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Research Note

Molecular Taxonomy of *Ganoderma cupreum* from Southern India Inferred from ITS rDNA Sequences Analysis

Malarvizhi Kaliyaperumal*

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

Abstract *Ganoderma* is a cosmopolitan wood-rot basidiomycete that has been extensively studied for its pathogencity and medicinal properties. Identification of *Ganoderma* based on macro-microscopic features led to large number of synonyms which resulted in 250 taxonomic names. A *Ganoderma* species collected from Courtallam, Tamil Nadu was identified as *G. cupreum*. Phylogenetic analysis inferred from internal transcribed spacer rDNA region resolved the Indian isolate MYC1 as *Ganoderma cupreum* which clustered with Australian and Asian "*cupreum*" clade with 85% bootstrap support BS and shared 99% and 98% nucleotide similarity with Malaysian and Australian '*cupreum*' respectively. This study represents the first molecular evidence of *G. cupreum* from Asian origin.

Keywords Ganodermataceae, Mycogeography, Polyporales, Taxonomy

The history of *Ganoderma* Karst. in India began in early 1900s. The species of *Ganoderma* play a vital role in causing a decline in productivity and death of several plant species including cash crops such as coconut, betel nut and tea, plantation trees such as *Acacia* and *Albizzia*, and trees in the natural forests including *Mesua ferrea*, *Dalbergia* sp., and *Grewia tiliifolia* [1]. Over 250 *Ganoderma* species have been described worldwide, most of them based on variable and overlapping characters. As a consequence, there are many synonyms and several species complexes have been recognized [2]. As per the current concept of *Ganoderma*, *G. lucidum* (Curtis : Fr.) P. Karst and *G. applanatum* (Pers.) Pat are probably the most poorly understood ones with frequent misnomers attributed to them [3]. There are many similar instances of controversies in the literature, and this clearly illustrates the prevailing confusion over species identities of *Ganoderma*. The identity of most of the species of *Ganoderma* recorded from India has been determined from morphological and cultural criteria alone, and particularly as *G. lucidum* and *G. applanatum* the names most commonly come across in India [4]. Most of the information available to date for the Indian isolates comes from the northern part of the country and the limited information available for the south is largely restricted to a few cash crops such as coconut. *Ganoderma cupreum* (Sacc.) Bres. a laccate species (subgen. *Ganoderma*) was earlier known as *G. chalceum* (Cooke) Stey. and was documented as a saprophytic species on an Asian oil palm [5]. The type specimens, *G. chalceum* and *G. cupreum* originated from the western Africa in Sierra Leone and Guinea, respectively. *Ganoderma cupreum* was mistakenly proposed as a synonym of *G. chalceum* [5] although the former has priority [2]. However, the binomial *G. chalceum* was still in use [6, 7]. *Ganoderma chalceum* has been considered to be a saprophytic species on *Elaeis guineensis* from Africa that might have migrated either naturally or through human activities into the Southeast Asia [5, 8]. The mycogeography of *G. cupreum* remains unclear still to date and only few investigations have been made with reference to *G. cupreum* taxonomy. The first evidence for the occurrence of *G. cupreum* was from Southeastern Australia based on molecular (internal transcribed spacer [ITS] rDNA) and biochemical markers (pectin methylesterase and polygalacturonase) [9, 10]. Besides the taxonomic aspect, antibacterial and pathogenecity of *G. cupreum* were also investigated [11, 12]. Literature survey indicated that only molecular evidence of Australian *G. cupreum* was

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***Corresponding author**
E-mail: malar.kaliyaperumal@gmail.com

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available whereas ITS rDNA sequences of *G. cupreum* from Malaysia and Cameroon were unpublished (but available in GenBank). In the present investigation, an unidentified *Ganoderma* isolate was identified as *G. cupreum* from Indian origin using ITS rDNA marker.

The isolate from native collection (Southern India, Tamil Nadu, India) examined in this study and sequences retrieved from NCBI for phylogenetic analysis are listed in Table 1. Basidiome corky, applanate-umbo, dark brown with pale yellow margin, 12~17 × 4~6 cm. Pileus highly laccate with concentric undulation with margin thin. Pore surface pale yellow to brown, 3~5.5 per mm. Context brown up to 13 mm thick, trimitic hypahe. Cutis claviform 19~55.8 × 3~14 μm. Basiospores brown, truncate, ovate to broadly ellipsoid, 6.8~11.4 × 5.0~10.2 μm, Chlamydospores were absent in context tissues. The strain MYC1 was isolated from unknown dead dicot stump, Courtallum, Tamil Nadu, India. *Ganoderma cupreum* is paleotropic in distribution from countries in Africa and Asia (India and Malysia), and Australia. The specimen was deposited at Botany Laboratory, University of Madras (voucher no. MUBL 375).

DNA isolation, PCR amplification, and PCR amplification and sequencing were described elsewhere [13]. The ITS rDNA regions were amplified with ITS1 (5'-TCCGTAGG-TGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTG-ATATGC-3') pair [14]. The PCR products were purified with QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced by the BigDye Terminator v.3.0 kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction; resolved on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All sequences were read bidirectionally. The sequences produced in this study have been deposited in the GenBank database. ITS accession numbers are listed in Table 1.

DNA sequences obtained from both strands were edited and contigs assembled using Sequencher ver. 4.2.2. (Gene Codes Corp., Ann Arbor, MI, USA). Nucleotide sequences were manually aligned in nexus file of PAUP* 4.0b 10 [15] configured for Windows. Gaps were introduced into sequences to increase their alignment similarity. Maximum parsimony analysis (MP) was performed using following settings: a branch swapping algorithm, tree-bisection-reconnection (TBR), addition of sequences set to random with 1,000 replicates, accelerated transformation (ACCTRAN), MULPARS=on, steepest descent not in effect, MAXTREES was set to 100, multistate taxa were interpreted as uncertainty, topological constraints were not enforced and gaps treated as missing data. Relative robustness of the clades were estimated by bootstrap analysis using 1,000 replicates were performed under the following parameters: TBR branch swapping with random addition sequences and estimated levels of homoplasy, retention indices and consistency indices were also determined in PAUP. The resulting phylogenetic trees were visualized with the TreeView 1.6.6 program [16]. *Amauroderma rude* JM/ASP.1 and *Ganoderma sinense* was used to root the phylogenetic tree.

Molecular genetic data provide powerful tools for assessing the effect of past and current events on the geographic distribution of species. *Ganoderma cupreum,* a laccate polypore has been reported earlier as *G. chalceum* by the classical methods [5, 8]. This study represents first molecular existence of *G. cupreum* from Southern Indian origin. The amplified product was ca. 650 bp. The native collection (MYC1) along with eight isolates of *G. cupreum* and the outgroup sequences *Amauroderma rude* and *G. sinense* were aligned in 612 positions in the ITS1-5.8S-ITS2 rDNA region with inclusion of conserved nucleotide signatures at both the 18S 3'-end (CATTA) and the 25S 5'-end

Table 1. List of isolates used in the present study

Species	Collection No.	Geographical origin	Host	GenBank accession No.
Amauroderma rude	JM/ASP.1[a]	Taiwan	NA	X78753/X78744
Ganoderma cupreum	DFP 3896[b]	Queensland, Australia	*Casuarina* sp.	AJ627586 /AJ627587
	DFP 4336[b]	New South Wales, Australia	Soft wood	AJ627588/AJ627589
	QFRI 8678.1[c]	Queensland, Australia	Dead wood	AY332532
	SUT H1[d]	Queensland, Australia	NA	AY569450
	MYC1[e]	Tamil Nadu, Southern India	Hard wood	DQ015906/DQ015907
	GanoTK4[f]	Cameroon	NA	JN105701
	GanoTK74[f]	Cameroon	NA	JN105702
	G133[g]	Malaysia	NA	JN596328
	K24[g]	Malaysia	NA	JN596329
G. sinense	ZHANG1734[h]	China	NA	Z37066/Z37103

[a]JM, Collection of J.M. Moncalvo, University of Toronto, Canada.

[b]DFP, Commonwealth Scientific and Industrial Research Organization, Division of Forest Products, Melbourne, Australia.

[c]QFRI, Queensland Forest Research Institute, Department of Primary Industries Forestry, Indooroopilly, Queensland.

[d]SUT H1, Swinburne University, Hawthorn.

[e]MYC1, Collection of M. Kaliyaperumal, CAS in Botany, University of Madras.

[f]Department of Plant and Animal Science, University of Buea, Cameroon.

[g]Department of Plant Science and Environmental Ecology, University of Malaysia, Malaysia.

[h]ZHANG, X.Q. Zhang, Beijing, China.

(GACCT) to anchor the alignment. The data matrix with 11 sequences along with two outgroups was included in MP analysis. The length of the ITS1 and ITS2 region ranged from 201~202 and 195~196, respectively. A total of 429 characters were included in analysis, of which 377 were constant, 31 variable characters were parsimony uninformative and 21 were parsimony informative. MP resulted in a single parsimony tree with tree length, 56; consistency index, 0.9821; homoplasy index, 0.0179; and retention index, 0.9688. A heuristic search with parsimony criterion resulted in well resolved *G. cupreum* clade (85% Bootstrap support). They strongly corresponded to the geographic origin of the isolates composed of collection from Australia, Southeast Asia (Malaysia), India and Cameroon. The Indian isolate MYC1 clustered with Malay-Australian '*cupreum*' with high BS (97%) (Fig. 1). The

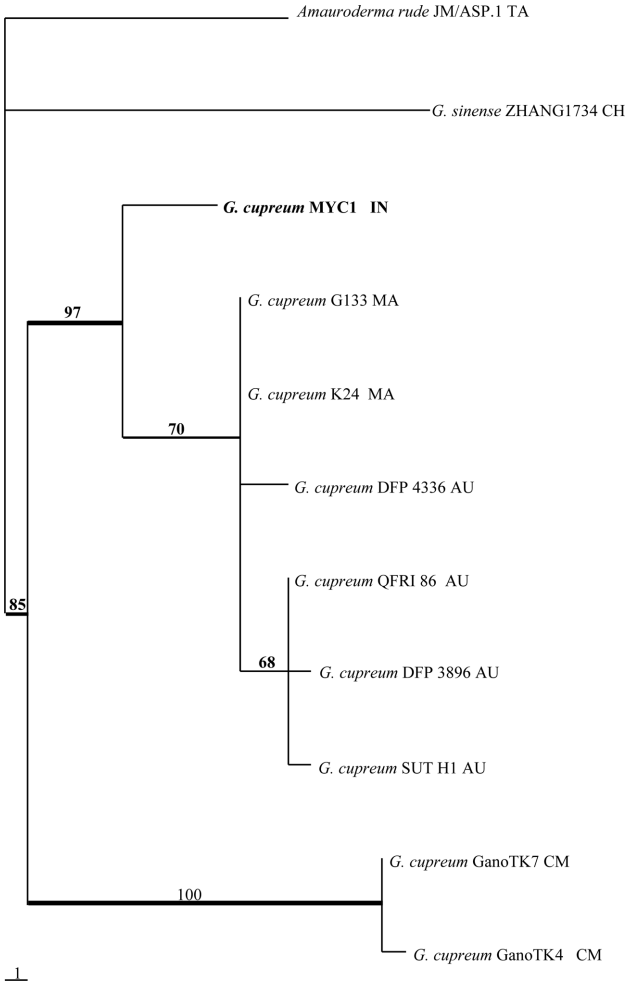

Fig. 1. Maximum parsimony tree derived from internal transcribed spacer 1/2 sequence data. Isolate sequenced in this study are shown in bold. The bold abbreviated codes after the isolates name indicate the geographical locations; AU, Australia; CH, China; CM, Cameroon; IN, India; MA, Malaysia; TA, Taiwan. Bootstrap values of ≥ 70% or more, based on 1,000 replicates, are indicated above the branches. The tree was rooted with *Amauroderma rude*.

nucleotide variation among the '*cupreum*' was 0.25% to 4.75%. Indian isolates exhibited 0.75 to 1% with Australian '*cupreum*'; 0.5% with Malaysian isolates and 3.75% to 4% with Cameroon isolates. The two Cameroon isolates TK7 and TK4 formed sister clade to Asia-Australian *G. cupreum* with 85% BS. The isolate MYC1 was named as *Ganoderma cupreum* to avoid more confusion to the existing *Ganoderma* taxonomy, moreover *G. cupreum* was given nomenclatural priority over *G. chalceum* [2]. The morphotaxonomic characters of the present collection are similar to *G. chalceum* reported from Maharashtra, India based on classical taxonomy [7]; however the specimen was not accessible for the present molecular studies. Earlier, *Polyporus chalceus* Cooke, *P. cupreum* Fr. and *G. polymorphum* Cleland were other possible synonyms of *G. chalceum* [17]. *G. polymorphum* (AD 1357 and AD 1361) was considered to be a synonym for *G. resinaceum* [18] and was reconsidered as *G. cupreum* [17]. The former particular specimen alone was collected from an arid environment which might be thriving vegetatively as a saprophyte on timber with the basidiomata being produced at extremely wet conditions.

The phylogenetic classification inferred from the sequence data was considered superior to the use of sequence variation statistics for identifying taxa, although the latter was of some use in supporting taxonomic decisions. The use of sequence variation alone as a basis for species delineation, especially between allopatric populations, might be more useful if supported by independent measures of genetic diversity. The three Australian strains viz., DFP 4336, DFP 3896, and QFRI 8678.1 were previously identified as *G. cupreum* however published as *G. chalceum* [5]; the later name was applied by few authors [6, 7] which has created more confusion. Apart from its taxonomic disputes, antibacterial terpenoids have been isolated from *G. cupreum* [11]. *Ganoderma cupreum* was reported to be less active in causing decay in heartwood of *Eucalyptus oblique* and *E. sieberi* [12].

The host relationship could not be ascertained in this study since only few collections were available from tropical Asia, Africa and Australia. Moreover, the climatic factors may play a key role in the distribution of this fungus and these isolates were collected from the angiosperm hosts. In order to study the host specificity and distribution of samples, more sampling and additional molecular data is very much required from oil palm infested *G. cupreum*. Examination of more samples from the other Asian or African countries and additional coding loci would provide interesting insights into the historical and contemporary forces that shape the mycogeographical distributions of *G. cupreum*.

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