Phylogenetic analyses and morphological characters reveal two new species of Ganoderma from Yunnan province, China

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Abstract
Ganoderma dianzhongense sp. nov. and G. esculentum sp. nov. are proposed as two new species based on both phenotypic and genotypic evidences. Ganoderma dianzhongense is characterized by the stipitate basidiomata, laccate and oxblood red pileus, gray white pore surface, duplex context and broadly ellipsoid basidiospores (9.0–12.5 × 6.5–9.0 μm) with coarse interwall pillars. Ganoderma esculentum is characterized by its basidiomata with slender stipe, white pore surface, homogeneous pileus context, and slightly truncate, narrow basidiospores (8.0–12.5 × 5.0–8.0 μm). Phylogenetic analyses were carried out based on the internal transcribed spacer (ITS), translation elongation factor 1-α (TEF1-α) and the second subunit of RNA polymerase II (RPB2) sequence data. The illustrations and descriptions for the new taxa are provided.

Keywords
Ganodermataceae, novel species, phylogeny, taxonomy
Introduction

Ganodermataceae was introduced by Donk (1948) which belongs to Polyporales and the latest studies indicated that it is a monophyletic group (Costa-Rezende et al. 2020). Currently, eleven genera viz. *Amauroderma* Murril, *Amaurodermellus* Costa-Rezende, *Cristataspora* Costa-Rezende, *Foraminispora* Robledo, *Costa-Rezende & Drechsler-Santos*, *Furtadoa* Costa-Rezende, *Haddowia* Steyaert, *Humphreya* Steyaert, *Magoderma* (Murrill) Steyaert, *Sanguinoderma* Y.F. Sun, D.H. Costa & B.K. Cui and *Tomophagus* Murrill are accepted in Ganodermataceae and supported by morphology and phylogeny (Steyaert 1972; Furtado 1981; Corner 1983; Zhao et al. 2000; Ryvarden 2004; Thametal 2012; Costa-Rezende et al. 2017; Costa-Rezende et al. 2020; Sun et al. 2020).

*Ganoderma* P. Karst (Ganodermataceae, Polyporales) was introduced to accommodate a laccate and stipitate fungus, *Ganoderma lucidum* (Curtis) P. Karst (Karsten 1881). *Ganoderma* is characterized by double-walled basidiospores with inter-wall protuberances (Karsten 1881; Moncalvo and Ryvarden 1997). There are 462 records in the Index Fungorum (http://www.Indexfungorum.org/; accessed date: 7 October 2021) and 506 records in MycoBank (http://www.mycobank.org/; accessed date: 7 October 2021). *Ganoderma* is one of the most taxonomically scrutinized genera among the Ganodermataceae and even in Polyporales (Richter et al. 2015; Costa-Rezende et al. 2020). Most *Ganoderma* species are wood decomposers, found in all temperate and tropical regions (Pilotti et al. 2004; Cao et al. 2012; Zhou et al. 2015).

*Ganoderma* has long been regarded as one of the most important medicinal fungi in the world (Paterson 2006); they have been used as medicine for over two millennia in China (Dai et al. 2009). Several *Ganoderma* species are known to be prolific sources of highly active bioactive compounds, especially polysaccharides, protein, sterols, and triterpenoids (Ahmadi and Riazipour 2007; Chan et al. 2007). These compounds are known to possess extensive therapeutic properties, such as antioxidant, antitumor, and antiviral agents, and improve sleep function (De Silva et al. 2013).

Species diversity of *Ganoderma* is abundant in China and more than 30 species have been described (Zhao et al. 2000; Wang et al. 2009; Cao et al. 2012; Li et al. 2015; Xing et al. 2016; Hapuarachchi et al. 2018; Liu et al. 2019; He et al. 2019; Wu et al. 2020). Yunnan province is considered as one of the hot-spots for studying biodiversity of polypores, and some new *Ganoderma* species have been described (Zhao 1989; Wang et al. 2010; Cao and Yuan 2013).

During our investigation into the diversity of *Ganoderma* in Yunnan province, several specimens of *Ganoderma* were collected from central and southern Yunnan. Phylogenetic analysis showed that the seven collections formed two distinct lineages and can be recognized as new species, hence two new species, namely *G. dianzhongense* and *G. esculentum* are introduced based on morphology and phylogeny.
Two new species of *Ganoderma*

**Materials and methods**

**Sample collection**

Seven *Ganoderma* specimens were collected during the rainy season from July 2016 to August 2019 in Yunnan Province of China. The samples were then photographed and transported back to the laboratory where their fresh macroscopic details were described. The specimens were deposited in the herbarium of Kunming Institute of Botany Academia Sinica (KUN-HKAS).

**Morphological studies**

Macro-morphological characters were described based on fresh material field notes, and the photographs provided here. Color codes are from Kornerup and Wanscher (1978). Micro-morphological data were obtained from the dried specimens and observed by using a microscope following Li et al. (2015). Sections were studied at magnification of up to 1000× using a NiKon E400 microscope and phase contrast illumination. Microscopic features and measurements were made from slide preparations stained with 5% potassium hydroxide (KOH) and 2% Melzer’s reagent. Basidiospore features, hyphal system, color, sizes and shapes were recorded and photographed. Measurements were made using the Image Frame work v.0.9.7 to represent variation in the size of basidiospores, 5% of measurements were excluded from each end of the range and extreme values are given in parentheses.

The following abbreviations are used: IKI = Melzer’s reagent, IKI– = neither amyloid nor dextrinoid, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = Cyanophilous (Xing et al. 2018). The abbreviation for basidiospores measurements (n/m/p) denote “n” basidiospores measured from “m” basidiomata of “p” specimens. Basidiospore dimensions (and “Q” values) are given as (a) b–av–c (d), where “a” represents the minimum, “d” the biggest, “av” the average “b” and “c” covers a minimum of 90% of the values. “Q”, the length/width ratio of a spore in side view, and “Q_m” for the average of all basidiospores ± standard deviation (Wang et al. 2015).

**DNA extraction, PCR amplification, and sequencing**

Total genomic DNA was extracted from dried pieces of pileus with tubes with modified CTAB protocol Doyle (1987). The genes ITS, TEF1-α and RPB2 were amplified by polymerase chain reaction (PCR) technique. The primers ITS1F / ITS4, TEF1-983 / TEF1-1567, and RPB2-6f / fRPB2-7cR were used to amplify the ITS, TEF1-α, RPB2 region, respectively (White et al. 1990; Liu et al. 1999; Matheny et al. 2007). PCR reactions (25 μL) contained mixture: 2.5 μL PCR reaction buffer, 2.5 μL 0.2% BSA, 2 μL dNTP (2.5 mm), 0.5 μL each of primer, 0.2 μL 5 U/μL Taq DNA polymerase, 1–1.5 μL DNA solution and 16 μL sterilized distilled H₂O. The PCR cycling for ITS was as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles at
94 °C for 30 sec, 53 °C for 30 sec and 72 °C for 50 sec and a final extension of 72 °C for 10 min. The PCR cycling for TEF1-α was as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 50 sec and a final extension of 72 °C for 10 min. The PCR cycling for RPB2 was as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for 50 sec and a final extension of 72 °C for 10 min. The PCR products were visualized via UV light after electrophoresis on 1% agarose gels stained with ethidium bromide. Successful PCR products were sent to Sangon Biotech Limited Company (Shanghai, China), using forward PCR primers. When sequences have heterozygous INDELS or ambiguous sites, samples were sequenced bidirectionally to make contigs of the amplified regions or verify the ambiguous sites (Wang et al. 2015). Raw DNA sequences were assembled and edited in Sequencher 4.1.4 and the assembled DNA sequences were deposited in GenBank (Table 1).

Sequencing and sequence alignment

Sequence data of three partial loci Internal transcribed spacer region (ITS), RNA polymerase II subunit 2 (RPB2), and translation elongation factor 1-alpha (TEF1-α) were used in the phylogenetic analyses. Besides the sequences generated from this study, other reference sequences were selected from GenBank for phylogenetic analyses (Table 1). Sequences were aligned using the online version of MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013) and adjusted using BioEdit v.7.0.9 by hand (Hall 1999) to allow maximum alignment and minimize gaps. Ambiguous regions were excluded from the analyses and gaps were treated as missing data. The phylogeny website tool “ALTER” (Glez-Peña et al. 2010) was used to convert the alignment fasta file to Phylip format for RAxML analysis and AliView and PAUP 4.0b 10 were used to convert the alignment fasta file to a Nexus file for Bayesian analysis (Swofford 2003). Phylogenetic analyses were obtained from Maximum Likelihood (ML) and Bayesian analysis (BI).

Molecular phylogenetic analyses

The maximum likelihood (ML) and Bayesian inference (BI) methods were used to analyze the combined dataset of ITS, TEF1-α and RPB2 sequences. Maximum likelihood analysis was conducted with RAxML-HPC2 on the CIPRES Science Gateway (Miller et al. 2010), involved 100 ML searches; all model parameters were estimated by the program. The ML bootstrap values (ML-BS) were obtained with 1000 rapid bootstrapping replicates. Maximum likelihood bootstrap values (ML) equal to or greater than 70% are given above each node (Figure 1).

Bayesian analysis was performed with MrBayes v3.2 (Ronquist et al. 2012), with the best-fit model of sequence evolution estimated with MrModeltest 2.3 (Nylander et al. 2008) to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo (MCMC) sampling. Six simultaneous Markov chains were run for 10,000,000 generations, trees were sampled every 500th generation, and 2,000 trees were obtained. The first 5000 trees, represent-
### Table 1. Species, specimens, geographic origin and GenBank accession numbers of sequences used in this study.

| Species | Voucher/strain | Origin | GenBank accession numbers | Reference |
|---------|----------------|--------|---------------------------|-----------|
| **G. aridicola** | Dai 12588 (Type) | South Africa | ITS: KU572491, TEF1-α: KU572502 | Xing et al. 2016 |
| **G. adspersum** | GACP15061220 | Thailand | ITS: MK345425, TEF1-α: MK371431, RPB2: MK371437 | Hapuarachchi et al. 2019 |
| | MFLU 19-2178 | Thailand | ITS: MN396653, TEF1-α: MN423149, RPB2: MN423114 | Luangharn et al. 2021 |
| **G. angustiporum** | Cui 13817 (Type) | Fujian, China | ITS: MG279170, TEF1-α: MG367563, RPB2: MG367507 | Xing et al. 2018 |
| | Cui 14578 | Guangdong, China | ITS: MG279171, TEF1-α: MG367564 | Xing et al. 2018 |
| **G. austral** | CMW 47785 | South Africa | ITS: MH571686, TEF1-α: MH576276 | Tchoumi et al. 2018 |
| | CMW 48146 | South Africa | ITS: MH571685, TEF1-α: MH576278 | Tchoumi et al. 2018 |
| **G. austroafricanum** | CBS138724 (Type) | South Africa | ITS: KM507324 | Coetzee et al. 2015 |
| **G. aff. austroafricanum** | CMW25884 | South Africa | ITS: MH571673, TEF1-α: MH576296 | Tchoumi et al. 2019 |
| **G. bambusicola** | Wu 1207-151 (Type) | Taiwan, China | ITS: MN957781, TEF1-α: LC517941, RPB2: LC517944 | Wu et al. 2020 |
| | Wu 1207-152 | Taiwan, China | ITS: MN957782, TEF1-α: LC517942, RPB2: LC517945 | Wu et al. 2020 |
| **G. boninense** | WD 2028 | Japan | ITS: KJ143905, TEF1-α: KJ143924 | Zhou et al. 2015 |
| | WD 2085 | Japan | ITS: KJ143906, TEF1-α: KJ143925 | Zhou et al. 2015 |
| **G. calidophilum** | MFLU 19-2174 | Yunnan, China | ITS: MN398337 | Luangharn et al. 2021 |
| | H36 | Yunnan, China | ITS: MW750241' | this study |
| **G. carnosum** | MJ 21/08 | Czech Republic | ITS: KU572492 | Xing et al. 2016 |
| | JV 8709/8 | Czech Republic | ITS: KU572493 | Xing et al. 2016 |
| **G. carocalcareus** | DMC 322 (Type) | Cameroon | ITS: EU089969 | Douanla and Langer 2009 |
| | DMC 513 | Cameroon | ITS: EU089970 | Douanla and Langer 2009 |
| **G. casuarinicola** | Dai 16336 (Type) | Guangdong, China | ITS: MG279173, TEF1-α: MG367563, RPB2: MG367508 | Xing et al. 2018 |
| | Dai 16339 | Guangdong, China | ITS: MG279176, TEF1-α: MG367568 | Xing et al. 2018 |
| **G. curtii** | CBS 100131 | NC, USA | ITS: JQ781848, TEF1-α: JQ781849 | Zhou et al. 2015 |
| | CBS 100132 | NC, USA | ITS: JQ781849, TEF1-α: JQ781850 | Zhou et al. 2015 |
| **G. destructans** | CBS 139793 (Type) | South Africa | ITS: NR132919 | Tchoumi et al. 2018 |
| | Dai 16431 | South Africa | ITS: MG279177, TEF1-α: MG367560 | Xing et al. 2018 |
| **G. donum** | CMW 42157 (Type) | South Africa | ITS: MG020249, TEF1-α: MG020228 | Tchoumi et al. 2019 |
| | CMW 42150 | South Africa | ITS: MG020249, TEF1-α: MG020228 | Tchoumi et al. 2019 |
| **G. eucadoriense** | ASL799 (Type) | Ecuador | ITS: KU128524 | Crous et al. 2016 |
| | PMC126 | Ecuador | ITS: KU128525 | Crous et al. 2016 |
| **G. eickeri** | CMW 49692 (Type) | South Africa | ITS: MH571690, TEF1-α: MH576287 | Tchoumi et al. 2019 |
| | CMW 50325 | South Africa | ITS: MH571689, TEF1-α: MH576290 | Tchoumi et al. 2019 |
| **G. ellipsoideum** | GACP1408966 (Type) | Hainan, China | ITS: MH106867 | Hapuarachchi et al. 2018 |
| | GACP14081215 | Hainan, China | ITS: MH106886 | Hapuarachchi et al. 2018 |
| **G. enigmaticum** | Dai 15970 | Africa | ITS: KU572486, TEF1-α: KU572496 | Xing et al. 2016 |
| | Dai 15971 | Africa | ITS: KU572487, TEF1-α: KU572497 | Xing et al. 2016 |
| **G. esculentum** | L4935 (Type) | Yunnan, China | ITS: MW750242, TEF1-α: MW838998 | this study |
| | L4946 | Yunnan, China | ITS: MW750243 | this study |
| **G. flexipes** | Wei 5494 | Hainan, China | ITS: JN383979 | Cao and Yuan 2013 |
| | MFLU 19-2198 | Yunnan, China | ITS: MN398340 | Luangharn et al. 2021 |
| **G. gibsonum** | MFLU 19-2176 | Thailand | ITS: MN396311 | Luangharn et al. 2021 |
| | MFLU 19-2190 | Laos | ITS: MN396310 | Luangharn et al. 2021 |
| **G. heohnelianum** | Dai 11995 | Yunnan, China | ITS: KU199988, TEF1-α: MG367550 | Song et al. 2016 |
| | Cui 13982 | Guangxi, China | ITS: MG279178, TEF1-α: MG367570 | Xing et al. 2018 |
| **G. hochiminhense** | MFLU 19-2224 (Type) | Vietnam | ITS: MN398324, TEF1-α: MN423176 | Luangharn et al. 2021 |
| | MFLU 19-2225 | Vietnam | ITS: MN396622, TEF1-α: MN423177 | Luangharn et al. 2021 |
| **G. knysnamense** | CMW 47755 (Type) | South Africa | ITS: MH571681, TEF1-α: MH576261 | Tchoumi et al. 2019 |
| | CMW 47756 | South Africa | ITS: MH571684, TEF1-α: MH576274 | Tchoumi et al. 2019 |
| **G. leucocontextum** | GDGM 44303 | Xizang, China | ITS: KJ027607 | Li et al. 2015 |
| | GDGM 44305 | Xizang, China | ITS: KJ027609 | Li et al. 2015 |
| **G. longzhi** | Cui 9166 | China | ITS: KJ143907, TEF1-α: JX029974 | Cao et al. 2012 |
| | Dai 12574 | Liaoning, China | ITS: KJ143908, TEF1-α: JX029977 | Cao et al. 2012 |
| **G. lobatum** | JV 1008/31 | USA | ITS: KF605671, TEF1-α: MG376553 | Xing et al. 2018 |
| | JV 1008/32 | USA | ITS: KF605670, TEF1-α: MG376554 | Xing et al. 2018 |
G. lucidum  K 175217  UK  KJ143911  KJ143929  KJ143971  Zhou et al. 2015
MT 26/10  Czech Republic  KJ143912  KJ143930  –  Zhou et al. 2015

G. martinicense  231NC  NC, USA  MG654182  MG754736  –  Loyd et al.2018
246TX  TX, USA  MG654185  MG754737  MG754858  Loyd et al.2018

G. mbrekobenum  UMN7-3 GHA (Type)  Ghana  KX000896  –  –  Crous et al. 2016
UMN7-4 GHA  Ghana  KX000898  –  –  Crous et al. 2016

G. mexicanum  MUCL 49453 SW17  Martinique  MK31811  MK31825  MK31836  Cabarroi-Hernández et al. 2019
MUCL 55832  Martinique  MK31815  MK31839  –  Cabarroi-Hernández et al. 2019

G. mizoramense  UMN-MZ4 (Type)  India  KY643750  –  –  Crous et al. 2017
UMN-MZ5  India  KY643751  –  –  Crous et al. 2017

G. multiplex  CWN 04670  Taiwan, China  KJ143913  KJ143931  KJ143972  Zhou et al. 2015
Dai 9447  Hainan, China  KJ143914  –  KJ143973  Zhou et al. 2015

G. multiplex  SP9  Brazil  KU569553  –  –  Bolatios et al. 2016
URM 83346  Brazil  JX310823  –  –  Bolatios et al. 2016

G. mutabile  CLZhao 982  Yunnan, China  MG231527  –  –  GenBank
Yuan 2289(Type)  Yunnan, China  JN383977  –  –  GenBank

G. myanmarense  MFLU 19-2167 (Type)  Myanmar  MN396329  –  –  Luangharn et al. 2021
MFLU 19-2169  Myanmar  MN396330  –  –  Luangharn et al. 2021

G. nasale  GACP17060211  (Type)  Laos  MK345441  –  –  Hapuarachchi et al. 2019
GACP17060212 Laos  MK345442  –  –  Hapuarachchi et al. 2019

G. neojaponicum  FFPR1 WD-1285  Tokyo, Japan  MN957784  –  –  Wu et al. 2020
FFPR1 WD-1532  Chiba, Japan  MN957785  –  –  Wu et al. 2020

G. orbiforme  Cui 13918  Hainan, China  MG279186  MG367576  MG367522  Xing et al. 2018
Cui 13880  Hainan, China  MG279187  MG367577  MG367523  Xing et al. 2018

G. parvulum  MUCL 47096  Cuba  MK554783  MK554721  MK554742  Cabarroi-Hernández et al. 2019
MUCL 52655  French Guiana  MK554770  MK554717  MK554755  Cabarroi-Hernández et al. 2019

G. phillipi  Cui 14443  Hainan, China  MG279188  MG367578  MG367524  Xing et al. 2018
Cui 14444  Hainan, China  MG279189  MG367579  MG367525  Xing et al. 2018

G. resinaceum  Rivoire 4150  France, Europe  KJ143915  –  –  Zhou et al. 2015
CBS 19476  Netherlands, Europe  KJ143916  KJ143934  –  Zhou et al. 2015

G. ryvardenii  HKAS 58053 (Type)  South Africa  HM138670  –  –  Kinge et al. 2011
HKAS 58054  South Africa  HM138671  –  –  Kinge et al. 2011

G. sessile  111TX  TX, USA  MG654306  MG754747  MG754866  Loyd et al.2018
113FL  FL, USA  MG654307  MG754748  MG754867  Loyd et al.2018

G. sianense  BJTC FM432(Type)  Shansi, China  MK764268  MK783937  MK783940  Liu et al. 2019
HSA 539  Shansi, China  MK764269  –  MK789861  Liu et al. 2019

G. sichuanense  HMAS42798 (Type)  Sichuan, China  JQ781877  –  –  Cao et al. 2012
Cui 7691  Guangdong, China  JQ781878  –  –  Cao et al. 2012

G. sinense  Wei 5327  Hainan, China  KF494998  KF494976  MG367529  Xing et al. 2018
Cui 13835  Hainan, China  MG279193  MG367583  MG367530  Xing et al. 2018

G. steytaertanum  MEL:2382783  Australia  KP012964  –  –  GenBank
6 WN 20B Indonesia  KJ654462  –  –  Glen et al. 2014

G. thaiandicum  HKAS 104640 (Type)  Thailand  MK384868  MK875829  MK875831  Luangharn et al. 2019
HKAS 104641  Thailand  MK384862  MK875830  MK875832  Luangharn et al. 2019

G. tropicum  He 1232  Guangxi, China  KF495000  KF494975  MG367531  Xing et al. 2016
HKAS 97486  Thailand  MH823539  –  MH883621  Luangharn et al. 2021

G. trognet  UMMN:120  MI, USA  MG654324  MG754764  –  Loyd et al.2018
UMMN:130  MI, USA  MG654326  MH025362  MG754871  Loyd et al.2018

G. tuberculatum  GVL-21  Veracruz, Mexico  MT232639  –  –  Espinosa-García et al. 2021
GVL-40  Veracruz, Mexico  MT232634  –  –  Espinosa-García et al. 2021

G. weberianum  CBS 128581  Taiwan, China  MK603805  MK636693  MK611971  Cabarroi-Hernández et al. 2019
CBS 219,36  Philippines  MK603804  MK611974  MK611972  Cabarroi-Hernández et al. 2019
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**Results**

**Phylogenetic analyses**

The dataset composed of ITS, TEF1-α and RPB2 genes, comprising a total of 2092 characters including gaps, ITS (1–656 bp), TEF1-α (657–1192 bp) and RPB2 (1193–2092 bp), including 57 taxa with *Tomophagus colossus* (Fr.) C.F. Baker as the out-group taxon (Wang et al. 2009; Cao et al. 2012). Best model for the combined 3-gene dataset estimated and applied in the Bayesian analysis was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The phylogenetic analysis of ML and BI produce similar topology. The combined dataset analysis of RAxML generates a best-scoring tree (Figure 1), with the final ML optimization likelihood value of -13861.891117. The aligned matrix had 993 distinct alignment patterns, with 38.83% completely undetermined characters or gaps. The base frequency and rate are as follows: A = 0.215319, C = 0.266028, G = 0.260220, T = 0.258433; rate AC = 0.885915, AG = 5.586021, AT = 0.936363, CG = 1.205084, CT = 6.595971, GT = 1.000000; gamma distribution shape: α = 0.246210. Bootstrap support values with a maximum likelihood (ML) greater than 70%, and Bayesian posterior probabilities (BPP) greater than 0.95 are given above the nodes (Figure 1).

Phylogenetic analysis showed that five collections clustered together with high bootstrap support, forming a clade sister to *G. shanxiense* with strong bootstrap support (ML-BS = 96%, BPP = 1.00, Figure 1). Two other collections clustered with *G. aridicola*, *G. bambusicola*, *G. casuarinicola*, *G. calidohilum*, *G. enigmaticum* and *G. thailandicum* (ML-BS = 100%, BPP = 1.00), but forming as a distinct lineage.
Figure 1. Phylogeny of the new *Ganoderma* species and related taxa based on ITS, TEF1-α and RPB2 sequence data. Branches are labeled with bootstrap values (ML) higher than 70%, and posterior probabilities (BPP) higher than 0.95. The new species are shown in bold red.
Two new species of *Ganoderma*

*Ganoderma dianzhongense* J. He, H.Y. Su & S.H. Li, sp. nov.
Index Fungorum number: 558822
MycoBank No: 841408
Figure 2

**Diagnosis.** *Ganoderma dianzhongense* is characterized by its mesopodal basidiomata, oxblood red to violet brown pileus surface, melon seed kernel-shaped and broadly ellipsoid basidiospores.

**Holotype.** CHINA. Yunnan Province, Kunming City, Luquan County, on the rotten broad-leaved trees, alt. 2480 m, Shu-Hong Li, 8 Sept. 2016, L4331 (HKAS 110005).

**Etymology.** The epithet ‘dianzhong’ refers to central Yunnan province in Chinese, where the holotype was collected.

**Description.** Basidiomata annual, stipitate, sub-mesopodal to mesopodal or with the back sides fused, coriaceous to woody. Pileus single, suborbicular to reniform, up to 4.8–13.1 cm diam., 1.1 cm thick, weakly to strongly laccate, glossy and shiny, oxblood red (9E7) to violet brown (11F8), smooth, and covered by a thin hard crust, concentrically zonate or azonate. Margin distinct, slightly obtuse. Stipe 9.0–17.7 × 1.1–1.9 cm, central, cylindrical, strongly laccate, dark red brown (11C8) to purplish (14A8) or almost blackish red-brown (10F4), fibrous to woody. Context up to 0.4 cm thick, duplex; lower layer dark brown (8F8), fibrous, composed of coarse loose fibrils; upper layer putty (4B2); corky to woody, bearing distinct concentric growth zones, without black melanoid band. Tubes woody hard, grayish brown, up to 0.9 cm long, unstrati-fied. Pore 4–6 per mm, round to angular, dissepiments slightly thick, entire; pore surface grey white to lead gray (2D2), turning light buff when dust (5D1).

**Hyphal system trimitic.** Generative hyphae 2.0–3.5 μm in diameter, colorless, thin-walled, clamp connections present; skeletal hyphae 3.0–6.0 μm in diameter, subthick-walled to solid, non-septate, arboriform with few branches, yellowish to golden-yellow; binding hyphae 1–2.5 μm in diameter, thick-walled, frequently branched, interwoven, hyaline to yellowish, scarce; all the hyphae IKI–, CB+; tissues darkening in KOH.

**Pileipellis** a crustohymeniderm, cells 20–45 × 5.5–7.5 μm, clavate to cylindrical, entire or rarely with one lateral protuberance, thick-walled, without granulations in the apex, golden-yellow to yellowish-brown, thick-walled, moderately amyloid at maturity.

**Basidiospores** (80/6/3) (9.0) 10–11.0–12.0 (12.5) × (6.5) 7.0–7.9–8.5 (9.0) μm, Q = (1.12) 1.25–1.55 (1.63), Qm = 1.40±0.09 (including myxosporium); holotype: (40/2/1) 10.0–10.9–12 × 7.0–7.9–8.5 (9.0) μm, Q = (1.20) 1.25–1.52, Qm = 1.39±0.08 (including myxosporium). mostly melon seed-shaped at maturity to broadly ellipsoid, usually with one end tapering and obtuse at maturity, with apical germ pore, yellowish to medium brown, IKI–, CB+, inamyloid; perisporium wrinkled, double-walled, with coarse interwall pillars. **Basidia** widely clavate to utriform, hyaline, with a clamp connection and four sterigmata, 11–19 ×10–13μm; basidioles pear-shaped to fusiform, 10–15 × 8–12 μm.
Figure 2. *Ganoderma dianzhongense* (HKAS 110005, holotype) A basidiomata B upper surface C cut side of pileus D pore surface E sections of pileipellis (LM) F skeletal hyphae from context (LM) G bing- ing hyphae from tubes (LM) H generative hyphae from tubes (LM) I-J basidia and basidioles (LM) K-L basidiospores (LM) M-N basidiospores (SEM) O-P culture after incubation at 28 °C for 8 days. Scale bars: 20 mm (O, P); 10 μm (E-L); 5 μm (M, N). Photographs Jun He.
Two new species of *Ganoderma*

**Habit.** Scattered, during fall, decaying wood of broad-leaved trees including *Quercus* sp. Currently, only known from central Yunnan province, China.

**Additional specimens examined.** **CHINA** Yunnan province, Shilin County, alt. 2109m, Jun He, 28 Aug., 2019, L4969 (HKAS 112719); Songming County, alt. 2204m, Shu-Hong Li, 8 Jul., 2016, L4230 (HKAS 112716); Wuding County, alt. 2295m, Shu-Hong Li, 24 Jul., 2019, L4737 (HKAS 112717); ibid, alt. 2432m, Jun He, 26 Jul., 2019, L4759 (HKAS 112718).

*Ganoderma esculentum* J. He & S.H. Li, sp. nov.
Index Fungorum number: 558823
MycoBank No: 841409
Figure 3

**Diagnosis.** *Ganoderma esculentum* is characterized by its strongly laccate chocolate brown pileus surface, slender stipe and narrow ellipsoid basidiospores.

**Holotype.** **CHINA.** Yunnan Province, Honghe City, Mengzi County, on a decaying wood log, alt. 1370 m, Jun He, 26 Aug., 2019, L4935 (HKAS 110006).

**Etymology.** The epithet ‘esculentum’ refers to this species named after a food.

**Description.** *Basidiomata* annual, stipitate, pleuropodal, laccate, woody-corky.

**Pileus** single, sub-orbicular to reniform to spatulate, up to 2.8–8.0 × 2.0–4.5 cm diam, 0.75 cm thick at the base, slightly convex to planate; surface glabrous, rugose to radially rugose, strongly laccate, not cracking, with a hard crust, difficult to penetrate with the fingernail; surface brownish-black (6C8) to chocolate brown (6F4), almost homogeneous in the adult. **Margin** grayish orange(6B5) to concolorous, entire, acute to obtuse, smooth to sulcate. **Stipe** 10.0–17.5 × 0.5–1.0 cm, dorsally lateral to nearly dorsal, sub-cylindrical, solid, surface smooth, very shiny, dark brown (8F8) almost black, darker than pileus, fibrous to woody. **Context** up to 0.2 cm thick, composed of coarse loose fibrils, dark brown (8F8), with black melanoid band. **Tubes** 0.2–0.5 cm long, dark brown, woody hard, unstratified. **Pore** 5–8 per mm, circular or sub-circular, woody; pore surface white when fresh, darkening to soot brown(5F5) when aging and drying.

**Hyphal system trimitic.** Generative hyphae 1.5–3.0 μm in diameter, colorless, thin-walled, clamp connections present; skeletal hyphae 3.5–5.5 μm in diameter, thick-walled to solid, non-septate, arboriform or not, non-branched or with a few branches in the distal end, golden brown; binding hyphae 1.0–3.0 μm in diameter, thick-walled, much-branched, arboriform, hyaline to yellowish, scarce; all the hyphae IKI–, CB+; tissues darkening in KOH.

**Pileipellis** a crustohymeniderm, cells 20–55 × 10–15 μm, narrowly clavate to tubular, generally smooth, slightly thick-walled to thick-walled with a wide lumen, occasionally expanded at the apex, without granulations, entire, yellowish to leather brown, weakly to strongly amyloid.

**Basidiospores** (40/3/2) (8.0) 9.0–**10.6**–12.5 × (5.0) 5.5–**6.6**–7.5 (8.0) μm, Q = (1.15) 1.34–**1.62**–2.01 (2.06), Q_m = 1.62±0.19 (including myxosporium); holo-
Figure 3. *Ganoderma esculentum* holotype (HKAS 110006) A basidiomata B upper surface C lower surface D cut side of pileus E pore surface F sections of pileipellis (LM) G, H skeletal hyphae from context (LM) I binging hyphae from tubes (LM) J generative hyphae from tubes (LM) K–M basidiospores (LM) N, O basidiospores (SEM). Scale bars: 20 μm (H); 10 μm (F, G, I–M); 5 μm (N, O). Photographs Jun He.
Two new species of *Ganoderma*

**Discussion**

Ganodermataceae is a large family of polypores, and has received great attention from mycologists for over many decades. However, species identification and circumscriptions have been unclear and taxonomic segregation of the genera has been controversial because of different viewpoints among mycologists (Moncalvo et al. 1995; Moncalvo and Ryvarden 1997; Costa-Rezende et al. 2020). Ganodermataceae was treated as a synonym of Polyporaceae and classify the genus *Ganoderma* into Polyporaceae by Justo et al. (2017). Later, Cui et al. (2019) excluded *Ganoderma* from Polyporaceae, due to *Ganoderma* having unique double-walled basidiospores. In addition, recent studies have clarified some uncertainties of generic delimitation and classification of polypores with ganodermatoid basidiospores, and proved that Ganodermataceae is a monophyletic group (Costa-Rezende et al. 2020). More collections of this family are needed in order to estimate the attributes of this taxon better.

In the phylogenetic inferences, *Ganoderma dianzhongense* is sister to *G. shanxiense*, which is known from the northern Shanxi province in China (Figure 1). Morphologically, both species share similar characters of the mesopodal basidiomata, suborbicular to reniform pileus, and broadly ellipsoid basidiospores (Table 2). However, *G. shanxiense* differs from *G. dianzhongense* in having a red to reddish-brown pileus surface, wider basidiospores (11.0–13.0 × 8.0–9.5 μm), and narrower skeletal hyphae (2.5–5.0 μm, Liu et al. 2019).

*Ganoderma dianzhongense* resembles *G. sinense* and *G. orbiforme* in having suborbicular pileus (Table 2). However, *G. sinense* is characterized by wider basidiospores (9.5–13.4 × 7.0–10.2 μm) and slightly longitudinally crested basidiospores (Wang and Wu 2007) and a uniformly brown to dark brown context. *Ganoderma orbiforme* has a purplish black to light brown pileus, a variably brown context, irregularly digitated pileipellis cells, and ellipsoid to ovoid basidiospores (6.9–10.6 × 3.6–5.7 μm) with fine and short echinulae, and a subtropical to tropical distribution (Wang et al. 2014). *Ganoderma orbiforme* is also phylogenetically unrelated (Figure 1).

In our multi-locus phylogeny analysis (Figure 1), *G. aridicola*, *G. bambusicola*, *G. casuarinicola*, *G. calidohilum*, *G. enigmaticum*, *G.mbrekobenum*, *G.thailandicum* and *G. esculentum* formed a distinct lineage, and was clearly separated from other *Ganoderma* species. It is easy to distinguish them from the morphological characteristics. *Gano-
derma bambusicola has a longer pileipellis (35–65 × 8–16 μm) and wider basidiospores than those of *G. esculentum* (10.0–13.0 × 6.5–8.0 μm, Wu et al. 2020). *Ganoderma aridicola* can be easily distinguished from *G. esculentum* by the sessile basidiomata and a fuscous to black pileus surface (Xing et al. 2016). *Ganoderma casuarinicolana* differs from
Two new species of *Ganoderma* by the latter has smaller basidiospores (8.3–11.5 × 4.5–7.0 μm, Xing et al. 2018), grayish brown longer pores and sectorial to shell-shaped pileus. *Ganoderma enigmaticum* mainly differs from *G. esculentum* by its golden yellow pileus surface, narrower basidiospores (8.0–11.0 × 3.5–6.0 μm, Coetzee et al. 2015) and causes root and butt rot of living and dead trees. *Ganoderma thailandicum* can be distinguished from *G. esculentum*, by its brownish-red pileus surface without radially rugose, narrowly clavate pileipellis cells with tuberculate and smaller basidiospores (6.8–10.2 × 5.8–7.7 μm, Luangharn et al. 2019). *Ganoderma mbrekobenum* can be differentiated from *G. esculentum* by its woody to corky texture when dried, with ovoid basidiospores (25.0–57.0 × 6.0–12.0 μm, Crous et al. 2016). *Ganoderma calidophilum* has a larger diameter binding hypha (2.4–5.2 μm) than *G. esculentum* (1.0–3.0 μm) and *G. calidophilum* has larger basidiospores (7.3–14.6 × 5.3–9.6 μm, Zhao et al. 1979; Luangharn et al. 2021) than *G. esculentum* (including myxosporium).

Morphologically, *G. esculentum* resemble *G. kunmingense* by radially rugose, the pileus and slender stipe (Table 2). However, *G. kunmingense* has narrower hyphae, tissues not darkening in KOH, and broadly ellipsoid to sub-globose basidiospores (7.5–10.5 × 6.0–9.0 μm, Zhao et al. 1989). In addition, *G. esculentum* shares also similarities with *G. neojaponicum* but the latter has a double-layered context with the paler layer near the pileus surface and wider basidiospores than those of *G. esculentum* (9.1–13.5 × 5.7–8.9 μm, Imazeki et al. 1939; Hapuarachchi et al. 2019).

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Supplementary material 1

Phylogenetic sequence dataset

Authors: Jun He

Data type: phylogenetic data

Explanation note: Sequence data of three partial loci internal transcribed spaces region (ITS), RNA polymerase II subunit 2 (RPB2), and translation elongation factor 1-alpha (TEF1-α) were used in the phylogenetic analyses.

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