.11646/phytotaxa.406.2.
[13] Wang DM, Wu SH, Su CH, et al. *Ganoderma multipileum*, the correct name for "*G. lucidum*" in tropical Asia. Bot Stud. 2009;50:451–458.
[14] Wu SH, Chen CL, Wei CL, et al. *Ganoderma bambusicola* sp. nov. (Polyporales, Basidiomycota) from Southern Asia. Phytotaxa. 2020;456(1):75–85. doi: 10.11646/phytotaxa.456.1.5.
[15] Xing JH, Song J, Decock C, et al. Morphological characters and phylogenetic analysis reveal a new species within the *Ganoderma lucidum* complex from South Africa. Phytotaxa. 2016;266(2):115–124. doi: 10.11646/phytotaxa.266.2.5.
[16] Zhao JD, Zhang XQ. Resources and distribution of Ganodermataceae. Mycosystema. 1992;1:55–62. doi: 10.13346/j.mycosystema.1992.01.010.
[17] Wu XL, Dai YC, Lin LH. Study on the Ganodermataceae of China I. Guizhou Sci. 2004;22(2):27–34.
[18] Luangharn T, Karunarathna SC, Khan S, et al. Antibacterial activity, optimal culture conditions and cultivation of the medicinal *Ganoderma australe*, new to Thailand. Mycosphere. 2017;8(8):1108–1123. doi: 10.5943/mycosphere/8/8/11.

[19] Ridgeway R. Color standards and color nomenclature. Washington (DC): Ridgeway; 1912. p. 12–225.
[20] Zheng HF, Huang FC, Liu B, et al. *Fulvifomes nonggangensis* and *F. tubogeneratus* (*Hymenochaetales*, *Basidiomycota*): two new species from Southern China based on morphological and molecular evidences. Mycobiology. 2021;49(3):213–222. doi: 10.1080/12298093.2021.1932162.
[21] Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 1990;172(8):4238–4246. doi: 10.1128/jb.172.8.4238–4246.1990.
[22] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al., editors. PCR protocols: guide to methods and applications. New York: Academic Press; 1990. p. 315–322. doi: 10.1016/B978-0-12-372180-8.50042-1.
[23] Kumar S, Stecher G, Li M, et al. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547–1549. doi: 10.1093/molbev/msy096.
[24] Swofford DL. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0 beta version. Sunderland (MA): Sinauer Associates; 2002.
[25] Darriba D, Taboada GL, Doallo R, et al. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012;9(8):772–772. doi: 10.1038/nmeth.2109.
[26] Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001;17(8):754–755. doi: 10.1093/bioinformatics/17.8.754.
[27] Larget B, Simon DL. Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol Biol Evol. 1999;16(6):750–759. doi: 10.1093/oxfordjournals.molbev.a026160.
[28] Rambaut A. FigTree version 1.4.3; 2012. Available from: http://tree.bio.ed.ac.uk/software/soft-ware/figtree/
[29] Stöver BC, Müller KF. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinf. 2010;11:7.
[30] Luangharn T, Karunarathna SC, Mortimer PE, et al. A new record of *Ganoderma tropicum* (Basidiomycota, Polyporales) for Thailand and first assessment of optimum conditions for mycelia production. MycoKeys. 2019;51:65–83. doi: 10.3897/mycokeys.51.33513.
[31] Qian K, Wu DM, Wang H, et al. Biological characteristics and antioxidant activity of wild *Ganoderma sichuanense*. Mycosystema. 2022;41(4):601–617. doi: 10.13346/j.mycosystema.210330.
[32] Xing JH. Species diversity, taxonomy and phylogeny of *Ganoderma*. Beijing: Beijing Forestry University; 2019.
[33] Simonic J, Stajic M, Glamoclija J, et al. Optimization of submerged cultivation conditions for extra- and intracellular polysaccharide production by medicinal Lingzhi or Reishi mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae). Int J Med Mushr. 2008;10(4):351–360. doi: 10.1615/IntJMedMushr.v10.i4.80.
[34] Chen XD, Zeng NK, Lan J. Cultural characteristics of mycelia of *Ganoderma gibbosum*. China J Chin Mater Med. 2010;35(15):1939–1942. doi: 10.4268/cjcmm20101505.
[35] Ma B, Wang K, Zhou S, et al. Identification, biological characteristics and lignocellulose-degrading enzyme activity assay of a wild *Ganoderma* sp. strain BSU01. Genomics Appl Biol. 2019;38(3):1109–1116. doi: 10.13417/j.gab.038.001109.
[36] Li MJ, Yuan HS. Type studies on *Amauroderma* species described by J.D. Zhao et al. and the phylogeny of species in China. Mycotaxon. 2015;130:79–89. doi: 10.5248/130.79.
[37] Jargalmaa S, Eimes JA, Park MS, et al. Taxonomic evaluation of selected *Ganoderma* species and database sequence validation. PeerJ 2017;5:e3596. doi: 10.7717/peerj.3596
[38] Arulpandi I, Kalaichelvan PT. *Ganoderma adspersum* and *Ganoderma cupreum* from South India, first report based on molecular phylogeny. Int J Curr Microbiol Appl Sci. 2013;2:693–702.
[39] Zhou LW, Cao Y, Wu SH, et al. Global diversity of the *Ganoderma lucidum* complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. Phytochem. 2015;114:7–15. doi: 10.1016/j.phytochem.2014.09.023.
[40] Bolaños AC, Bononi VLR, Gugliotta AM, et al. New records of *Ganoderma multiplicatum* (Mont.) Pat. (Polyporales, Basidiomycota) from Colombia and its geographic distribution in South America. Check List 2016;12(4):1–7. doi: 10.15560/12.4.1948.
[41] Xing JH, Sun YF, Han YL, et al. Morphological and molecular identification of two new *Ganoderma* species on *Casuarina equisetifolia* from China. MycoKeys 2018;34:93–108. doi: 10.3897/mycokeys.34.22593.
[42] Cao Y, Wu SH, Dai YC. Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi". Fungal Divers. 2012;56:49–62. doi: 10.1007/s13225-012-0178-5.
[43] Wang XC, Xi RJ, Li Y, et al. The species identity of the widely cultivated *Ganoderma*, 'G. lucidum' (Ling-zhi), in China. PLOS ONE 2012;7(7):1-12. doi: 10.1371/journal.pone.0040857.
[44] Loyd AL, Held BW, Barnes CW, et al. Elucidating 'lucidum': distinguishing the diverse laccate *Ganoderma* species of the United States. PLoS One 2018;13(7):e0199738. doi: 10.1371/journal.pone.0199738.
[45] Cabarroi-Hernández M, Villalobos-Arámbula AR, Torres-Torres MG, et al. The *Ganoderma weberianum-resinaceum* lineage: multilocus phylogenetic analysis and morphology confirm *G. mexicanum* and *G. parvulum* in the Neotropics. MycoKeys 2019;59:95–131. doi: 10.3897/mycokeys.59.33182.