Phylogenetic and microscopic studies in the genus *Lactifluus* (*Basidiomycota*, *Russulales*) in West Africa, including the description of four new species

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**Abstract:** Despite the crucial ecological role of lactarioid taxa (*Lactifluus, Lactarius*) as common ectomycorrhiza formers in tropical African seasonal forests, their current diversity is not yet adequately assessed. During the last few years, numerous lactarioid specimens have been sampled in various ecosystems from Togo (West Africa). We generated 48 ITS sequences and aligned them against lactarioid taxa from other tropical African ecozones (Guineo-Congolean evergreen forests, Zambezian miombo). A Maximum Likelihood phylogenetic tree was inferred from a dataset of 109 sequences. The phylogenetic placement of the specimens, combined with morpho-anatomical data, supported the description of four new species from Togo within the monophyletic genus *Lactifluus*: within subgen. *Lactifluus* (*L. flavellus*), subgen. *Russulopsis* (*L. longibasidius* and *L. pectinatus*), and subgen. *Edules* (*L. melleus*). This demonstrates that the current species richness of the genus is considerably higher than hitherto estimated for African species and, in addition, a need to redefine the subgenera and sections within it.

**Key words:** Cryptic species, Distribution, *Lactifluus*, *Lactarius*, Molecular phylogeny, Taxonomy

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**INTRODUCTION**

Although more attention is now being paid to tropical fungi, the species richness of tropical macrofungi remains unclear. The tropical African mycobiota (including the West African) remains under-collected (Rivièr et al. 2007, Maba et al. 2013, 2014). Continued scientific collecting is therefore essential to raise our knowledge of tropical macrofungi, not least in poorly collected African ecosystems.

**Russulaceae** are among the commonest ectomycorrhizal macrofungi in West African forest ecosystems (Verbeken & Buyck 2001, van Rooij et al. 2003, Rivière et al. 2007, Verbeken & Walleyn 2010, Bâ et al. 2012, Maba et al. 2013, 2014, Sanon et al. 2014). Recent progress in molecular investigations within *Russulaceae* has not only led to the separation of the monophyletic genera *Lactifluus* and *Lactarius*, but also indicated the necessity of including West African taxa for any meaningful assessment of the diversity within this group (Verbeken et al. 2011). Still, recent mycological investigations using West African specimens have yielded new *Lactifluus* and *Lactarius* species (van Rooij et al. 2003, van de Putte et al. 2009, Maba et al. 2013, 2014). Similarly, previous studies have supported the high species diversity within *Lactifluus* in the region, compared to *Lactarius*, and indicated that many previously undescribed species might be expected in the region (Buyck et al. 2008, Stubbe et al. 2010, Verbeken et al. 2011, Maba et al. 2013, 2014).

Here, we combine morpho-anatomical studies and molecular phylogenetic analyses of rDNA ITS sequences from recent collections from Togo, and describe four new species within *Lactifluus*.

**MATERIAL AND METHODS**

**Specimens sampling**

Specimens were collected from 2011 to 2013 in caesalpinioïd- and phyllantoid-dominated woodlands of the Fazao-Malfakassa National Park and in the western forest area of Togo. Sampling methods and morphological data recording, as well as specimen handling and conservation, were as described in Maba et al. (2013, 2014). The colour data were recorded from fresh material using Kornerup & Wanscher (1978).

**Microscopy and scanning electron microscopy**

Microscopic studies followed the protocol in Maba et al. (2013, 2014), and SEM micrographs were prepared as detailed in Maba et al. (2013). Preliminary identification of

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specimens was made using the results of investigations in similar ecosystems (van Rooij et al. 2003). Additionally, we used the monograph of Verbeken & Walleyen (2010) on tropical African Lactarius s. l. species.

DNA Extraction, sequencing and PCR amplification
DNA was retrieved from dried specimens following the protocol used by Maba et al. (2013, 2014). The nrDNA ITS (including the ITS1, ITS2 and 5.8S regions) was amplified using the fungal specific primer ITS1-F in combination with the basidiomycete specific primer ITS4-B (Gardes & Bruns 1993). The 48 new ITS sequences obtained, including seven from the newly described species (specimens MD108, MD140, MD141, MD156, MD157, MD393, and MD397) have been deposited in the European Nucleotide Archive (ENA).

Sequence editing, analyses, and molecular phylogenetic inference
We first checked and downloaded the most similar sequences of fully identified taxa (up to species) and unidentified (up to genus) using BLASTN (Altschul et al. 1997) in public sequence databases (NCBI, ENA, and UNITE). Sequences with a minimum of 90 % of similarity to each of our sequences were considered and downloaded. To maximize the alignment, preference was given to tropical Africa sequences (already compiled in Maba et al. 2013, 2014). Then, closely related sequences from elsewhere were used to further populate the dataset.

The raw sequences were assembled and edited using BioEdit v. 7.2.5 (Hall 2005, update 12 Nov. 2013). Our final ITS dataset (Table 1) comprised 107 Russulaceae sequences including 81 of Lactifluus, 14 of Lactarius, five of Multifurca, and seven of Russula, along with two out-group sequences (one Gloeocystidiellum and one Hericium, both Russulales). A multiple alignment was performed using the online version of MAFFT v. 7.130b (Katoh & Toh 2008, update 27 Sept. 2013), by applying the “best accurate” option for the alignment. The resulting alignment was corrected manually by removing ambiguously aligned regions as well as mismatched and common empty columns. Our final sequence dataset was composed of 109 rDNA ITS sequences and had a total length of 700 bp.

The Maximum Likelihood (ML) tree was inferred in MEGA v. 6 (Tamura et al. 2013) by applying the General Time Reversible (GTR + G + I) (Nei and Kumar 2000) nucleotide substitution model. The rate variation among sites was modeled with a Gamma distribution, shape parameter = 6. The Subtree-Pruning-Regrafting Extensive (SPR level 5) with a very strong branch swap filter was applied as the ML heuristic method for Tree Inference Option. The initial ML tree was obtained automatically by NJ/BioNJ; and the phylogeny was tested using the bootstrap method with 1000 replicates (Felsenstein 1985).

RESULTS
ITS sequence analyses
The inferred phylogenetic tree (Fig. 1) had distinct and supported clades representing the accepted subgenera within Lactifluus (I to VIII), as well as other genera in Russulales (groups A to E). Within clade A, the first well supported (99 %) clade (I) constitutes Lactifluus subgen. Lactariopsis, with 13 sequences of nine taxa. Clade II encompasses sequences of unidentified samples from Togo (LK392607) and Benin (LK392604, LK392605, and LM999911). The sequences belonging to Lactifluus subgen. Russulopsis form clade III, which includes six of our newly generated sequences, five of which are from Togo and one from Guinea (LK392608). This last subgeneric clade (III) is phylogenetically supported within Lactifluus by a 60 % bootstrap value. The fourth clade (IV) represents Lactifluus subgen. Edules with 11 sequences corresponding to nine taxa. This clade is supported by 77 % bootstrap value. Lactifluus subgen. Lactifluus encompasses clades V (21 taxa) and VI (three taxa) and is the largest, with 36 sequences (including 17 of ours) representing 24 taxa. Clades V and VI are supported by 91 and 53 % bootstrap values, respectively. The Lactifluus subgens Gerardii and Piperati (until now unknown from African ecosystems) are represented by clades VII and VIII respectively. All six subgenera (Lactariopsis, Russulopsis, Edules, Lactifluus, Gerardii and Piperati) included in our analysis, are supported monophyletically and together form the genus Lactifluus.

Multifurca, with five samples, is a monophyletic group of its own (62 % bootstrap support) and constitutes clade B. The genus formed a sister clade to Lactifluus (clade A) and Lactarius (clade C). Clade C encompassed 14 Lactarius sequences, monophyletically well-supported by a 98 % bootstrap value. Sequences of Russula (five samples, clade D), and of Lactifluus and Lactarius, as well as those of Multifurca, formed a well supported (100 %) monophyletic group, the Russulaceae clade. In this analysis, Russula deviates and forms a separate monophyletic clade with 97 % bootstrap support. The sister clade (composed of a Gloeocystidiellum and a Hericium species, both Russulales) to all Russulaceae sequences formed the outgroup (clade E) in this analysis.

The sequences of MD157 (LK392597) and MD108 (LK392598) belong to the same species (99 % of bootstrap support) and are placed within Lactifluus subgen. Edules, within a well supported (98 %) clade together with three unnamed species. The phylogenetic placement of both specimens in this subgenus is supported by morpho-anatomical features (see below).

Within the Lactifluus subgen. Russulopsis clade, both sequences of MD141 (HG426473) and MD156 (LK392596) cluster as a single species (99 %), and together form a sister (100 % of bootstrap value) to MD140 (LK392599). Together, the clade formed by the above mentioned sequences and the one containing Lactifluus longipes (two samples), and an unnamed one (MD224 = LK392608), clustered as a monophyletic clade (61 % of bootstrap) that corresponds to Lactifluus subgen. Russulopsis. Similarly, the sequences of MD393 (LK392594) and MD397 (LK392595) appear as sister taxa (100 % support value) of the same species. They nested within Lactifluus subgen. Lactifluus clade with 96 % of bootstrap support and as sister to several named species and un-named collections.

The combination of morpho-anatomical features (see descriptions below) from each sample and the molecular
Table 1. ITS sequences generated in this study.

| Species                        | Collection numbers | Countries         | ENA Acc. No  |
|--------------------------------|--------------------|-------------------|--------------|
| Lactifluus annulatoangustifolius| MD145              | Togo              | HG426475     |
| Lactifluus densifolius         | C2362              | Togo              | HG917385     |
| Lactifluus edulis              | C2168              | Togo              | HG917384     |
| Lactifluus emergens            | DPM04              | Togo              | HG426467     |
| Lactifluus flammans            | MD124              | Togo              | HG426471     |
| Lactifluus flavellus           | MD393              | Togo              | LK392594     |
| Lactifluus flavellus           | MD397              | Togo              | LK392595     |
| Lactifluus fazaensis           | MD152              | Togo              | HG426477     |
| Lactifluus foetens             | MD150              | Togo              | HG917381     |
| Lactifluus foetens             | MD359              | Burkina Faso     | LK392603     |
| Lactifluus gymnocarpus         | MD125              | Togo              | HG426472     |
| Lactifluus gymnocarpoides      | MD301              | Benin             | LK392601     |
| Lactifluus gymnocarpoides      | MD318              | Benin             | LK392600     |
| Lactifluus heimi              | C2016              | Togo              | LK392612     |
| Lactifluus longibasidius       | MD141              | Togo              | HG426473     |
| Lactifluus longibasidius       | MD156              | Togo              | LK392596     |
| Lactifluus longipes            | ADK4315            | Togo              | HG917383     |
| Lactifluus longipes            | C2445              | Togo              | HG917391     |
| Lactifluus luteopus            | MD102              | Togo              | LK392602     |
| Lactifluus luteopus            | AV94-463           | Burundi           | LK392611     |
| Lactifluus aff. medusae        | MD142              | Togo              | HG426474     |
| Lactifluus melleus             | MD108              | Togo              | LK392598     |
| Lactifluus melleus             | MD157              | Togo              | LK392597     |
| Lactifluus nonpiscis           | MD101              | Togo              | HG426468     |
| Lactifluus pectinatus          | MD140              | Togo              | LK392599     |
| Lactifluus rubiginosus         | MD389              | Togo              | HG917386     |
| Lactifluus sudanicus           | MD105              | Togo              | HG426469     |
| Lactifluus sudanicus           | MD148              | Togo              | HG426476     |
| Lactifluus sp.                 | C2349              | Togo              | HG426478     |
| Lactifluus sp.                 | MD123              | Togo              | HG426470     |
| Lactifluus sp.                 | MD304              | Benin             | LK392604     |
| Lactifluus sp.                 | MD317              | Benin             | LK392605     |
| Lactifluus sp.                 | MD131              | Togo              | LK392606     |
| Lactifluus sp.                 | MD154              | Togo              | LK392607     |
| Lactifluus sp.                 | MD224              | Guinea            | LK392608     |
| Lactifluus sp.                 | MD355              | Burkina Faso     | LK392609     |
| Lactifluus sp.                 | MD234              | Guinea            | LK392610     |
| Lactifluus sp.                 | MD160              | Togo              | LK931501     |
| Lactifluus sp.                 | C1819              | Togo              | LM999910     |
| Lactifluus sp.                 | MD326              | Togo              | LM999911     |
| Lactifluus sp.                 | C2157              | Togo              | HG426466     |
| Lactifluus sp.                 | MD355B             | Burkina Faso     | LN651269     |
| Lactarius kabansus             | AV99-179           | Zimbabwe          | HG917390     |
| Lactarius miniatescens         | MD132              | Togo              | HG917376     |
| Lactarius subbalophaeus        | MD151              | Togo              | HG917374     |
| Lactarius tenellus             | MD100              | Togo              | HG917372     |
| Lactarius tenellus             | MD149              | Togo              | HG917373     |
Fig. 1. Maximum Likelihood (ML) tree showing the placement of the four newly described species (L. flavellus, L. longibasidius, L. melleus and L. pectinatus) within the Russulaceae. Bootstrap values higher than 50% are shown above/below the branches.
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phylogenetic analyses, support the description of four new species: *Lactifluus melleus* (MD157 and MD108), *L. longibasidius* (MD156 and MD141), *L. pectinatus* (MD140), and *L. flavellus* (MD393 and MD397).

**TAXONOMY**

*Lactifluus flavellus* Maba & Guelly, sp. nov.
MycoBank MB808850
(Figs 2–4)

*Etymology:* Refers to the yellowish, small and slender basidiome.

*Diagnosis:* Pileus concave then plano-convex to depressed; pellis dry, entirely and strongly striate; pastel yellow to light yellow, or yellowish white. Lamellae adnate, broadly decurrent to subdecurrent, widely spaced, unequal, regular. Stipe very long stipe, the longest known within African lactarioids. Context white, fleshy, thin in the pileus but thick in the stipe; latex white and unchanging; taste spicy. Basidiospores with ridged amyloid ornamentation, ridges mostly interconnected.

*Anatomy:* marginal cells of lamellae irregular, cylindrical to subclavate, septate and thin-walled; pleurocystidia densely abundant and conspicuously emergent, subcylindrical to narrowly fusiform; pileipellis palisade-like, with a suprapellis composed of cylindrical to subcylindrical cells; stipitipellis hymenoderm-like to subcellular.

*Type:* Togo: Plateaux region, Prefecture of Wawa, Béna Eglekoutsè, 7°31’6.6” N 0°54’7.41” E, on soil in gallery forest dominated by *Uapaca guineensis*, 17 July 2013, Dao Maba MD393 (TOGO – holotype). ENA accession no. LK392594.

*Description:* Pileus 15–50 mm diam, concave when young then plano-convex when older, slightly depressed in the centre; pellis entirely and strongly striate even in young basidiomes, very slightly smooth in the centre, dry, pastel yellow to light yellow (3A4-5) when young, yellowish white to pastel yellow when older (3A3-4 to 3A5). Margin strongly incurved in young

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**Fig. 2. Lactifluus flavellus** (MD393) basidiome. A. Lamellae and the pileus. B. Pileus and stipe detail. C. Stipe. Bars = 10 mm.

**Fig. 3. Lactifluus flavellus** (MD393) light microscopy. A. Pleuropseudocystidia. B. Pileipellis. C. Basidia. D. Basidiospores. E. Marginal cells. F. Pleurocystidia. Bars = 10 μm.

**Fig. 4. Lactifluus flavellus** (MD393) SEM of basidiospores. A, C. Dorsal view. B, D. Lateral and detail showing the plage.
specimens, straight and involuted and strongly striate in old specimen up to 2/3 from the edges to the centre. Lamellae adnate, broadly decurrent to subdecurrent, widely spaced, unequal, regular with 3 lamellulae between 2 lamellae (L+i = 4–6/cm), very brittle, yellowish white to pale yellow (3A2–3); edge entire. Stipe 40–95 × 7–12 mm, smooth, soft, central, cylindrical and slightly tapering downwards, medulla solid, concolorous to pileus, but paler at the base. Context white, fleshy, thin in the pileus while thick in the stipe, brittle. Latex
not abundant, white and unchanging, taste slightly spicy.

**Basidiospores** (Figs 3D, 4A–D) globose, subglobose to ellipsoid (7.0–7.5–8.5–9.0) × (6.5–7.0–7.5–8.0) μm (Q = (1.08–1.15–1.25–1.30(–1.35); n = 75), amylloid ornamentation of ridges to 0.5 μm in height, mostly connected, forming almost a complete reticulum; amylloid spot present in a distinct plage. Basidia (Fig. 3C) 4-spored, 50–60 × 18.5–11 μm, subclavate; sterigmata 4–6 × 1.5–2 μm. Lamella edge sterile. Marginal cells (Fig. 3E) 25–50 × 5–6(–7) μm, irregular, cylindrical to subclavate, sometimes tapering downwards, septate; thin-walled. Hymenophoral trama cellular, composed of sphaerocytes and numerous laticiferous hyphae. Pleurocystidia (Fig. 3F) dense, abundant, 55–80(–85) × 6–11 μm, emergent to 30 μm above the hymenium, subcylindrical to narrowly fusiform, sometimes septate. Pleuropseudocystidia (Fig. 3A) not abundant, 5–10(–11) μm diam, subcylindrical, rarely tortuous, sometimes bifurcate, tapering upwards, with a slightly moniliform to mucronate apex; thin-walled, with pale brown contents; incrustations and oleiferic drops present in the hymenium. Pileipellis (Fig. 3B) palisade-like, terminal elements of suprapellis 25–70(–85) × 3–4(–6) μm, composed of cylindrical to subcylindrical cells, scarce thick-walled terminal hyphae present; subpellis composed of irregularly spherical and isodiametric cells. Stipitipellis hymenoderm-like to subcellular, terminal elements subcylindrical to subclavate, thin-walled, rarely bifurcate. Clamps absent.

**Distribution:** Known only from Bèna Eglekoutô, Togo.

**Notes:** Lactifluus flavellus (MD393 and MD397) clusters within the clade containing sequences of L. longisporus (DQ421971 from Zimbabwe), L. aff. medusae (HG426474 from Togo), and L. gymnocarpoides (LK392600 and LK392601 both from Benin), from which it differs morphologically and anatomically (Verbenken & Walley 2010). Lactifluus gymnocarpoides has both a lampropalisade structure as the pileipellis and basidiospores that are ellipsoid to strongly elongate.

**Additional specimen examined: Togo:** Plateaux region, Prefecture of Wawa, Bèna Eglekoutô, 7°31′.18″ N 0°54′.77″ E, on soil, gallery forest dominated by Uapaca guineensis, 17 July 2013, Dao Maba MD397 (TOGO). ENA accession no. LK392595.

**Lactifluus longibasidius** Maba & Verbeken, sp. nov. MycoBank MB808851 (Figs 5–7)

**Etymology:** After the shape and the size of the basidia.
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yellowish to whitish, very thin at the margin; stipe firm and whitish. Latex copious, milky white and unchanging; taste not special.

Basidiospores (Figs 6G, 7A–D) globose to subglobose, sometimes ellipsoid, 7.5–8.5–9.0 × 6.5–7–7.5 (Q = 1–1.15–1.29; n = 82), ornamentation of distinguishable amyloid warts (0.5 µm high), finely and partially interconnected; no amyloid spot present in the plage. Basidia (Fig. 6C) 4-spored, long and slender, 70–95–120(–130) × 7–8(–9) µm, sometimes tortuous with sterigmata 6–8(–9) × 2–3 µm. Lamella edge sterile. Marginal cells (Fig. 6F) 30–66(–72) × 3–5 µm, subcylindrical to cylindrical, sometimes branched; septate. Hymenophoral trama composed of a mixture of sphaerocytes, numerous laticiferous and filamentous hyphae. Pleurocystidia (Fig. 6D) 75–80 × 6–7–7.5 µm, abundant, emergent, thin-walled, subcylindrical to cylindrical, sometimes tapering upwards, capitate or conical. Pleuropseudocystidia (Fig. 6A) abundant, 5–7(–8) µm diam, not always emergent, with brown needle-like contents. Pleipeilopsis (Fig. 6B) lamprotrichoderm-like, terminal elements 2–3(–5) µm wide, subcylindrical to fusiform, with distinctly tapering apex, septate and rather bifurcate, slender, thick-walled elements present. Stipitipellis a trichoderm with terminal elements 2–3(–4) µm wide, irregular, septate, bifurcate, longer than in the pileipellis. Clamps absent.

Distribution: Known only from Fazao-Malfakassa National Park, Togo.

Notes: Lactifluus longibasidius (MD141 and MD156) fits phylogenetically within the Lactifluus subgen. Russulopsis clade, but has some microscopic features that recall Lactifluus subgen. Lactariopsis sect. Chamaeleontini (Verbeken & Walleyn 2010).

Additional specimen examined: Togo: Central region: Prefecture of Thaoudjo, Fazao-Malfakassa National Park, 8°42’21” N 0°46’22” E, on soil in woodland dominated by Uapaca togoensis and Isoberlinia doka, 19 June 2011, Dao Maba MD141 (TOGO). ENA accession no. HG426473.
**Lactifluus melleus** Maba, sp. nov.
MycoBank MB808852 (Figs 8–10)

*Etymology:* The epithet recalls the honey-coloured basidiome.

*Diagnosis:* Pileus concave then plano-concave, strongly depressed in the centre, sometimes subinfundibuliform; pellis velvety and soft, smooth when freshly harvested, striate when dehydrated. Recognized by the dry, honey-coloured, light yellow to deep yellow or warm yellow pileus, slightly more pronounced in the centre. *Lamellae* adnate, broadly subdecurrent to decurrent, moderately spaced, unequal, irregular; context of pileus brittle, slightly thick in the centre, fleshy, white in the pileus as well as in the stipe; latex scarce, white, and unchanging. *Basidiospores* with well developed amyloid warts connected by fine lines; pleurocystidia absent; an ixocutis to a trichoderm pileipellis with a suprapellis composed of irregular, cylindrical to subclavate cells, septate, interwoven; dermatocystidia with a moniliform to mucronate apex; marginal cells of lamellae irregular, cylindrical to subclavate, thin-walled.

*Type:* Togo: Central region: Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 8°30' 56''N 0°54'44.1''E, on soil in woodland dominated by *Uapaca togoensis*, *Isoberlinia doka* and *I. tomentosa*, 19 June 2011, Dao Maba MD157 (TOGO – holotype; GENT – isotype). ENA accession no. LK392597.

*Description:* Pileus 40–60 mm diam, concave when young then plano-concave, strongly depressed in the centre,
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Distribution: Known only from Fazao-Malfakassa National Park, Togo.

Notes: Lactifluus melleus (MD108 and MD157) nested phylogenetically within Lactifluus subgen. Edules, but it has some microscopic features (not all), as mentioned above, that recall Lactifluus subgen. Lactariopsis sect. Chamaeleontini and also Lactifluus subgen. Russulopsis (Verbeken & Walley 2010).

Additional specimen examined: Togo: Central region: Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 8°42'27"N 0°45'13"E, on soil in woodland dominated by Isoberlinia doka and Uapaca togoensis, 19 June 2011, Dao Maba MD140 (TOGO – holotype; GHENT – isotype). ENA accession no. LK392599.

Description: Pileus 25–75 mm diam, concave when young, later plano-concave and slightly depressed in the centre when older; dry, tomentose, striate to 1/3 from the margin, slightly sticky, shortly sulcate and broadly pectinate near the margin, smooth in the centre, pale yellow to light yellowish (3A4-5) and light orange to deep orange (5A6-8). Margin strongly crenulated, strongly involuted when young, becoming incurved to slightly straight when old. Lamellae adnate, broadly subdecurrent to decurrent, spaced to very distant (L+ = 4–5/cm), unequal, irregular, yellowish to pale yellow (3A4-5). Stipe 23–30 × 8–12 mm, central, cylindrical, tapering downwards, dry, smooth and concolorous with the pileus. Context of pileus fragile, very brittle and thin near the margin; margin, slightly thicker in the centre; stipe firm and yellowish. Latex abundant; white, unchanging.

Basidiospores (Figs 12C, 13A–C) globose to subglobose, sometimes ellipsoid, (6.5–)7.5–8.5–9.5(–10.0) × (6.0–)6.5–7.0–7.5(–8) µm (Q = 1.03–1.12–1.16; n = 75), ornamentation of distinctly amyloid warts, to 0.5 µm high, interconnected at the base and forming a complete reticulum. Pleurocytistidia abundant, emergent, cylindrical to subcylindrical, fusiform, sometimes septate, thin-walled. Also recognized by an ixotrichopalisade-like pileipellis with a suprapellis composed of subcylindrical to subclavate cells, mixed with numerous swollen and subglobose to globose cells; marginal cells of the lamellae cylindrical to fusiform, septiculate.

Type: Togo: Central region: Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 8°42'27"N 0°45'13"E, on soil in woodland dominated by Isoberlinia doka and Uapaca togoensis, 19 June 2011, Dao Maba MD140 (TOGO – holotype; GHENT – isotype). ENA accession no. LK392599.

Diagnosis: Pileus concave, plano-concave to depressed; pileis dry, tomentose, striate near the margin, sticky, shortly sulcate and broadly pectinate near the margin; pale yellow to light yellowish and light orange to deep orange. Lamellae adnate, broadly subdecurrent to decurrent, spaced to distant; context yellowish, fragile, very brittle and thin near the margin, slightly thicker in the centre; latex abundant, white, unchanging. Basidiospores with amyloid warts ornamentation interconnected at the base and forming a complete reticulum. Pleurocytistidia abundant, emergent, cylindrical to subcylindrical, fusiform, sometimes septiculate, thin-walled. Also recognized by an ixotrichopalisade-like pileipellis with a suprapellis composed of subcylindrical to subclavate cells, mixed with numerous swollen and subglobose to globose cells; marginal cells of the lamellae cylindrical to fusiform, septiculate.

Notes: Lactifluus melleus (MD108 and MD157) nested phylogenetically within Lactifluus subgen. Edules, but it has some microscopic features (not all), as mentioned above, that recall Lactifluus subgen. Lactariopsis sect. Chamaeleontini and also Lactifluus subgen. Russulopsis (Verbeken & Walley 2010).

Additional specimen examined: Togo: Central region: Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 8°42'27"N 0°45'27", on soil in woodland dominated by Uapaca togoensis, Isoberlinia doka and I. tomentosa, 19 June 2011, Dao Maba MD108 (TOGO, GENT). ENA accession no. LK392598.

Lactifluus pectinatus Maba & Yorou, sp. nov. MycoBank MB808853 (Figs 11–13)

Etymology: Recalling the pectinate shape of the pileus.

Diagnosis: Pileus concave, plano-concave to depressed; pileis dry, tomentose, striate near the margin, sticky, shortly sulcate and broadly pectinate near the margin; pale yellow to light yellowish and light orange to deep orange. Lamellae adnate, broadly subdecurrent to decurrent, spaced to distant; context yellowish, fragile, very brittle and thin near the margin, slightly thicker in the centre; latex abundant, white, unchanging. Basidiospores with amyloid warts ornamentation interconnected at the base and forming a complete reticulum. Pleurocytistidia abundant, emergent, cylindrical to subcylindrical, fusiform, sometimes septiculate, thin-walled. Also recognized by an ixotrichopalisade-like pileipellis with a suprapellis composed of subcylindrical to subclavate cells, mixed with numerous swollen and subglobose to globose cells; marginal cells of the lamellae cylindrical to fusiform, septiculate.

Notes: Lactifluus melleus (MD108 and MD157) nested phylogenetically within Lactifluus subgen. Edules, but it has some microscopic features (not all), as mentioned above, that recall Lactifluus subgen. Lactariopsis sect. Chamaeleontini and also Lactifluus subgen. Russulopsis (Verbeken & Walley 2010).
subcylindrical to subclavate, mixed with numerous swollen and subglobose to globose elements, thick-walled elements scarce. Stipitipellis a palisade, terminal elements 20–35 × 3–4(–5) µm, cylindrical, fusiform; subpellis composed of isodiametrical cells. Clamps absent.

Distribution: Known only from Fazao-Malfakassa National Park, Togo.

Notes: *Lactifluus pectinatus* (MD140), as well as *L. longibasidius*, is supported phylogenetically within the *Lactifluus* subgen. *Russulopsis* clade, but, as noted above, they present some microscopic features that recall *Lactifluus* subgen. *Lactariopsis* sect. *Chamaeleontini* (Verbeken & Walleyn 2010).

**DISCUSSION**

*Lactifluus longibasidius* and *L. pectinatus*, are morpho-anatomically clearly different from previously described species, and have microscopic features that confirm both their separateness and phylogenetic positions (Fig. 1). In addition to the presence of pleurocystidia in both species (although with different shapes and sizes, see Figs 6 and 12), they have basidiospores with a well-developed amyloid ornamentation, composed of distinguishable obtuse, finely and partially interconnected warts; they have no amyloid spot.
in the plaque. These features characterize some known species of Lactifluus subgen. Russulopsis, as well as L. subgen. Lactariopsis sect. Chamaeleontini (Verbeken & Walleyn 2010). However, L. longibasidius, with two representative collections, differs considerably from L. pectinatus, as well as from the known Lactifluus species in these groups, in the shape and size of the pleurocystidia (to 130 µm) when present, and the marginal cells (to 72 µm). The species is recognizable by the surprisingly long basidia (to 135 µm) that usually emerge to 50 µm above the hymenium. These features have never been reported for any known species from tropical Africa (van Rooij et al. 2003, Buyck 2007, Verbeken & Walleyn 2010). In addition, both L. pectinatus and L. longibasidius have no dermatocystidia, such as those observed in L. ruvubuensis and L. longipes.

Lactifluus melleus, represented by two samples, is phylogenetically well supported within Lactifluus subgen. Ectodules. It has a velvety and soft pellis, appearing smooth in fresh specimens, but is striate in dehydrated samples; moderately spaced, unequal, irregular, and subdecurrent to decurrent lamellae; and an ixocutis to trichoderm moderately spaced, unequal, irregular, and subdecurrent to decurrent lamellae; and an ixocutis to trichoderm. In fresh specimens, basidiospores have no dermatocystidia, such as those observed in L. ruvubuensis and L. longipes.

Lactifluus flavellus conforms to Lactifluus subgen. Lactifluus. Comparative microscopical studies with the closely related L. uapacae, show that the new species has some distinctivel anatomical characters. It has a very long stipe (to 95 mm), the longest yet reported within tropical African ecosystems, [42x674]in West Africa, and particularly in West Africa, remain crucial for a real assessment of the extent of tropical African mycodyversity, and will thereby help to highlight the evolutionary traits within milkcaps.

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