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A new ethanol-based macrochemical test combined with a cultural character in the process of identification of the cosmopolitan wood-decayer, *Ganoderma resinaceum* Boud. (Basidiomycota)

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A new macrochemical test using ethanol drops was set up and described here as a safer, quicker and more reliable substitute for the previously used match flame to reveal yellow resin on the pileus of *Ganoderma resinaceum*, no matter its geographical origin. Four concentrations (30, 70, 90 and 99%) of ethanol (CH_3-CH_2OH) were tested with distilled water as negative control, as a substitute to the old match flame test in the process of identification of this species. The positive control test was performed on 18 other species of *Ganoderma* including *Ganoderma lucidum*. All control tests were negative, ethanol concentrations ranging between 90-99% revealed a ± bright and lasting yellowish resin oozing from the pileus of *G. resinaceum.* Observations from laboratory cultures showed that in this genus, only mycelium of *G. resinaceum* so far turns yellowish as earlier established in other studies on strains of the species identified at molecular (ITS-rDNA) level. Therefore, in this very wide genus where the boundaries between numerous species are still poorly circumscribed, the new positive ethanol test combined with the occurrence of yellowish zones in mycelial cultures bring more accuracy in the identification process of *G. resinaceum,* prior to confirmation by additional taxonomic investigations.

Key words: *Ganoderma resinaceum*, identification process, ethanol, pileus, mycelial culture, yellow resin.

INTRODUCTION

Ganoderma Karst with over 250 species (Chang and Buswell, 1999; Ryvarden, 1992) is a cosmopolitan genus recorded worldwide; in tropical as well as in temperate climates. It belongs to the family Ganodermataceae with

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key characters, the shape and size of basidiospores and the texture of the pileus (Furtardo, 1965; Ryvarden, 2000). Several species are found in tropical Africa and numerous studies (Bresadola, 1890; Futardo, 1965; Hjortstam et al., 1993; Kengni Ayissi and Mossebo, 2014a; Kengni Ayissi et al., 2014b; Kinge and Mih, 2014; Kinge, 2012; Moncalvo and Ryvarden, 1997; Mossebo et al., 2014; Roberts and Ryvarden, 2006; Ryvarden and Johansen, 1980; Steyaert, 1967, 1972, 1980; Zoberi, 1972) have so far been carried out in this genus due to its importance in various scientific domains such as agriculture, forestry pathology and medicine.

In spite of other numerous studies in the taxonomy and molecular phylogeny carried out in the genus *Ganoderma* (Adaskaveg and Giltbertson, 1986; Furtardo, 1967; Kinge and Mih, 2011; Mohanty et al., 2011; Moncalvo et al., 1995; Ryvarden, 2004) also known as wood decay fungi causing white rot, the boundaries between the over 250 taxa of *Ganoderma* so far described are still poorly circumscribed and not universally accepted due to variations and inconsistencies in macro- and micromorphological characters of several species including *Ganoderma resinaceum* which was termed by Steyaert (1980) with *Ganoderma parvelum* as a complex due to these variations and inconsistencies. With regards to *G. resinaceum* in particular, an extensive study carried out by Kengni Ayissi and Mossebo (2014), Kengni Ayissi et al. (2014b) and Mossebo et al. (2014) on a large number of specimens collected in several countries of tropical Africa, showed that specimens of this species are highly variable in their macro- and micro-morphology, size and colour. The above mentioned studies brought more light on these variations and inconsistencies and most importantly clues for a better identification of potential collections of this species which is cosmopolitan considering that it grows as well in the cold northern hemisphere and the generally hot tropical climates of the southern hemisphere.

Etymologically and according to Boudier (1889) who first described *G. resinaceum*, the specific epithet "*resinaceum*" means "resinous" and refers to a hard-setting sticky liquid which is resin oozing mostly from fresh damaged or scratched basidiomes. According to some specific peer-reviewed URL for *Ganoderma* [(www.first-nature.com/fungi/ganoderma-resinaceum.php) in Sept. 2014], this liquid is confirmed as being yellow and oozes from the fungus when it is cut, before setting rapidly to form a shiny varnished pileus surface. Contrary to the above mentioned features, other peer reviewed URL [(www.mycocharentes.fr) in March 2011] of macrofungi from western Europe show numerous clearly identified specimens of *G. resinaceum* with a laccate pileus, however showing no shiny varnished appearance and no yellow resin oozing on the pileus, feature also rare or inconspicuous on specimens from the tropics (Kengni Ayissi and Mossebo, 2014a; Kengni Ayissi et al., 2014b; Mossebo et al., 2014). *G. resinaceum* is also described in the above mentioned URL as showing on scratch a yellowish layer made of resin and about this resin layer, Ryvarden and Melo (2014), Ryvarden (2000, 2004), Nŭnez and Ryvarden (2000) and Breitenbach and Kränzlin (1986) said that "basidiocarps are with age more reddish brown to bay and dull due to an excreted resinous layer that becomes yellowish when crushed and melts in a match flame".

The above mentioned remarks made by various authors presume that in addition to its laccate and shiny varnished pileus, *G. resinaceum* could be readily identified even on the field of collection just by cutting or scratching the pileus looking for a yellow layer underneath or rather a yellow liquid setting on fresh basidiocarps and reported as melting in a match flame. However, trials carried out on pileus of numerous specimens of *G. resinaceum* (Ambit, 2011; Kengni Ayissi and Mossebo, 2014a; Mossebo et al., 2014) collected in several tropical countries of central Africa (Table 1) showed that the above mentioned taxonomic test (cutting or scratching pileus to let resin flow over the pileus or using a match flame to melt it on the pileus) used to reveal resin hardly work on these tropical specimens. In fact, the monitoring of several tropical specimens - identified by authors (Kengni Ayissi and Mossebo, 2014a; Mossebo et al., 2014) and cross-checked by Ryvarden (University of Oslo, Norway, personal communication) as being *G. resinaceum* - shows that they barely or not at all excrete a resinous layer as it rarely appears on the pileus of very few strains of the specimens collected. This leads to the conclusion that resin and its above mentioned features used as an indicator in the process of identification of *G. resinaceum* very likely apply mostly on specimens growing in climates of the northern hemisphere where most of the descriptions are done, and rather rarely or inconspicuously on specimens growing in hot climates of tropical Africa and the southern hemisphere in general. Therefore, considering all the above mentioned difficulties in conspicuously observing yellow resin on the pileus of *G. resinaceum* and the inconveniences in using the "match flame" in order to reveal resin in the process of identification of *G. resinaceum* which is so far the only species in the genus *Ganoderma* known to produce yellow resin on the pileus, this study aims to develop other methods in order to clearly and unequivocally reveal resin on basidiomes of *G. resinaceum* from tropical Africa (south of Sahara) and more generally from all regions of the world, irrespective of the climate and environment in which these basidiomes appear.

About the cultural characters, Mohanty et al. (2011) described the first record of *G. resinaceum* and *G. weberianum* from north India based on ITS-rDNA molecular phylogeny and reported that only *G. resinaceum* showed yellowish zones on mycelium in artificial cultures, whereas this yellowish colour was reported by the authors to be totally absent in cultures of *G. weberianum* and *G. lucidum*, the latter being the reference species in the genus *Ganoderma*. Therefore, inspired by Mohanty et al. (2011),

Table 1. Geographical coordinates (GPS) of collection areas, collection date and growth substrates of specimens of *G. resinaceum* tested (Source: Kengni Ayissi and Mossebo, 2014a).

S/N	Herbarium number	Date of collection	Area of collection *(i)*	GPS coordinates	Substrate
1	HUY1-DM 45B	28/06/1996	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of angiosperm
2	HUY1-DM 47D	15/07/1996	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of oil palm tree (*Elaeis guineensis*)
3	HUY1-DM 72(ART)	3/10/2009	Mbengwi/Cameroon	N 05° 55' 18" E 10° 08' 32"	Stump of angiosperm
4	HUY1-DM 85	26/08/1996	Campus University of Yaoundé 1 (UY1)/Cameroon	N 03° 51' 31,6" E 11° 29' 59"	Stump of angiosperm
5	HUY1-DM 104	13/07/1997	UY1/Cameroon	N 03° 51' 31,6" E 11° 29' 59	Stump of angiosperm
6	HUY1-DM 105B	23/05/2000	Yaoundé/Cameroon	N 03° 52' 26" E 11° 32' 06	Stump of angiosperm
7	HUY1-DM 418	18/11/2004	Yaoundé/Cameroon	N 03° 52' 26" E 11° 32' 06"	Tree trunk in decay
8	HUY1-DM 506	07/04/2007	Dja Biosphere reserve/Cameroon	N 03° 23' 39" E 12° 43' 25"	Tree trunk in decay
9	HUY1-DM 509	08/04/2007	Dja Biosphere reserve/Cameroon	N 03° 23' 39" E 12° 43' 25"	Tree trunk in decay
10	HUY1-DM 524	12/05/2011	Yaoundé/Cameroon	N 03° 51' 38" E 11° 30' 07"	Stump of angiosperm
11	HUY1-DM 538	10/04/2007	Dja Biosphere reserve/Cameroon	N 03° 23' 39" E 12° 43' 25"	Tree trunk in decay
12	HUY1-DM 612A	12/07/2009	Yaoundé/Cameroon	N 03° 52' 28" E 11° 31' 04"	Stump of angiosperm
13	HUY1-DM 612B	June 2008	Yaoundé/Cameroon	N 03° 52' 28" E 11° 31' 04"	Tree trunk in decay
14	HUY1-DM 612 C	12/07/2009	Yaoundé/Cameroon	N 03° 52' 28" E 11° 31' 04"	Stump of angiosperm
15	HUY1-DM 617	13/06/2008	UY1/Cameroon	N 03° 51' 31,6" E 11° 29' 59	Stump of angiosperm
16	HUY1-DM 619A	12/06/2008	Yaoundé/Cameroon	N 03° 51' 23" E 11° 31' 08"	Tree trunk in decay
17	HUY1-DM 619B	13/06/2008	Yaoundé/Cameroon	N 03° 51' 28" E 11° 30' 09"	Stump of angiosperm
18	HUY1-DM 619C	13/06/2008	Yaoundé/Cameroon	N 03° 51' 28" E 11° 30' 09"	Stump of angiosperm
19	HUY1-DM 619 E	08/03/2013 21/07/2013	UY1/Cameroon	N 03° 51' 31,6" E 11° 29' 59	Stump of angiosperm
20	HUY1-DM 622	15/06/2008	Lobaye Forestry domain/ RCA	N 04° 22' 20" E 19° 27' 18"	Tree trunk in decay
21	HUY1-DM 629	09/06/2008	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Tree trunk in decay
22	HUY1-DM 660	13/06/2008	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of angiosperm
23	HUY1-DM 696	20/08/2009	Yaoundé/Cameroon	N 03° 51' 33" E 11° 31' 02"	Trunk of oil palm tree (*Elaeis guineensis*) in decay
24	HUY1-DM 709	23/08/2011	Kpangbala Ndeke in RCA	N 04° 27' 17" E 19° 32' 28"	Tree trunk in degradation

Table 1. Contd

25	HUY1-DM 711	21/08/2008	Lobaye Forestry domain in RCA	N 04° 22' 20" E 19° 27' 18"	Roots of an angiosperm stump in decay
26	HUY1- DM 784	23/03/2013	Ipassa-Makokou in Gabon	N 0° 30' 05'' E 12° 47' 42''	Tree trunk in dacay
27	HUY1-DM 785	26/03/2013	Ipassa-Makokou in Gabon	N 0° 30' 11'' E 12° 47' 40.5''	Tree stump
28	HUY1 -DM 786	27/04/2013	Kisangani in Congo (Kinshasa)	N 0° 31' 59'' E 25° 1146.14''	Stump of *Elaeis guineensis*
29	HUY1-DM 731	30/01/2012	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	trunk of a oil palm tree (*Elaeis guineensis*) in decay
30	HUY1-DM 732	22/11/2011	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of angiosperm
31	HUY1-DM 750	23/04/1998	UY1/Cameroon	N 03° 51'31,6" E 11° 29' 59	Stump of angiosperm
32	HUY1-DM 760A	26/05/2012	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of angiosperm
33	HUY1-DM 763	03/05/2011	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of angiosperm
34	HUY1-DM 764	06/10/2012	UY1/Cameroon	N 03° 51'31,6" E 11° 29' 59	Trunk of a living angiosperm
35	HUY1-DM 769	11/11/2009	Mbengwi/Cameroon	N 05° 55' 18" E 10° 08' 32"	trunk of angiosperm
36	HUY1-DM 777	08/11/2012	Nkoabang (suburbs of Yaoundé/Cameroon)	N 03° 53' 18" E 11° 32' 06"	Stump of angiosperm

(i) Collection areas were forestry domains and reserves as well as savannahs, urban centres and their outskirts showing various types of vegetations in several countries of central Africa.

mycelial culture tests on several species of *Ganoderma* from our collections were also set up in addition to the ethanol test as a taxonomic indicator in the process of identification of *G. resinaceum*.

MATERIALS AND METHODS

Morphological identification

Before carrying out the macrochemical test properly said, each specimen of *Ganoderma* collected was first thoroughly scrutinized in taxonomy according to the features described by Kengni Ayissi and Mossebo (2014a) in order to determine whether it actually belongs to *G. resinaceum* or to other species that could be used for the positive control test. However, some dry specimens found in the Herbarium of the Mycology Section of the Royal Botanic Gardens in Kew had been previously identified after collection in UK as *G. resinaceum* by Kew taxonomists and therefore used as such in the test as strains from temperate (cold) countries. For tropical specimens collected mostly in central Africa, the macro- and micro-morphological features were first described according to various protocols used for polypores description (Mossebo and Ryvarden, 1997, 2003; Mossebo, 2005; Mossebo et al., 2007) and more specific protocols for *Ganoderma* (Kengni Ayissi and Mossebo, 2014a; Kinge and Mih, 2014; Kinge and Mih, 2011; Kinge, 2012; Mohanty et al., 2011; Mossebo et al., 2014; Gilbertson and Ryvarden, 1986; Ryvarden and Johansen, 1980; Ryvarden, 2000, 2004; Nŭnez and Ryvarden, 2000). These features were thereafter compared with the summary presented in Table 3 and to those of existing taxa of *Ganoderma* described in the most reliable taxonomic studies (Kengni Ayissi and Mossebo, 2014a;Kinge, 2012; Mohanty et al.,2011; Mossebo et al., 2014; Nŭnez and Ryvarden, 2000; Ryvarden and Johansen,1980; Ryvarden, 2000, 2004), in order to determine whether the specimen belonged to *G. resinaceum*. Double of our specimens were sent to Ryvarden (University of Oslo, Norway, personal communication) to cross-check our preliminary identifications. They were thereafter registered in the Herbarium of the Department of Botany of the University of Oslo with voucher material conserved in the mycological herbarium of the University of Yaoundé 1 in Cameroon under the HUY1-DMx herbarium number (Table 1).

Macrochemical test using ethanol to reveal resin on basidiomes of *Ganoderma resinaceum*

Freshly collected basidiocarps or exsicatta of tropical strains of *G. resinaceum* described by Kengni Ayissi and Mossebo (2014a) (Table 1) were used in the test inspired from Charbonnel (1995) who reported ethanol test on the pileus of some Agaricales species for taxonomic purposes. Some other collections [K(M) 21513-7; K(M) 21513-18; K(M) 21513-47] of *G. resinaceum* from UK found at the herbarium of the Mycology Section of the Royal Botanic

Gardens in Kew-UK were also tested as strains from temperate (cold) countries. The pileus as test surface was first cleaned whenever necessary, using a clean piece of fabric or sponge in order to get rid of dust, spore prints or any dirty remains (soil, grass, insects etc) from the collection area. Thereafter, four concentrations (30, 70, 90 and 99%) of ethanol (CH_3-CH_2OH) were prepared and filled in plastic droppers of 30 ml each. The 0% concentration was simple distilled water used as negative control. The positive control consisted of tests of the same concentrations of ethanol carried out on eighteen (18) species of *Ganoderma* collected over the study period. They were gradually collected mostly in the tropics in the same collection areas as mentioned in Table 1. Seven (7) were not clearly identified at species level whereas the 11 others were clearly identified *as Ganoderma australe, Ganoderma applanatum, Ganoderma baudonii, Ganoderma carocalacreus, Ganoderma colossum, Ganoderma hildebrandii, Ganoderma lobenense, Ganoderma ryvardense, Ganoderma weberianum*, *Ganoderma zonatum* and *Ganoderma lucidum* which is the reference species in this genus. It must be underscored that the 7 unidentified species were included in the positive control test because in the process of their identification, although their specific names were not clearly determined, most of their macroscopic and microscopic features as well as the preliminary BLAST SEARCH of their ITS-rDNA sequences (not shown here) were first entirely different from each other, and all different from those of *G. resinaceum* and the 11 other species tested as positive control. In order to perform the test, the test surface was first virtually subdivided in two equal parts using a special design on a piece of white paper on which two windows were cut opened as presented on Figures 1A, B, F and 1G, one designed for the ethanol test and the other for water control. The paper design was thereafter attached to the pileus using a tailor needle so as to show the two test windows ready to receive drops of the reagent. Twenty to thirty drops of a given concentration (30, 70, 90 and 99%) of ethanol were thereafter poured on the “test area” (test surface) of the pileus and the same number of drops of distilled water (0% ethanol) on the “water control area” (Figures 1A, B, F and 1G) of the same pileus. For each collection, three (3) replicates of each test were performed using 3 different basidiomes of the collection and the test was gradually upon collection, extended to all specimens presented in Table 1 and other more recent collections used as positive control. The basidiomes tested were thereafter closely monitored. In the case of positive reaction, resin was revealed on fresh basidiomes either as a yellowish to yellow spot (Figures 1B and 1G) clearly occurring 5 to 15 min after pouring the drops on the “test area” of the pileus. On exsiccata, the test surface either gradually changed to show just a ± conspicuously shiny varnished sticky liquid (Figure 1A), or a shiny varnished spot oozing resin as a yellow sticky liquid (Figure 1C). For each test carried out, the resin colour (Figures 1B, C and 1G) recorded was coded according to the colour chart of Kornerup and Wansher (1978) and for the positive control, the basidiomes of *G. lucidum* (Figure 1E and F) and other 17 fresh and dry basidiomes of other species were handled in the same manner as described above for *G. resinaceum*.

These series of tests were launched in Cameroon in 2005, continued in 2006-2007 at the Mycology Section of the Royal Botanic Gardens in Kew (UK), and thereafter extended, cross-checked and finalized from 2008 to 2014 at the University of Yaoundé 1 in Cameroon as specimens (Table 1) were gradually collected or received from colleagues from other countries.

Test of resin production in mycelial culture of *G. resinaceum*

Along the ethanol test on basidiocarps of *G. resinaceum*, mycelium raised from tiny pieces of context-tissue from fresh basidiocarps plated on malt extract agar (MEA) according to Mossebo (2002) and Mohanty et al. (2011) was monitored (Figure 1D) for 7 to 28 days in order to find out whether mycelium in artificial culture produces or exudes resin in comparison with results of ethanol test on the same basidiocarps. As control test here, pieces of context-tissue of fresh basidiocarps of *G. lucidum* and most of the other species (Table 2) tested as positive control were also plated on MEA and monitored for the same time period.

RESULTS

The following key remarks could be drawn from the detailed results presented in Table 2:

1. Ethanol at concentrations ranging from 90 to 99% reveals a ± conspicuous bright and lasting yellowish to yellow resin on pileus of fresh basidiocarps of *G. resinaceum.*
2. On dry basidiocarps (exsiccata), the same concentrations of ethanol most often just brighten ± conspicuously the pileus and sometimes also ooze resin.
3. The bigger the concentration of ethanol between 90 to 99% concentrations, the faster and more conspicuous is the basidiocarp response to the test.
4. At a concentration of 70%, a faint and evanescent yellowish resin appears on the pileus of fresh basidiocarps of *G. resinaceum.*
5. Concentrations of ethanol inferior (<) to 70% do not react or barely exude a very faint and evanescent yellowish resin on pileus.
6. The above mentioned reactions are recorded in average 5 to 15 min after pileus receives drops of ethanol, but in some cases, up to 30 min could be necessary in order to observe a conspicuous and clear reaction regardless of the ethanol concentration.
7. Distilled water as negative control does not exude resin from the pileus of *G. resinaceum.*
8. *Ganoderma lucidum*, *G. applanatum, G. australe, G. baudonii, G. carocalacreus, G. colossum, G. hildebrandii, G. lobenense, G. ryvardense, G. weberianum*, *G. zonatum* and 7 other unidentified species of *Ganoderma,* all tested as positive control do not exude resin with ethanol at the above mentioned concentrations which on some species rather ± fade the brightness of the pileus depending on the level of concentration used.
9. Already tested spots on the pileus of a basidiocarp should not be retested and eventual additional tests on the same basidiocarp must be carried out on different spots.
10. In addition to a positive ethanol test, the occurrence of yellowish zones on parts of mycelium in culture of the presumed specimen of *G. resinaceum* constitutes another major step forward since it brings more accuracy in the identification process of this species, considering that, so far, only specimens that produced these yellowish zones (Figure 1D) in mycelial cultures - colour earlier described by Mohanty et al. (2011) as occuring only in *G. resinaceum* - also responded positively to the ethanol test, indicating a positive relationship between these two taxonomic parameters in *G. resinaceum.*

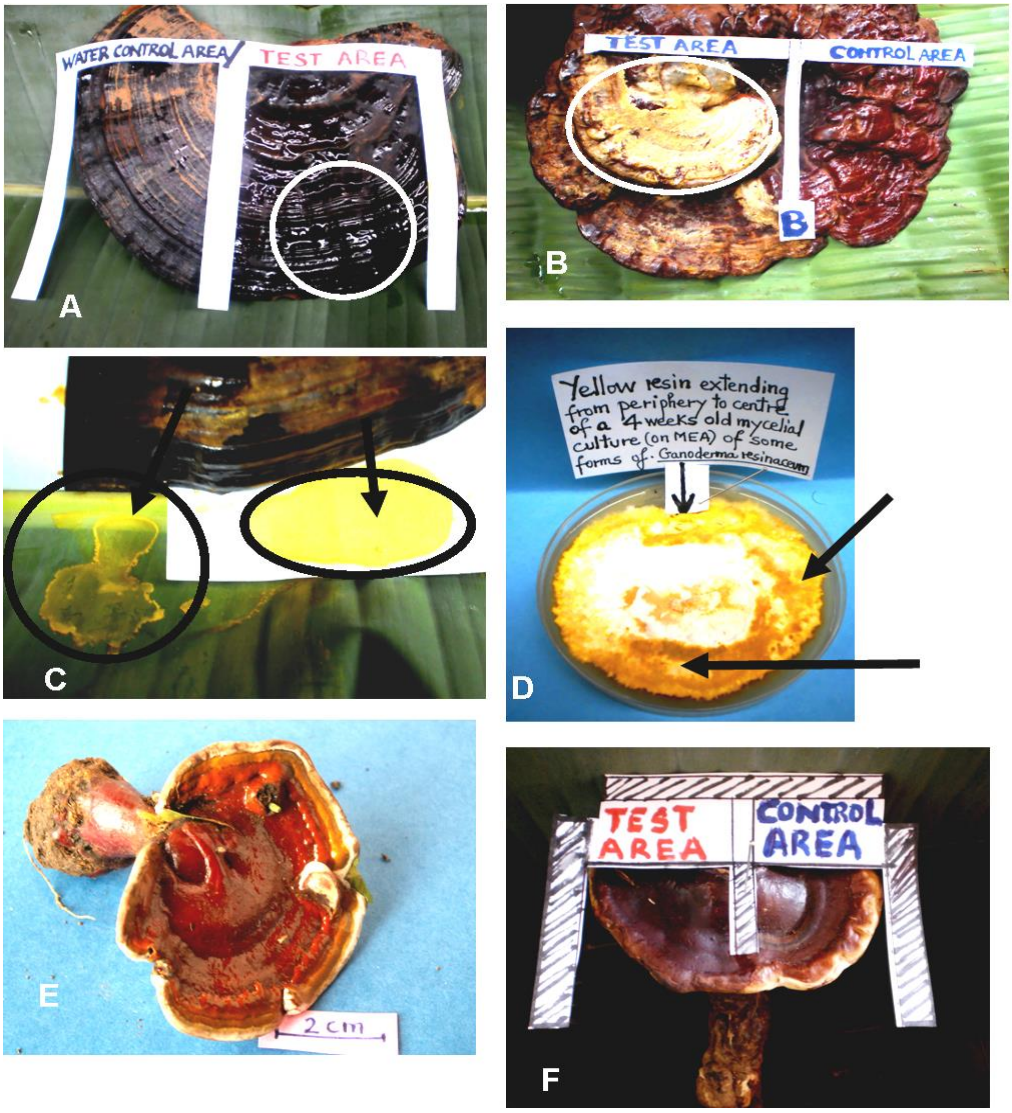

Figure 1. A: Ethanol test exuding a ± bright shiny varnished and sticky resin ("test area") on pileus of a dry basidiocarp (exsicattum) of *G. resinaceum*; **B:** ethanol test showing a spot of yellowish resin ("test area") on fresh basidiocarp of *G. resinaceum;* **C:** ethanol test exuding yellowish to yellow resin on exsicattum of *G. resinaceum;* **D:** yellowish zones occurring in mycelial cultures as a key cultural feature of *G. resinaceum*; E: a strain of *G. lucidum* from central Africa tested; F: a different strain of *G. lucidum* (exsicattum) tested as positive control.

The above mentioned results clearly show that ethanol could henceforth be considered as a safer, quicker and more reliable substitute to the "match flame" test previously described by several authors including Ryvarden and Melo (2014), Ryvarden (2000, 2004), Nũnez and Ryvarden (2000) and Breitenbach and Kränzlin (1986), as a reagent to reveal yellowish resin on the pileus of *G. resinaceum*. Therefore, a positive ethanol test combined with the above mentioned mycelial culture features stand as outstanding in the process of identification of *G. resinaceum*, but however still need additional macro- and micromorphological investigations of the specimens tested in order to confirm identification.

DISCUSSION

Results of this study show that positive ethanol test at concentrations ranging from 90 to 99% that reveal yellowish resin on the pileus of fresh and dry basidiocarps combined with the occurrence of yellowish zones (Mohanty et al., 2011) in mycelial cultures could be considered as an outstanding step in the process of identification of *G. resinaceum* since it brings more accuracy in this process. Both tests considered together therefore constitute a reliable taxonomic orientation test and a major progress in the taxonomy of *Ganoderma*, a genus in which the boundaries of several of the over 250

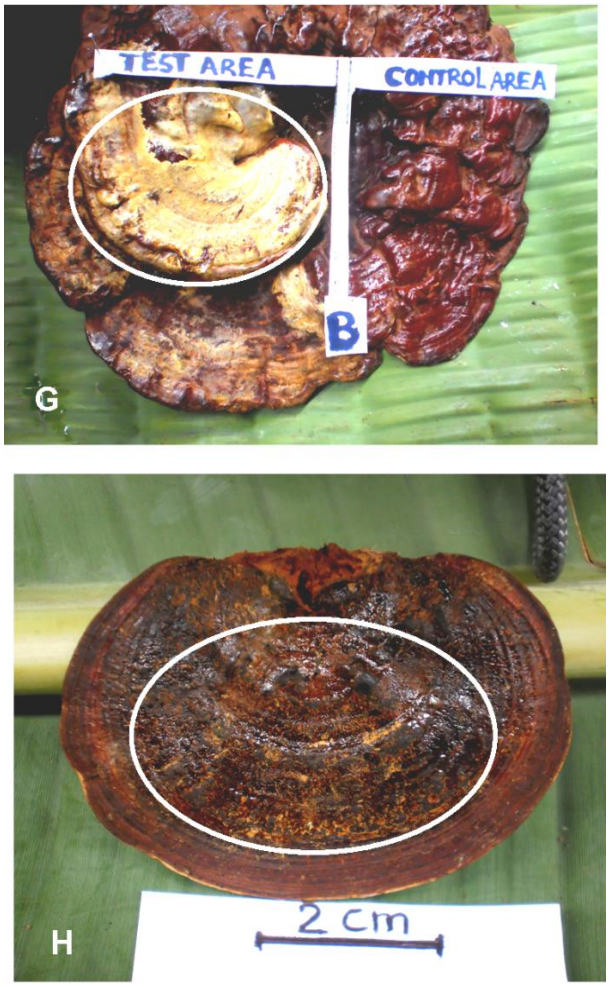

Figure 1. Contd. G: (zoom on Figure 1B) Pileus of a fresh specimen of *G. resinaceum* showing on the "control area" of the pileus surface with no trace of yellowish resin revealed rather on the "test area" by drops of ethanol at 90-99%; **H:** a different specimen of *G. resinaceum* naturally exuding on pileus and on scratch traces of coagulated yellowish resin as a key character conspicuously appearing on some strains of the species.

taxa are still poorly circumscribed in spite of numerous studies carried out by various authors (Gilbertson and Ryvarden, 1986; Kengni Ayissi and Mossebo, 2014a; Kinge and Mih, 2011, 2014; Kinge, 2012; Mohanty et al., 2011; Nŭnez and Ryvarden, 2000; Ryvarden and Johansen, 1980; Ryvarden, 2000, 2004; Steyaert 1967, 1980) in the taxonomy and molecular phylogeny of this genus. In fact, these tests save time and brings more accuracy in the process of identification of *G. resinaceum*, in that when for instance for one reason or another we are in search of *G. resinaceum* in particular among numerous specimens of *Ganoderma* collected on the field, it is only when the test reveals a ± conspicuous spot of yellowish to yellow resin on fresh specimens (Figures 1B & 1G) or a lasting shiny varnished and ± sticky spot [Figure 1A "Test Area")] on dry sporocarps, all these combined to the occurrence of yellowish zones in mycelial cultures - the latter feature being linked to the genus only to *G. resinaceum* identified at molecular level (Mohanty et al., 2011)- that taxonomic investigations matching those described by various authors (Giltbertson and Ryvarden, 1986; Kengni Ayissi and Mossebo, 2014a; Kinge and Mih, 2014; Kinge and Mih, 2011; Kinge, 2012; Mohanty et al., 2011; Mossebo et al., 2014; Mossebo et al., 2007; Mossebo, 2005; Mossebo and Ryvarden, 1997, 2003; Nŭnez and 304 Ryvarden, 2000; Ryvarden, 2000, 2004; Ryvarden and Johansen, 1980) and summarized in Table 3 could be used to confirm identification. Otherwise, further steps in the identification process should be directed rather towards other species of *Ganoderma* different from *G. resinaceum*. The test also constitutes a major progress in taxonomic mycology because it is easier, quicker and safer to perform, and the result easier to read and interpret than the match flame so far used and not always successfully, to melt yellowish resin on the pileus as previously described by several authors including Ryvarden and Melo (2014), Ryvarden (2000, 2004), Nŭnez and Ryvarden (2000), Ryvarden and Giltbertson (1993) and Breitenbach and Kränzlin (1986). Additional tests having been carried out successfully on specimens of *G. resinaceum* from the northern hemisphere and precisely at the Herbarium of the Department of Mycology at the Royal Botanic Gardens in Kew as mentioned earlier, this new test can also be considered as fully efficient in detecting yellowish resin on potential specimens of *G. resinaceum*, irrespective of their geographical origin.

With regards to interactions between ethanol and resin, for specimens of *G. resinaceum* naturally oozing yellowish resin (Figure 1H) readily visible on parts of the pileus surface or on scratch, as for those showing no trace of yellow resin on the pileus [Figure 1A, B and 1G ("control area")], it is obvious that ethanol at 90 to 99% concentrations essentially acts as a solvent that dissolves resin either coagulated on the pileus surface (Figure 1H) or rather embedded in the cuticle cells of *G. resinaceum* and oozes it over the pileus surface [(Figures 1A, B and 1G ("test area") and Figure 1C] where it becomes conspicuously visible. Ethanol used as a solvent for resin was already earlier mentioned in some peer reviewed URL [Ref. (www.nature-energie-vitalite.com/tag/sante) in September 2014] where it is said that solvents as alcohol in general including ethanol are used to dilute "propolis" which is a kind of yellow honey-like resin produced by bees, alcohol being used here as a solvent to dilute it and easily get rid of wax and other impurities in order to obtain pure honey after filtration. Ethanol at 90% was also used by Charbonnel (1995) as a differential test between some species of *Agaricus* including *Agaricus* of the group *xanthoderma*, *Agaricus campestris*, *Agaricus arvensis*, *Agaricus silvicola* and *Agaricus romanegsii*, whereby the pileus of some species either reacted as

Table 2. Ethanol test to reveal resin on pileus and resin production in mycelial cultures of *G. resinaceum.*

Parameter		Tests results on *G. resinaceum* and other species of *Ganoderma* as positive control		
		Fresh basidiocarp of *G. resinaceum*	**Dry basidiocarp (exsiccata) of *G. resinaceum***	**Positive control: *G. lucidum, G. applanatum, G. australe, G. baudonii, G. carocalacreus, G. colossum, G. hildebrandii, G. lobenense, G. ryvardense, G. weberianum, G. zonatum, G.* sp1, *G.* sp2, *G.* sp3, *G.* sp. 4, *G.* sp. 5, *G.* sp. 6, *G.* sp. 7**
Results of ethanol tests	**Natural colour of the pileus**	Variable, reddish-brown (8F5-8 and 7F7-8 to 10F8) (Figure 1B : Control Area) to bluish black (18F6-8) on parts of the lower side around the stipe, or shiny varnished brown (Figure 1H) (5E4-8, 5F6-8, 6E5-7, 6F7-8) on other specimens	Dark-grayish (10F1-2, 11F1-2, 12F1-2) to dark violaceous (16F6-8) or blackish violaceous (18F6-8) or darker (Figure 1A: Water Control Area and Figure 1C)	Bright reddish-brown (7F7-8 to 8F5-8) (Figure 1E/1F: Control Area) for dry basidiocarps of *G. lucidum*; different colours for other species
	30%	Natural colour of pileus unchanged	Natural colour of pileus unchanged	Natural colour of pileus unchanged
	70%	Pileus slightly brightening and most often showing exudates of faint and evanescent yellowish (2A2-4) resin	Pileus brightening just slightly and occasionally showing exudates of faint and evanescent yellowish (2A2-4) resin	Natural colour of pileus unchanged or ethanol slightly degrades pileus colour or brightness without revealing yellow resin
	90%	Pileus ± conspicuously brightening (Figure 1A: Test Area) and most often also oozing exudates of bright and lasting yellowish to yellow (3B6-8 to 3B7-8) resin (Figure 1B & G: Test Area)	Pileus ± conspicuously brightening, and sometimes oozing ± sticky exudates (Figure 1A: Test Area) of yellowish to yellow resin (2B6-8) (Figure 1C)	No exudates on pileus rather absorbing ethanol without colour change or ethanol slightly degrades pileus colour or brightness without revealing yellow resin, therefore absent in *G. lucidum* (Figure 1E/1F: Test Area) and other 17 species tested as positive control
	99%			
Water control test	**Distilled water**	Natural colour of pileus Unchanged	Natural colour of pileus unchanged	Natural colour of the pileus Unchanged
	Mycelial culture	Mycelium exudes yellowish to yellow (3A7-8) resin in 2 to 4 weeks old cultures (Figure 1D)	//	No exudates of yellow resin. Mycelium remains mat white to cream in 2 to 4 weeks old cultures.

Figures combined with alphabets and dashes in brackets refer to the colour code in Kornerup and Wansher (1978).

yellow, yellowish or red to ethanol drops and that of other species did not react at all. Furthermore and according to the biochemist of the team of co-authors and to some specialists (Matthews and Mumford, personal communication at Silwood Park station of the Imperial College of Science and Medicine in London) in organic chemistry question on this issue in the UK during our research stay at Kew, resin is said to contain numerous resin acids which are acid components of resin with high molecular weight and which are insoluble in water and soluble in organic solvents

Table 3. Summary of the key macroscopic and microscopic features of *G. resinaceum* (Source: Kengni Ayissi and Mossebo, 2014a).

I- Key macroscopic features

-**Basidioma** (6)8 − 40 × 4 − 30 × 0,8 − 7 cm, most often semi-circular, sometimes dimidiate, rarely fan-shaped to spathuliform with a ± extended stalked base, or exceptionally imbricate around a central stalk.

-Pileus colour appearing as a succession of: whitish (5A1 to 6A1) (± thin or absent)/yellowish to shiny orange-yellow (5A3-4 to 6C4-6) (± thin or absent) / hiny orange-reddish-brown to violaceous-brown (8F8 to 11F8), from margins towards the attachment point to the substrate on young basidioma, sometimes covered by a brownish to chocolate-brown (8F5-7) spore powder and most often turning to a fading brownish to dark brown colour (7E6-8 to 7F7-8) with blackish tints on ageing basidioma; or the pileus sometimes rather shows a ± uniform brown (7F7-8 to 8F8 or darker), brownish or reddish brown (8F7-8 to 11F8) colour sometimes appearing rather as a succession of : brownish to brown (16F4-6) (± thin or absent) / dark-violaceous (16F6-8 or darker) or blackish-violaceous (18F6-8 or darker), from margins towards the attachment point to the substrate; sometimes showing ± conspicuously traces of coagulated yellowish resin over the pileus surface of rare specimens, or rather yellowish resin layer underneath revealed on scratch . Pileus sometimes rather uniformly coloured dark-violaceous (16F6-8 or darker) to blackish-violaceous (18F6-8 or darker), or rather showing on young basidioma an additional ± thin whitish margins; concentric zones ± conspicuous, sometimes crossed by radial ridges. Pileus consistency of mature sporocarps relatively hard under finger press, not soft, neither spongy as in some species of *Ganoderma*.

-Pore surface most often whitish (2A1-2) (or brownish on finger touch or when bruised) on fresh basidioma (sometimes brownish or yellowish on dried basidioma), pores circular, 4 − 5 (6) per/mm.

-Junction between pore surface and pileus margins either: rounded to applanate on mature basidioma, generally not clearly limited as well on young as on mature basidioma, and showing either an inconspicuous single line bordering both parts, or most often rather two merging structures, or: clearly limited on most mature basidiocarps by a single or several (2 to 8) ± conspicuous lines bordering both parts.

-Context built either in a distinct two layers profile with an upper 1 − 3 cm light brown (5E8 to 7E7-8) layer and a 0.5 – 1.3 cm darker, wood to chocolate-brown (7F6-8) lower layer, both layers however showing ± vertically oriented mycelial strands, or rather built in a single (0.5 – 1.8 cm thick) light brown to brown (8E4-5 to 9F5-6) layer, but sometimes showing underneath a very thin (≈ 1 mm) whitish brown (6C3 to 8C2 or 8D2-3) layer corresponding to the pore surface whenever the colour of the latter differs from that of the tube layer

-**Tube layer** generally a pale to greyish brown (7F4-6 on fresh basidioma and 7D5-6 on exsicatta) upper layer (0.2 – 1.3 cm thick) and a very thin (≈ 1 mm) whitish brown (6C-3) lower layer corresponding to the pore surface

II- Key microscopic features

-Basidiospores (5) 8 – *11.15* – 12 (18 – 20) × (4.5) 6 – *7* – 8 (11 – 12) µm, ± densely echinulate, ellipsoid or narrowly to broadly ellipsoid (Qm = 1.46 – *1.6* – 1.68) with truncated and non-truncated apices , some showing a ± conspicuous apiculus with oil drop(s) in others.

-Cuticle cells (14) 15 − 50 (60) × (5) 7 − 10 (15) µm, polymorphic, mostly clavate or subcylindrical to cylindrical, apically lobed in some specimens and showing ± numerous lateral and/or apical outgrowths and protuberances in others, thick-, thin-, or thin- and thick-walled with large and narrow lumen, sometimes dichotomously branched from the base.

-Hyphal system dimitic, generative hyphae hyaline, thin-walled, 1.6 – 5.2 µm diam. with clamp connections at septa level; skeletal hyphae abundant, ± branched, thick-walled, clampless

Figures combined with alphabet letters and dashes in brackets refer to the colour code in Kornerup and Wansher (1978).

such as ethanol (CH_3-CH_2OH). Their affirmation matches our results, since it clearly explains the negative reactions observed in all control tests carried out with distilled water and the positive reactions obtained with ethanol (Table 2). It is however worth mentioning that resinous-like pigments or secondary metabolites in some specimens different from *G. resinaceum* might eventually also react with ethanol at the above mentioned concentrations. However, in this case and whatever is the concentration of ethanol used, these reactions are generally faint to very faint and/or fugacious and evanescent, disappearing almost entirely on the pileus shortly after the reaction, be it with fresh or dry basidiocarps and should therefore not be confused with the conspicuous positive reactions presuming *G. resinaceum* as shown in Figures 1A, 1B, 1G ("test area") and 1C. This accounts for the necessity to first obtain both positive tests, that is, a positive ethanol test on pileus and yellowish zones in mycelial cultures (Mohanty et al., 2011), before confronting the taxonomic descriptions to existing literature in order to confirm identification of *G. resinaceum*.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Adaskaveg JE, Giltbertson R (1986). Cultural studies and genetics of sexuality of Ganoderma lucidum and G. tsugae in relation to the taxonomy of the G. lucidum complex. Mycologia 78:694-705. http://dx.doi.org/10.2307/3807513

Ambit RT (2011). Contribution to the taxonomic and ethnomycological study of Macromycetes growing in Mbengwi (North-West region) and surrounding areas in Cameroon. M.Sc thesis, Faculty of Science, University of Yaoundé 1, Cameroon, 87 p.

Boudier EJL (1889). Etude descriptive d'une nouvelle espèce de Ganoderma de France: Ganoderma resinaceum sp.nov. Bull. Soc. Mycol. Fr. 5:72.

Breitenbach J, Kränzlin F (1986). Champignons de Suisse. Tome 2. Champignons sans lames : Hétérobasidiomycètes, Aphyllophorales, Gastéromycètes. Edt. Mykologia, CH-6000 Lucerne 9. 412 p.

Bresadola J (1890). Fungi kamerunenses. Bull. Soc. Mycol. Fr. 6: XXXII- XLIX.

Chang ST, Buswell JA (1999). Ganoderma lucidum (Curt.; Fr.) P. Karst. (Aphyllophoromycetideae). – A mushrooming medicinal mushroom. Int. J. Med. Mushroom 1:139-146. http://dx.doi.org/10.1615/IntJMedMushrooms.v1.i2.30

Charbonnel J (1995). Les réactifs mycologiques. Tome 1. Les réactifs macrochimiques. Edité par l'auteur. 344 p.

Furtardo JS (1965). Relation of microstructure to the taxonomy of the Ganodermataceae (Polyporaceae) with special reference to the structure of the cover of the pilear surface. Mycologia 57: 688-711.

Furtardo JS (1967). Some tropical species of Ganoderma (Polyporaceae) with pale context. Persoonia 4:379-389.

Gilbertson RL, Ryvarden L (1986). North American Polypores, Vol. 1. Abortiporus–Lindtneria. Fungiflora, Oslo, Norway. 433 pp.

Hjortstam K, Ryvarden L, Watling R (1993). Preliminary checklist of non-agaricoid macromycetes in the Korup National Park, Cameroon and surrounding area. Edinb. J. Bot. 50 (1):105-119. http://dx.doi.org/10.1017/S0960428600000743

Kengni Ayissi MB, Mossebo DC, Machouart MC, Kansci G, Tsigaing TF, Dogang LR, Metsebing BP, Djifack NM (2014b). A new method by correlation to forecast the optimal time of spore-prints production and collection on sporocarps of Ganoderma resinaceum Boud. (Basidiomycota) on natural substrate. Mycosphere 5(6): 758-767.

Kengni Ayissi MB, Mossebo DC (2014a). Some noteworthy taxonomic variations in the complex wood-decayer Ganoderma resinaceum (Basidiomycota) with reference to collections from tropical Africa. Kew Bull. 69(4):1-14. http://dx.doi.org/10.1007/s12225-014-9542-9

Kinge TR (2012). Basal Stem Rot Disease of Oil palm and Identification of species of Ganoderma in South Western Cameroon. Ph.D Thesis, University of Buea, Cameroon. 218 p.

Kinge TR, Mih A (2011). Ganoderma ryvardense sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon. Mycosphere 2(2):179-188.

Kinge TR, Mih A (2014). Ganoderma lobenense (Basidiomycetes), a new species from oil palm (Elaies guineensis) in Cameroon. J. Plant Sci. 2(5): 242-245.

Kornerup A, Wansher JH (1978). Methuen handbook of colour. 3rd edn. Eyre Methuen, London. 252 p.

Mohanty PS, Harsh NSK, Pandley A (2011). First report of Ganoderma resinaceum and G. weberianum from north India based on ITS sequence analysis and micromorphology. Mycosphere 2(4): 469-474.

Moncalvo JM, Ryvarden L (1997). A nomenclatural Study of the Ganodermataceae Donk. Synopsis fungorum 11. 114 p.

Moncalvo JM, Wang H, Hseu RS (1995). Phylogenetic relationships in Ganoderma inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87(2): 223-238. http://dx.doi.org/10.2307/3760908

Mossebo DC (2002). Growth of wood-inhabiting Lentinus species from Cameroon in laboratory culture. Mycologist 16(4):168-171. http://dx.doi.org/10.1017/S0269915X02004068

Mossebo DC (2005). Contribution à la connaissance de la flore mycologique tropicale : Inventaire, taxonomie et systématique des collections de Basidiomycètes (Macromycètes) du Cameroun et d'Afrique centrale. Mémoire d'Habilitation à Diriger des Recherches (HDR), Université de Lille 2, France. p. 1-127.

Mossebo DC, Ryvarden L (1997). Fomitopsis africana sp. nov. (Polyporaceae, Basidiomycotina). Sydowia 49 (2): 147-149.

Mossebo DC, Ryvarden L (2003). The Genus Mycorrhaphium in Africa. Mycotaxon 88:229-232.

Mossebo DC, Njouonkou AL, Courtecuisse R, Amougou A (2007). Enzymatic activities and decay characteristics in some wood-rotting Basidiomycetes from Cameroon and determination of the time-dependant activity of syringaldazine in spot tests. Cryptogamie-Mycologie 28(2):107-121.

Mossebo DC, Kengni Ayissi MB, Ambit RT (2014). New taxa and potential pharmacological properties of some Ganodermataceae (Basidiomycota) from Cameroon and central Africa. In: abstracts book (Scripta Botanica Belgica 52, 291) of the Proceedings of the XXth AETFAT Congress, Stellenbosch, South Africa, 13th-17th January 2014.

Nŭnez M, Ryvarden L (2000). East Asian Polypores. Volume 1. Ganodermataceae and Hymenochataceae. Synopsis Fungorum 13. Fungiflora, Oslo, Norway. 168 p.

Roberts P, Ryvarden L (2006). Poroid fungi from Korup National Park, Cameroon. Kew Bull. 61: 55-78.

Ryvarden L (1992). Genera of Polypores; nomenclature and taxonomy. Synopsis Fungorum 5. 292 p.

Ryvarden L (2000). Studies in neotropical polypores 2: A preliminary key to neotropical species of Ganoderma with a laccate pileus. Mycologia 92(1):180-191. http://dx.doi.org/10.2307/3761462

Ryvarden L (2004). Neotropical polypores, Part 1. Introduction, Ganodermataceae & Hymenochaetaceae. Synopsis Fungorum 19. 227 p.

Ryvarden L, Melo I (2014). Poroid fungi of Europe. Synopsis Fungorum 31. 455 p. Fungiflora, Oslo, Norway.

Ryvarden L, Johansen I, (1980). A Preliminary Polypore Flora of East Africa. Oslo, Fungiflora. 636 p.

Steyaert RL (1967). Les Ganoderma palmicoles. Bull. J. Bot. Nat. Belg. 37 (4):465-492. http://dx.doi.org/10.2307/3667472

Steyaert RL (1972). Species of Ganoderma and related Genera, mainly of the Bogor and Leiden Herbaria. Persoonia 7: 55-118.

Steyaert RL (1980). Study of some Ganoderma species. Bull. J. Bot. Nat. Belg. 50:135-186. http://dx.doi.org/10.2307/3667780

Zoberi MH (1972). Tropical macrofungi. Some common species. The MacMillan Press Ltd, London and Basingstoke.