First Record of Ganoderma multipileum Associated with Delonix regia (Boj. ex Hook.) Raf. Die-back in Vietnam

Trang Thi Thu Nguyen
University of Science
Hoang Duc Nguyen
University of Science
Anh Tu Bui
University of Medicine and Pharmacy at Ho Chi Minh City
Linh Thuoc Tran
University of Science
Khanh Huyen Thi Pham
The University of Danang
Kim Thuong Pham Van
The University of Danang
Manh Hung Tran (tmhung@smp.udn.vn)
The University of Danang

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Abstract

Ganoderma multipileum was initially discovered in Taiwan as a wood decay fungus on many host plants. However, the detailed taxonomy of this genus has suffered from a lack of in-depth investigation. In this study, the classification of these fungi was described based on their morphology, the phylogeny of three regions (ITS, RPB2, and TEF1 sequences), and the assessment of scanning electron microscope photographs of basidiospores. In addition, the similarities and differences in the characteristics of this mushroom in comparison to related species were analyzed and discussed from morphological and phylogenetic perspectives. The results indicate that *G. multipileum* was newly recorded in Vietnam, and its relation to ornamental plant die-back is noted.

Introduction

The genus *Ganoderma* belongs to the family Ganodermataceae and has more than 418 epithets (www.indexfungorum.org, accessed July 30, 2022). *Ganoderma* has been used as a medicinal herb for thousands of years in many Asian countries. These species are found in many regions around the world in diverse ecosystems. Species in the genus *Ganoderma* mostly grow on substrates of decayed wood or fibrous materials, where they degrade the lignocellulose in wood and are white-rot fungi. However, some parasitic species are present on the stems or roots of trees in the Northern and Southern Hemispheres. Basal stem rot on oil palm is caused by *Ganoderma*, resulting in a yield loss of 23.8 fresh fruit brunches t ha^{-1} (Ariffin et al., 2000). This has affected millions of farmers in Malaysia, Indonesia, and other countries, which annually produce 17.8 million tons of oil palms worldwide. *Ganoderma* species have also been documented attacking *Acacia* species in India, with many *Acacia* species serving important economic roles, such as growing pulp and wood. *G. applanatum* and *G. lucidum* have also been reported in Pakistan (Nasir, 2005) and India on 144 crop perennials hosts such as *Acacia, Arnacardium, Artocarpus, Bamboo, Cocos, Coffea, Dalbergia, Pinus, Quercus* and other species (Sankaran et al., 2005). Stem, butt, and root rot diseases are typical results of *Ganoderma* attacks. Before a stressed tree dies from such an infection, its symptoms may include branch die-back and the loss of foliage. The main entry points for diseases may be through soil roots, vegetative spread, or introduction by spores through wounds (Kües et al., 2015a).

Among the *Ganoderma* species, *G. multipileum* was discovered on a stump of decaying wood for the first time in Taiwan (D. M. Wang et al., 2009). Since then, *G. multipileum* has been described based on morphology and phylogeny in subtropical and tropical countries such as China (Cao et al., 2012; X. C. Wang et al., 2012; Zhou et al., 2015), India (Bhatt et al., 2018), Pakistan (Umar et al., 2022), and Thailand (Luangharn et al., 2021). In previous reports, *Ganoderma multipileum* was described as a white-rot fungus that mainly decays dead softwood such as *Sterculia nobilis*, *Delonix regia* (D. M. Wang et al., 2009), and *Pinus merkusii* (Luangharn et al., 2021), as well as hardwood plants such as *Dalbergia sissoo* and *Vachellia nilotica* (Umar et al., 2022).

Delonix regia is a flowering plant of the Fabaceae family and is widely grown in Vietnam and other tropical countries. Various parts of this plant have been used in folk medicine (Modi et al., 2016; Singh & Naresh, 2014). A decoction of the bark is used to treat malaria, bloating, and rheumatism (Modi et al., 2016), while the leaves can treat lower blood pressure (L. S. Wang et al., 2016). In addition, phenolic compounds effectively increase antioxidant and antimicrobial activities, which hold potential for functional food and medicinal therapies (Shabir et al., 2011). In Vietnam, the tree is also cultivated as an ornamental avenue tree along streets or near schools

and offices. It is considered a summer symbol and is deeply rooted in Vietnamese culture. Notably, a recent document from Vietnam showed that this broadleaf tree host had *Ganoderma colossum* reported on a stump as a wood decay mushroom (Kleinwächter et al., 2001). In addition, tree protection in urban areas is significant because most plants that grow in metropolitan areas are woody (Tello et al., 2005). If urbanized trees are damaged by attacking pathogens, they can potentially harm citizens. Therefore, understanding the harmful pathogens of woody plants is necessary to determine and conduct control measures.

In this study, a new pathogenic species of *Ganoderma* was found in *D. regia* samples collected in Ho Chi Minh City, South Vietnam. Its morphological characteristics and molecular sequence data are described and discussed below.

Material And Methods

Sample collection and mycelium isolation

Fresh specimens were collected in District 5, Ho Chi Minh City (Vietnam) in July 2022 by Nguyen, T.T.T of the Faculty of Biology and Biotechnology, University of Science. The voucher specimens were deposited at the Laboratory of Microbiology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University Ho Chi Minh City, Vietnam, with code VNHCM1805. The mycelium was grown by transferring tissue samples from fresh fruiting bodies to potato dextrose agar (PDA; HiMedia, Mumbai, India) in Petri dishes (STRIPLAN, Germany) under sterile conditions and cultivating them at 25 ± 2°C. For additional studies, mycelium was stored in 13 x 100 mm tubes (Pyrex, USA) at 4°C.

Morphological characterization

The specimens were stored at the Department of Microbiology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University Ho Chi Minh City, Vietnam. Macroscopic features were observed on field notes and digital images (Canon EOS 7D, Japan). Kornerup and Wanscher's (1978) color standards were used to aid in identification. Mushroom samples were dried at 50°C and stored in resealable plastic bags. The samples were cut into small pieces, inserted into a 5% KOH solution, stained with 1% cotton blue and Melzer reagent solution, and observed using a stereomicroscope (Meiji EMZ 127212, Saitama, Japan). Fruiting bodies were observed at 1000x magnification using an optical microscope (S/N EU1611034 microscope and CMEX-10 PRO USB 3.0 microscope camera, Euromex, Arnhem, Netherlands). The description of the basidiomata of *Ganoderma* followed Karst (1881).

Dimensions for basidiospores are given using the notation of the form (a) b–c (d). The b–c range contains a minimum of 90% of the measured values. Extreme values (a and d) are provided in parentheses. L_m and W_m indicate the average basidiospore length and width (± standard deviation) for the measured basidiospores. Q implies the "length/width ratio" of a basidiospore in the side view; Q_{avg} means the average Q of all specimens ± the standard deviation of the sample. A size of at least 50 basidiospores was measured. A JSM-IT100 InTouchScope™ Scanning Electron Microscope (SEM) (JEOL, Ltd, Tokyo, Japan) was used to observe endospore ornamentation in detail.

DNA extraction, PCR, and sequencing

Mycelia were extracted using the phenol:chloroform:isoamyl alcohol (Merck, Darmstadt, Germany) technique (Elkins, 2013) with TE buffer (Tris HCl pH 8.0, 1.0 mM EDTA). RNAse A (60 µg) was added following extraction to obtain only DNA. Total DNA was determined using NanoVue Plus (GE Healthcare, Gillingham, United Kingdom) and stored in a refrigerator at -20°C after purification and concentration. Primers ITS1F and ITS4 were used to amplify nuclear ribosomal internal transcribed spacers (ITSs; ITS1,5.8S, ITS2). Two protein-coding genes, translation factor 1α (TEF1) and RNA polymerase II's second largest submit (RPB2), were amplified using primer pairs EF1-983F/EF1-2218R and fRPB2-5F/bRPB2-7R2. The PCR reaction components (total volume, 25 µL) included the following: 4 µL sample DNA, 2.5 µL Taq buffer, 2.5 µL 2 mM dNTP, 0.125 µL primers, 0.25 µL Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 1.68 µL $MgCl_2$ with optimized buffer, and 13.95 µL sterile Milli-Q water. The PCR cycle included a 5-minute initial denaturation step, then 35 cycles as follows: denaturation at 95°C for 30 s, 55°C for 30 s, and extension at 72°C for 1 minute to amplify the ITS region and for 2 minutes to amplify TEF1 and RPB2. The final elongation step was 5 minutes at 72°C. PCR products (2 µL) were electrophoresed in Tris-acetate EDTA buffer on a 2% agarose gel stained with EtBr (Lai & Herr, 1992), then observed and photographed in a UV lightbox. Sanger sequencing was then conducted by Macrogen (Seoul, Korea).

Table 1
Primers used in this study.

No.	Primers	Target gene	Direction	Primers sequences (5'→3')	References
1	ITS1F	ITS	Forward	CTTGGTCATTTAGAGGAAGTAA	(Gardes & Bruns, 1993)
	ITS4		Reverse	TCCTCCGCTTATTGATATGC	(White et al., 1990)
2	EF1-983F	TEF1	Forward	GCYCCYGGHCAYCGTGAYTTYAT	(Rehner & Buckley, 2005)
	EF1-2218R		Reverse	ATGACACCRACRGCRACRGTYTG	(Rehner & Buckley, 2005)
3	fRPB2-5F	RPB2	Forward	GAYGAYMGWGATCAYTTYGG	(Liu et al., 1999)
	bRPB2-7R2		Reverse	ACYTGRTTRTGRTCNGGRAANGG	(Matheny & Bougher, 2006)

Phylogenetic tree construction

According to the corresponding Sanger sequencing chromatograms, misleading data from the ends of raw sequencing fragments were manually trimmed and assembled into consensus sequences using BioEdit v7.2. All newly obtained sequences have been submitted to GenBank (Sayers et al., 2019). Results for related genera on studies or searched and downloaded from the GenBank database [https://www.ncbi.nlm.nih.gov/] (Table 2) were acquired using BLAST via MAFFT v7.487 [https://mafft.cbrc.jp/alignment/software/] (Katoh & Standley, 2013) to align the obtained sequences. The alignments were edited and manually corrected using AliView (Larsson, 2014). The best fit model was determined by jModelTest v2.1.10 (Darriba et al., 2012) based on the corrected Akaike information criterion (AICc). Maximum likelihood (ML) analyses were performed using IQ-TREE v2.0.3 on CIPRES Science Gateway v3.3 (Miller & Schwartz, 2010) with 1000 ultrafast bootstrap replicates. For Bayesian analysis, the best-fit nucleotide model, HKY + I + G, was selected by jModelTest v2.1.10 and posterior probabilities (PPs) were determined by Markov chain Monte Carlo (MCMC) using MrBayes v3.2.7a (Ronquist &

Huelsenbeck, 2003). Three different runs with 1 million generations and four chains were executed until the split deviation frequency value was < 0.01 and sampled every 100th generation. The initial 25% of sample trees were discarded as burn-in. The MCMC runs were checked to reach convergence with all ESS values above 200 by using Tracer v1.7.2 (Rambaut et al., 2018). The ML bootstrap values (BS) ≥ 70% and PP ≥ 0.95 were presented on topologies from ML analyses, respectively. *Tomophagus colossus* TC-02 was selected as the outgroup for both ML and Bayesian analyses. Phylogenetic trees were visualized and modified using FigTree v.1.4.4 [http://tree.bio.ed.ac.uk/software/figtree/].

Table 2
GenBank accession numbers of *Ganoderma* species sequences used in the phylogenetic analysis.

Species	GenBank accession No.			Voucher	Locality	References
	ITS	*RBP2*	*TEF1*			
Ganoderma boninense	KJ143905	KJ143964	KJ143925	WD 2028	Japan	(Zhou et al., 2015)
Ganoderma boninense	KJ143906	KJ143965	KJ143924	WD 2085	Japan	(Zhou et al., 2015)
Ganoderma curtisii	JQ781848	KJ143966	KJ143926	CBS 100131	USA	(Zhou et al., 2015)
Ganoderma curtisii	MG654167	MG754854	-	223FL	USA	(Loyd, Barnes, et al., 2018)
Ganoderma curtisii	MG654097	MG754856	MG754728	UMNFL28	USA	(Loyd, Barnes, et al., 2018)
Ganoderma flexipes	MZ354925	MZ245403	MZ221657	Dai 20461	China	(Sun et al., 2022)
Ganoderma flexipes	MZ354924	MZ245402	MZ221656	Cui 13863	China	(Sun et al., 2022)
Ganoderma leucocontextum (Holotype)	KF011548	-	-	GDGM 40200	China	(Li et al., 2015)
Ganoderma leucocontextum	KU572485	MG367516	KU572495	Dai 15601	China	(Xing et al., 2018)
Ganoderma lingzhi (Holotype)	JQ781858	JX029980	JX029976	Wu1006-38	China	(Cao et al., 2012)
Ganoderma lingzhi	KY364245	KY393267	KY393279	SFC20150624-06	Korea	(Jargalmaa et al., 2017)
Ganoderma lingzhi	JQ781864	JX029978	JX029974	Dai12479	China	(Cao et al., 2012)
Ganoderma lucidum	KJ143911	KJ143971	KJ143929	K 175217	UK	(Zhou et al., 2015)
Ganoderma lucidum	MK554777	MK554765	MK554730	MULC:31549	France	(Cabarroi-Hernández et al., 2019)
Ganoderma lucidum	KJ143912	-	KJ143930	MT 26/10	Czech Republic	(Zhou et al., 2015)

Species	GenBank accession No.			Voucher	Locality	References
	ITS	*RBP2*	*TEF1*			
Ganoderma multipileum Ding Hou (Holotype)	KJ143913	KJ143972	KJ143931	CWN 04670	Taiwan, China	(D. M. Wang et al., 2009)
Ganoderma multipileum	**MZ706463**			**VNHCM1805**	**Vietnam**	**This study**
Ganoderma multipileum	MG279184	MG367521	MG367575	Cui 14373	China	(Xing et al., 2018)
Ganoderma multipileum	KJ143914	KJ143973	KJ143932	Dai 9447	China	(Zhou et al., 2015)
Ganoderma multipileum	MN401406	MN423142	MN423172	MLU 19-2166	Thailand	(Luangharn et al., 2021)
Ganoderma oregonense	JQ781875	KJ143974	KJ143933	CBS 265.88	USA	(Loyd, Barnes, et al., 2018)
Ganoderma oregonense	MG654190	MG754740	-	UMNAK1	USA	(Loyd, Barnes, et al., 2018)
Ganoderma resinaceum	KJ143916	-	KJ143934	CBS 194.76	The Netherlands	(Zhou et al., 2015)
Ganoderma resinaceum	MG706249	MG837820	MG837857	LGAM 448	Greece	(Sun et al., 2022)
Ganoderma resinaceum	MK554786	MK554764	MK554737	MUCL:52253	France	(Cabarroi-Hernández et al., 2019)
Ganoderma sessile	MG654306	MG754866	MG754749	111TX	USA	(Loyd, Barnes, et al., 2018)
Ganoderma sessile	MG654319	MG754750	MG754869	228DC	USA	(Loyd, Barnes, et al., 2018)
Ganoderma sessile	KJ143936	-	KJ143936	JV 1209/9	USA	(Zhou et al., 2015)
Ganoderma sichuanense	MN396324	MN423130	MN423163	MFLU 19-2164	Thailand	(Luangharn et al., 2021)
Ganoderma sichuanense	KT693253	-	-	B4 13106 13304 13309 clone 2	USA	(Raja et al., 2017)

Species	GenBank accession No.			Voucher	Locality	References
	ITS	*RBP2*	*TEF1*			
Ganoderma tropicum	MG279194	MG367532	MG367585	Dai 16434	China	(Xing et al., 2018)
Ganoderma tropicum	KF495000	MG367531	MG367584	He 1232	China	(Xing et al., 2018)
Ganoderma tsugae Murill	KJ143919	KJ143977	KJ143939	Dai 12751b	USA	(Zhou et al., 2015)
Ganoderma tsugae	DQ206985	DQ408116	DQ059048	AFTOL-ID 771	USA	(Matheny et al., 2007)
Ganoderma tsugae	MG654345	-	MG754766	UMNWI23	USA	(Loyd, Barnes, et al., 2018)
Tomophagus colossus	KJ143923	-	KJ143943	TC-02	Vietnam	(Zhou et al., 2015)

Results

Phylogeny

Phylogenetic analyses were conducted using the combined dataset, including the ITS, RPB2, and TEF1 sequences of 36 taxa. These sequences belong to the genus *Ganoderma* and were grouped into six clades. Reference sequences were obtained from GenBank (from samples collected in the USA, UK, Czech Republic, France, Italy, Korea, China, Japan, Netherlands, Thailand, and Vietnam) with holotypes (*G. lingzhi*, *G. leucocontextum*, and *G. multipileum*). *Tomophagus colossus* (Fr.) Murill 1905 (TC-02, Vietnam) is the outgroup of the taxonomic tree. The dataset comprised 2176 sites with gaps (558 sites for ITS, 946 sites for RPB2, and 672 sites for TEF1). Tree topologies resulting from the ML analysis were similar to the Bayesian analysis. The best-scoring ML tree and Bayesian analysis are shown in Fig. 1. The results showed that specimens collected from Vietnam clustered significantly, forming a monophyletic group with the *G. multipileum* taxon, including the holotype (CWN 04670) and other sequences from China (Cui 14373, Dai 9447) and Thailand (MLU 19-2166), with high ML/PP support values (100% and 1.00, respectively).

Taxonomy

Habitat: *G. multipileum* VNHCM1805 lived on the roots and trunk of a royal poinciana tree (*D. regia*, Fabaceae). When the basidiocarps were observed in November 2018, the tree bloomed thereafter in May 2019. In contrast, the tree was no longer flowering in May 2020 and its leaves were lost, with branches dying back until May 2021 (Fig. 2).

Basidiome

annual, stipitate, laccate, woody. *Pileus* 15–25 × 7–10 cm up to 2.2 cm thick at the base, flabelliform, upper surface brownish orange (6C8), orange-rooted to reddish brown (8D8) at maturity, weakly to strongly laccate, reniform, dimidiate, pilei expanding, sometimes with pilei growing from the lower pilei or growing together, up to 36 cm, and 54 cm in overall width from the lower pilei, curly and wavy edges; lower surface yellowish white (4A2) to brownish gray at maturity. The young basidiocarp's upper surface brownish orange (6C8), orange-rooted to reddish brown (8D8), somewhat lacquered, obviously sulcate, rugged and non-rough, margin obtuse or not, white to orange-yellow. *Context* 0.1–1.8 cm thick, light brown (6D6) to dark brown (6F8), occasionally melanoid in KOH, corky. *Hymenophore* whitish cream on the surface while young, straw or light brown (6D6) at maturity. Pores, subcircular to circular, 25–110 µm thickness, 60–80 µm per mm, 60–220 µm in diameter, 3–5 spores/mm. *Stipe* 1.5–3.5 cm in length, up to 0.7–3.0 cm in width, sub-cylindrical to cylindrical, strong laccate, reddish brown (9D7), and brownish red (10D6) at maturity.

Hyphal structure

hyphae system trimetric, colored, generative hyphae; 2.0–5.2 µm in diameter (n = 30), colored, thin-walled; skeletal hyphae 4.3–7.1 µm broad (n = 30), yellowish brown to reddish brown in KOH; binding hyphae, 1.2–2.0 µm in diameter, few, wall thick, very ramified. *Basidiospores* ellipsoid, mostly truncated, double walls, brownish orange (7C4) to brown (7E5), (7.3)8.0–11.5(12.2) × (5.3)5.5–7.8(8.3) µm, (with Q_{avg} = 1.79 ± 0.28, L_m = 10.63 ± 0.7µm, W_m = 6.81 ± 0.54 µm (with myxosporium), (6.6)7.3–10.9(11.3) × (4.9)5.1–7.2(7.7) µm, (with Q_{avg} = 1.47 ± 0.34, L_m = 8.73 ± 0.63µm, W_m = 6.2 ± 0.35 µm (without myxosporium). Exosporium (outer wall), hyaline, light brown to brownish orange (6A4, 6C8), endosporium (inter-wall) with conspicuous echinulae, fine dark brown (7F) eursporium. *Pileipellis* cutis, consisting of clavate cells, 13–426.0–13.6 µm, dextrinoid to mildly or severely amyloid.

Discussion And Conclusion

The genus *Ganoderma* and the species *G. lucidum* were described in 1881 by Finnish mycologist P. A. Karsten (Karsten, 1881). The taxonomy of the *G. lucidum* complex is chaotic when using morphology classification due to its distribution spanning across many parts of the world, which has led to morphological variability. Recent developments in DNA sequence analysis represent effective methods used by taxonomists to combine data. In 1950, Ding Hou et al. discovered *G. multipileum* and separated this mushroom into a new species in the *G. lucidum* complex in Taiwan. Then, D. M. Wang et al. (2009) sequenced the nrDNA of this species. Based on morphological and nrDNA ITS region molecular data, this fungus is present in tropical areas. To date, it has been described and illustrated in four countries and territories (Taiwan, China, Thailand, and Pakistan). In previous research, it decayed various types of wood from gymnosperms (Merkus pine, *Pinus merkusii*) and angiosperms (*Sterculia nobilis* – Chinese chestnut, *Dalbergia sissoo* – North Indian rosewood, *Vachellia nilotica* – gum arabic tree). Most host plant angiosperms of *G. multipileum* are in the Fabaceae family (*D. regia*, *D. sissoo*, *V. nilotica*).

In the present study, *G. multipileum* was described and illustrated using morphology and phylogeny from a host plant in Ho Chi Minh City, Vietnam, based on three loci: ITS, TEF1, and RBP2 sequences. Among the *Ganoderma* species, *G. adspersum*, *G. applanatum*, *G. resinaceum*, and *G. pfeifferi* are the most harmful fungi in Europe's urban landscapes (Michalíková et al., 2021), while *G. boninense* mainly affected oil palm plantations in Southeast Asia (Ariffin et al., 2000; Bharudin et al., 2022). In the United States, *G. appnalatum*, *G. boninense*, the

G. lucidum complex, *G. sessile*, and *G. zanatum* are pathogens in landscape trees (Kües et al., 2015b; Kunta et al., 2010; Loyd, Linder, et al., 2018)

The literature has reported that *Ganoderma* spp. are mainly found near the stumps and roots of trees and that this genus is one of the most common plant pathogens (Grinn-Gofroń et al., 2021; Loyd, Linder, et al., 2018; Náplavová et al., 2021; Sadyś et al., 2014). Species in this genus are white-rot fungi and possess lignin- and cellulose-degrading enzymes that allow *Ganoderma* spp. to decompose up to 99% of lignin in various types of wood (Dill & Kraepelin, 1986). Therefore, *Ganoderma* species parasitize old trees and rarely young trees. Airborne fungal spores infect the bark through open wounds in the trunk and root (Sadyś et al., 2014). Thus, the xylem cells are necrotic and cannot conduct water and minerals to leaves and other parts of the plant (Pearce, 1991). Herein, *G. multipileum* were collected in Ho Chi Minh City, Vietnam, where there is a crowded, high-density population of this species (Yue et al., 2021). Over the approximately 3-year observation period for royal poinciana tree symptoms following *G. multipileum* parasitization, the tree lost its leaves and died. Some reports show that stresses and aging are also agents affecting parasites of *Ganoderma* species in plants (Kües et al., 2015; Michalíková et al., 2021). This study contributed to the discovery of *G. multipileum*. Notably, biotic pathogens such as polypore mushrooms can affect woody trees in urban areas.

Declarations

Ethics approval and Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The data generated during the current study are available from the corresponding author on reasonable request.

Competing interests The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Author contribution All authors contributed to the study conception and design. Material preparation and description were performed by TTT Nguyen, HD Nguyen and AT Bui. Fungal remains were analyzed and interpreted by TTT Nguyen, KHT Pham, KTV Pham, LT Tran and MH Tran. The first draft of the manuscript was written by TTT Nguyen and MH Tran. All authors read and approved the final manuscript.

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References

1. Ariffin, D., Idris, A. S., & Singh, G. (2000). Status of Ganoderma in Oil Palm. In J. Flood, P. D. Bridge, & M. Holderness (Eds.), *Ganoderma Diseases of Perennial Crops* (pp. 49–68).

2. Bharudin, I., Ab Wahab, A. F. F., Abd Samad, M. A., Xin Yie, N., Zairun, M. A., Abu Bakar, F. D., & Abdul Murad, A. M. (2022). Review Update on the Life Cycle, Plant–Microbe Interaction, Genomics, Detection and Control Strategies of the Oil Palm Pathogen Ganoderma boninense. Biology, *11*, 251. https://doi.org/10.3390/biology11020251
3. Bhatt, M., Mistri, P., Joshi, I., Ram, H., Raval, R., Thoota, S., Patel, A., Raval, D., Bhargava, P., Soni, S., Bagatharia, S., & Joshi, M. (2018). Molecular survey of basidiomycetes and divergence time estimation: An Indian perspective. PLOS ONE, *13*(5), e0197306. https://doi.org/10.1371/JOURNAL.PONE.0197306
4. Cabarroi-Hernández, M., Villalobos-Arámbula, A. R., Torres-Torres, M. G., Decock, C., & Guzmán-Dávalos, L. (2019). The Ganoderma weberianum- resinaceum lineage: multilocus phylogenetic analysis and morphology confirm G. mexicanum and G. parvulum in the Neotropics. MycoKeys, *59*, 95–131. https://doi.org/10.3897/MYCOKEYS.59.33182
5. Cao, Y., Wu, S. H., & Dai, Y. C. (2012). Species clarification of the prize medicinal Ganoderma mushroom Lingzhi. Fungal Diversity, *56*(1), 49–62. https://doi.org/10.1007/S13225-012-0178-5
6. Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, *9*(8), 772. https://doi.org/10.1038/NMETH.2109
7. Dill, I., & Kraepelin, G. (1986). Palo Podrido: Model for Extensive Delignification of Wood by Ganoderma applanatum. Applied and Environmental Microbiology, *52*(6), 1305–1312. https://doi.org/10.1128/AEM.52.6.1305-1312.1986
8. Elkins, K. M. (2013). DNA Extraction. In K. M.Elkins (Ed.), *Forensic DNA Biology* (1st ed., pp. 39–52). Elsevier Inc. https://doi.org/10.1016/B978-0-12-394585-3.00004-3
9. Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Molecular Ecology, *2*(2), 113–118. https://doi.org/10.1111/J.1365-294X.1993.TB00005.X
10. Grinn-Gofroń, A., Bogawski, P., Bosiacka, B., Nowosad, J., Camacho, I., Sadyś, M., Skjøth, C. A., Pashley, C. H., Rodinkova, V., Çeter, T., Traidl-Hoffmann, C., & Damialis, A. (2021). Abundance of Ganoderma sp. in Europe and SW Asia: modelling the pathogen infection levels in local trees using the proxy of airborne fungal spore concentrations. Science of The Total Environment, *793*, 148509. https://doi.org/10.1016/J.SCITOTENV.2021.148509
11. Jargalmaa, S., Eimes, J. A., Park, M. S., Park, J. Y., Oh, S. Y., & Lim, Y. W. (2017). Taxonomic evaluation of selected Ganoderma species and database sequence validation. PeerJ, *5*(7), e3596. https://doi.org/10.7717/PEERJ.3596
12. Karsten, P. A. (1881). Enumeratio Boletinearum et Polyporearum Fennicarum, Systemate novo dispositarum. Reviews of Mycology, *3*, 16–19. https://cir.nii.ac.jp/crid/1573387449823898368
13. Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution, *30*(4), 772–780. https://doi.org/10.1093/MOLBEV/MST010
14. Kleinwächter, P., Anh, N., Kiet, T. T., Schlegel, B., Dahse, H. M., Härtl, A., & Gräfe, U. (2001). Colossolactones, New Triterpenoid Metabolites from a Vietnamese Mushroom Ganoderma colossum§. Journal of Natural Products, *64*(2), 236–239. https://doi.org/10.1021/NP000437K
15. Kornerup, A., & Wanscher, J. H. (1978). *Methuen handbook of colour* (3rd ed.). London, Methuen & Co. Ltd.

16. Kües, U., Nelson, D. R., Liu, C., Yu, G.-J., Zhang, J., Li, J., Wang, X.-C., & Sun, H. (2015a). Genome analysis of medicinal Ganoderma spp. with plant-pathgenic and saprotrophic life-styles. Phytochemistry, *114*, 18–37.
17. Kües, U., Nelson, D. R., Liu, C., Yu, G. J., Zhang, J., Li, J., Wang, X. C., & Sun, H. (2015b). Genome analysis of medicinal Ganoderma spp. with plant-pathogenic and saprotrophic life-styles. Phytochemistry, *114*, 18–37. https://doi.org/10.1016/J.PHYTOCHEM.2014.11.019
18. Kunta, M., Miao, H., Shigaki, T., Perez, J. L., & Skaria, M. (2010). Ganoderma Infecting Citrus in Texas is a Unique Taxon within the G.lucidum Complex: Evidence from the ITS Region. Pest Technology, *4*(1), 62–64.
19. Lai, J. S., & Herr, W. (1992). Ethidium bromide provides a simple tool for identifying genuine DNA-independent protein associations. Proceedings of the National Academy of Sciences of the United States of America, *89*(15), 6958. https://doi.org/10.1073/PNAS.89.15.6958
20. Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics, *30*(22), 3276–3278. https://doi.org/10.1093/BIOINFORMATICS/BTU531
21. Li, T. H., Hu, H. P., Deng, W. Q., Wu, S. H., Wang, D. M., & Tsering, T. (2015). Ganoderma leucocontextum, a new member of the G. lucidum complex from southwestern China. Mycoscience, *56*(1), 81–85. https://doi.org/10.1016/J.MYC.2014.03.005
22. Liu, Y. J., Whelen, S., & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution, *16*(12), 1799–1808. https://doi.org/10.1093/OXFORDJOURNALS.MOLBEV.A026092
23. Loyd, A. L., Barnes, C. W., Held, B. W., Schink, M. J., Smith, M. E., Smith, J. A., & Blanchette, R. A. (2018). Elucidating “lucidum”: Distinguishing the diverse laccate ganoderma species of the United States. In *PLoS ONE* (Vol. 13, Issue 7). https://doi.org/10.1371/journal.pone.0199738
24. Loyd, A. L., Linder, E. R., Anger, N. A., Richter, B. S., Blanchette, R. A., & Smith, J. A. (2018). Pathogenicity of ganoderma species on landscape trees in the Southeastern United States. In *Plant Disease* (Vol. 102, Issue 10, pp. 1944–1949). https://doi.org/10.1094/PDIS-02-18-0338-RE
25. Luangharn, T., Karunarathna, S., Dutta, A. K., Paloi, S., Lian, C. K., Huyen, L. T., Pham, H. N., Hyde, K. D., Xu, J., & Mortimer, P. (2021). Ganoderma (Ganodermataceae, Basidiomycota) species from the Greater Mekong Subregion. Journal of Fungi, *7*, 819. https://doi.org/10.21203/rs.3.rs-45287/v1
26. Matheny, P. B., & Bougher, N. L. (2006). The new genus Auritella from Africa and Australia (Inocybaceae, Agaricales): Molecular systematics, taxonomy and historical biogeography. Mycological Progress, *5*(1), 2–17. https://doi.org/10.1007/S11557-005-0001-8/FIGURES/5
27. Matheny, P. B., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Henrik Nilsson, R., Hughes, K. W., Hofstetter, V., Ammirati, J. F., Schoch, C. L., Langer, E., Langer, G., McLaughlin, D. J., Wilson, A. W., Frøslev, T., Ge, Z. W., Kerrigan, R. W., Slot, J. C., Yang, Z. L., … Hibbett, D. S. (2007). Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution, *43*(2), 430–451. https://doi.org/10.1016/J.YMPEV.2006.08.024
28. Michalíková, A., Beck, T., Gáper, J., Pristaš, P., & Gáperová, S. (2021). Can wood-decaying urban macrofungi be identified by using fuzzy interference system? An example in Central European Ganoderma species. Scientific Reports, *11*, 13222. https://doi.org/10.1038/s41598-021-92237-5
29. Miller, M. A., & Schwartz, T. (2010). The CIPRES science gateway: A community resource for phylogenetic analyses Sustainable Software for Science View project CIPRES View project. *Proceedings of the Gateway*

Computing Environments Workshop (GCE), 1–8. https://doi.org/10.1145/2016741.2016785

30. Modi, A., Mishra, V., Bhatt, A., Jain, A., Mansoori, M. H., Gurnany, E., & Kumar, V. (2016). Delonix regia: historic perspectives and modern phytochemical and pharmacological researches. Chinese Journal of Natural Medicines, *14*(1), 31–0039. https://doi.org/10.3724/SP.J.1009.2016.00031
31. Náplavová, K., Beck, T., Gáper, J., Pyszko, P., Gáperová, S., & 2. (2021). Synanthropic Process Evaluation (with Factors Affecting Propensity to Parasitism) and Host Range within the Genus Ganoderma in Central Europe. *Forests*, *12*(11), 1437. https://doi.org/https://doi.org/10.3390/f12111437
32. Nasir, N. (2005). Diseases caused by Ganoderma spp. on perennial crops in Pakistan. In *Mycopathologia* (Vol. 159, Issue 1, pp. 119–121). https://doi.org/10.1007/s11046-004-4433-5
33. Pearce, R. B. (1991). Reaction zone relics and the dynamics of fungal spread in the xylem of woody angiosperms. Physiological and Molecular Plant Pathology, *39*(1), 41–55. https://doi.org/10.1016/0885-5765(91)90030-L
34. Raja, H. A., Baker, T. R., Little, J. G., & Oberlies, N. H. (2017). DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification? Food Chemistry, *214*, 383–392. https://doi.org/10.1016/J.FOODCHEM.2016.07.052
35. Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology, *67*(5), 901–904. https://doi.org/10.1093/SYSBIO/SYY032
36. Rehner, S. A., & Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia, *97*(1), 84–98. https://doi.org/10.3852/MYCOLOGIA.97.1.84
37. Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, *19*(12), 1572–1574. https://doi.org/10.1093/BIOINFORMATICS/BTG180
38. Sadyś, M., Skjøth, C. A., & Kennedy, R. (2014). Back-trajectories show export of airborne fungal spores (Ganoderma sp.) from forests to agricultural and urban areas in England. Atmospheric Environment, *84*, 88–99. https://doi.org/10.1016/J.ATMOSENV.2013.11.015
39. Sankaran, K. V., Bridge, P. D., & Gokulapalan, C. (2005). Ganoderma diseases of perennial crops in India-an overview. Mycopathologia, *159*, 143–152. https://doi.org/10.1007/s11046-004-4437-1
40. Sayers, E. W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K. D., & Karsch-Mizrachi, I. (2019). GenBank. Nucleic Acids Research, *47*(D1), D94–D99. https://doi.org/10.1093/NAR/GKY989
41. Shabir, G., Anwar, F., Sultana, B., Khalid, Z. M., Afzal, M., Khan, Q. M., & Ashrafuzzaman, M. (2011). Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of gold mohar [Delonix regia (Bojer ex Hook.) Raf.]. Molecules, *16*(9), 7302–7319. https://doi.org/10.3390/molecules16097302
42. Singh, S., & Naresh, S. (2014). A review: Introtruction to genus Delonix. Sumitra Singh et Al. World Journal of Pharmacy and Pharmaceutical Sciences, *3*(6), 2042–2055. www.wjpps.com
43. Sun, Y. F., Xing, J. H., He, X. L., Wu, D. M., Song, C. G., Liu, S., Vlasák, J., Gates, G., Gibertoni, T. B., & Cui, B. K. (2022). Species diversity, systematic revision and molecular phylogeny of Ganodermataceae (Polyporales, Basidiomycota) with an emphasis on Chinese collections. Studies in Mycology, *101*, 287–415. https://doi.org/10.1016/s0166-0616(14)60217-7

44. Tello, M. L., Tomalak, M., Siwecki, R., Gáper, J., Motta, E., & Mateo-Sagasta, E. (2005). Biotic urban growing conditions-threats, pests and diseases. In C. Konijnendijk, K. Nilsson, T. Randrup, & J. Schipperijn (Eds.), *Urban Forests and Trees: A Reference Book* (pp. 325–365). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-27684-X_13/COVER
45. Umar, A., Ahmed, S., Guzman-Davalos, L., & Cabarroi-Hernandez, M. (2022). Ganoderma multipileum and Tomophagus cattienensis-new records from Pakistan. Mycotaxon, *137*(1), 135–151. https://doi.org/10.5248/137.135
46. Wang, D. M., Wu, S. H., Su, C. H., Peng, J. T., Shih, Y. H., & Chen, L. C. (2009). Ganoderma multipileum, the correct name for “G. lucidum” in tropical Asia. Botanical Studies, *50*(4), 451–458.
47. Wang, L. S., Lee, C. T., Su, W. L., Huang, S. C., & Wang, S. C. (2016). Delonix regia Leaf Extract (DRLE): A Potential Therapeutic Agent for Cardioprotection. PLOS ONE, *11*(12), e0167768. https://doi.org/10.1371/JOURNAL.PONE.0167768
48. Wang, X. C., Xi, R. J., Li, Y., Wang, D. M., & Yao, Y. J. (2012). The species identity of the widely cultivated ganoderma, “G. lucidum” (ling-zhi), in China. PLoS ONE, *7*(7). https://doi.org/10.1371/journal.pone.0040857
49. White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols, a guide to methods and applications*. Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
50. Xing, J. H., Sun, Y. F., Han, Y. L., Cui, B. K., & Dai, Y. C. (2018). Morphological and molecular identification of two new Ganoderma species on Casuarina equisetifolia from China. MycoKeys, *34*(34), 93–108. https://doi.org/10.3897/MYCOKEYS.34.22593
51. Yue, W., Chen, Y., Thy, P. T. M., Fan, P., Liu, Y., & Zhang, W. (2021). Identifying urban vitality in metropolitan areas of developing countries from a comparative perspective: Ho Chi Minh City versus Shanghai. Sustainable Cities and Society, *65*, 102609. https://doi.org/10.1016/J.SCS.2020.102609
52. Zhou, L. W., Cao, Y., Wu, S. H., Vlasák, J., Li, D. W., Li, M. J., & Dai, Y. C. (2015). Global diversity of the Ganoderma lucidum complex (Ganodermataceae, Polyporales) inferred from morphology and multilocus phylogeny. Phytochemistry, *114*, 7–15. https://doi.org/10.1016/J.PHYTOCHEM.2014.09.023

Figures

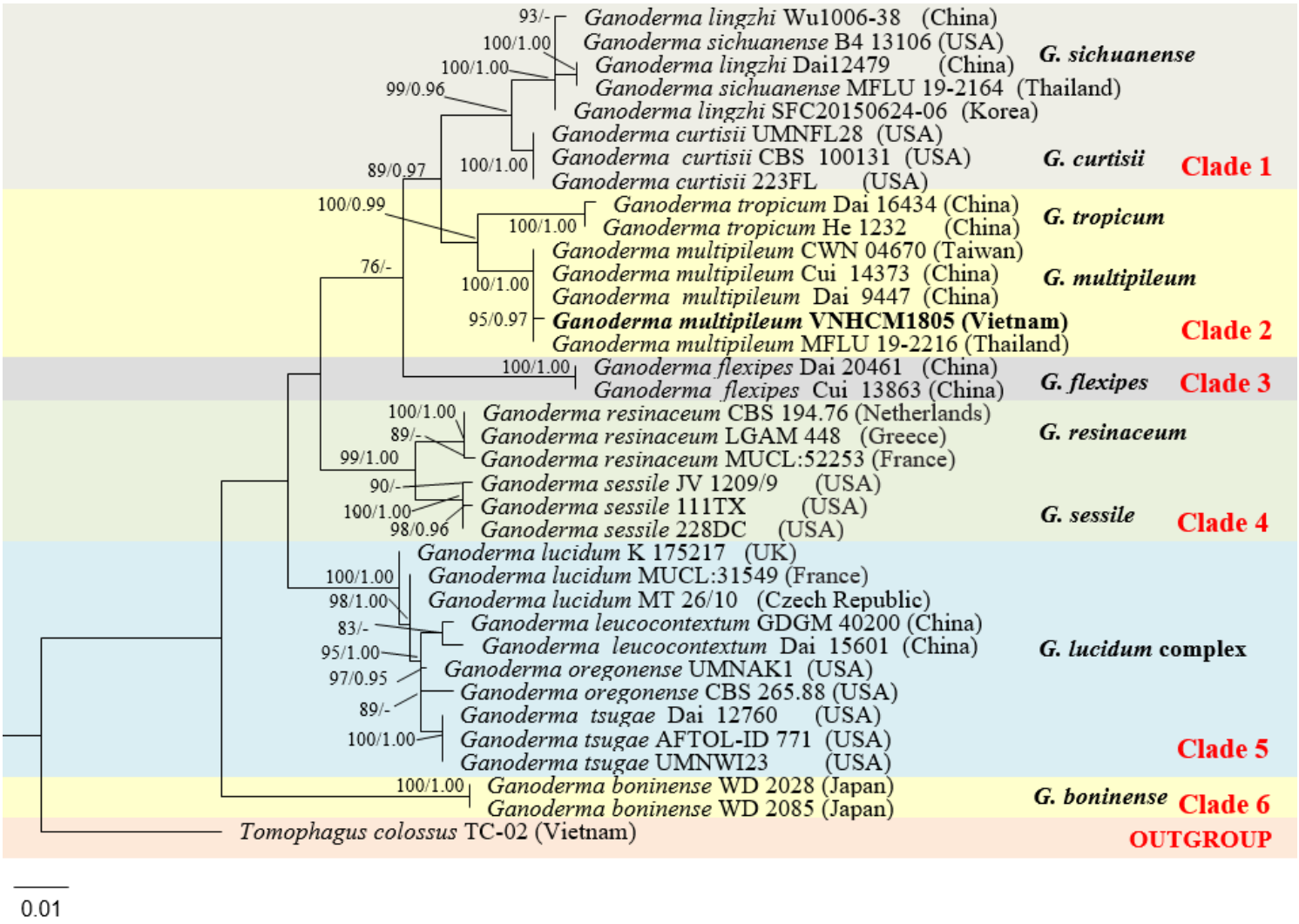


Figure 1

Phylogeny of *Ganoderma*based on data from ITS, *RPB2*, and *TEF1*sequences. Bootstrap values were **obtained from maximum likelihood above 70% and posterior probabilities equal to or greater than 0.95 (given above branches). The tree is rooted in** *Tomophagus colossus* TC-02 (Vietnam). New species were collected in Vietnam in bold (black).

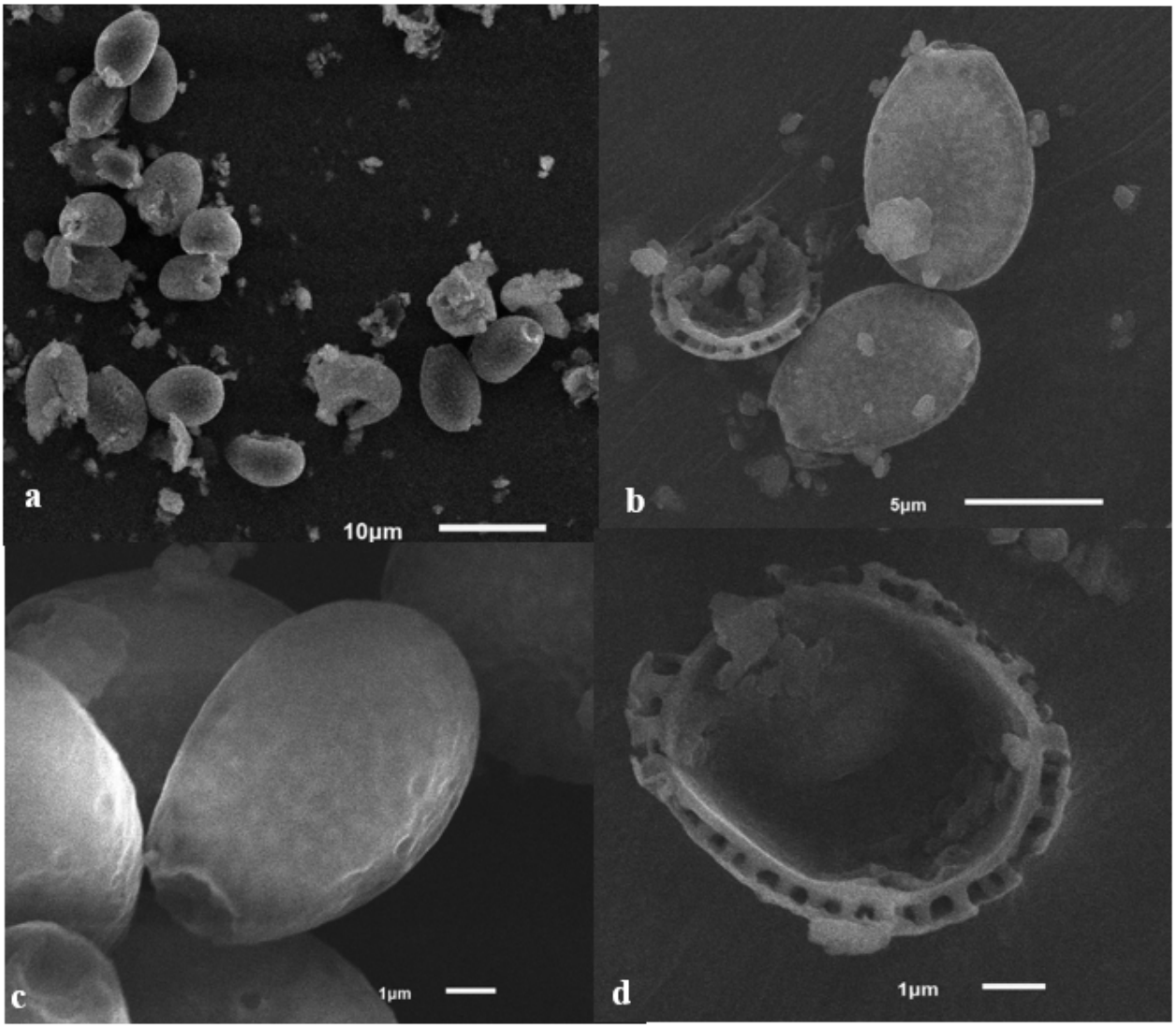


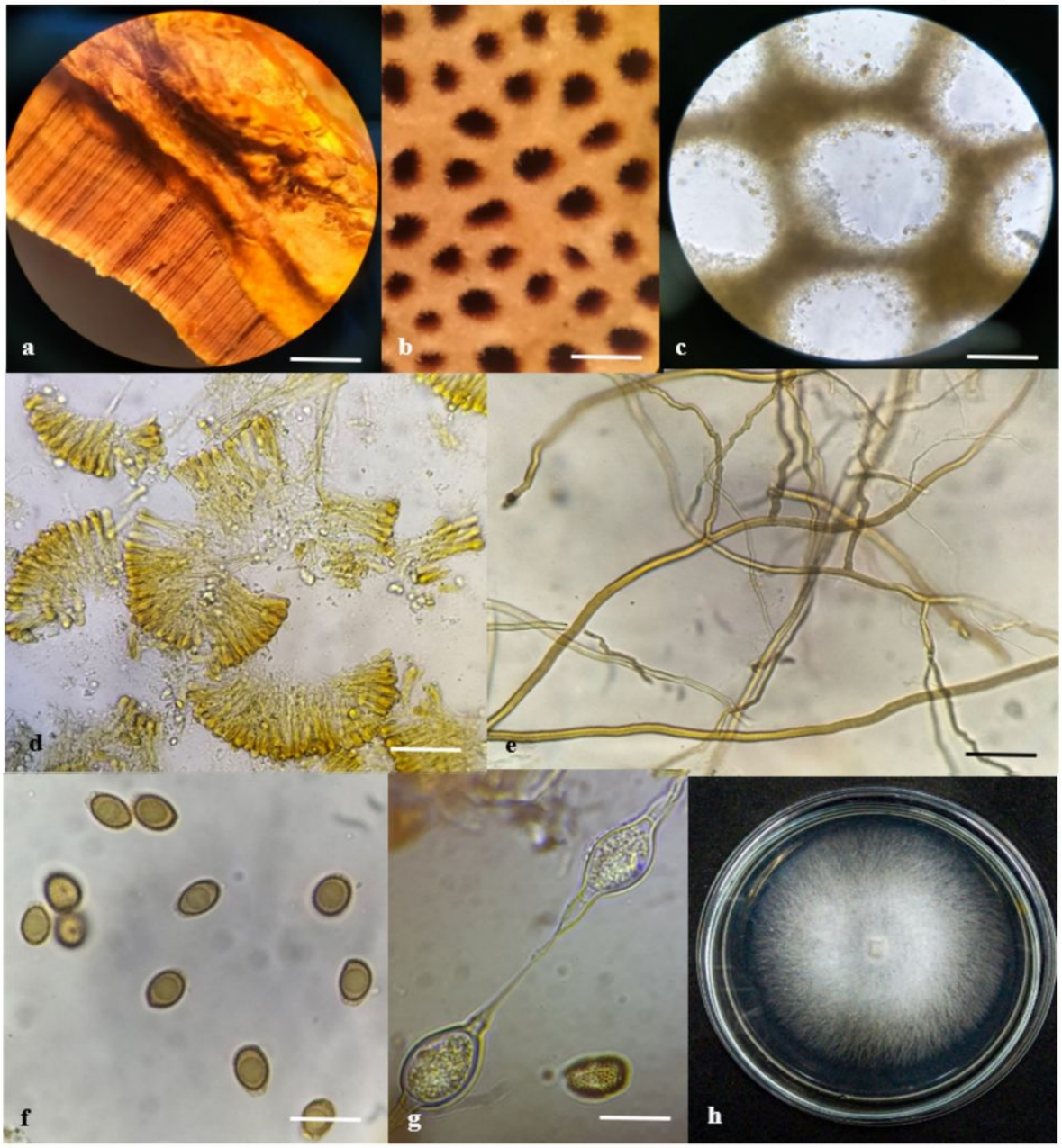
Figure 2

Basidiospores of *G. multipileum* VNHCM1805 observed via a SEM. a-view at 1500x, b-view of exosporium and endosporium at 5000x, c-view of exosporium at 10000x, d-detail in endospore ornamentation at 10000x.

Figure 3

D. regia disease symptoms and the macroscopic structuresof *G. multipileum* VNHCM1805 basidiomes. a-*D. regia* tree in May 2019; b-The same tree in May 2020; c-The same tree in May 2021, d- Basidiocarps growing at the base of the same living tree; e-Lower surface of a basidiome; f-Young basidiocarp on tree roots; g-Cutting part of the bark from the same living tree where the young basidiocarp was growing; f-Section of basidiome. Scale bars: d: 20 cm; e–h: 2 cm.

Colonies: mycelia white (4A1), pale yellowish white (4A2) to grayish yellow (4B6) after 7 days old, round, growth in the radiating direction, thick in the center and thinner outward at 25 ± 2°C on PDA medium, 5 days old. Hyphae hyaline, clamp connections, branched. Chamydospores



presented after 5–7 days old, club-shaped, thick-walled, staining with 1% cotton blue, 5.5–8.5 x 9.0 x 11.0 µm.

Figure 4

Microscopic structures of the basidiome *G. multipileum* VNHCM1805. a-Section of pileus (2x); b-Pores in lower surface (4x); c-A pore (40x); d-Section of *Pileipellis* (100x); e–f-Hyphae of context (100x); f-basidiospores (100x); g-Chlamydospores (100x); h-Culture after incubation at 25 ± 2°C for 7 days. Scale bars: a: 1 cm, b: 1 mm, c: 50 µm, d: 20 µm, e–g: 10 µm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupportinginformationRPB2TEF1alpharawsequences.txt