Phylogenetic relationships among species of *Ganoderma* (Ganodermataceae, Basidiomycota) from Cameroon

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Abstract

Ganoderma is an important genus of the Polyporales in the tropics. Identification of tropical species has mainly been based on morphology, which has led to misidentification. This study aimed to elucidate the diversity and phylogenetic relationships of *Ganoderma* isolates from different hosts in Cameroon using morphological and molecular techniques. Analyses of basidiocarp morphology and the internal transcribed spacer and mitochondria small subunit were undertaken for 28 isolates from five plant species. The results show that the isolates belong to eight species. Three of the species were identified to species level; of these only *G. ryvardense* has been previously described from Cameroon while *G. cupreum* and *G. weberianum* are new records. The five remaining species did not match with any previously described species and have been designated as *Ganoderma* with different species affinities.

Keywords: Morphological, Molecular, phylogenetics, Ganodermataceae, mtSSU, ITS

Introduction

The genus *Ganoderma* (Polyporales, Basidiomycota) includes species that occur on various host tree species in tropical and temperate regions of the world, where they may survive as

saprophytes, decomposing lignin of hardwood, or as pathogens of living trees (Singh, 1991; Paterson, 2007). Several species are responsible for root and butt rots of commercially important crops such as tea (*Camillia sinensis* (L.) Kuntze, rubber (*Hevea brasiliensis* Muell. Arg.), temperate hardwoods, coconut (*Cocos nucifera* L. and betelnut palms (*Areca catechu* L.) (Singh, 1991). A number of species cause basal stem rot of oil palm (*Elaeis guineensis*), (Idris, 1999; Tengoua & Bakoume, 2005), and other tropical forest trees (Masuka & Nyoka,1995). Due to the fact that *Ganoderma* species differ in their life strategies, it is important to have comprehensive knowledge regarding the taxonomy of the species in Cameroon. This knowledge will aid in developing diagnostic methods to efficiently and accurately identify potential pathogenic species.

The taxonomy of *Ganoderma* dates back to 1881 during which Karsten erected the genus. At that time only one species, *Polyporus lucidus* (*G. lucidum* (Curtis : Fr.) P. Karst.), was included in the genus. Today more than 300 species reside in this genus, circumscribed by their unique double walled basidiospores (Moncalvo & Ryvarden, 1997). Due to difficulties associated with the taxonomy of *Ganoderma*, the exact number of species is, however, not clear. Moncalvo & Ryvarden (1997) included 118 names for species in the genus *Ganoderma* after revising species described in the last 200 years.

The traditional taxonomy of *Ganoderma* species is based mainly on morphological characteristics of their fruiting bodies (Seo & Kirk, 2000). The two most important morphological characters are the shape and size of basidiospores and cuticle cells (Steyaert, 1972). However, many taxa with similar basidiocarp morphology are now separated in different

species based on their host specificity or discovered to represent different biological or phylogenetic species (Adaskaveg & Gilbertson, 1986; Gottlieb & Wright 1999a; Smith & Sivasithamparam, 2003; Pilotti *et al*., 2004). The sole use of basidiocarp macro and micro-morphology in the taxonomy of *Ganoderma* species has resulted in many synonyms, species complexes and possible misidentifications of species (Steyaert, 1972; Bazzalo & Wright, 1982; Adaskaveg & Gilbertson, 1986). For example, *G. tsugae* was considered to be a synonym of *G. lucidum* based on basidiospore morphology and host specificity (Steyaert, 1972). Later studies, however, showed that these are not only separate species, but also that *G. lucidum* does not occur in North America (Steyaert, 1980). For these reasons, the taxonomy of the genus *Ganoderma* is considered to be in a state of crisis (Ryvarden, 1994).

The majority of molecular phylogenetic studies that address fungal systematics at deeper taxonomic levels used a single gene or intergenic regions from the nuclear ribosomal DNA operon (e.g. Lutzoni *et al.,* 2004). Phylogenies derived from sequence data from the internally transcribed spacer region of the nuclear rDNA operon have often been used to identify distinct lineages among closely related species or species complexes, and has become a popular method to detect fungal phylogenetic species (Hibbett *et al.,* 1995; Moncalvo *et al.,* 1995; Zervakis *et al.,* 2004). However, combined sequence data from gene regions such as the nuclear and mitochondrial rDNAs have also been used in several studies on basidiomycetes (Binder & Hibbett, 2002). The internally transcribed spacer region has a higher degree of variation than genes within the rDNA operon, and is now perhaps the most widely sequenced region for fungi, including for *Ganoderma* species (Smith & Sivasithamparam, 2000; Guzelday & Colack, 2007; Douanla-Meli & Langer, 2009)

Several studies employed alternative methods in an attempt to overcome the difficulties associated with basidiocarp morphology to identify *Ganoderma* species. These studies used cultural characteristics (Adaskaveg & Gilbertson, 1986; Pilotti *et al*., 2003), differences in isozyme profiles (Gottlieb *et al.,* 1998; Smith & Sivasithamparam, 2000b) and DNA-based techniques (Moncalvo *et al.,* 1995a, b; Gottlieb *et al.,* 2000; Smith & Sivasithamparam, 2000a; Hong & Jung, 2004). Of these, methods utilizing DNA sequence data became powerful tools in taxonomic studies of *Ganoderma* species and resolved some of the taxonomic problems (Moncalvo *et al.*, 1995a, b; Kinge & Mih, 2011).

DNA sequence data, mainly from the internally transcribed spacers (ITS1 and ITS2) and 5.8S gene of the rDNA operon (collectively referred to as the ITS region) and mitochondria small subunit (mtSSU) gene, has successfully been used to distinguish isolates belonging to genera within the *Ganodermataceae*. The ITS region was shown to be a suitable molecular marker for inferring phylogenies of species within *Ganoderma* (Moncalvo *et al.,* 1995a; Smith & Sivasithamparam, 2000). In addition, Hong & Jung (2004) used mitochondrial small subunit gene sequences to differentiate and infer the phylogeny of *Ganoderma* species from South Korea. More recently, Douanla-Meli & Langer (2009) described a new species, *G. carocalcareus*, from Cameroon based on morphological characteristics as well as ITS and mtSSU DNA sequence data. Besides, the study of Douanla-Meli & Langer (2009), no other study from Africa utilized DNA sequences from both the ITS and mtSSU regions to describe new species of *Ganoderma*.

The species diversity of *Ganoderma* and other polypores in Africa received very little attention. It is therefore reasonable to suggest that a wealth of information is waiting to be discovered. Moncalvo & Ryvarden (1997) listed 49 *Ganoderma* species from Africa of which were named solely based on morphological characters. Few contributions to the taxonomy of *Ganoderma* were made from Cameroon, in spite of its importance in this country. Turner (1981) reported the occurrence of *G. tornatum* var *tornatum,* followed by Nunez & Daniels (1999) who identified *G. hildebrandii*, *G. lucidum* and *G. cf. multiplicatum* from the Dja biosphere reserve. Recently, Douanla-Meli (2007) described *G. hildebrandii, G. lucidum and G. resinaceum* from the Mbalmayo forest reserve in Cameroon. *Ganoderma carocalcareus* was described as a new species from the Mbalmayo forest reserves by Douanla-Meli & Langer (2009). Most recently, Kinge & Mih (2011) reported a new species, *G. ryvardense,* associated with basal stem rot disease of oil palm plantations in Southwest and Littoral regions of Cameroon. The identification of most of these species was based solely on morphological characteristics. As very little is known about the diversity of *Ganoderma* in Cameroon, the aim of this study was to identify and infer phylogenetic relationships among species of *Ganoderma* from this country.

Materials and Methods

Origin and Sampling of *Ganoderma* Basidiocarps

Fresh fruiting bodies resembling *Ganoderma* were collected during mycological surveys in 2010 and 2011. Samples were collected from Lobe, Idenau, Bota, Mondoni, Mungo and Beneo estates as well as from forested areas in Buea, Idenau, Ekona and Bafia in the Mount Cameroon Region (Figure 1). Two samples collected from Yaounde, which had the same morphological characters

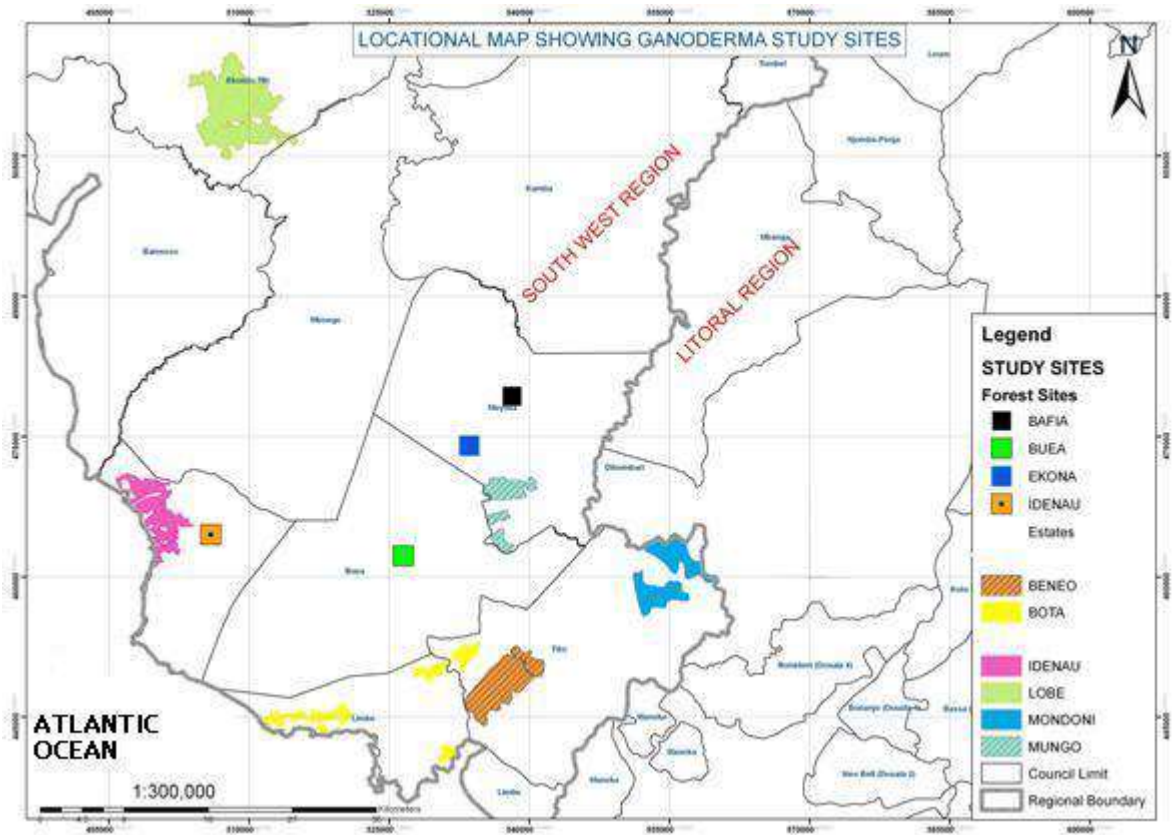

Figure 1. Locational map of study sites of *Ganoderma* in Cameroon.

with some samples from the other study areas, were included in the analysis for comparative purposes. Samples were preserved by oven-drying at 50 ^{o} C for 24 hrs at the end of each day. Small pieces of the hymenophore from each of the samples were dried with silica gel and preserved for subsequent DNA sequence analyses. Representative samples were deposited in the ARC herbarium in Pretoria, South Africa with accession numbers PREM 60576- PREM 60596.

Morphological Characterization

Macro-morphological features were recorded from fresh material, characters that were considered included: a laccate or non-laccate appearance, type of basidiocarp (stipitate/sessile/dimidiate, imbricate, number of concentric zones), margin shape (lobed,

fertile/sterile, rounded/acute) and colour (brown, white, reddish). Micro-morphological characters included pore morphology (colour, pores per mm, angular/rounded, diameter, dissepiments and axes), context (colour and length), and spore characteristics (length, width). The morphological characters were compared with that of *Ganoderma* species described by various authors (Ryvarden & Johansen 1980, Corner 1983, Ryvarden 1995, Moncalvo & Ryvarden 1997, Ryvarden, 2000; Kinge & Mih, 2011).

Samples were prepared for micro-morphological analyses by slicing free-hand thin sections of dried basidiocarps, passing through the hymenium. The sections were mounted in 5% KOH and observed under a compound microscope. Twenty measurements were taken for pore diameter, thickness of dissepiments and the distance between axes of pores. Twenty randomly selected basidiospores from each specimen were measured and ornamentation was noted. The results were coded as n/m/p, where n is the number of basidiospores measured, m the number of basidiomata involved and p the number of collections. Spore dimension is given in the following notation: (a) b–c (d) which takes into account the lowest (a) and highest (d) extreme values and the range b–c that contains a minimum of 90% of the values. The length: diameter ratio, Q, was calculated and the mean (Q_m) and standard deviations were estimated.

DNA extraction

DNA was extracted from the dried herbarium material following the protocol outlined by Raeder & Broda (1985), but with some modifications. Fruiting bodies were surface sterilized with 3.5%

commercial Jik (NaOCl), air dried and sliced open. Small piece of the hymenophore were transferred to eppendorf tubes to which preheated (60-65^{o}C) DNA extraction buffer (5% W/v cetytrimethylammonium bromide (CTAB), 1.4M NaCl, 0.2% v/v 2 mercaptoethanol, 20 mM EDTA pH 8, 10mM Tris- HCl pH 8, and 1% v/v polyvinylpolyrrolidone) was added together with sterile ceramic beads. The hymenophore material was then homogenized for 60 seconds using a FastPrep (FP120 BIO 101 SAVANT) cell disrupter (Southern Cross Biotechnology, Cape Town, South Africa). The cell – buffer suspension was incubated at 65^{o}C for 30 min. Cells were disrupted again and the suspension incubated for 30 min at the same temperature. Cell debris was removed by centrifugation (10000 rpm, 30 min), followed by several chloroform: octanol 24:1 (1:1 v/v) extractions through centrifugation (10000 rpm, 15 min) to remove proteins. This was repeated until a clear interphase was obtained. A final chloroform extraction was done to remove the residual octanol. Quantification of DNA was done using a Nanodrop ND-1000 spectrometer (Nanodrop Technologies, Rockland, DE, USA).

PCR amplification of the ITS and mtSSU region

The ITS region (including the ITS-1, 5.8S gene and ITS-2) was amplified using primers ITS1 and ITS4 (White *et al.,* 1990). Primers BMS05 and BMS113 (Hong & Jung, 2004) were used to amplify a region of the mtSSU gene. All PCR mixtures consisted of 100 ng genomic DNA, reaction buffer supplied with the polymerase (Roche Diagnostics, Randburg, South Africa), 2.5 uM of each dNTP (Fermentas Life Sciences, Pretoria, South Africa), 0.4 uM of each primer, and 0.5 units of Taq polymerase (FastStart Taq). PCR cycles consisted of an initial denaturation at 95^{o}C for 5 min, followed by 35 cycles of denaturation at 95^{o}C for 30 s, annealing for 30 s at

62°C (ITS) or 50°C (mtSSU) and extension at 72°C for 30 s (ITS) or 2 min (mtSSU). A final extension at 72°C for 7 min (ITS) or 10 min (mtSSU) was included to complete the reaction.

PCR products were analyzed by electrophoreses on either 1% or 2% agarose gels and visualization under ultra violet light illumination. Amplicon sizes were estimated by comparing the PCR product against a 100 bp-ladder DNA marker (Promega Corporation, Madison, MI, USA) or DNA molecular weight Marker III (Lambda DNA digested with EcoRI and HindIII restriction enzymes, Fermentas Life Sciences,Pretoria South Africa).

DNA sequencing

The PCR products were purified prior to sequencing using a PCR purification kit according to the manufacturer's instructions (Invitek). PCR products were sequenced in both directions using the same set of primers for the respective PCR reactions. Sequence reactions were performed using an ABI Prism® BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS (Perkin Elmer, Warrington,UK) following the protocol supplied by the manufacturer. DNA sequences were obtained using an ABI PRISM 3130x1 Genetic Analyser (Applied Biosystems, Foster City, USA). The resulting sequences of each strand were edited individually and assembled into contigs using the software CLC Bio Main Workbench version 6 (www.clcbio.com). All sequences generated during this study have been deposited in GenBank (Table 1).

Table 1: **ITS and mtSSU GenBank accession numbers of isolates from Cameroon only used in this study, along with their origins, hosts and source**

ITS/mtSSU GenBank number	Species	Geographical origin	Host	Reference
GanTK10/ JN105691	*Ganoderma* sp.	Cameroon/Ekona	Oil palm	This study
GanTK74/ JN105692	*Ganoderma* sp.	Cameroon/Mungo	Oil palm	This study
GanTK30/ JN105696	*Ganoderma* sp.	Cameroon/Mondoni	Oil palm	This study
TK31/ JN105693	*Ganoderma* sp.	Cameroon/Mondoni	Oil palm	This study
GanTK41/ JN105699	*Ganoderma* sp.	Cameroon/Mungo	Oil palm	This study
GanTK52/ JN105694	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK43/ JN105695	*Ganoderma* sp.	Cameroon/Mungo	Oil palm	This study
GanTK32/ JN105698	*Ganoderma* sp.	Cameroon/Idenau	Oil palm	This study
GanTK13/ JN105697	*Ganoderma* sp.	Cameroon/Lobe,Beneo	Oil palm	This study
HM138670	*Ganoderma ryvardense*	Cameroon/Lobe,Dibombari	Oil palm	This study
TK33/ JN105700	*Ganoderma* sp.	Cameroon/Idenau	Oil palm	This study
TK57/ JN105706	*Ganoderma* sp.	Cameroon/Bota	Oil palm	This study
TK4/ JN105701	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
RT7/ JN105702	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK25/ JN105707	*Ganoderma* sp.	Cameroon/Mungo	Oil palm	This study
TK01/ JN105708	*Ganoderma* sp.	Cameroon/Buea	*Pinus slyvestris*	This study
TK24/ JN105710	*Ganoderma* sp.	Cameroon/Yaounde	*Pinus slyvestris*	This study
GanTK35/ JN105709	*Ganoderma* sp.	Cameroon/Ekona	Hardwood	This study
TK09/ JN105711	*Ganoderma* sp.	Cameroon/Bafia	*Cassia* sp.	This study
GanTK50/	*Ganoderma* sp.	Cameroon/Yaounde	*Cassia* sp.	This study

JN105712				
TK53/ JN105713	*Ganoderma* sp.	Cameroon/Lobe	Oil palm	This study
TK38/ JN105714	*Ganoderma* sp.	Cameroon/Lobe	Oil palm	This study
TK15/ JN105715	*Ganoderma* sp.	Cameroon/Idenau	Hardwood	This study
TK68/ JN105716	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK36/ JN105717	*Ganoderma* sp.	Cameroon/Buea	Hardwood	This study
TK06/ JN105703	*Ganoderma* sp.	Cameroon/Buea	*Acacia* sp.	This study
TK17/ JN105705	*Ganoderma* sp.	Cameroon/Ekona	hardwood	This study
TK16/ JN105704	*Ganoderma* sp.	Cameroon/Ekona	hardwood	This study
TK50/JN105718	*Ganoderma* sp.	Cameroon/Yaounde	*Cassia sp.*	This study
TK1 / JN105719	*Ganoderma* sp.	Cameroon/Buea	*Pinus sylvestris*	This study
TK24 / JN105720	*Ganoderma* sp.	Cameroon/Yaounde	*Pine sylvestris*	This study
TK6/ JN105721	*Ganoderma* sp.	Cameroon/Buea	*Acacia* sp.	This study
TK40/ JN105722	*Ganoderma* sp.	Cameroon/Buea	Hardwood	This study
TK15/JN105723	*Ganoderma* sp.	Cameroon/Idenau	Hardwood	This study
TK68/ JN105724	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK2 / JN105725	*Ganoderma* sp.	Cameroon/Buea	*Acacia* sp.	This study
TK17 / JN105726	*Ganoderma* sp.	Cameroon/Ekona	hardwood	This study
TK35/ JN105727	*Ganoderma* sp.	Cameroon/Ekona	hardwood	This study
TK16 / JN105728	*Ganoderma* sp.	Cameroon/Ekona	hardwood	This study
TK32 / JN105729	*Ganoderma* sp.	Cameroon/Idenau	Oil palm	This study
TK7 / JN105730	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK52 / JN105731	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK4 / JN105732	*Ganoderma* sp.	Cameroon/Buea	*Cassia* sp.	This study
TK9 / JN105733	*Ganoderma* sp.	Cameroon/Bafia	hardwood	This study
TK25 / JN105734	*Ganoderma* sp.	Cameroon/Mungo	Oil palm	This study
TK31 / JN105735	*Ganoderma* sp.	Cameroon/Mondoni	Oil palm	This study
TK36 / JN105736	*Ganoderma* sp.	Cameroon/Buea	Hardwood	This study

DNA sequence comparisons and data sets

An initial identification of the isolates based on DNA sequence data was done by similarity searches of the ITS and mtSSU sequences using the BLASTn (Basic Local Alignment Search Tool for nucleotides) search algorithm against sequences in GenBank. DNA sequences from *Ganoderma* species showing the highest similarity and coverage (80% to 100%) with those from the Cameroon samples were downloaded and included in this study for comparative purposes

Table 2: ITS and mtSSU GenBank accession numbers of taxa used in this study, along with their origins, hosts and source

ITS/mtSSU GenBank number	Species	Geographical origin	Host	Reference
EU239386	*Ganoderma aff. steyaertayanum*	Indonesia and Malaysia	*Acacia mangium*	Smith and Sivasithanparam,2000a
EF016754	*Ganoderma sp.*	India	*Areca catechu*	Kumari *et al.,* 2006
HQ235634	*Ganoderma sinense*	China	unknown	Li, 2010
GU213482	*Ganoderma lucidum*	China	unknown	Huang *et al.,* 2009
AY593865	*Ganoderma japonicum*	Britain	unknown	Wang and Yao,2005
HM583824	*Ganoderma sp.*	New Zealand	*Eucalyptus* sp.	Hopkins *et al.,* 2010
AJ608709	*Ganoderma applanatum*	Indonesia	*Acacia mangium*	Bougher, 2003
AY884180	*G. australe*	Britain	unknown	Wang and Yao,2005
AY569450	*Ganoderma cupreum*	Australia	unknown	Roberts,2004
AJ627585	*G. mastoporum*	Indonesia/Malaysia	*Acacia mangium*	Bougher,2004
AJ537399	*G. sp.*	Indonesia	*Acacia mangium*	Bougher *et al*.,2003
AJ608710	*G. philippi*	Indonesia	*Acacia mangium*	Bougher *et al.*,2003
FJ392283	*G. pseudoferreum*	China	Rubber	Zhang *et al.,* 2008
EF188277	*G. lucidum*	China	unknown	Jia *et al.,* 2009
GQ249880	*G. lucidum*	India	unknown	Singh *et al.,* 2009
GQ249884	*G. lucidum*	China	unknown	Singh *et al.,* 2009
FR686556	*G. applanatum*	Germany	Urban trees	Schmidt *et al.,* 2010

EF060003	*G. lipsiense*	Finland	unknown	Terho *et al.*, 2006
EF059994	*G. lipsiense*	Finland	unknown	Terho *et al*., 2006
AY884176	*G. lucidum*	Britain	unknown	Wang and Yao,2005
AY636059	*G. lucidum*	India	unknown	Singh *et al.*, 2004
EF060007	*G. resinaceum*	Finland	unknown	Terho *et al.*, 2006
GU731560	*G. weberianum*	France	unknown	Favel *et al.*, 2010
EU089969	*G. carocalcareus*	Cameroon/Mbalmayo	*Anthocleista nobilis*	Douanla Meli and Langer, 2009
EU089970	*G. carocalcareus*	Cameroon/Mbalmayo Forest reserve	*Anthocleista nobilis*	Douanla Meli and Langer, 2009
AY569451	*G. weberianum*	Australia	unknown	Roberts,2004
AY593867	*G. neojaponicum*	Taiwan, China	unknown	Wang and Yao,2005
EF524049	*Trametes versicolor*	Germany	unknown	Hoegger *et al.*, 2007
EU863192	*Pycnoporus sanguineus*	Malaysia	unknown	Aziz *et al.*, 2008
AF248341	*Ganoderma lucidum*	South Korea	unknown	Hong and Jung,2004
AF248342	*Ganoderma lucidum*	South Korea	unknown	Hong and Jung,2004
AF214469	*Ganoderma oerstedii*	South Korea	unknown	Hong *et al.*, 1999
AF248343	*Ganoderma meredithae*	South Korea	unknown	Hong and Jung,2004
AF248344	*Ganoderma meredithae*	South Korea	unknown	Hong and Jung,2004
AF248345	*Ganoderma oregonense*	South Korea	unknown	Hong and Jung,2004
DQ661919	*Ganoderma sp.*	United Kingdom	unknown	Chouiter *et al.*, 2006
AF248349	*Ganoderma subamboinense,var laevisporum*	South Korea	unknown	Hong and Jung,2004
AF248348	*Ganoderma subamboinense*	South Korea	unknown	Hong and Jung,2004
AF214472	*Ganoderma resinaceum*	South Korea	unknown	Hong *et al.*, 1999

DQ661918	*Ganoderma adspersum*	United Kingdom	unknown	Chouiter *et al.*, 2006
AF248336	*Ganoderma applanatum*	South Korea	unknown	Hong and Jung,2004
AF214474	*Ganoderma valesiacum*	South Korea	unknown	Hong *et al.*, 1999
AF214473	*Ganoderma tsugae*	South Korea	unknown	Hong *et al.*, 1999
AF248353	*Ganoderma valesiacum*	South Korea	unknown	Hong and Jung,2004
AF248352	*Ganoderma tsugae*	South Korea	unknown	Hong and Jung,2004
AF214470	*Ganoderma oerstedii*	South Korea	unknown	Hong *et al.*, 1999
AF248346	*Ganoderma oregonense*	South Korea	unknown	Hong and Jung,2004
EU089967	*Ganoderma carocalcareus*	Cameroon	*Anthocleista nobilis*	Douanla-Meli and Langer,2009
F248322	*Ganoderma lobatum*	South Korea	unknown	Hong and Jung,2004
U27080	*Trametes versicolor*	USA	unknown	Hibbett and Donoghue,1995
U27059	*Pycnoporus cinnabarinus*	USA	unknown	Hibbett and Donoghue,1995

(Table 2). *Trametes versicolor* (EF524049) and *Pycnoporous sanguineus* (EU863192) were included as the outgroup taxa for the ITS dataset while *Trametes versicolor* (U27080) and *Pycnoporus cinnabarinus* (U27059) were used as outgroup taxa for mtSSU dataset.

The sequences from samples collected in Cameroon were first treated separately and then in combination with sequences for *Ganoderma* from GenBank. Thus, four data matrices were generated and referred to as the ITS Cameroon and mtSSU Cameroon Matrices (both matrices included sequences only from the Cameroon isolates) as well as the ITS *Ganoderma* and mtSSU *Ganoderma* Matrices (both including sequences from the isolates from Cameroon and sequences

downloaded from GenBank). The sequences for the two gene regions were aligned using Multiple sequence Alignment based on Fast Fourier Transform (MAFFT version 5, Katoh *et al.*, 2005) and the alignments were evaluated manually in MEGA version 4 (Tamura *et al.*, 2007). Alignments will be deposited in TreeBASE (www.treebase.org). Nucleotide substitution models were determined for each of the data matrices using MrModelTest 2.2 for Bayesian analysis (http://www.abc.se/nylander) and jModelTest version 0.1.1 for Maximum Likelihood (Posada, 2008).

Phylogenetic analyses

Phylogenetic trees were generated based on parsimony, maximum likelihood and Bayesian inference of phylogenies. Parsimony analyses were done using PAUP* (Phylogenetic Analysis Using Parsimony and other Methods) version 4.0b10 (Swofford, 2003). The most parsimonious trees were obtained by employing a heuristic tree search algorithm with random addition of sequences (1000 replicates) and tree bisection-reconnection branch swapping (TBR). Tree branches with zero length were collapsed. Parsimony-uninformative and ambiguous characters were excluded prior to the analyses and gaps were treated as fifth character. Tree lengths (TL), retention indexes (RI), consistency indexes (CI) and homoplasy indexes (HI) were determined after the heuristic searches were completed. Statistical support for the nodes were obtained using bootstrap (1000 replicates) (Felsenstein, 1985) in PAUP* with the same tree search settings, but with the addition of sequences set to closest.

Maximum likelihood (ML) analyses were conducted using PhyML 3.0 (Guindon & Gascuel, 2003). A HKY85+G model was incorporated for the ITS Cameroon and ITS *Ganoderma* matrices. A TPMuf+G model was used for the mtSSU Cameroon Matrix and a TVM+G model for the mtSSU *Ganoderma* Matrix.

Phylogenetic trees based on Bayesian inferences (BI) were generated using MrBayes version 3.1 (Ronquist & Huelsenbeck, 2003). For this purpose, a HKY+I+G model was used for the two ITS matrices and GTR+I+G model was incorporated in the analyses for the two mtSSU matrices. Tree searches were done using a Markov Chain Monte Carlo (MCMC) algorithm with four Markov chains. The number of generations was set to $1x10^6$ and every 100th generation was sampled. After completion of the analyses, the log likelihood scores of each tree was plotted against the generation time to determine the number of trees that should be excluded before the stationary point is reached. A majority rule consensus tree was generated from the remaining trees and posterior probability values for the nodes were calculated in MrBayes.

RESULTS

Morphological characteristics

Basidiocarps were place into eight groups based on their clustering in phylogenetic trees and morphological differences (Table 3). The morphological characteristics of these groups are presented below:

Table 3: Identity and grouping of species of *Ganoderma* based on morphological and molecular data

Collection numbers	Species identity and groupings
GanoTK10, GanoTK74, GanTK30, TK31, GanTK41, GanTK52, TK43,GanTK32,GanTK13,TK33,TK57	*G. ryvardense* Group1
TK4, RT7	*G. cupreum* Group 2
TK06,TK17 and TK16	*G. weberianum* Group 7
TK25, TK01, TK24, GanTK35	*G.* sp. 1 Group 3
TK09 and TK50	*G.* sp. 2 Group 4
TK53 and TK38	*G.* sp. 3 Group 5
TK15, TK68,TK36,TK40	*G.* sp. 4 Group 6
TK2	*G.* sp. 5 Group 8

Group 1 (G. *ryvardense*)

Basidiocarp laccate, sessile and dimidiate, pileus colour brown, reddish brown, brown, reddish black, 2-8 concentric zones. Margin colour varied from brown to white, lobed and rounded. Context colour varied being black, brown, white, brown and reddish brown. Tube length 0.4-0.8 cm. Diameter of pileus 13-32 cm, pore diameter 150-275 µm, pore dissepiments 50-175 µm, pore axes 200-375 µm, 2-5 pores per mm, length of spores 9-13 µm, width= 6-8 µm, spores are ellipsoid with slightly truncated apices, Q = 1.43-2.17 and Qm = 1.64±0.18. This group included samples labeled TK10, 13, 74, 30, 43, 31, 52, 41, 33, 57 and 32.

Group 2 (*G. cupreum*)

Basidiocarp laccate, sessile, dimidiate and stipitate, stipe up to 3 cm long and 2.5 cm wide, and laterally attached. Pileus colour reddish brown, 2-6 cm, upper surface strongly laccate. Margin thin and incurved with 5-8 concentric zones. Pore surface cream white. Pores 3-5 per mm, rounded, diameter 85-220 µm, dissepiments 35-175 µm, pore axes 155-350 µm. Context colour white and length range from 3.5-4 cm, tube length 1-1.5 cm and light brown. Length of spores 9-11 µm, width = 5-7 µm, Q = 1.5-2 and Qm = 1.71±0.16. This group comprised of samples labeled TK4 and RT7.

Group 3 (*Ganoderma* sp. 1)

Basidiocarp laccate, sessile, dimidiate and stipitate. Stipe 5cm by 2.5cm. Pileus colour light red with 3-5 concentric zones. Margin colour yellowish and rounded. Context colour light brown and varies from 2.5-4cm. Tube length 1.2-1.4 cm, diameter of pileus 11.5-15 cm, pore diameter 125-250 µm, pore dissepiments 75-190 µm, pore axes 225-310 µm, 2-4 pores per mm. Length of spores 9-13 µm, width= 6-8 µm, Q = 1.25-1.86 and Qm = 1.51±0.18. This group comprised of samples labeled TK25, 01, 24 and 35.

Group 4 (*Ganoderma* sp. 2)

Basidiocarp laccate and sessile, pileus dark brown in colour with 3-4 concentric zones. Margin brown and lobed, context colour brown and varies from 0.4-0.5 cm. Tube length 1.5-1.7 cm, diameter of pileus 20.5-22 cm. Pore diameter 125-250 µm, pore dissepiments 50-150 µm, pore

axes 200-350 µm, 2-4 pores per mm. Length of spores 9-13 µm, width= 6-8 µm, Q =1.44 and Qm= 1.64±0.15. This group comprised of samples labeled TK09 and TK50.

Group 5 (*Ganoderma* sp. 3)

Basidiocarp laccate, sessile, colour of pileus brown with 2-4 concentric zones zones. Margin of pileus white and lobed. Context colour brown and varies from 0.6-0.8 cm. Tube length 0.5-0.7 cm, diameter of pileus 26-30 cm, pore diameter 150-250 µm, pore dissepiments 50-135 µm, pore axes 225-375 µm, 2-4 pores per mm. Length of spores 10-15 µm, width= 6-7 µm, Q = 1.57-2.17 and Qm = 1.77±0.17. This group comprised of samples labeled TK53 and TK38.

Group 6 (*Ganoderma* sp. 4)

Basidiocarp laccate,sessile,dimidiate and stipitate, stipe 3.5 by 3 cm. Pileus brown to reddish brown with 2-8 concentric zones. Margin of pileus black and rounded. Context colour white and varies from 3.5-4.5 cm. Tube length range from 1-1.6 cm, diameter of pileus range 13.5-25 cm, pore diameter 125-265 µm, pore dessipiments 60-160 µm, pore axes 150-280 µm and 3-5 pores per mm. Length of spores 9-13 µm, width= 5-8 µm, Q = 1.43-2 and Qm = 1.69±0.15. This group comprised of samples labeled TK15, 36, 68 and 40.

Group 7 (*G. weberianum*)

Basidiocarp strongly laccate, stipitate, stipe up to 4.5 cm long and 2.5 cm wide, pileus colour reddish brown, yellowish red, 1-3 concentric . Margin thin and incurved, margin colour range from white to yellow, margin lobed and rounded. Context colour brown and light yellow, one resinous layer present in the woody context of the pileus. Tube length 0.5-0.7 cm. Diameter of pileus range from 5-10 cm. Pore surface cream white, pores 2-5 per mm, circular, 125-240 µm diameter, dessipiments is 70-190 µm thick. Context 4 cm long and light brown with few gasterospores. Length of spores 7.5-10 µm, width= 4-6 µm, Q 1.5-2 and Qm = 1.75±0.16. This group includedsamples labeled TK06, 16 and 17.

Group 8 (*Ganoderma* sp. 5)

Basidiocarp laccate,dimidiate and stipitate, stipe 3.5 by 3 cm. Pileus reddish brown with 4 concentric zones. Margin of pileus white and rounded. Context colour light yellow and varies from 3.5 cm. Tube length range from 1 cm, diameter of pileus range 25 cm, pore diameter 125-265 µm, pore dessipiments 60-125 µm, pore axes 150-250 µm and 3-4 pores per mm. Length of spores 9-12 µm, width= 6-7 µm, Q = 1.5-1.83 and Qm = 1.64±0.10. This group comprised of a single sampled labeled TK2.

PCR amplification:

DNA amplicons of the ITS were successfully obtained from 40 samples used in this study, while the mtSSU region could be amplified only for 19 samples. Most amplifications yielded a single

fragment for the samples, but multiple fragments were obtained for some of the ITS and mtSSU amplifications. PCR products that showed multiple fragments were discarded from further analyses. The amplicon size for the ITS regions was approximately 800 base pairs (bp), while the size of the mtSSU was approximately 1000 bp.

DNA sequence comparisons

BLASTn comparisons of the ITS sequence data from the Cameroon isolates against those from GenBank revealed high similarity with various species of *Ganoderma*. Isolates from oil palm (TK10, TK13) had the highest similarity (100%) with *G. ryvardense* (HM138670) from Cameroon. Isolates from *Cassia* sp. (TK4, TK7) showed highest similarity with *G. cupreum* (AY569450) from Australia (97%). Isolates from *Acacia* sp. (TK6, TK16) and hardwood (TK17,) showed similarity with *G. weberianum* (AY569451, 95%) from Australia and *G. carocalcareus* (EU089969, 98%). Isolates from *Pinus slyvestris* (TK1, TK24), Cassia sp. (TK9, TK50, TK68) and hard wood (TK35, TK15, TK36) showed similarity with *G. neojaponicum* (AY593867) from Taiwan, China but with low similarity (90%).

BLASTn comparisons of the mtSSU sequence data from the Cameroon isolates with those from GenBank revealed high similarity with various species of *Ganoderma*. Isolates from oil palm (TK32) had the highest similarity (94%) with *G. meredithae* (AF248343) from South Korea. Isolates from *Cassia* sp.(TK4) showed highest similarity with *G. valesiacum* (AF248353) from South Korea (94%). Isolates from *Acacia* sp. (TK6) and hardwood (TK16) showed similarity with *G. lucidum* (AF248341, 97%) from South Korea and *G. subamboinense* (AF248349, 96%). Isolates from *Pinus slyvestris* (TK1, TK24) and hard wood (TK35) showed similarity with *G.*

lucidum (AF248342) from Taiwan and *G. meredithae* (AF248344) form South Korea, all with 96% support.

Data matrices and phylogenetic analyses

The ITS Cameroon Matrix included 30 taxa, of which two represented the outgroup taxa. Of the 730 characters, 529 were constant and 201 were parsimony informative. Heuristic searches yielded eight parsimonious trees with a tree length of 345 steps, with CI = 0.756 and RI = 0.905. Trees generated from this dataset clustered the Cameroon isolates into seven groups (Groups 1 – 7) (Figure 2). Group 1 consisted of isolates from oil palm together with one isolate from *Cassia* sp., (100% MLB and 100% BP). Group 2 comprised of isolates from *Cassia* sp. all from Buea with 100% MLB, 99% PB and 100% PP values. Groups 3 – 6 formed a strongly supported monophyletic group (100% for MLB, PB PP values). Group 3 included isolates from *Pinus slyvestris* and hardwood (MLB: 87%, PB: 90% and PP: 100%) and one isolate from oil palm (64% PB and 100% PP support). Group 4 comprised of isolates from *Cassia* sp. from Bafia and Yaounde with strong statistical support (PB: 100%, PP: 100%). Group 5 consisted of isolates from oil palm from Lobe (PB: 63%, PP: 99%). Group 6 included isolates from hardwood and *Cassia* sp. (MLB: 99%, PB: 98% and PP: 100%). Group 7 consisted of isolates from hardwood and *Acacia* sp. from Buea (100% for MLB, PB and PP).

The ITS *Ganoderma* Matrix consisted of 55 ingroup taxa and two outgroup taxa. The total number of characters equaled 725, of which 490 characters were constant and 235 characters were parsimony informative. Heuristic searches yielded eight parsimonious trees with a TL =

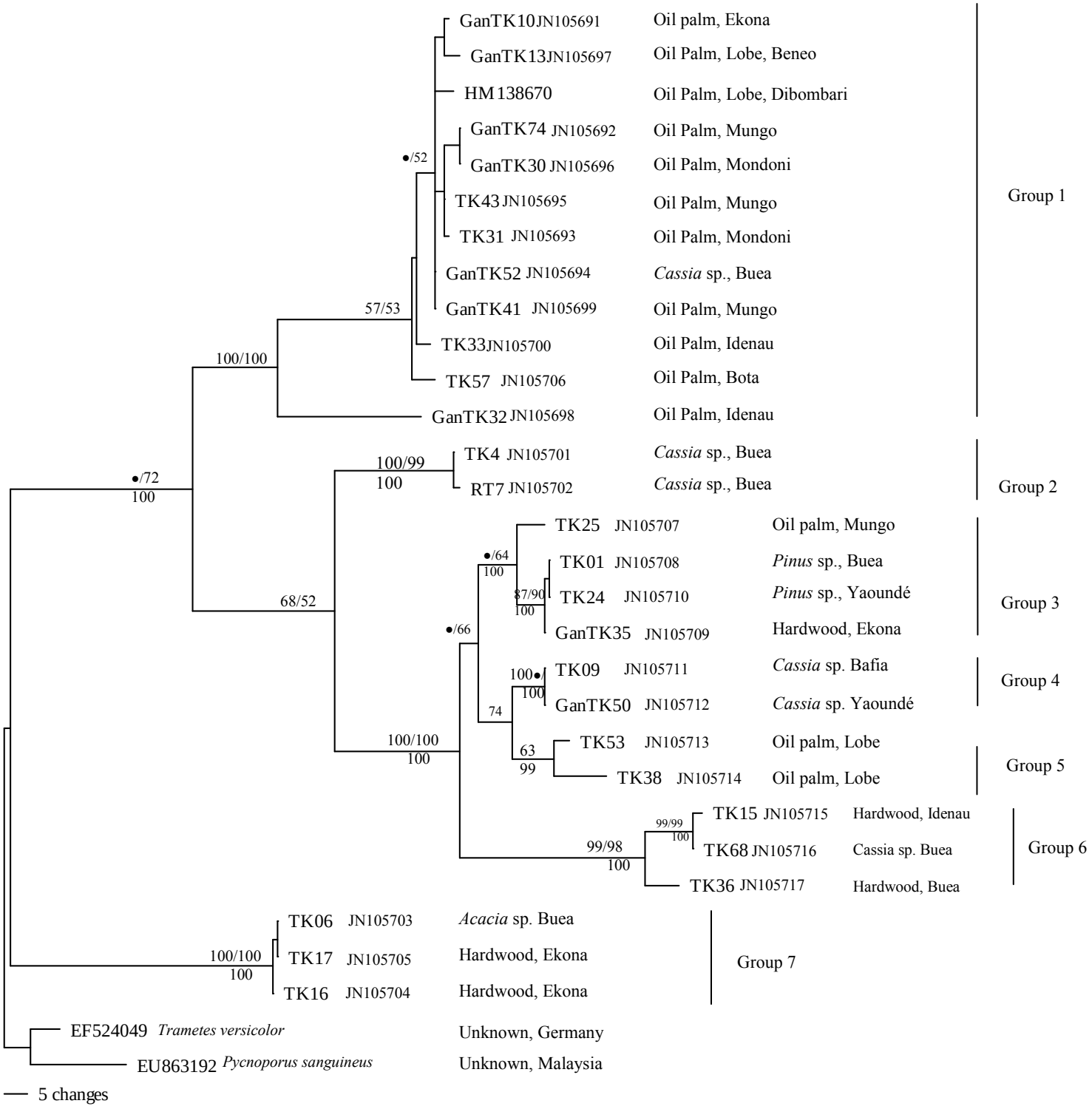

Figure 2: Phylogenetic tree generated from the ITS Cameroon matrix, based on parsimony GenBank accession number, tree host and origin are indicated next to the sample numbers. Maximum likelihood bootstraps (MLB) > 50%, Parsimony bootstrap (PB) values > 50% are indicated at the above the branches and Posterior Probability (PP) values > 95% below the branches. (● depicts MLB and PB values < 50 %)

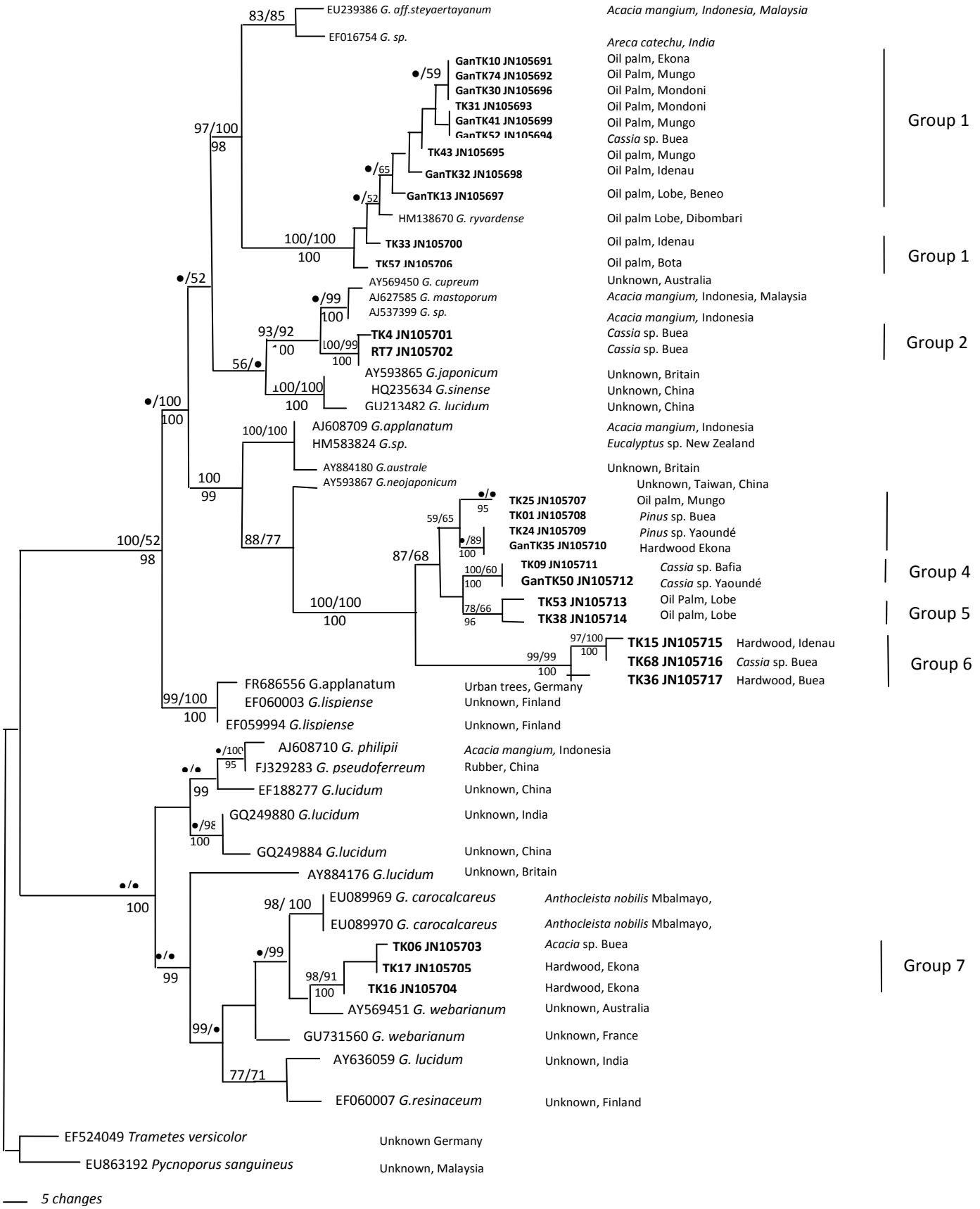

Figure 3: Phylogenetic tree generated from the ITS *Ganoderma* matrix using parsimony. GenBank accession number, tree host and origin are indicated next to the sample numbers. Maximum likelihood bootstraps (MLB) > 50%, Parsimony bootstrap (PB) values > 50% are indicated at the above the branches and Posterior Probability (PP) values > 95% below the branches. (● depicts MLB and PB values < 50 %)

588 steps, CI = 0.556 and RI = 0.836. Phylogenetic trees generated from this dataset placed Group 1 in a monophyletic group with a sequence representing *G. ryvardense* (100% MLB, PB and PP) (Figure 3). Group 2 formed a sister group with a monophyletic group that included sequences of *G. cupreum* from Australia, *G. mastoporum* from Indonesia and Malaysia and a *Ganoderma* sp. from Indonesia (MLB: 93%, PB: 92% and PP: 100%). Groups 3 – 6 were place sister to *G. neojaponicum*, but with low statistical support (MLB: 88% and PB: 77%). Group 7 was placed in a group that included sequences from *G. lucidum* from India, *G. carocalcareus* from Cameroon, *G. weberianum* from Australia and France, *G. lucidum* from Britain and *G. resinaceum* from Finland, but with statistical support only from the Maximum Likelihood analysis (MLB: 99%)

The mtSSU Cameroon Matrix 19 ingroup and two outgroup taxa. Isolates belonging to Group 5 from trees generated based on the ITS Cameroon matrix were not included because the sequences were heterogeneous. The matrix consisted of 986 with 946 characters being constant and only 40 characters were parsimony informative. Heuristic searches yielded eight parsimonious tree with a TL = 63, CI = 0.719 and RI = 0.812. Phylogenetic trees generated from this data set suffered from low resolution, several of the groups identified from the ITS Cameroon Matrix were not supported by the trees generated from this matrix (Figure 4). The monophyly of isolates placed in Groups 1, 2, 3 and 6 from the ITS Cameroon Matrix, were not resolved on the trees generated from the mtSSU Cameroon Matrix. Phylogenetic trees generated by PB clustered Groups 3 – 7 into a major group (PB: 68%, PP: 99). Within this group, isolates belonging to Group 4 formed a monophyletic group (MLB: 92%, PB: 86%) and clustered with

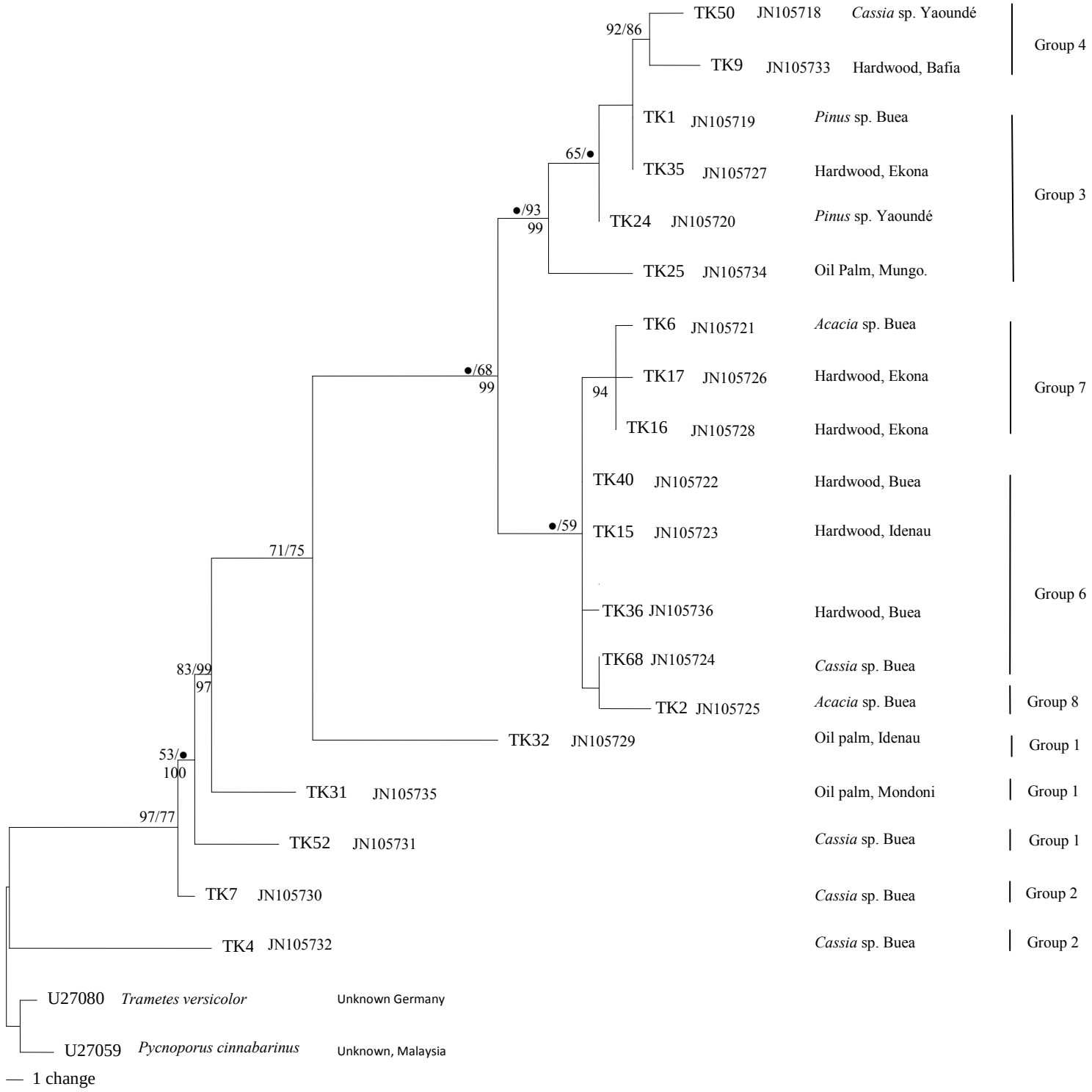

Figure 4: Phylogenetic tree generated from the mtSSU Cameroon matrix, based on parsimony. GenBank accession number, tree host and origin are indicated next to the sample numbers. Maximum likelihood bootstraps (MLB) > 50%, Parsimony bootstrap (PB) values > 50% are indicated at the above the branches and Posterior Probability (PP) values > 95% below the branches. (● depicts MLB and PB values < 50 %)

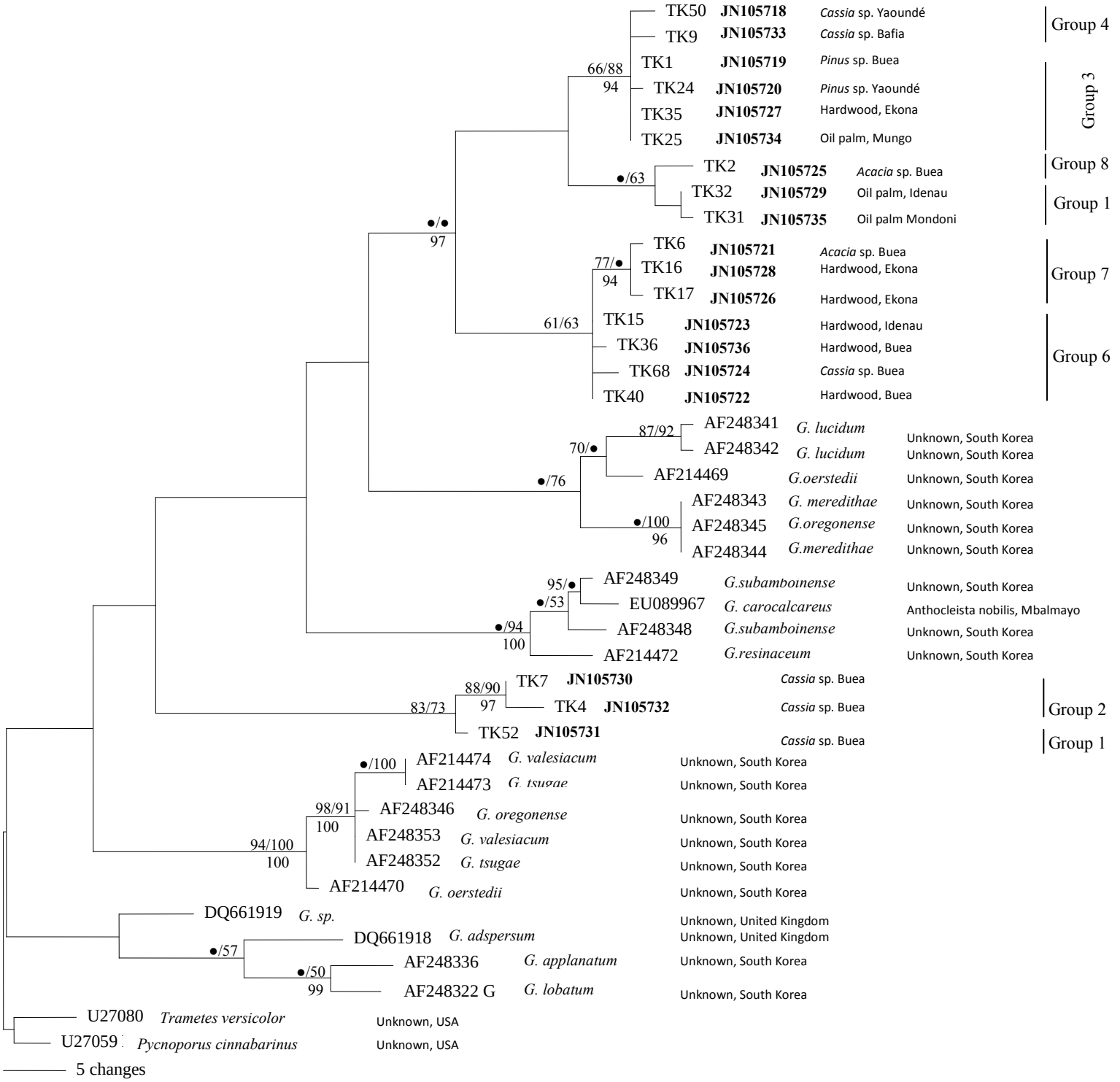

Figure 5: Phylogenetic tree generated from the mtSSU *Ganoderma* matrix, based on parsimony. GenBank accession number, tree host and origin are indicated next to the sample numbers. Maximum likelihood bootstraps (MLB) > 50%, Parsimony bootstrap (PB) values > 50% are indicated at the above the branches and Posterior Probability (PP) values > 95% below the branches. (● depicts MLB and PB values < 50 %)

isolates from Group 3 (PB: 93%, PP: 99%). Isolates belonging to Group 7 formed a monophyletic group based on their marginal posterior probability value (PP: 94%) (Figure 4).

The mtSSU *Ganoderma* Matrix included 39 ingroup and two outgroup taxa. Of the 987 total characters included in this matrix, 888 characters were constant and 99 were parsimony informative. Heuristic searches yielded eight parsimonious trees, with a TL = 270 steps, CI = 0.489 and RI = 0.747. Phylogram based on mtSSU of the combined isolates from Cameroon and those from GenBank resulted into eight major groups. Trees generated from this matrix did not reveal any supported phylogenetic relationship with *Ganoderma* species that showed a high mtSSU DNA sequence similarity from the BLASTn searches (Figure 5).

DISCUSSION

Eight groups of *Ganoderma* were identified during this study from different hosts and areas in Cameroon based on their morphological characteristics. Phylogenetic analyses placed these morphological groups into seven monophyletic groups based on ITS DNA sequence data. Phylogenetic trees generated from mtSSU sequence data suffered from low bootstrap and posterior probability values, and generally did not support most of the monophyletic groups identified from the ITS sequence matrices. Three of the groups were identified as belonging to *G. ryvardense* (Group 1) *G. cupreum* (Group 2) and *G. weberianum* (Group 7), respectively, based on their phylogenetic relationships with these species. The remaining groups represent distinct species that are probably new to science.

Group 1 was identified as *G. ryvardense*. This species was described as a new species by Kinge & Mih (2011) and reported to be pathogenic to oil palms in Cameroon. During the current study basidiocarps belonging to this species were collected from *Cassia* sp., and hence broadening the known host range in Cameroon. Basidiocarps could be differentiated from other species based on their ellipsoid basidiospores with slightly truncated apices and larger size. *Ganoderma ryvardense* formed a sister group with *G. steyaertayanum* based on ITS sequence data, supporting the findings of Kinge & Mih (2011).

The second species group (Group 2) showed a close association with *G. cupreum* and *G. mastoporum*. Basidiocarps residing in this group are morphologically distinct by having laccate crust which partially extends over the pore layer, spores that are truncated, ovate to broadly elliptical with smaller basidiospores compared to other species, including *G. weberianum*. The basidiocarp in this group showed morphological similarity with *G. cupreum*. What is currently known as *G. cupreum*, was also named *G. chalceum* by Steyaer (1972), Corner (1983) and Hood *et al.* (1996). However, the name *G. cupreum* takes priority as this is the older name (Moncalvo & Ryvarden, 1997). Corner (1983) suggested that *G. boninense, G. lamaoense, G. leytense, G. malayanum, G. multiplicatum, G. sarasinii* and *G. subfornicatum* should be synonymized with *G. cupreum*. Corner (1983) and Quanten (1997) reported minor morphological differences between *G. polymorphum* and *G. cupreum*. More recently, Smith & Sivasithamparam, (2003) noted the morphometric and macromorphology similarity of these two species. *Ganoderma cupreum* is found in the *G. chalceum* complex, a species complex pantropical in distribution and related to the *G. lucidum* group (Moncalvo & Ryvarden, 1997). In the current study, *G. cupreum* and *G. mastoporum* isolates from GenBank could not be differentiated based on ITS sequences,

and cluster together with high bootstrap support and posterior probability. However, the morphological characters of isolates from Cameroon were similar to *G. cupreum* and the BLASTn resulted in high similarity with *G. cupreum* from Australia.

Basidiocarps belonging to Group 7 were identified as *G. weberianum* based their morphological and ITS sequence similarity with this species. Basidiocarp of this species were collected on *Acacia sp.* and hardwood from Buea and Ekona, respectively. *Ganoderma weberianum* can be differentiated from other species by the presence of resinous layer in the woody context of the pileus and stipe, and also the presence of few gasterospores in the context. Also, the basidiospores are smaller in size compared to *G. ryvardense* and *G. weberianum*. Moncalvo & Ryvarden (1997) suggested, based on morphology and mating tests, that *G. rivulosum* and *G. lauterbachii* are synonymous with *G. weberianum*. Moncalvo *et al.,* (1995a, 1995b) found that isolates of *G. weberianum* from Australia could be differentiated from *G. microsporum* using ITS rDNA sequence data. However, isolates of *G. weberianum* from Asian could not be separated separated from those representing *G. microsporum*. Smith & Sivasithamparam, (2003) later showed that the two species could be separated by the presence of gasterospores in *G. weberianum.* In the current study, gasteropores were observed for the basidiocarps residing in Group 7 and ITS sequences from this group had high similarity with sequences belonging to *G. weberianum* from Australia (AY569451). Based on these results, we believe that this group belongs to *G. weberianum*.

Morphological and ITS Groups 3, 4, 5 and 6 could not be identified to species level using morphological and DNA sequence characteristics. Similarly, morphological group 8 found on

the mtSSU tree for which an equivalent ITS group was not found, could not be identified. For simplicity, these groups are referred to as *Ganoderma* sp. 1 to 5.

Ganoderma sp. 1 (Group 3) was collected on *Pinus* sp. and hardwood from Bafia, Ekona and Yaounde. Basidiocarps of this species are light red with a rounded and yellowish margin and can be separated from the other taxa only by ITS sequences. *Ganoderma* sp. 2 (Group 4) was collected on *Cassia* sp. from Bafia and Yaounde. The pileus of the basidiocarps is dark brown and pileus margin is brown and lobed. This species can differentiated from other species only by ITS sequences. *Ganoderma* sp. 3 (Group 5) was collected on oil palm from Lobe estate, it has brown pileus with lobed and white margin, it is distinguished from other taxa by larger basidiospores. *Ganoderma* sp. 4, which belong to morphological group 6, was collected on *Acacia* sp., *Cassia* sp. and hardwood from Buea and Idenau. Basidiospore is brown to reddish brown. Basidiocarps belonging to *Ganoderma* sp. 5 (Group 8) were collected on *Acacia* sp. from Buea. This species has a characteristic reddish black pileus and margin is white and lobed. This species can be separated from other taxa based on their morphology and mtSSU sequence only.

In this study different species of *Ganoderma* were found on oil palms in plantations from Cameroon. *Elaeis guineensis, Acacia* sp., *Cassia* sp., *Pinus slyvestris* and some unidentified hardwood were found to be the major hosts to *Ganoderma* species with the highest records from oil palm plantations which showed high incidence of infection causing basal stem rot disease. At least 15 species of *Ganoderma* have been reported to be associated with basal stem rot disease on oil palms (Turner, 1981). In Indonesia and Malaysia oil palms were reported to be affected by a single species, namely *G. boninense* Pat. (Utomo *et al.*, 2005). In Papua New Guinea this

disease is caused by *G. boninense* and *G. tornatum* (Pilotti *et al.*, 2004). In this study the clades representing isolates from oil palm were very diverse and placed at different position on the phylograms, suggesting that different species of *Ganoderma* occur on oil palm and cause basal stem rot disease.

The main use of basidiocarps morphology complicated the taxonomy of species of *Ganoderma.* (Steyaert, 1972; Bazzalo & Wright, 1982; Adaskaveg & Gilbertson, 1986). This is because different species often share the same basidiocarp characteristics, which make it difficult to distinguish between them. Therefore such species appear similar while they represent different species. Furthermore, basidiocarps are produced seasonally and in fast grown plantations basidiocarps may be absent on disease trees (Lee, 2000), which makes identification of the fungus very difficult or impossible. Most basidiocarp characters are affected by environmental conditions during development, which result in high level of intra-species variation in some species caused by the morphological plasticity (Lee, 2000) .

In this study a high level of variability was observed in morphological characters among basidiocarps within species of *Ganoderma* from oil palm. This is in concordance with studies that showed morphological variation among conspecific isolates due to environmental conditions as isolates from oil palm were from different localities that varied in latitude and altitude. Steyaert (1975a), for example, demonstrated that the basidiospore size of *G. tornatum* (Pers.) Bres varies with latitude and altitude. Similarly the context colour of *G. lucidum* was darker in collections from the more Southern latitudes on the European continent than northern latitudes (Steyaert, 1972). Results from this study together with others reinforce the views of Ryvarden

(1995) & Moncalvo (2000) that basidiocarp morphology in the Ganodermataceae is highly plastic. It is therefore concluded that species of *Ganoderma* are highly variable and consist of many species complexes which was evident in this study in the *G. lucidum* and *G. resinaceum* groups.

Multi-copy genes, such as the tandem repeated rDNA operon, often presents intra-strain heterogeneous copies of the genes. This phenomenon has been recognized in various fungal species (e.g. in *Fusarium* sp. O'Donnell *et al.,* 1998 and *Armillaria* from Bhutan, Coetzee *et al.*, 2005). In this study, some basidiocarps yielded multiple ITS PCR fragments making sequencing impossible. Furthermore, sequence heterogeneity was observed within some individual samples. In *Ganoderma,* intrastrain heterogeneity in ITS sequences was previously reported for *G. applanatum var. gibbosum, G. fornicatum, G. japonicum and G. neojaponicum* strains (Wang & Yao, 2005) and *G. phillippii* from Indonesia (Coetzee *et al.*, 2011). However, Coetzee et al. (2011) observed heterogeneity only in the ITS-1 region; the ITS-2 region could therefore be used for species identification (Coetzee *et al.*, 2011). In the current study, heterogeneity was mostly observed in the ITS-1 region. However, since the complete ITS region (ITS1, 5.8S and ITS2) was needed for phylogenetic analyses, isolates with heterogeneous sequences were discarded.

The mtSSU yielded much lower resolution than the ITS sequences in this study. This is in contrast to Hong *et al.,* (2002) who showed that mtSSU rDNA sequences had 3.3 times more information than the ITS sequences among the species of *Ganoderma*. However, the lower resolution in the mtSSU it is supported by Douanla-Meli & Langer, (2009) who observed that the

ITS region contains 2.5 times more information than the mtSSU region for the species of *Ganoderma* that they investigated. For this reason we consider sequences from the ITS region to be a more useful diagnostic marker to distinguishing between species of *Ganoderma*, specifically from Cameroon.

Phylogenies were constructed using the ITS and mtSSU regions from a wide collection of species of *Ganoderma* isolated from different hosts from Cameroon. A general close relationship was observed among isolates of *Ganoderma* from Cameroon and those from diverse origins such as Australia, China, Britain and Finland. Also, a close relationship was observed among the Cameroon and South Korean isolates of species of *Ganoderma* and an isolate from Cameroon when the mtSSU gene region was used, this might imply that only few sequences of *Ganoderma* species based on mtSSU gene region are present in GenBank and those present are from South Korea. Hence, there is need to increase the mtSSU sequence database.

This study presents the identification and phylogeny of *Ganoderma* from different host and regions in Cameroon. Three known species, *G. ryvardense*, *G cupreum* and *G. weberianum* were recognized, of which the two latter species are new records to Cameroon. Five species designated *Ganoderma* sp. 1 – 5, were identified and awaits species descriptions from future studies. To the best of our knowledge this is the first comprehensive study differentiating species of *Ganoderma* from Cameroon based on both morphological and molecular data.

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