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GANODERMA TSUGAE (POLYPORALES; GANODERMATACEAE), A NEW RECORD FROM PAKISTAN

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Abstract

Under the family Ganodermataceae, genus *Ganoderma* is distributed globally which causes white rot to a variety of tree species. Including Pakistan, many species of genus *Ganoderma* have been reported from different parts of the world. In this research, *Ganoderma tsugae* is reported from Punjab, Pakistan as a new record. This species is included in the *Ganoderma lucidum* complex which has been characterized by stipitate and solitary basidiocarps, shiny, varnished and laccate pileus with bald surface, echinuate subglubose to ovoid basidiospores, cutis elements of 19-34 × 7-11 µm in size and trimitic hyphal system. This paper presents the first morphological and anatomical description of *Ganoderma tsugae* with the help of colored micrographs and manual illustrations along with two comparison tables of different species of genus *Ganoderma* reported from Pakistan, has also been provided.

Key words: *Ganoderma tsugae*, Macrofungi, New record, Punjab, White rot, Wood rotting

Introduction

Fungi is the most divergent group of living organisms estimated to be around 3.5–5.1 million (Hawksworth, 1991; Blackwell, 2011; Hawksworth & Lucking, 2017; Aman *et al*., 2022) having both macrofungal and microfungal groups with great importance. For the identification of different species, the morphological, physiological and molecular markers are used (Wang *et al*., 2016; Aman *et al.*, 2022). Macro fungi, the microscopic spore carrying fruiting bodies, have significant ecological and economic importance as saprobes, parasites, symbionts and pathogens along with great medicinal and nutritional values (Mueller *et al*., 2007; Torres-Torres *et al*., 2013; Kinge *et al*., 2020; Ahmed *et al.*, 2023).

One of the variegated groups of fungi is the family Ganodermataceae that was described by Donk in 1948. The most characteristic feature of this family is the bilayered basidiospores (Karsten, 1881; Moncalvo & Ryvarden, 1997*;* Gottlieb & Wright, 1999; Aman *et al*., 2022). According to Gottlieb and Wright (1999) and Kirk *et al*. (2008), Ganodermataceae (order Aphyllophorales) is a great polyporales family with the included the genera; *Amauroderma, Foraminispora, Furtadoa, Ganoderma, Haddowia, Humphreyam and Polyporopsis*. Under the division of the basidiomycetes, the family Ganodemataceae has the genus named *Ganoderma* (Zhou *et al*., 2015; Thawthong *et al*., 2017; Konara *et al.*, 2022; Ahmed

et al., 2023). The generic name *Ganoderma* was customized by Karsten (1881), Moncalvo and Ryvarden (1997) and Gottlieb and Wright (1999). The classification of genus *Ganoderma* is polyphyletic (Ahmed *et al*., 2023). Almost 80 species of *Ganoderma* are found in the world according to Kirk *et al*. (2008) and Luangharn *et al*. (2021) reports. But Index Fungorum and MycoBank have 459 and 503 records, respectively (http://www.indexfungorum.org; http://www.mycobank.org).

Genus *Ganoderma* has mushrooms of great medicinal importance (Dai *et al*., 2009; Hapuarachchi *et al*., 2018a) and are good sources of bioactive compounds like proteins, polysaccharides, triterpenoids and steroids (Osinska-Jaroszuk *et al*., 2014). *Ganoderma* species are parasites called as facultative parasites present on existing, rotting and dead trees (Moncalvo & Ryvarden, 1997; Pilotti *et al*., 2004; Torres-Torreset *et al*., 2013; Zhou *et al*., 2015; Nguyen *et al*., 2023). As they are white rot fungi, they degenerate lignocellulose present in wood (Sumic *et al*., 2017; Lu *et al*., 2020). For instance, the stem rot on the oil palm has resulted in the 23.8 tons of fresh fruit brunches loss per hectare as investigated by Pilotti (2005) and Nguyen *et al*. (2023).

This genus has been classified on the basis of type of mycelia, color of fruiting bodies, host range, presence of stipes, length or thickness of stipes, shape of pileus and size or shape of pores (Ito, 1955; Kim *et al*., 2001). The macromorphological characters are the interwall pillars, stipitate fruiting bodies with double-walled basidiospores (Karsten, 1881; Moncalvo & Ryvarden, 1997; Gottlieb & Wright, 1999; Luangharn *et al*., 2021), and micromorphological characters have also been studied by (Haddow & Haddow, 1931; Steyaert, 1967a, 1967b, 1972, 1975, 1977; Adaskaveg & Gilbertson, 1988; Patouillard, 1889; Torres-Torres & Guzman-Davalos, 2012).

According to the investigations of Wang *et al*. (2012) and Hapuarachchi *et al*. (2018b), genus *Ganoderma* has been distributed worldwide in the regions such as Africa, America, Asia and Europe. In Pakistan, nineteen species of this genus have been reported; *Ganoderma applanatum* (Pers.) Pat., *Ganoderma australe* (Fr.) Pat., *Ganoderma ahmadii* Steyaert, *Ganoderma boninense* Pat., *Ganoderma curtisii* (Berk.) Murrill, *Ganoderma colossus* (Fr.) C.F. Baker, *Ganoderma chalceum* (Cooke) Steyaert, *Ganoderma flexipes* Pat., *Ganoderma gibbosum* (Blume & T. Nees) Pat., *Ganoderma lucidum* (Curtis) P. Karst., *Ganoderma leucocontextum* T.H. Li, W.Q. Deng, Sheng H. Wu, Dong M. Wang & H.P. Hu, *Ganoderma multipileum* Ding Hou, *Ganoderma multistipitatum* S. Ahmed, M. Awais, M.M. Sadiq, A. Umar, L. Dufosse, M.T. Khan, J. Alkahtani & R.M. Mahmoud, *Ganoderma multiplicatum* (Mont.) Pat., *Ganoderma multicornum* Ryvarden, *Ganoderma resinaceum* Boud., *Ganoderma pakistanicum* Umar, A., Ahmed, S., & Gafforov, Y., *Ganoderma tsugae* Murrill (current study), *Ganoderma tornatum* (Pers.) Bres. in different reports.

In this study, we are dealing with the first occurrence of *G. tsugae*, collected from Pakistan. The main objective of this research was to study the collected *Ganoderma* sp., and to describe it novelty status from Pakistan. This taxon was found as a new record on the basis of morphological criteria of identification. Morpho-anatomical characterization and analysis of *Ganoderma tsugae* are essential for its proper taxonomic placement and its differentiation from closely related species.

Materials and methods

Collection and observation of specimen

G. tsugae was collected during rainy seasons in 2021. The site for this collection was Dharamkot, Feroz Wala Road, district Gujranwala, Punjab, Pakistan. The sample was solitary and attached with the base of tree trunk. Photography and tagging with specific code and labeling (i.e. location, date and host tree name) was done in the field. The sample was brought in Mycology laboratory, GCU Lahore and surface sterilized with alcohol. The sample was then air dried. Along with the tag, the dried sample was placed in a zip locked file.

Macroscopic analysis

For the macroscopic characterization, the shape and color, surface and texture, length and width of fruiting body along with the margins of stipe and pileus and hymenthial surface of basidiocarp were analyzed. Taste and odor were also observed.

Slide preparation and micrometry

For the anatomical analysis, microscopic slides were prepared. For this reason, small pieces of the dried specimen were soaked separately for 5 minutes in alcohol and put onto the glass slide. The fine section of sample was sharply cut and buried under cover slip. 5% KOH was utilized as mounting media. For staining, 1% Congo red stain was used. Under light microscope, the prepared slide was observed at different magnification powers of Compound microscope.

Micrometry was also performed to take into account the length and width, shape, quotient value and range of basidiospores, basidiole, cutis elements and skeletal hyphae. Manual illustrations were made by observing hymenial section. These microscopic features were further used in identification of the species.

Results

***Ganoderma tsugae* Murrill, Bulletin of the Torrey Botanical Club. 29; 601 (1902)**

Macroscopic Characterization

Basidiocarp; 8-15 cm, imbricate, subdimidiate, stipitate, annual, solitary. **Pileus;** 3-5×1-2.5 cm, creamy, reniform, irregular, spathulate to uneven, undulate, bald surface. **Pileus surface;** strongly laccate at maturity, tough or hard texture, imbricate, shallow sulcate, spathulate, umbonate or uneven, convex, radial furrowed, shiny, glossy, incised, irregularly rugose, when young to age have smooth layers at the center, thin crust covering the context, radial at the center and outspread toward the margin, irregularly ruptured. **Context;** homogeneous, thick near the stipe, soft and fibrous, covered with thin crust, brownish-orange to brown brownish-red at the upper layers when dried. **Pore surface;** up to 6 pore per mm, circular or angular, whitish at young stage, turn to medium dark brown at maturity. **Tube;** 0.3-1.6 cm long, light brown. **Stipe;** 8-12×4-6 cm, central, cylindrical, brownish to reddish brown, broad and thick at the base, uneven towards base, bald, rough surface, solid, unequal, covered irregularly ruptured crust, when mature laccate, strong laccate with soft and sometimes wavy margins at old. **Rhizomorphs;** absent. **Odor;** pleasant.

Microscopic Characterization

Basidiospores; 10-14 × 7-9 µm, Q = 1-1.4, Q_{avg} = 1.2, sub glubose to ovoid, brown, oil droplets absent, double layered, echinulate, having turgid vesicular appendix, apically truncate, coated by hyaline, orange brown in 5% KOH. **Trimitic hyphal system;** present. **Generative hyphae;** 2.85-4.2 µm in diameter, rusty brown, branched, aseptate, broad, flexuous, thin to thick walled, with clamp connections. **Skeletal**

hyphae; 2.85-5.9 µm, yellow to greenish, highly branched, septate, broad, thick walled and clamped. **Binding hyphae;** 2.85-5.7 µm, pale yellow, septate, thick walled and branched. **Stipe hyphae;** 3-6 µm, light golden yellowish, aseptate, thick walled, wide and unbranched. **Basidia;** 19-45 × 7.1-8.5 µm, cylindrical clavate, bisporic, yellowish green, double walled, clamped at base, outer thick walled, without oil droplets. **Cutis element**; 19-34 × 7-11 µm, bottle shaped, clavate to elongated, thick walled, golden yellow to greenish in color, smooth, without oil droplet.

COLLECTION EXAMINED: PAKISTAN: Punjab, District Gujranwala, Tehsil Gujranwala, Feroz Wala Road, Dharamkot, solitary, attached with base of tree trunk, 226 meters (744 ft.) a.s.l., 24th September 2021, collected by Saliha Rukhsar (voucher code: GM-91).

Literature Reviewed for Species Identification: Description of this species was confirmed by reviewing the published literature and descriptions of *G. tsugae* reported worldwide by Torres-Torres *et al*. (2015) and Luangharn *et al*. (2021).

DISCUSSION

Ganoderma tsugae is a flat polypore mushroom also called Hemlock Varnish Shelf, Hemlock Reishi, or Eastern Reishi. This species in *Ganoderma* genus has been characterized by varnished appearance of the pileus which distinguishes it from *Ganoderma lucidum*, and is laccate with resinous bands having shelf-like growth pattern. Subglubose to ovoid shaped, thick walled, echinuate basidiospores with size range of 10-14×7-9 µm and a timitic hyphal system are the distinctive characters which make this species of *Ganoderma* different from other species (Torres-Torres *et al.*, 2015; Luangharn *et al*., 2021).

G. lucidum has been used as a synonym for *Ganoderma tsugae* in past (Atkinson, 1908; Haddow & Haddow, 1931; Steyaert, 1977; Luangharn *et al*., 2021) as both have similar appearance. But phylogenetic analysis proved *G. tsugae* independent and distinct species from *G. lucidum*. The major difference lies in their habitat as *Ganoderma lucidum* generally grows on angiosperms and gymnosperms but *G. tsugae* prefers coniferous trees exclusively on hemlocks (Zhou *et al*., 2015; Luangharn *et al*., 2021). Worldwide, this species of genus *Ganoderma* is extensively distributed across USA (Gilbertson & Ryvarden, 1986; Loyd *et al*., 2018; Luangharn *et al*., 2021).

G. tsugae resembles *G. oregonense* as both have rough basidiospores, white context tissue, associated with decaying conifers (Torres-Torres *et al*., 2015; Loyd *et al*., 2018; Luangharn *et al*., 2021) and laccate pileal surface. To distinguish them, *G. tsugae* have reniform pileus with white margins (Umar *et al*., 2021), resinous bands in the context and concentrically zonate surface, but *G. oregonense* have rounded-flabelliform pileus with rusty brown margins, no resinous bands and azonate to slightly zonate surface. The length and thickness of the tubes are used as key characters to distinguish these two species according to Overholts (1953). Besides this, *G. tsugae* has smaller basidiospores (9-11 × 6-8 µm) (Torres-Torres *et al*., 2015; Zhou *et al*., 2015) as compared to *G. oregonense* (13-17 × 8-10 µm) (Gilbertson & Ryvarden, 1986). In *G. tsugae* slightly darker layer is present next to the tubes in the context (Overholts, 1953; Gilbertson & Ryvarden, 1986) while homogeneous context in *G. oregonense* (Gilbertson & Ryvarden, 1986).

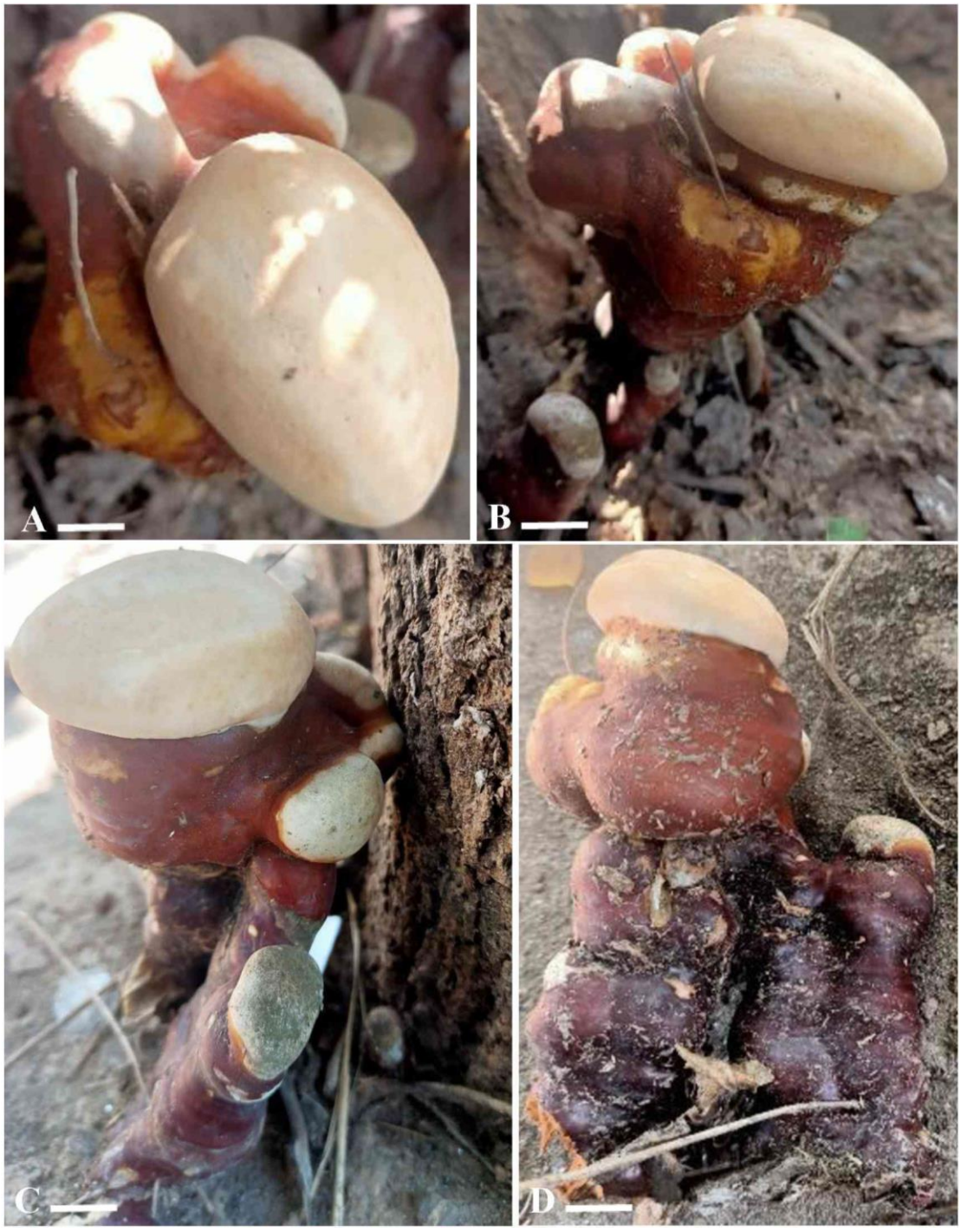

Figure 1. Morphology of basidiomata of *Ganoderma tsugae* (GM-91). **A-D.** Different views of basidioma. **Scale bars: A.** 0.6 cm. **B.** 1.3 cm. **C&D** 1.2 cm.

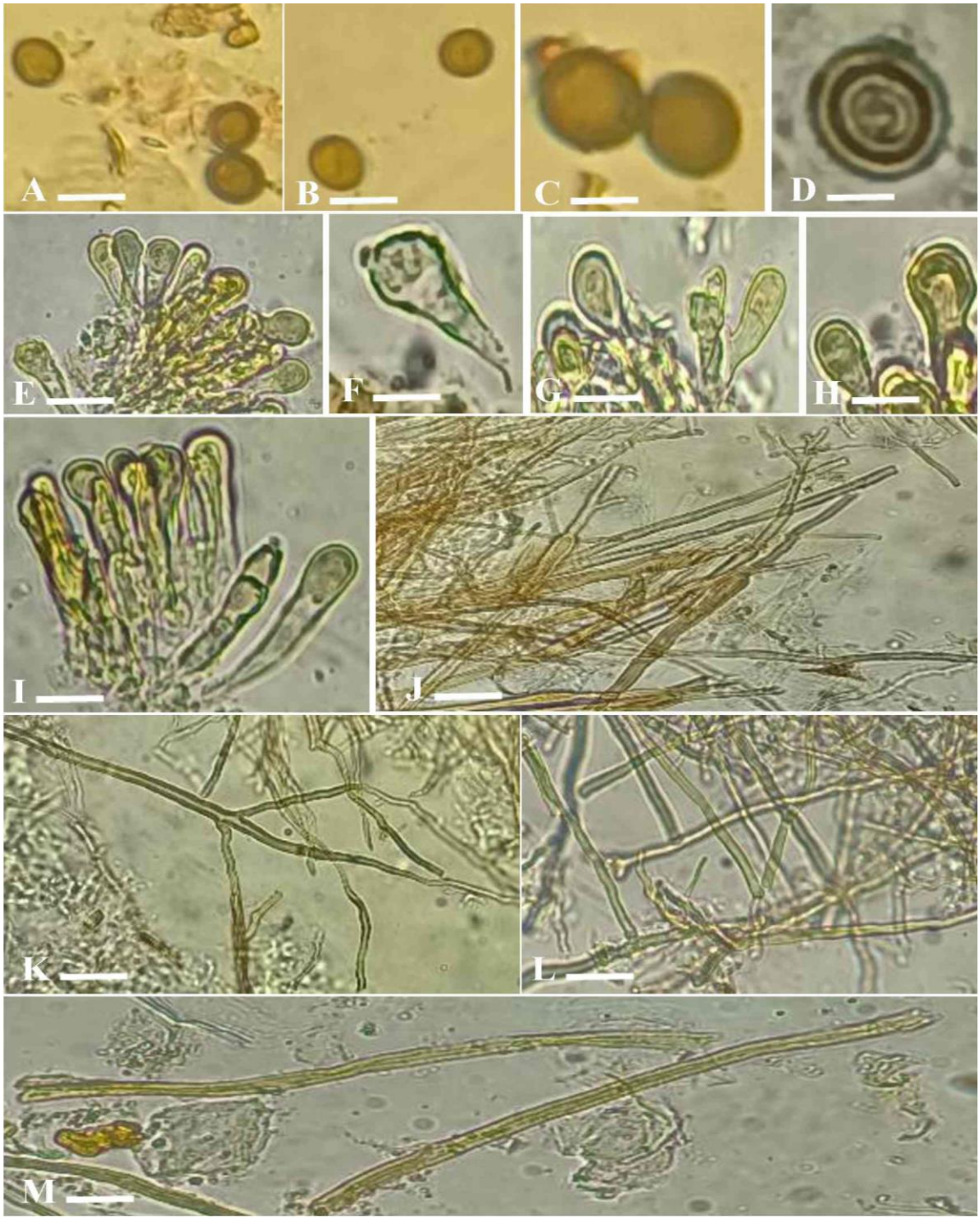

Figure 2. Light micrographs of microscopic features of *Ganoderma tsugae* (GM-91). **A-D.** Basidiospores. **E & F.** Basidia. **G-I.** Cutis elements. **J.** Generative hyphae. **K.** Skeletal hyphae**. L.** Binding hyphae. **M.** Stipe hyphae. **Scale bars: A & B.** 20 µm. **C.** 7.5 µm. **D.** 4 µm. **E.** 16 µm. **F.** 8.8 µm. **G.** 12 µm. **H.** 11.3 µm. **I.** 7.5 µm. **J.** 16 µm. **K.** 21 µm. **L.** 42 µm. **M.** 22 µm.

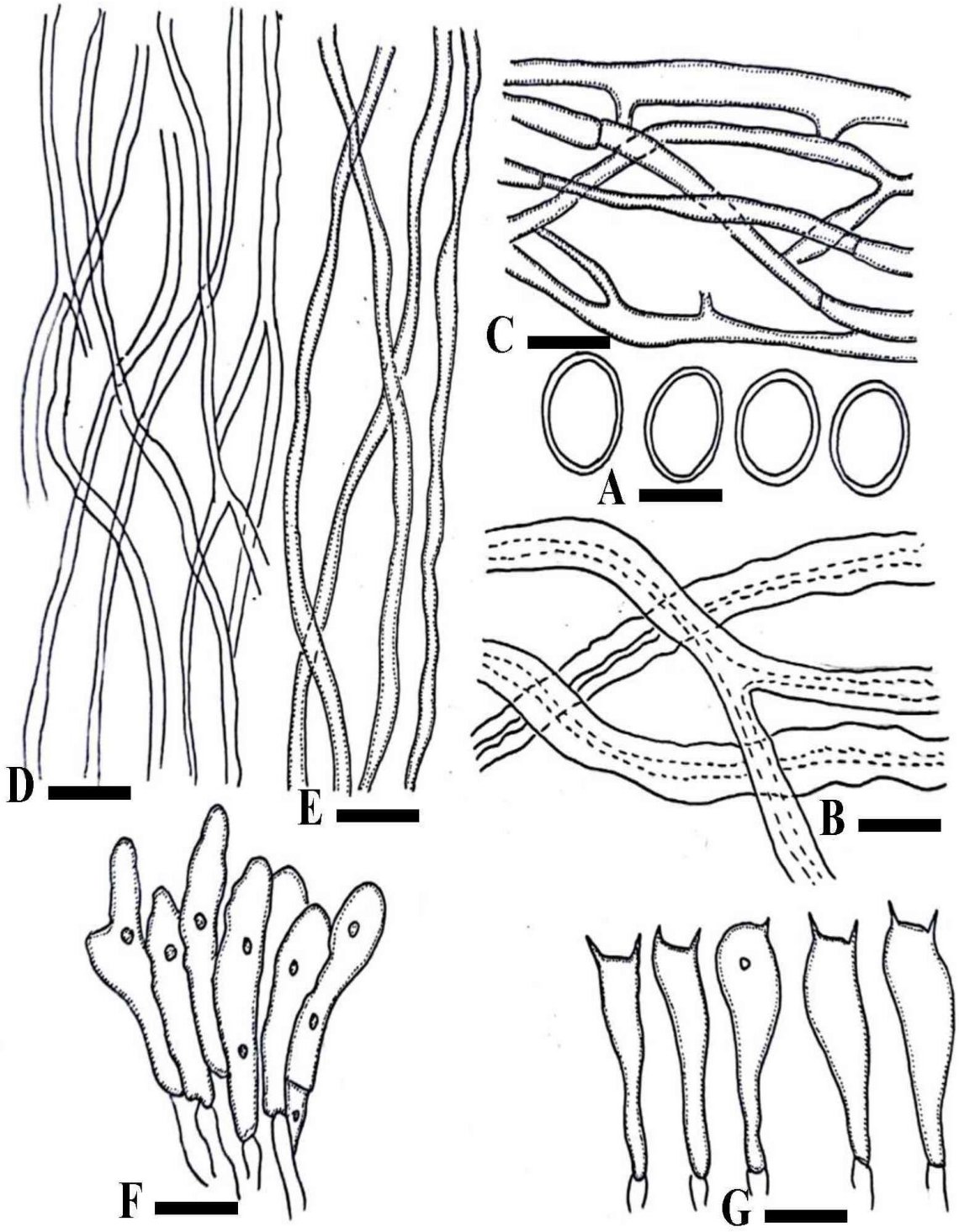

Figure 3. Illustrations of microscopic features of *Ganoderma tsugae* (GM-91). **A.** Basidiospores. **B.** Stipe hyphae. **C.** Binding hyphae. **D.** Generative hyphae. **E.** Skeletal hyphae. **F.** Cutis elements. **G.** Basidia. **Scale bars: A.** 26 µm. **B.** 9 µm. **C.** 14 µm. **D.** 17 µm. **E.** 21 µm. **F.** 9.4 µm. **G.** 14 µm.

Among the species in *G. lucidum* complex, *G. tsugae* resembles *G. leucocontextum* in macroscopic appearance as both species have reniform and laccate pileus, resinous bands in the context, have white margins and are concentrically zonate, but they are settled apart by their habitat and microscopic features. *G. tsugae* and its varieties are associated with coniferous wood and *G. leucocontextum* is found to be growing on wood of deciduous trees and the distinguishing microscopic characters is; *G. tsugae* have larger basidiospores (13-15×7.5-8.5 mm) (Gilbertson & Ryvarden, 1986; Li *et al*., 2014) and sub-glubose to ovoid in shape as compared to *G. leucocontextum* with smaller basidiospores (9.5-12.5×7-9 µm) (Li *et al*., 2014) and ellipsoidal shape.

G. tsugae is closely related to *G. lucidum* as both species have some similarities in macroscopic and microscopic characters; both species have reddish brown to dark brown pileus with laccate and concentrically zonate surface, *G. tsugae* have resinous bands with creamy to pink-buff context (Steyaert 1980; Umar *et al*., 2021) but there are no resinous bands with deep rusty brown context in *G. lucidum* (Ryvarden & Gilbertson, 1993; Umar *et al*., 2021). These two species are distinguished on the basis of size of basidiospores, and according to previous literature *G. tsugae* has spores size 10.9- (11.2)-12.5 × 6.6- (7 .8)- 8.5 µm as compared to 10.6- (11.5)-11.8 × 6.8- (7.4)-7.8 in *G. lucidum*, defining that there is a difference in the width in relation to lengths in basidiospores sizes. The mean values of the spore index of *G. tsugae* (1.57) is significantly different from *G. lucidum* mean value of spore index of 1.50. The inter-wall pillars in *G. tsugae* between inner and outer walls are very distinct as compared to unobvious pillars in *G. lucidum* and the vacuoles in *G. tsugae* are inconspicuous as compared to conspicuous in spores of *G. lucidum* (Adaskaveg and Gilbertson, 1986). *G. tsugae* have circular pores (Umar *et al*., 2021) but in *G. oregonense*, the pores are circular to irregular (Umar *et al*., 2021) and subcircular in *G. leucocontextum* and *G. lucidum* (Cao *et al*., 2012; Umar *et al*., 2021).

Some species under the genus *Ganoderma* such as *G. tsugae*, *G. flexipes*, *G. lingzhi*, *G. multipileum* and *G. sichuanense* resemble *G. tropicum* in pileus surface being reddish-brown, dark-brown context and ellipsoidal and echinulate basidiospores along with irregular cuticle cells (Cao *et al*., 2012; Luangharn *et al*., 2021). *Ganoderma tsugae* and *G. lucidum* have some matching characters, and *G. tsugae, G. tropicum, G. sichuanense, G. multipileum and G. flexipes* resemble *G. lingzhi* as they have same reddish brown pileal surface, basidiospores, and cuticle cells (Cao *et al*., 2012; Luangharn *et al*., 2021). Two tables of comparison of different *Ganoderma* species with our specimen is given in Table 1&2.

Uses of *Ganoderma tsugae*

Under natural conditions, *G. tsugae* causes delignification, exhibiting degradation pattern similar to cellulase-deficient mutants observed in *Phanerochaete chrysosporium* (Eriksson *et al*., 1980; Ruel *et al*., 1981; Blanchette, 1984; Galappaththi *et al*., 2024).

In medical field, *G. tsugae* and *G. lucidum* extracts hamper the colorectal cancer cells growth *in vitro* (Hsu *et al*., 2008). The anti-tumorigenic activities have been reported from species of *Ganoderma* in which *Ganoderma tsugae and Ganoderma lucidum* have been used in the Asia for medicinal purposes. There are many biologically active compounds such as immune-modulatory proteins, polysaccharides and triterpenes that show antitumor effects (Hsu *et al*., 2008).

Table 1: A comparison of macroscopic characters of different species of genus *Ganoderma* reported from Pakistan

Sr. no.	Taxa	Macroscopic characterization								Source
		Pileus							Stipe Length (cm)	
		Size (cm)	Shape	Color (at maturity)	Resinous Bands/ Non Resinous Bands	Laccate/ Non Laccate	Concentric Zones (on the upper surface of the pileus)	Context Thickness (cm)		
1.	***G. applanatum***	19.5-21× 9.3-16	bean or kidney shape	orange-grey	no resinous bands	non-laccate	concentrically zonate	1.2- 1.9	(mostly absent) 2.5-3.8	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
2.	***G. australe***	12-32×14-28	umbonate	reddish-orange to brownish orange	resinous bands	non-laccate	concentrically zonate	0.5-2	absent	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
3.	***G. ahmadii***	7 × 5	circular	brownish red	resinous bands	laccate	concentrically zonate	Very thin 0.003	2	Steyaert, 1972
4.	***G. boninense***	15 × 10	convex	brownish-orange	resinous bands	laccate	concentrically zonate	0.4-1	absent	Steyaert, 1967b; Luangharn *et al*., 2021; Mukhtar, 2019
5.	***G. curtisii***	1.3-1.5 × 1- 1.2	semicircular to kidney shaped	brownish red to reddish brown with purple hues	resinous bands	laccate	concentrically zonate	0.5-2	6.8- 7.8	Haddow and Haddow, 1931; Mukhtar, 2019
6.	***G. colossus***	16-23.5 × 10-12	bracket-shaped	reddish brown	resinous bands	laccate	concentrically azonate	2	absent	Steyaert, 1972; Elshafie *et al*., 2004; Moradali *et al*., 2007

7.	***G. chalceum***	7.5×7	semi-circular	reddish brown	resinous bands	laccate	concentrically azonate	1.5	7-8	Westphalen *et al*., 2010; Kaliyaperumal, 2013 Mukhtar, 2019
8.	***G. flexipes***	0.5-3.2 × 0.5-3	sub-reniform to reniform	reddish-brown to dark brown	resinous bands	laccate	concentrically Zonate	0.5-2	7- 10	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
9.	***G. gibbosum***	9.0–9.4 × 4.5–4.8	convex	grayish brown	resinous bands	non-laccate	concentric zonate	1.5	absent	Umar *et al*., 2023
10.	***G. lucidum***	12.2-14.3 × 13-16.2	tree like or irregular	red brown color to dark brown-black color plus yellowish-brown center	resinous bands	laccate	concentrically Zonate	1.8	5-6	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
11.	***G. leucocontextum***	10-18 × 5-9	reniform	blackish red-brown	resinous bands	laccate	concentrically Zonate	2	6-7	Li *et al*., 2014; Luangharn al., 2021; Umar *et al*., 2021
12.	***G. multipileum***	13-16.2×12.2-14.3	tree like or irregular	red brown color to dark brown-black color plus yellowish-brown center	resinous bans	laccate	concentrically zonate	1.5-2.2	5-9	Cao *et al*., 2012; Li *et al*., 2014; Luangharn *et al*., 2021
13.	***G. multistipitum***	24-26 × 10-11	reniform	reddish brown to	resinous bans	laccate	azonate	1.4-1.6	8.5-8.7	Ahmed *et al*., 2023

				dark brown						
14.	***G. multiplicatum***	5-7 × 4-12	round-flabelliform	dark violet-brown	resinous bans	laccate	concentrically zonate	0.6-0.9	absent	Ryvarden, 2000; Torres-Torres *et al*., 2013;
15.	***G. multicornum***	8 × 7	conical	creamish white	resinous bans	laccate	concentrically zonate	1.3	absent	Mukhtar, 2019
16.	***G. pakistanicum***	we were unable to locate any information about size and shape of pileus *Ganoderma pakistanicum*		yellowish brown to brown	no information was found about resinous bands of this species	laccate	we were unable to locate any information concentric zones and context thickness of *Ganoderma pakistanicum*		absent	Umar *et al*., 2022
17.	***G. resinaceum***	2.4-11.1 × 1.2-6.3	round-flabelliform, dimidiate, reniform	violet brown to orange-red	no resinous bands	laccate	concentrically zonate	1.3-1.9	2.5 - 3.1	Gottlieb, and Wright, 1999; Nagadesi, 2018; Luangharn *et al*., 2021
18.	***G. tsugae***	3-5×1-2.5	reniform	reddish-brown	resinous bans	laccate	concentically zonate	1- 1.8	8-12	Current study, Luangharn *et al*., 2021; Umar *et al*., 2021; Li *et al*., 2014
19.	***G. tornatum***	6.2-10.5×4.5-7.0	circular or subcircular	grayish white to brown	resinous bands	laccate	concentrically zonate	0.1- 4	absent	Steyaert, 1975

Table 2: A comparison of microscopic characters of different species of genus *Ganoderma* reported from Pakistan

Taxa	Microscopic Characterization								Source
	Basidiospores		Type of Hyphal system	Hyphal system			Basidia (µm)	Cutis elements (µm)	
	Size (µm)	Shape		Generative Hyphae (µm)	Skeletal Hyphae (µm)	Binding Hyphae (µm)			
G. applanatum	6.5-9.8×4.5-7.5	broadly Ellipsoidal or Ovoid	trimitic	0.7-2.9	2.85-8.55	1.4-3.5	12-22×7.6-11.2	30×14-18	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
G. australe	8.4-12×6-7.2	ellipsoidal or Ovate	trimitic	2.2-3.8	2.9-4.2	2.6-4.0	10-25×6.6-12.2	18-35 × 8-11	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
G. ahmadii	8-9.6-11 × 5.5-6.4-7	ovoid	trimitic	we were unable to locate any information about the diameter of generative hyphae	3-4	we were unable to locate any information about the diameter of binding hyphae and size of basidia		25 × 3-4	Steyaert, 1972
G. boninense	9.5 × 5.2	ellipsoidal	dimitic	1.4-5.0	2.6-5.6	1.2-4.8	14-27 × 7-12	30-50 × 5-10	Steyaert, 1967b; Luangharn *et al*., 2021; Mukhtar, 2019
G. curtisii	9.5-11.2×4.8 - 6.3	broadly Ellipsoidal	trimitic	2.4-4	2.85-5.7	2- 3.1	12-27×5.6-11	30 ×10-12	Haddow and Haddow, 1931; Mukhtar, 2019
G. colossus	14-16 × 9-11	ovate	dimitic	2-4	3-5	___	20-30 × 13-17	40 × 7-8	Steyaert, 1972; Elshafie *et al*., 2004; Moradali *et al*., 2007
G. chalceum	8.5-10-11.5 × 4.5-5.9- 7.5	ovoid	dimitic	1.5-3.0	2.0–7.0	____	not seen	19-55.8 × 3-14	Westphalen *et al*., 2010; Kaliyaperumal, 2013 Mukhtar, 2019
G. flexipes	(8.5-)9-10.3(-11) × (5-) 5.3-7	broadly Ellipsoidal	trimitic	(1.8-)2.2-2.9-3.4(-3.8)	(3.0-)3.8-4.8-5.4(-6.2)	(2.2-)2.8-3.8-4.5(-5.1)	21-33×5.7-9	15-30 × 6.6-9	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
G. gibbosum	7.5–10.2 × 4.6–5.9	elongate to ellipsoid,	dimitic	1.6-2.7	4.8-5.2	___	not seen	not seen	Umar *et al*., 2023

G. lucidum	(8-) 9-12 (-13) ×6-9	ellipsoidal or Ovate	trimitic	(1.8-) 2.0-2.3-2.7(-3.0)	(3.2-)4.3-5.4-6.1(-6.8)	(2.4-)2.9-4.4-5.0(-5.9)	25.65-28.5×5.7-11.4	20-40 × 7-15	Li *et al*., 2014; Luangharn *et al*., 2021; Umar *et al*., 2021
G. leucocontextum	10.4-12.2 × 6.4-10.2	ellipsoidal	trimitic	2.3-5.2	2.6-5.5	1.8-4.2	24-30×3.85-5.7	30-58 × 9-11	Li *et al*., 2014; Luangharn al., 2021; Umar *et al*., 2021
G. multipileum	(8-)8.8-10.4(-11.3) × (5-) 5.6-6.9(-7.2)	ellipsoidal or ovoid	trimitic	3.3-4.5	5.7	3.2-4.3	8.9-11.4 × 5.3-5.7	26-44 × 5.8-11.6	Cao *et al*., 2012; Li *et al*., 2014; Luangharn *et al*., 2021
G. multistipitum	10.2-11.2 × 5.3-5.4	ellipsoidal	trimitic	we were unable to locate any information about diameter of hyphal system and size of basidia of *Ganoderma multistipitum*				25.7-32.9 × 7.59-6.9	Ahmed *et al*., 2023
G. multiplicatum	8-9.9 × 6.2-6.8	ellipsoid	dimitic	2-3.8	2.2-3.8	____	not seen	38.5-62 × 5.6-10	Ryvarden, 2000; Torres-Torres *et al*., 2013;
G. multicornum	6.8 × 3.4	obviate	dimitic	2-3	5-10	____	16-24 × 12-15	20-50 × 6-18	Mukhtar, 2019
G. pakistanicum	8.2-8.9 × 5.0-5.8	broadly ellipsoid to ovoid	we were unable to locate any information about hyphal system, size of basidia and cutis elements of *Ganoderma pakistanicum*						Umar *et al*., 2022
G. resinaceum	9.3-13.5 × 5.2-8.0	broadly Ellipsoidal	trimitic	2.3-4.8	2.85-5.7	2.4-5.2	28.5-45.6 ×8.55-11.4	88-103 × 6-12	Gottlieb, and Wright, 1999; Nagadesi, 2018; Luangharn *et al*., 2021
G. tsugae	10-14 × 7-9	sub glubose to Ovoid	di-trimitic	2.85-4.2	2.85-5.9	2.85-5.7	19-45 × 7.1–8.5	19-34 × 7-11	Current study, Luangharn *et al*., 2021; Umar *et al*., 2021; Li *et al*., 2014

Conclusion

Based on morphological and anatomical data of our research, it was confirmed that *Ganoderma tsugae* was a new record in the myco-flora of Pakistan. This species was described in this article by manual illustrations in conjunction with high resolution macro and micro-morphological colored photographs. Furthermore, two exclusive comparison tables featuring all the species reported from Pakistan was an integral component of this scientific contribution. To further validate the accuracy of morpho-anatomical identification, it is recommended for the future that molecular techniques such as DNA barcoding (e.g., ITS nrDNA sequencing) should be done for the confirmation of phylogenetic placement of *Ganoderma tsugae* within the *Ganoderma* genus.

Conflict of interest

All the authors declare that there is no conflict of interest.

Author's Contribution

All authors have equal contribution to the work reported here.

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