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High phenotypic plasticity of *Ganoderma sinense* (Ganodermataceae, Polyporales) in China

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Abstract

Ganoderma is a typical polypore genus which has been used in traditional medicines for centuries and is increasingly being used in pharmaceutical industries worldwide. The genus has been extensively researched due to its beneficial medicinal properties and chemical constituents. However, the taxonomy of *Ganoderma* remains unclear, and has been plagued by confusion and misinterpretations. During an extensive survey of *Ganoderma* we were found 20 *G. sinense* specimens from China. The specimens were identified as *G. sinense* with the aid of molecular phylogenetic evidence. The studied specimens exhibit phenotypic plasticity due to extrinsic factors, even though they possess identical nucleotide sequences. Seven sequences derived from this study have a single nucleotide polymorphism located at position 158 in ITS1 region and all *G. sinense* collections clustered in the same species clade. The specimens are each described, illustrated with coloured photographs and compared to similar taxa. We provide a phylogenetic tree for *Ganoderma* based on ITS, SSU, LSU and RPB2 sequence data and the taxonomic status of *G. sinense* is discussed.

Key words – Environment factors – Morphological characteristics – Phylogeny – Taxonomy

Introduction

The genus *Ganoderma* was established by Karsten (1881) with *G. lucidum* (Curtis) P. Karst. as the type species. It is a diverse genus that occurs throughout the world. About 450 epithets have been listed (Index Fungorum, accessed 27 February 2019). *Ganoderma* species grow as facultative parasites that can also live as saprobes on decaying stumps or associated with roots of living and dead trees (Singh et al. 2007). They are not edible, since the basidiomes are always corky, tough and bitter in taste (Hapuarachchi et al. 2015). Two types of basidiomes are produced, laccate with a

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shiny upper surface, and non-laccate with a dull upper surface (Smith & Sivasithamparam 2003, Pilotti et al. 2004). *Ganoderma* species have been broadly utilized as traditional medicines for millennia in Asia (Zhou et al. 2015, Hapuarachchi et al. 2018a) and, because of their highly prized medicinal value are widely researched (De Silva et al. 2012a, b, 2013, Hapuarachchi et al. 2016a, b, 2017, 2018c). *Ganoderma* species are economically important not only because of their medicinal properties, but also because of their phytopathogenicity (Dai et al. 2007, 2009).

Ganoderma is characterized by having ellipsoid to ovoid basidiospores, with a truncate apex and an endosporium with columnar ornamentations (Costa-Rezende et al. 2017). *Ganoderma* is morphologically the most complex polypore genus and characteristics such as shape, colour of the fruit body, host-specificity and geographical origin, are used to identify species (Szedlay 2002, Hapuarachchi et al 2018b). Species identification is complicated and has been widely debated (Moncalvo et al. 1995a, Wang et al. 2009, Cao et al. 2012, Yao et al. 2013, Richter et al. 2015, Zhou et al. 2015). *Ganoderma* species are genetically heterogeneous because of geographical differentiation and high genetic diversity (Miller et al. 1999, Pilotti et al. 2003). This high genetic diversity is associated with substantial morphological variation, even within species and hence, naming a species within this genus is often ambiguous (Hong et al. 2001, 2002). High phenotypic plasticity at the macroscopic level and the uniformity of microscopic characteristics, subjective interpretation of various features, lack of keys and accessible type specimens, and presence of single nucleotide polymorphism have resulted in numerous unnecessary species names (Seo & Kirk 2000, Zhang et al. 2017). Hence, DNA sequence data play a crucial role in identifying species (Cao et al. 2012, Yang & Feng 2013, Pawlik et al. 2015). Phylogenetic analyses have proved that extensive convergence or parallelism of morphological characteristics has occurred during evolution of *Ganoderma* (Hong & Jung 2004, Moncalvo 2005). The specific interhybridization and genetic background of *Ganoderma* remains relatively unclear and the genetic distance between *Ganoderma* species has not been evaluated (Zheng et al. 2009). *Ganoderma* was first reported from China by Teng (1934) and later, many researchers extensively studied this genus and about 100 species were recorded (Zhao & Zhang 2000, Wu & Dai 2005, Cao et al. 2012). *Ganoderma sinense* was originally described from China by Zhao et al. (1979) and is characterized by slightly longitudinally crested basidiospores and a uniformly brown to dark brown context. This species has been morphologically confused with *G. lucidum* (Curtis) P. Karst., *G. japonicum* (Fr.) Sawada (= *G. dimidiatum* (Thunb.) V. Papp) and *G. orbiforme* (Fr.) Ryvarden but, later researchers have distinguished them from *G. sinense* (Pegler & Yao 1996, Wang et al. 2014).

Phenotypic plasticity is the development of different phenotypes from a single genotype, depending on the environment (Aubin-Horth & Renn 2009). It is an extensive feature of life observed in various traits and is often contended to be the result of natural selection. Phenotypic plasticity includes an ecological and an evolutionary perspective, however, recent development in large-scale gene expression technology makes it feasible to study plasticity from a molecular perspective (Aubin-Horth & Renn 2009). This phenomenon has been observed commonly in ascomycetes, due to the influence of environmental or cultural factors, and could easily result in misidentification as new taxa based on minor and statistically insignificant variation of morphological characteristics (Jeewon & Hyde 2016). However, phenotypic plasticity in macroscopic fungi has been poorly studied and only general aspects have been described (Ramírez-López et al. 2013).

The development of basidiomes is influenced by the interaction of both intrinsic (genetic and physiological) and extrinsic (environmental) factors (Moore-Landecker 1996, Moore et al. 2008, 2011). In basidiomycetes, the development of basidiomes of *Coprinus* sp., *Panus* sp., *Morchella* sp., *Pleurotus* sp. and *Typhula* sp. is affected by environmental factors including the availability of nutrients, temperature, humidity, light, and pH (Morimoto & Oda 1973, Bujakiewicz 1992, Kost 1992, Boulianne et al. 2000, Straatsma et al. 2001, Salerni et al. 2002, Kawakami et al. 2004, Singh et al. 2004, Ramírez-López et al. 2013). In general, the taxonomy of *Ganoderma* is considered uncertain due to the high phenotypic plasticity of the basidiomes at macroscopic level and uniformity of microscopic features (Sankaran et al. 2005). Gottlieb & Wright (1999) have reported

phenotypic plasticity in both micro- and macro-morphological characteristics of South American species of *Ganoderma*. Wu & Dai (2005) mentioned that the morphology of *Ganoderma* species varies greatly due to influence of climate, nutrition, vegetation, and geographical environment and it is not in association with the genetic material of a particular species. Wu & Dai (2005) described phenotypic plasticity of *G. sinense* in China based on different macro- and uniform micro-morphological characteristics along with descriptions and colour photographs. In this study, we support the claims of Wu & Dai (2005) based on strong molecular evidence together with morphological data. We describe 20 *G. sinense* specimens having different macro-morphological characteristics to each other based on combined ITS, LSU, SSU and RPB2 analyses, colour photographs and illustrations.

Materials & Methods

Sample collection

Ganodermataceae were collected from Hainan during August 2014, and from Guizhou in September to October 2017. Collected materials were dealt with as described by Cao et al. (2012) and deposited at Guizhou University (GACP) and Mae Fah Luang University (MFLU) herbaria.

Macroscopic and microscopic characterization

Macro-morphological characteristics were described based on fresh materials, and on the photographs provided here. Colour codes (e.g. 6C8) are from Kornerup & Wanscher (1978). Specimens were dried and placed separately in plastic bags. For micro-morphological observations, basidiomes were examined using a stereo dissecting microscope (Motic SMZ 168 series). Sections were cut with a razor blade and mounted in 5% KOH, and then observed, measured and illustrated using a Nikon ECLIPSE 80i compound microscope equipped with a camera (Canon 600D). Measurements were made using Tarosoft (R) Image Frame Work v. 0.9.7. At least 20 basidiospores were measured from each mature specimen, except for very scanty materials. The basidiospore size was measured both with and without the myxosporium, but only spore sizes with myxosporium were used for comparisons. Basidiospore dimensions are given as (a–) b–*c*–d (–e), where a represents the minimum, b (mean average-standard deviation), *c* the average, d (mean average+standard deviation) and e the maximum. $\boldsymbol{Q}$, the length/width ratio (L/W) of a spore in side view and $\boldsymbol{Q_m}$ is the average, smallest and largest $\boldsymbol{Q}$ values given as *Q*. Pellis sections were taken from the mature pileus portion and mounted in Melzer's reagent for observation.

DNA extraction, PCR and sequencing

Dried samples of basidiomes were used to extract genomic DNA using an EZgene TM Fungal gDNA Kit (Biomiga, CA, USA) and following the manufacturer's instructions. DNA concentrations were estimated visually in agarose gel by comparing band intensity with a DNA ladder 1Kb (Invitrogen Biotech). Reaction mixtures (50 µl) contained 2 µl template DNA (ca. 10 ng), 19 µl distilled water, and 2 µl (10 µM) of each primer and 25 µl 2x BenchTopTM Taq Master Mix (Biomigas). Amplification conditions were 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min for all DNA fragments. The ITS rDNA regions were amplified using the universal primer pair ITS4 and ITS5 and the 18S and 28S rDNA genes were amplified using the universal primer pair NS1 and NS4 and primer pair LROR and LR5, respectively (Vilgalys & Hester 1990, White et al. 1990, Rehner & Samuels 1994). Protein coding gene, RNA polymerase II gene (RPB2) was amplified using primer pair fRPB26f/7CR (Liu et al. 1999). Amplified PCR products were verified by 1% agarose gel electrophoresis stained with ethidium bromide in 1x TBE. The PCR products were sequenced by SinoGenoMax Co. (Beijing).

Sequence alignment and phylogenetic analysis

Other sequences used in the analyses (Table 1) were retrieved from GenBank based on ITS BLAST searches (Benson et al. 2017) and recently published data. Sequences that had possibly been contaminated were unnamed species (such as those with aff. in the species name) were discarded, ambiguous regions were excluded and gaps were treated as missing data in the analyses (Nilsson et al. 2012). Seventy-four nucleotide sequences representing 22 species of Ganodermataceae from Africa, Asia, America and Europe were retrieved and all sequences were aligned with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh & Standley 2013). The resulting alignment was improved manually when necessary using BioEdit v. 7.0.5.2 (Hall 1999). Maximum likelihood (ML) analyses were performed using RAxML-HPC2 (Stamatakis 2014) on the CIPRES Science Gateway V. 3.3 (Miller & Blair 2009), with default settings, except that the number of bootstrap replicates was set to 1,000. Maximum parsimony (MP) analysis was performed with PAUP version 4.0b10 (Swofford 2002) using a heuristic search and TBR (tree bisection–reconnection) swapping for 1000 random replicates. Gaps were set as "missing" data and the characters were specified as unordered and equally weighted for bootstrap analysis (Hillis & Bull 1993) performed with 1000 replications with simple addition sequences to obtain estimates of reliability for nodes. For Bayesian analysis (BY), the GTR+I+G model of nucleotide evolution was selected with the help of MrModeltest 2.2 (Nylander 2004) as the best-fit model and posterior probabilities (PP) (Rannala & Yang 1996) were determined by Markov Chain Monte Carlo sampling (BMCMC) using MrBayes v3.1.2 (Ronquist et al. 2012). BY analyses were conducted with six simultaneous Markov chains and trees were summarized every 100th generation. The analyses were stopped after 5,000,000 generations when the average standard deviation of split frequencies was below 0.01. The convergence of the runs was checked using TRACER v1.6 (Rambaut et al. 2013). The first 25% of the resulting trees were discarded as burn-in, and PP were calculated from the remaining sampled trees. In both ML and BY analyses, *Coriolopsis trogii* was selected as the outgroup. ML, MP bootstrap values and BY posterior probabilities greater than or equal to 70% and 0.95, respectively, were considered as significant support. The phylogenetic tree was visualized with FigTree version 1.4.0 (Rambaut 2012, available at http://tree.bio.ed.ac.uk/software/figtree/).

Table 1 Sequences used in the phylogenetic analysis

Species	Voucher /strain	Origin	ITS	LSU	RPB2	SSU	Reference
G. adspersum (Schulzer) Donk	SFC20141001 22	Korea	KY364 252	-	KY393 271	-	Jargalmaa et al. 2017
G. adspersum	SFC20141001 16	Korea	KY364 251	-	KY393 270	-	Jargalmaa et al. 2017
G. angustisporum J.H. Xing, B.K. Cui & Y.C. Dai	Cui 13817 (holotype)	Fujian, China	MG279 170	-	MG367 507	-	Xing et al. 2018
G. angustisporum	Cui 14578	Guangdong, China	MG279 171	-	-	-	Xing et al. 2018
G. applanatum (Pers.) Pat.	SFC20150930 02	Korea	KY364 258	-	KY393 274	-	Jargalmaa et al. 2017
G. applanatum	Dai8924	China	KU219 987		-	-	Song et al. 2016
G. aridicola J.H. Xing & B.K. Cui)	Dai12588 (holotype)	Durban, South Africa	KU572 491	-	-	-	Xing et al. 2016
G. australe (Fr.) Pat.	K(M)120828	UK	AY884 183	-	-	-	Arulpandi & Kalaichelvan 2013
G. australe	GDGM25344	China	JX195 198	-	-	-	GenBank

Table 1 Continued.

Species	Voucher /strain	Origin	ITS	LSU	RPB2	SSU	Reference
G. austroafricanum M.P.A. Coetzee, M.J. Wingf., Marinc. & Blanchette	CMW41454 (holotype)	South Africa	KM507324	KM507325	-	-	Coetzee et al. 2015
G. casuarinicola **J.H.** Xing, B.K. Cui & Y.C. Dai	Dai 16336 (holotype)	Guangdong, China	MG279173	MG367508	MG279173		Xing et al. 2016
G. casuarinicola	Dai 16338	Guangdong, China	MG279175	-	MG367510	-	Xing et al. 2016
G. destructans M.P.A. Coetzee, Marinc. & M.J. Wingf.	CMW43670 (holotype)	South Africa	KR183856	-	-	-	Coetzee et al. 2015
G. destructans	CMW43671	South Africa	KR183857	-	-	-	Coetzee et al. 2015
G. ecuadoriense A. Salazar, C.W. Barnes & Ordoñez	ASL799 (holotype)	Ecuador	KU128524	-	-	-	Crous et al. 2016
G. ecuadoriense	PMC126	Ecuador	KU128525	-	-	-	Crous et al. 2016
G. gibbosum (Cooke) Pat.	SFC20150630-23	Korea	KY364264	-	KY393276		Jargalmaa et al. 2017
G. japonicum (Fr.) Sawada (1931)	AS5.69 type 1	China	AY593864	-	-	-	GenBank
G. japonicum	AS5.69 type 2	China	AY593865	-	-	-	GenBank
G. japonicum	Gja-1	China	GU213475	-	-	-	GenBank
G. japonicum	G22	China	KX055529	-	-	-	GenBank
G. leucocontextum T.H. Li, W.Q. Deng, Dong M. Wang & H.P. Hu	Dai15601	China	KU572485	-	-	-	Xing et al. 2016
G. leucocontextum	GDGM40200 (holotype)	China	KF011548	-	-	-	Li et al. 2015
G. lobatum (Cooke) G.F. Atk.	JV1212/10J	USA	KF605676	-	-	-	GenBank
G. lobatum	JV0409/13J	USA	KF605675	-	-	-	GenBank
G. lucidum (Curtis) P. Karst.	K175217	UK	KJ143911	-	KJ143971	-	Zhou et al. 2015
G. lucidum	MT 26/10 (BRNM)	Czech Republic	KJ143912	-	-	-	Zhou et al. 2015
G. mebrekobenum E.C. Otto, Blanchette, Held, C.W. Barnes & Obodai	UMN7-3 GHA(holotype)	Ghana	KX000896	-	-	-	Crous et al. 2016
G. mebrekobenum	UMN7-4GHA (holotype)	Ghana	KX000898	-	-	-	Crous et al. 2016
G. mizoramense Zothanz.,	UMN-MZ4 (holotype)	India	KY643750	-	-	-	Crous et al. 2017

Table 1 Continued.

Species	Voucher /strain	Origin	ITS	LSU	RPB2	SSU	Reference
Blanchette, Held & C.W. Barnes							
G. mizoramense	UMN-MZ5	India	KY643751	KY643751	-	KY643751	Crous et al. 2017
G. multipileum Ding Hou	CWN 04670 (TNM)	Taiwan, China	KJ143913	-	KJ143972	KJ143931	Zhou et al. 2015
G. multipileum	Dai 9447 (IFP)	Hainan, China	KJ143914	-	KJ143973	-	Zhou et al. 2015
G. multiplicatum (Mont.) Pat.	Dai 13122	China	KU572488	-	-	-	Xing et al. 2016
G. multiplicatum	Dai 13710	China	KU572489	-	-	-	Xing et al. 2016
G. multiplicatum	GACP14081328	Hainan, China	MH106879	-	-	-	Hapuarachchi et al. 2018b
G. orbiforme (Fr.) Ryvarden	GACP14080953	Hainan, China	MK313108	-	-	-	This study
G. orbiforme	GACP14081185	Hainan, China	MK313109	-	-	-	This study
G. podocarpense J.A. Flores, C.W. Barnes & Ordoñez	QCAM6422 (holotype)	Ecuador	MF796661	MF796660	-	-	Crous et al. 2017
G. sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang	GACP17092520	Sandu, China	MK313110	-	-	-	This study
G. sinense	GACP17092522	Sandu, China	MK313111	-	-	-	This study
G. sinense	GACP17092530	Sandu, China	MK313112	-	-	MK341557	This study
G. sinense	GACP17092533	Sandu, China	MK313113	MK336399	-	MK341558	This study
G. sinense	GACP17092543	Sandu, China	MK313114	MK336400	MK371431	MK341559	This study
G. sinense	GACP17092547	Sandu, China	MK313115	-	-	-	This study
G. sinense	GACP17092548	Sandu, China	MK313116	-	-	-	This study
G. sinense	GACP17092567	Sandu, China	MK313117	MK336401	-	MK341560	This study
G. sinense	GACP17092581	Sandu, China	MK313118	-	-	-	This study
G. sinense	GACP17092588	Sandu, China	MK313119	MK336402	MK371432	MK341561	This study
G. sinense	GACP17092592	Sandu, China	MK313120		-	-	This study
G. sinense	GACP17092510 9	Sandu, China	MK313121	-	-	-	This study
G. sinense	GACP17092512 8	Sandu, China	MK313122	-	-	-	This study
G. sinense	GACP17092513 0	Sandu, China	MK313123				
G. sinense	GACP17101242	Kaili, China	MK313124	-	-	-	This study
G. sinense	GACP17101254	Kaili, China	MK313125	MK336403	-	MK341562	This study

Table 1 Continued.

Species	Voucher /strain	Origin	ITS	LSU	RPB2	SSU	Reference
G. sinense	GACP17101260	Kaili, China	MK313126	MK336404	-	MK341563	This study
G. sinense	GACP17101270	Kaili, China	MK313127	MK336405	-	MK341564	This study
G. sinense	GACP17101223 9	Kaili, China	MK313128	-	-	-	This study
G. sinense	GACP14081236	Hainan, China	MH106882	-	-	-	Hapuarachchi et al. 2018b
G. sinense	Wei5327	Hainan, China	KF494998	KF495008	MG367529	-	GenBank
G. sinense	Cui13835	Hainan, China	MG279193	-	MG367530		Xing et al. 2018
G. sinense	GDGM25829	China	KC415760				GenBank
G. sinense	GS96	China	DQ424990	-	-	-	GenBank
G. sinense	GS92	China	DQ424982	-	-	-	GenBank
G. sinense	GS175	China	DQ425014	-	-	-	GenBank
G. sinense	GS111	China	DQ424995	-	-	-	GenBank
G. sinense	XZ-G-C1	China	HQ235633		-		GenBank
G. sinense	XZ-G-C2	China	HQ235634		-		GenBank
G. sinense	ZZ	China	KM249933	-	-	-	GenBank
G. tropicum (Jungh.) Bres.	Dai9724	China	JQ781879	-	-	-	Cao et al. 2012
G. tropicum	GACP14081518	China	MH106884	-	-	-	Hapuarachchi et al. 2018b
G. wiiroense E.C. Otto, Blanchette, C.W. Barnes & Held	UMN-20 (holotype) GHA	Ghana	KT952361	-	-	-	Crous et al. 2015
G. wiiroense	UMN-21-GHA (para type)	Ghana	KT952363	-	-	-	Crous et al. 2015
Coriolopsis trogii (Berk.) Domański	RLG4286sp	USA	JN164993	-	JN164867	-	Jargalmaa et al. 2017

Results

Phylogeny

The tree topologies obtained from ML, MP and BY were identical, therefore, only the ML tree is shown (Fig. 1). Our twenty collections of *Ganoderma sinense* from China clustered with all *G. sinense* and *G. japonicum* sequences, in a well-supported clade (ML = 100%, MP = 99%, BPP = 1.00).

Taxonomy

Ganoderma P. Karst., Rev. Mycol. (Toulouse) 3: 17 (1881)

= *Dendrophagus* Murrill, Bull. Torrey bot. Club 32(9): 473 (1905)

= *Elfvingia* P. Karst., Bidr. Känn. Finl. Nat. Folk 48: 333 (1889)
= *Friesia Lázaro Ibiza*, Revista Real Acad. Ci. Madrid 14: 587 (1916)
= *Ganoderma* subgen. *Trachyderma* Imazeki, Bull. Tokyo Sci. Mus.1: 49 (1939)
= *Tomophagus* Murrill, Torreya 5: 197 (1905)
= *Trachyderma* (Imazeki) Imazeki, Bull. Gov. Forest Exp. Stn Tokyo 57: 97 (1952)

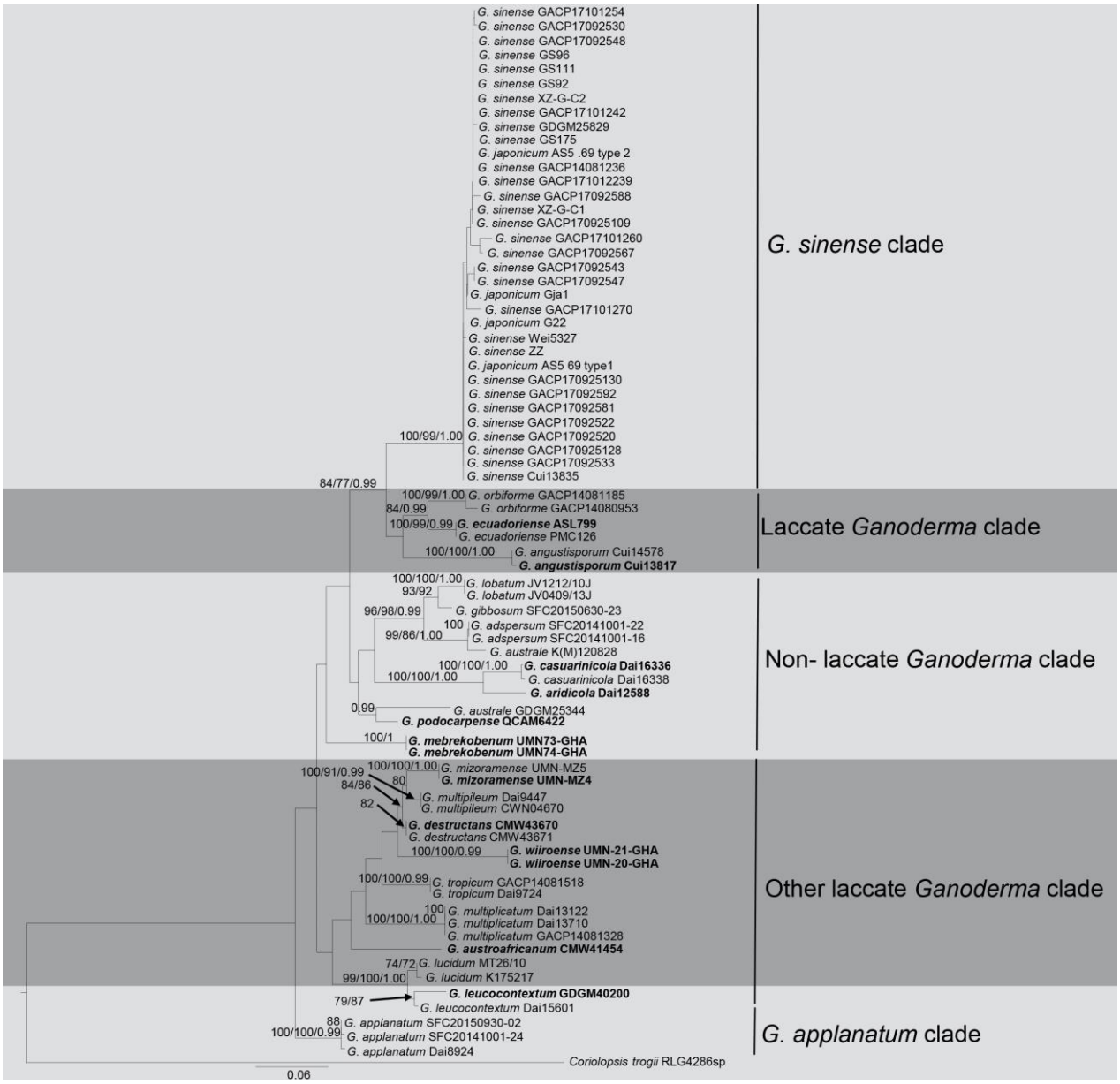

Fig. 1 – Phylogram for *Ganoderma* generated from maximum likelihood analysis of ITS, LSU, SSU and RPB2 sequence data. Bootstrap support values for maximum likelihood and maximum parsimony, greater than 70% and posterior probabilities from Bayesian inference ≥ 0.95 are given above branches. The tree is rooted with *Coriolopsis trogii*. Type species are indicated in bold.

Description (from Ryvarden 2004).

Basidiomes annual or perennial, stipitate to sessile; pileus surface with a thick, dull cuticle or shiny and laccate with a thin cuticle or cuticle of clavate end cells; context cream coloured to dark purplish brown, soft and spongy to firm-fibrous; pore surface cream coloured, bruising brown, the pores regular, 4–7 per mm; tube layers single or stratified, pale to purplish brown; stipe when present central or lateral; hyphal system dimitic; generative hyphae with clamps; skeletal hyphae hyaline to brown, non-septate, often with long, tapering branches; basidia broadly ellipsoid,

tapering abruptly at the base; cystidia absent; basidiospores broadly to narrowly ellipsoid with a truncate apex and apical germ pore, wall two-layered, endosporium brown and separated from the hyaline exosporium by inter-wall pillars, negative in Melzer's reagent, 7–30 μm long.

Type species – *Ganoderma lucidum* (Leyss: Fr.) Karst.

Ganoderma sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang, Acta Microbiol. Sin. 19(3): 272 (1979) Figs 2–19

= *Ganoderma formosanum* T.T. Chang & T. Chen, Trans. Or. Mycol. Soc. 82: 731 (1984).

Misapplications:

= *Ganoderma japonicum* (Fr.) Lloyd in Teng, Fungi of China: 447 (1963); Tai, Syll. Fung. Sin.: 469 (1979); Teng, Fungi of China: 326 (1996), non *Polyporus japonicus* Fr., Epicrisis: 442 (1838) (*Ganoderma japonicum* (Fr.) Lloyd, Mycol. Writ. 3: Syn. Stip. Polyp.: 102(1912).

= *Ganoderma lucidum* (Leyss.) P. Karst. var. *japonicum* (Fr.) Bres. in Teng, Sinensia 5: 199 (1934). non *Polyporus japonicus* Fr., Epicrisis: 442 (1838).

Description (Wang et al. 2005): Basidiomes annual, stipitate, corky-woody. Pileus 2.5–0.5 × 5.2–9 cm. 9.2–1.2 cm thick in nature, 2.5–6.5 × 3.5–12 cm. 0.5–1.5 cm thick when cultivated, dimidiate; upper surface usually purplish black to black, laccate, concentrically sulcate or not, radially rugose; margin often subtruncated. Pore surface pale brown to dark brown: tubes up to 1.4 cm long, grey-brown; pores 5–6 per mm, circular, 50–180 μm diam., dissepiments 40–160 μm thick. Stipe 6–19 cm long, 0.5–1.0 cm thick, lateral, dorsolateral or eccentric, cylindrical or flattened: concolorous with the pileus, laccate. Context 1–5 mm thick, uniformly brown or red brown near the tube layer or with whitish streaks and patches near cutis; hyphal system trimitic; generative hyphae 3–5 μm diam., hyaline, thin-walled, with clamp connections; skeletal hyphae 4.5–7 μm diam., golden brown in 5% KOH solution, dextrinoid in Molder's reagent; ligative hyphae 1–2.5 μm diam., thick-walled, much branched. Basidiospores 10.5–13.5 × 7–9 μm including endosporium and 8–9 × 5.5–7. μm, excluding myxosporium: ovoid; brown with a dark brown eusporium bearing few and thick echinulae, overlaid by a hyaline myxosporium, truncate or not at the apex. Basidia not seen. Cutis hymeniodermic elements 20–60 × 4–8 μm, clavate, amyloid in Melzer's reagent.

Annotated list of *Ganoderma sinense* specimens with different morphological characteristics

We have collected 20 morphologically different *G. sinense* specimens from China and collection sites details are summarized in Table 3. Detailed morphological descriptions of each specimen are as follows.

Specimen no. 1

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 7–8.5 × 6–8 cm, up to 1 cm thick at the base, orbicular, upper surface brownish black (6C8) to brown (6E4) pale brown (5A5) at the margin, concentrically sulcate zones, irregularly ruptured crust overlying the context; margin 1 mm thick, blunt, pale brown (5A5); lower surface pale brown (5A5). *Hymenophore* up to 12 mm long, indistinctly stratose; pores brownish grey (6D2), pores circular, sub circular or isodiametric, 4–5 per mm. *Context* up to 1.5 cm thick, duplex, dry, upper layer dark brown (7F8), corky; lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils, woody. *Stipe* central, sub cylindrical, concolorous with the pileus, 8 × 13 cm, 1.5 cm at the base. *Basidiospore***s** (n = 20) (8.7–)10.1–*11.5*–13(14.1–) × (–5.3)7–*7.9*–8.9(–9.2) μm (Q_m = 1.5, Q= 1.2–2.1, with myxosporium). (6.9–)8.1–*9.2*–10.2(–10.7) × (3.4–)5.3–*6.4*–7.5(–7.8) μm (Q_m = 1.4, Q = 1.2–2.1, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brown (6E4), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.8–*1.1*–1.6) μm in width, colorless, thin-walled; skeletal hyphae (n = 20) (2.8–*3.4*–3.8) μm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding hyphae (n = 20) (3.2–*4.4*–5.4) μm in width, thick walled, branched, nearly solid, brown (6E4) (Fig. 2)

Specimen no. 2

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 12–15.5 × 10–12.5 cm, up to 1.5 cm thick at the base, subreniform; upper surface reddish brown (8E7) to brownish orange (6C8), distinctly concentrically sulcate zones, irregularly ruptured crust overlying the context, swollen at the point of attachment; margin 2 mm thick, blunt, concolorous with the pileus; lower surface pale brown (5A5). *Hymenophore* up to 12 mm long, indistinctly stratose; pores brownish grey (6D2), circular or sub circular, 3–5 per mm. *Context* up to 1 cm thick, duplex, dry; upper layer dark brown (7F8), corky; lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils, woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 8 × 15 cm, 1 cm at the base. *Basidiospores* (n = 25) (10.6–)11.5–*12.5*–13.5(14.3–) × (–7.1)7.7–*8.3*–8.9(–11.7) µm Q_m = 1.5, *Q* = 1.1–1.8, with myxosporium). (6.8–)9.2–*10.2*–11.1(–11.7) × (5.4–)6.1–*6.8*–7.7(–8.4) µm (Q_m = 1.5, *Q* = 1.1 – 1.8, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brown (6E4), clavate like cells. *Context* trimitic, generative hyphae (n = 25) (0.5–*1.6*–3.3) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (3.3–*4.5*–5.2) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding hyphae (n = 20) (3.4–*4.6*–5.5) µm, thick walled, branched, nearly solid, brown (6E4) (Fig. 3).

Specimen no. 3

Basidiome annual, stipitate, laccate, corky. *Pileus* 5.5–8 × 3–4.5 cm, up to 0.5 cm thick at the base, sub orbicular; upper surface brown (6E4) to brownish black (6C8), yellowish brown (5D8) at the margin, slightly radially rugose, irregularly ruptured crust overlying the context; margin 1 mm thick, soft, yellow brown (5D8); lower surface light brown (6D6). *Hymenophore* up to 12 mm long, indistinctly stratose; pores brownish grey (6D2), circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 3 mm thick, duplex, dry; upper layer dark brown (7F8), corky; lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils; woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 5.5 × 12 cm, 1 cm thick at the base. *Basidiospores* (n = 20) (7.5–)8.7–*10.2*–11.6(12.2–) × (–5.2)6.2–*7.2*–8.3(–8.9) µm (Q_m = 1.4, *Q* = 1.2–1.7, with myxosporium). (5.7–)6.5–*7.7*–9(–9.5) × (3.7–)4.5–*5.4*–6.3(–6.5) µm (Q_m = 1.4, *Q* = 1.2–1.7, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5), eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brown (6E4), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (1.3–*2.1*–3.5) µm in width, colourless, thin walled without clamp connections; skeletal hyphae (n = 20) (1.8–*3.9*–6.1) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding hyphae (n = 20) (2.7–*4.2*–6.5) µm in width, thick walled, branched, nearly solid, brown (6E4) (Fig. 4).

Specimen no. 4

Basidiome annual, stipitate, strongly laccate, branched, corky. *Pileus* 6–10 × 5–8 cm, up to 1 cm thick at the base, orbicular; upper surface brown (6E4) to brownish black (6C8), yellowish brown (5D8) at the margin, irregularly ruptured crust overlying the context; margin blunt, yellow brown (5D8); lower surface light brown (6D6). *Hymenophore* up to 20 mm long, indistinctly stratose; pores brownish grey (6D2), circular, 2–4 per mm. *Context* up to 1 cm thick, duplex, dry, upper layer dark brown (7F8), corky; lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils; woody. *Stipe* central, sub cylindrical, concolorous with the pileus, 5 × 8 cm, 1.5 cm at the base. *Basidiospores* (n = 20) (8–)9.7–*11.6*–11.2(13.4–) × (–6.3) 7.3–*8.1*–8.8(–9.6) µm (Q_m = 1.4, *Q* = 1.1–1.6, with myxosporium). (6.6–)8.2–*9.6*–10.9(–11.4) × (5.8–)7.3–*6.7*–7.5(–8.5) µm (Q_m = 1.4, *Q* = 1.2–1.7, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brown (6E4), clavate like cells, dextrinoid. *Context* trimitic; generative hyphae (n = 20) (0.3–*1.3*–2.2) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (2.6–*3.6*–5.1) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding

hyphae (n = 20) (0.9–*2.6*–4.5) μm in width, thick walled, branched, nearly solid, pale brown (5A5) (Fig. 5).

Fig. 2 – *Ganoderma sinense* specimen no. 1 (GACP17092567). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Skeletal hyphae (100×). k Generative hyphae (100×). l Binding hyphae (100×). Scale bars: f–i = 10 μm, j–l = 5 μm.

Fig. 3 – *Ganoderma sinense* specimen no. 2 (GACP17092533). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–h Spores (100×). i Generative hyphae (40×). j Skeletal hyphae (40×). k Binding hyphae (40×). Scale bars: f–h = 10 μm, i–k = 5 μm.

Fig. 4 – *Ganoderma sinense* specimen no. 3 (GACP17092522). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–h Spores (100×). i Skeletal hyphae (100×). j Generative hyphae (100×). k Binding hyphae (100×). Scale bars: f–h = 10 μm, i–k =5 μm.

Fig. 5 – *Ganoderma sinense* specimen no. 4 (GACP17092530). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–h Spores (100×). i Generative hyphae (100×). j Skeletal hyphae (100×). k Binding hyphae (100×). Scale bars: f–h = 10 μm, i–k = 5 μm.

Specimen no. 5

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 7–9 × 7–8 cm, up to 1.5 cm thick at the base, spathulate, suborbicular to irregular shape; upper surface brownish black (6C8), irregularly ruptured crust overlying the context; margin 3 mm thick, blunt, concolourous with the pileus; lower surface light brown (6D6). *Hymenophore* up to 20 mm long, indistinctly stratose; pores brownish grey (6D2), pores circular, 3–5 per mm. *Context* up to 1 cm thick, duplex, dry, upper layer dark brown (7F8); lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils; woody corky. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 8 × 12 cm, 2.5 cm at the base. *Basidiospores* (n = 25) (9.5–)11.1–*12.3*–13.5(14.5–) × (–7.1)8–*8.6*–9.3(–9.9) µm (Q_m = 1.4, Q = 1.1–1.7, with myxosporium). (7.5–)9–*9.8*–10.7(–11) × (4.8–)6.3–*7.1*–7.8(–9.1) µm (Q_m = 1.4, *Q* = 1.1–1.9, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5), eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C4), clavate like cells, dextrinoid. *Context* trimitic; generative hyphae (n = 20) (0.7–*1.1*–1.3) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (2.1–*3.6*–2.9) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding hyphae (n = 22) (1.7–*2.6*–3.8) µm in width, thick walled, branched, nearly solid, brown (6E4) (Fig. 6).

Specimen no. 6

Basidiome annual, stipitate, strongly laccate, branched, corky. *Pileus* 6–10 × 5–8 cm, up to 1 cm thick at the base, subreniform; upper surface grey (8F1) to brownish grey (8E2), radially rugose, concentrically sulcate zone, irregularly ruptured crust overlying the context, margin; 3 mm thick, blunt, concolourous with the pileus, lower surface; reddish brown (8E6). *Hymenophore* up to 20 mm long, indistinctly stratose; pores initially brownish grey (8D2), reddish brown (8E7) to dark brown (8F6), fibrous, composed of coarse loose fibrils, corky; lower layer brownish red (8C8), woody. *Stipe* eccentric, sub cylindrical, dichotomous to trichotomous to irregularly branched, concolorous with the pileus, 5 × 8 cm, 1.5 cm from the base. *Basidiospores* (n = 20) (9.2–)9.8–*11.2*–12.5(12.6–) × (–6.4)7.3–*8.1*–8.7(–8.8) µm, (Q_m = 1.4, *Q* = 1.1–1.4, with myxosporium). (n = 20) (7.2–)7.8–*9.1*–10.3(–10.8) × (5.5–)6.2–*6.7*–6.8(–7.4) µm, (Q_m = 1.3, *Q* = 1.2–1.5, without myxosporium), broadly ellipsoid, brown (6D6), with a light brown (6D6) eusporium bearing thick, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C8), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (1.2–*1.5*–1.8) µm in width, colorless, thin-walled; skeletal hyphae (n = 25) (2.1–*2.8*–3.8) µm in width, thick walled, nearly solid, sometimes branched, brown (6D6); binding hyphae (n = 20) (2.1–*2.8*–4.1) µm in width, thick walled, branched, nearly solid, brown (6D6) (Fig. 7).

Specimen no. 7

Basidiome annual, stipitate, laccate, corky. *Pileus* 6–8 × 3.5–5.5 cm, up to 1 cm thick at the base, sub reniform, upper surface; brownish grey (6C2) to black, radially rugose with turberculate bumps and ridges and rivulose depressions, distinctly concentrically sulcate zones, irregularly ruptured crust overlying the context, swollen at the point of attachment; margin blunt, concolourous with the pileus, 3mm thick; lower surface light brown (6D6). *Hymenophore* up to 15 mm long, indistinctly stratose; pores initially brownish grey (6D2), bruising dark brown (6F6), pores circular or sub circular, 4–6 per mm. *Context* up to 1 cm thick, dry, duplex; upper layer brown (6E6) to dark brown (6F6), fibrous/pithy, composed of coarse loose fibrils; lower layer brown (6D8), woody. *Stipe* dorsally lateral to nearly dorsal, sub cylindrical, concolorous with the pileus, 6 × 10 cm, 1 cm at the base. *Basidiospores* (n = 25) (8.7–)9.9–*11.0*–12.1(12.7–) × (–5.6)6.8–*7.5*–8.1(–8.2) µm, (Q_m = 1.5, *Q* = 1.2–2.6, with myxosporium). (n = 25) (6.1–)7.2–*8.2*–9.2(–9.7) × (4.1–)4.9 –*5.6*–6.4(–6.8) µm, (Q_m = 1.5, *Q* = 1.1–1.8, without myxosporium), ellipsoid, brownish orange (6C8) with a brown (6E6), eusporium bearing thick, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C8), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.5–*1.2*–3.1) µm in width, hyaline, thin walled without clamp

connections; skeletal hyphae (n = 20) (1.3–*2*–3.1) μm in width, thick walled, nearly solid, sometimes branched, brown (6E6) to dark brown (6F6); binding hyphae (n = 30) (0.9–*2.2*–4.6) μm in width, thick walled, branched, nearly solid, brown (6E6) to dark brown (6F6) (Fig. 8).

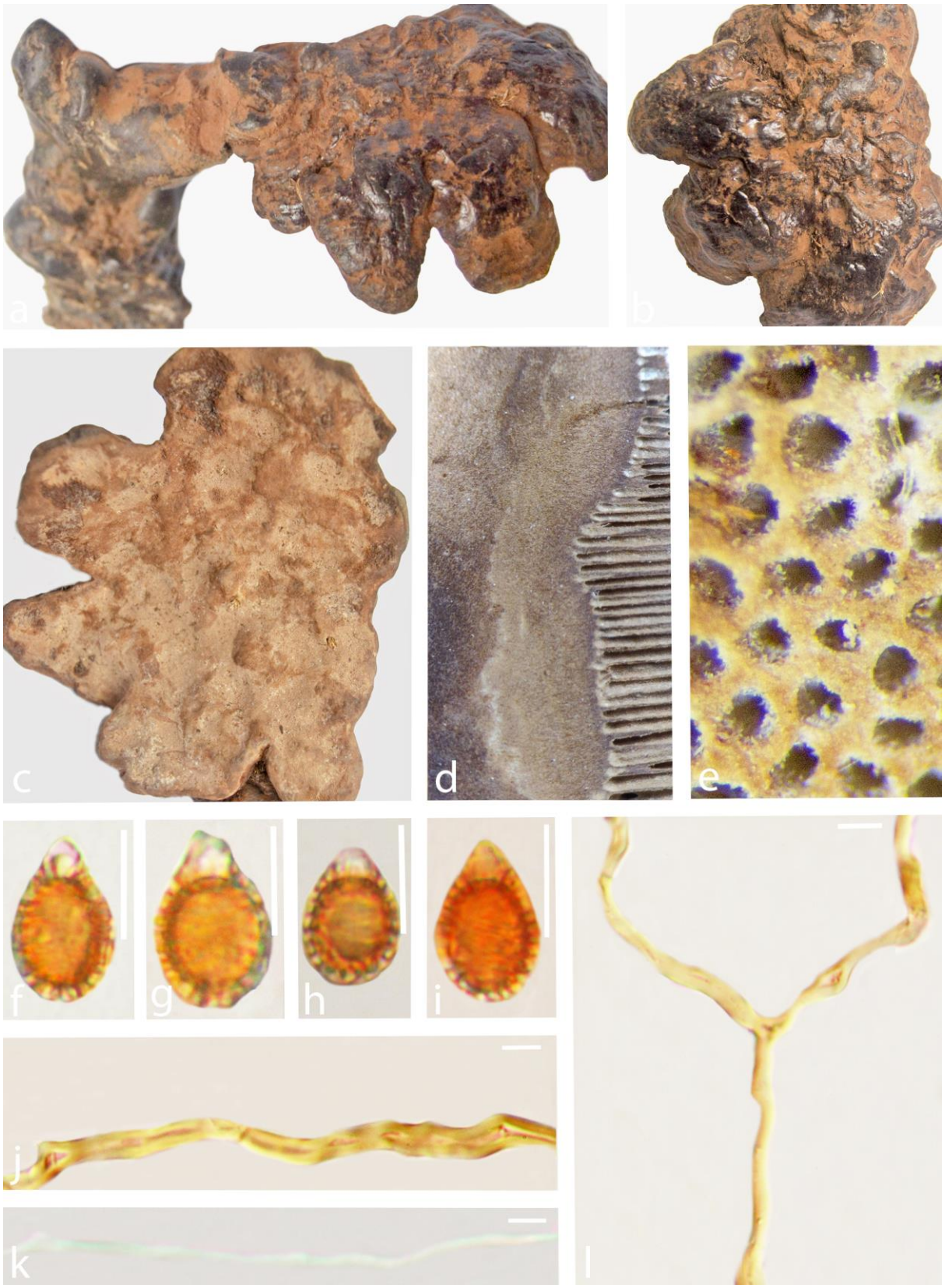

Fig. 6 – *Ganoderma sinense* specimen no. 5 (GACP170925128). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i spores (100×). j Skeletal hyphae (100×). k Generative hyphae (100×). l Binding hyphae (100×). Scale bars: f–i = 10 μm, j–l = 5 μm.

Fig. 7 – *Ganoderma sinense* specimen no. 6 (GACP17092592). a Upper surface. b Lower surface. c Cut surface. d Pores in the lower surface (5×). e–h Spores (100×). i Skeletal hyphae (100×). j Binding hyphae (100×). k Generative hyphae (100×). Scale bars: e–h = 10 μm, i–k = 5 μm.

Fig. 8 – *Ganoderma sinense* specimen no. 7 (GACP171012239). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Cuticle cells (100×). k Skeletal hyphae (100×). l Generative hyphae (100×). m Binding hyphae (100×). Scale bars: f–j = 10 μm, k–m = 5 μm.

Specimen no. 8

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 7–10 × 6–6.5 cm, up to 1 cm thick at the base, subreniform; upper surface reddish brown (8E7) to brownish orange (6C8), radially

rugose, slightly concentrically sulcate, irregularly ruptured crust overlying the context; margin 3 mm thick, blunt, grayish yellow (4B4); lower surface light brown (6D6). *Hymenophore* up to 15 mm long, indistinctly stratose; pores initially brown (6D7), bruising dark brown (6F6), pores circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 1 cm thick, triplex, dry; upper layer light brown (6D6), fibrous, composed of coarse loose fibrils, corky; middle layer dark brown (6F6), fibrous, corky; lower layer dark brown (6E6), woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 8 × 9 cm, 1 cm on the base. *Basidiospores* (n = 25) **(**10.4–)11.3–*12.2*–13.1(14.4–) × (–6.7)7.8–*8.5*–9.2(–9.6) µm, *(*Q_m = 1.4, *Q* = 1.3–1.7, with myxosporium). (n = 25) (8.9–)9.5–*10.2*–10.8(–11.7) × (5.4–)6.5–*7.3*–8.1(–8.5) µm (*Q* = 1.2–1.9, Q_m = 1.4, without myxosporium), ellipsoid, brown (6E6), with a brown (6E6) eusporium bearing thick, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C8), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.2–*1.2*–1.7) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (2.8–*3.5*–4.3) µm in width, thick walled, nearly solid, sometimes branched, brownish orange (6C6); binding hyphae (n = 20) (2.4–*3.3*–4.3) µm in width, thick walled, branched, nearly solid, brownish orange (6C6) (Fig. 9).

Specimen no. 9

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 7–8 × 5–5.5 cm, up to 0.5 cm thick at the base, reniform, sub-dimidiate; upper surface brownish grey to black (8F8), radially rugose, concentrically sulcate, with irregularly ruptured crust overlying the pellis; margin blunt, concolorous with the pileus; lower surface brown (7E8). *Hymenophore* up to 15 mm long, indistinctly stratose; pores initially dark brown (8F8), bruising brown (7E8), pores circular or sub-circular, 4–5 per mm. *Context* up to 1 cm thick, duplex, dry; lower layer brown (7D8), fibrous, composed of coarse loose fibrils; upper layer dark brown (8F8), corky. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 8 × 13 cm. *Basidiospores* (n = 25) (10.6–)11.5–*12.5*–13.5(–14.3) × (7.1–)7.7–*8.3*–8.9(–11.7) µm (*Qm* = 1.5, *Q* = 1.1–1.8 with myxosporium). (6.8–)9.2–*10.2*–11.1(–11.7) × (5.4–)6.1–*6.8*–7.7(–8.4) µm (*Qm* = 1.5, *Q* = 1.1–1.8, without myxosporium), ellipsoid, brownish orange (7C8) to reddish orange (7B8) with a brown eusporium bearing fine, short, and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (7C8), clavate like cells, dextrinoid. *Context* dimitic; generative hyphae (n = 25) (0.5–*1.6*–3.3) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (3.3–*4.5*–5.2) µm thick-walled, nearly solid, sometimes branched, orange (5A6); binding hyphae (n = 20) (3.4–*4.6*–5.5) µm, thick-walled, branched, nearly solid, light brown (5A5) (Fig. 10).

Specimen no. 10

Basidiome annual, stipitate, strongly laccate, branched, corky. *Pileus* 6–16 × 9–11 cm, up to 1.0 cm thick at the base, suborbicular; upper surface dark brown (8F8) to brownish black (6C8), slightly concentrically sulcate, radially rugose, irregularly ruptured crust overlying the context; margin blunt, yellow brown (5D8); lower surface brown (7E8). *Hymenophore* up to 25 mm long, indistinctly stratose; pores initially dark brown (8F8), bruising brown (7E8), pores circular or sub circular, 3–5 per mm. *Context* up to 1 cm thick, duplex, dry, lower layer brown (7D8), fibrous, composed of coarse loose fibrils; upper layer dark brown (8F8), corky. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 5 × 7 cm, 1.5 cm at the base. *Basidiospores* (n = 25) (9.5–)10.8–*12.2*–13.4(13.7–) × (–6.6)7.5–*8.2*–8.9(–9.1) µm (Q_m = 1.5, *Q* = 1.2–1.7, with myxosporium). (7.5–)9.3–*10.4*–11.5(–12) × (5.4–)6–*6.8*–7.7(–7.9) µm (Q_m = 1.5, *Q* = 1.2–1.9, without myxosporium), ellipsoid, brownish orange (7C8) to reddish orange (7B8) with a brown (7D8), eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (7C8), clavate like cells. *Context* trimitic, generative hyphae (n = 20) (1.1–*2*–2.9) µm, colorless, thin-walled; skeletal hyphae (n = 25) (3.3–*3.9*–5.7) µm, thick walled, nearly solid, sometimes branched, orange (5A6); binding hyphae (n = 20) (2.5–*3.9*–5.1) µm, thick walled, branched, nearly solid, orange (5A6) (Fig. 11).

Fig. 9 – *Ganoderma sinense* specimen no. 8 (GACP17092588). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Binding hyphae (100×). k Skeletal hyphae (100×). l Generative hyphae (100×). Scale bars: f–i = 10 μm, j–l = 5 μm.

Fig. 10 – *Ganoderma sinense* specimen no. 9 (GACP14081236). a Upper surface. b Lower surface. c Cut surface. d Pores in the lower surface (5×). e–g Spores (100×). h Generative hyphae (100×). i Skeletal hyphae (100×). j Binding hyphae (100×). Scale bars: e–g = 10 μm, h–j = 5 μm.

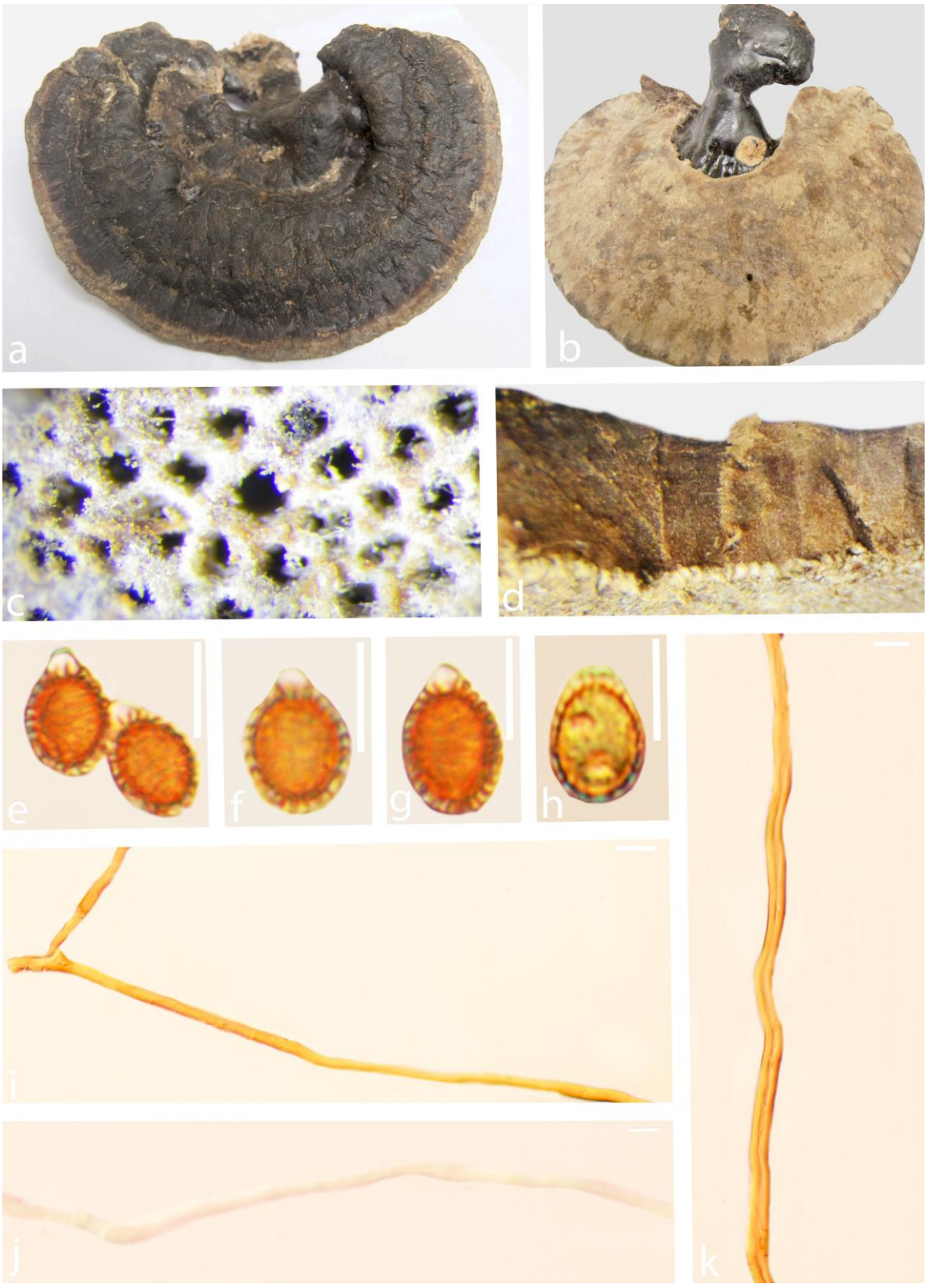

Fig. 11 – *Ganoderma sinense* specimen no. 10 (GACP17092548). a Upper surface. b Lower surface. c Pores in the lower surface (5×). d Cut surface. e–h Spores (100×). i Binding hyphae (100×). j Generative hyphae (100×). k Skeletal hyphae (100×). Scale bars: e–h = 10 μm, i–k = 5 μm.

Specimen no. 11

Basidiome annual, stipitate, laccate, corky. *Pileus* 2.5–4 × 1.5–2.5 cm, up to 0.5 cm thick at the base, sub orbicular to sub reniform; upper surface brownish black (6C8), radially rugose with

turberculate bumps and ridges, slightly concentrically sulcate, irregularly ruptured crust overlying the context; margin 2 mm thick, soft, concolorous with the pileus; lower surface light brown (6D6). *Hymenophore* up to 12 mm long, indistinctly stratose; pores brownish grey (6D2), pores circular or sub circular, 3–5 per mm. *Context* up to 4 mm thick, duplex, dry; upper layer dark brown (7F8); lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils; woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 3 × 8 cm, 1 cm at the base. *Basidiospores* (n = 20) (12.6–)13.1–*13.8*–14.5(13.6–) × (–7.8)8.5–*9.1*–9.7(–10.3) µm (Q_m = 1.5, Q = 1.4–1.8, with myxosporium). (10.5–)11.3–*12.0*–12.7(–13.4) × (6.1–)6.7–*7.4*–8.1(–8.5) µm (Q_m = 1.6, Q = 1.4–2.0, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C4), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.8–*1.2*–1.6) µm in width, colourless, thin walled without clamp connections; skeletal hyphae (n = 20) (3.4–*4.6*–5.2) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4); binding hyphae (n = 20) (3.4–*4.2*–5.9) µm in width, thick walled, branched, nearly solid, brown (6E4) (Fig. 8) (Fig. 12).

Specimen no. 12

Basidiome annual, stipitate, laccate, corky. *Pileus* 1.5–4.5 × 1–2.5 cm, up to 0.5 cm thick at the base, subreniform to sub orbicular; upper surface brownish black (6C8), radially rugose with turberculate bumps and ridges, distinctly concentrically sulcate, irregularly ruptured crust overlying the context, swollen at the point of attachment; margin 2 mm thick, soft, concolourous with the pileus; lower surface light brown (6D6). *Hymenophore* up to 12 mm long, indistinctly stratose; pores initially brownish grey (6D2), bruising dark brown (6F6), pores sub circular or isodiametric, 2–4 per mm. *Context* up to 3 mm thick, duplex, dry; upper layer dark brown ((7F8); lower layer light brown (6D6), fibrous, composed of coarse loose fibrils, woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 4 × 8 cm, 0.5 cm at the base. *Basidiospores* (n = 20) **(**10.1–)11.4–*12.5*–13.5(14.4–) × (–6.9)7.8–*8.5*–9.1(–9.7) µm (Q_m = 1.4, Q = 1.2–1.7, with myxosporium). (8.5–)9.7–*10.8*–11.9(–12.8) × (5.1–)6–*6.8*–7.6(–8.2) µm (Q_m = 1.6, Q = 1.2–2, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5), eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C4), clavate like cells. *Context* dimitic; generative hyphae not observed; skeletal hyphae (n = 20) (2.5–*3.3*–4.2) µm in width, thick walled, nearly solid, sometimes branched, pale brown (5A5); binding hyphae (n = 20) (2.6–*3.3*–4.1) µm in width, thick walled, branched, nearly solid, brownish orange (6C4) (Fig. 13).

Specimen no. 13

Basidiome annual, stipitate, laccate, corky. *Pileus* 2.5–5 × 1–2.5 cm, up to 0.4 cm thick at the base, reniform; upper surface brownish black (6C8), slightly concentric, not sulcate, radially rugose with turberculate bumps and ridges, irregularly ruptured crust overlying the context; margin 2 mm thick, soft, concolorous with the pileus; lower surface light brown (6D6). *Hymenophore* up to 12 mm long, indistinctly stratose; pores initially brownish grey (6D2), bruising dark brown (6F6), pores circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 8 mm thick, triplex, dry; upper layer dark brown ((7F8); middle layer light brown (6D6); lower layer brown (6E4) to dark brown (7F8), fibrous, composed of coarse loose fibrils, woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 3.5 × 8 cm, 1 cm thick at the base. *Basidiospores* (n = 20) (10.5–)11.8–*12.9*–13.9(14.3–) × (–8.1)8.3–*8.9*–9.5(–10) µm *(*Q_m = 1.4, Q = 1.2–1.7, with myxosporium). (8.8–)10.1–*11.2*–12.3(–12.9) × (5.5–)6.5–*7.3*–8.1(–8.4) µm *(*Q_m = 1.5, Q = 1.2–1.9, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C4), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.4–*1.1*–1.5) µm in width, colourless, thin walled without clamp connections; skeletal hyphae (n = 20) (4–*4.8*–5.9) µm in

width, thick walled, nearly solid, sometimes branched, brownish orange (6C4); binding hyphae (n = 20) (1.2–*2.6*–3.9) μm in width, thick walled, branched, nearly solid, pale brown (5A5) (Fig. 14).

Fig. 12 – *Ganoderma sinense* specimen no. 11 (GACP17101242). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–h Spores (100×). i Generative hyphae (100×). j Skeletal hyphae (100×). k Binding hyphae (100×). Scale bars: f–h = 10 μm, i–l = 5 μm.

Fig. 13 – *Ganoderma sinense* specimen no. 12 (GACP17101254). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Binding hyphae (100×). k Skeletal hyphae (100×). Scale bars: f–i = 10 μm, j–k = 5 μm.

Specimen no. 14

Basidiome annual, stipitate, laccate, corky. *Pileus* 2–4 × 1–1.5 cm, up to 0.5 cm thick at the base, flabelliform; upper surface reddish brown (8E7) to brownish black (6C8), radially rugose

with turberculate bumps and ridges, irregularly ruptured crust overlying the context; margin 2 mm thick, soft, yellowish brown (5D8); lower surface light brown (6D6). *Hymenophore* up to 12 mm long, indistinctly stratose; pores initially brownish grey (6D2), bruising dark brown (6F6), pores circular, 3–4 per mm. *Context* up to 1 cm thick, duplex, dry; upper layer dark brown (7F8), lower layer brown (6E4) to dark brown ((7F8), fibrous, composed of coarse loose fibrils, woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 5.5 × 10 cm, 1 cm thick at the base. *Basidiospores* (n = 20) (10.3–)11.5–*12.8*–14.2(15.6–) × (–8.1)8.5–*9.2*–9.8(–10.9) µm (Q_m = 1.4, *Q* = 1.1–1.7, with myxosporium). (8.3–)9.7–*11.1*–12.5(–13.8) × (6.4–)6.9–*7.7*–8.5(–10.1) µm (Q_m = 1.4, *Q* = 1.1–1.8, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (6C4), clavate like cells, dextrinoid. *Context* trimitic; generative hyphae (n = 20) (0.4–*1*–1.5) µm in width, colourless, thin walled without clamp connections; skeletal hyphae (n = 20) (2.1–*3.9*–3.9) µm in width, thick walled, nearly solid, sometimes branched, brown (6E4) to brownish orange (6C4); binding hyphae (n = 20) (1.4–*2.7*–3.7) µm in width, thick walled, branched, nearly solid, pale brown (5A5) (Fig. 15).

Specimen no. 15

Basidiome annual, stipitate, laccate, corky. *Pileus* 8–12 × 7–9 cm, up to 1.5 cm thick at the base, spathulate; upper surface dark brown (8F8) to brownish black (6C8), radially rugose with turberculate bumps and ridges and rivulose depressions, concentrically sulcate zones, irregularly ruptured crust overlying the context; margin blunt to truncate, concolorous with the pileus; lower surface brown (7E8). *Hymenophore* up to 15 mm long, indistinctly stratose; pores initially dark brown (8F8), bruising brown (7E8), pores circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 8 mm thick, duplex, dry, lower layer brown (7D8), fibrous, composed of coarse loose fibrils; upper layer dark brown (8F8), corky. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 6 × 11 cm, 2.5 cm thick at the base. *Basidiospores* (n = 25) (9.8–)10.6–*11.3*–11.9(12.1–) × (–6.9)7.3–*8*–8.6(–9.3) µm (Q_m = 1.4, *Q* = 1.2–1.6, with myxosporium). (7.6–)8.2–*8.8*–9.5(–10.3) × (4.3–)5.3–*6.1*–6.9(–7.6) µm (Q_m = 1.5, *Q* = 1.1–1.9, without myxosporium), ellipsoid, brownish orange (7C8) to reddish orange (7B8) with a brown (7D8) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipelli***s** a hymeniderm, brownish orange (7C8), clavate like cells. *Context* trimitic; generative hyphae (n = 20) (0.6–*1.4*–2.4) µm, hyaline, colourless, thin walled without clamp connections; skeletal hyphae (n = 20) (0.9–*2.1*–3.5) µm, thick walled, nearly solid, sometimes branched; binding hyphae (n = 20) (2.7–*3.8*–5.3) µm, thick walled, branched, nearly solid, orange (5A6) (Fig. 16).

Specimen no. 16

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 4–5.5 × 3–4 cm, up to 1 cm thick at the base, spathulate; upper surface dark brown (8F8) to brownish black (6C8), radially rugose, irregularly ruptured crust overlying the context; margin blunt, concolorous with the pileus; lower surface brown (7E8). *Hymenophore* up to 15 mm long, indistinctly stratose; pores initially dark brown (8F8), circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 1 cm thick, duplex, dry; lower layer brown (7D8), fibrous, composed of coarse loose fibrils; upper layer dark brown (8F8), corky. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 8 × 13 cm, 1 cm at the base. *Basidiospores* (n = 20) (9.9–)10.5–*11.3*–12.1(12.8–) × (–6.6) 7.5–*8.2*–8.6 (–9.1) µm (Q_m = 1.4, *Q* = 1.2–1.5, with myxosporium). (8.1–)8.7–*9.4*–10(–10.4) × (5.8–) 6.3–*6.7*–7.3 (–7.6) µm (Q_m = 1.4, *Q* = 1.2–1.6, without myxosporium), ellipsoid, brown, with a brown eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, orange (5A6), clavate like cells, dextrinoid. *Context* trimitic; generative hyphae (n = 20) (0.5–*1*–1.5) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (2.2–*3.4*–5.4) µm thick walled, nearly solid, sometimes branched, orange (5A6); binding hyphae (n = 20) (1.1–*3.3*–5.2) µm in width, thick walled, branched, nearly solid, orange (5A6). (Fig. 17).

Fig. 14 – *Ganoderma sinense* specimen no. 13 (GACP17101260). a Upper surface. b Lower surface. c Cut surface. d Pores in the lower surface (5×). e–h Spores (100×). i Skeletal hyphae (100×). j Binding hyphae (100×). k Generative hyphae (100×). Scale bars: e–h = 10 μm, i–k = 5 μm.

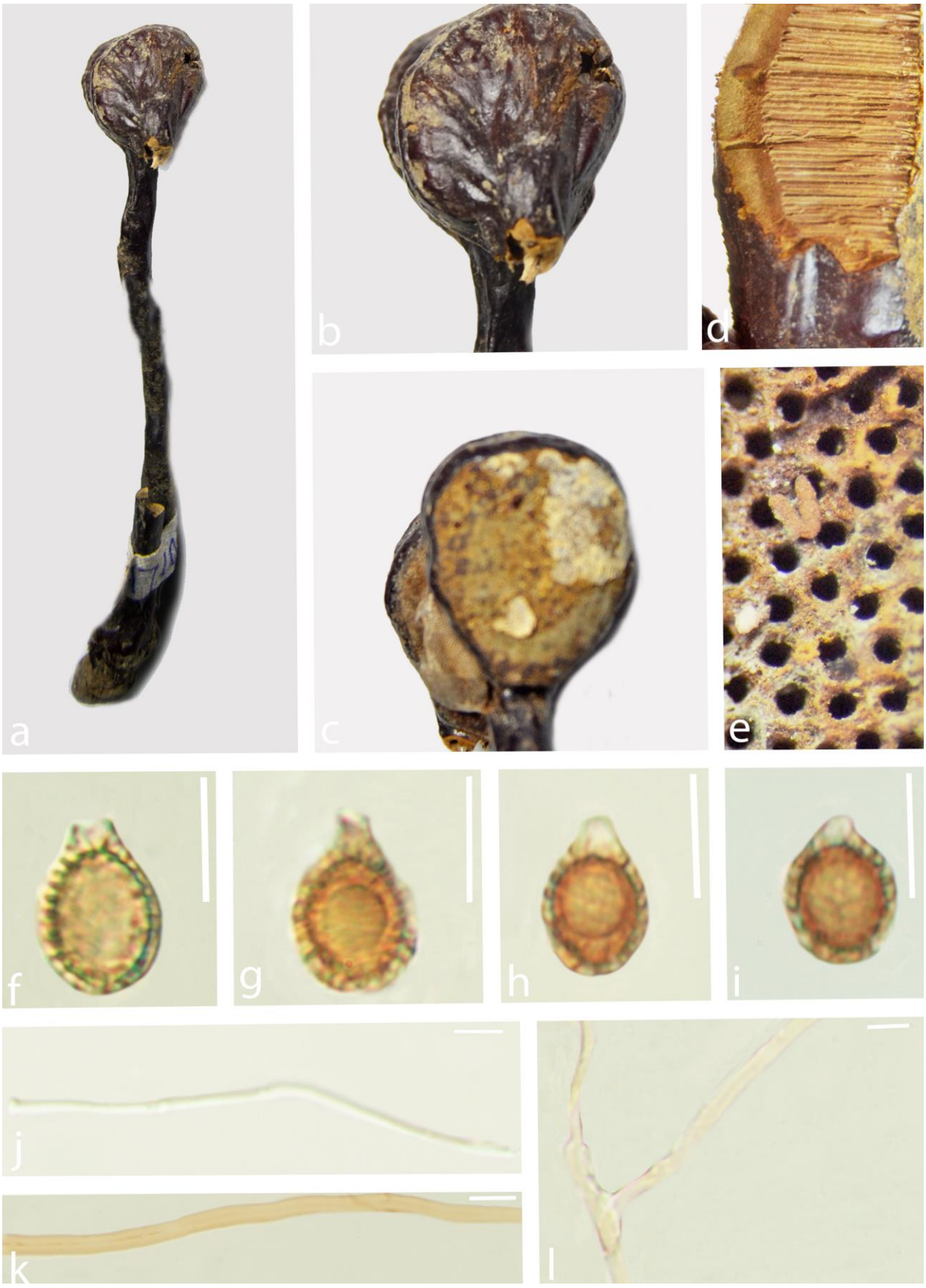

Fig. 15 – *Ganoderma sinense* specimen no. 14 (GACP17101270). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Generative hyphae (100×). k Skeletal hyphae (100×). l Binding hyphae (100×). Scale bars: f–I = 10 μm, j–l = 5 μm.

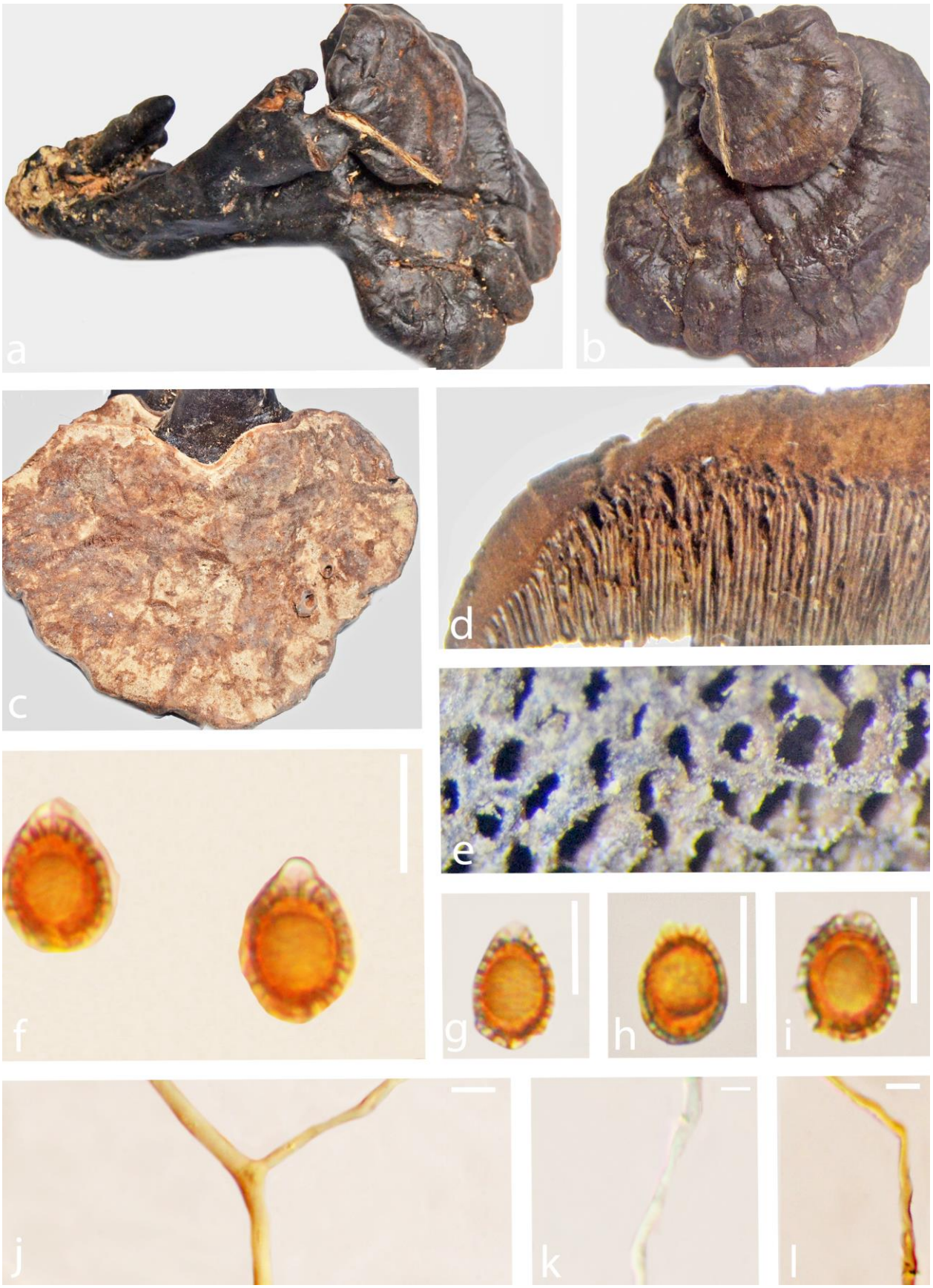

Fig. 16 – *Ganoderma sinense* specimen no. 15 (GACP17092581). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). i Binding hyphae (100×). j Generative hyphae (100×). k Skeletal hyphae (100×). Scale bars: f–i = 10 μm, j–l = 5 μm.

Fig. 17 – *Ganoderma sinense* specimen no. 16 (GACP17092520). a Upper surface. b Lower surface. c Cut surface (5×). d Pores in the lower surface. e–g spores (100×). h Generative hyphae (40×), i Binding hyphae (40×). j Skeletal hyphae (40×). Scale bars: e–g = 10 μm, h–j = 5 μm.

Specimen no. 17

Basidiome annual, stipitate, laccate, corky. *Pileus* 7.5–11 × 6–7.5 cm, up to 1.5 cm thick at the base, reniform; upper surface dark brown (8F8) to brownish black (6C8), radially rugose, concentrically sulcate zones, irregularly ruptured crust overlying the context; margin blunt or wavy, concolorous with the pileus, lower surface brown (7E8). *Hymenophore* up to 20 mm long, indistinctly stratose; pores initially dark brown (8F8), bruising brown (7E8) pores circular, sub circular or isodiametric, 4–5 per mm. *Context* up to 5 mm thick, duplex, dry, lower layer brown (7D8), fibrous, composed of coarse loose fibrils; upper layer dark brown (8F8), corky. *Stipe* eccentrically stipitate, sub cylindrical, concolorous with the pileus, 5 × 7 cm, 1.5 cm at the base. *Basidiospores* (n = 25) (8.5–)9.1–*10.2*–11.3(12.3–) × (–4.7)6.2–*7*–7.8(–8.3) µm (Q_m = 1.5, Q = 1.1–1.9, with myxosporium). (6.2–)6.6–*7.5*–8.3(–8.9) × (3.2–)4.2–*5*–5.7(–6.2) µm (Q_m = 1.5, Q = 2.1–1.2, without myxosporium), ellipsoid, brownish orange (7C8) to reddish orange (7B8) with a brown (7D8) eusporium bearing fine, short and distinct echinulae overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, orange (5A6), clavate like cells, dextrinoid. *Context* trimitic; generative hyphae (n = 30) (0.8–*1.3*–1.8) µm in width, colourless, thin walled without clamp connections; skeletal hyphae (n = 30) (2.6–*4.1*–5.8) µm in width, thick walled, nearly solid, sometimes branched, orange (5A6); binding hyphae (n = 20) (1.3–*1.7*–2.4) µm in width, thick walled, branched, nearly solid, orange (5A6) (Fig. 18).

Specimen no. 18

Basidiome annual, stipitate, strongly laccate, corky. *Pileus* 7–10 × 6–8 cm, up to 1 cm thick at the base, subreniform; upper surface brown (6E4) to brownish black (6C8), yellowish brown (5D8) at the margin, slightly concentrically sulcate zones, radially rugose, irregularly ruptured crust overlying the context; margin 2 mm thick, blunt, yellow brown (5D8); lower surface pale brown (5A5). *Hymenophore* up to 10 mm long, indistinctly stratose; pores initially brownish grey (6D2), pores circular, sub circular or isodiametric, 3–5 per mm. *Context* up to 1 cm thick, duplex, dry, upper layer dark brown (7F8), corky; lower layer pale brown (5A5), fibrous, composed of coarse loose fibrils, woody. *Stipe* eccentric, sub cylindrical, concolorous with the pileus, 5 × 8 cm, 1.5 cm at the base. *Basidiospores* (n = 20) (9.6–)10.3–*11.4*–12.6(13.5–) × (–6.7)7.3–*7.8*–8.4(–8.9) µm, (Q_m = 1.5, Q = 1.1–1.7, with myxosporium). (7.8–)8.4–*9.4*–10.4 (–11.5) × (4.6–)5.6–*6.2*–6.8(–7.4) µm (Q_m = 1.5. Q = 1.2–1.8, without myxosporium), ellipsoid, brown (6E4), with a pale brown (5A5) eusporium bearing fine, short and distinct echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brown (5D8), clavate like cells. *Context* trimitic, generative hyphae (n = 20) (0.6–*1.3*–1.8) µm in width, colorless, thin-walled; skeletal hyphae (n = 20) (3.8–*5.2*–6.5) µm in width, thick walled, nearly solid, sometimes branched, pale brown (5A5); binding hyphae (n = 20) (2.2–*3.2*–4.4) µm in width, thick walled, branched, nearly solid, brown (6E4) (Fig. 19).

Notes – *Ganoderma sinense* was originally described from Hainan Province, China by Zhao et al. (1979) and is characterized by slightly longitudinally crested basidiospores and a uniformly brown to dark brown context. Although the holotype of this species is now very scanty and difficult to observe, our *G. sinense* collections agree with that of the holotype described by Wang & Wu (2007), and with descriptions given by Bi et al. (1993), Zhao & Zhang (2000), Wu & Dai (2005). *Ganoderma sinense* was long considered to be *G. lucidum* or *G. japonicum* by Chinese authors (Teng 1934, 1939, 1963). *Ganoderma lucidum* is characterized by cream or pinkish buff to clay buff colour basidiome, cuticle composed of clavate like cells and ellipsoid to broadly pear-shaped, coarsely echinulated basidiospores (Zhou et al. 2015). However, *G. sinense* differs from *G. lucidum* by having thin-fleshed basidiomes, with long, slender stipes, rarely branched skeletal hyphae with bovista type binding hyphae (Pegler & Yao 1996). *Ganoderma japonicum* (= *G. dimidiatum*) has a yellow pileus when young, red-brown or rusty brown at maturity and off-white context (Pegler & Yao 1996), but *G. sinense* has purplish black to black pileus and brown context. *Ganoderma formosanum*, introduced from Taiwan by Chang & Chen (1984) has very similar morphology to *G. sinense*. However, Zhao & Zhang (2000) considered *G. formosanum* as distinct based on its duplex context and ovoid basidiospores, and this conclusion was followed by Wu & Dai (2005). Wang &

Wu (2007) studied both holotypes of *G. formosanum* and *G. sinense* and confirmed that both species share similar morphological characteristics. Furthermore, it was concluded that these species are synonyms based on molecular and morphological data and the earliest valid name to be used is *G. sinense* (Moncalvo et al. 1995a, Wang & Wu 2007).

Fig. 18 – *Ganoderma sinense* specimen no. 17 (GACP170925130). a upper surface. b lower surface. c pores in the lower surface (5×). d cut surface. e–g spores (100×). h generative hyphae (100×). i skeletal hyphae (100×). j binding hyphae (100×). Scale bars: e–g = 10 µm, h–j = 5 µm.

Fig. 19 – *Ganoderma sinense* specimen no. 18 (GACP17092543). a Upper surface. b Lower surface. c Cut surface. d Pores in the lower surface (5×). e–g Spores (100×). h Skeletal hyphae (40×). i Generative hyphae (40×). j Binding hyphae (40×). Scale bars: e–g = 10 μm, h–j = 5 μm.

Ganoderma sinense is morphologically similar to *G. orbiforme* in having a purplish black to black laccate pileus, uniformly brown context or whitish streaks or patches near the cuticle, a dorsally lateral or lateral stipe and subtropical-tropical distribution (Wang et al. 2014). However, *G.*

sinense can easily be distinguished from *G. orbiforme* since *G. sinense* bears an erect stipe, cuticle composed of clavate cells, and ovoid basidiospores with few, long and thick echinulae (Wang et al. 2014). Also, these 2 species have been clearly separated based on molecular data (Wang et al. 2014, Hapuarachchi et al. 2018b, this study).

In Pharmacopoeia of People's Republic of China 2000 edition (part one) the general term "Lingzhi" covers both "Chizhi (*G. lucidum*)" and "Zizhi" (*G. sinense*). Nevertheless, with the aid of molecular data, *G. lucidum* and *G. sinense* are clearly distinguished as two different taxa (Zhou et al. 2008, Liao et al. 2015, this study). Based on chemotaxonomical data, oxygenated lanostane-type triterpenes have been proven as suitable markers to differentiate *G. lucidum* and *G. sinense* (Fu et al. 2008).

Discussion

In this study, we retrieved all currently available ITS, LSU, SSU and RPB2 gene sequences of *G. sinense* and *G. japonicum* from GenBank. We observed, identical sequences of each gene separately; four *G. sinense* (Wei5327, Cui13835, ZZ, GACP14081236) and three *G. japonicum* (G22, Gja-1, AS5.69 type 1) ITS sequences obtained from GenBank together with 13 ITS sequences from our analysis (GACP17092520, GACP17092522, GACP17092530, GACP17092533, GACP17092543, GACP17092547, GACP17092581, GACP17092588, GACP17092592, GACP170925130, GACP17101242, GACP17101260, GACP17101270), one LSU sequence (Wei5327) accessed from GenBank and eight LSU sequences from this study (GACP17092533, GACP17092543, GACP17092567, GACP17092588, GACP17092512239, GACP17101254, GACP17101260, GACP17101270), eight SSU sequences from our analysis (GACP17092530, GACP17092533, GACP17092543, GACP17092567, GACP17092588, GACP17101254, GACP17101260, GACP17101270) and two RPB2 sequences, retrieved from GenBank (Wei5327 and Cui13835) and two sequences from our study (GACP17092543, GACP17092588), respectively. However, we noticed (reference sequence: *G. sinense* voucher Wei5327), seven *G. sinense* (GS92, GS96, GS111, GS175, GDGM25829, XZ-G-C1, XZ-G-C2) and one *G. japonicum* (AS5.69 type 2) ITS sequences retrieved from GenBank, together with six *G. sinense* ITS sequences from this study (GACP17092567, GACP17092588, GACP170925109, GACP170925130, GACP17101242, GACP17101254) show single-nucleotide polymorphism (SNP) located at position 158 in ITS 1 region (Fig. 20). However, they all grouped together to form a monophyletic clade in the phylogenetic tree (Fig. 1). Furthermore, there are no observed SNP's in 13 *G. sinense* sequences from this study and three of each *G. sinense* and *G. japonicum* nucleotide sequences retrieved from GenBank. SNP leads to phenotypic plasticity of species of *Ganoderma* and as such could easily lead to specimens being misidentified as new taxa. This phenomenon has been previously observed in *G. orbiforme* (Wang et al. 2014) and *G. lucidum* (Zhang et al. 2017), and this is the first time to record SNP in *G. sinense*.

As previously mentioned, *G. japonicum* (= *G. dimidiatum*) and *G. sinense* are two different species based on morphological evidence. Nevertheless, all *G. japonicum* and *G. sinense* sequences clustered together in our analysis. *Ganoderma japonicum* was introduced as *Boletus dimidiatus* from Japan (Thunberg 1784) based on laterally stipitate basidiocarp with usually undulate, ferruginous and shiny upper surface, yellow margin and white pore surface. Fries (1821) synonymized this species with *Polyporus lucidus* (Curtis) Fr., and Fries (1838) assigned the epithet "*japonicus*" for *B. dimidiatus* and accepted it as a separate taxon. Both *P. japonicus* Fr. and *P. lucidus* var. *japonicus* Fr. names can be accepted for this species since Fries did not number this taxon in the same page (Papp 2016). Bresadola (1912) combined *P. japonicus* in *Ganoderma* and introduced it as a variety of *G. lucidum*. Sawada (1931) suggested that *P. japonicus* is a separate species and referred to it as *G. japonicum* (Fr.) Sawada by observing the darker form of *G. lucidum*. Imazeki (1939) suggested that *P. japonicus* sensu Fries is a synonym of *Ganoderma lucidum*. The original descriptions and drawings of both *Boletus dimidiatus* and *P. japonicus*, suggest that they represent a member of the *G. lucidum* species complex (Dai et al. 2017). However, *G. lucidum sensu stricto* is distributed in northern and southern Europe, and probably extends to China

(Moncalvo et al. 1995a, b). Further studies confirmed that the species named *G. lucidum* from both Europe and mainland China are not conspecific based on analyses of ITS and 25S ribosomal DNA sequences. Later, many other authors confirmed the same opinion (Pegler & Yao 1996, Smith & Sivasithamparam 2000, Hong & Jung 2004). The specimen examined by Fries has probably been lost (Moncalvo & Ryvarden (1997) and *P. japonicus* Fr. is based on the type of *Boletus dimidiatus*, which was cited by Fries in 1838 (Papp 2016). Since the names published in Fries (1838) were not sanctioned, the binomial *P. japonicus* is illegitimate and is a superfluous name for *B. dimidiatus*. Hence, the new combination, *Ganoderma dimidiatum* (Thunb.) V. Papp was proposed as the earliest valid name to use for this species (Papp 2016).

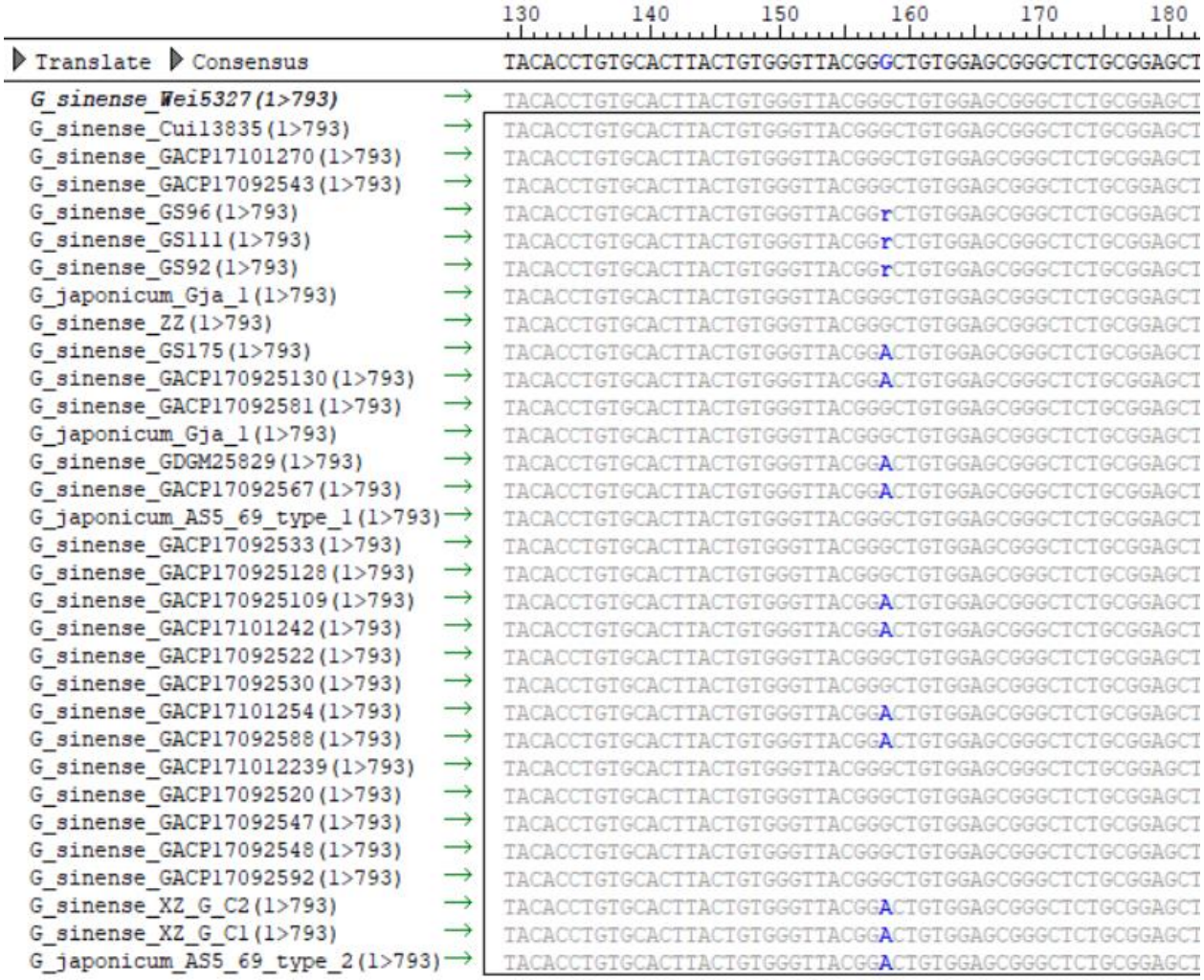

Fig. 20 – Multiple-alignment of *Ganoderma sinense* and *G. japonicum* ITS1 sequences. A single-nucleotide polymorphism located at positions 158 is indicated in blue (Reference sequence: *G. sinense* voucher Wei5327). R stands for 'AG'.

Since, *G. japonicum* is a blackish, laccate, and stipitate fungus, it was generally synonymized with the traditional Chinese medicinal mushroom *G. sinense* distributed in subtropical areas (Teng 1964, Zhao 1989). However, it is most likely a misapplication, since specimens of *G. japonicum* from China and Japan are not conspecific as *G. japonicum* has a yellow pileus when young, red brown or rusty brown at maturity and off-white context, therefore is clearly distinguished from the purplish black to black pileus and brown context in *G. sinense* (Pegler & Yao 1996, Dai et al. 2017). Moreover, in our collections we observed a brown context in every specimen, which in turn concluded them as *G. sinense*. Liao et al. (2015) suggested that *G. sinense* and *G. japonicum* should be considered as synonyms based on their high sequence similarity in ITS data. We also observed

this scenario in our analysis, not only ITS (except SNP showing sequences), but with LSU, SSU and RBP2 sequence data. Despite that, all the available sequences of *G. japonicum* (Wang & Yao 2005) in GenBank were obtained from Chinese specimens (descriptions unavailable) and were most likely wrongly identified. Also, still there are no molecular data available under the new authentic name "*G. dimidiatum*" in GenBank. Subsequently, we could not assure the authenticity of the distribution of *G. dimidiatum* in China since lack of molecular evidence from the type locality (Japan). Hence, based on molecular data, we conclude that the above studied materials from China are conspecific and represent one taxon; *G. sinense*. We would recommend recollecting fresh collections and reference collections from the type locality to clarify the taxonomy of *G. dimidiatum*. In addition, to obtain a better understanding of the evolution of *G. dimidiatum* and *G. sinense*, a phylogeny with more genes, and in particular single-copy nuclear genes such as TEF1 or β-tubulin would be recommended. Furthermore, a combination of morphological, chemotaxonomic and molecular methods would be more appropriate to develop a more stable taxonomy *G. dimidiatum* and *G. sinense*.

Our study reveals that the collection of 20 *G. sinense* specimens exhibit great variability in macro-morphology, yet the micro-morphological characteristics (hyphal system, basidiospore size and shape) do not show significant variation except one specimen; dimitic hyphal system (GACP17101254), pores circular, subcircular or isodiametric in shape and basidiospore size varies from 10–14 × 7–9 μm with ellipsoid shape (Table 2).

Among our 20 *G. sinense* specimens, 14 were from Sandu Shui County and five from Rongjiang County, Kaili, Guizhou Province, China. One specimen was from its type locality, Hainan Province, China (Table 3); this island has subtropical to tropical vegetation (Dai et al. 2011). Sandu Shui and Rongjiang Countries have a subtropical monsoon climate and subtropical humid monsoon climate, respectively (National Meteorological Information Center, China, 2000). As previously described, environmental factors such as temperature, humidity and air pressure vary according to the elevation of mountains (Table 3). Researchers have shown that the range and duration of light exposure play important roles in the formation of basidiomes in fungi such as *Coprinus* sp., *Typhula ishikariensis* and *Clavicorona pyxidata* (Morimoto & Oda 1973, James & McLaughlin 1988, Boulianne et al. 2000, Kawakami et al. 2004). The high frequency of *G. sinense* specimens found in southwestern Guizhou Province (Sandu and Kaili) in the rainy summer suggests that temperature and humidity are important factors in the formation and maturation of *G. sinense*. However, the temperature effect is not likely to be direct, as specimens were most commonly found under closed canopies where they reach greater length (GACP17092533, GACP17092592). Few researchers described the relationship between the extent of canopy coverage and light incidence in tropical zone mushrooms (Lodge & Cantrell 1995, Suárez-Duque 2004, Gibertoni et al. 2007, Lodge et al. 2008). Steep slopes enhance basidiome development of *Thelephora* sp. due to factors related to humidity and nutrient requirements (Lodge et al. 2008). In this study, *G. sinense* specimens were found predominantly on steeper slopes due to improved nutrient uptake and with lower humidity. Hence, environmental factors such as air pressure, elevation, humidity, nutrient uptake, pH and temperature significantly vary in Hainan, Kaili and Sandu mountain areas which in turn affect basidiome development in *G. sinense*. In addition, slope, orientation and vegetation types in the collected area greatly affect the phenotypic plasticity (Ramírez-López et al. 2013).

Based on a combined analysis of the molecular results, related micro morphological data and environmental factors, we conclude that all 20 specimens examined in this study are conspecific even though the macro-morphology shows great variability. We suggest establishing *Ganoderma sinense* as a species complex based on molecular data. However, we need epitypes, reference collections and fresh collections with multigene nucleotide sequence data (with TEF1 or β-tubulin) to determine the taxonomy and evolution of *G. sinense*. A combination of morphological, chemotaxonomic, molecular and extrinsic factor determination methods would be suitable to establish a more stable taxonomy for *G. sinense*. Maximum taxon sampling, roles of conductivity

and distribution of nutrients, structure and type of soils data will be needed to better understanding the development and distribution of this species.

Table 2 Morphological characteristics comparison for 20 *Ganoderma sinense* specimens observed in this study

Specimen voucher	**Basidiome upper surface**								**Context**	**Hyphal system**	**Basidiospore**	
	Stipe orientation	**Shape**	**Size** (cm)	**Colour**	**Margin colour**	**Laccate/non laccate**	**Concentric zones**	**Radially rugose**			**Shape**	**Average size** (μm)
GACP17092567	Centrally stipitate	Orbicular	7–8.5 × 6–8	Yellowish brown to pale brown	Pale brown	Strongly laccate	Weakly concentrically sulcate	Not observed	Duplex	Trimitic	Ellipsoid	11.5 × 7.9
GACP17092533	Eccentrically stipitate	Reniform	12–15.5 × 10–12.5	Yellowish brown to pale brown when young, becoming reddish brown to brownish orange when mature	Concolorous with the pileus	Strongly laccate	Distinctly concentrically sulcate	Not observed	Duplex	Trimitic	Ellipsoid	12.5 × 8.3
GACP17092522	Eccentrically stipitate	Suborbicular	5.5–8 × 3–4.5	Yellowish brown to pale brown	Yellow brown	Laccate	Not observed	Slightly radially rugose	Duplex	Trimitic	Ellipsod	10.2 × 7.2
GACP17092530	Centrally stipitate	Orbicular	6–10 × 5–8	Yellowish brown to pale brown when young, becoming reddish	Yellow brown	Strongly laccate	Weakly concentrically sulcate	Not observed	Duplex	Trimitic	Ellipso-id	11.6 × 8.6

Table 2 Continued.

Specimen voucher	Basidiome upper surface								Context	Hyphal system	Basidiospore	
	Stipe orientation	Shape	Size (cm)	Colour	Margin colour	Laccate/non laccate	Concentric zones	Radially rugose			Shape	Average size (μm)
				brown to brownish orange when mature								
GACP170925128	Eccentrically stipitate	Spathulate	7–9 × 7–8	Yellowish brown to pale brown when young, becoming reddish brown to brownish orange when mature	Concolorous with the pileus	Strongly laccate	Not observed	Not observed	Duplex	Trimitic	Ellipso-id	12.3 × 8.6
GACP17092592	Eccentrically stipitate, having dichotomous, trichotomous or irregular branches	Subreniform	6–10 × 5–8	Grey to brownish grey	Concolorous with the pileus	Strongly laccate	Distinctly concentrically sulcate	Slightly radially rugose	Duplex	Trimitic	Broadly ellipsoid	11.2 × 8.1
GACP171012239	Eccentrically stipitate	Reniform	6–8 × 3.5–5.5	Brownish grey to black	Concolorous with the pileus	Laccate	Distinctly concentrically sulcate	Observed	Duplex	Trimitic	Ellipso-id	11.0 × 7.5
GACP17092588	Eccentrically stipitate	Subreniform	7–10 × 6–	Reddish brown to brownish	Grayish yellow	Strongly laccate	Slightly concentrically sulcate	Observed	Triplex	Trimitic	Ellipso-id	12.2 × 8.5

Table 2 Continued.

Specimen voucher	Basidiome upper surface								Context	Hyphal system	Basidiospore	
	Stipe orientation	Shape	Size (cm)	Colour	Margin colour	Laccate/non laccate	Concentric zones	Radially rugose			Shape	Average size (μm)
			6.5	orange								
GACP14081236	Eccentrically stipitate	Reniform	7–8 × 5.0–5.5	Brownish grey to black	Concolorous with the pileus	Strongly laccate	Distinctly concentrically sulcate	Slightly radially rugose	Duplex	Dimitic	Ellipsoid	12.3 × 8.5
GACP17092543	Eccentrically stipitate	Subreniform	7–10 × 6–8	Brown to brownish black	Yellow brown	Strongly laccate	Slightly concentrically sulcate	Observed	Duplex	Trimitic	Ellipsoid	11.4 × 7.8
GACP17092548	Eccentrically stipitate	Suborbicular	6–16 × 9–11	Dark brown to brownish black	Yellow brown	Strongly laccate	Slightly concentrically sulcate	Slightly radially rugose	Duplex	Trimitic	Ellipso-id	12.2 × 8.2
GACP17101242	Eccentrically stipitate	Subreniform	2.5–4 × 1.5–2.5	Brownish black	Concolorous with the pileus	Laccate	Slightly concentrically sulcate	Observed	Duplex	Trimitic	Ellipso-id	13.8 × 9.1
GACP17101254	Eccentrically stipitate	Subreniform	1.5–4.5 × 1.0–2.5	Brownish black	Concolorous with the pileus	Laccate	Distinctly concentrically sulcate	Observed	Duplex	Dimitic	Ellipso-id	12.5 × 8.5
GACP17101260	Eccentrically stipitate	Reniform	2.5–5 × 1.0–2.5	Light brown	Concolorous with the pileus	Laccate	Slightly concentric, not sulcate	Observed	Triplex	Trimitic	Ellipso-id	12.9 × 8.9
GACP17101270	Eccentrically stipitate	Flabelliform	2–4 × 1–1.5	Reddish brown to brownish black	Yellowish brown	Laccate	Not observed	Slightly radially rugose	Duplex	Trimitic	Ellipso-id	12.8 × 2.9
GACP17092581	Eccentrically stipitate	Spathulate	8–12 × 7–9	Dark brown to brownish black	Concolorous with the pileus	Laccate	Observed	Slightly radially rugose	Duplex	Trimitic	Ellipso-id	11.3× 8.0

Table 2 Continued.

Specimen voucher	Basidiome upper surface								Context	Hyphal system	Basidiospore	
	Stipe orientation	Shape	Size (cm)	Colour	Margin colour	Laccate/non laccate	Concentric zones	Radially rugose			Shape	Average size (μm)
GACP17092520	Eccentrically stipitate	Spathulate	4–5.5 × 3–4	Dark brown to brownish black	Concolorous with the pileus	Strongly laccate	Not observed	Observed	Duplex	Trimitic	Ellipso-id	11.3 × 8.2
GACP17092513	Eccentrically stipitate	Reniform	7.5–11 × 6–7.5	Dark brown to brownish black	Concolorous with the pileus	Laccate	Observed	Observed	Duplex	Trimitic	Ellipso-id	10.2 × 7.0

Table 3 Collection site details of *Ganoderma sinense* specimens in China

Province	Collection site	Collection date	Collec-tor	*Ganoderma sinense* specimen voucher	Longitudes and latitudes	Average annual rain fall (mm)	Average annual temperature (°C)	Height (m)	Relati-ve Humi-dity (%)	Substrate-	Reference
Hainan	Jiangfengling Mountain, Le Dong County	2014/08/12	T.C. Wen	GACP14081236	108° 51′-109° 02′ E, 18° 44′ 18° 52′N	2650	29	838	57	On rotten wood, in dry dipterocarp forest and in upper mixed deciduous forest or growing up from soil	Dai et al. 2011
Guizhou	Sandu Shui Autonomous County	2017/09/25	T.C. Wen	GACP17092520, GACP17092521, GACP17092522, GACP17092523,	107°40'-108°14'E, 25°30'-	1326	18	560	80		National Meteorological Information Center,

Table 3 Continued.

Province	Collection site	Collection date	Collec-tor	*Ganoderma sinense* specimen voucher	Longitudes and latitudes	Average annual rain fall (mm)	Average annual temperature (°C)	Height (m)	Relati-ve Humi-dity (%)	Substrate-	Reference
				GACP17092533, GACP17092534	26°10'N						China, 2000
				GACP17092530, GACP17092532				570			
				GACP17092539, GACP17092540,				600			
				GACP17092592, GACP17092595				580			
				GACP17092567, GACP17092568				520			
				GACP17092581, GACP17092582, GACP17092588, GACP17092589				620			
				GACP17092543, GACP17092547, GACP17092548, GACP170925109, GACP17092520, GACP170925128, GACP170925129, GACP170925130, GACP170925132				650			
				GACP17101242, GACP17101245				560			
				GACP17101254, GACP17101255				580			
				GACP17101260, GACP17101262				600			

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