

Taxonomy and Molecular Phylogeny of *Ganoderma tropicum* (Jungh.) Bres.: A New Record from Thiruvananthapuram District, Kerala, on a Previously Unreported Host, *Artocarpus hirsutus* (Lam.).

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ABSTRACT

Ganoderma tropicum (Jungh.) Bres., a laccate polypore fungus with considerable ecological and medicinal significance, is widely distributed across tropical Asia. In the course of a systematic survey of poroid fungi in Thiruvananthapuram District, Kerala, India, a stipitate *Ganoderma* species with a distinctly laccate pileus was collected from the dead stump of *Artocarpus hirsutus* (Lam.). Detailed morphological examination revealed taxonomically important features including a trimitic hyphal system, ellipsoid double-walled basidiospores with interwall pillars, and a hymeniodermic pileus crust, which are consistent with the diagnostic characteristics of *G. tropicum*. To support species identification and confirm phylogenetic placement, the Internal Transcribed Spacer (nrITS) region of rDNA was amplified, sequenced, and compared with reference sequences retrieved from GenBank. Phylogenetic analysis using the Maximum Likelihood (ML) method revealed that the Kerala material nested within a strongly supported clade (97% bootstrap) comprising sequences of *G. tropicum* from China, India, Taiwan, and Thailand. This integrative approach confirms the taxonomic identity of the specimen as *G. tropicum*, marking its first record from Thiruvananthapuram District and the first known occurrence on the endemic host *Artocarpus hirsutus*. This study contributes valuable data on the geographic distribution, host range, and genetic relationships of *G. tropicum*, and emphasizes the importance of coupling classical taxonomy with molecular tools for resolving species boundaries and uncovering fungal diversity in tropical regions.

Keywords: Agaricomycetes, Kerala, Polyporales, Taxonomy, White rot

INTRODUCTION

The genus *Ganoderma* was established by Karsten in 1881, designating *Polyporus lucidus* (Curtis) Fr. (currently accepted as *Ganoderma lucidum* (Curtis) P. Karst.) as the type species (Moncalvo and Ryvarden, 1997). Members of this genus are widely acknowledged for their phytopathogenic behavior, frequently implicated in the onset of wood decay and various forms of rot in host plants (Cao and Yuan, 2013; Coetzee *et al.*, 2015). The genus demonstrates a cosmopolitan distribution, thriving in diverse ecosystems spanning tropical and temperate climates across continents such as Africa, the Americas, Europe, and Asia (Wong *et al.*, 2012; Cao and Yuan, 2013). In addition to their phytopathological relevance, *Ganoderma* species are of substantial economic importance due to their well-documented medicinal properties. Several *Ganoderma* species have been employed for centuries in traditional East Asian medicine,

particularly in China, Japan, and Korea, where it is valued for its reputed ability to enhance longevity, promote general health, and modulate immune responses (Hapuarachchi *et al.* 2018)

Ganoderma is characterized by distinctive laccate or non-laccate, sessile to stipitate basidiomata and double-walled basidiospores with interwall pillars (Karsten 1881; Moncalvo and Ryvarden 1997). There are 498 records in the Index Fungorum (<http://www.indexfungorum.org/>; accessed date: 10th April 2025).

During our ongoing studies on poroid fungi of Thiruvananthapuram District, Kerala, India, an interesting stipitate *Ganoderma* species with laccate pileus was observed on the dead stump of *Artocarpus hirsutus*. It was identified as *Ganoderma tropicum* (Jungh.) Bres., based on macro-micro morphology and nrITS based molecular phylogeny, and is described here in detail.

MATERIALS AND METHODS

Morphological studies

Gross morphological descriptions are based exclusively on fresh materials collected from Thiruvananthapuram District, Kerala State, India. Microscopic characters were studied on dried material using hand cut sections of basidiomata stained with 1% Congo Red and mounted in 3% solution of KOH and examined under an Olympus CX43 optical microscope (Olympus, Japan). For evaluation of the range of spore size, 20 basidiospores were measured for length and width. Spore quotient ($Q = L/W$) was also determined. Color coding follows that of Kornerup and Wanscher (1978). Microphotographs were made with a Magcam DC10 digital camera attached to the same microscope (Olympus, Japan). The

Specimen is deposited in the Mycological Herbarium of Postgraduate and Research Department of Botany, Government College for Women, Thiruvananthapuram.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh specimen following protocols in Izumitsu *et al.* (2012). The Internal Transcribed Spacer region (nrITS) was amplified and sequenced from the presented species. PCR reactions were performed with the primer pair ITS1, ITS4 (White *et al.*, 1990). The protocols for PCR amplification and sequencing followed Kumar *et al.* (2018). The newly generated sequence was deposited in GenBank. Sequences retrieved from GenBank and obtained in this study are listed (**Table 1**).

Table 1. Species, Identification numbers, GenBank accession numbers and their geographic location used for nrITS based phylogenetic analyses in the present study

Species	Voucher/Strain	GenBank No.	Geographic origin
<i>Amauroderma calcitum</i>	FLOR:50931	KR816528	Brazil
<i>Ganoderma applanatum</i>	SFC20141001-24	KY364255	South Korea
<i>Ganoderma applanatum</i>	Wei5787a	KF495001	China
<i>Ganoderma australe</i>	HUEFS:DHCR417	MF436676	Australia
<i>Ganoderma destructans</i>	CBS139793	NR132919	South Africa
<i>Ganoderma destructans</i>	CMW43670	KR183856	South Africa
<i>Ganoderma enigmaticum</i>	CBS139792	NR132918	South Africa
<i>Ganoderma enigmaticum</i>	Ghana2/938397	KR014265	Ghana
<i>Ganoderma gibbosum</i>	UB1	KU569556	Brazil
<i>Ganoderma gibbosum</i>	SPC2	KU569547	Colombia
<i>Ganoderma lingzhi</i>	Dai12441	JQ781869	China
<i>Ganoderma lingzhi</i>	Wu1006-38	JQ781858	China
<i>Ganoderma lingzhi</i>	Li245	JQ781863	China
<i>Ganoderma lucidum</i>	K175217	KJ143911	UK
<i>Ganoderma lucidum</i>	BR4195	KJ143909	France
<i>Ganoderma lucidum</i>	Dai2272	JQ781851	Sweden
<i>Ganoderma lucidum</i>	Dai11593	JQ781852	Finland
<i>Ganoderma multipileum</i>	CWN04670	KJ143913	China
<i>Ganoderma multipileum</i>	Dai9447	KJ143914	China
<i>Ganoderma multipileum</i>	HMAS242384	JF915409	China
<i>Ganoderma orbiforme</i>	URM83332	JX310813	Brazil
<i>Ganoderma orbiforme</i>	URM83334	JX310814	Brazil
<i>Ganoderma orbiforme</i>	URM83335	JX310815	Brazil
<i>Ganoderma parvulum</i>	URM83339	JX310817	Brazil
<i>Ganoderma parvulum</i>	URN83340	JX310818	Brazil
<i>Ganoderma pfeifferi</i>	K(M)120818	AY884185	UK
<i>Ganoderma pfeifferi</i>	JV0511/11	KF605660	Unknown

Species	Voucher/Strain	GenBank No.	Geographic origin
<i>Ganoderma resinaceum</i>	HSBU200830	KT343303	Iran
<i>Ganoderma resinaceum</i>	HMAS86599	AY884177	UK
<i>Ganoderma sessile</i>	UMNMN8	MG654281	USA
<i>Ganoderma sichuanense</i>	MFU16-2670	KY404119	Thailand
<i>Ganoderma sichuanense</i>	MFU16-2668	KY244062	Thailand
<i>Ganoderma sichuanense</i>	MFU16-2667	KY244061	Thailand
<i>Ganoderma sichuanense</i>	HMAS251145	JF915400	China
<i>Ganoderma tropicum</i>	HKAS76644	KC222317	China
<i>Ganoderma tropicum</i>	HUMCC18-0046a	MH823539	Thailand
<i>Ganoderma tropicum</i>	HKZT2022102301	QR140836	China
<i>Ganoderma tropicum</i>	JZT-VL	PQ119874	India
<i>Ganoderma tropicum</i>	Wu0407-2	EU021458	Taiwan
<i>Ganoderma tropicum</i>	BTYDZT2023061103	OR764801	China
<i>Ganoderma tropicum</i>	ZGCVN429	MT364498	India
<i>Ganoderma tropicum</i>	HMDGF001	OM920538	China
<i>Ganoderma tropicum</i>	BCRC37122	EU021457	Taiwan
<i>Ganoderma tropicum</i>	He1232	KF495000	China
<i>Ganoderma tropicum</i>	Dai9724	JQ781879	China
<i>Ganoderma tropicum</i>	HMAS263143	JF915410	China
<i>Ganoderma tropicum</i>	MHGCW006	PV382975	India
<i>Ganoderma tropicum</i>	RB1	MK851524	India
<i>Ganoderma tropicum</i>	AP17	FJ491960	India
<i>Ganoderma tropicum</i>	ZGCVN873	MT364499	India
<i>Ganoderma valesiacum</i>	CBS428.84	JQ520218	USA

Sequence alignment and phylogenetic analysis

The nrITS sequences of *Ganoderma* species from present study (GenBank number PV382975) along with species of *Ganoderma* retrieved from GenBank based on BLAST results in NCBI and previous studies (Laungharn *et al.*, 2019, 2023; Sun *et al.*, 2022) were aligned using MAFFT web tool (www.ebi.ac.uk/Tools/msa/mafft/) with default settings. The final aligned data matrix of nrITS sequences from 50 taxa, including one species of *Amauroderma* as outgroup (**Table 1**), was then imported into BioEdit v7.2.6.1 (Hall, 1999) for manual adjustment. *Amauroderma calcitum* was selected as outgroup following Laungharn *et al.*, (2020). Maximum likelihood (ML) analysis was performed in the web based platform <http://iqtree.cibiv.univie.ac.at> (Trifinopoulos *et al.*, 2016) with 1000 ultrafast bootstrap replicates. Kimura 2- parameter with Gamma variable (K2P+G4) was selected automatically as the best fit substitution model in the same web platform with the least BIC score (4327.9902). The phylogram inferred from ML analysis is displayed with MEGA 11.0.13 (Tamura *et*

al., 2021).

RESULTS

Molecular phylogeny

The phylogeny inferred from ML analysis of the data matrix of nrITS sequences is provided in **Figure 1**. Sequence of *Ganoderma* species from the present study was nested in a clade containing sequences of *G. tropicum* from China, India, Taiwan and Thailand with a strong ML bootstrap (97 %) supporting its identity as *G. tropicum*.

Taxonomy

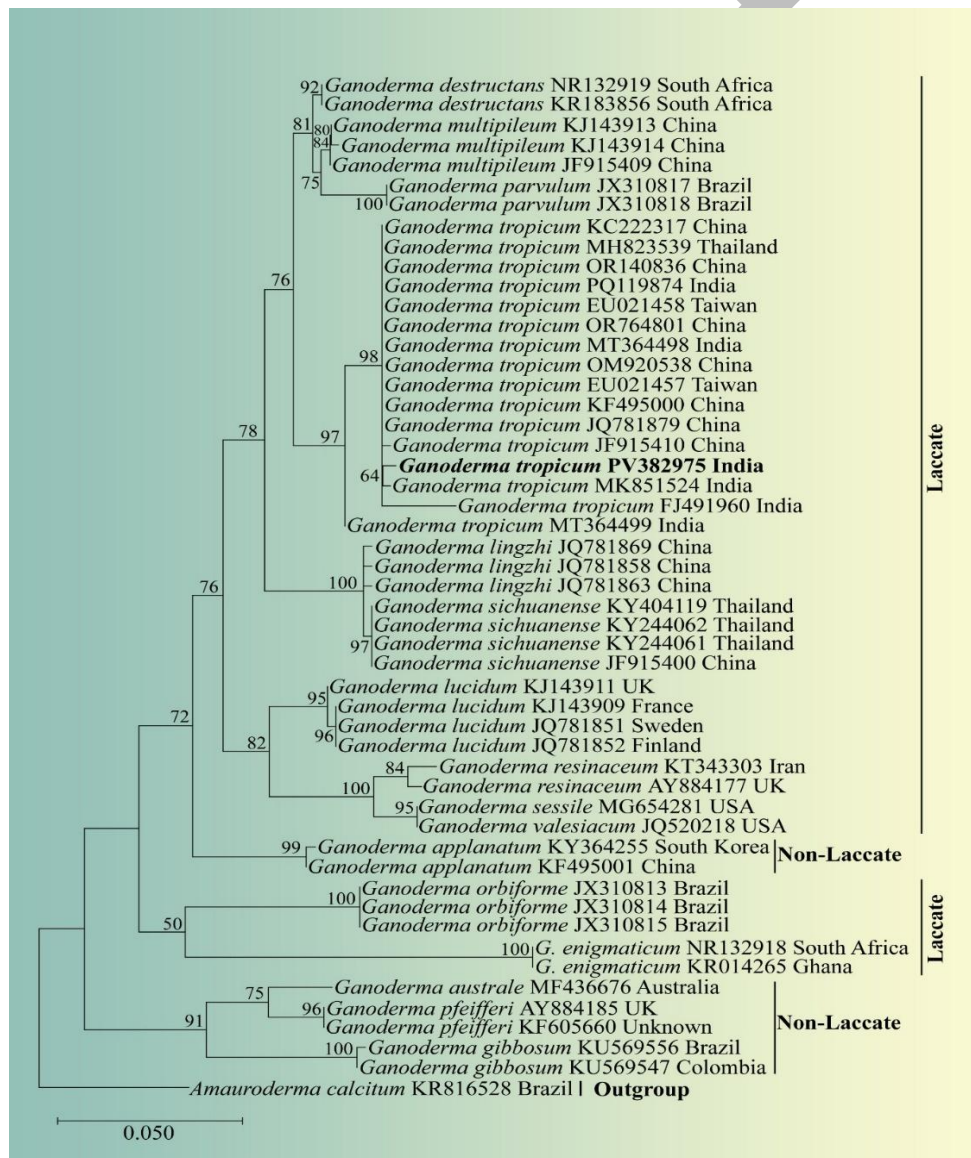
***Ganoderma tropicum* (Jungh.) Bres., *Annales Mycologici* 8(6): 586 (1910)**

Figure 2

Basidiomes: Basidiomata annual, solitary, tough when fresh, becoming harder and woody on maturing, light weighted on drying, laccate, stipitate. *Pileus* upto 3.5 cm in length, 11 cm broad, and 2.5 cm thick; *Pileus shape* irregular, nodulose, with uneven lobes, often with a stretched palm like appearance; *Pileus surface* sulcate, smooth, shiny, laccate, glabrous; *Pileus color* white towards margin, maize (yellow) to

orange (4A6/5A6/5A7) towards stipe and violet brown to dark magenta (10E8/11E8/13F6) further down; *Pileus margin* smooth, entire, obtuse, white. *Hymenophore* poroid; *Pores* 5–6 in number per mm, round to ovoid, irregular at some parts, absent near margin; *Dissepiments* upto 60 µm thick; *Pore surface* white, turning light to dark brown (6D8/6F7) on bruising; *Pore tube* 5–13 mm in length, non-stratified. *Context* 1–4 cm, tough, corky, brown (6E4). *Stipe* central, compressed, woody, upto 9.2 x 6 cm. *Stipe surface* dark magenta (13F6), shiny, laccate, glabrous, *Stipe tissue* slightly fistulose, light brown (6D8). ***Pileal crust***: hymeniodermis; *cuticular elements* 20–50 × 5–8 µm, cylindroclavate, clavate, regular or irregularly branched or lobed, thick walled, smooth,

brownish yellow (5C8), forming a palisade. ***Hyphal structure***: hyphal system trimitic; *generative hyphae* upto 2.6 µm broad, hyaline, and unbranched, thin-walled, with clamp connections; *skeletal hyphae* upto 5 µm broad, occasionally with arboriform branching, light brown, aseptate, thick-walled; *skeleto-ligative hyphae* upto 2.6 µm broad, branched, brown, aseptate, thick-walled. ***Basidia***: Not observed. ***Basidiospores***: (6.5–) 7–11 (–12) × (4–) 4.5–6.5 (–7) without exosporium, AvL=9, AvW=5.5, Q=1.4–1.6, ellipsoid to ovoid, truncate at the apex, acyanophilous, inamyloid, exosporium thin, subhyaline, smooth, endosporium thick, brown, with inter-wall pillars. ***Odor***: not distinctive. ***Spore print***: not obtained.



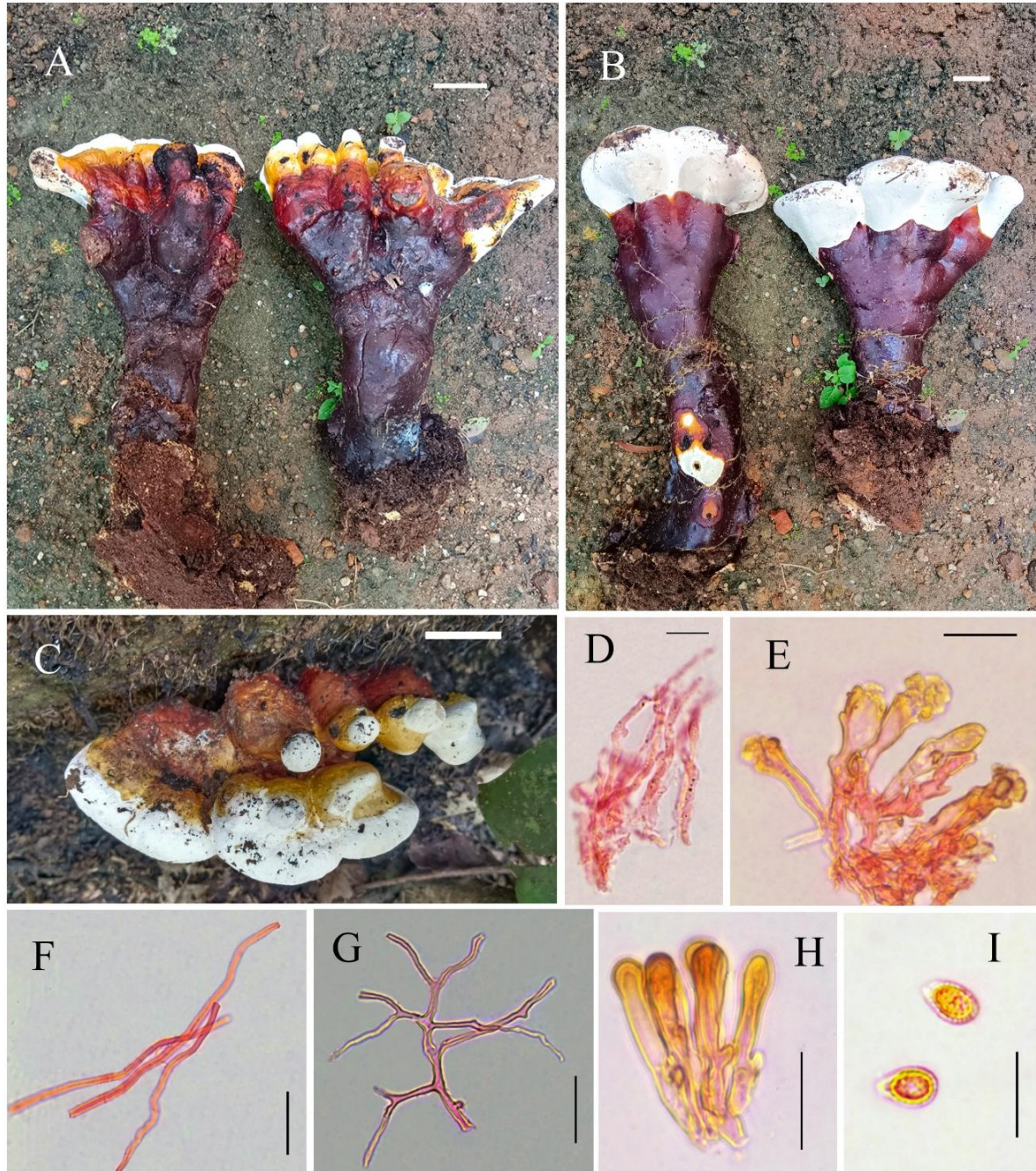


Figure 2: *Ganoderma tropicum*. A, Habit; B, Hymenophore view; C, Pileus top view; D, Generative hyphae with clamp; E and H, Cuticular elements; F, Skeletal hyphae; G, Arboriform skeletoligative hyphae; I, Basidiospores. Scale bars: A-C = 20 mm; D and I = 10 μ m; E-H = 20 μ m

Habitat: On dead stump of *Artocarpus hirsutus* (Lam.), at its base, causing white-rot.

Specimens examined: INDIA. Kerala State, Thiruvananthapuram district, Palode, 04 July 2024, MHGCWT006; Palode, 13 September 2024, MHGCWT007.

DISCUSSION

Ganoderma tropicum is a widely distributed laccate

species reported from several tropical regions worldwide (Luangham *et al.*, 2019). In India, its occurrence has been documented in Himachal Pradesh (Kaur *et al.*, 2023), Kerala (Vinjusha and Kumar, 2022), Maharashtra (Manohar *et al.*, 2024), Tamil Nadu (Kaliyaperumal and Kalaichelvan, 2007), and the Union Territory of Chandigarh (Kaur *et al.*, 2023). Within Kerala, it has been previously reported from Kozhikode and Palakkad

districts, though not from Thiruvananthapuram district. The species exhibits considerable macro-morphological variability across its range (Luangharn *et al.*, 2019). Even though it is morphologically similar to other laccate *Ganoderma* species such as *G. flexipes*, *G. lingzhi*, *G. multipileum*, *G. sichuanense*, and *G. tsugae*, all of which share features like a reddish-brown pileus, ellipsoid basidiospores, and the presence of cuticle cells, molecular data and phylogenetic analyses warrants its segregation as a distinct species.

Ganoderma tropicum is known to cause white-rot on several angiosperm hosts across tropical regions. In China, it has been reported on *Acacia confusa*, *Casuarina* sp., and *Diospyros bejandii* (Dai *et al.*, 2004; Sun *et al.*, 2022). In Thailand, its occurrence has been recorded on *Anthocephalus chinensis* and *Dipterocarpus* sp. (Luangharn *et al.* 2019, 2023), while in Indonesia, it was reported on *Elaeis guineensis* (Turner, 1981). From India, the fungus has been previously documented on *Cocos nucifera* (Vinjusha and Kumar, 2022) and *Tamarindus indica* (Manohar *et al.* 2024). The present study adds to this host range by reporting *G. tropicum* on a novel host, *Artocarpus hirsutus*, from Thiruvananthapuram District, Kerala.

Ganoderma tropicum is recognized for its high nutritional and medicinal value and is cultivated and commercialized in countries like China and Thailand (Luangharn *et al.*, 2019). It is listed in the Chinese Pharmacopeia (Wu *et al.*, 2013) and contains lanostanoid-type triterpenes of chemotaxonomic and therapeutic interest (Ríos *et al.*, 2012; Da Silva *et al.*, 2013; Zhang *et al.*, 2015). Despite its presence in various regions of India, the species remains underexplored in terms of its potential applications, highlighting the need for further research and sustainable utilization strategies.

CONCLUSION

The present study documents the first record of *Ganoderma tropicum* (Jungh.) Bres. from Thiruvananthapuram District, Kerala, expanding both its geographical and host range within India. Detailed morphological examination combined with molecular phylogenetic analysis based on nrITS sequences confirms its identity. The species was found fruiting on a previously unreported host, *Artocarpus hirsutus* (Lam.), thereby contributing a

new host association to the known biology of *G. tropicum*. This finding adds valuable data to the fungal biodiversity of the region and underscores the importance of continued mycological surveys coupled with integrative taxonomic approaches for better understanding the diversity and ecological roles of wood-decaying fungi in tropical ecosystems.

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