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Full Length Research Paper

Evaluation of antifungal activity of snake venoms from the Amazon forest

Marcelo dos Santos Neves[1], Diego Rayan Teixeira de Sousa[3], Maria do Perpétuo Socorro Borges Carriço Ferreira[2], Maria Zeli Moreira Frota[2], João Vicente Braga Souza[3*] and Jorge Luis López Lozano[1]

[1]Universidade do Estado do Amazonas/Fundação de Medicina Tropical- Dr Heitor Vieira Dourado, Brazil.
[2]Federal University of Amazonas / UFAM, Brazil.
[3]Mycology Laboratory of the National Institute of Amazonian Research / INPA, Brazil.

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In recent years, many antimicrobial peptides have been found in the venoms of animals from different sources and have been intensively studied to elucidate their ability to inhibit the growth of potential pathogenic microorganisms. The aim of this study was to characterize and evaluate the *in vitro* antifungal activity of crude venom from two amazonian snakes: *Bothrops atrox* and *Crotalus durissus ruruima*. The molecular profile of representative proteins from the venom samples was obtained by reversed-phase high-performance liquid chromatography (RP-HPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Fungal inhibition was investigated by microdilution assays against two *Candida albicans* strains. Based on the chromatography and electrophoresis analyses, the venom from *B. atrox* and *C. durissus ruruima* were characterized. In addition, the venoms (400 µg/mL) were not able to cause significant inhibition (> 50%) of the growth of *C. albicans* KL-07, at only 9.09% (200 µg/mL) and 7.88% (400 µg/mL), respectively, and neither presented any influence on the growth of strain *C. albicans* ATCC 36232.

Key words: Antifungal, activity, snake and Amazon.

INTRODUCTION

The venoms of animals have evolved to generate a broad group of peptide toxins for capture and defense. These peptides are directed against a wide variety of pharmacological targets and represent sources of prototype drugs. Some of these peptides have been used for *in vivo* studies to prove their effectiveness, in preclinical or clinical trials, for developing treatments for pain, diabetes, multiple sclerosis and cardiovascular disease (Lewis and Garcia, 2003).

Most venoms comprise a mixture of highly complex peptides, usually showing diverse and selective pharmacology. Despite this diversity, venom peptides appear to have evolved from a relatively small number of structures that are particularly well adjusted to meet critical issues

*Corresponding authors. E-mail: joao.souza@inpa.gov.br. Tel: +55 (92) 9114-7815. Fax: +55 (92) 3643-3377.

of potency and stability (Lewis and Garcia, 2003). Studies have demonstrated that the peptides present in the venom from snakes, wasps, spiders and scorpions represent a new class of antifungal and antimicrobial proteins (Gomes et al., 2005; de Oliveira Junior et al., 2013).

In recent decades, an increasing number of human populations are becoming more susceptible to opportunistic fungal infections, which has led to a growing number of clinical cases involving emergent fungal species. Furthermore, the discovery of clinical isolates that exhibit inherent or developed resistance to drugs such as Amphotericin B, Clotrimazole, Econazole, Fluconazole, 5-Fluorocytosine, Itraconazole, Ketoconazole, Miconazole, and Nystatin presents a challenge when treating fungal infections. Therefore, studies aimed at the discovery of new antifungal drugs, particularly proteins derived from animal toxins, are fundamental and necessary to expand the therapeutic options, thus ensuring greater efficiency and control in the treatment of these infections (Arango et al., 2004).

Although it is important to evaluate the peptides from Brazilian snakes (Nunes et al. 2011; Okubo et al. 2012), studies on the antifungal activity and molecular characterization of proteins and peptides from snake venoms obtained from the Amazon as well as the in vitro growth inhibition of yeasts of clinical interest are scarce in the literature. This situation makes such work an important initiative for this line of research in the Amazon region (Núñez et al., 2009; Calvete et al., 2011). The objective of this study was to characterize and evaluate the *in vitro* antifungal activity of crude venom from two amazonian snakes: *Bothrops atrox* and *Crotalus durissus ruruima*.

MATERIALS AND METHODS

Venoms

The venom samples were obtained from adult specimens of *B. atrox* and *C. durissus ruruima*, species belonging to the Snakebite Center "Professor Paul Friedrich Bührnhein" from the Foundation of Tropical Medicine Hietor Viera Dourado (FMT-HVD). The venom was collected through manual pressure on the venom glands after anesthetization of the snakes with carbon dioxide (CO_2). The samples were centrifuged (5000 x g for 15 min), and the supernatant was filtered (0.45 µm), lyophilized and stored at -20°C.

Characterization of venom

The venoms from B. atrox and C. durissus ruruima were characterized by chromatographic and electrophoresis methods

Reversed-phase high-performance liquid chromatography: The protein fractions of the venoms used in inhibition tests were obtained using the conventional chromatographic method of reversed-phase fractionation using organic solvents and buffer solutions (López-Lozano et al. 2002). The detection of the molecular profile of the protein constituents of the venoms was achieved using reversed-phase high-performance liquid chromatography (RP-HPLC) with a semipreparative ODS column (Shim-Pack C18, 10 mm x 250 mm, 10 mM) equilibrated with 0.1% trifluoroacetic acid (TFA; solution A). The elution of the venom constituents was started with a continuous gradient flow of solution A for 10 min and 0.1% TFA in acetonitrile (solution B) from 0 to 60% in 70 minutes (min). The flow rate for the elution of the constituents was 2.5 ml/min using an analytical monitoring detector at 216 nm. For each chromatographic process, 4 mg of each sample was applied, with two chromatographic analyses each (Ali et al. 2010).

Electrophoresis

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique described by Laemmli (1970) used was. The running gel concentration was 15%, and the stacking gel was 4%. The venoms were diluted (volume to volume) with sample buffer (0.125 M Tris, 2% SDS, 10% glycerol and 0.05% bromophenol blue, with or without β-mercaptoethanol), resulting in reduced and non-reduced fractions, respectively. The samples were heated for 5 min at 100°C (reduced) or 40°C for 30 min (non-reduced). *B. atrox* at a concentration of 0.2 µg/µl and *C. durissus ruruima* at a concentration of 0.4 µg/µl, along with two controls for race *B. atrox* and *C. durissus ruruima,* both at a concentration of 10 µg/µL, were electrophoresed at a constant current of 20 mA/gel using Tris-glycine, pH 8.3 (0.025 M Tris, 0.192 M glycine, 0.1% SDS) as the running buffer. The gels were then stained with silver nitrate, as described (Babaie et al., 2013).

Evaluation of antifungal activity of venoms

In vitro tests were used to evaluate the antifungal activity of the venoms. A *Candida albicans* strain, identified with code KL-07, isolated from a patient with a clinical and laboratory diagnosis of chronic or recurrent vulvovaginal candidiasis that showed clinical resistance to conventional therapy at the referral center for Diseases sexually Transmitted Infections - Foundation of Tropical Dermatology and Venereology "Alfredo da Matta" (FUAM) was used. The standard strain of *C. albicans*, ATCC 36232, provided the Mycology Laboratory of the National Institute for Amazonian Research (INPA) was also used.

The initial concentration (or stock) of *B. atrox* and *C. durissus ruruima* venoms used in the tests was 20 and 10 mg/ml, respectively. The MICs of the venoms were determined according to CLSI M27-A2. The final concentrations ranged from 400 to 0.8 µg/ml for both venoms, and 64 to 0.06 µg/ml ketoconazole was implemented as a control. Microdilution trays containing 100 µL of twofold serial dilutions of the antifungal in standard RPMI 1640 broth were inoculated with 100 µl of the fungi at $2.5x10^3$ CFU/ml and incubated in ambient air at 35°C for 24 to 48 h. Reference MICs were defined as the lowest drug concentration that showed 50% of growth inhibition compared with the control (Pfaller et al,. 2013).

Statistical analysis

All experiments were performed in triplicate, and the data were used to calculate the average, median and standard deviation (SD) of the readings. In comparing the medians of different dilution levels, the test Non-parametric Kruskal-Wallis test with a significance level of 5% was used. Epi-Info 3.3 software for Windows, developed and distributed by the CDC (www.cdc.org / epiinfo), was used in the analysis.

RESULTS

In order to characterize the venoms, HPLC and electro-

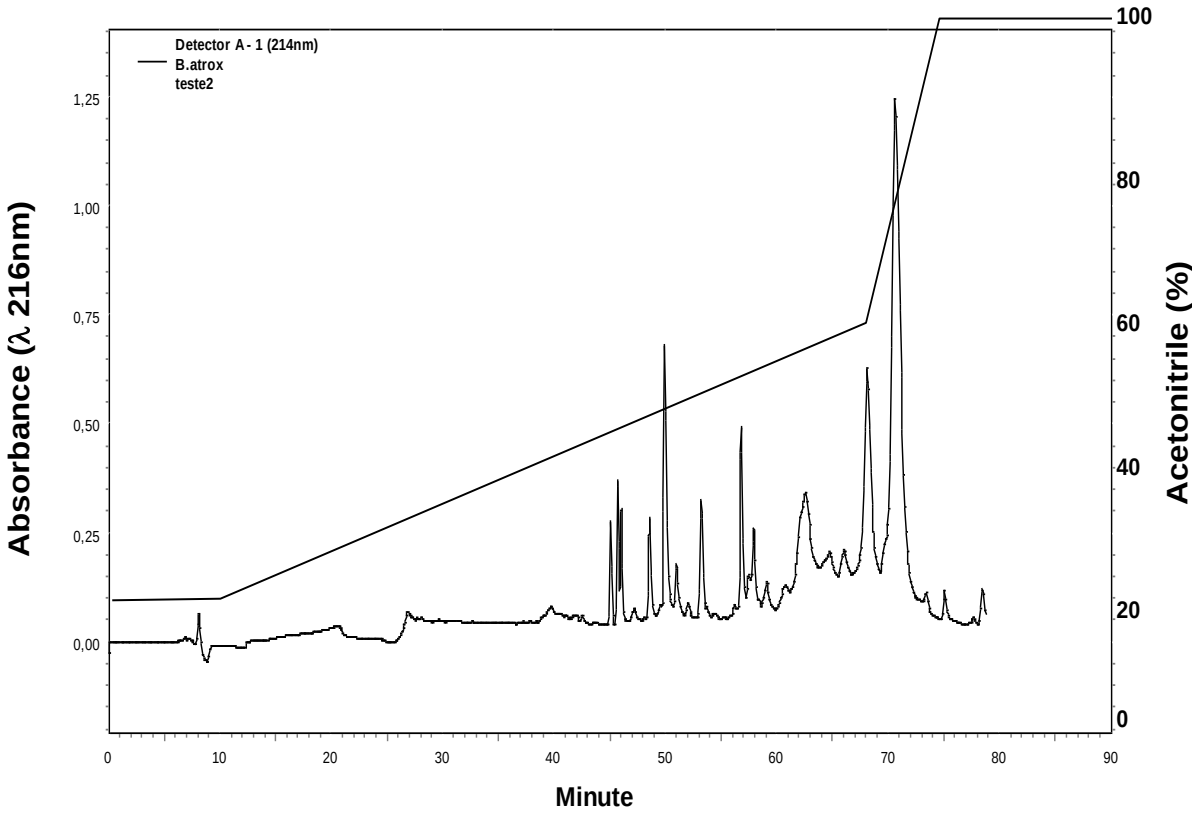

Figure 1. HPLC chromatographic profile of venom from *B. atrox* using a flow rate of 1 mL/minute. Solution A - 0.1% TFA; solution B - 0.1% TFA in acetonitrile; Column - C18 RP.

phoresis analysis were carried out. The chromatographic profiles (HPLC-RP-C18) for the *B. atrox* and *C. durissus ruruima* venoms are shown in Figures 1 and 2, respectively. The profiles presented differences in peak number, peak intensity and peaks/time interval.

The electrophoresis (SDS-PAGE) profiles of the venoms were different under reducing and non-reducing conditions (presence or absence of β-mercaptoethanol) (Figures 3). In addition, the predominant bands in both venoms were of approximately 14, 23 and 50 kDa .

The antifungal activity of the venoms was investigated. In Figure 4 is shown the influence of different contents of the venom of *B. atrox* and *C. durissus ruruima* on the growth of *C. albicans* KL-07.

The venoms (400 µg/mL) were not able to cause a significant inhibition (> 50%) of the growth of *C. albicans* KL-07, at 9.09% (200 µg/mL) and 7.88% (400 µg/mL), respectively. Furthermore, neither venom presented any influence on the growth of *C. albicans* strain ATCC 36232.

DISCUSSION

In recent years, many antimicrobial peptides have been found in the venoms of animals from different sources and have been intensively studied to elucidate their ability to inhibit the growth of potential pathogenic microorganisms (Liu et al., 2013; Bahar and Ren, 2013; He et al., 2013). The data presented in the present work constitute important preliminary information about the characteristic and antifungal activity of the venom from *B. atrox* and *C. durissus ruruima.*

Our characterization (chromatography and electrophoresis assays) demonstrated that the venoms have distinctive profiles of expected protein fractions in a snake venom (Liu et al., 2013; Bahar and Ren, 2013; He et al., 2013). The chromatograms and SDS-PAGE profiles presented here are important for comparisons with future works intended to investigate the characterization and/or biological function of these venoms.

By investigating the influence of the venoms on the growth of *C. albicans* KL-07, only slight activity was observed for both, with a poor correlation between the venom content and growth inhibition. Similar results were previously described in bioassays with venoms (Bustillo et al., 2008; Afc et al., 2010). One explanation for this situation is the existence of an optimal concentration for inhibition or that interactions between the venom compounds at different concentrations cause interference with growth inhibition (Haeberli et al., 2000; Kuhn-Nentwig et al., 2012; Ciscotto et al., 2009). Previous studies corroborate the last explanation in that the peptide

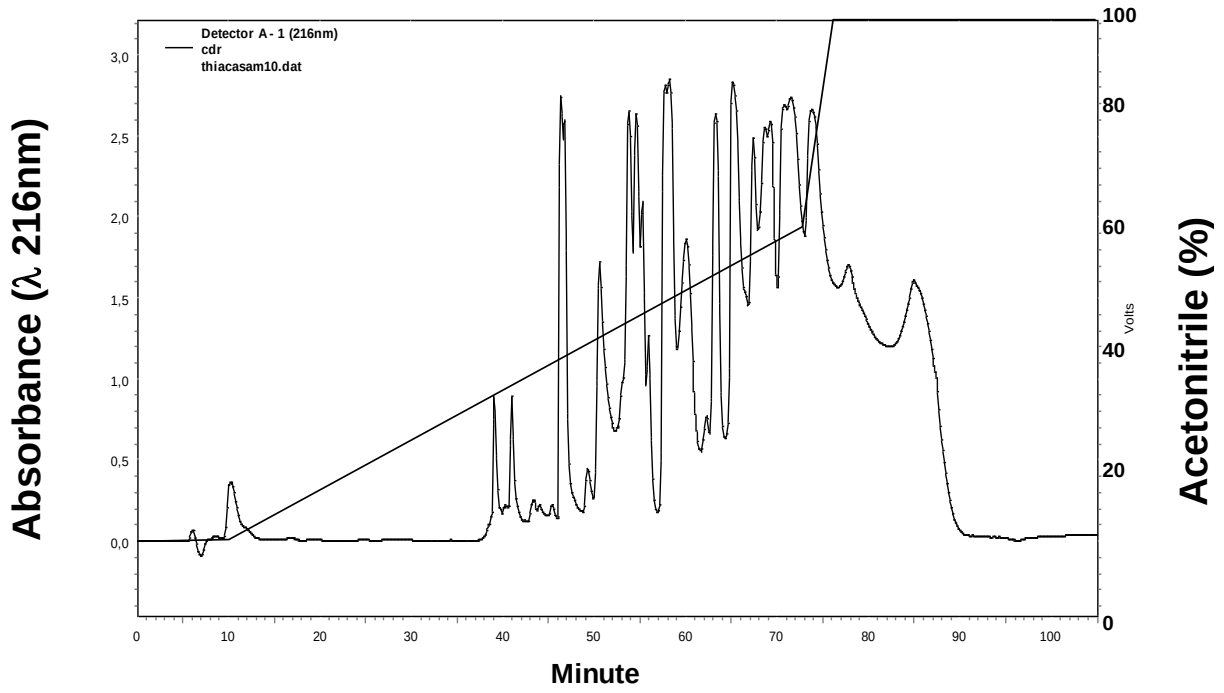

Figure 2. HPLC chromatographic profile of venom from *C. durissus ruruima* using a flow rate of 1 mL/minute. Solution A - 0.1% TFA; solution B - 0.1% TFA in acetonitrile; Column - C18 RP.

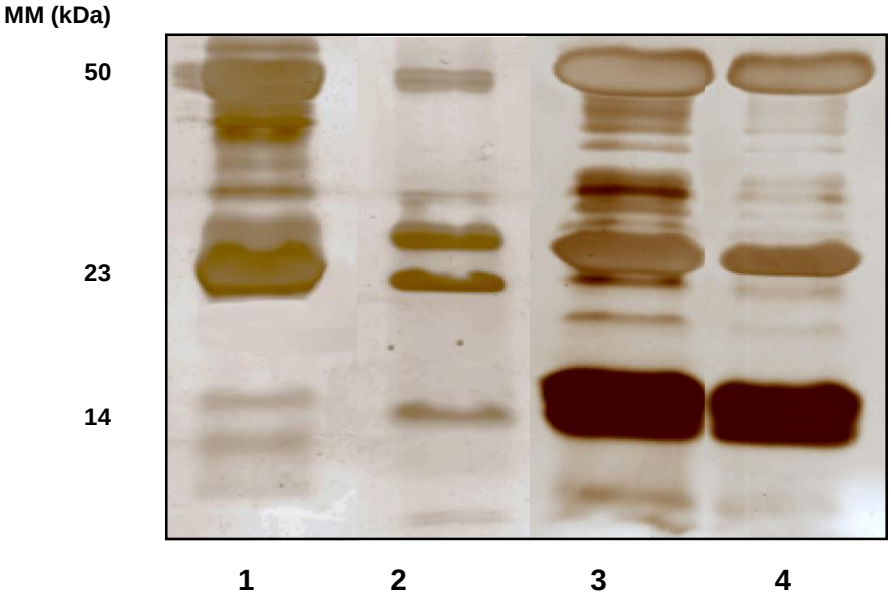

Figure 3. SDS-PAGE profile of the venom (10 µg/µl) from *B. atrox* (lanes 1 and 3) and *C. durissus ruruima* (lanes 2 and 4) under non-reducing (lanes 1 and 2) and reducing (lanes 3 and 4) conditions.

fraction of the venom from *Bothrops jararaca* showed higher inhibitory activity on the growth of *Candida albicans* than the unfractionated venom (Gomes et al., 2005). According to the data obtained, the venoms may have also suffered from proteolysis by *C. albicans* proteases (Castro and Lima, 2012; Demitto et al., 2012).

The absence of inhibition against *C. albicans* ATCC 36232 demonstrated a difference between the two strains,

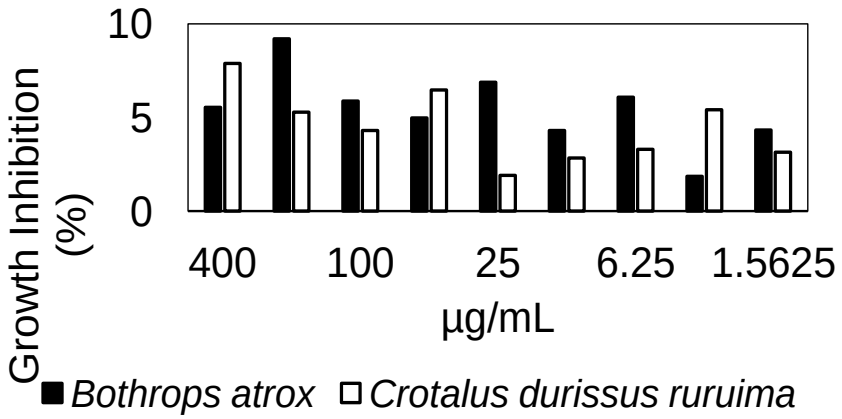

Figure 4. Influence of different contents of *Bothrops atrox* and *Crotalus durissus ruruima* venom on the growth of *Candida albicans* KL-07.

and mechanisms that prevent the binding of specific proteins to the fungal cell membrane is a possible explanation (Maróti et al., 2011; Barbosa et al., 2011).

Future works should avoid self-degradation caused by proteases from the venom (Schneiter and Di Pietro, 2013; Röhm et al., 2013) because both venoms presented high concentrations of metalloproteases (Calvete et al., 2011).

Conflict of interests

The authors did not declare any conflict of interest.

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Diversity and distribution of species of *Ganoderma* in south western Cameroon

Tonjock Rosemary Kinge[1]* and Afui Mathias Mih[2]

[1]Department of Biological Sciences, Faculty of Science, University of Bamenda, P. O. ox 39, North West Region, Cameroon.
[2]Department of Botany and Plant Physiology, Faculty of Science, University of Buea, P. O. Box 63, South West Region, Cameroon.

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The genus *Ganoderma* is one of the most important group of Basidiomycetes due to their medicinal effects and also because they cause decay in a very wide range of tree species all over the world. Opportunistic sampling was used to collect 57 samples of *Ganoderma* from oil palm and other hosts for identification using comparative morphology and supported by molecular studies of the ITS and mtSSu gene regions. The objectives were to identify the species associated with different hosts, and to generate a checklist of species of *Ganoderma* in south western Cameroon. Morphological and molecular characterization of the 57 specimens showed that they belonged to 17 species of *Ganoderma*. Two species, *Ganoderma tornatum* and *Ganoderma chalceum* are known records for Cameroon. Four species, *Ganoderma weberianum*, *Ganoderma cupreum*, *Ganoderma steyaertanum* and *Ganoderma zonatum* are new records for Cameroon. The remaining 11 species *Ganoderma ryvardense*, *Ganoderma lobenense*, and *Ganoderma* species 1–9 with different affinities might be new to science. Six plant species were identified as hosts to different species of *Ganoderma*. They are *Elaeis guineensis*, *Cassia* sp., *Acacia* sp., *Pinus sylvestris*, *Avocado* sp. and unidentified hardwoods, with *E. guineensis*, hosting the highest number of species. With supplementary literature survey, a check-list of 23 species was established.

Key words: Host tree species, morphology, mushroom, taxonomy.

INTRODUCTION

The genus *Ganoderma*, a member of Aphyllophorales, was described by Karsten in 1881. The correct citation of the type species is written as *Ganoderma lucidum* (Curt; Fr) P. Karst., (Karsten, 1981). *Ganoderma* can degrade lignin component of wood while leaving white cellulose exposed, or as pathogens of living trees such as oil palm, rubber, tea and wood rot of forest trees, thereby causing diseases (Singh, 1991; Paterson, 2007). Several species are responsible for root and butt rots of commercially important crops such as tea [*Camillia sinensis* (L.) Kuntze],

*Corresponding authors. E-mail: rosemary32us@yahoo.com. Tel: +237 67462339.

rubber (*Hevea brasiliensis* Muell. Arg.), temperate hardwoods, coconut (*Cocos nucifera* L.) and betel nut palms (*Areca catechu* L.) (Singh, 1991). Several species cause basal stem rot of oil palm (*Elaeis guineensis*) (Kinge et al., 2012), and other tropical forest trees. The fruit body of *Ganoderma*, for its perceived health benefits, has gained wide popular use as a dietary supplement in China, Japan, North America and other regions of the world, including Cameroon. *Ganoderma* species are also used in folk medicine to cure various diseases, and strains are commercially cultivated for the preparation of health tablets or drinks. As a kind of health food, it has also been used to prevent and treat immunological diseases, such as hypertension, tumorigenesis, etc. (Liu et al., 2002; Kinge et al., 2011). The many medicinal benefits of *Ganoderma* were reviewed by Jong and Birmingham (1992). On the other hand, some *Ganoderma* species play an important role in plant pathogens. Several species cause severe diseases in plantations or in forests (Steyaert, 1967; Bakshi et al., 1976). However, some of them have been shown to selectively delignify wood and are recognized as a potentially important source of lignin-degrading enzymes (Otjen et al., 1987).

The genus *Ganoderma* was divided into two distinct groups. The laccate including the *Ganoderma lucidum* complex is characterised by the presence of a cutex layer on the outer surface of the fruiting body that renders it shiny. The non-laccate lacks the cutex layer and is referred to as *Ganoderma applanatum* complex. Over 290 taxonomic names have been published in the genus *Ganoderma*, indicating that the genus is morphologically complex (Ryvarden, 2000). This led Ryvarden (1991) to describe the state of *Ganoderma* taxonomy as being in crisis. Traditional identification of *Ganoderma* species has been based on morphological features, physiological and developmental characters and chemical components such as secondary metabolites (Takamatsu, 1998). Species concept in the genus *Ganoderma* is thus not universally accepted neither well established (Gottlieb et al., 2000).

It has been shown that morphology and culture characteristics of species from the same genus can be greatly affected by growth conditions (Moncalvo, 2000). This signifies that a large number of synonyms may exist due to the number of species that have been identified based on morphology (Moncalvo, 2000). The shape of basidiocarp (fruiting body) has been demonstrated to be greatly influenced by the environment (Chen, 1993), and the basidiospores by latitude and altitude (Steyaert, 1975). In some species, the context colour was darker in collections from southern latitudes than northern latitudes on the European continent (Steyaert, 1972). Age and environment have been shown to have a marked effect on the colour, size and brightness of the fruiting body, as well as length of stipe (Moncalvo, 2000). Identification of *Ganoderma* based on these characteristics have contributed greatly to the confusion in the naming of species within this genus, and have resulted in traditional taxonomic methods being inconclusive for establishing a stable classification system for *Ganoderma* species (Hong et al., 2002; Hseu et al., 1996). Traditional identification methods are being supplemented with new identification methods such as restriction fragment length polymorphism (RFLP) (Miller et al., 1999), sequence analysis (Hong et al., 2002; Moncalvo et al., 1995a, b; Smith and Sivasithamparam, 2000a) and isoenzyme electrophoresis (Gottlieb et al., 1998; Gottlieb and Wright, 1999; Smith and Sivasithamparam, 2000b). It is the phylogenetic analysis of amino acid or DNA sequences that is known to have the highest resolving power (Bruns et al., 1991). These modern techniques have helped to clarify the distribution of different species complexes in the genus *Ganoderma,* and have revealed some instances of misidentification (Gottlieb et al., 1998; Moncalvo et al., 1995a, b).

Despite advances in taxonomic techniques, the species diversity of *Ganoderma* and other polypores in Africa have received very little attention. In Cameroon, the following species have been reported: *Ganoderma tornatum* var. *tornatum*, *Ganoderma hildebrandii*, *G. lucidum*, *Ganoderma* cf. *multiplicatum*, *Ganoderma resinaceum*, *Ganoderma carocalcareus* and *Ganoderma ryvardense* (Turner, 1981; Nunez and Daniels, 1999; Douanla-Meli and Langer, 2009; Kinge, 2012). Moncalvo and Ryvarden (1997) listed 49 *Ganoderma* species from Africa. Apart from the work published by Douanla-Meli and Langer (2009), Kinge (2012) and Kinge et al. (2012) all other reports based their identification on morphology alone. It is therefore reasonable to suggest that a wealth of information is waiting to be discovered. As very little is known about the diversity of *Ganoderma* in Cameroon. In order to understand the diversity of *Ganoderma* species, the knowledge of their distribution and association with their substrates are essential. The substrates such as dead and decaying wood and its associated fungi and invertebrates are vital elements of the forest ecosystem and their decay processes represent a key path for nutrient and carbon recycling (Bobiec et al., 2005). Thus, the objectives of this research were as follows: to identify the different hosts of species of *Ganoderma* in the study area, to prepare a check-list of species of *Ganoderma* in the study area and to produce a species distribution map.

MATERIALS AND METHODS

Study area and sampling

Collection trips were done between 2008-2011 from Lobe, Bai, Dibombari, Idenau, Bota, Mondoni, Mungo and Beneo estates as well as on forest areas in Buea, Idenau, Ekona and Bafia in the Mount Cameroon Region all in South Western Cameroon (Figure 1). An opportunistic sampling method was used in collecting the samples. Collection sites were geo-referenced by GPS points using the Garmin Etrex Venture GPS.

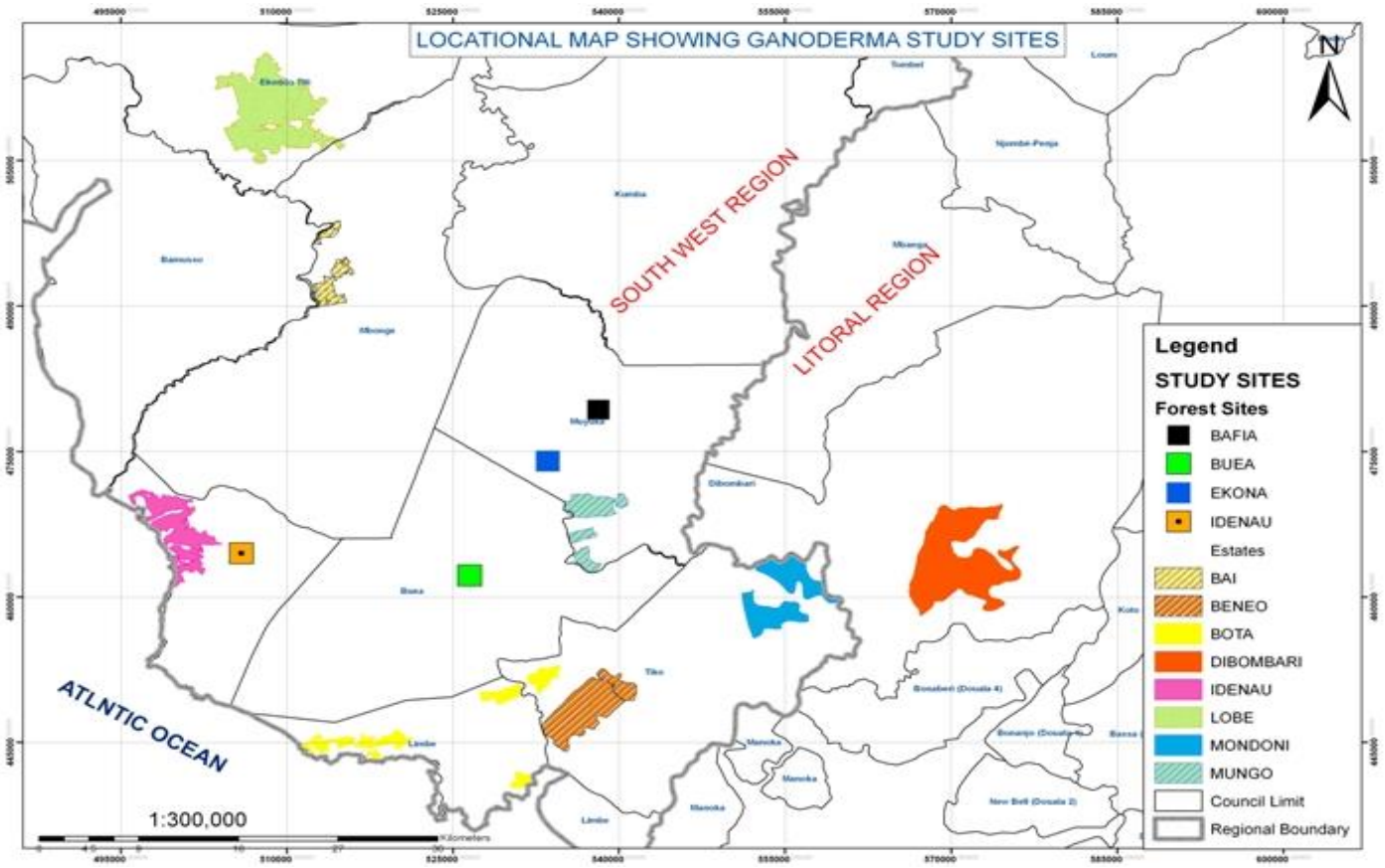

Figure 1. Sampling sites for diversity studies of species of *Ganoderma* in south western Cameroon.

Morphological characterization

External and internal morphology

Prior to examination of the basidiomata, specimens were photographed from above as per Steyaert (1972). External and internal morphological characters and confirmation by molecular methods have been described by Kinge et al. (2012).

After identification, a species distribution map based on the presence or absence of species was produced using GIS software (ArC GIS 9.3). A thorough literature search was also made to supplement data from the present field survey and a checklist for species of *Ganoderma* in Cameroon was produced.

RESULTS

Species diversity

A total of 57 samples were examined morphologically representing 17 species. Of the 17 identifiable entities, 6 were identified with known species. The 11 others had affinities with existing species and appeared to be new to science. Two of these, *G. ryvardense* and *G. lobenense* have been described as a new species (Kinge, 2012; Kinge and Mih, 2014).

Disease symptoms of *Ganoderma* on different hosts

Symptoms of *Ganoderma* disease was found on different hosts in the study area. This was evident with the presence of basidiocarp at the base of the stem in some cases. In oil palm, external symptoms observed included a one sided yellowing, or mottling of the lower fronds, followed by necrosis. The newly unfolded leaves were shorter than normal and chlorotic, and additionally the tips were necrotic in some plants. Also, with the progression of the disease within the plant, an overall pale appearance, with retarded growth was noticed and the spear leaves remain unopened.

Dead desiccated fronds droop at the point of attachment to the trunk or fracture at some point along the rachis and hang down to form a skirt of dead leaves. There is also the creation of bole at the base of the trunk, after which the palm breaks and falls over. In *Cassia* sp., wilting of the leaves was observed followed by yellowing of the leaves, defoliation and finally dieback was observed in the crown. In *Acacia* sp., there was wilting of the leaves, death branches and finally death of the tree. Only basidiocarp formation was observed on *Pinus sylvestris*, *Avocado* sp. and several unidentified hardwood (Figure 2).

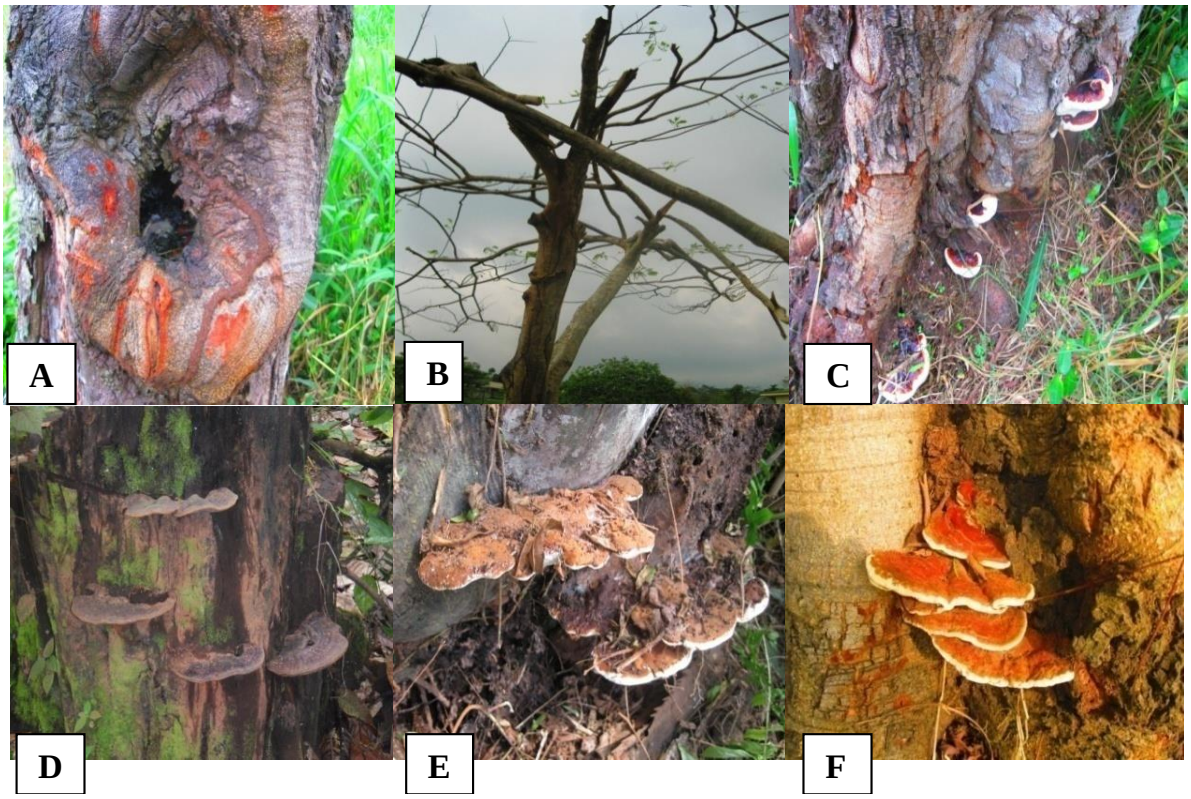

Figure 2. Symptoms of basal stem rot on different host caused by different *Ganoderma* species (A) *Acacia* sp., (B) *Cassia* sp., (C) *Cassia* sp., (D) *Avocado* sp., (E) oil palm, (F) HARDWOOD.

Table 1. Diversity of hosts and species of *Ganoderma* in south western Cameroon

Hosts	Species of *Ganoderma*
Elaeis guineensis	*G. ryvardense, G. lobenense, G. chalceum, G. steyartanum, G. tornatum, G. zonatum*, *Ganoderma* sp. 3
Cassia sp.	*G. cupreum*, *G. ryvardense, G. weberianum*, *Ganoderma* sp. 2, *Ganoderma* sp. 4
Acacia sp.	*Ganoderma* sp. 1
Pinus sylvestris	*Ganoderma* sp. 5
Avocado sp.	*Ganoderma* sp. 8
Unidentified hardwood	*Ganoderma* sp. 5, *Ganoderma* sp. 2, *Ganoderma* sp. 4, *Ganoderma* sp. 6, *Ganoderma* sp. 7, *Ganoderma* sp. 8, *Ganoderma* sp. 9

Natural range and distribution of species of *Ganoderma*

The plant species that were host to various species of *Ganoderma* are shown on Table 1. Of the 17 species obtained from the study area, eight were restricted to oil palm and nine species to ornamentals and forest trees. The species were well distributed except for species like *G. lobenense*, *G. weberianum* and *Ganoderma* species 1-9 with different affinities which were restricted to specific locations (Figure 3). There were six species reported in literature of which just one species, *Ganoderma tornatum,* was found in the present study.

Checklist of species of *Ganoderma* in Cameroon

Apart from the 17 species of *Ganoderma* collected from the study area, a review of the literature identified 8 other species from Cameroon, giving a total of 25 species of *Ganoderma* in Cameroon (Table 2).

DISCUSSION

The present study shows that 17 species of *Ganoderma* can be discerned based on comparative morphology. Comparative morphology remains the cheapest and most

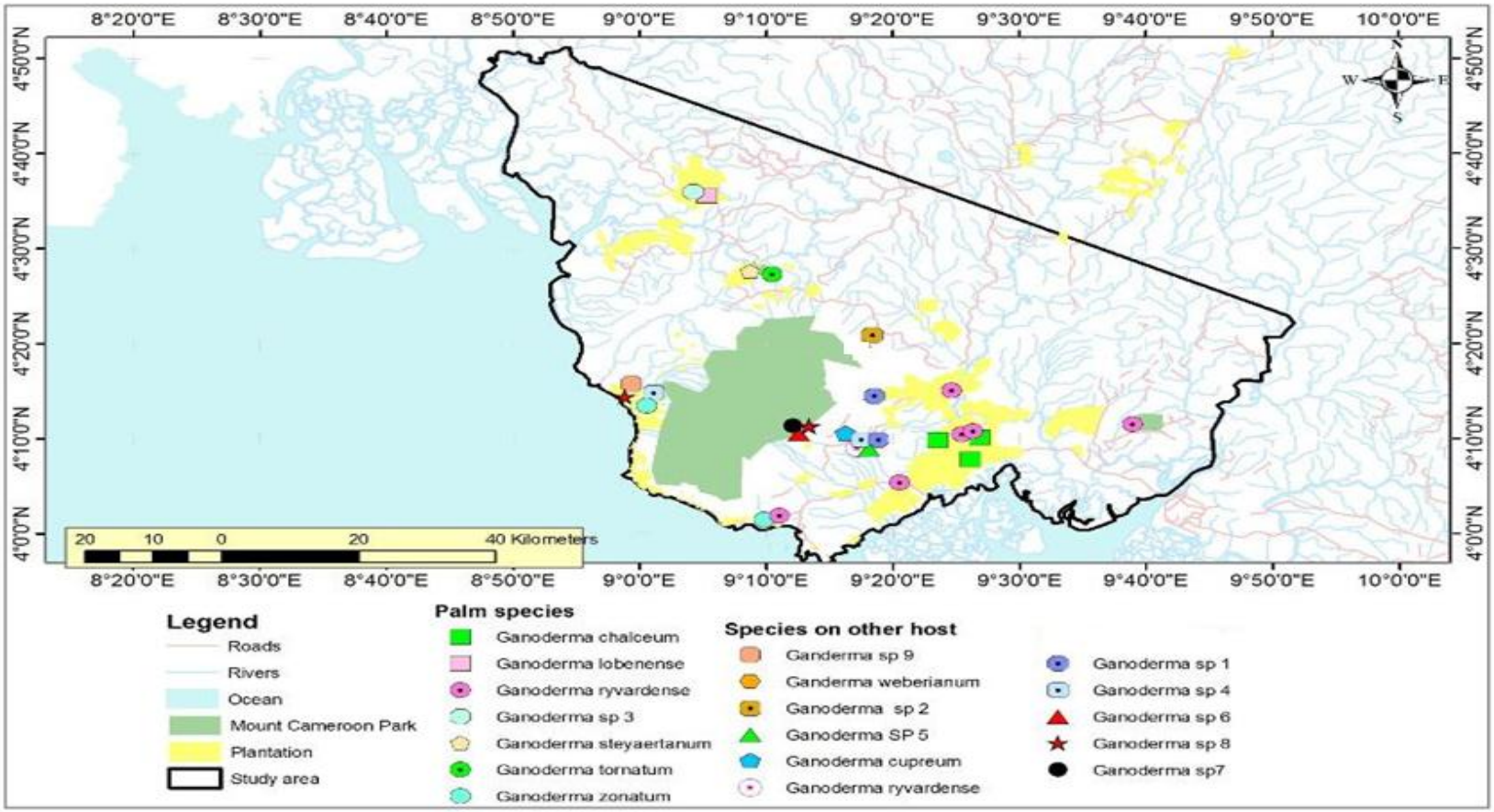

Figure 3. Distribution map of species of *Ganoderma* in south western Cameroon.

available tool for identification in the developing economies. This is evident with many authors who have used comparative morphology to study the taxonomy of *Ganoderma* from Cameroon. For example, Turner (1981) reported the occurrence of *G. tornatum* var *tornatum* from Cameroon but without specifying the locality. Nunez and Daniels (1999) identified *Ganoderma hildebrandii*, *G. lucidum* and *G. cf. multiplicatum* from the Dja biosphere reserve and recently, Douanla–Meli (2007) described *G. hildebrandii, G. lucidum and Ganoderma resinaceum* and *Ganoderma australe* from the Mbalmayo forest reserve.

This survey represents the first major investigation establishing a checklist of species of *Ganoderma* in Cameroon. A small number of collections have already been published, principally by different authors and are compared with the present study. We checked and studied a total of 57 collections representing 17 species of *Ganoderma* from the study area. Understanding

Table 2. Checklists of species of *Ganoderma* in Cameroon.

Species name	Annotation	Host	Locality	Voucher no.	Reference
Species identified in this study					
Ganoderma chalceum	–	*Elaeis guineensis*	Bota	HKAS 58056	Kinge , 2012
Ganoderma cupreum	–, #	*Cassia* sp.	Buea	PREM 60577	Kinge, 2012
Ganoderma lobenense	+	*Elaeis guineensis*	Lobe	HKAS 58059	Kinge, 2012
Ganoderma ryvardense	+	*Elaeis guineensis*, *Cassia* sp.	Dibombari, Mungo, Beneo, Bota, Ekona, Mondoni, Buea	HKAS 58053 PREM 60590	Kinge, 2012
Ganoderma steyaertanum	–, #	*Elaeis guineensis*	Bai	HKAS 58052 PREM 60589	Kinge, 2012
Ganoderma tornatum	–	*Elaeis guineensis*	Bai	HKAS 58057	Kinge, 2012 Turner, 1981
Ganoderma weberianum	–, #	*Cassia* sp.	Ekona, Buea	PREM 60587	Kinge, 2012
Ganoderma zonatum	–, #	*Elaeis guineensis*	Lobe, Idenau	HKAS 58060	Kinge, 2012
Ganoderma sp. 1	+	*Elaeis guineensis, Pinus sylvestris*	Ekona, Buea	PREM 60592	Kinge, 2012
Ganoderma sp. 2	+	*Cassia* sp., unidentified hardwood	Bafia	PREM 60582	Kinge, 2012
Ganoderma sp. 3	+	*Elaeis guineensis*	Lobe	PREM 60588	Kinge, 2012
Ganoderma sp. 4	+	*Cassia* sp., *Acacia* sp., hardwood	Buea	PREM 60595	Kinge, 2012
Ganoderma sp. 5	+	*Acacia* sp.	Buea	PREM 60576	Kinge, 2012
Ganoderma sp. 6	+	Unidentified hardwood	Buea	PREM 60593	Kinge, 2012
Ganoderma sp. 7	+	Unidentified hardwood	Buea	PREM 60594	Kinge, 2012
Ganoderma sp. 8	+	Unidentified hardwood, *Avocado* sp.	Idenau, Bafia	PREM 60581	Kinge, 2012
Ganoderma sp. 9	+	Unidentified hardwood	Idenau	PREM 60596	Kinge, 2012
Species in earlier studies					
Ganoderma hildebrandii	–	Unidentified hardwood, on the ground	Dja, Mbalmayo forest reserves	MA38255	Nunez and Daniels, 1999 Douanla – Meli, 2007
Ganoderma lucidum	–	Unidentified hardwood	Dja, Mbalmayo forest reserves	MA38189	Nunez and Daniels, 1999
Ganoderma cf. *multiplicatum*	–	Unidentified hardwood	Dja, Mbalmayo forest reserves	MA38262	Nunez and Daniels, 1999
Ganoderma resinaceum	–	Unidentified hardwood	Mbalmayo forest reserves	NS	Douanla – Meli, 2007
Ganoderma australe	–	Unidentified hardwood	Mbalmayo forest reserves	NS	Douanla – Meli, 2007
Ganoderma carocalcareus	–	*Anthocleista nobilis*	Mbalmayo forest reserves	DMC 322	Douanla – Meli, 2007
Ganoderma colossum	–	Unknown	Yaounde forest	DCM	Mossebo, 2012
Ganoderma baudonii	–	Unknown	Yaounde forest-	DCM	Mossebo, 2012

Species are annotated by: + = New species, – = known species and # = new records, NS = not stated.

the taxonomic status of species of *Ganoderma* in Cameroon, it is confirmed that till date, 25 valid species, some with affinities have been reported from Cameroon of which 17 species are reported in the present study. The work facilitates the understanding of species diversity of *Ganoderma* from Cameroon. Due to the high variability of morphological characters, the genus *Ganoderma* has been described as a fairly character poor genus by Moncalvo and Ryvarden (1997) and Douanla-Meli and Langer (2009).

As per the study, *E. guineensis, Cassia* sp., unidentified hardwood*, Acacia* sp*.* and *P. sylvestris* were found to be most susceptible hosts to species of *Ganoderma*. Oil palms of the CDC plantation were the most susceptible and showed high incidence of infection causing threat to the plantation. Seven species of *Ganoderma* (*G. ryvardense*, *G. lobenense*, *G. tornatum*, *G. chalceum*, *G. steyaertanum, G. zonatum* and *Ganoderma* sp. 3) were found associated with basal stem rot disease of oil palm. Six species out of the seven species were found to be host specific and found only in association with oil palm while *G. ryvardense* had dual host distribution because it was found to be pathogenic on oil palm and *Cassia* sp. Other species of *Ganoderma* encountered in the study area on landscape plants such as (*Acacia* sp., *Cassia* sp., *P. sylvestris*), unidentified hardwood, and forest trees are *G. cupreum, G. weberianum* and *Ganoderma* species with different affinities.

Species of *Ganoderma* have traditionally been reported to be a problem on oil palm and thus of economic importance to agriculture (Utomo and Niepold, 2000; Utomo et al., 2005). The present study showed that members of this genus can be of significant importance in horticulture, infecting landscape plants (*Pinus* sp., *Acacia* sp., *Cassia* sp.) and fruit trees (*Avocado*). They can also be of importance in forestry. Whereas some species were host-specific; others attached more than one host. This showed that the diseases caused by *Ganoderma* could rise to epidemic proportions as was observed in Mungo. Our study and observations strongly suggested that there is a dearth of information on *Ganoderma* species diversity and distribution in Cameroon, emphasizing the point made by Douanla-Meli and Langer (2009) about the poor state of knowledge on macrofungi in Cameroon. The checklist presented in this study contains only those *Ganoderma* taxa for which a reasonably confident identification has been obtained. *G. tornatum* and *G. chalceum* are known records for Cameroon. *Ganoderma weberianum, Ganoderma cupreum, Ganoderma steyaertanum* and *Ganoderma zonatum* are new records for Cameroon*. Ganoderma ryvardense, Ganoderma lobenense, and Ganoderma* species 1-9 with different affinities might be new to science and some are supported with molecular data.

The distribution of some species of *Ganoderma* at certain sites and hosts and not in others may be due to dispersal, if spores have just begun and they have not had enough time to expand their range into other habitats. It might also be because the species are habitat specific or because the viability of their spore is short. The species were well distributed except for species like *viz*. *G*. *lobenense*, *G*. *weberianum* and *Ganoderma* species with different affinities which are restricted to specific locations and hosts. The abundance of certain species such as *G. ryvardense* might be due to the fact that spores have acquired the capacity to remain dormant during unfavourable periods. Symptoms of basal stem rot disease on different hosts were evident on *Cassia* sp., *Acacia* sp., *Avocado* sp., *E. guineensis*, *Pinus sylvestris* and many unidentified hardwood. Different species of *Ganoderma* have been reported as the causal agents for basal stem rot disease of oil palm in Malaysia, Indonesia, Papua New Guinea and Cameroon. The most widely reported fungi associated with root- rot disease of tropical *Acacia* are species of *Ganoderma* (Glen et al., 2006). In Papua New Guinea an unnamed *Ganoderma* species was associated with root and butt rot in a plantation of *Acacia mangium* (Arentz and Simpson, 1988) and a sporocarp of *G. weberianum* (Bres. & Henn. ex Sacc.) Steyaert was also collected from a decayed stump in this plantation. This shows that species of *Ganoderma* have diverse hosts.

Conflict of interests

The authors did not declare any conflict of interest.

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