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)

Claude Mossebo Dominique, T Ambit Rose, Machouart Marie Claire, Kansci Germain

To cite this version:


HAL Id: hal-01482089
https://hal.univ-lorraine.fr/hal-01482089

Submitted on 3 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
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)

Dominique Claude MOSSEBO1,2*, Rose T. AMBIT1, Marie-Claire MACHOUART3 and Germain KANSCI4

1Mycological Laboratory, University of Yaoundé 1, B.P. 1456 Yaoundé, Cameroon.
2Mycology Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, England.
3Laboratoire Stress Immunité Pathogène – EA 7300 – Parasitologie-Mycologie – Université de Lorraine, 9 avenue de la forêt de Have, 54511 Vandoeuvre les Nancy, France.
4Department of Biochemistry, Faculty of Science, University of Yaoundé 1, B.P. 812 Yaoundé, Cameroon.

Received 23 February, 2015; Accepted 27 March, 2015

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 (CH3-CH2OH) 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...
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, 1967; 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 Gilbertson, 1986; Futardo, 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 parvum 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.mycocarentes.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), Núñez and Ryvarden (2000) and Breitenbach and Kränzlin (1986) said that “basidioecarps 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 basidioecarps 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).

<table>
<thead>
<tr>
<th>S/N</th>
<th>Herbarium number</th>
<th>Date of collection</th>
<th>Area of collection (i)</th>
<th>GPS coordinates</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HUY1-DM 45B</td>
<td>28/06/1996</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 21&quot; E 11° 31' 03&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>2</td>
<td>HUY1-DM 47D</td>
<td>15/07/1996</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 21&quot; E 11° 31' 03&quot;</td>
<td>Stump of oil palm tree (<em>Elaeis guineensis</em>)</td>
</tr>
<tr>
<td>3</td>
<td>HUY1-DM 72(ART)</td>
<td>3/10/2009</td>
<td>Mbengwi/Cameroon</td>
<td>N 05° 55' 18&quot; E 10° 08' 32&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>4</td>
<td>HUY1-DM 85</td>
<td>26/08/1996</td>
<td>Campus University of Yaoundé 1 (UY1)/Cameroon</td>
<td>N 03° 51' 31,6&quot; E 11° 29' 59&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>5</td>
<td>HUY1-DM 104</td>
<td>13/07/1997</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 26&quot; E 11° 32' 06&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>6</td>
<td>HUY1-DM 105B</td>
<td>23/05/2000</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 26&quot; E 11° 32' 06&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>7</td>
<td>HUY1-DM 418</td>
<td>18/11/2004</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 23' 39&quot; E 12° 43' 25&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>8</td>
<td>HUY1-DM 506</td>
<td>07/04/2007</td>
<td>Dja Biosphere reserve/Cameroon</td>
<td>N 03° 51' 31,6&quot; E 11° 31' 04&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>9</td>
<td>HUY1-DM 509</td>
<td>08/04/2007</td>
<td>Dja Biosphere reserve/Cameroon</td>
<td>N 03° 23' 39&quot; E 12° 43' 25&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>10</td>
<td>HUY1-DM 524</td>
<td>12/05/2011</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 51' 38&quot; E 11° 30' 07&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>11</td>
<td>HUY1-DM 538</td>
<td>10/04/2007</td>
<td>Dja Biosphere reserve/Cameroon</td>
<td>N 03° 23' 39&quot; E 12° 43' 25&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>12</td>
<td>HUY1-DM 612A</td>
<td>12/07/2009</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 28&quot; E 11° 31' 04&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>13</td>
<td>HUY1-DM 612B</td>
<td>June 2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 28&quot; E 11° 31' 04&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>14</td>
<td>HUY1-DM 612 C</td>
<td>12/07/2009</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 28&quot; E 11° 31' 04&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>15</td>
<td>HUY1-DM 617</td>
<td>13/06/2008</td>
<td>UY1/Cameroon</td>
<td>N 03° 51' 31,6&quot; E 11° 29' 59&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>16</td>
<td>HUY1-DM 619 A</td>
<td>12/06/2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 51' 23&quot; E 11° 31' 08&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>17</td>
<td>HUY1-DM 619 B</td>
<td>13/06/2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 51' 28&quot; E 11° 30' 09&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>18</td>
<td>HUY1-DM 619 C</td>
<td>13/06/2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 51' 28&quot; E 11° 30' 09&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>19</td>
<td>HUY1-DM 619 E</td>
<td>08/03/2013</td>
<td>UY1/Cameroon</td>
<td>N 03° 51' 31,6&quot; E 11° 29' 59&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>20</td>
<td>HUY1-DM 619 F</td>
<td>21/07/2013</td>
<td>Lobaye Forestry domain/ RCA</td>
<td>N 04° 22' 20&quot; E 19° 27' 18&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>21</td>
<td>HUY1-DM 622</td>
<td>15/06/2008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>HUY1-DM 629</td>
<td>09/06/2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 21&quot; E 11° 31' 03&quot;</td>
<td>Tree trunk in decay</td>
</tr>
<tr>
<td>23</td>
<td>HUY1-DM 660</td>
<td>13/06/2008</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 52' 21&quot; E 11° 31' 03&quot;</td>
<td>Stump of angiosperm</td>
</tr>
<tr>
<td>24</td>
<td>HUY1-DM 696</td>
<td>20/08/2009</td>
<td>Yaoundé/Cameroon</td>
<td>N 03° 51' 33&quot; E 11° 31' 02&quot;</td>
<td>Trunk of oil palm tree (<em>Elaeis guineensis</em>) in decay</td>
</tr>
</tbody>
</table>
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 at 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; Núñez 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; Núñez 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 (CH₃CH₂OH) 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 nyvadense, 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 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 basidiocarps 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 Konnerup 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 (exsiccatas), 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. G. resinaceum, G. applanatum, G. australe, G. baudonii, G. carocalacreus, G. colossum, G. hildebrandii, G. lobenense, G. nyvadense, 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 occurring 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 (exsiccatum) 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 exsiccatum 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* (exsiccatum) 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), Núñez 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...
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; Núñez and Ryvarden, 2000; Ryvarden and Johansen, 1980; 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), Núñez 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 yellowish resin as a key character conspicuously appearing on some strains of the species.

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 yellowish resin as a key character conspicuously appearing on some strains of the species.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fresh basidiocarp of <em>G. resinaceum</em></th>
<th>Dry basidiocarp (exsiccata) of <em>G. resinaceum</em></th>
<th>Positive control: <em>G. lucidum</em>, <em>G. applanatum</em>, <em>G. australre</em>, <em>G. baudonii</em>, <em>G. carocalacreus</em>, <em>G. colossom</em>, <em>G. hildebrandii</em>, <em>G. lobenense</em>, <em>G. ryvardense</em>, <em>G. weberianum</em>, <em>G. zonatum</em>, <em>G. sp1</em>, <em>G. sp2</em>, <em>G. sp3</em>, <em>G. sp. 4</em>, <em>G. sp. 5</em>, <em>G. sp. 6</em>, <em>G. sp. 7</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural colour of the pileus</td>
<td>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</td>
<td>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)</td>
<td>Bright reddish-brown (7F7-8 to 8F5-8) (Figure 1E/1F: Control Area) for dry basidiocarps of <em>G. lucidum</em>; different colours for other species</td>
</tr>
<tr>
<td>30%</td>
<td>Natural colour of pileus unchanged</td>
<td>Natural colour of pileus unchanged</td>
<td>Natural colour of pileus unchanged</td>
</tr>
<tr>
<td>70%</td>
<td>Pileus slightly brightening and most often showing exudates of faint and evanescent yellowish (2A2-4) resin</td>
<td>Pileus brightening just slightly and occasionally showing exudates of faint and evanescent yellowish (2A2-4) resin</td>
<td>Natural colour of pileus unchanged or ethanol slightly degrades pileus colour or brightness without revealing yellow resin</td>
</tr>
<tr>
<td>90%</td>
<td>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 &amp; G: Test Area)</td>
<td>Pileus ± conspicuously brightening, and sometimes oozing ± sticky exudates (Figure 1A: Test Area) of yellowish to yellow resin (2B6-8) (Figure 1C)</td>
<td>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 <em>G. lucidum</em> (Figure 1E/1F: Test Area) and other 17 species tested as positive control</td>
</tr>
<tr>
<td>99%</td>
<td>Water control test</td>
<td>Distilled water</td>
<td>Mycelium exudes yellowish to yellow (3A7-8) resin in 2 to 4 weeks old cultures (Figure 1D)</td>
</tr>
</tbody>
</table>

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), 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 planatate 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 (6C3-2) 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 (CH₃CH₂OH). 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 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.

ACKNOWLEDGEMENTS

The authors are very grateful to emeritus Professor Leif Ryvarden of the University of Oslo in Norway for cross-checking the preliminary identifications of *G. resinaceum* and other species of *Ganoderma* used in the macrochemical tests. The first author is particularly grateful to the Commonwealth Scholarship Commission (CSC) for the grant of a Commonwealth Fellowship [CMCF-2006-21] thanks to which part of this research work and others were carried out at the Mycology Section of the Royal Botanic Gardens, Kew, UK. The first author is also very grateful to Dr. Brian Spooner, the then head of the Mycology Section at Kew for all the logistical support put at the authors disposal during the research stay in Kew, UK.

REFERENCES


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.


