Delimitation of some neotropical laccate *Ganoderma* (Ganodermataceae): molecular phylogeny and morphology

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Abstract: *Ganoderma* includes species of great economic and ecological importance, but taxonomists judge the current nomenclatural situation as chaotic and poorly studied in the neotropics. From this perspective, phylogenetic analyses inferred from ribosomal DNA sequences have aided the clarification of the genus status. In this study, 14 specimens of *Ganoderma* and two of *Tomophagus* collected in Brazil were used for DNA extraction, amplification and sequencing of the ITS and LSU regions (rDNA). The phylogenetic delimitation of six neotropical taxa (*G. chalceum*, *G. multiplicatum*, *G. orbiforme*, *G. parvulum*, *G.* aff. *oerstedtii* and *Tomophagus colossus*) was determined based on these Brazilian specimens and found to be distinct from the laccate *Ganoderma* from Asia, Europe, North America and from some specimens from Argentina. Phylogenetic reconstructions confirmed that the laccate *Ganoderma* is distinct from *Tomophagus*, although they belong to the same group. The use of taxonomic synonyms *Ganoderma subamboinense* for *G. multiplicatum*, *G. boninense* for *G. orbiforme* and *G. chalceum* for *G. cupreum* was not confirmed. However, *Ganoderma parvulum* was confirmed as the correct name for specimens called *G. stipitatum*. Furthermore, the name *G. lucidum* should be used only for European species. The use of valid published names is proposed according to the specimen geographical distribution, their morphological characteristics and rDNA analysis. Rev. Biol. Trop. 62 (3): 1197-1208. Epub 2014 September 01.

Key words: Agaricomycetes, phylogenetic taxonomy, rDNA sequences, species delimitation, neotropics.

Ganoderma P. Karst. (Ganodermataceae, Agaricomycetes) is one of the largest genera of Polyporales and was described by Karsten (1881) based on *Polyporus lucidus* (Curtis) Fr. from Europe, a species with a laccate (shiny varnished looking) surface. The genus is characterized by double-walled basidiospores with truncate apex and ornamented endospore (Moncalvo & Ryvarden, 1997). The genus includes 80 species of wide geographic distribution with several tropical species and others restricted to temperate areas (Ryvarden, 2000; Kirk, Cannon, Minter, & Stalpers, 2008). Ryvarden (2004) reported the presence of 20 species in the neotropics, although Torres-Torres, Guzmán-Dávalos & Gugliotta (2012) and Gugliotta, Abrahão & Gibertoni (2013) listed 18 and 28 species, respectively, only in Brazil.

Being well known as decomposers and pathogens in tropical forests (Zakaria, Ali, Salleh, & Zakaria, 2009), species of this genus, mainly of the *G. lucidum* complex, produce bioactive compounds widely studied for preventing and relieving human diseases, such as several types of tumors and cancers, gastric ulcers, diabetes mellitus, hypertension and viral infections (Zhou et al., 2007). Besides, some medicinal effects were reported in manuscripts of the Chinese civilization more than 2 000 years ago (Hong & Jung, 2004; Seo & Kirk, 2000).

Although of significant ecological and biotechnological importance, the taxonomy

of laccate *Ganoderma* has been questioned in recent years and poorly studied in the neotropics. Currently, taxonomists consider the nomenclatural situation as chaotic and suggest the necessity for a global revision due to the existence of multiple names for single species (Ryvarden, 1991; Ryvarden, 2004; Moncalvo & Ryvarden, 1997; Postnova & Skolotneva, 2010).

Usually, different morphological characteristics of the basidiospores (dimensions) and basidiomata (thickness of the cuticle and presence or absence of resinaceous deposits in the context) are widely used in an attempt to identify laccate species of *Ganoderma* (Seo & Kirk, 2000; Ryvarden, 2004; Torres-Torres & Guzmán-Dávalos, 2012; Torres-Torres, Guzmán-Dávalos, & Gugliotta, 2012). However, the use of molecular data, especially for phylogenetic studies based on ribosomal DNA sequences, combined with morphological studies has helped many authors to clarify the status of the genus (Moncalvo, Wang, & Hseu, 1995a; Moncalvo, Wang, & Hseu, 1995b; Gottlieb, Ferrer, & Wright, 2000; Smith & Sivasithamparam, 2000; Hong & Jung, 2004; Kaliyaperumal & Kalaichelvan, 2008; Cao, Wu, & Dai, 2012; Yang & Feng, 2013).

In Brazil, there are only a few studies regarding *Ganoderma* (Torrend, 1920; Loguercio-Leite, Groposo, & Halmenschlager, 2005; Torres-Torres et al., 2012) and none of these include a phylogenetic analysis. Thus, the aim of the present study was to determine phylogenetic relationships of the laccate *Ganoderma* based on sequence variation of the Internal Transcribed Spacer (ITS) and Large subunit (LSU) rDNA and to delimit the species' occurrence within Brazilian territory.

MATERIAL AND METHODS

Morphological analysis: Thirty-one laccate specimens of *Ganoderma* and four of *Tomophagus* colossus (Fr.) Murrill were used in this study (Table 1). To observe the characteristics of the basidiospores, the hyphal system and the cuticle hyphae, free-hand thin sections of dried material were mounted in 5% KOH to ensure rehydration. Melzer's reagent was used to test the amyloid reaction of the cuticle hyphae. Our data were then compared to the available literature (Bresadola, 1911; Gottlieb & Wright, 1999; Núñez & Ryvarden, 2000; Ryvarden, 2000; Ryvarden, 2004; Welti & Courtecuisse, 2010; Tham et al., 2012, Torres-Torres et al., 2012) and discussed in the text. All studied specimens were deposited in the herbarium Pe. Camille Torrend (URM), Department of Mycology, Universidade Federal de Pernambuco, Brazil.

Genomic DNA extraction, polymerase chain reaction and sequencing: DNA was extracted using fragments of basidiomata (30-50mg) ground with a pestle in a porcelain mortar containing liquid nitrogen. The resulting powder was transferred to a tube containing 700µL of extraction buffer [CTAB 2%, 100mM Tris-HCl pH8, 1.4M NaCl, 20mM EDTA, 1% PVP (Rogers & Bendich, 1985)] and incubated at 65ºC for 30-40 min. DNA was purified with 700µL of chloroform-isoamyl alcohol (24:1), gently precipitated in 600µL of isopropanol and washed with 1mL of ethanol. Finally, the pellet was suspended in 50µL of ultrapure water (Góes-Neto, Loguercio-Leite, & Guerrero, 2005). The reaction mix and parameters for PCR amplification of the full ITS regions was according to Smith & Sivasithamparam (2000) using the primers ITS1 and ITS4 (White, Bruns, Lee, & Taylor, 1990). For LSU rDNA region, the amplification was performed with parameters and reagent concentrations following Góes-Neto, Loguercio-Leite & Guerrero (2005) using the primer pair LR0R and reverse LR7 (Moncalvo, Lutzoni, Rehner, Johnson, & Vilgalys, 2000). Negative controls containing all components of the reaction mix, except DNA, were used in each procedure to detect possible contamination. The amplification products of the sixteen laccate specimens (Table 1) were purified using the PureLink PCR Purification Kit (Invitrogen) and the purified products were sequenced at the Human Genome Research Center of the Universidade

TABLE 1
List of specimens collected from different areas in Brazil

Species	Geographic origin Locality (city, State)	Number of deposit in the Herbarium	GenBank accession number (ITS/LSU)
Ganoderma chalceum	Sítio Carro Quebrado (Triunfo, Pernambuco)	URM 80457	JX310812/JX310826
G. chalceum	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 81393	*
G. chalceum	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 82131	*
G. multiplicatum	Mineradora Millennium (Mataraca, Paraíba)	URM 83346	JX310823/ JX310837
G. multiplicatum	Parque Nacional Municipal de Porto Velho (Porto Velho, Rondônia)	URM 81081	*
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83332	JX310813/ JX310827
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83334	JX310814/ JX310828
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83335	JX310815/ JX310829
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83336	JX310816/ JX310830
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83337	*
G. orbiforme	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 83338	*
G. orbiforme (as *G. stipitatum*)	RPPN Carnijó, (Moreno, Pernambuco)	URM 81398	*
G. parvulum	Campus UFPE (Recife, Pernambuco)	URM 83339	JX310817/ JX310831
G. parvulum	Campus UFPE (Recife, Pernambuco)	URM 83340	JX310818/ JX310832
G. parvulum (as *G. resinaceum*)	Recife, Pernambuco	URM 2948#	JX310821/ JX310835
G. parvulum (as *G. stipitatum*)	Estação Experimental do IPA (Caruaru, Pernambuco)	URM 80765	JX310822/ JX310836
G. parvulum	Mataraca, Brazil	URM 83343	JQ618246/ JX310810
G. parvulum	Parque Ecológico Riacho do Meio (Barbalha, Ceará)	URM 83344	JX310819/ JX310833
G. parvulum	Missão Velha, Ceará	URM 83345	JX310820/ JX310834
G. parvulum (as *G. stipitatum*)	Estação Experimental do IPA (Caruaru, Pernambuco)	URM 80297	*
G. parvulum (as *G. stipitatum*)	Estação Experimental do IPA (Serra Talhada, Pernambuco)	URM 80332	*
G. parvulum (as *G. resinaceum*)	Fazenda Sr. Rudá (São José das Taperas, Alagoas)	URM 80416	*
G. stipitatum	RPPN Carnijó, (Moreno, Pernambuco)	URM 81426	*
G. stipitatum	Parque Estadual de Dois Irmãos (Recife, Pernambuco)	URM 81427	*
G. parvulum (as *G. stipitatum*)	Parque Ecológico Prof. João de Vasconcelos Sobrinho (Caruaru, Pernambuco)	URM 81394	*
G. parvulum (as *G. stipitatum*)	Parque Ecológico Prof. João de Vasconcelos Sobrinho (Caruaru, Pernambuco)	URM 81395	*
G. parvulum (as *G. stipitatum*)	Parque Nacional Municipal de Porto Velho (Porto Velho, Rondônia)	URM 81051	*
G. aff. *oerstedii* (as *G. resinaceum*)	Campus UFPE (Recife, Pernambuco)	URM 83400	JX310824/ JX310838
G. aff. *oerstedtii* (as *G. resinaceum*)	Estação Experimental do IPA (Serra Talhada, Pernambuco)	URM 80336	*
G. aff. *oerstedtii* (as *G. resinaceum*)	Estação Experimental do IPA (Caruaru, Pernambuco)	URM 80831	*
G. aff. *oerstedtii*(as *G. resinaceum*)	Estação Ecológica de Seridó (Seridó, Rio Grande do Norte)	URM 80848	*
Tomophagus colossus (as *G. colossus*)	Mineradora Millennium (Mataraca, Paraíba)	URM 83330	JQ618247/ JX310811
T. colossus	Parque Nacional do Catimbau (Buíque, Pernambuco)	URM 80450	JX310825/ JX310839
T. colossus (as *G. colossus*)	Mazagão, Amapá	URM 48437	*
T. colossus (as *G. colossus*)	Mazagão, Amapá	URM 48437	*

Culture collection URM of the Department of Mycology of the Universidade Federal de Pernambuco.
* Specimens without sequences available.

de São Paulo (USP, Brazil) in an ABI-310 Capillary Sequencer (PerkinElmer, Wellesley Massachusetts, USA). Cycle sequencing was carried out with primers ITS1 and ITS4 for ITS region and LR0R and LR5 for LSU region (Moncalvo et al., 2000). All sequences were deposited in GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA).

Phylogenetic analysis: Sixteen ITS and LSU rDNA sequences (14 of *Ganoderma* and two of *T. colossus*) of laccate Ganodermataceae were compared with other sequences retrieved

from GenBank (Table 2). These sequences were aligned using ClustalX (Larkin, 2007), manually edited in BioEdit (Hall, 1999) and realigned to obtain the final alignment. Phylogenetic analyses and tree construction were performed separately for each locus. Neighbor joining (NJ) distances, maximum parsimony (MP) and maximum likelihood (ML) analyses were carried out using PAUP* version 4.0b10 (Swofford, 2002) and the support was evaluated using 1 000 bootstrap replicates. The NJ and ML analysis were based on HKY+G (ITS) and TIM+I (LSU) obtained from ModelTest 3.7 (Posada & Crandall, 1998), which computed the most likely patterns of phylogenetic evolution. Sequences from *Amauroderma rude* var. *intermedium* J. S. Furtado were used as outgroup for phylogenetic reconstruction based on ITS sequences and two specimens of *T. colossus* were used as outgroup for the LSU analysis.

RESULTS

The ITS1-5.8S-ITS2 regions sequenced in this study varied in length from 548 to 571 nucleotides. The size of the ITS1 region did not differ markedly among the studied specimens, ranging from 199 to 205 nucleotides. This small variation was also observed for the ITS2 region and ranged between 192 and 201 nucleotides. The final alignment (ITS1 + ITS2) included 419 sites, with 266 constant sites (63%) and 153 variable (36%), of which 120 (28%) were parsimony informative.

The size of LSU sequences ranged from 1 320 to 1 322 nucleotides and aligned at 1 323 positions. Of these, 1 278 characters were constant, 21 characters were variable but parsimony uninformative, and 24 characters were parsimony informative. Although this study provided new sequences within the ITS and LSU regions, it is as yet impossible to perform multigene analysis due to the lack of other neotropical gene sequences from the species analyzed here.

The phylogenetic reconstruction performed with NJ, MP and ML analyses for ITS sequences showed basically the same topology and few differences in bootstrap values (Fig. 1). The same was observed for LSU analysis (Fig. 2). These results confirm that laccate *Ganoderma* is a monophyletic group although with low statistical support based on ITS analysis (NJ 70%; MP < 50% and ML 60%) and with high bootstrap values based on LSU analysis (NJ, MP and ML 100%). In the phylogenetic reconstruction based on ITS regions, seven clades were delimited (A, B, C, D, E, F and G). The Brazilian specimens of the six taxa studied (*G. chalceum*, *G. multiplicatum*, *G. orbiforme*, *G. parvulum*, *G.* aff. *oerstedtii* and *T. colossus*) were recovered in clades A, B, D and G, discussed below.

DISCUSSION

The clade A formed a monophyletic lineage distinct from the laccate *Ganoderma* with strong statistical support (NJ, MP and ML = 100%) and included two species, *T. colossus* and *T. cattienensis*. *Tomophagus colossus* from Brazil grouped with representatives of the Asian species (NJ 98%, MP 99% and ML 90%), confirming that they are of the same species, but distinct from *T. cattienensis*. The genus *Tomophagus* was established first by Murrill (1905) and in the following decades was contested, being considered a confusing group. Later, molecular phylogenetic studies confirmed the genus as group well established in Ganodermataceae (Moncalvo, Wang, Wang, & Hseu, 1995c; Hong & Jung, 2004; Tham et al., 2012).

However, recent papers still mention *Tomophagus* as belonging to *Ganoderma* (Welti & Courtecuisse, 2010; Cao et al., 2012). *Tomophagus* currently has two species, *T. colossus* (= *G. colossus*) and *T. cattienensis*, both sharing the laccate (shiny) pilear surface, pale context with slightly dextrinoid skeletal hyphae, large basidiospores, and the striking chlamydospores, providing a unique combination of characters (Ryvarden, 2000; Ryvarden, 2004; Tham et al., 2012). *Tomophagus colossus* differs morphologically from *T. cattienensis* by having yellowish pilear surface while *T. cattienensis* has red to light brown pilear surface.

TABLE 2
Origin and GenBank accessions of the strains used in this study

Species	Geographic Origin	Strain/specimen number	GenBank accession number
ITS sequences			
Amauroderma rude var. intermedium	Taiwan	JMM ASP.1	X78753&X78774
G. boninense	Taiwan	RSH RS	X78749&X78770
G. cupreum	Australia	DFP 3896	AJ627586& AJ627587
G. cupreum	Australia	DFP 4336	AJ627588& AJ627589
G. lingzhi (as *G. lucidum*)	Ibaraki, Japan	WD-565	EU021455
G. lingzhi (as *G. lucidum*)	Ibaraki, Japan	WD-2038	EU021456
G. lingzhi (as *G. lucidum*)	China	ACCC 5.65	Z87354&Z87364
G. lingzhi (as *G. lucidum*)	China	HMAS 60537	Z37050& Z37074
G. lucidum	France	CBS 270.81	Z37049& Z37099
G. lucidum	Argentina	BAFC 33621	AF170007/ AF170008
G. lucidum	USA	CBS 430.84	Z37051& Z37075
G. lucidum	Argentina	BAFC 33631	AF170009& AF170010
G. lucidum	Norway	RYV 33217	Z37096& Z37073
G. multipileum (as *G. lucidum*)	India	BCRC 36123= ATCC 32471	EU021459
G. multipileum (as *G. lucidum*)	Nantou, Taiwan	BCRC 37033	EU021462
G. multipileum (as *G. lucidum*)	Taitung, Taiwan	BCRC 37043	EU021460
G. multipileum (as *G. lucidum*)	Pingtung, Taiwan	CWB 01740	EU021461
G. multipileum (as *G. lucidum*)	Philippines	JMM P93-1	X78745& X78766
G. multipileum (as *G. lucidum*)	India	ATCC 32472	X87351& X87361
G. parvulum	Mataraca, Brazil	URM 83343	JQ618246
G. resinaceum	Netherlands	CBS 194.76	X78737& X78758
G. resinaceum	UK	CBS 152.27	Z37062 & Z37085
G. subamboniense var. laevisporum	Argentina	ATCC 52419	X78736& X78757
G. tuberculosum	Argentina	BAFC 33599	AF170011& AF170012
T. colossus	Philippines	CBS 216.36	Z37071& Z37091
T. colossus	India	kk-02	AJ749970
T. colossus	Vietnam	ANH s.n.(=TRTC 157076	JN184395
T. colossos	Vietnam	HCMC10 (TRTC 161190)	JN184396
T. cattienensis	Vietnam	CT99 (TRTC 161191)	JN184397
T. cattienensis	Vietnam	CT119	JN184398
LSU sequences			
G. boninense	Taiwan	RSH RS	X78777
G. lucidum	Taiwan	n.a	X78776
G. lucidum	Korea	IUM 00298	DQ208410
G. lucidum	Korea	IUM 01122	DQ208411
G. lucidum	Korea	C-1 (Wild)	DQ208412
G. lucidum	Korea	C-2 (Wild)	DQ208413

n.a= not available.

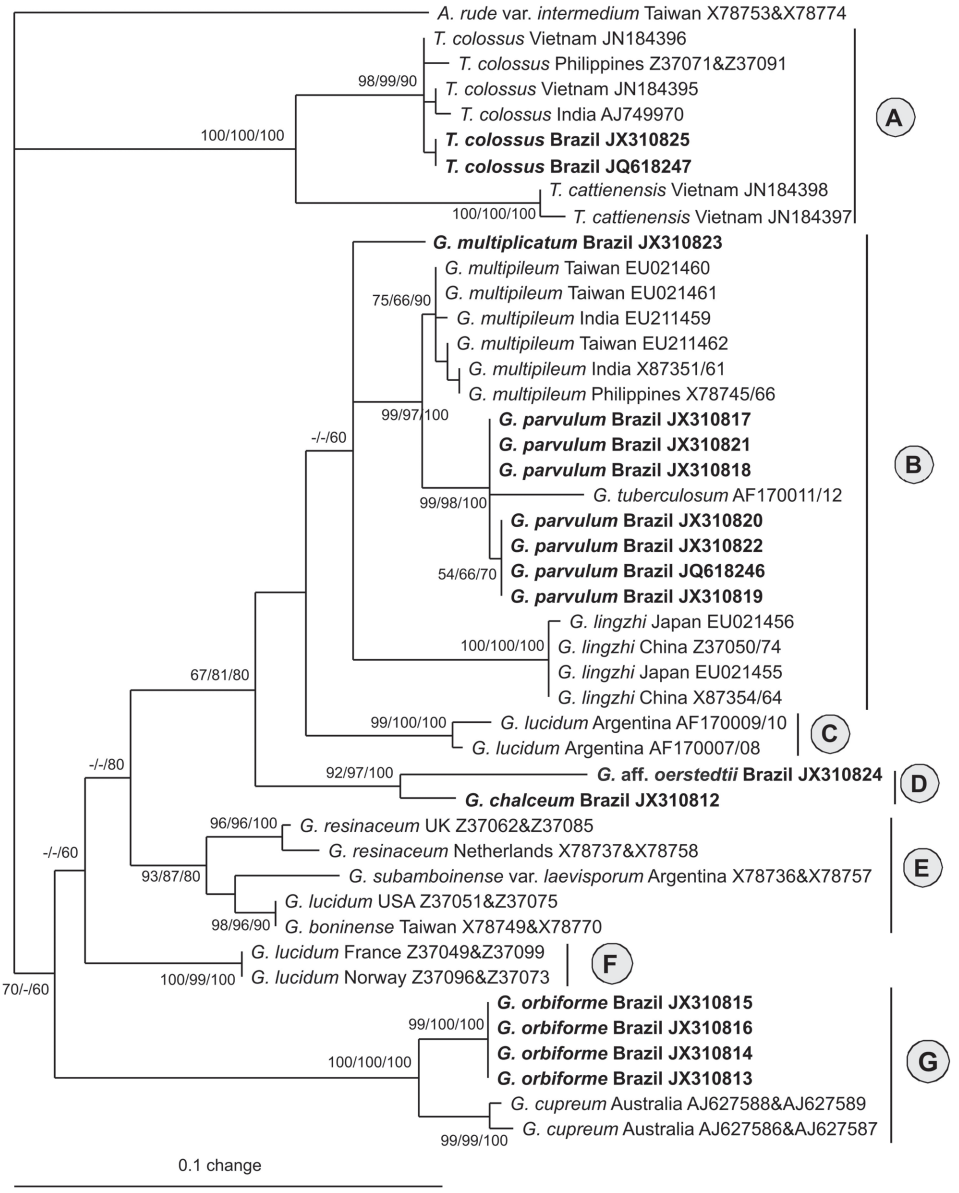

Fig. 1. Phylogenetic reconstruction of the laccate *Ganoderma* based on alignment of 419 nucleotides of the ITS region. Bootstrap values (%) were generated from neighbor joining method with distances of HKY+G, maximum parsimony and maximum likelihood (ML) analysis (1 000 bootstraps), respectively. Values above 50% are shown. One specimen of *Amauroderma rude* var. *intermedium* was used as outgroup. For maximum parsimony: Consistency Index (CI) = 0.6690 and Retention Index (RI) = 0.8898.

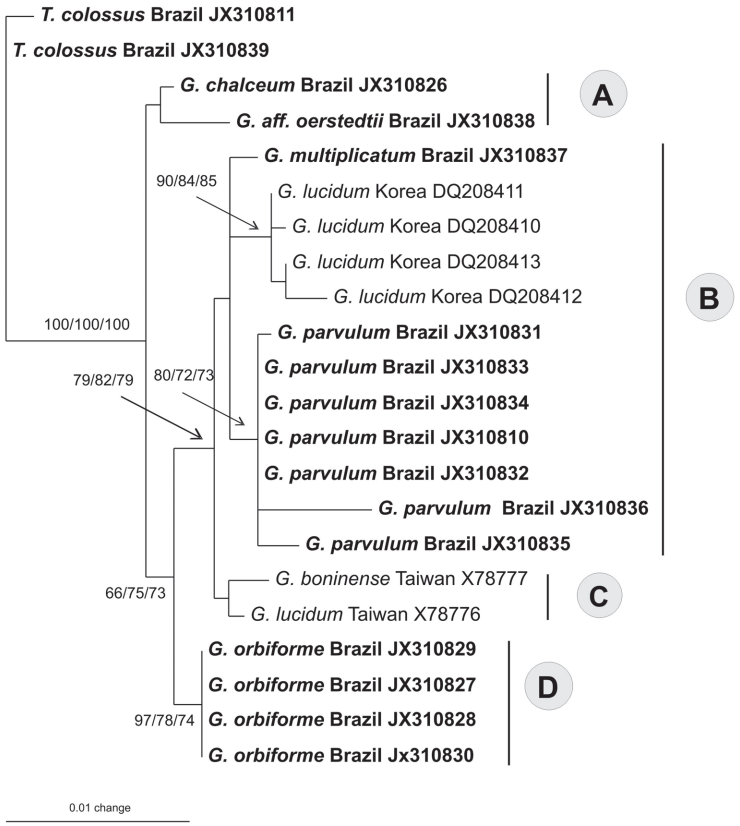

Fig. 2. Phylogenetic reconstruction of the laccate *Ganoderma* from Brazil based on alignment of the 1 323 nucleotides of the LSU region. Bootstrap values (%) were generated from neighbor joining method with distances of TIM+I, maximum parsimony and maximum likelihood analysis (1 000 bootstraps), respectively. Values above 50% are shown. Two specimen of *T. colossus* were used as outgroup. For maximum parsimony: Consistency Index (CI) = 0.7966 and Retention Index (RI) = 0.8519.

Furthermore, *T. cattienensis* has a context that turns pale brown upon drying, instead of remaining creamy white as *T. colossus*, and shows slightly larger basidiospores [17.5-21.5 x 11.5-14.5µm (Tham et al., 2012) versus 16-19 x 10.5-12.5µm in *T. colossus* from Brazil. This size is consistent with Ryvarden's (2004) descriptions].

Clade B was composed of specimens of *G. multiplicatum*, *G. multipileum*, *G. parvulum*, *G. tuberculosum* and *G. lingzhi*. Similar topology was also observed in the phylogenetic analysis based on LSU sequences for the available species (*G. multiplicatum*, "*G. lucidum*", *G. parvulum*) (Fig. 2).

Ganoderma multiplicatum is a neotropical species described from Venezuela and is characterized by having amyloid, slightly tuberculate hyphal ends in the cuticle, and subglobose to ellipsoid basidiospores (7.5-8.5 x 5-6µm). The Brazilian specimens studied agree with the descriptions of Gottlieb & Wright (1999), Ryvarden (2000, 2004) and Torres-Torres, Guzmán-Dávalos, & Gugliotta (2012). Ryvarden (2000) recognized *G. subamboinense* Henn. as a synonym of *G. multiplicatum*, originally described from Brazil. Although morphologically similar [context with two or more black, resinous layers, hyphal ends in the cuticle generally amyloid and basidiospores

8-10 x 6-7µm (Gottlieb & Wright, 1999)], *G. subamboinense* Henn var. *laevisporum* Bazzalo & J.E. Wright ATCC 52419 (with a single sequence available for the species) were shown here to be distantly related (clade E), and thus should not be considered as synonyms.

Of the specimens here identified as *G. parvulum* by morphology and phylogenetic analysis, one was deposited in Herbarium URM as *G. stipitatum* (URM 80765) and another in the Culture Collection URM as *G. resinaceum* (URM 2948). For a long time, *G. parvulum* was considered synonym of *G. stipitatum* (Moncalvo & Ryvarden, 1997; Ryvarden, 2004). We agree that these are the same species, however, we follow the opinion of Torres-Torres et al. (2012) that the name *G. parvulum* Murrill 1902 should be used in preference to *G. stipitatum* (Murrill) Murrill 1908 (*Fomes stipitatus* Murrill 1903) as it was described earlier. Steyaert (1980) cited a wrong reference for the *G. parvulum* protologue and this was followed by Ryvarden (2004).

Ganoderma resinaceum URM 2948 is probably *G. parvulum* incorrectly identified. The basidiomata of *G. resinaceum* used for the original identification of the strain was not deposited in any Herbarium, preventing re-identification of the specimen. These two species are macromorphologically similar, and, microscopically, they both have smooth, weakly amyloid hyphal ends of the cuticle. However, *G. resinaceum* has larger basidiospores [9-11.5 x 5-7µm according to Ryvarden (2004) and 11.2-12.5 x 6.5-7.4µm according to Torres-Torres et al. (2012) versus 8-10 x 5-6µm in *G. parvulum* from Brazil)] and no black, resinous layers in the pale brown context (Ryvarden, 2004; Torres-Torres et al., 2012). The basidiospores of the Brazilian specimens of *G. parvulum* were similar to the description in Ryvarden (2004, as *G. stipitatum*). Furthermore, *G. resinaceum* was described on the basis of a specimen from France and *G. parvulum* was originally described from Brazil.

Similarly, the Argentinean specimen described as *G. tuberculosum* (BAFC 33599) (Gottlieb & Wright, 1999) also corresponds to *G. parvulum*. *Ganoderma tuberculosum* is characterized by a black, thick resinose band in the context similar to *G. parvulum*, but has longer basidiospores [10-11 x 7.5-9µm according to Welti & Courtecuisse (2010) versus 8-10 x 5-6µm in *G. parvulum* from Brazil]. Besides, *G. tuberculosum* does not have amyloid cuticle elements unlike *G. parvulum*, whose elements are distinctly amyloid. Gottlieb & Wright (1999) commented that the specimen BAFC 33599 was preliminarily identified as *G. resinaceum*, but was later revised to *G. tuberculosum*. However, they observed cuticle hyphal ends distinctly amyloid characteristic of *G. parvulum* and different from what was observed in the type of *G. tuberculosum* deposited in the Herbarium of the New York Botanical Garden in the study by Welti & Courtecuisse (2010). Yet, Gottlieb & Wright (1999) noted basidiospores ovoid to ellipsoid, 10-12 x 6-9µm, in three Argentinian specimens, including BAFC 33599, similar to the observed in *G. tuberculosum*. It is likely that the size of basidiospores is not a taxonomic criterion relevant for separating these two species, but the presence or absence of amyloid reaction of the cuticle elements is.

The specimens of *G. parvulum* and *G. multiplicatum* proved to be distinct from the specimens previously listed as *G. lucidum* for Asia (*G. multipileum* and *G. lingzhi* in figure 1; *G. lucidum* in Fig. 2) and from specimens still regarded as *G. lucidum* in South America, North America and Europe (clades C, E and F, respectively, in Fig. 1). Due to the high phenotypic plasticity of the subgenus *Ganoderma*, several species have been mistaken for *G. lucidum strictu sensu*. Torres-Torres et al. (2012) commented that Brazilian specimens of *G. multiplicatum* and *G. parvulum* have been mistaken for *G. lucidum*.

Although reports of *G. lucidum* in the neotropics are still found in the literature (Torres-Torres et al., 2012; Vasco-Palacios & Franco-Molano, 2013), in Herbaria records (http://emuweb.fieldmuseum.org/botany/crfResultsList.php, http://splink.cria.org.br) and in online databases (http://www.cybertruffle.

org.uk/venefung/eng/index.htm, http://www.mycokey.com/Ecuador/EcuadorDB.htm, Gugliotta et al., 2013), we are of the opinion that this is a species restricted to Europe, as advocated by different authors (Moncalvo & Ryvarden, 1997; Cao et al., 2012). Thus, non-European *G. lucidum* corresponds to other species. Wang et al. (2009), using morphological and molecular data based on ITS sequences, observed that the specimens from tropical Asia identified preliminarily as *G. lucidum* were *G. multipileum*, whose type specimen is originally Asian. In a similar study, Cao, Wu, & Dai (2012), also using morphological and molecular studies based on ITS sequences and including strains widely cultivated in China, observed that *G. lingzhi* is the correct name for *G. lucidum* in East Asia. Similar approaches should be taken for all non-European *G. lucidum*.

In clade D, the specimen of *G. chalceum* grouped with one Brazilian specimen of *G.* aff. *oerstedtii* (URM83400) (NJ 92%, MP 97% and ML 100%, for ITS analysis), both belonging to the *G. resinaceum* complex. This clade can also be observed in the phylogenetic reconstruction based on LSU sequences, although with low statistical support (NJ, MP an ML < 50%). Basidiospores are smaller in *G. chalceum* [10-12 x 5-7µm versus 12-15 x 8-10µm in *G.* aff. *oerstedtii* (Ryvarden, 2004)] and the black resinous layer in the context is absent in *G.* aff. *oerstedtii* (present in *G. chalceum*). Thus, in this clade, the size of basidiospores and presence of resinous deposits in the context seem to be important characters for species delimitation. *Ganoderma* aff. *oerstedii* URM83400 was initially identified as *G. resinaceum*, but the use of *G. resinaceum* for South America specimens is not appropriate since two specimens of *G. resinaceum* (type locality: France) from Europe (clade E) are distinctly related. The material of *G.* aff. *oerstedtii* (URM83400) is scarce and more collections are desirable in order to confirm this species.

Clade G is composed by specimens of *G. orbiforme* and *G. cupreum*. All specimens of *G. orbiforme* clustered with high statistical support (ITS analysis: NJ 99%, MP and ML 100%; LSU analysis: NJ 97%, MP 78% and ML 74%) and were clearly distinct from the other laccate species.

The Index Fungorum databases and Ryvarden (2004) recognize *G. boninense* as synonym of *G. orbiforme*. However, the Mycobank database considers *G. boninense* as a distinct species, a conclusion supported by our study (*G. boninense*: clade E in ITS analysis, clade C in LSU analysis; *G. orbiforme*: clade G in ITS analysis, clade D in LSU analysis). Both species have similar basidiospore size [9-11µm in the Brazilian *G. orbiforme* versus 10-12µm (Núñez & Ryvarden, 2000) and 8.5-12µm (Chang, 1992) in *G. boninense*], and irregular, amyloid cuticle hyphal ends, but resinous layers are present in *G. orbiforme* and apparently absent in *G. boninense* (Chang, 1992; Núñez & Ryvarden, 2000). *Ganoderma orbiforme* was originally described from Guinea in Africa and also recorded in the neotropics, while *G. boninense* was originally described from Bonin Island in Japan and has been reported throughout the Pacific Islands and Sri Lanka, Australia, Taiwan, Japan and China (Chang, 1992; Moncalvo et al., 1995b). Apparently, the geographical distribution of the species was not considered relevant when the synonym was proposed.

Similarly, the Index Fungorum and MycoBank databases consider *G. chalceum* as a synonym for *G. cupreum* (clade E in ITS analysis). Both species have similar basidiospore size [(10-12 × 5-7µm in *G. chalceum* versus 8-11 × 5-7µm in *G. cupreum* (Bresadola, 1911)] and were originally described from western Africa. Contrary to *G. cupreum*, *G. chalceum* has been reported to the neotropics (Ryvarden, 2000; Ryvarden, 2004; Torres-Torres et al., 2012). The sequences of *G. cupreum* are of Australian origin and Smith & Sivasithamparam (2000) commented that more research was needed to verify that the isolates were correctly named. Moreover, there are no sequences of *G. cupreum* and *G. chalceum* originating from the type locality for better comparison.

In this study, we delimit six laccate taxa of Ganodermataceae collected in Brazil based

on rDNA analyses with the support of morphological characters, mostly size of basidiospores, presence/absence of dark, resinous layers in the context, and presence/absence of amyloid hyphal ends in the cuticle. In addition, geographical distribution is also considered relevant as all Brazilian species differ from the previously known laccate *Ganoderma* from Asia, Europe, North and South America.

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RESUMEN

Delimitación de algunos *Ganoderma* (Ganodermataceae) lacados neotropicales: filogenia molecular y morfología. *Ganoderma* incluye especies de gran importancia económica y ecológica, sin embargo, su nomenclatura actual es caótica y poco estudiada en el neotrópico. En este estudio se utilizaron 14 muestras de *Ganoderma* y dos de *Tomophagus* recolectados en Brasil para la extracción de ADN, amplificación y secuenciación de las regiones ITS y LSU. La delimitación filogenética de seis táxones neotropicales fue discutida con base en especímenes brasileños y secuencias del GenBank. Estas especies mostraron ser distintas de los *Ganoderma* lacados de Asia, Europa, América del Norte y de algunos ejemplares de Argentina. Las reconstrucciones filogenéticas confirman que los *Ganoderma* lacados son distintos de *Tomophagus*, aunque pertenecen al mismo grupo. No se confirman los sinónimos de *G. subamboinense* a *G. multiplicatum*, de *G. boninense* a *G. orbiforme* y *G. chalceum* a *G. cupreum*. *G. parvulum* se confirma como el nombre correcto para *G. stipitatum*. *G. lucidum* sólo se debe utilizar para especies europeas. Por lo tanto, se propone el uso de nombres publicados válidamente de acuerdo con la distribución geográfica de las muestras, características morfológicas y análisis de ADNr.

Palabras clave: Agaricomycetes, taxonomía filogenética, secuencias de ADNr, delimitación de especie, neotrópico.

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