Ganoderma ryvardense sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon

Kinge TR and Mih AM*

Department of Plant and Animal Sciences, Faculty of Science, University of Buea, P.O. Box 63 Buea, South West Region, Cameroon.

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A species of *Ganoderma*, which could not be identified with any known species, was found during a survey of fungi associated with basal stem rot disease of oil palm in the littoral and south western areas of Cameroon. Morphological and molecular characterization showed that it is closely related to *G. steyaertanum* and *G. boninense*, but distinct from these in having ellipsoidal basidiospores with slightly truncated apices. Elucidation of the phylogenetic relationship with other species of *Ganoderma*, using internally transcribed rDNA sequences (ITS1, 5.8S and ITS2) showed that it is a distinct species, in the oil palm clade that is new to science. This species has been named *Ganoderma ryvardense* R.K. Tonjock & A.M. Mih, with the specific epithet in honor of Lief Ryvarden, a renowned mycologist who has contributed immensely to the African mycobiota and to the genus *Ganoderma*. The holotype (HKAS 58053) is lodged at the HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences) while all new sequences generated during the study are deposited in GenBank.

Key words – Ganodermataceae – Molecular – Morphological – Taxonomy

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*Corresponding author: Afui M Mih – e-mail – afuimih@yahoo.com

Introduction

Oil palm, *Elaeis guineensis* Jacq., is an important estate crop in Cameroon. It is produced mainly in the rain forest belt stretching along the Cameroon coast. The total area under production is estimated at 625,000 ha (Faostat. fao.org/site). Production is mainly by multinational corporations, although small holdings also contribute significantly to the national output (Bakoume et al. 2002). Despite the large area under production, the yields are still below world means (Tengoua & Bakoume 2005). Pests and diseases are major production constraints faced by agro industries as well as smallholders. Among oil palm diseases, basal stem rot caused by a soilborne fungus, *Ganoderma* sp., is next to vascular wilt caused by another soilborne fungus, *Fusarium oxysporum* f. sp. *elaeidis* (Tengoua & Bakoume 2005). The basal stem rot (BSR) is found throughout oil palm growing areas of Cameroon and is one of the major factors responsible for the low yields (Tengoua & Bakoume 2005). The disease has also been recorded in Malaysia, Indonesia, Nigeria, Ghana, Zaire, Angola, Tanzania and North Mozambique (Turner 1981). Several species of *Ganoderma* have been reported to be pathogenic on oil palm in different countries (Wakefield 1920, Steyaert 1967, Turner 1981). The BSR disease was first reported in Malaysia in 1930 and the causal agent was identified as *G. lucidum* (W. Curt.) Karst. (Turner 1981). Six additional species were later identified associated with BSR in oil

palms in Malaysia and Indonesia (Sumatra): *G. boninense* Pat., *G. miniatocinctum* Steyaert, *G. chalceum* (Cooke) Steyaert, *G. tornatum* (Pers.) Bers., *G. zonatum* Murill and *G. xylonoides* Steyaert (1967). Adaskaveg & Gilbertson (1986) and Miller (1995) identified the major pathogen in Malaysia as *G. boninense*. However, Idris (1999) reported another species, *G. zonatum* as being weakly pathogenic on oil palm in addition to *G. boninense*. Pilotti et al. (2004) identified two species associated with BSR disease of oil palm in Papua New Guinea, namely *G. boninense* and *G. tornatum*. The cause of the disease in Cameroon has simply been attributed to *Ganoderma* sp. (Turner 1981, Tengoua & Bakoume 2005).

The genus *Ganoderma* (type species *G. lucidum*) is easily recognised by its unique double-walled basidiospores, a feature diagnostic for the genus. The taxonomy of the genus is, however, poorly circumscribed, not universally accepted, and has been described as being in a state of chaos (Ryvarden 1991). The inadequacy of using only morphological features for identification and characterization of *Ganoderma* species has been recognised (Bazzalo & Wright 1982, Gilbertson & Ryvarden 1986). Moncalvo (2000) has used rDNA sequence data to demonstrate this inadequacy and noted that the oil palm clade of *Ganoderma* was highly heterogeneous, containing various taxa. We made a survey of oil palm plantations in Cameroon between 2008 and 2009 to identify the species diversity of *Ganoderma* associated with the BSR disease. We describe one of the species encountered that is new to science.

Materials and methods

Sampling and macro-morphological identification

Ganoderma basidiocarps were collected from two oil palm estates in Cameroon, namely, Lobe and Dibombari. Morphological characters such as laccate and non-laccate, type of basidiocarp (stipitate/sessile/dimidiate, imbricate, concave, number of concentric zones, etc.), margin shape (lobed, fertile/sterile, rounded/acute) and colour (brown, white, reddish, etc.), pores (colour, pores per mm^{-1}, angular/rounded), pore diameter, dissepiments and axes; tube size and colour, context, and spore characters, which are confirmative with the species of *Ganoderma* described by various authors (Ryvarden & Johansen 1980, Steyaert 1980, Corner 1983, Ryvarden 1995, Moncalvo & Ryvarden 1997, Gottlieb & Wright 1999, Ryvarden 2000) were considered for identification of the species in the present study.

Micro-morphological identification

For internal morphology, free-hand thin sections of dried basidiocarps, passing through the hymenium, were prepared. The sections were mounted in 5% KOH and observed under a compound microscope. Twenty measurements were taken of pore diameter, thickness of dissepiments and the distance between axes of pores. Twenty randomly selected basidiospores, from each specimen were measured, inclusive and exclusive of ornamentation. The results were coded as n/m/p, where n is number of basidiospores measured, m number of basidioma involved and p number of collections. The dimension of spores is given with the notation form (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 estimated. All materials examined are deposited in HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences) with voucher numbers HKAS58055, HKAS58053 and HKAS58054.

Molecular identification

DNA extraction, amplification and sequencing

Genomic DNA was isolated from 40–60 mg of dried herbarium material following modified extraction procedures of Jasalavich et al. (2000) using CTAB-extraction buffer (1% (w/v) CTAB; 0.7 M NaCl, 0.05 M tris-HCl, pH 8.0; 10 mM EDTA; 1% (w/v) PVP.40; 1.5% (v/v) ß-mercaptoethanol, chloroform-isoamylalcohol (24:1 v/v) and phenol-chloroform-isoamylalcohol (25:24:1 v/v/v). DNA amplifications via polymerase chain reactions (PCRs) were performed in a 96-well TGradient Thermocycler (Applied Biosystems, U.S.A). The complete internal transcribed spacer (ITS1-

5.8S-ITS2) was amplified with primer pairs ITS1/ITS4 and ITS5/ITS4B (White et al. 1990), respectively. The PCR was performed on 25 µL solution composed of 24 µm Master mix and 1 µL of extracted DNA. The 24 ml Master mix was composed of 15 µL H2O, 2.5 µL of 10× PCR-buffer with mM MgCl2 inclusive, 1 µL each of ITS1, ITS4 or ITS4B and ITS 5, respectively (5µM), 2.5 µL dNTPMix (200µM), 1 µL of BSA and 0.3 µL taq-polymerase (5 U/ µL, BM). PCR program was planned as follow: pre-denaturing: 94°C for 3–4 min, continued for 35 cycles with the following 3 steps: denaturing at 94°C for 40 seconds, annealing at 50°C for 40 seconds, and extension at 72°C for 40 seconds followed with one last extension in 72°C for 8 min. PCR products were checked on 1% agarose gel in 0.5× TBE buffer and stained with 0.5 µg/mL ethidium bromide. Purification was done with bioteke's Purification Kit (Bioteke Corporation, Beijing, China), according to the manufacturer's instructions. Purified PCR products were sequenced with an ABI 3730 DNA analyzer and an ABI bigdye3.1 terminator cycle sequencing kit (Sangon Co., Shanghai, China).

Sequence alignment and phylogenetic analyses

New sequences generated in this study are deposited in GenBank (ITS rDNA). A sequence homology search in the Gene Bank Nucleotide database of the National Center for Biotechnology Information (NCBI), Bethesda (www.ncbi.nlm.nih.gov/) was performed using the program Fasta 3.0. Sequences found during this search and which were included in this study for the purpose of sequence comparison to our data are listed in Table 1. These sequences of the combined ITS region (ITS1-5.8S-ITS2) were constructed and analyzed. Alignments were performed using ClustalX (Thompson et al. 1997) and manually optimized by eye. Phylogenetic analyses were performed by the maximum parsimony analyses (MP) in PAUP* 4.0b10 (Swofford 2002), and by the Bayesian inference (BI) analysis in MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003). Parsimony analyses used unweighted characters defined as unordered, and gaps were treated as missing data. Maximum parsimony analysis (MP) was performed using the following settings: a branch swapping algorithm, tree-bisection-reconnection (TBR), addition of sequences set to random with 1000 replicates, accelerated transformation, steepest descent not in effect, maxtrees was set to 100, multistate taxa were interpreted as uncertainty, topological constraints were not enforced and gaps treated as missing data. Relative robustness of the clades was estimated by bootstrap analysis using 1000 replicates (Felsenstein 1985). The estimated levels of homoplasy, retention and consistency indices were also determined in PAUP. The resulting phylogenetic trees were visualized with the TreeView 1.6.6 program.

Bayesian posterior probabilities (Zhaxybayeva & Gogarten 2002) were calculated with Markov Chain Monte Carlo (MCMC) algorithm by running simultaneously four Markov chains with the settings determined by MrModeltest. The Markov chains started from a random tree and ran for 1,000,000 generations, saving every 100th tree. The first 8,000 samples representing the burn-in phase of the analysis were discarded and the consensus tree was constructed on 12,000 samples.

Results

Macromorphological features

Taxonomic description

Ganoderma ryvardense R.K. Tonjock & A.M. Mih **sp. nov.** Fig. 1

MycoBank 519307

Etymology – The specific epithet refers to the renowned mycologist, Lief Ryvarden, who has contributed greatly to the African mycobiota and to the genus *Ganoderma*.

Basidiomata annuua, dimidiata, circularis, concava, circa 13 cm longa, circa 8.5 cm lata, superficies pilealis rubella, obscure undulata, tribus circulis concentricorum munita, margine rubello, undulato. Hymenophorus ligneus, brunneus. Contextus rubello-brunneus, 24 mm profundus. Tuba 7 mm profunda. Superficies pororum flavida. Pori angulares, 2–4/mm, 150–250 µm diam., dissepimentis 65–150 µm crassis, axibus 185–375 µm distantibus. Basidiosporae ellipsoidales vel stilloformes, apice subtruncato, ovoido vel plano; ornamentum apici ovoidi nullum; cum ornamento (9–)10–13(–14) × (5–)6–8 µm, ornamento excluso 9–12(–13) × (4–)5–7 µm.

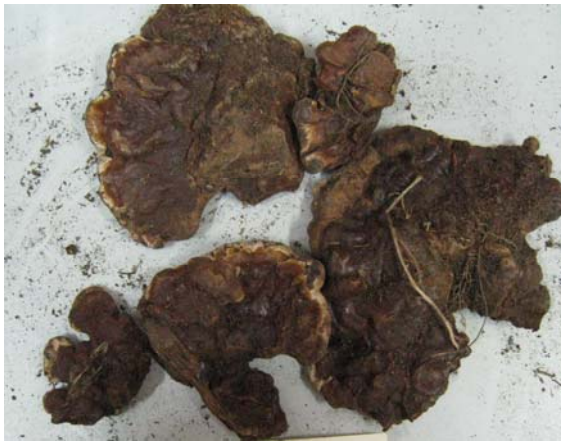

Fig. 1 – *Ganoderma ryvardense* from oil palm plantations in Cameroon.

Endosporae valde ornatae, distincte echinulae. Cellulae pilleipellis clavatae vel subclavatae, 6.5–10 µm diam.

Holotypus – Tonjock and Mih (HKAS 58053) 13 November 2008, Lobe Estate, South West Region, Cameroon.

Fruitbodies – Basidiocarp annual, dimidate, pileus concave and circular. Upper surface of pileus up to 13 cm in length and 8.5 cm wide. Pileus is reddish as well as the margin of the pileus. Margin pattern of the pileus is waved and the surface pattern is shallow waved with three concentric rings (Fig. 1). Hymenophore is woody dark brown and context is deep reddish brown. The context and tube depths are 24 and 7 µm, respectively. Pore surface light yellowish; pores angular; 2–4 per mm; pore diameter 150–250 µm; dissepiments 65–150 µm and axes 185–375 µm.

Basidiomata – (n/m/p) 20/1/1 including ornamentation (9–)10–13(–14) × (5–)6–8 µm; Q = 1.38–1.93(–2); Q_m = 1.71±0.17 and excluding ornamentation 9–12(–13) × (4–)5–7 µm; Q = 1.43–2(–2.25); Q_m = 1.80±0.20. Basidiospores ellipsoid and slightly truncated at base, apex is slightly truncated; pale yellow; wall 0.5 µm thick, strongly ornamented and distinctly echinulate at the base (Fig. 2).

Pileipellis – A palisade of hypae elements with terminal elements clavate, subclavate and branched; 6.5–10 µm (Fig. 3).

Habitat and known distribution – Pathogenic on oil palm, so far only known from Lobe in the South West and Dibombari in the Littoral Regions of Cameroon.

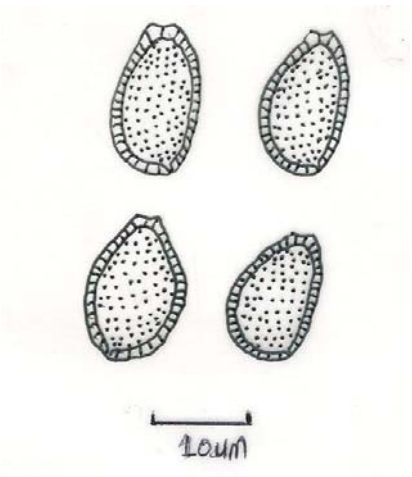

Fig. 2 – Basidiospores of *Ganoderma ryvardense* from oil palm in Cameroon.

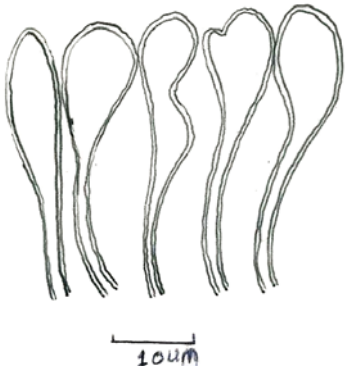

Fig. 3 – Pelipellis of *Ganoderma ryvardense* from oil palm in Cameroon

Analyses of ITS rDNA sequences

Ganoderma sequences generated in this study have been deposited in GenBank with accession numbers HKAS58055, HM138670, HKAS58053 HM13867, HKAS58054 and HM138672. The data set consisted of 46 taxa with *Pycnoporus sanguineus* and *Trametes versicolor* selected as outgroup taxa. A total of 743 characters were used for the analysis. After ambiguous positions were removed, 536 characters were constant, 157 characters were parsimony informative, and 50 variable characters were parsimony-uninformative. Gaps were treated as "missing", while multistate taxa were interpreted as uncertainty. Unweighted parsimony analyses produced one tree island with 404 steps, CI, RI, RC and HI were 0.656, 0.875, 0.574 and 0.344, respectively. All consensus trees were identical with regards to the grouping of *Ganoderma* ITS sequences.

Table 1 Species of *Ganoderma* used for phylogenetic analysis in this study.

ITS 1 and ITS 2 GenBank No.	Species	Locality	Hosts	Authors
AF255095	*Ganoderma* sp.	USA	unknown	Moncalvo,J.M. and Buchanan,P.K.
AF255183	*Ganoderma* sp.	unknown	unknown	Moncalvo,J.M. and Buchanan,P.K.
AF255188	*Ganoderma* sp.	unknown	unknown	Moncalvo,J.M. and Buchanan,P.K.
AH008110	*G. zonatum*	Australia	unknown	Gottlieb,A.M., Ferrer,E. and Wright,J.E.
AJ608712	*Ganoderma* sp.	Indonesia	*Acacia mangium*	Bougher,N.L.
AJ608713	*G. philippii*	Indonesia	*A. mangium*	Bougher,N.L.
AJ627585	*G. mastoporum*	Indonesia & Malaysia	*A. mangium*	Bougher,N.L
AY220537	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220539	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220540	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220541	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220542	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220543	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY220544	*Ganoderma* sp.	Indonesia	oil palm	Utomo,C., Werner,S., Niepold,F. and Deising,H.B.
AY569450	*G. cupreum*	Australia	forest trees	Roberts,L.M.
AY593860	*G. fornicatum*	China	unknown	Wang,D.-M. and Yao,Y.-J.
AY593864	*G. japonicum*	China	unknown	Wang,D.M. and Yao,Y.J.
AY636059	*G. lucidum*	India	unknown	Singh,S.K. and Kamal,S
AY884175	*G. carnosum*	Britain	unknown	Wang,D.-M. and Yao,Y.-J.
AY884178	*G. applanatum*	Britain	unknown	Wang,D.-M. and Yao,Y.-J.
DQ425009	*G. applanatum*	China	unknown	Su,C.L., Zhang,J.S. and Pan,Y.J.
DQ425011	*G. oerstedii*	China	unknown	Su,C.L., Zhang,J.S. and Pan,Y.J.
EF016754	*Ganoderma* sp.	India	*Areca catechu*	Sheena Kumari,T.K., Rohini,I., Gunasekaran,M. and Rajesh,M.K.
EF060005	*G. lipsiense*	Finland	trees	Terho,M., Hantula,J. and Hallaksela,A.-M.
EF060007	*G. resinaceum*	Finland	trees	Terho,M., Hantula,J. and Hallaksela,A.-M.
EF524049	*Trametes versicolor*	Germany	unknown	Hoegger,P.J., Bobekova,E., Pfeffer,A. & Kuees,U.
EU021457	*G. tropicum*	Taiwan	unknown	Wang,D.-M. and Wu,S.-H.
EU021458	*G. tropicum*	Taiwan	unknown	Wang,D.-M. and Wu,S.-H.
EU239386	*G. steyaertanu*	Australia	*Acacia*	Smith,B.J. and Sivasithamparan,K.
EU273513	*G. gibbosum*	China	unknown	Jiang,J., Ju,X., Cao,X., Sun,Y., Yin,T. and Miao,Q.
EU486458	*G. carnosum*	Canada	unknown	Denis,M.W. and Berbee,M.L.
FJ379262	*G. lucidum*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
FJ392284	*G. pseudoferreum*	China	rubber tree	Zhang,X., Xie,Y., Qi,Y., Pu,J. and Zhang,H.
FJ478088	*G. fulvellum*	China	unknown	Jiang,J., Ju,X., Cao,X., Sun,Y., Yin,T. and Miao,Q.

Table 1 (Continued) Species of *Ganoderma* used for phylogenetic analysis in this study.

ITS 1 and ITS 2 GenBank No.	Species	Locality	Hosts	Authors
FJ582638	*G. gibbosum*	China	unknown	Jiang,J., Ju,X., Cao,X., Sun,Y., Yin,T. and Miao,Q.
FJ805250	*G. resinaceum*	France	unknown	Lesage-Meessen,L., Haon,M., Favel,A., Taussac,S. and Navarro,D.
FJ810182	*Pcynoporus sanguineus*	China	unknown	Jiang,J., Zhang,S., Cao,X., Li,C., Yin,T., Miao,Q., Li,C., Mei,K. and Hu,Y.
GU213472	*G. applanatum*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
GU213474	*G. australe*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
GU213475	*G. japonicm*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
GU213479	*G. lucidum*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
GU213486	*G. mastoporum*	China	unknown	Huang,L.H., Wu,Q.P. and Yang,X.B.
GU731555	*G. adspersum*	France	unknown	Favel,A., Navarro,D., Haon,M., Taussac,S. and Lesage-Meessen,L.
HKAS58053 HKAS58054 HKAS58055	*G. ryvardense*	Cameroon	oil palm	Tonjock,R.K and Mih,A.M

The 50% majority consensus Bayesian tree and MPTs showed identical topology with both the bayesian and the parsimony analyses well supported. One MPT is shown (Fig. 4) with BSS and BPP. Phylograms showed seven major clades, of which the reasonably supported (62% BSS and 82% BPP) *G. steyaertanum* group contains *G. ryvardense*. Sequences in this group were resolved in two subclades. The two sequences of *G. ryvardense* form a distinct clade with a relatively confirmed branch (100% BSS and 100% BPP) sister to the branch with sequences of *Ganoderma* sp. identified as *G. boninense* and other *Ganoderma* spp. from oil palm hosts with *G. steyaertanum* from acacia plant. The monophyletic origin of the *G. ryvardense* was highly supported with 100% bootstrapping (Fig. 4).

Discussion

Ganoderma ryvardense was identified by examination of material at different growth stages. The consistency in the anatomical evolution of the basidiomata enhanced the appreciation of the ontogeny. *G. ryvardense* was found growing on living oil palm directly attached to the stem. Morphologically, *G. ryvardense* is characterized by having ellipsoid basidiospores with a slightly truncated apex and a truncated base. The context layer is three times larger than the tube layer. Based on these characters and its distinct phylogenetic placements, *G. ryvardense* is described as new to science.

The present species is similar to *G. boninense* morphologically in having clavate pellipelis and similar spore shape. Steyaert (1967) gave basidiospores of *G. boninense* as 8.5–13.5 × 4.5–7.5 µm, dissepiments 10–140 µm, axes 175–345 µm while Ryvarden (1984) gave 10–12 × 7–8 µm. Pilotti et al. (2004) stated that *G. boninense* basidiospores are narrowly ellipsoid, measuring 9–11 × 4.2–5.6 µm and dark reddish brown but the type specimen of *G. boninense* basidiospores measured 8.2–13.5 × 5–8.6 µm, pore diameter was 90–207 µm, dissepiments 22–141 µm and pore axes was 185–340 µm (Smith & Sivasithamparam 2003). However, *G. ryvardense* differs from *G. boninense* in that the latter has ellipsoid spores which are slightly truncated at the sides and the apices with slightly different basidiospore measurements. Morphologically, *G. ryvardense* is also similar to *G. hildebrandii* in having ellipsoid basidiospores that are slightly truncated at the apex.

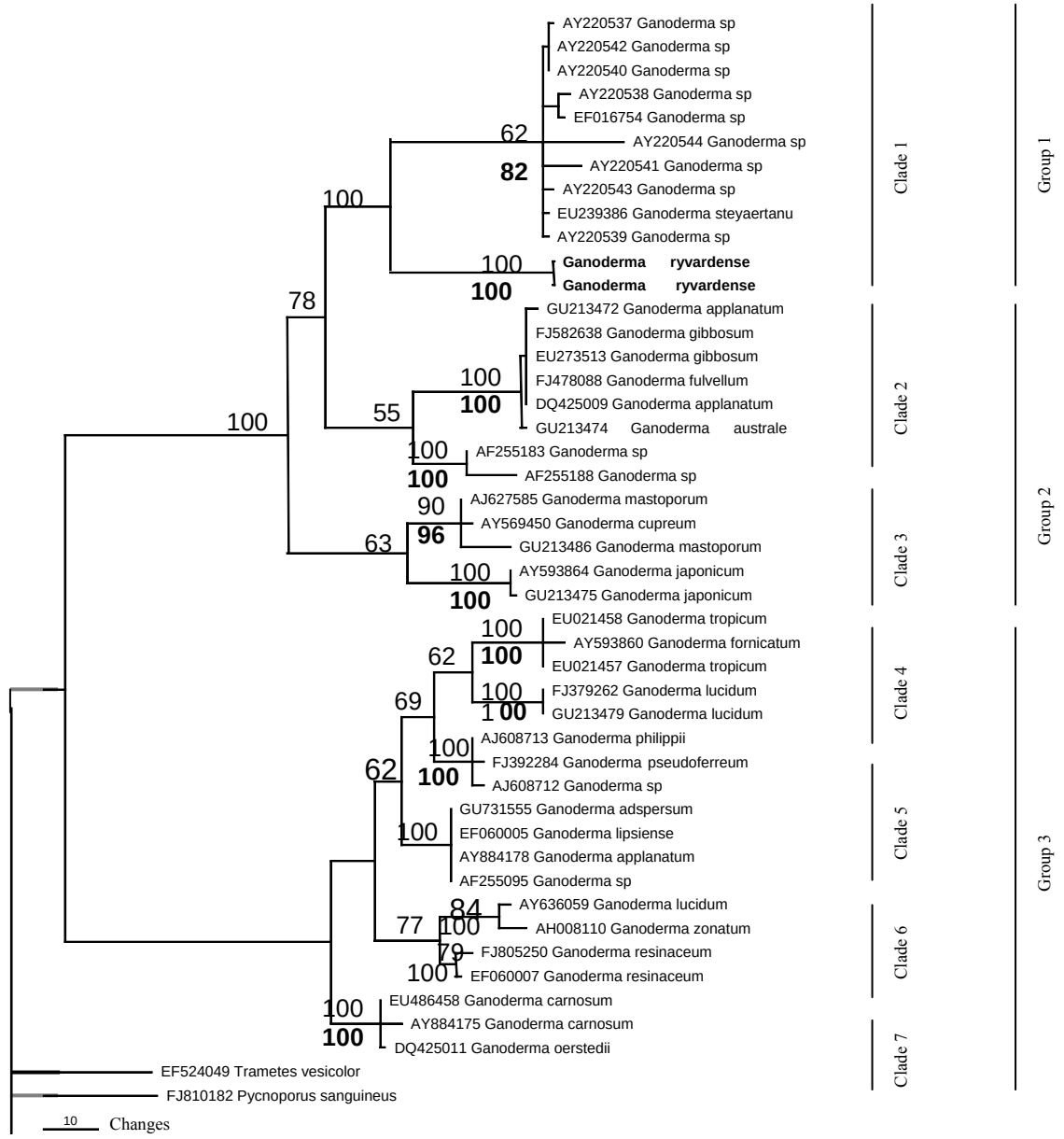

Fig. 4 – Phylogenetic placement of *Ganoderma ryvardense*. From unweighted parsimony analyses of ITS1-5.8S-ITS2 sequences using 1,000 random stepwise addition replications and branch swapping TBR (length of the tree = 404 steps, CI = 0.656, RI = 0.875). Bootstrap values (> 50%) from 1,000 replicates are indicated above line in regular type. Numbers below branches in boldface are Bayesian posterior probabilities (> 80%) from Bayesian analysis, analysis searched for 1,000,000 generations using 4 chains. sequenced in this study are shown in bold. The tree was rooted to *Pycnoporus sanguineus* and *Trametes versicolor*.

However, in all other aspects such as length and width of basidospores, pilleipellis and macro structure, *G. hildebrandii* is different since its basiodiospores are drop-shaped (Moncalvo & Ryvarden 1995).

Phylogenetically, *G. ryvardense* formed a sister group with *G. steyaertanum* but morphologically they are different species. The latter differs in having basidiospores that are echinulate, truncate, ovate and elliptical with measurements of 7.3–12.7 × 5 × 9.5 µm, dissepiments 22–163 µm, pore 136–225 µm and 3–5 pores mm (Smith & Sivasithamparam 2003).

The higher level of nucleotide substitution in the ITS rDNA resulted in seven clades

that correspond to the geographical origin and the different host ranges. Clade 1 consists of collections from India and Indonesia (*Ganoderma* spp.), Australia (*G. steyaertanum*) (82% bootstrapping), and *G. ryvardense* from Cameroon. Clade 2 consists of *G. applanatum*, *G. gibbosum*, *G. fulvellum*, *G. australe* and *Ganoderma* sp. Clade 3 consists of *G. mastoporum*, *G. cupreum* and *G. japonicum*. Clade 4 consists of *G. tropicum*, *G. lucidum* and *G. fornicatum*. Clade 5 consists of *G. philippii*, *G. pseudoferreum*, *Ganoderma* sp., *G. adspersum*, *G. lipsiense* and *G. applanatum*. Clade 6 consists of *G. lucidum*, *G. zonatum* and *G. resinaceum*. Clade 7 consists of *G. carnosum* and *G. oerstedii* (100% bootstrapping). Clade 1 belongs to group 1, which is the oil palm clade, clades 2 and 3 belong to group 2, while clades 4, 5, 6 and 7 belong to group 3.

Internal transcribed spacer phylogeny of Moncalvo (2000) indicated a close relationship between *G. boninense* and *G. zonatum* but inthis study *Ganoderma* species from Indonesia identified as *G. boninense* by Utomo et al. (2005) did not cluster with any species of *G. zonatum*; instead it formed a phylogenetic clade with *G. steyaertanum* and *G. ryvardense*. This might be due to the fact that *Ganoderma* sp. from Indonesia may have being misidentified.

Hong & Jung (2004) found that mitochondrial rDNA sequences had 3.3 times more information than ITS sequences among the species of *Ganoderma*. In contrast, Douanla-Meli & Langer (2009) found that ITS data had about 2.5 times more information than mtSSU. So in the presen study, only ITS data were used. It is evident that some African species of *Ganoderma* do not have any generated ITS sequences, so care was taken in including all described African species of *Ganoderma* for comparison with the new species. Analyses of ITS sequences have shown that the new African species, *G. ryvardense*, is related to *Ganoderma* sp., thought to be *G. boninensis* in the original publication, and to G. *steyaertanum*, which form a species group, specifically a phylogenetic clade. *G. ryvardense* belongs in this group, but can be discriminated by its morphology and supported by its phylogenetic placement.

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