



Diversity of *Lactifluus* (*Basidiomycota*, *Russulales*) in West Africa: 5 new species described and some considerations regarding their distribution and ecology

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Abstract

The genus *Lactifluus* is one of the common ectomycorrhizal fungal taxa in tropical African forest ecosystems. Recent morphological and anatomical mycological studies based on specimens we sampled from 2007 to 2013 in West African forest ecosystems, including dry, dense, riparian forests and woodlands, enable to assess the diversity and the occurrence of *Lactifluus* species in the Guineo-Sudanian domain. A total of 51 ITS rDNA sequences generated from our samples were aligned against tropical African and worldwide *Lactifluus* sequences available in GenBank. A Maximum Likelihood phylogenetic tree was inferred from 113 sequences. The phylogenetic placement of the species, combined with our morpho-anatomical data, supported the description of five new species distributed among *Lactifluus* species. Our data further confirm that the species richness of the genus *Lactifluus* is high and partly unexplored in the Guineo-Sudanian domain, and confirmed that, in both the Guineo-Sudanian and the Congo-Zambeian domain many common species occur. Patterns of occurrence of the recorded *Lactifluus* species from Guineo-Sudanian ecozones are also highlighted.

Key words – Guineo-Sudanian – milkcaps – morpho-anatomy – molecular phylogeny – taxonomy

Introduction

Although there has been remarkable progress in tropical mycological investigations for the last twenty years, tropical African ecozones, and particularly West Africa, remain very poorly explored (van Rooij et al. 2003, Rivière et al. 2007, Diédhiou et al. 2013). In Guineo-Sudanian forest ecosystems, the genera *Scleroderma*, *Tomentella*, *Russula*, *Lactarius* (*L.*) and *Lactifluus* (*Lf.*) are among the most studied ectomycorrhizal fungi (ECM) (van Rooij et al. 2003, Yorou et al. 2011, 2012, Verbeken & Walley 2010, Sanon et al. 2013, Maba et al. 2013, 2014, 2015, Sanon et al. 2014). Recent progress in molecular phylogenetic analyses and morpho-anatomical investigations within lactarioid taxa (Buyck et al. 2008, Verbeken & Walley 2010, Van de Putte et al. 2010, Stubbe et al. 2010, Verbeken et al. 2011, De Crop et al. 2014, Maba et al. 2013, 2014, 2015)

highlighted the high genetic diversity of the genus *Lactifluus*, with numerous new species and cryptic species described from tropical Africa (Verbeken et al. 2008, Van de Putte et al. 2009, De Crop et al. 2012, Maba et al. 2013, 2015). In order to better circumscribe species limits and the ecological plasticity of *Lactifluus* species, mycological investigations have been undertaken from specimens collected in West African forest ecosystems (Maba et al. 2013, 2014, 2015).

Maba et al. (2015) undertook molecular analysis of *Lactifluus* taxa, including unidentified specimens from West Africa. In addition to considerations regarding ecology, the present study provides morpho-anatomical support for new *Lactifluus* species identification. Thus, from numerous specimens we sampled, five *Lactifluus* specimens of which the molecular phylogenetic positions are supported by Maba et al. (2015), are described hereby as new species to science, based on morpho-anatomical differences with most closely related species. The newly described *Lactifluus* species are accommodated in subgenera *Lactariopsis* (two species), *Russulopsis* (one species) and *Edules* (two species). Chorological patterns of recorded *Lactifluus* species in West Africa and their putative host trees are discussed.

Material and Methods

Specimens were sampled between 2007 and 2013 in various West African forest ecosystems, including the northern Guinean seasonal, dry, dense, riparian and open forests, woodlands and savannas following a megatranssect through five countries (Benin, Togo, Burkina Faso, Mali, and Guinea). The specimens described here were sampled from DAN riverside forest in south-western part of Burkina Faso (MD355); in Malouwaita rainforest of Guinea (MD219B, MD224 and MD234). Specimens (C2349, MD123 and MD131) were sampled in Fazao-Malfakassa National Park, whereas specimens DPM05, C2157 and C2163 were sampled in Aledjo Reserve forest, all in central Togo. Sampling techniques, records of preliminary morphological data as well as specimens' preparation for conservation are detailed previously (Maba et al. 2013). Colours were recorded following Kornerup & Wanscher (1978). Holotypes of the new species are conserved in TOGO herbarium and isotypes in GENT and M (Thiers 2012).

Light and Scanning Electron Microscopy

Microscopical studies were performed focusing on the lactarioids anatomical diagnostic features, following Verbeken & Walley (2010) and Maba et al. (2013, 2014, 2015). Measurements are given referring to Buyck (1991) and detailed by Maba et al. (2013). Comparative microscopic studies also integrated specimens of *Lactifluus zenkeri* (A MA. 20) and *L. sesemotani* (AV94-471 and AV94-82) received from Ghent University as loans. SEM micrographs were obtained using the procedures explained by Maba et al. (2013). Preliminary identification of specimens were made using the *Lactarius* s. l. study based on material collected in similar ecosystems in the neighboring country Benin (van Rooij et al. 2003) and the monograph of Verbeken & Walley (2010) about tropical African *Lactarius* s. l.

DNA Extraction, sequencing and PCR amplification

Genomic DNA extraction, sequencing and PCR amplification were undertaken by Maba et al. (2015). The internal transcribed spacer regions (ITS) of the nuclear ribosomal DNA including ITS1, ITS2 and 5.8S regions were amplified using the fungal specific primer ITS1F in combination with the basidiomycete specific primer ITS4B (Gardes & Bruns 1993). A total of 51 ITS sequences were obtained and the sequences of the newly described species have been deposited at European Nucleotide Archive/ENA (Table 1).

Sequence editing, analyses and molecular phylogenetic inference

We refer to Maba et al. (2015) for sequence editing, analyses and phylogenetic inference (Supplement). Four new sequences (three newly generated and one obtained from GenBank) were added to the sequence dataset compiled in Maba et al. (2015). The multiple sequence alignment and procedure for phylogenetic tree inference refer to Maba et al. (2015) and the Maximum Likelihood

Table 1 – List of our generated and public Genbank sequences included in phylogenetic analyses

Species	ENA, accession numbers	Localities
<i>Lactifluus</i> (81 sequences)		
<i>Lactifluus allardii</i>	KF220017, KF220015	USA
<i>Lactifluus annulatoangustifolius</i>	HG426475	Togo
<i>Lactifluus annulatoangustifolius</i>	AY606981	Madagascar
<i>Lactifluus annulatoangustifolius</i> sp. nov.*	HG426470, LK392606	Togo
<i>Lactifluus atrovolutinus</i>	GU258231	Malaysia
<i>Lactifluus burkinabeii</i> sp. nov.*	LK392609	Burkina Faso
<i>Lactifluus brunneocarpus</i> sp. nov.*	LK392608	Guinea
<i>Lactifluus chamaeleontinus</i>	AY606980	Zambia
<i>Lactifluus chiapanensis</i>	GU258297	Mexico
<i>Lactifluus clarkeae</i>	HQ318283	Australia
<i>Lactifluus clarkeae</i>	GU222280	New Zealand
<i>Lactifluus crocatus</i>	HQ318265, Q318248, HQ318266	Thailand
<i>Lactifluus denigricans</i>	AY606983	Benin
<i>Lactifluus densifolius</i>	HG917385	Togo
<i>Lactifluus edulis</i>	HG917384	Togo
<i>Lactifluus emergens</i>	HG426467	Togo
<i>Lactifluus emergens</i>	AY606979	Zimbabwe
<i>Lactifluus fazaoensis</i>	HG426477	Togo
<i>Lactifluus flammans</i>	HG426471	Togo
<i>Lactifluus flammans</i>	UDB016931	Benin
<i>Lactifluus flavellus</i>	LK392594, LK392595	Togo
<i>Lactifluus flocktonae</i>	JX2666621, JX2666622	Australia
<i>Lactifluus foetens</i>	HG917381	Togo
<i>Lactifluus foetens</i>	LK392603	Burkina Faso
<i>Lactifluus genevievae</i>	GU258294	Australia
<i>Lactifluus glaucescens</i>	KF220117	Italy
<i>Lactifluus glaucescens</i>	KF220094	Belgium
<i>Lactifluus glaucescens</i>	KF220075	France
<i>Lactifluus guellii</i> sp. nov.*	HG426466	Togo
<i>Lactifluus gymnocarpoides</i>	LK392601	Benin
<i>Lactifluus gymnocarpoides</i>	LK392600	Benin
<i>Lactifluus gymnocarpus</i>	HG426472	Togo
<i>Lactifluus heimii</i>	LK392612	Togo
<i>Lactifluus hygrophoroides</i>	JN129397	China
<i>Lactifluus inversus</i>	AY606976	Guinea
<i>Lactifluus longibasidius</i>	LK392596, HG426473	Togo
<i>Lactifluus longipes</i>	HG917391, HG917383	Togo
<i>Lactifluus longipilus</i>	HQ318235, HQ318258, KF432958	Thailand
<i>Lactifluus longisporus</i>	DQ421971	Zambia
<i>Lactifluus luteopus</i>	LK392602	Togo
<i>Lactifluus luteopus</i>	LK392611	Burundi
<i>Lactifluus medusae</i>	HG426474	Togo
<i>Lactifluus madagascariensis</i>	AY606977	Madagascar
<i>Lactifluus melleus</i>	LK392598, LK392597	Togo
<i>Lactifluus membranaceus</i> sp. nov.*	LK392610	Guinea
<i>Lactifluus membranaceus</i> sp. nov.*	HG426478	Togo
<i>Lactifluus nodosicystidiosus</i>	AY606975	Madagascar
<i>Lactifluus nonpiscis</i>	HG426468	Togo
<i>Lactifluus pectinatus</i>	LK392599	Togo
<i>Lactifluus piperatus</i>	KF220122, KF220120	France
<i>Lactifluus pelliculatus</i>	AY606978	Madagascar
<i>Lactifluus phlebophyllus</i>	AY606074	Madagascar
<i>Lactifluus pseudoluteopus</i>	HQ318286	Thailand
<i>Lactifluus rubroviolascens</i>	AY606984	Zambia
<i>Lactifluus rubroviolascens</i>	AY606985	Madagascar
<i>Lactifluus rubiginosus</i>	HG917386	Togo
<i>Lactifluus sudanicus</i>	HG426469, HG426476	Togo
<i>Lactifluus velutissimus</i>	AY606982	Zimbabwe

Species	ENA, accession numbers	Localities
<i>Lactifluus volemus</i>	HQ318279, HQ318275	Thailand
<i>Lactifluus volemoides</i>	UDB016930	Benin
<i>Lactifluus</i> sp.	LK392607	Togo
<i>Lactifluus</i> sp.	LK931501	Togo
<i>Lactifluus</i> sp.	LK392604	Benin
<i>Lactifluus</i> sp.	LK392605	Benin
<i>Lactifluus</i> sp.	LM999911	Benin
<i>Lactifluus</i> sp.	LN651269	Burkina Faso
<i>Lactifluus</i> sp.	LM999910	Togo
<i>Lactifluus</i> sp.	UDB014027	Cameroon
Lactarius (17 sequences)		
<i>Lactarius baliophaeus</i>	GU258277	Zambia
<i>Lactarius kabansus</i>	HG917376	Togo
<i>Lactarius kabansus</i>	HG917390	Zimbabwe
<i>Lactarius miniatescens</i>	HG917375	Burkina Faso
<i>Lactarius miniatescens</i>	HG917374	Togo
<i>Lactarius tenellus</i>	HG917373	Togo
<i>Lactarius saponaceus</i>	HG917379	Guinea
<i>Lactarius saponaceus</i>	HG917378	Togo
<i>Lactarius subbaliophaeus</i>	HG917372	Togo
<i>Lactarius</i> sp.	UDB013804	Zambia
<i>Lactarius</i> sp.	UDB015091	Gabon
<i>Lactarius</i> sp.	UDB018664	Zambia
<i>Lactarius</i> sp.	UDB018662	Zambia
<i>Lactarius</i> sp.	UDB013845	Zambia
<i>Lactarius</i> sp.	UDB013930	Cameroon
<i>Lactarius</i> sp.	UDB016860	Zambia
<i>Lactarius</i> sp.	UDB013836	Zambia
Multifurca (5 sequences)		
<i>Multifurca zonaria</i>	DQ422000, DQ421990	Thailand
<i>Multifurca furcata</i>	DQ421995, DQ421994	USA
<i>Multifurca ochricompacta</i>	DQ421984	USA
Russula (8 sequences)		
<i>Russula cremeirosea</i>	EU819424	USA
<i>Russula congoana</i>	HG917387	Togo
<i>Russula congoana</i>	UDB016932	Benin
<i>Russula compressa</i>	UDB016985	Benin
<i>Russula discopus</i>	JQ902046	Burundi
<i>Russula discopus</i>	JQ902050	Senegal
<i>Russula lipida</i>	JF908663	Italy
<i>Russula xerampilina</i>	KF386758	USA
Out group (2 sequences)		
<i>Gloeocystidiellum</i> sp.?	KJ140715	USA
<i>Hericium erinaceum</i>	EU784265	Kew

(*) Newly described species

(ML) tree obtained has included a total of 113 ITS sequences (Tab. 1, Supplement). The alignment is submitted to TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S17549>).

Results

ITS sequence analyses

Sequences of the target specimens (MD123, MD131, MD224, MD234, MD355, C2157, and C2349) are supported in the clade that encompasses representatives of *Lactifluus* subgenera *Lactariopsis*, *Edules* and *Russulopsis* (Fig. 1). The sequences of two newly described species (MD123 and MD131, C2349 and MD234) cluster within the *Lf.* subg. *Lactariopsis* subclade, with 98% and 57% of bootstrap support respectively, with already known species (subclade I). The subclade II encompasses sequences of unidentified specimens from Togo (MD154) and Benin

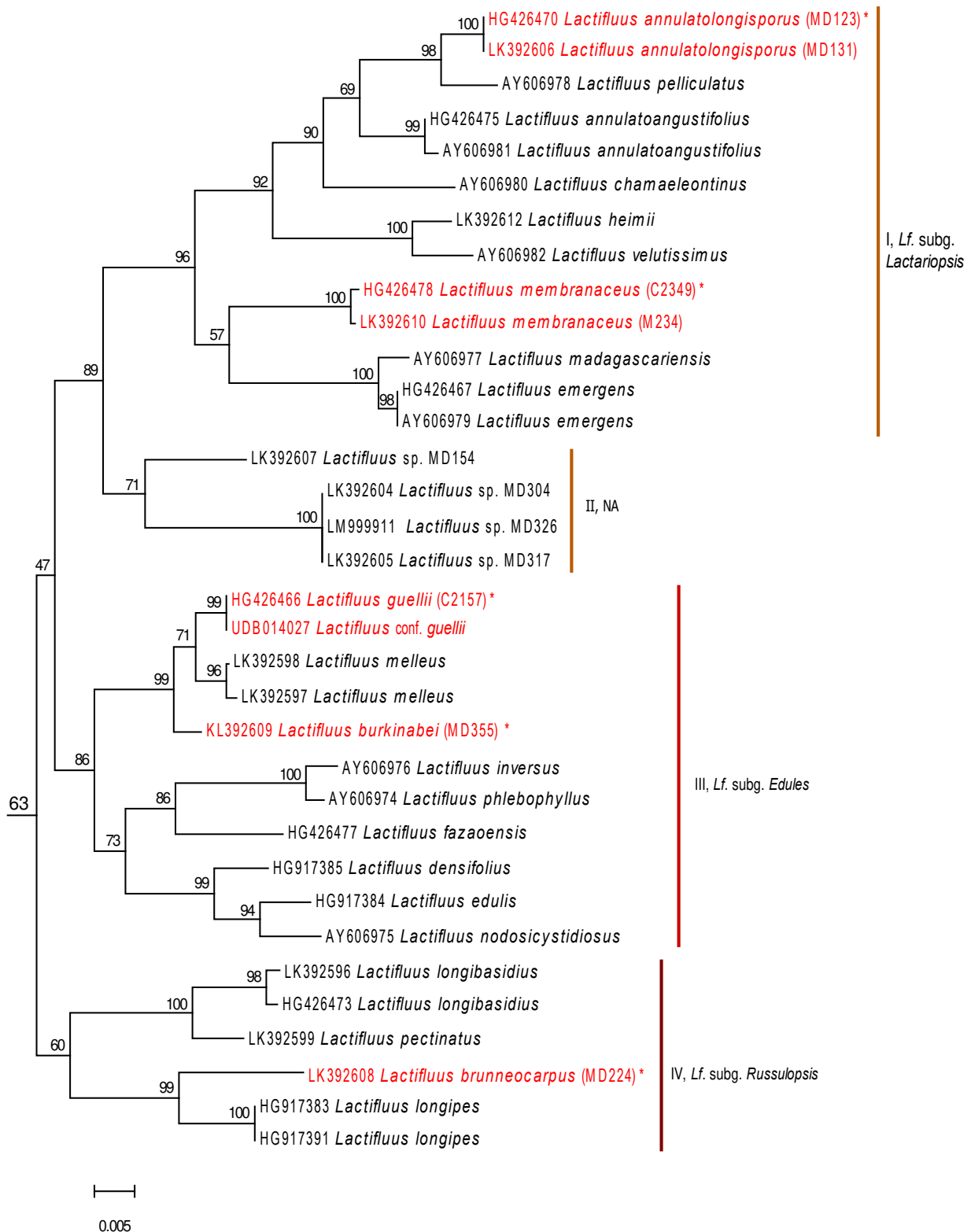


Fig. 1 – Phylogenetic tree obtained from ITS sequences. Bootstrap values (in %) are from maximum likelihood (ML) analyses (1000 bootstraps). *Lactifluus* (subgenera *Lactariopsis*, *Edules* and *Russulopsis*) subtree showing the five newly described species: *Lf. annulatolongisporus*, *Lf. brunneocarpus*, *Lf. burkinabei*, *Lf. guellii* and *Lf. membranaceus*. (*) is mentioned in front for the holotypes.

(MD304, MD317, and MD326). At the same time the sequences of the specimens C2157 and MD355 (two newly described species), fit in *Lf.* subg. *Edules*, with respectively 71 % and 99% of bootstrap support with already known species (subclade III). In *Lf.* subg. *Russulopsis* subclade (IV), nested the sequence of one new described species (MD224), supported by 99% of bootstrap value as sister species of *Lf. longipes*.

The morpho-anatomical analyses reveal deviating features between specimen MD123, MD131, MD219B, MD224, MD234, MD355, C2157, C2163, C2349 and DPM05, and their morphological closely related species. These deviating features, coupled with the phylogenetic placement of the specimens, accommodates them into five new species, notably: *Lactifluus annulatolongisporus* (specimens MD123 and MD131) and *Lactifluus membranaceus* (C2349, DPM05 and MD234) within *Lf.* subg. *Lactariopsis*; *Lactifluus brunneocarpus* (MD219B and MD224) in *Lf.* subg. *Russulopsis*, and *Lactifluus burkinabei* (MD355) and *Lactifluus guellii* (C2157 and C2163), both within *Lf.* subg. *Edules*.

Taxonomy

Lactifluus annulatolongisporus Maba, sp. nov.

Figs 2 – 4

Mycobank MB811601,

Facesoffungi Number: FoF 01642

Genbank ENA, accession number HG426470

Etymology – Referring to the presence of an annulus and the basidiospores that are strongly elongate.

Pileus (Fig. 2A-C) 40-65 mm diam., plano-convex, depressed to umbilicate, slightly subinfundibuliform; remnants of secondary/partial velum forming an evanescent annulus; margin first incurved then crenulate; pellis pruinose, dry, not dehiscent; whitish, orange white at the margin, pale orange in the center, appearing zonate-like. Lamellae slightly crowded, broadly adanate to subdecurrent, irregular, unequal; 1 or 3 lamellulae between 2 lamellae ($L+l=7-10/cm$). Stipe 30-40 × 10-15 mm; cylindrical, tapering downwards; fleshy and firm. Context whitish to fleshy, firm; thick in the center of the pileus and thin near margin. Latex not abundant, whitish and unchanging.

Basidiospores (Fig. 3C, 4A-C) strongly elongate, rarely ellipsoid (9)10-10.5-11(11.5) × (5.5)6.0-7.0-7.5(8) μm ($Q=1.3-1.4-1.5-1.6-1.7$; $n=120$); ornamentation amyloid, composed of very short warts, fine lines, thicker in their middle part, not clearly distinguishable under light microscope; very low warts slightly connected (as seen in scanning electron microscope); amyloid spot in the plage distinctly present (Fig. 3C). Basidia (Fig. 3B) 40-65 × 10-12 μm, subcylindrical and four-spored. Pleurocystidia absent. Pleuropseudocystidia (Fig. 3D) 6-30 μm diam., very abundant, clavate to conical, rarely subcylindrical, sometimes bifurcate, apex micronate or capitate, sometimes emergent up to 50 μm above the hymenium, contents needle-like. Lamellar edge sterile. Hymenophoral trama composed of a mixture of filamentous hyphae, sphaerocytes and lactifers. Marginal cells (Fig. 3E) 25-60 × 4-11 μm, utriform, tortuous, dichotomously branched. Pileipellis (Fig. 3B) a lamprotrichopalisade, terminal elements thick-walled very slender, up to 240 μm long, septate, sometimes forked or branched. Stipitipellis identical to pileipellis. Clamps absent.

Material examined – Togo, Central region, Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, N08°42'58" E00°46'22", on soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 18 June 2011, (collector) D.L. Maba, MD123 (TOGO, holotype), AV11-147 (GENT, Isotype); Togo, Central region, Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, N08°42' 24" E00°45' 08", on soil in woodland dominated by *Isoberlinia doka*, and *Uapaca togoensis*, 18 June 2011, (collector) D.L. Maba, MD131 (TOGO, holotype), ENA acc. no. LK392606.

Known distribution – Togo, Fazao-Malfakassa National Park.

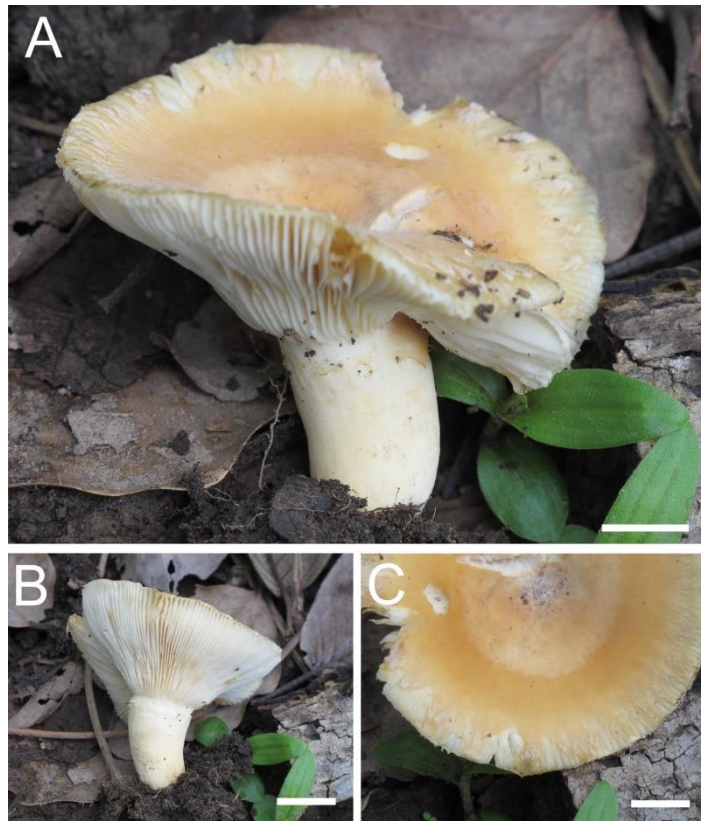


Fig. 2 – Basidiome of *Lactifluus annulatolongisporus* (MD123). A. Detailed view. B. Lamellae and stipe detailed. C. Pileus view, pellis detailed. – Scale Bars = 10 mm.

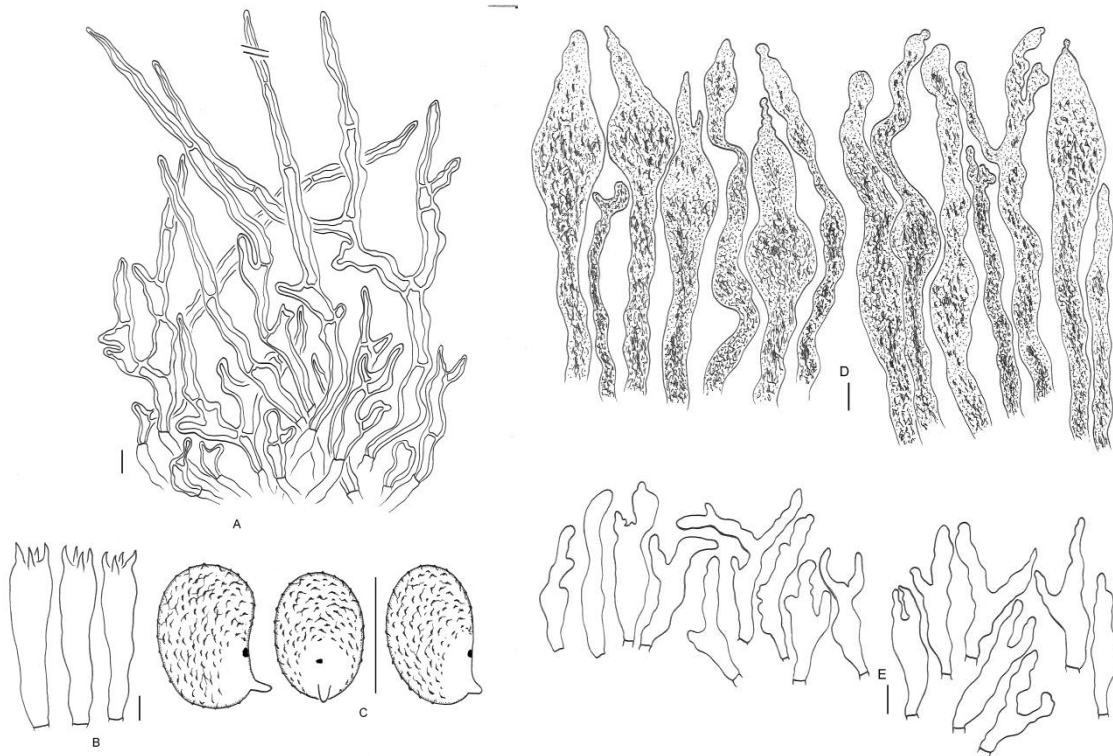


Fig. 3 – Light microscopy of *Lactifluus annulatolongisporus*. A. Pileipellis B. Basidia. C. Basidiospores D. Pleuropseudocystidia, MD123 (left); MD131 (right). E. Marginal cells, MD131 (left); MD123 (right). – Scale Bars = 10 μ m. Section line drawing

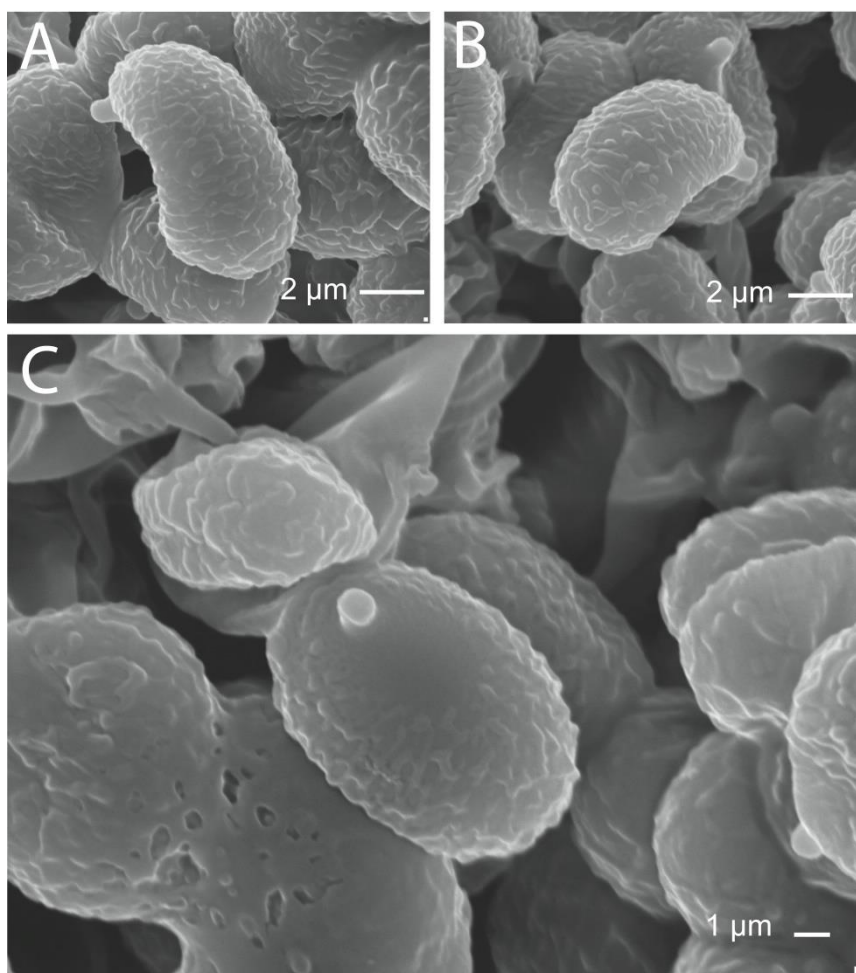


Fig. 4 – SEM of *Lactifluus annulatolongisporus* (MD123). Basidiospores: A-B. Dorsal view. C. Lateral and detailed view showing the plage.

Lactifluus brunneocarpus Maba, sp. nov.

Figs 5 – 7

Mycobank MB811602,

Facesoffungi Number: FoF 01643

GenBank ENA, accession number LK39260

Etymology – Referring to the brown (5B5-8 to 5C6-8) coloration of the basidiome.

Pileus (Fig. 5A-D) 60-100 mm diam., very thin-fleshed, plano-convex and depressed when young, then infundibuliform when older; pellis wet, indehiscent, strongly striate near margin, smooth in the center; brownish orange to brownish yellow, darker in the center (5B5-8 to 5C6-8). Margin incurved to straight, finally uprolled. Lamellae adnate to subdecurrent, distinctly distant or spaced, rarely forked at the margin, very fragile and very brittle, unequal, regular pattern with 3 lamellulae between 2 lamellae, (L+l= 4-5/cm), whitish to pale orange. Stipe concolorous to the pileus, 35-50 × 10-13 mm, cylindrical, central, tapering downwards, wet mat, fleshy and firm. Context of pileus very thin, slightly thick in the center, very fragile and brittle, stipe firm; whitish to pale orange (5A2-4). Latex abundant, whitish, changing slightly to green, taste and smell not special.

Basidiospores (Fig. 6E, 7A-B) broadly ellipsoid, $7.0\text{-}8.5\text{-}9.5 \times 6.0\text{-}6.5\text{-}7.0 \mu\text{m}$ ($Q=1.17\text{-}1.2\text{-}1.25\text{-}1.3\text{-}1.35$; $n=75$), ornamentation amyloid, composed of well-developed irregular, conical or rounded, and isolated warts; amyloid spot in the plage absent. Basidia (Fig. 6C) 50-75 × 9-10 μm ; 4-spored; subcylindrical to subclavate, tapering downwards; sterigmata 3-5 × 1.5-2.5 μm , well developed. Pleurocystidia (Fig. 6D) very abundant, 45-80 × 7-10 μm ; irregularly shaped, subcylindrical, tortuous, much branched and commonly diverticulate, thin-walled, septate, apex tapering. Pleuropseudocystidia (Fig. 6B) not abundant, 6-10 μm diam.; emergent, irregularly

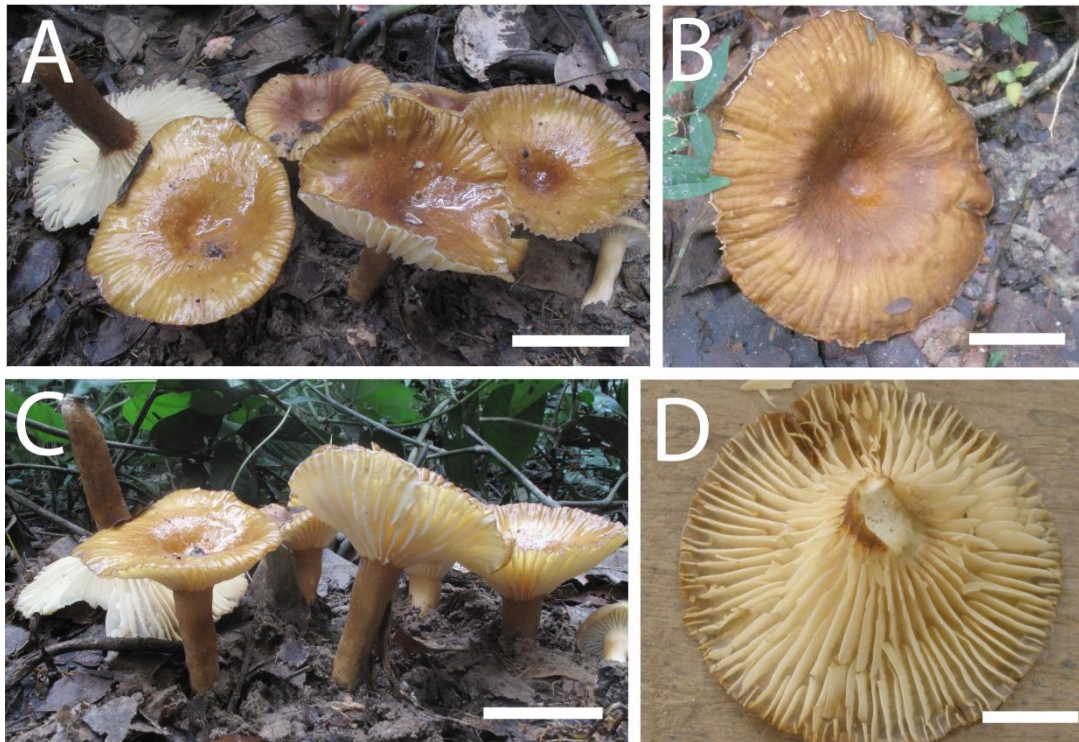


Fig. 5 – Basidiome of *Lactifluus brunneocarpus*. A, C. General detailed view (MD219B). B. Pileus view, pellis detailed (MD224). D. View of lamellae, detailed (MD224). – Scale Bars = 10 mm.

subcylindrical, apex tapering sometimes inflated, content brown, needle-like. Hymenophoral trama mostly filamentous composed of a mixture of hyaline hyphae, sphaerocytes, and lactifers. Lamellae edge sterile. Marginal cells (Fig. 6F) $40\text{-}60 \times 2\text{-}5 \mu\text{m}$, subcylindrical to cylindrical, sometimes septate sometimes frocked at apex. Pileipellis (Fig. 6A) a cutis to ixocutis-like, mono-layered, composed of interwoven horizontal, slightly ascending hyphae, thin-walled, septate, and often branched, in mixture with lactifers; terminal element $3\text{-}5 \mu\text{m}$ diam., cylindrical to subcylindrical. Stipitipellis identical to pileipellis. Clamps absent.

Material examined – Guinea, Malouwaita, N10°32'7.7" W09°22'8.6", on soil in rainforest dominated by *Uapaca heudelotii*, 18 July 2011 (collector) D.L. Maba, MD224 (TOGO, holotype), Isotype Munich (M); Guinea, Malouwaita, N08°19'7.3" W09°13'20.1", on soil in rainforest dominated by *Uapaca heudelotii*, 18 July 2011, (collector) D.L. Maba, MD219B (TOGO).
Known distribution – Guinea, Malouwaita.

Lactifluus burkinabei Maba, sp. nov.

Figs 8 – 10

Mycobank MB811603

Facesoffungi Number: FoF 01644

Genbank ENA, accession number LK392609

Etymology – Referring to Burkina Faso, the country where the holotype was found.

Pileus (Fig. 8A-C) 60-105 mm diam., firm, fleshy and thick, plano-convex and depressed to infundibuliform, pellis wet, smooth, sticky; orange to deep orange (5A7-8). Margin smooth, inflected to downrolled. Lamellae broadly, decurrent, irregular, unequal ($L+l= 8\text{-}9\text{/cm}$), very commonly forked, strongly anastomosing at the insertion of the stipe, widely spaced, light yellow to orange yellow (4A6-8). Stipe 15-40 \times 10-15 mm, cylindrical, central and tapering downwards, dry matt, firm. Context fleshy and firm, pale yellow to butter yellow (3A5-4A5), thick in the center, and slightly thinner at the margin, stipe fleshy and firm. Latex slightly abundant, whitish, unchanging, taste bitter and spicy, smell not special.

Chemical reaction – changing to brown with FeSO_4 on the context.

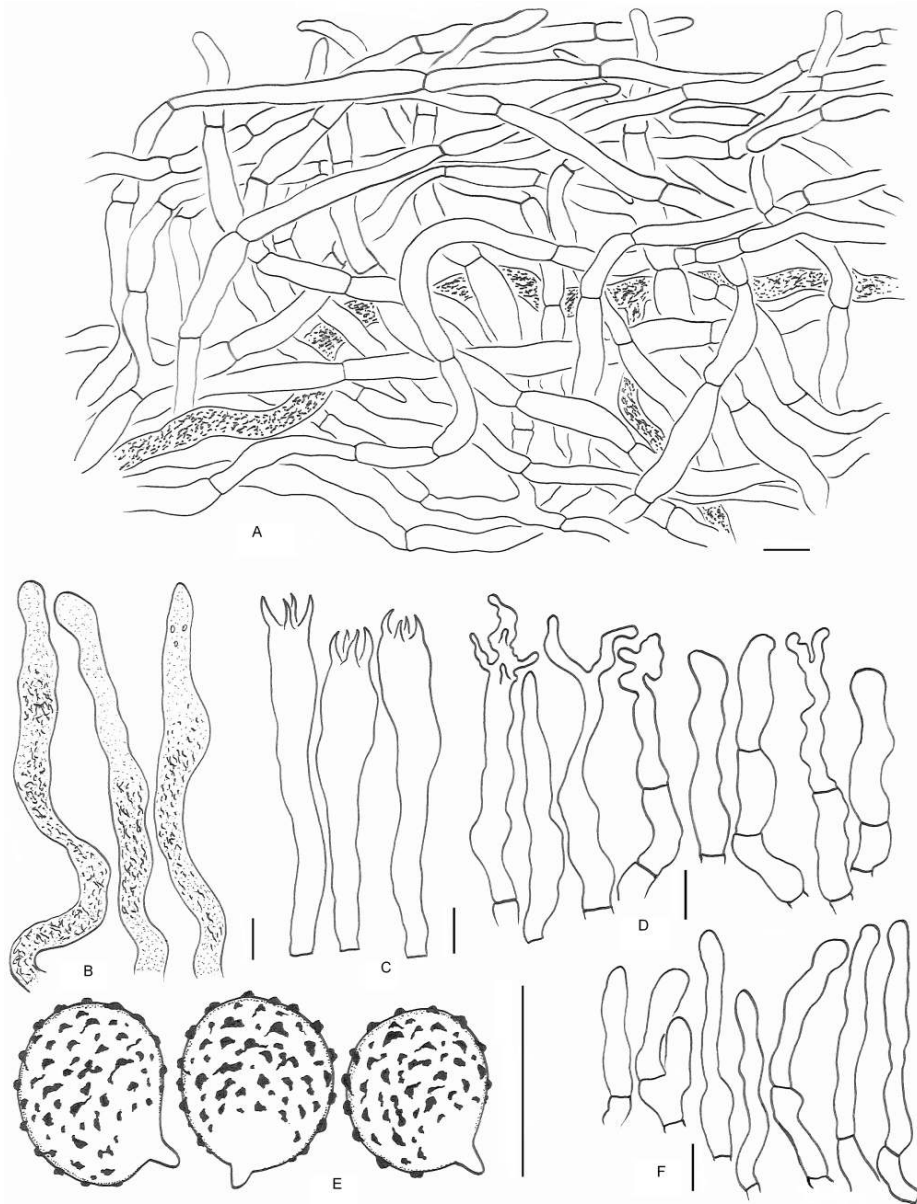


Fig. 6 – Light microscopy of *Lactifluus brunneocarpus* (MD224). A. Pileipellis. B. Pleuropseudocystidia. C. Basidia. D. Pleurocystidia. E. Basidiospores. F. Marginal cells. – Scale Bars = 10 μ m. Section line drawing

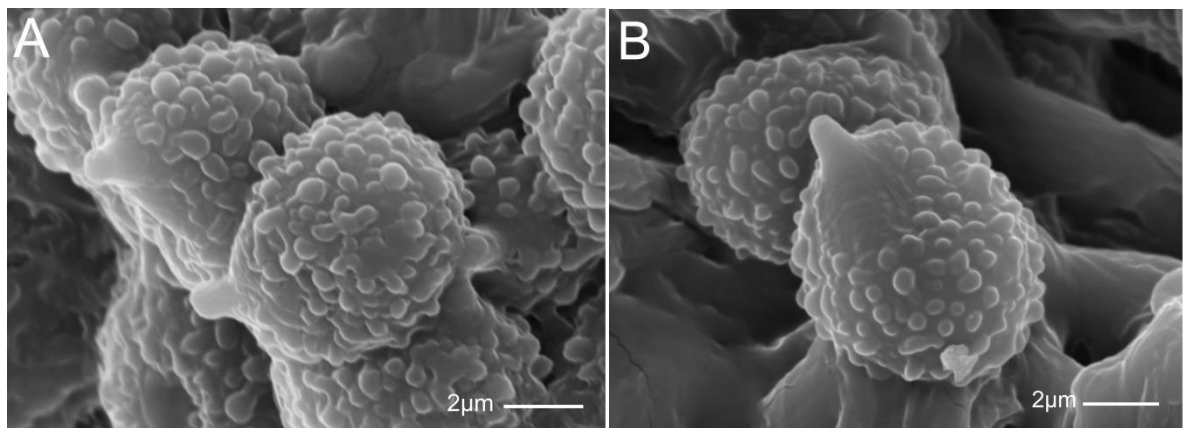


Fig. 7 – SEM of *Lactifluus brunneocarpus* (MD224). Basidiospores: A. Lateral front view. B. Proximal profile view showing the plage



Fig. 8 – Basidiome of *Lactifluus burkinabei* (MD355). A. Pileus detailed. B. View of lamellae and stipe, detailed. C. View of context change with FeSO_4 reagent. – Scale Bars = 10 mm.

Basidiospores (Fig. 9G, Fig. 10B-C) subglobose to ellipsoid, $8.0\text{-}9.0\text{-}10 \times 7.0\text{-}7.5\text{-}8.0 \mu\text{m}$ ($Q=1.06\text{-}1.15\text{-}1.25$; $n=75$); ornamentation amyloid, composed of irregularly shaped warts, almost interconnected forming reticulum, and seldom isolated; plage with a strong amyloid spot. Basidia (Fig. 9C) $55\text{-}75 \times 10\text{-}12$ (13) μm ; variable, two- and four-spored; subcylindrical to subclavate; sterigmata $6\text{-}12 \times 2\text{-}3 \mu\text{m}$, sometimes with irregular shape, apex sometimes bulging or appearing swollen. Pleuroleptocystidia (Fig. 9E) very abundant, $60\text{-}75 \times 10\text{-}13 \mu\text{m}$; subcylindrical to subclavate, thin-walled, apex often tapering upwards, almost mucronate to rostrate, emergent. Pleuropseudocystidia (Fig. 9B) very abundant, $8\text{-}25 \mu\text{m}$ diam.; irregularly subcylindrical, mostly inflated, apex capitate to mucronate; very emergent and projecting up to $40 \mu\text{m}$ above the hymenium; with irregular, needle-like, brown contents. Hymenophoral trama cellular composed of a mixture of sphaerocytes, and lactifers. Lamellae edge sterile. Marginal cells (Fig. 9D) $15\text{-}35 \times 5\text{-}6(8) \mu\text{m}$, very variable in shape, cylindrical to subcylindrical, sometimes fusiform, sometimes septate; apex distinctly mucronate or flared. Pileipellis (Fig. 9A) an ixotrichopalisade to trichopalisade, with abundant clavate to subglobose cells (up to $50 \mu\text{m}$ diam.), suprapellis elements subcylindrical sometimes fusiform, irregularly branched, septate. Dermatocystidia (Fig. 9F) abundant, $40\text{-}65 \times 4\text{-}7 \mu\text{m}$, with mucronate to subcapitate apex, with needle-like contents. Stipitipellis a trichopalisade to lamprotrichopalisade, suprapellis composed of irregular elements, subcylindrical to subclavate, sometimes tortuous, septate; thick-walled elements present, in mixture with numerous interwoven lactifers. Clamps absent.

Material examined – Burkina Faso, Bobodioulasso, Orodara, DAN, $\text{N}10^{\circ}53'6.9''$ $\text{E}04^{\circ}50'27.9''$, on soil in gallery forest dominated by *Berlinia grandifolia* and *Uapaca guineensis*, 12 July 2013, (collector) D.L. Maba, MD355 (TOGO, holotype), Isotype Munich (M).

Known distribution – Burkina Faso, Orodara.

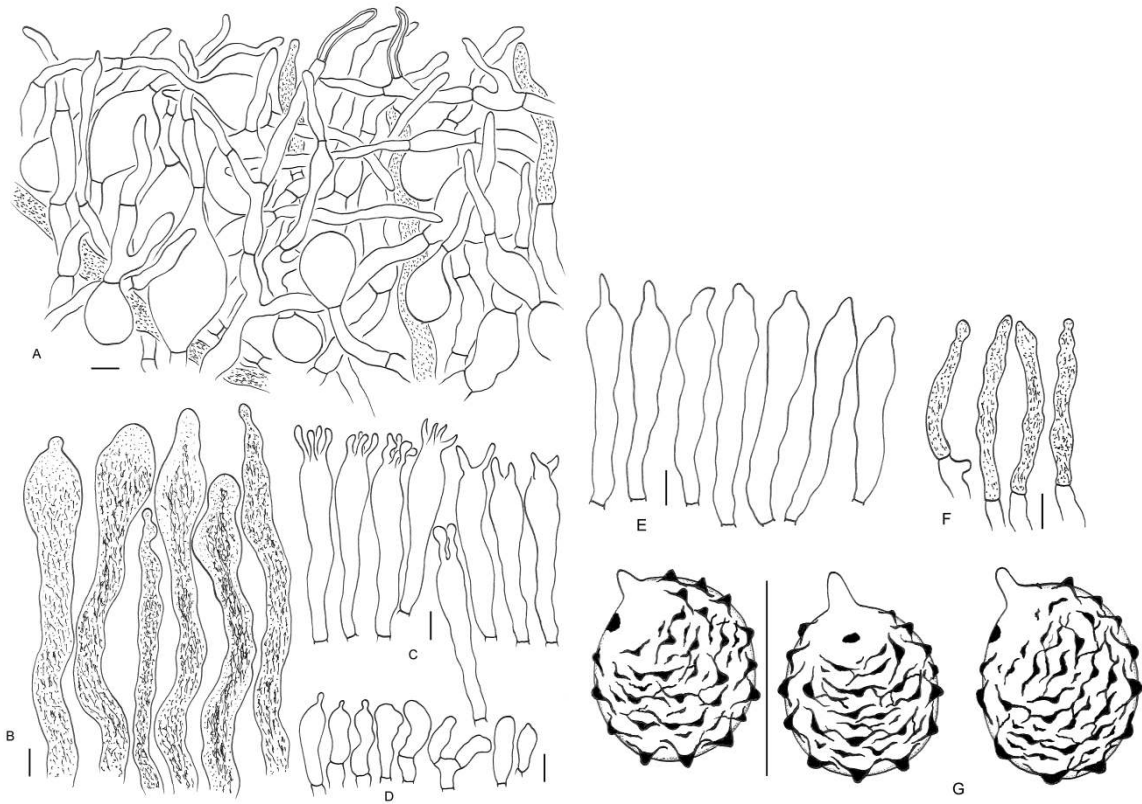


Fig. 9 – Light microscopy of *Lactifluus burkinabei* (MD355). A. Pileipellis. B. Pleuropseudocystidia. C. Basidia. D. Marginal cells. Hymenium. E. Pleurocystidia. F. Dermatocystidia. G. Basidiospores. – Scale Bars = 10 μ m. Section line drawing.

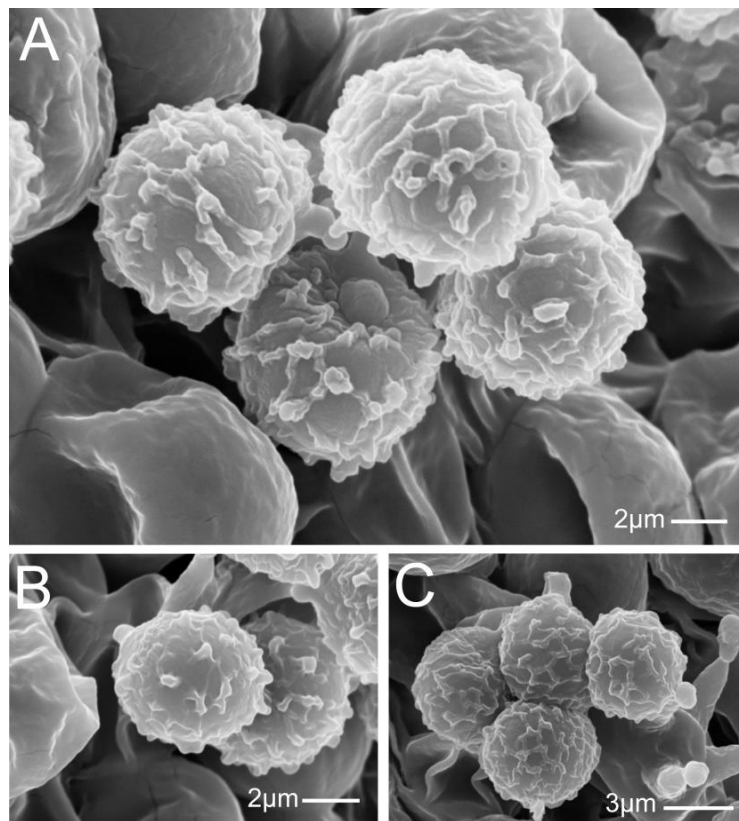


Fig. 10 – SEM of *Lactifluus burkinabei* (MD355). Basidiospores: A. Overview and detailed view showing the plage with amyloid spot. B. Lateral/dorsal view. C. Dorsal view.

Lactifluus guellii Maba, sp. nov.

Mycobank MB811604

Facesoffungi Number: FoF 01645

Genbank ENA, accession number HG426466

Figs 11 – 13

Etymology – In honour to Prof. Atsu Guelly, from the University of Lomé (Togo) for initiating and promoting studies on macromycetes in Togo.

Pileus (Fig. 11A-C) 35-60 μm diam., plano-convex, depressed to subinfundibuliform; pellis dehiscent, sticky, faintly striate when young and strongly striate near margin when older; orange white, pale orange to light orange (5A2-5) darker in center. Margin strongly striate, at first incurved then straight, crenulated to uprolled. Lamellae adnate to subdecurrent, spaced, unequal, irregular; 3 or 5 lamellulae between 2 lamellae ($L+l=5-7/\text{cm}$), sometimes bifurcate or forked at the margin (up to $\frac{1}{3}$ from margin), orange white. Stipe 25-30 \times 10-15 μm , cylindrical, tapering downwards, smooth, orange white. Context thin near margin, fleshy and firm in the center of the pileus and the stipe; whitish. Latex not abundant, whitish and unchanging.

Basidiospores (Fig. 12B, 13A-D) broadly ellipsoid to elongate, 7.5-9.5-10.5(11.5) \times 6.5-7.5-8(8.5) μm ($Q=1.2-1.30-1.45$; $n=65$); ornamentation amyloid; composed of well-developed blunt warts ($>0.5\mu\text{m}$ high) connected by fine lines; plage with amyloid spot (Fig. 12, 13). Basidia (Fig. 12E) 45-70 \times 10-12 μm , subcylindrical, four-spored. Pleurocystidia (Fig. 12F) rather abundant, 45-75 \times 5-8 μm , irregularly shaped, subcylindrical, mostly tortuous to fusiform, thin-walled. Pleuropseudocystidia (Fig. 12D) very abundant, 4-15 μm diam., tortuous to fusiform, tapering upwards, mucronate, emergent, contents needle-like and granular. Lamellar edge sterile. Hymenophoral trama cellular, mixture of sphaerocytes and laticifers. Marginal cells (Fig. 12C) 10-35 \times 4-8 μm , broadly clavate, subcylindrical to fusiform, thin-walled, bifurcate and septate. Pileipellis (Fig. 12A) lamprotrichoderm-like with abundant swollen hyphae; terminal hyphae sometimes thick-walled; pseudocystidia abundant, 5-8 μm diam., with needle-like contents. Stipitipellis mixed ixocutis-like, composed of subclavate, subcylindrical to fusiform hyphae, and interwoven hyphae with sometimes thick-walled apex. Clamps absent.

Material examined – Togo, Central region: Prefecture of Assoli, Reserve Forest of Aledjo N09°16'53.7", E001°13'41.2", gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis* 26 May 2008, leg. A.K. Guelly, C2157 (TOGO, Holotype); Togo, Central region: Prefecture of Assoli, Reserve Forest of Aledjo N09°16'53.7", E001°13'41.2", gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis* 26 May 2008, leg. A.K. Guelly, C2163 (TOGO).

Known distribution – Togo, Aledjo Reserve Forest.

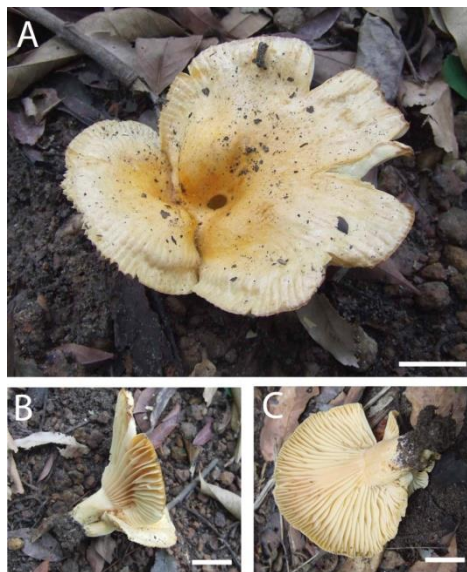


Fig. 11 – Basidiome of *Lactifluus guellii* (C2157). A. Pileus detailed. B. Lateral view of the lamellae and stipe. C. Detailed view lamellae and stipe. – Scale Bars = 10 mm.

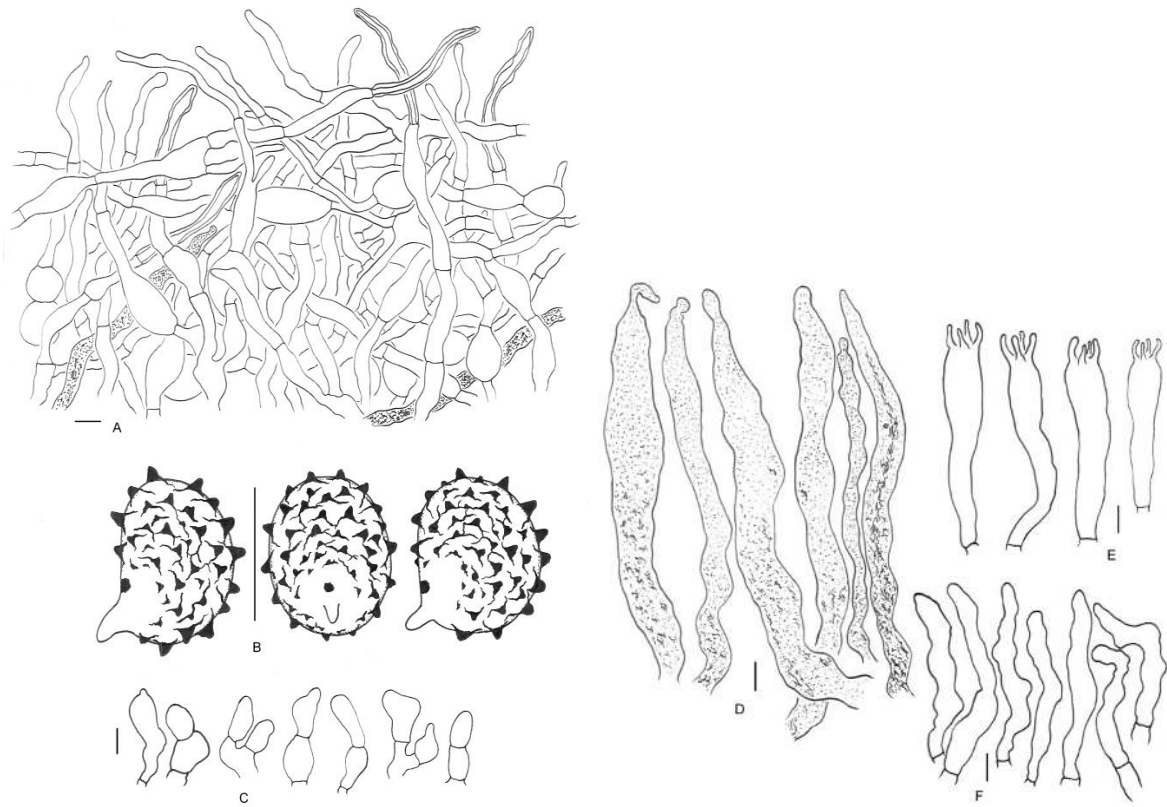


Fig. 12 – Light microscopy of *Lactifluus guellii* (C2157). A. Pileipellis B. Basidiospores. C. Marginal cells. D. Pleuropseudocystidia. E. Basidia. F. Pleurocystidia. – Scale Bars = 10 μm . Section line drawing.

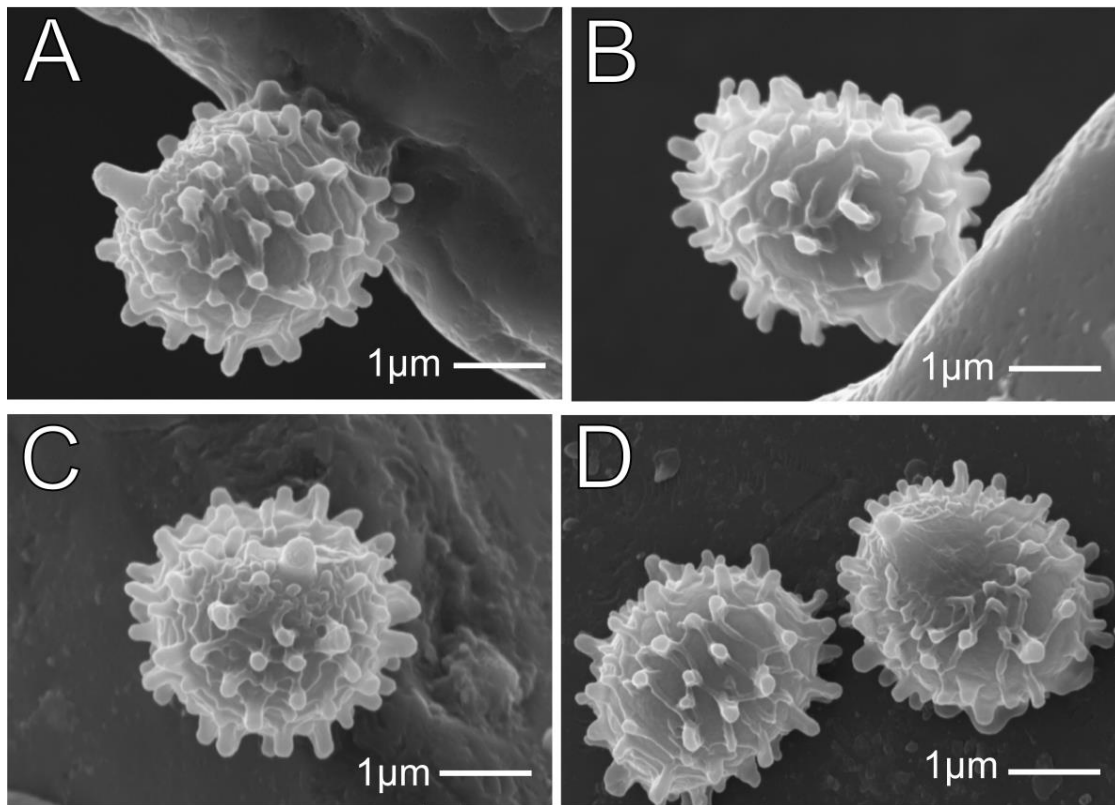


Fig. 13 – SEM of *Lactifluus guellii* (C2157). Basidiospores: A. Proximal view (left), lateral view (right). B. Dorsal view. C. Proximal view.

Lactifluus membranaceus Maba, sp. nov.

Figs 14 – 16

Mycobank MB811605

Facesoffungi Number: FoF 01646

Genbank ENA, accession number HG426478

Etymology – Referring to the remaining membranous-like velum that covers the pileus.

Pileus (Fig. 14A-C) 50-65 mm diam., convex when young, then plano-convex and depressed in the center, covered by a universal veil which initially enclosed the young basidiome; dry, velvety, uniform, membranous, smooth; beige (4BC3), orange white to yellow orange (4B5-8 to 5B4-6); remnants of secondary velum forming thin, and an evanescent annulus. Margin enrolled to incurved with remnants of secondary velum at margin. Lamellae thin, broadly decurrent, irregular, unequal ($L+1 = 6-7-8/cm$), very commonly forked, slightly dense, yellowish white to pale orange (4A4-5 to 5A3). Stipe 40-55 × 14 mm, cylindrical, central, tapering downwards, velvety, dry matt, full and firm. Context of pileus pale orange, to orange white, thick in the center, slightly thinner at the margin, solid in the stipe. Latex not abundant or scarce, whitish, unchanging; taste and smell not special.

Basidiospores (Fig. 15E, 16A-D) globose to subglobose, sometimes ellipsoid, $7.0-7.5-8.0(8.5) \times (5.5)6.0-7.0-7.5 \mu m$, ($Q = (1.04)1.06-1.12-1.15(1.18)$; $n=75$). Weak amyloid ornamentation composed of very short, fine lines-like, not well distinctive under light microscope; very low developed warts slightly connected under SEM; plage distinct with amyloid spot. Basidia (Fig. 15D) $35-57 \times 8-10 \mu m$, four-spored, subcylindrical to subclavate; sterigmata $7-8 \times 1.5-2 \mu m$. Pleurocystidia absent. Pleuroseuodocystidia (Fig. 15C) very abundant, 6-15 (20) μm diam.; irregularly subcylindrical, slightly inflated, tapering upwards, very rarely tortuous, apex mucronate; projecting up to 40 μm above the hymenium; with irregular dense brown, slightly needle-like contents. Hymenophoral trama heteromerous, composed of a mixture of filamentous hyphae, sphaerocytes, and laticifers. Lamellae edge sterile. Marginal cells (Fig. 15A) $20-35 \times 5-6 \mu m$, distinctly cylindrical to subcylindrical, slightly fusiform, septate. Pileipellis (Fig. 15B) a lamprotrichopalisade, hyphae very thick-walled (2-3 μm), suprapellis elements 60-150 × 3-5 μm ; slender, irregularly branched to diverticulate, very tortuous to fusiform, tapering upwards. Stipitipellis a lamprotrichopalisade, identical to pileipellis. Clamps absent.

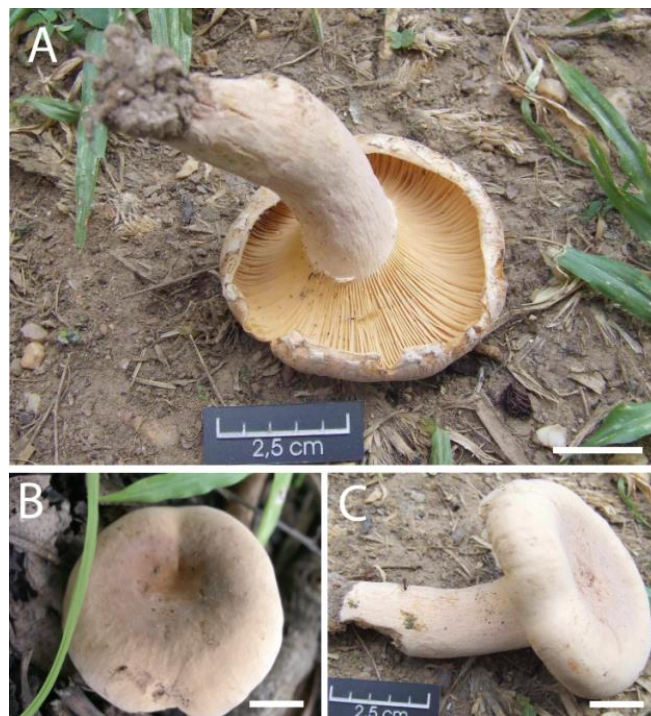


Fig. 14 – Basidiome of *membranaceus*. A. View of the lamellae and stipe (C2349). B-C. Pileus and stipe view, pellis detailed (B: MD234; C: C2349). – Scale Bars = 10 mm.

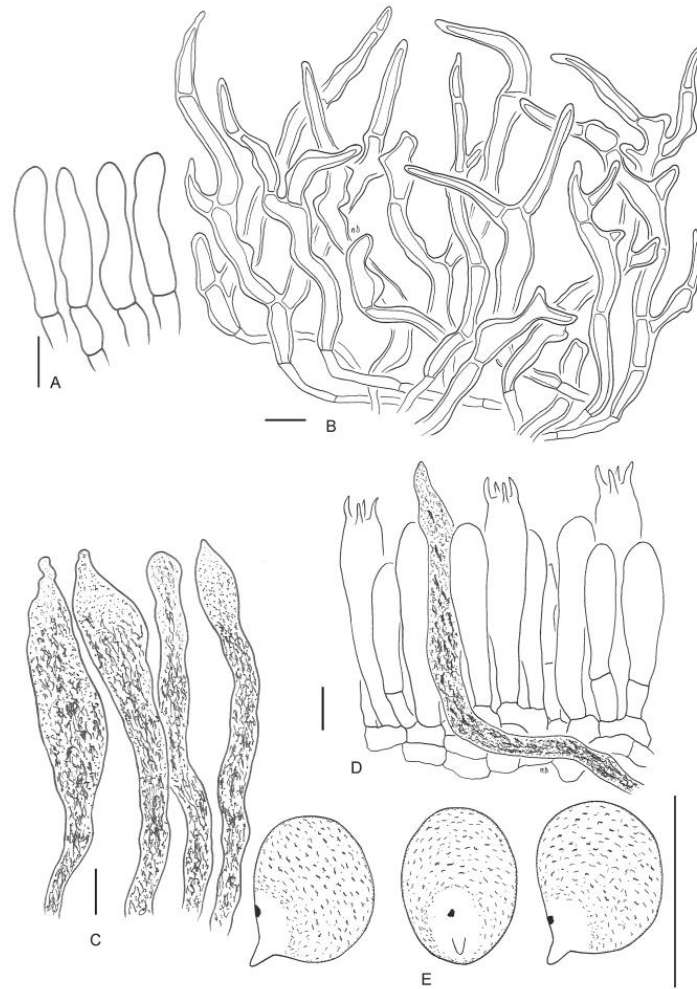


Fig. 15 – Light microscopy of *Lactifluus membranaceus* (C2349). A. Marginal cells. B. Pileipellis. C. Pleuropseudocystidia. D. Hymenium. E. Basidiospores. – Scale Bars = 10 μ m. Section line drawing.

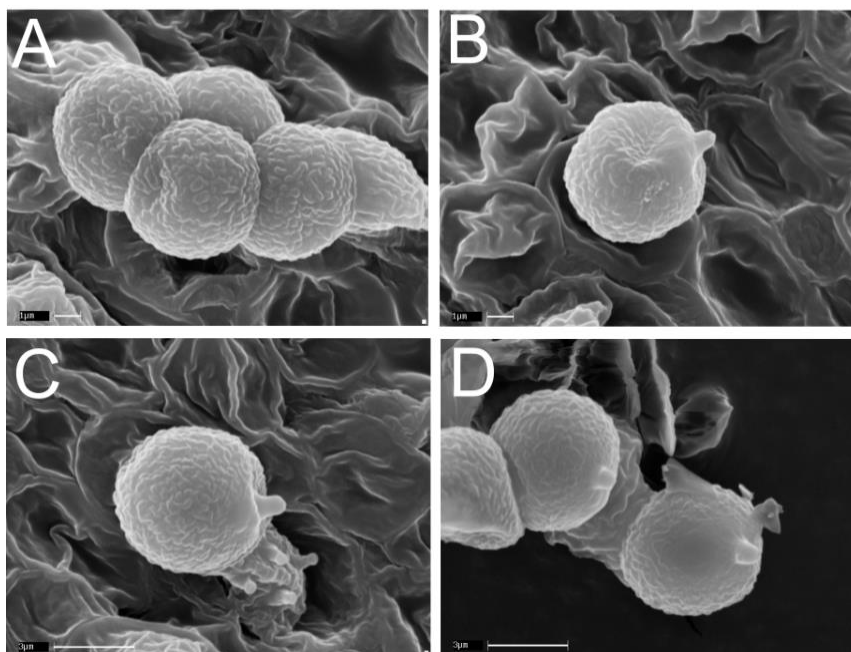


Fig. 16 – SEM of *Lactifluus membranaceus* (C2349). Basidiospores: A. Overview B-C-D Front.

Material examined – Togo, Central region, Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, N08°42'58" E00°46'22", on soil in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 8 June 2008, (collector) D.L. Maba, C2349; (TOGO, holotype); Guinea, Malouwaita, N10°32'5.7" W9°22'8.6", on soil in rainforest dominated by *Uapaca heudelotii*, 18 July 2011, (collector) D.L. Maba, MD234 (TOGO), ENA accession no. LK392610; Togo, Central region, Prefecture of Assoli, Reserve Forest of Aledjo N09°13.9'8.1" E01°11.4'42", on soil in woodland dominated by *Isoberlinia tomentosa* and *Uapaca togoensis* 12 July 2008, (collector) D.L. Maba, DPM05 (TOGO).

Known distribution – Togo, Fazao-Malfakassa National Park and Aledjo Reserve Forest; Guinea, Malouwaita

Discussion

Lactifluus subgenus *Lactariopsis*

Species *Lactifluus annulatolongisporus* and *Lf. membranaceus* described here fit the traditionally delimited *Lf.* subg. *Lactariopsis* (Fig. 1) that encompasses all tropical African annulate lactarioid taxa, but also several without annulus. Both species present the following morpho-anatomical characters that support their phylogenetic placement within *Lf.* subg. *Lactariopsis*: remnants of the secondary/partial velum forming an annulus, basidiome coloration (yellowish brown, yellowish orange, ochraceous, brownish orange, pale orange), pileus with dry and indehiscent pellis, latex scarce and unchanging, lack of pleurocystidia, and presence of a lamprotrichopalisade as pileipellis and stipitipellis (Verbeken & Walley 2010). *Lactifluus annulatolongisporus* is in some respect close to *Lactifluus zenkeri* due to its marginal cells that are branched, dichotomously bifurcate and tortuous (Fig. 3E); and to *Lf. heimii* (Verbeken) Verbeken, which has ellipsoid to elongate basidiospores up to 11.6 µm long, and 1.64 for ratio (Verbeken & Walley 2010). *Lf. annulatolongisporus* differs considerably from all hitherto examined members of *Lf.* subg. *Lactariopsis* possessing an annulus (Verbeken & Walley 2010), by the presence of amyloid spot in the plage of its basidiospores; unlike *Lf. heimii* and *Lf. zenkeri*. The specimens examined (MD123 and MD131), present strongly bulbous to clavate pleuropseudocystidia, distinctly bulged in the middle, tapering up- and downward, sometimes branched or bifurcate, and are therefore different from those of *Lf. zenkeri* and *Lf. heimii*. Although morphologically different to *Lf. zenkeri*, also by the basidiospores (size, ornamentation and presence of amyloid spot), *Lactifluus annulatolongisporus* and *Lf. zenkeri* have branched, dichotomously bifurcate and tortuous marginal cells, unlike *Lf. heimii*, which has the same elements that are shortly cylindrical to clavate (Verbeken & Walley 2010). In addition, species including *Lactifluus annulatoangustifolius* (Verbeken) Verbeken, *Lf. heimii*, *Lf. velutissimus* (Verbeken) Verbeken, *Lf. zenkeri* and the newly described *Lf. annulatolongisporus*, have lamprotrichopalisade to lampropropalisade as pileipellis structure, which differs only by the sizes of terminal elements (Verbeken & Walley 2010). Thus, the combination of morpho-anatomical characters of *Lf. annulatolongisporus* distinguishes it from the other annulate species.

Lactifluus zenkeri is the one, in some respect, morpho-anatomically closest to the newly described *Lactifluus membranaceus* (specimens C2349, MD234 and DPM05). Both species have a velvety, indehiscent and dry pellis; remnants of secondary velum are membranous-like, forming a fragile and thin annulus. However, the pellis of *Lactifluus membranaceus* is beige, orange white to yellow orange colored, while it is whitish, yellowish brown to pale ochraceous, darker in the center for *Lactifluus zenkeri* (Verbeken & Walley 2010). The microscopic examinations conducted have confirmed the dissimilarity between both species. *Lactifluus zenkeri* has utriform or tortuous to conical, dichotomously branched marginal cells, close to *Lf. annulatolongisporus*, scarce pleuropseudocystidia, basidiospores mostly ellipsoid, amyloid spot mostly absent in plage (Verbeken & Walley 2010). On the contrary, *Lf. membranaceus* has distinctly cylindrical to

subcylindrical, septate marginal cells (Fig. 15A), rarely fusiform, very abundant pleuropseudocystidia (Fig. 15C), and basidiospores (Fig. 15E, 16A-D) mostly globose to subglobose, with distinctly amyloid spot present in plage. Both *Lf. annulatolongisporus* and *Lf. membranaceus* fit *Lf.* subg. *Lactariopsis* and their sequences are well supported in this subclade.

Lactifluus* subgenus *Russulopsis

Lactifluus brunneocarpus (specimens MD219B and MD224) has a brown colored pellis of the pileus, darker in the center and a stipe of similar colour, Latex changing slightly to green, basidiospore ornamentation composed of irregular, rounded, and obtuse warts isolated, and fits therefore *Lf.* subg. *Russulopsis* that encompasses some known species including *Lf. ruvubuensis* (Verbeken) Verbeken and *Lf. longipes* (Verbeken) Verbeken (Verbeken & Walley 2010), with closely related characters. Morphologically *Lf. brunneocarpus* presents wet, indehiscent, very fragile and brittle pellis, a strongly striate pileus of up to 2/3 from the margin, very fragile, adnate to subdecurrent lamellae, with regular pattern of 3, distinctly spaced lamellulae in between. In contrast, *Lf. longipes* is morphologically identifiable by smooth, slightly concentrically zonate, radially wrinkled pileus, a long and slender stipe and very dense and frequently forked lamellae (Verbeken & Walley 2010), while *Lf. ruvubuensis* has a thick basidiome with dehiscent, tomentose and finely fibrose towards the margin pellis, and unequal, strongly decurrent lamellae, according to Verbeken & Walley (2010). Moreover, unlike *Lf. longipes*, which has long stipe (up to 80 mm high), *Lf. brunneocarpus* has a stipe with measurements comprised between 35 and 50 mm high (for 11 different stipes sized). Microscopically, *Lf. brunneocarpus* differs from the two above mentioned closest species by its strongly diverticulate, branched and tortuous pleurocystidia (Fig. 6D) that are absent in *Lf. longipes*. But, in addition, pleuropseudocystidia are much branched and tortuous in both, *Lf. ruvubuensis* and *Lf. longipes* (Verbeken & Walley 2010), while they are subcylindrical and slightly tapering upwards in *Lf. brunneocarpus* (Fig. 6B). The marginal cells of *Lf. brunneocarpus* (Fig. 6F) are subcylindrical and septate, and the basidiospores (Fig. 6E) present well-developed irregular, rounded to conical, and isolated amyloid warts as ornamentation unlike *Lf. ruvubuensis*. *Lf. brunneocarpus* even morpho-microscopically different from *Lf. ruvubuensis* and *Lf. longipes* as mentioned above, fits *Lf.* subg. *Russulopsis* and its sequence forms a subclade with two sequences of *Lf. longipes*, supported by 99%.

Lactifluus* subgenus *Edules

The sequences of the newly described species *Lf. burkinabei* (specimen MD355), and *Lf. guellii* (specimen C2157), cluster within *Lf.* subg. *Edules*. Morphologically, *Lf. burkinabei* has firm, fleshy and thick pileus, wet and smooth pellis, very decurrent, commonly forked, and lamellae strongly anastomosing at the insertion of the stipe; its margin is smooth, inflected to downrolled. It differs thereby considerably to known *Lactifluus* species from tropical Africa (van Rooij and al. 2003; Verbeken & Walley 2010). Microscopically, *Lf. burkinabei* has subglobose to ellipsoid basidiospores (Fig. 9G, 10A-C), with irregularly shaped amyloid warts, interconnected and forming a reticulum, seldomly isolated. It has additionally, two types of basidia (Fig. 9C): two-spored (about 1/4 to 1/3 of the basidia) as those observed for *Lf. inversus* (Verbeken & Walley 2010) and four-spored ones that both are subcylindrical to subclavate. Its pleuroleptocystidia (Fig. 8E) present almost mucronate to rostrate, often upwards tapering apices, closely related to those observed for *Lf. indusiatus* Verbeken (Verbeken & Walley 2010) of *Lf.* sect. *Chamaeleontini* Verbeken. *Lf. burkinabei* presents an ixotrichopalisade to trichopalisade (Fig. 9A) pileipellis, composed of very abundant clavate to subglobose cells, in mixture with mucronate to subcapitate dermatocystidia (Fig. 9F); this pileipellis feature has never been observed for any known African lactarioids (van Rooij et al. 2003; Verbeken & Walley 2010). Conversely, *Lf. guellii* (specimens C2157 and C2163) has indehiscent and very sticky; strongly striate pellis when old, and smooth in the center when young; strongly striate, incurved then straight, crenulated to uprolled margin;

adnate to subdecurrent and spaced lamellae that are sometimes forked at the margin. Microscopically it has ellipsoid to elongate basidiospores (Fig. 12B, 13A-D), with strong, well-developed blunt amyloid warty ornamentation (>0.5µm high), finely interconnected at the base, closely related to those observed in *Lf. melleus* Maba (Maba et al. 2015). The pleurocystidia of *Lf. guellii* (Fig. 12F) are irregularly shaped, mostly tortuous to fusiform in contrast to the pleuroleptocystidia of *Lf. burkinabei* (Fig. 9E) that are almost mucronate to rostate with often upwards tapering apices. These features are unlike that of *Lf. melleus*. Pleuroseudocystidia of *Lf. guellii* (Fig. 12D) are emergent, fusiform, tortuous and mucronate, closely related to those of *Lf. corbula* R. Heim & Gooss.-Font. This latter mentioned species has a cutis-like pileipellis, cylindrical to subclavate marginal cells, and ellipsoid basidiospores (up to 10.4 µm high, and up to 1.35 as ratio; n=60) with no amyloid spot in place (Verbeken & Walleyn 2010). *Lf. guellii* on the contrary has lamprotrichoderm-like pileipellis, with abundant swollen hyphae; its marginal cells are broadly clavate, subcylindrical to fusiform, bifurcate and septate, and its basidiospores are broadly ellipsoid to elongate (up to 11.5 µm high, and up to 1.45 as ratio; n=65), with strong amyloid spot in place. In the phylogeny analyses, *Lf. guellii* is supported (97%) as same species with one unidentified from Cameroon (UDB014027), with that it forms a terminal clade; together with *Lf. melleus* a clade with 71% of bootstrap support. At the same time the sequence of *Lf. burkinabei* is well supported by 99% as a subclade within subgenus *Edules*, the subgenus itself is supported by 86% of bootstrap support value.

Ecology and occurrence of *Lactifluus* species in the Guineo-Sudanian domain

Table 2 Occurrence of *Lactifluus* species in Sudanian woodlands, riverside and/or rain forests. +...: frequency of collections studied (based on collections from 2007 to 2013)

Species	Occurrence	
	Woodlands	Gallery forest
<i>Lactifluus annulatoangustifolius</i>	+++	++
<i>Lf. annulatolongisporus</i>	++	
<i>Lf. brunneocarpus</i>		++
<i>Lf. burkinabei</i>		+
<i>Lf. chamaeleontinus</i>		++
<i>Lf. densifolius</i>		++++
<i>Lf. edulis</i>	+	+
<i>Lf. emergens</i>		++
<i>Lf. flavellus</i>		++
<i>Lf. fazaoensis</i>	+	
<i>Lf. flammans</i>	+++++	++
<i>Lf. foetens</i>	+++	++
<i>Lf. guellis</i>		++
<i>Lf. gymnocarpus</i>	++	++
<i>Lf. gymnocarpoides</i>	+++++++	
<i>Lf. heimii</i>		+
<i>Lf. inversus</i>		+
<i>Lf. longibasidius</i>	++	
<i>Lf. longipes</i>		++++
<i>Lf. luteopus</i>	+++++	++++
<i>Lf. medusae</i>	++	
<i>Lf. melleus</i>	++	
<i>Lf. membranaceus</i>	++++	
<i>Lf. nonpiscis</i>	+	+++
<i>Lf. pectinatus</i>		+
<i>Lf. rubiginosus</i>		++++
<i>Lf. sudanicus</i>	+++	++++

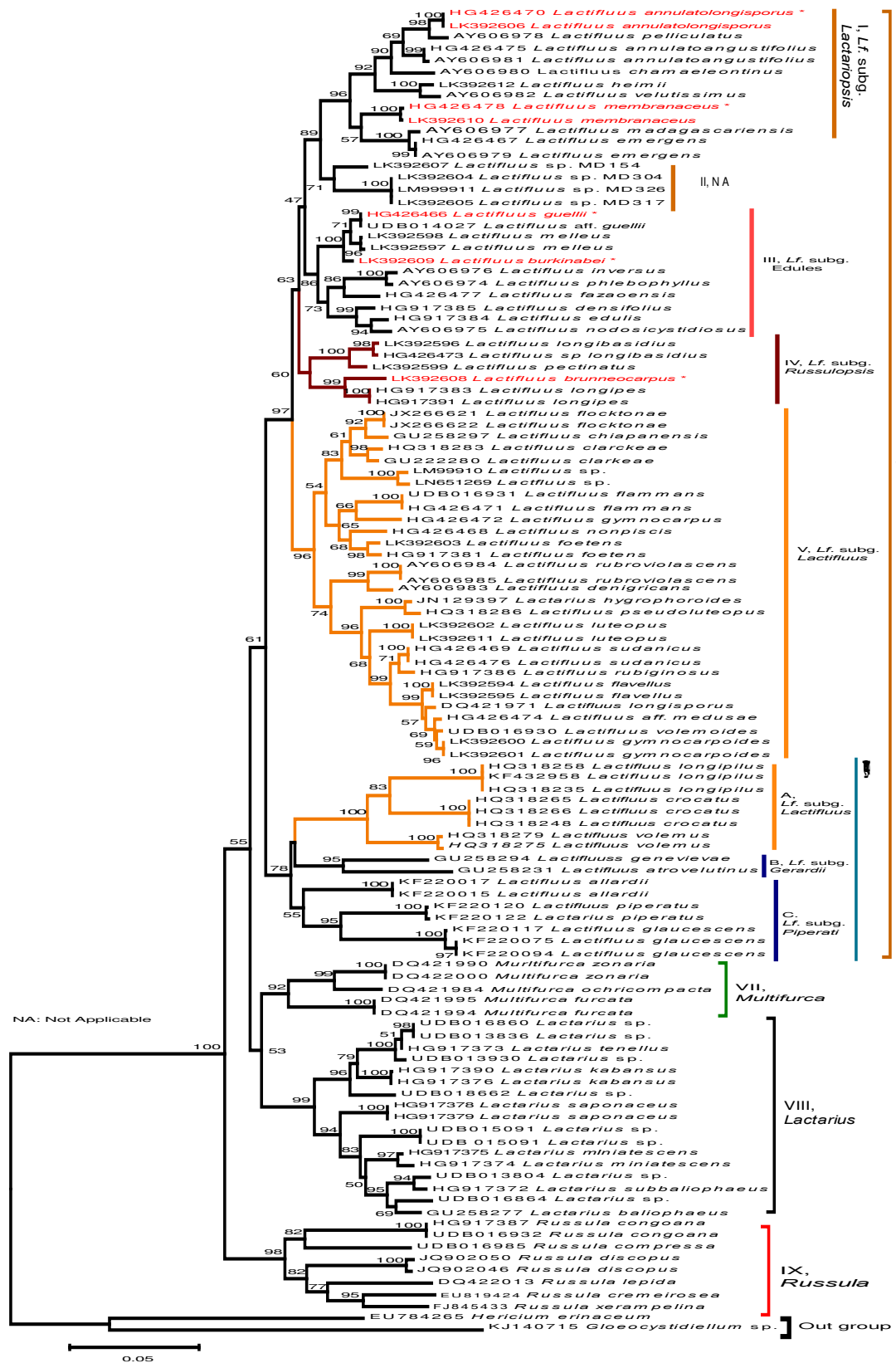


Fig. 17 – General phylogenetic tree obtained from 113 ITS sequences. Bootstrap values (in %) are from maximum likelihood (ML) analyses (1000 bootstraps). Traditional taxa including genera and subgenera are demarcated. The Phylogenetic placement of our five newly described species: *Lf. annulatolongisporus*, *Lf. brunneocarpus*, *Lf. burkinabei*, *Lf. guellii* and *Lf. membranaceus* is showing within the genus *Lactifluus*. (*) is mentioned in front of each holotype.

Species of the genus *Lactifluus* are widespread in Guineo-Sudanian ecosystems and occur preferentially earliest between the end of May to July and latest between the end of August and September (Verbeken and Buyck 2001; van Rooij et al. 2003; Verbeken & Walley 2010; Maba et al. 2013, 2014, 2015). In collections that have been continuously sampled since 2007 in various ectomycorrhizal dominated ecosystems, certain *Lactifluus* species including *Lf. annulatoangustifolius*, *Lf. edulis*, *Lf. foetens*, *Lf. gymnocarpus*, *Lf. luteopus*, *Lf. nonpiscis* and *Lf. sudanicus* have shown no preference regarding vegetation type, as they had been collected in both woodlands and riverside/rain forests (Table 2). *Lactifluus brunneocarpus*, *Lf. chamaeleontinus*, *Lf. densifolius*, *Lf. guellii*, *Lf. rubiginosus*, *Lf. longipes*, and *Lf. flavellus* were collected only in riverside forests. Whereas *Lf. annulatoangustifolius*, *Lf. emergens*, *Lf. gymnocarpoides*, *Lf. medusae*, *Lf. membranaceus*, and *Lf. melleus* were collected only in woodlands (supplement), *Lf. flammans* is collected mostly in woodlands (five times), but also in riverside forest (twice). *Lactifluus burkinabei*, *Lf. fazaoensis*, *Lf. heimii*, *Lf. inversus*, *Lf. pectinatus* were collected once, either in woodlands or in riverside forests. Thus, future additional mycological investigations including new inventories, should therefore contribute for better understanding of their ecological status including their distribution/occurrence, and phenology, and will also highlight whether any species are endemic in the Sudanian domain.

In contrast, some species including *Lf. medusae*, *Lf. densifolius*, *Lf. edules*, *Lf. heimii*, *Lf. velutissimus* are not restricted to Zambezi domain as suggested by Verbeken and Buyck (2001), as the recent mycological investigations have provided collections from Sudanian domain (Maba et al. 2013, 2015). Additionally, the occurrence in both Guineo-Sudanian and Congo-Zambezi domain, in woodland or in riverside forests, of numerous *Lactifluus* species including *Lf. annulatoangustifolius*, *Lf. aurantiifolius*, *Lf. chamaeleontinus*, *Lf. carmineus*, *Lf. densifolius*, *Lf. edulis*, *Lf. emergens*, *Lf. flammans*, *Lf. gymnocarpus*, *Lf. gymnocarpoides*, *Lf. heimii*, *Lf. inversus*, *Lf. luteopus*, *Lf. medusae*, *Lf. nonpiscis*, *Lf. longipes*, *Lf. longisporus*, *Lf. pelliculatus*, *Lf. pumilus*, *Lf. rubiginosus*, *Lf. ruvubuensis*, *Lf. sesemotani*, *Lf. velutissimus*, *Lf. volemoides* and *Lf. zenkeri* (Table 2), confirm that in both the Guineo-Sudanian and the Congo-Zambezi domain, several common species occur as mentioned by Verbeken and Buyck (2001). Clearly, as suggested by the latter cited authors, many common *Lactifluus* and *Lactarius* species still need to be described from both domains.

Species of the genus *Lactifluus* are common and widespread in Guineo-Sudanian forest ecosystems, and display important anatomical features (Maba et al. 2013, 2015) of taxonomic relevance. The present study and the previously undertaken (Maba et al. 2013, 2015) support the high species richness of the genus *Lactifluus* in tropical Africa. This study provided additional new *Lactifluus* species of tropical African domains, and in some respect supports those of Van de Putte et al. (2009, 2010), and De Crop et al. (2014), which have suggested that the genus *Lactifluus* contains cryptic and/or semi-cryptic species, based respectively on investigations undertaken within *Lf.* subg. *Lactifluus*, section- *Lactifluus*, and *Lf.* subg. *Piperati*, section *Piperati*. Evidently, a combination of anatomical and molecular analyses is the best way for interspecific discrimination, as well as species richness assessment by providing relevant arguments for supporting or denying traditional morphological diagnosis for species identification. In addition, West African forests ecosystems remain very poorly investigated. Thus, continuous specimen sampling/collecting as well as accelerated DNA sequencing and anatomical characterization of ectomycorrhizae, will contribute to a better understanding of ecological process within this genus.

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References

- Buyck B. 1991 – The study of microscopic features in *Russula*. 1. Spores and Basidia. *Russulales News* 1, 8–26.
- Buyck B, Hofstetter V, Eberhardt U, Verbeken A & Kauff F. 2008 – Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect. *Ochricompectae*. *Fungal Diversity* 28, 15–40.
- De Crop E, Nuytinck J, Van de Putte K, Lecomte M, Eberhardt U, Verbeken A. 2014 – *Lactifluus piperatus* (*Russulales*, *Basidiomycota*) and allied species in Western Europe and a preliminary overview of the group worldwide. *Mycological Progress* 13, 493–511.
- De Crop E, Tibuhwa D, Baribwegure D, Verbeken A 2012 – *Lactifluus kigomaensis* sp. nov. from Kigoma Province, Tanzania. *Cryptogamie Mycologie* 33(4): 421–426.
- Diédhiou AG, Ebenye HCM, Selossé MA, Awana NO, Bâ AM 2013 – Diversity and community structure of ectomycorrhizal fungi in mixed and monodominant African tropical rainforest. In *Tropical and Neotropical forest*. (eds) Bâ AM, McGuire KL and Diédhiou AG. 2013 – CRC Press.
- Gardes M and Bruns TD. 1993 – ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizas and rusts. *Molecular Ecology* 2, 113–118.
- Kornerup A & Wanscher JH 1978 – *Methuen Handbook of Colour*. Methuen, London.
- Maba DL, Guelly AK, Yorou NS, Verbeken A & Agerer R. 2013 – Two New *Lactifluus* species (*Basidiomycota*, *Russulales*) from Fazao Malfakassa National Park (Togo, West Africa), *Mycological Progress* 13, 513–524.
- Maba DL, Guelly AK, Yorou NS, De Kesel A, Verbeken A & Agerer R. 2014 – The genus *Lactarius* s. str. (*Basidiomycota*, *Russulales*) in Togo (West Africa): phylogeny and a new species described. *IMA Fungus* 5, 39–49.
- Maba DL, Guelly AK, Yorou NS, Verbeken A & Agerer R. 2015 – Phylogeny and microscopic studies within the genus *Lactifluus* (*Basidiomycota*, *Russulales*) in West Africa including four new species. *IMA fungus* 6, 13–24.
- Rivière T, Diédhiou AG, Diabaté M, Senthilarasu G, Hatarajan K, Verbeken A, Buyck B, Dreyfus B, Béna G, Bâ AM. 2007 – Genetic diversity of ectomycorrhizal Basidiomycetes from African and Indian tropical rain forests. *Mycorrhiza* 17, 415–428.
- Sanon E, Guissou KM-L, Yorou NS & Buyck B. 2014 – Le genre *Russula* au Burkina Faso (Afrique de l'Ouest): quelques espèces nouvelles de couleur brunâtre. *Cryptogamie, Mycologie* 35, 377–397.
- Sanon KB, Bâ AM and Duponnois R. 2013 – Diversity and Function of Ectomycorrhiza between *Scleroderma* and *Afzelia* species in Burkina Faso. In *Ectomycorrhizal symbioses*. In *Tropical and Neotropical forest*. (eds) Bâ AM, McGuire KL and Diédhiou AG. 2013 – CRC Press
- Stubbe D, Nuytinck J, Verbeken A. 2010 – Critical assessment of the *Lactarius gerardii* species complex (*Russulales*). *Fungal Biology* 114, 271–283.
- Thiers B. 2012 – *Index Herbariorum: a global directory of public herbaria and associated staff*. New York Garden's Virtual Herbarium. <<http://sweetgum.nybg.org/ih/>> access 19 January 2012
- Van De Putte K, De Kesel A, Nuytinck J & Verbeken A. 2009 – A new *Lactarius* species from Togo with an isolated phylogenetic position. *Cryptogamie Mycologia* 30, 39–44.
- Van de Putte K, Nuytinck J, Stubbe D, Le HT & Verbeken A. 2010 – *Lactarius volemus* sensu lato (*Russulales*) from northern Thailand: morphological and phylogenetic species concepts explored. *Fungal Diversity* 45, 99–130.
- van Rooij P, De Kesel A & Verbeken A. 2003 – Studies in tropical African *Lactarius* species (*Russulales*, *Basidiomycota*) 11. Records from Benin. *Nova Hedwigia* 77, 221–251.
- Verbeken A, Buyck B. 2001 – Diversity and ecology of tropical ectomycorrhizal fungi in Africa. In: *Tropical Mycology* (Watling R, Frankland JC, Ainsworth AM, Isaac S, Robinson G, eds): 11–24. Wallingford: CABI Publishing.

- Verbeken A, Stubbe D, Nuytinck J. – 2008 Two new *Lactarius* species from Cameroon. *Cryptogamie Mycologie*. 29, 137-143.
- Verbeken A & Walley R. 2010 – Monograph of *Lactarius* in Tropical Africa. National Botanic Garden of Belgium *Fungus Flora of Tropical Africa*, Vol. 2. 161 p.54 pl
- Verbeken A, Nuytinck J & Buyck B. 2011 – New combinations in *Lactifluus*. 1. *Lactifluus* subgenera *Edules*, *Lactariopsis*, and *Russulopsis*. *Mycotaxon* 118, 447–453.
- Verbeken A, Van De Putte K & De Crop E. 2012 – New combinations in *Lactifluus*. 3. *Lactifluus* subgenera *Lactifluus* and subgenera *Piperati*. *Mycotaxon* 120, 443–450.
- Verbeken A & Nuytinck J. 2013 – Not every milkcap is a *Lactarius*. *Scripta Botanica Belgica* 51, 162–168.
- Yorou NS, Diabaté M, Agerer R. 2011 – Phylogenetic placement and anatomical characterization of two new West African *Tomentella* (*Basidiomycota*, *Fungi*) species. *Mycological Progress* 11, 171–180.
- Yorou NS, Gardt S, Diabaté M, Guissou M-L, Agerer R. 2012 – Three new *Tomentella* species from West Africa identified by anatomical and molecular data. *Mycological Progress* 11, 449–462.