Cultural diagnosis of *Ganoderma lucidum* complex from southern India

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Abstract. *Ganoderma lucidum* and allied species are widespread and cause white rot diseases on economically important crops, hardwoods and forest trees. An attempt has been made to distinguish *G. lucidum* complex by cultural characteristics. This study showed that the *G. lucidum* complex in a native collection is represented by *G. lucidum, G. resinaceum, G. tropicum, G. weberianum,* and *Ganoderma* sp. Most of the collections were confined to hardwood and rarely found on palm host. All the five species produced chlamydospores with varying shape and size. *Ganoderma resinaceum* and *G. lucidum* had an optimum growth rate at 30-35 °C; the former produced larger chlamydospores in culture than the later. *Ganoderma tropicum* produced cylindrical chlamydospores and had average growth rate at 20-25 °C. *Ganoderma weberianum* produced both chlamydospores and gastrospores in cultures with optimum growth rate at 30-35 °C. *Ganoderma* sp. produced both amyloid and inamyloid chlamydospores in culture and had optimum growth temperature of 20-25 °C. All the eight isolates showed positive reaction to acid aniline test.

Key words: aniline acid test, chlamydospores, extracellular oxidase, *Ganoderma lucidum* complex, southern India

Introduction

Karsten (1881) established the genus *Ganoderma,* with *G. lucidum* (Curtis : Fr.) P. Karst. as a type species. Several chemical strains for cultural diagnosis have been highlighted by Davidson *et al*. (1938), Nobles (1948), and Stalpers (1978). The species unable to degrade lignin were not considered to be *Ganoderma* (Davidson *et al.* 1938; Nobles 1948). Nobles (1948) developed a key with 11 characters referring to the cultural characters for the 126 wood inhabiting basidiomycetes. Nobles (1953) studied the differences in cultural characteristics of *G. lucidum, G. tsugae,* and *G. oregonense.* Davidson *et al*. (1942) and Sen (1973) supported cultural information to discriminate *G. lucidum* complex. Stalpers (1978) presented a descriptive analytical key with 96 characters for the identification of wood inhabiting *Aphyllophorales*. Bazzalo & Wright (1982) showed that the *G. lucidum* complex is represented in Argentina by *G. lucidum* s. str., *G. oerstedii, G. resinaceum, G. zonatum,* and *G. subamboinense* var. *laevisporum* based on macro-morphological and cultural studies. Temperature required for growth, chlamydospore production, and shape was considered as exceptionally valuable culture character to differentiate morphologically similar species and/or species complexes (Adaskaveg & Gilbertson 1986, 1989; Hseu 1990; Wang & Hua 1991; Moncalvo *et al.* 1995b; Hong & Jung 2004). Adaskaveg & Gilbertson (1988) suggested that cultural characteristics are important to distinguish species within *G. lucidum* complex. Adaskaveg & Gilbertson (1989) discriminated four North America species, viz. *G. lucidum, G.*

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colossum, G. zonatum, and *G. meredithiae* based on cultural characteristics. The cultural characters are less polymorphic than the macro-morphological characters but, however not suitable for the identification of biological species (Moncalvo *et al.* 1995b; Kaliyaperumal 2006). In the woods infested with *Ganoderma* species, chlamydospore production appears to be important for the long time survival than mycelia per se in soil (Chang 2003). In India, 15 *Ganoderma* spp. have been reported so far, and among them 12 falls into the subgen. *Ganoderma* (Sankaran *et al.* 2005). Identifications have largely been made from traditional morphological characters and cultural characters. The present investigation is to discriminate the *G. lucidum* complex from Tamil Nadu, southern India, by study of cultural characters which would be of easiest and an alternative method for the identification other than classical taxonomy and expensive molecular methods.

Material and Methods

Isolations, description of cultures and temperature studies for growth

Ten isolates were used in this study. Table 1 shows the isolates, host, and geographical origin. Cultures were raised from context tissue of the fresh basidiomata and grown on 2 % malt extract agar (MEA) medium at 25 °C. The actively growing mycelia from pure cultures were used as an inoculum for all the experiments. The presences or an absence of extracellular oxidase was determined by Aniline Agar Medium (Alberto & Wright 1997). The oxidation reactions were rated based on the brown halo color developed around the inoculum and they were measured (mm) and coded as follows: negative (0); very weak (upto 5 mm, +); weak (5-10 mm, ++); strong (10-20 mm, +++), and very strong (over 20 mm, ++++). Duplicates were maintained. Optimum growth temperatures for each isolate were determined. The inoculated plates were incubated at 6 temperatures ranging from 15-40 °C at 5 °C intervals along with duplicates.

Chlamydospore characters

The chlamydospore characters (shape, size, and reaction to Melzer's reagent) were observed for the discrimination of *G. lucidum* complex. Actively growing mycelia incubated for 2 weeks were stained with Melzer's reagent and observed under a stereomicroscope (20×). The occurrence, abundance, shape, size, and reaction to Melzer's reagent were recorded. The cultures were identified using cultural atlas of *Ganoderma* (Wang & Hua 1991).

Results and Discussion

All the ten isolates of *G. lucidum* complex produced extracellular oxidase with varying intensity. In all cases no growth of mycelium was observed throughout the incubation period. The intensity of brown halos greatly varied with among the 10 isolates (Table 2). The differences in magnitude and intensity of the halos among the isolates of the duplicates were always identical. Five strains of *G. lucidum* (G01, I5, ANN1, MYC4, and GKMV) and *G. tropicum* (MYC6) produced very strong reactions (over 20 mm) followed by *Ganoderma* sp. CN2 (10-20 mm), whereas *G. resinaceum* (PTK3 & K42) and *G. weberianum* (19) produced weak reactions (5-10 mm) in aniline agar medium (Table 2). This test will be useful in delimiting the genus *Ganoderma* from other white rot fungi. Albertó & Wright (1997) reported that aniline acid test as a powerful tool in the separation of white rot fungi. *Ganoderma lucidum, G. resinaceum,* and *G. weberianum* are mesophilic with optimum growth rate at 30-35 °C whereas temperature required for the average growth of *G. tropicum* and *Ganoderma* sp. CN2 were 25-30 °C.

Chlamydospore characters

The characters useful in distinguishing between the *Ganoderma* cultures are chlamydospore production (Adaskaveg &

Table 1. Isolates of *Ganoderma lucidum* complex and their host and geographical origin

Species	Source and collection number	Geographical origin	Host
G. lucidum	I5	Chennai	hardwood
G. lucidum	ANN1	Chennai	hardwood
G. lucidum	GKMV	Chennai	*Cassia roxburghii*
G. lucidum	MYC4	Chennai	*Delonix regia*
G. lucidum	G01	Chennai	hardwood
G. resinaceum	PTK3	Chennai	*Tamarindus indica*
G. resinaceum	K42	Chennai	*Cassia siamea*
G. tropicum	MYC6	Coutrallum	*Albizzia lebbeck*
G. weberianum	19	Vandalur	*Polyalthia* sp.
Ganoderma sp.	CN2	Coonoor	hardwood

Table 2. Cultural characteristics and optimum growth temperature of *G. lucidum* complex from southern India

Species	Acid aniline test	Temperature optima (°C)	Chlamydospores characters		
			Reaction to Melzer's reagent	Shape	Size (µm)
G. lucidum	++++	30-35	amyloid	globose, subglobose or elliptical	(4.5–) 6-15 (–18) × (2–) 2.5-4 (–4.5)
G. resinaceum	++	30-35	dextrinoid	ellipsoid or subglobose	(4–) 5-14.5 (–16) × (2.5–) 3-3.5 (–4)
G. tropicum	++++	25-30	inamyloid	cylindrial	(12–) 13.5-15 (–16) × (4–) 5-6 (–6.5)
G. weberianum	++	30-35	amyloid	subglobose or elliptical, non-striated	(3.5–) 4-6 (–7) × (3.5–) 4-6 (–7)
				spherical or elliptical with echinulate*	(4–) 4.5-6.5 (–10) × (2–) 2.5-3 (–3.5)*
Ganoderma sp.	+++	25-30	amyloid and inamyloid	ellipsoid	(3.5–) 5.5-12 (–12.5) × (1.5–) 2-2.5 (–3)

*Shape and size of gastrospores in *G. weberianum;* absent in other species

Gilbertson 1988, 1989; Wang & Hua 1991; Moncalvo *et al.* 1995b; Hong & Jung 2004).

The chlamydospores and/or gastrospores produced by *G. lucidum* complex were listed in Table 2. Abundant chlamydospores were observed in cultures of *G. resinaceum* (PTK3 and K42). They were ellipsoidal or subglobose, hyaline, smooth; the contents gave a dextrinoid reaction in Melzer's reagent; the size ranged from (4–) 5-14.5 (–16) × (2.5–) 3-3.5 (–4) µm; and intercalary and/or rarely terminal in position. Code no.: 2, 3, 8, 10, 34, 36, 37, 39, 40, 42, 53. Stalpers (1978), Bazzalo & Wright (1982), Adaskaveg & Gilbertson (1986), and Wang & Hua (1991) investigations of cultural studies agreed with the description of southern Indian *G. resinaceum.* In *G. weberianum,* chlamydospores were abundant and (3.5–) 4-6 (–7) × (3.5–) 4-6 (–7) µm in size; subglobose or elliptical, non-striated in shape; amyloid; intercalary or terminal in position. Code no.: 2, 3, 8, 10, 34, 36, 37, 38, 42, 53, 54. Two types of chlamydospores, viz. ellipsoid-striated and ellipsoid-non-striated were reported in *G. weberianum* (Wang & Hua 1991). The latter type was found in southern Indian and also reported in a Taiwanian strains by Wang & Hua (1991). In addition, spherical or elliptical with echinulate and fissured gastrospores, with the size ranging from (4–) 4.5-6.5 (–10) × (2–) 2.5-3 (–3.5) were also observed in southern Indian *G. weberianum.* The presence of gastrospores alone was reported in Australian (Smith & Sivasithamparam 2003) and Indonesian collections (Steyaert 1972). Furtado (1965) reported abundant gastrospores in *G. rivulosum* Pat. & Har., a synonym of *G. weberianum* (Steyaert 1972; Corner 1983). The gastrospores play a significant role in the propagation of the fungus; they may vary to a large extent in size and shape (Steyaert 1972). The absence of the character must be considered carefully, since some specimens yield abundant gastrospores while some sections yielded few or none (Steyaert 1972). Smith & Sivasithamparam (2003) revealed that these spores do not occur in all specimens and even routine sampling may not be helpful to detect the gastrospores in the samples. Hseu *et al.* (1989) considered gastrospores as the only character to delimit the *G. weberianum* and *G. microsporum.* Therefore, careful examination is required before naming the specimen thereby avoiding synonyms or misnaming. *Ganoderma* sp. CN2 produced both inamyloid and amyloid chlamydospores in cultures. They were (3.5–) 5.5-12 (–12.5) × (1.5–) 2-2.5 (–3) µm in size; ellipsoid in shape; intercalary or terminal in position. Code no.: 2, 3, 8, 10, 34, 36, 37, 39, 40, 43, 53, 54. Chlamydospores were larger than those of *G. weberianum* and smaller than *G. resinaceum* and the strain lacked gastrospores in culture. The temperature requirement for growth and unique chlamydospore characters clearly indicated that the isolate CN2 is a distinct species which could be allied taxa of *G. resinaceum* rather than *G. weberianum.*

Ganoderma tropicum alone produced distinctive abundant narrow, cylindrical, inamyloid, and thin walled chlamydospores. The size ranged from (12–) 13.5- 15 (–16) × (4–) 5-6 (–6.5) µm. Code no.: 2, 3, 8, 10, 34, 36, 37, 39, 40, 43, 54 (55). Moncalvo *et al.* (1995a) reported similar findings in Asian cultures whereas chlamydospores were reported to be absent in Indonesian (Steyaert 1972) and Argentinean (Gottileib & Wright 1999) collections. *Ganoderma lucidum* produced dexrinoid chlamydospores in culture. They were globose, subglobose or elliptical; terminal or intercalary; (4.5–) 6-15 (–18) × (2–) 2.5-4 (–4.5) µm in size. Code no.: 2, 3, 8, 10, 34, 36, 37, 38, 42, 53, 54. Nobles (1965) had corrected the name of *G. lucidum* and *G. sessile* for the cultures with chlamydopores. Chlamydospores were reported to be absent in Argentinean and European *G. lucidum* (Bazzalo & Wright 1982; Wang & Hua 1991; Ryvarden 2000). However, Asian *G. lucidum* produced abundant chlamydospores in cultures (Moncalvo *et al.* 1995b).

Chlamydospores of *Ganoderma* in woody debris enhance the resistance of the fungi to environmental stresses such as

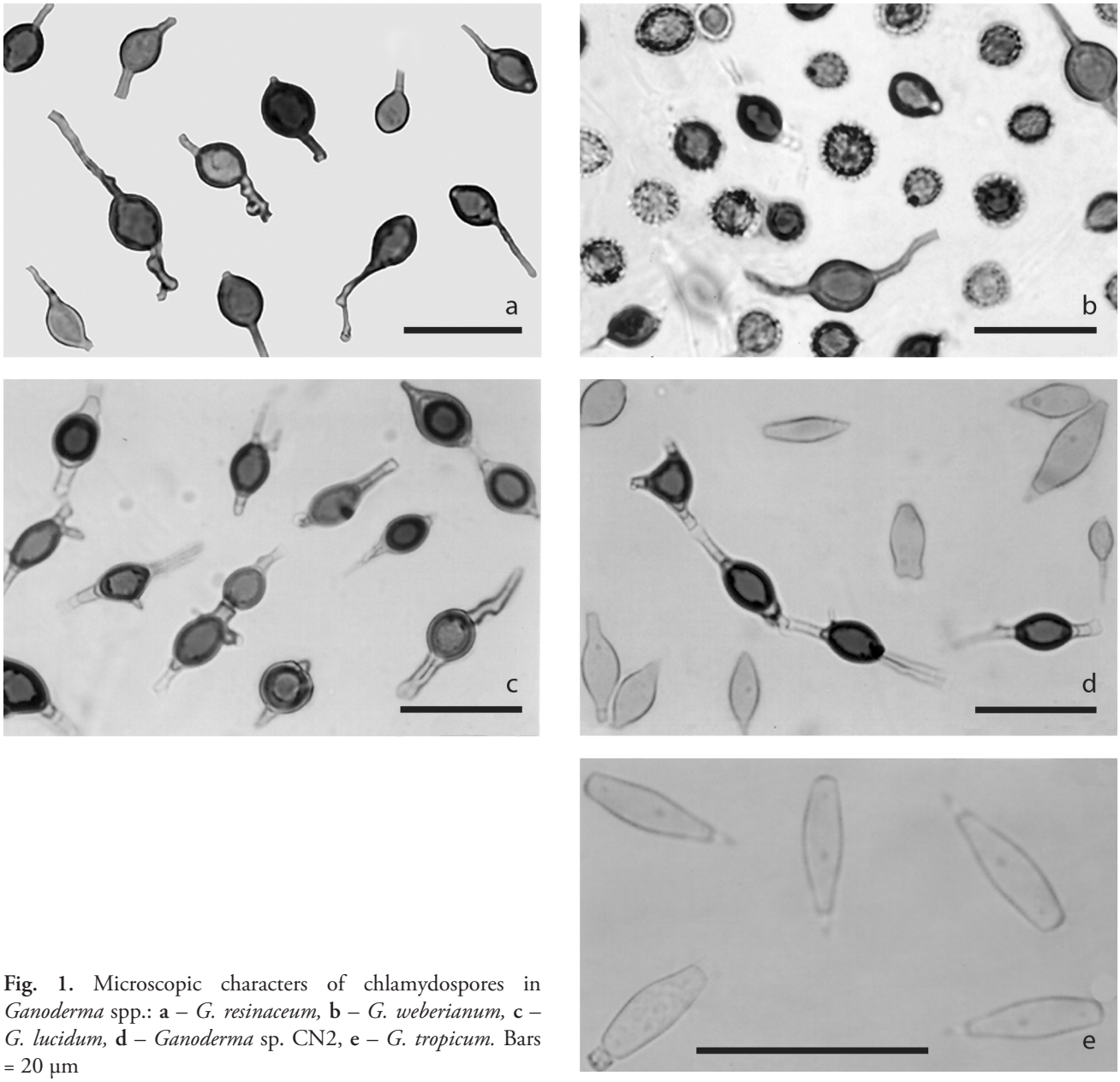

Fig. 1. Microscopic characters of chlamydospores in *Ganoderma* spp.: **a** – *G. resinaceum,* **b** – *G. weberianum,* **c** – *G. lucidum,* **d** – *Ganoderma* sp. CN2, **e** – *G. tropicum.* Bars = 20 µm

flooding. Flooding infested fields may help control those wood inhabiting fungi such as *G. australe* and *G. boninense* that do not produce chlamydospores (Chang 2003). The presence of chlamydospore in cultures plays a significant role in the identification of *Ganoderma* spp.

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