Diversity, Molecular phylogeny, Ecology and Distribution of the genera *Lactifluus* and *Lactarius* (*Russulales, Basidiomycota*) in West Africa

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ERKLÄRUNG

Hiermit versichere ich ehrenwörtlich, dass meine Dissertation selbständig und ohne unerlaubte Hilfsmittel angefertigt worden ist.
Die vorliegende Dissertation wurde weder ganz, noch teilweise bei einer anderen Prüfungskommission vorgelegt.
Ich habe noch zu keinem früheren Zeitpunkt versucht, eine Dissertation einzureichen oder an einer Doktorprüfung teilzunehmen.

Dao Lamèga Maba, München, 20 May 2015
(Unterschrift)

To Germaine Maba,
my wife, who endured a 3-year period of my absence and of physical separation.
To my son, Prince Klebert Harssa, who I have missed during this period.
To my father Ernest Maba and my mother Affi Apetovi

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Abbreviations and acronyms

°C	Degree Celsius
bp	base pairs
BR	Bruxelle
CA	California
CTAB	3D cetyltrimethylammonium bromide
GS	Guineo-Sudanian
CZ	Congo-Zambezian
dH_2O	distilled H_2O
DNA	desoxyribonucleic acid
dNTP	desoxynucleoside triphosphate
ECM	ectomycorrhiza
ENA	European Nucleotides Archives
G	Ghent
GPS	Global Positioning System
G + I	Gamma distribution + Invariant site
GTR	General Time Reversible substitution
i.e.	id est (= in other words)
INSD	International Nucleotide Sequence Database
ITS	Internal Transcribed Spacer(s) of the nuclear ribosomal DNA
K(M)	Royal Botanic Gardens Kew-Mycology
L	*Lactarius*
Lf	*Lactifluus*
ML	Maximum Likelihood
MCL	Maximum Composite Likelihood
N. A.	Not Applicable
NCBI	National Center for Biotechnology Information
nrDNA	nuclear ribosomal DNA
rDNA	ribosomal desoxyribonucleic acid
PCR	Polymerase Chain Reaction
s. l.	sensu lato
s. str.	sensu stricto

sect.	section
sect. nov.	section nova (=new section)
SEM	Scanning Electron Microscopy
sp. nov.	species nova (= new species)
SPR	Subtree-Pruning-Regrafting Extensive
subg.	subgenus (subgenera)
TA	Tropical Africa
UV	Ultra violet
WA	West Africa

List of original publications

I **Maba DL,** Guelly AK, Yorou NS, Verbeken A and Agerer R (2013) Two New *Lactifluus* species (*Basidiomycota*, *Russulales*) from Fazao Malfakassa National Park (Togo, West Africa). Mycological Progress 13: 513–524

II **Maba DL,** Guelly AK, Yorou NS, De Kesel A, Verbeken A and 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

III **Maba DL,** Guelly AK, Yorou NS, Verbeken A and Agerer R (2015a) Phylogenetic and microscopic studies in the genus *Lactifluus* (*Basidiomycota, Russulales*) in West Africa including the description of four new species. IMA Fungus 6(1): 13–24

IV **Maba DL,** Guelly AK, Yorou NS, and Agerer R (2015b) Diversity of *Lactifluus* (*Basidiomycota*, *Russulales*) in West Africa: 5 new species described and some considerations regarding their distribution and ecology. Mycological Progress (Under review).

V **Maba DL,** Sanon E, Verbeken A, Kamou H and Agerer R (2015c) The genus *Lactifluus* (*Basidiomycota Russulales*) from West Africa: What do we know? Diversity, morpho-anatomy, molecular phylogeny and new species described presenting thromboplera and knobs. (In review by co-author).

Table 1: Author's contribution to each paper (I-V) (%)

	I	II	III	IV	V
Collecting trips and specimen sampling	100	100	100	100	100
Microscopic investigations and line drawings	100	100	100	100	100
DNA extraction and PCR	100	100	100	100	100
Sequences analyses and phylogenetic studies	90	100	100	100	100
Drafting and improvement of Manuscripts	80	90	90	90	90

Chapter 1

General introduction: Outline of this thesis

Fungi constitute one important group of eukaryotes. Although mycological research continues to grow, fungi remain one of the understudied organisms. They form a large and diverse group, estimated at about 1.5 million species of which only about 128432 (7%) are described (Hawksworth 2004, http://www.catalogueoflife.org/col/browse/tree). Fungi can be ecologically divided into saprobes (decomposers), parasites (pathogens), and symbionts (lichens and mycorrhizae). Fungal morphology encompasses various forms, e.g. mushrooms, Bracket fungi, jelly fungi. Taxonomic studies of fungi are still in progress, and their evolutionary kinship/relationships remain unclear or are in many cases not adequately highlighted.

Although new species are being described exponentially, the real species richness of macrofungi remains not yet assessed. Moreover, although particular attention is being given to tropical mycology during the last decade, that of tropical Africa is still rather poorly understood. Evidently, fungi play an important and an irreplaceable role in ecology and micro-ecology of any ecosystem, and contribute to the health of living organisms in various ways. (Lange 2014). No doubt, lack of taxonomic consensus targeting for example at specific fungal genera, is problematic to ecology, biochemistry, biotechnology, medicine, and as well as biodiversity estimation.

This study focuses at the genera *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel, lactarioids from tropical West Africa. Both genera were formerly known as members of one large genus *Lactarius* Pers. sensu lato, until molecular investigations of Buyck et al. (2008). The former genus *Lactarius,* described by Christian Hendrik Persoon in 1797, with *L. piperatus* (L.) Pers. as the original type species, encompassed all worldwide known lactarioid taxa. Later, Henri François Anne de Roussel described the genus *Lactifluus* in 1806, using also the same *L. piperatus* as the type species.

Mycological investigations of *Russulales* (Kreisel ex P.M.Kirk) P.F.Cannon & J.C.David, and particularly lactarioids are subject of ongoing research. *Russulales* are systematically phylum *Basidiomycota* R.T. Moore, subphylum *Agaricomycotina* Doweld, class *Agaricomycetes* Doweld, and encompasses 13 accepted families (http://www.catalogueoflife.org/col/browse/tree), one of which *Russulaceae* Lotsy. About eight genera tend to be accepted within *Russulaceae*, of which *Boidinia* Stalpers & Hjortstam, *Cystangium* Singer & A.H. Sm., *Lactarius* Pers., *Macowanites* Kalchbr. ex Berk., *Multifurca* Buyck & V. Hofstetter, *Pseudoxenasma* K.H. Larss. & Hjortstam, *Russula* Pers., and *Zelleromyces* Singer & A.H. Sm. (Miller et al. 2006). Thus, the family embraces three major types of fruitbodies: agaricoid and pleurotoid forms with a cap, gills, and a stipe; gasteroid forms with closed or partially closed fruitbodies; secotioid fruitbodies, and corticioid, crust-like forms (Miller et al. 2006; Buyck et al. 2008).

Recent molecular analyses of *Russulaceae* based on multigene-analysis (Buyck et al. 2008) demonstrated that *Lactarius* s. l. formed a polyphyletic/paraphyletic group (Fig. 1). Lactarioids fell apart into three separate clades, into two super-clades and a small one. The latter showed a mixture of sequences of *Russula* species of the former subsection *Ochricompactae* Bills & O.K. Mill., and *Lactarius furcatus* Coker. This small clade was later described as the currently known genus *Multifurca* Buyck & V. Hofst. Afterwards, while the *Russula* clade was monophyletically supported, *Lactarius* s. l. appeared resolved and well supported in two separate clades, only when *Multifurca* representatives are excluded (Buyck et al. 2008; Verbeken et al. 2011). Thus, both clades were suggested to be separated for more convincing taxonomy in two genera (Buyck et al. 2008; Verbeken et al. 2011).

Systematic of *Lactarius* and *Lactifluus*

Kingdom: *Fungi*
Phylum: *Basidiomycota*
Subphylum: *Agaricomycotina*
Class: *Agaricomycetes*
Order: *Russulales*
Family: *Russulaceae*
Genera: *Lactarius* (more than 550 species), *Lactifluus* (more than 120 species).
Source: http://www.catalogueoflife.org/col/browse/tree

Lactarioids could for longtime easily be identified already in the field by their particular character of exuding latex (milk) from the basidiome when injured. This character is unknown from *Russula*. However, this character fails sometimes to separate lactarioid species to *Russula,* as in tropical Africa many lactarioid species occur with very scarce latex (Buyck et al. 2008). In addition, many lactarioid taxa also tend to have uniformly and dull-coloured caps and stipes as well as regularly inserted shorter lamellulae among the gills, as in some *Russula* (Buyck et al. 2008). Microscopically, lactarioids are distinguished from *Russula* Pers., by well-developed and branched laticiferous hyphae extending into the hymenium as pseudocystidia or pleuropseudocystidia (Buyck 1995; Verbeken 1997; Buyck et al. 2008; Verbeken & Nuytinck 2013), which could be emergent or not (Appendix 4). However some critical cases have been reported as *Lactifluus* (formerly *Lactarius*) *ruvubuensis* Verbeken (Verbeken), which does not show pseudocystidia in some specimens, although it exuded abundant latex (Vebeken 1996a, b, c).

The proposal to separate *Lactarius* s. str. and *Lactifluus* as autonomous genera that encompass all previously known/described lactarioid species was finally accepted (Buyck et al. 2010; Verbeken et al. 2011). Moreover, almost all formerly known temperate lactarioid species, and about 20% of tropical and subtropical ones are accepted as members of the amended genus *Lactarius* s. str., which encompasses therefore about 80% of *Lactarius* s. l. species. Thus, species of the former subgenera *Piperites* (Fr. ex J. Kickx f.) Kauffman, *Russularia* (Fr. ex Burl.) Kauffman, and *Plinthogali* (Burl.) Hesler & A.H Sm., were accommodated in the newly accepted genus *Lactarius*. On the contrary, the former *Lactarius* subg. *Lactifluus* (Pers.) Roussel was erected as new genus *Lactifluus* and encompasses about 20% of *Lactarius* s. l. species with a main distribution in the Southern hemisphere, which were formerly classified in *Lactarius* subg. *Lactarius* Pers., subg. *Lactifluus* (Burl.) Hesler & A.H. Sm., subg. *Russulopsis* Verbeken, subg. *Lactariopsis* (Henn.) R. Heim, subg. *Gerardii* (A.H. Sm. & Hesler), and the former sect. *Edules* Verbeken, which was not affiliated to any subgenus (Table 2). At the same time *L. torminosus* (Schaeff.: Fr.) Pers. was accepted as the new type species of the genus *Lactarius* s. str., while *Agaricus lactifluus* L., currently known as *Lactifluus volemus* (Fr.: Fr.) Kuntze was accepted as type species of the genus *Lactifluus* (Buyck et al. 2010; Verbeken et al. 2011; Verbeken et al. 2012). At current state of knowledge, about 80% of temperate species belong to *Lactarius* s. str. and 20% to *Lactifluus*, while about 80% of tropical and subtropical species belong to *Lactifluus* and 20% to *Lactarius* s. str. Afterwards, *Lactifluus* species have been affiliated to six subgenera including subgenera: subg. *Russulopsis* (Verbeken) Verbeken, subg. *Piperati* Verbeken, subg. *Lactariopsis* (Henn.) Verbeken, subg. *Lactifluus* (Pers.) Roussel, subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, and subg. *Edules* (Verbeken) Verbeken (Verbeken et al. 2011, 2012; Stubbe et al. 2012).

Table 2: Current and former subgenera in lactarioids (Verbeken et al. 2011, 2012, Stubbe et al. 2012)

Current subdivision		*Lactarius* s. l.	Distributions
Genus	Subgenera	Subgenera	
Lactarius	*Piperites*	*Piperites*	Temperate regions and in Tropical Africa
	Russularia	*Russularia*	Temperate regions and South-East Asia, Africa

	Plinthogali	*Plinthogali*	Temperate and Tropical regions
Lactifluus	*Edules*	-	Tropical Africa
	Gerardii	*Gerardii*	North America, Asia, Australia
	Lactariopsis	*Lactariopsis*	Tropical Africa, North America, Europe and Asia
	Lactifluus	*Lactifluus*	Africa, Australia, Asia, Europe, North, South and Central America.
	Russulopsis	*Russulopsis*	Tropical Africa
	Piperati	*Lactarius*	Europe, Asia, North America

A short history of tropical/West African lactarioids

The diverse vegetation types of West Africa (White 1983) harbor a great diversity of ectomycorrhizal fungi. The taxonomy, however, of this fungal diversity is scantily documented. Few investigations were undertaken among which Hennings (1893, 1895a, b, 1897), and Wakefield (1914) remain certainly among the oldest ones. Later, Heim (1936, 1955), Alibert (1944), Locquin (1954), Alasoadura (1966, 1967), Pegler (1962, 1966), and Holden (1970) gave brief taxonomic descriptions on some species collected in Benin, Nigeria, Ghana and Ivory Coast. More recent works are those of Ogundana & Fagade (1981), Thoen & Ducousso (1989), Yorou et al. (2011a-d, 2014), Yorou & De Kesel (2002), Ducousso et al. (2002), De Kesel et al. (2002, 2008, 2011), De Kesel & Guelly (2007), van Rooij et al. (2003), Guissou (2005), Rivière et al. (2007), Maba (2010), and of Sanon et al. (2014). All these works evidenced the high fungal diversity in West African forest ecosystems. However, except the works of van Rooij et al. (2003) and Guissou (2005), which provided complete monographs of *Lactarius* s. l. and macrofungi at national level (Benin and Burkina Faso, respectively), most of the works mentioned above give only scarce information on some fungal taxa.

The genera *Lactarius*, *Lactifluus*, and *Russula* are the most common *Russulaceae* in tropical Africa, documented through numerous contributions by Verbeken (1995, 1996a, b, c, 1997, 1998a, b, 2000, 2001), and Buyck (1988, 1989, 1994a, b, c, 1997, 1999, 2001), Buyck et al. (1996, 2007), van Rooij et al. (2003), van de Putte et al. (2009), Douanla-Meli and Langer (2009), Verbeken & Walleyn (2010), De Crop et al. (2012), and Sanon et al. (2014). But most of the taxonomical informations are available for specimens originating predominantly from Zambezian Endemism Center (Burundi, Democratic Republic of Congo, Zimbabwe, Tanzania and Zambia) and Guineo-Congolean Endemism Center (Cameroon, Gabon).

Although significantly economically and ecologically important, lactarioids, except for Benin (van Rooij et al. 2003; De Kesel et al. 2002), have been very poorly investigated in West Africa, whereas species richness within this group of fungi may approach those of the Zambezian domain. For example, a total of 22 species was inventoried in Benin by van Rooij et al. (2003), among which five species were described as new. These works, which address first monographs of lactarioids in Benin, confirm the commonness of this formerly large genus *Lactarius* s. l. in tropical African ecosystems. Moreover, an endemism rate of 5 % was mentioned by the authors from Sudanian region (van Rooij et al. 2003), thus evidencing a high probability detecting new species.

Apart from preliminary investigations made by Heim (1955), Verbeken & Walleyn (2010) provided a more or less comprehensive monograph of tropical African lactarioid taxa. All African lactarioids are classified in six different subgenera, and 17 sections (Table 3). In addition, seven lactarioid species are not classified (insufficiently known), while eight species are excluded, due to the former misidentification; they are no lactarioids or belong to other known species (Verbeken & Walleyn 2010).

Table 3: Former subgenera and sections of tropical lactarioids (Verbeken 2010)

Subgenera	Sections
Lactariopsis	*Lactariopsis*, (Henn.) Singer
	Chamaeleontini Verbeken
Russulopsis	*Russulopsidei* Verbeken
Not assigned	*Edules* Verbeken
Not assigned	*Aurantiifolii* Verbeken
Lactifluus	*Rubroviolascentini* (Singer) Verbeken
	Pseudogymnocarpi Verbeken
	Rugati Verbeken
	Polysphaerophori Singer
	Phlebonemi R. Heim ex Verbeken
Not assigned	*Chromospermi* Verbeken
Piperites	*Piperites* (Fr. ex J. Kickx f.) Burl. s.l.
Not assigned	*Amari* Verbeken

Russularia	*Russularia* Fr. ex Burl.
Plinthogali	*Nigrescentes* Verbeken
	Plinthogali (Burl.) Singer s.l.
	Pseudofulginosi Verbeken

Aims of the thesis

The genera *Lactarius* s. str. and *Lactifluus* in WA are characterized by a scarcity of the documentation relative to species richness, taxonomy, ecology and distribution, but also to the conservation status and their phylogenetic tendencies

The goal of the present study was to highlight the species richness of lactarioids in West African forest ecosystems, based on morpho-anatomical and molecular phylogenetic analysis. Thus, to examine what *Lactifluus* and *Lactarius* species occur in West Africa (WA), contribute to the knowledge of their niche differentiation, as well as species distribution. This study, focused at five main research topics:

Specifically: (1) Provide the state of knowledge of species richness (diversity) of *Lactifluus* and *Lactarius* in West African ecosystems: Species identification, description, and illustration. (2) Morpho-anatomical and molecular characterization of lactarioid taxa, (3) Knowledge of molecular phylogenetic tendencies of West African lactarioids in comparison to Congo-Zambezian and worldwide, (4) Analysis of ecology and distribution of common lactarioid species in West African forest ecosystems, (5) Addressing an identification key (long term) of lactarioid species of tropical Africa taking into account the newly revealed morpho-anatomical characters.

Chapter 2

Two New *Lactifluus* species (*Basidiomycota*, *Russulales*) from Fazao Malfakassa National Park (Togo, West Africa).

Maba DL, Guelly AK, Yorou NS, Verbeken A, Agerer R (2013) Mycological Progress 13: 513–524

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ORIGINAL ARTICLE

Two New *Lactifluus* species (*Basidiomycota*, *Russulales*) from Fazao Malfakassa National Park (Togo, West Africa)

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Abstract Macro- and micromorphologic study of two milkcaps from Fazao Malfakassa National Park of Togo is supported by molecular phylogenetic analysis of ITS sequences obtained by the extraction of ribosomal DNA of each sample. This has led to the description of two new *Lactifluus* species: *L. fazaoensis* in *L*. subg. *Edules* and *L. sudanicus* in *L*. subg. *Lactifluus*. The two newly proposed taxa are compared with other related African species. *Lactifluus fazaoensis* is closely related to *L*. *aureifolius* and *L*. *edulis*, but is characterized by the concave to infundibuliform pileus, with dry, smooth and orange to greyish orange pellis and the distant, pale yellow to pale orange lamellae. Microscopically, it shows a character that has not up to now been as distinctly observed in *Lactifluus*: pleurocystidia and mainly cheilocystidia are tortuous to very irregularly branched and commonly diverticulate. *Lactifluus sudanicus* is morphologically recognizable by its convex to plano-convex, slightly depressed pileus, with dry, tomentose, slightly pruinose and pale orange to yellowish pellis. It is closely related to *L. longisporus*, *L. flammans* and *L. volemoides*. Microscopically, it presents lampropalisadic pileipellis and stipitipellis, with elements of suprapellis very high and close to *L. medusae*. Its basidiospores are ellipsoid with irregular amyloid warts ornamentation up to 1 μm, partially connected. The combination of these features does not match any of the species phylogenetically and morphologically close.

Keywords *Lactifluus* · *Lactarius* · Molecular phylogeny · Tropical Africa · Togo · Anatomy

Introduction

Molecular data are widely used in fungal systematics and taxonomy, and constitute an important and indispensable tool in fungal diversity assessment, species delimitation and identification, conservation biology and ecology (Begerow et al. 1997; 2010; Kõljalg et al. 2005; James et al. 2006; Hibbett et al. 2007). In one of the most important groups of ectomycorrhizal fungi in many African ecosystems, the Russulales, recent molecular research has changed the generic concepts in the clade and the exact boundaries between russuloid genera have accurately assessed through numerous samples of new tropical taxa (Eberhardt and Verbeken 2004; Buyck et al. 2008). Furthermore the traditional and rather well-characterized genus *Lactarius* has recently been split into three separate genera: *Multifurca* Buyck & V. Hofstetter (with only very few and up to now no African representatives and also containing some of the former *Russula* species), *Lactarius* Pers. sensu novo and *Lactifluus* (Pers.) Roussel (Buyck et al. 2008; Stubbe et al. 2010; Verbeken et al. 2011). The new genus *Lactifluus*, which contains the former subgenera *Lactarius* subg. *Lactarius*, *L.* subg. *Lactariopsis*, *L.* subg. *Russulopsis*, *L.* subg. *Lactifluu*s, *L.* subg. *Gerardii*, and the former section *L.* section *Edules* includes only 20 % of the former milkcaps on a world-wide scale, but about 75–80 % of all known African species (Buyck et al. 2010; Verbeken et al. 2011, 2012).

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Although being the smallest clade as compared to *Lactarius* s.n., the genus *Lactifluus* has a very high genetic diversity and falls apart in very distant clades (Buyck et al. 2008, 2010; Stubbe et al. 2010; Verbeken et al. 2011, 2012). Since this genus has its major distribution in tropical Africa and contains many presumed species complexes (the genetic variation often being larger than the morphological one; Verbeken and Nuytinck 2013), it is evident that there remain many new African species to be discovered, and that molecular tools are necessary to reveal and support these new taxa.

Despite the recent progress in fungal systematics and taxonomy (Stajich et al. 2009), we are still far from knowing the full extent of fungal diversity, particularly at a global scale (Begerow et al. 2010; Hibbett et al. 2011). Only about 5 % of the estimated 1.5 million species of extant fungi have been described (Hawksworth 2001), and sequence data are available for about 1 % of the hypothesized number of fungal species (Nilsson et al. 2009b; Begerow et al. 2010). One of the explanations for this huge lack of knowledge is that large geographical areas, such as tropical Africa, are still underexplored. Though *Lactifluus* and *Lactarius* are very striking macrofungi and among the best studied in the area, there are many more new species to be expected. With the number of African milkcaps estimated to be at least 150 (Verbeken 2001), about 100 *Lactarius* and *Lactifluus* species have been recorded and described up to now (Van Rooij et al. 2003; Verbeken and Walleyn 2010; Douanla-Meli and Langer 2009; Van de Putte et al. 2009; De Crop et al. 2012), about 33 from the Sudanian domain (White 1983). The investigation of Van Rooij et al. (2003) has estimated an endemism rate of 5 % of African *Lactarius* and *Lactifluus* species of Sudanian region including Fazao Malfakassa National Park (compared to Zambezian domain), thus evidencing the high probability of detecting and describing many new species.

Gallery forests and woodlands of the Sudanian domain are rich in *Caesalpiniaceae* and *Phyllantaceae* ectomycorrhizal trees including, among others, *Uapaca togoensis* Pax, *U. guineensis* Müll.Arg., *U. esculenta* A. Chev. ex Aubrév. & Leandri, *U. heudelotii* Baill, *Isoberlinia tomentosa* (Harms) Craib & Stapf, *I. doka* Craib & Stapf, *Berlinia grandiflora* Hutch & Dalz, *Afzelia africana* Smith, *Anthonota fragrans* (Baker f.) Exell & Hillc. and *Paramacrolobium coeruleum* (Taub.) J.Léonard. Previous mycological investigations in Sudanian ecosystems have reported a remarkable high diversity of ectomycorrhizal (ECM) fungal taxa including the genera *Russula*, *Lactarius* and *Lactifluus* (Verbeken and Buyck 2001; Rivière et al. 2007; Buyck et al. 2008; Verbeken 1996a, b, c, d, 2000; Verbeken and Walleyn 2010; Diédhiou et al. 2010; Douanla-Meli and Langer 2009; Bâ et al. 2012), but also numerous ectomycorrhizal resipunate species (Yorou et al. 2011a, b; Gardt et al. 2011). The Fazao Malfakassa National Park contains both gallery forests and woodlands (Afidégnon et al. 2002), with a high predominance of ECM tree species. The diversity of ectomycorrhizal fungal taxa in this park has been repeatedly evidenced by the record of numerous *Lactarius* and *Lactifluus* species such as *Lactifluus nonpiscis* (Verbeken) Verbeken, *Lactifluus densifolius* (Verbeken & Karhula) Verbeken, *Lactifluus longipes* (Verbeken) Verbeken, *Lactifluus medusae* (Verbeken) Verbeken, *Lactarius kabansus* Pegler & Piearce and the species *Lactifluus cocosmus* Van de Putte & De Kesel, known only from Togo (Van de Putte et al. 2009). In the present paper, we describe two new *Lactifluus* species from Fazao Malfakassa National Park of Togo. In addition to morphological and microscopic evidence, molecular phylogenetic analysis of these new species of rDNA data obtained by the sequencing of the ITS1 and ITS2 from each sample support this conclusion.

Materials and methods

Specimen sampling

Specimens were collected during the rainy season from 2008 to 2011 in caesalpinioid- and phyllantioid-dominated vegetation types of the Fazao-Malfakassa national Park in central Togo. Representative basidiocarps along with root tips excavated from below the basidiocarps were collected under various native ECM trees. Geographic positions of collecting sites were recorded using a GPS (Garmin 60CS; International, Olathe, USA). Preliminary morphological data were recorded using fresh basidiocarps, after they have been photographed in situ and ex situ with a digital Camera Olympus CAMEDIA C7070 Wide Zoom (Olympus Imaging America, Melville, NY, USA). The color data were recorded on fresh material following Kornerup and Wanscher (1978). After preliminary records of morphological features, specimens were dried using a propane gas-heated field dryer (De Kesel 2001), and afterwards conserved as exsiccates in sealable plastic bags (type Minigrip) with following herbarium numbers Maba Dao 152/Verbeken 11–178 and Maba Dao 105/Verbeken 11–174. Nomenclatural aspects together with the authors of species names are checked in Index Fungorum. We follow Thiers (2012) for herbarium acronyms and original descriptions of both new species are deposited in Mycobank (Crous et al. 2004; Norvell and Redhead 2012). The holotypes and additional other studied material are deposited in TOGO (Thiers 2012). Isotypes are conserved in M and GENT and deposited at MycoBank.

Microscopy and scanning electron microscopy

Microscopic studies were performed on dried material following the protocol outlined by Verbeken and Walleyn (2010) with a microscope Leica DM LB2 equipped with a drawing tube (Wetzlar, Germany). Fine handsections of the basidiocarps were mounted and observed in 2.5 % KOH. All structures, except the basidiospores, were examined, measured and illustrated mainly in ammonia Congo-red (Horak 2005). Spore shape and ornamentation are described and illustrated as observed in Melzer's reagent (Horak 2005). Measurements were made in Congo red at a magnification of ×1,000 following the protocol of Buyck (1991) and Verbeken and Walleyn (2010). Whenever present, the contents of cystidia, laticiferous hyphae and intracellular pigmentation are highlighted in the illustrations by stippling. We assessed the density of the lamellae as follow: L+l=total number of lamellae (L) plus lamellulae (l) per cm at pileus margin. For procedures of SEM micrographs, we refer to Gardt et al. (2011). Preliminary identifications of our specimens were based on Verbeken and Walleyn (2010) and Van Rooij et al. (2003), as the latter monographed *Lactarius* s. l. species in similar ecosystems of the neighbouring country, Benin.

DNA extraction, sequencing and PCR amplification

DNA was extracted from small hymenium pieces (0.5 mm× 0.5 mm) from dry basidiocarps using the ChargeSwitch® gDNA Plant Kit (Invitrogen, Darmstadt, Germany) as outlined in the user's guide. The ITS region of the ribosomal DNA was amplified using the fungi-specific primer ITS1F in combination with the basidiomycetes-specific primer ITS4B (Gardes and Bruns 1993). Ingredients were added as recommended by the manufacturer of the Taq polymerase (Invitrogen) and sterile distilled water was added to obtain a final reaction volume of 25 µl. The PCR amplification started by initial denaturation step (94 °C for 2 min), followed by 5 cycles of denaturation (94 °C for 0.5 min), primer annealing (52 °C for 2 min), elongation (72 °C for 2 min), another 30 cycles with a decreased annealing temperature (50 °C for 1 min), and a final elongation (72 °C for 5 min). The amplicons were precipitated overnight at room temperature after addition of 16 µl of 100 % isopropanol and 2.3 µl 5 M NaCl. DNA sequencing was performed by the sequencing service of the Department of Biology, Genomic Service Unit, Ludwig-Maximilians-Universität München, Germany, using an ABI 3730 capillary sequencer. We obtained a total of 13 sequences of which three sequences of both new species are deposited at European Nucleotide Archive (ENA).

Sequence edition, analyses and molecular phylogenetic inference

The generated sequences were edited, processed and the consensus sequences were assembled using BioEdit v.7.0.5 (Hall 2005). For getting relevant sequences to use in a multiple alignment, a BlastN (Altschul et al. 1997) search was performed against the International Nucleotides Sequences Database (INSD; Benson et al. 2008) and the UNITE database (Abarenkov et al. 2010; Kõljalg et al. 2005) for supporting the taxonomic affinity of our specimens. In addition, sequences of African (Benin, Zambia, Cameroon, Madagascar, Zimbabwe, Guinea, Burundi; see Buyck et al. 2008; Tedersoo et al. 2011) species were retrieved from the UNITE database (http://unite.ut.ee/SearchPages.php) for a detailed pairwise nucleotide-based and phylogenetic analyses. The Full Multiple alignment was done in BioEdit v.7.0.5 (Hall 2005), using ClustalW Multiple alignment option (Thompson et al. 1994) by applying Bootstrap NJ Tree and 1,000 as the number of bootstraps. Sequence similarity values between our newly generated sequences and the anatomically close ones were obtained in BioEdit v.7.0.5, after sequences have been aligned automatically. A minimal alignment length of 650 bp is considered in the assessment of the genetic distance between close species.

From the top 50 best matches generated for each of our sequences, sequences of the fully identified taxa (up to species level) and unidentified but close to our sequence have been considered and downloaded. The final dataset submitted to analysis encompassed 33 ITS rDNA sequences (comprising our generated ones and those obtained from GenBank) for a total length of 755 characters. The most likely tree was calculated in the computer programme MEGA 5.1 (Tamura et al. 2011) by applying the General Time Reversible substitution model (GTR+G+I) with pairwise deletion of gaps, and the nearest-neighbor-interchange (NNI) as the Maximum Likelihood heuristic method for the Tree Inference Option. The support of branches was assessed by bootstrap analysis (Felsenstein 1985) of 1,000 replicates.

Results

ITS rDNA sequence analyses

Molecular phylogenetically, all 33 investigated species fall into three different and well-supported clades (Fig. 1). All three clades match well with the morphologically defined *Lactifluus* subgenera *Lactariopsis* (Clade I), *Edules* (Clade II) and *Lactifluus* (Clade III) with bootstrap supports of 98, 89 and 70 %, respectively. The sequence of the specimen Maba Dao 152/Verbeken 11–178 (accession number HG426477) clustered phylogenetically within *L*. subg. *Edules* as a sister species to one still unidentified *Lactifluus* (Maba Dao 141, accession number HG426473) from Togo with well-supported bootstrap value of 100 %. The additional closest species are composed of two unidentified sequences from Cameroon (UDB014027) and Togo (C2157, accession number HG426466). Based on morphological and microscopic comparisons (see below) as well as analyses of the molecular phylogenetic placement (clade II Fig. 1), we conclude that specimen Maba Dao 152/Verbeken 11–178 represents a new species, proposed here as *Lactifluus fazaoensis*. The clade formed by the three unidentified specimens mentioned above and *Lactifluus fazaoensis* deviates as external sister clade in the clade forming *Lactifluus subg. Edules* (Verbeken) Verbeken notably *L. edulis* (Verbeken & Buyck) Buyck, *L. nodosicystidiosus* (Verbeken & Buyck) Buyck, *L. densifolius* (Verbeken & Karhula) Verbeken, *L. phlebophyllus* (R. Heim) Buyck, and *L. inversus* (Gooss.-Font. & R. Heim) Verbeken, and two still unidentified sequences, with bootstrap values from 72 to 100 %.

In contrast, the sequence of the specimen Maba Dao 105/Verbeken 11–174 (accession number HG426469) is phylogenetically placed in **L**. subg. *Lactifluus*. In this last clade (Clade III Fig. 1), the species *Lactifluus rubroviolascens* (R. Heim) Verbeken (AY606980, from Zambia and AY606985, from Madagascar) and *Lactifluus denigricans* (Verbeken & Karhula) Verbeken, of the section *Rubroviolascentini* (Singer) Verbeken deviate and form an external clade with bootstrap 100 %. The sequence of our specimen Maba Dao 105/Verbeken 11–174 clustered as sister to sequences of a group of species composed of *Lactifluus longisporu*s (Verbeken) Verbeken (DQ421971), *Lactifluus volemoides* (Karhula) Verbeken (UDB016930) and *L.* aff. *medusae* (Maba Dao 142 from Togo, accession number HG426474) with 100 % bootstrap support. Also based on numerous macro- and micromorphological differences with these species, we propose Maba Dao 105/Verbeken 11–174 as a new species, named *Lactifluus sudanicus*.

Taxonomy

1 - ***Lactifluus fazaoensis*** Maba, Yorou & Guelly sp. nov. (Figs. 2, 3, 6) GenBank ENA, **accession number** HG426477, Mycobank **MB 805561**

Pileus 60–70 mm diam., firm, concave then slightly planoconcave, subinfundibuliform, slightly depressed, slightly striate near the margin when old; pellis not dehiscent, mat, dry, smooth, orange to greyish orange (5A5–4 to 5B5–6). **Margin** slightly thick and striate or crenulate, involute. **Lamellae** broadly adnate to subdecurrent, large (4–7 mm), distant, unequal, with 3 to 6 lamellulae between two lamellae (L+l=3–4/cm), very brittle, pale yellow to orange (4A5–4 to 5A5–4); edge entire. **Stipe** 32×15 mm, cylindrical, central, tapering downwards, concolorous to pileus; surface smooth, whitish brown when old. **Context** fleshy, brittle, firm and solid, whitish to whitish orange. **Latex** abundant, white and unchanging; taste not observed.

Basidiospores subglobose to ellipsoid (6.5) 7.4–7.8 8.6(9)×(5.6)6.2–6.8–7(7.5) µm (Q=(1.08)1.18–1.2–1.24(1.29); n=120); ornamentation amyloid, very low, composed of very short, weakly and fine lines (dashes-like), not distinctive under light microscope, very low developed warts slightly connected; plage distinct and with amyloid spot. **Basidia** 4-spored, 42–65(75)×7–9 µm, subclavate, sterigmata 4–5(7)×1–2(3) µm. **True pleurocystidia** abundant, 44–68(85)×5–7 µm, irregular, cylindrical, mucronate, moniliform to rounded at the apex, sometimes branched; content oleiferous. **Pleuropseudocystidia** rather abundant, 7–9(10) µm diam., cylindrical, with oleiferous, brown content, moniliform, tapering upwards, sometimes tortuous and projecting above the hymenium. Lamellar edge sterile. **Cheilocystidia** 38–65(85)×5–6(7) µm, tortuous, irregular, much branched and commonly diverticulate. **Hymenophoral trama** mostly cellular composed of sphaerocytes mixed with lactifers. **Pileipellis** as a trichoderm with transition to cutis; composed of cylindrical to subclavate, septate terminal cells of (1)2–3(5) µm diam., which are slender, irregular and interwoven. **Stipitipellis** an ixocutis to cutis; suprapellis slightly thick, composed of cylindrical and septate terminal cells and laticiferous hyphae; subpellis composed of isodiametric cells and laticiferous hyphae. **Clamps** absent.

Material studied

Togo, Central region, Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 08°42′25″N, 00°46′24″E, woodland dominated by *Uapaca togoensis*, *Isoberlinia doka* and *I. tomentosa*, 19 June 2011, leg. Maba Dao, herb. Maba Dao 152; Holotypus (TOGO), Isotype Munich (M) and GENT (Verbeken 11–178), GenBank acc. no. HG426477, Mycobank **MB 805561.**

Etymology

Referring to the origin area of the type material

2-***Lactifluus sudanicus*** Maba, Yorou & Guelly sp. nov. (Figs. 4, 5, 7) GenBank ENA, **accession number** HG426469, Mycobank **MB 805562**

Pileus 35–65 mm diam., convex when young, then planoconvex and slightly depressed when old, sometimes infundibuliform; surface dry, mat, indehiscent, tomentose, slightly pruinose, pale orange to yellowish (5A4–5 to 4A4–5). **Margin** thin, straight, then revolute. **Lamellae** decurrent, distant, unequal and irregular (L+l=5–6–7/cm), with 3 or 7 lamellulae between two lamellae, very brittle, white (2A1–1A1). **Stipe** 35–50×8–12 mm, cylindrical, central, tapering downwards, concolorous to the pileus in under 2/3 and yellowish white (1A2–2A2) in upper 1/3. **Context** white, thin, very brittle, and slightly thick in the center. **Latex** very abundant, distinctly white, unchanging, slightly viscose; taste not remarkable. Chemical reactions unknown.

Basidiospores ellipsoid, 7.4–7.6–8.2–8.4(9.2)×6–6.5–7–7.6 µm, (Q=1.1–1.2–1.29–1.31; n=85); ornamentation amyloid, composed of distinct irregular warts up to 1 µm high,

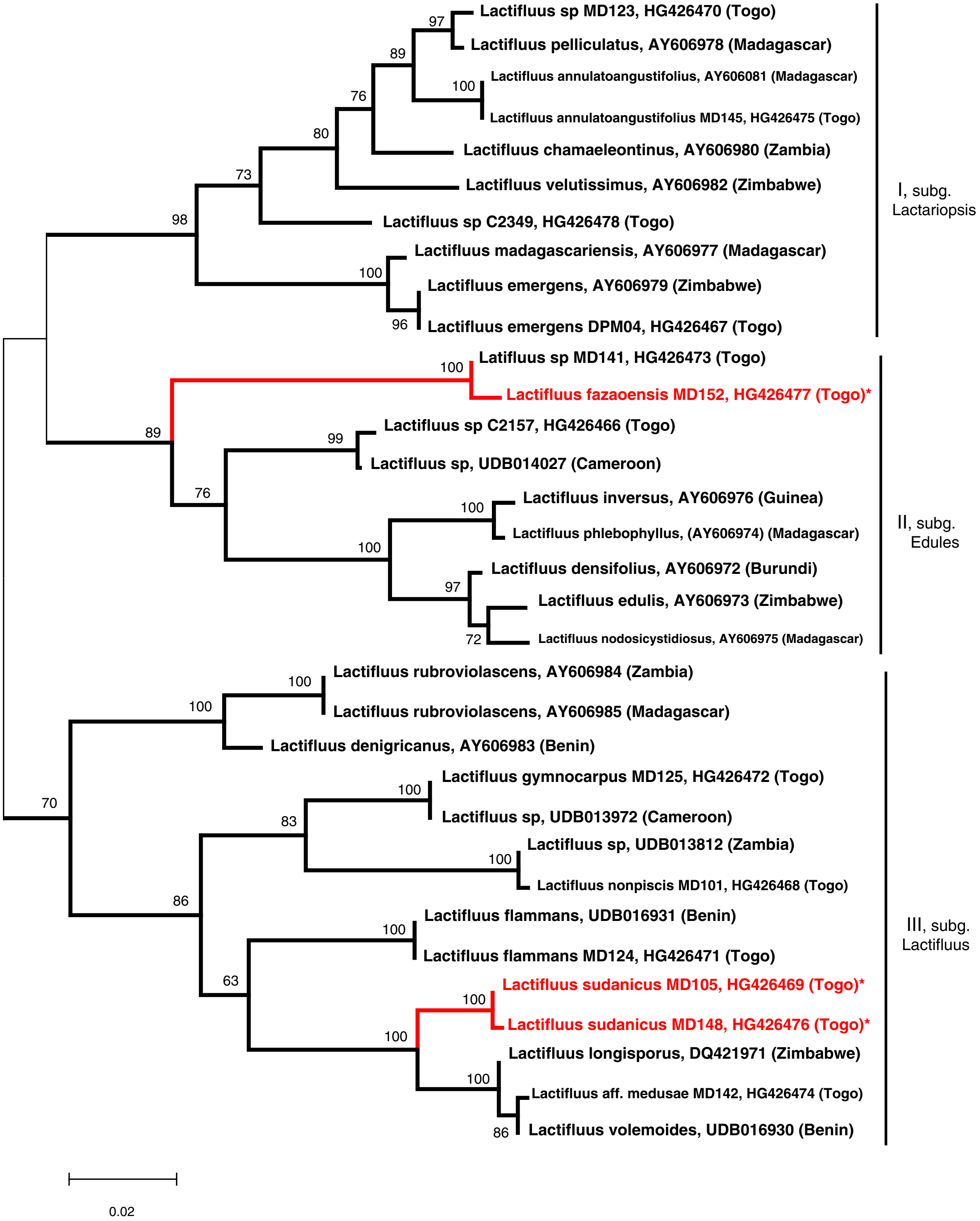


Fig. 1 Phylogeny tree. Best Maximum Likelihood tree showing the placement of *L. fazaoensis* and *L. sudanicus* within African *Lactifluus* species. Bootstrap values higher than 60 % are shown above the branches. GenBank (UNITE, NCBI and ENA) sequences names are followed by accession numbers and/or number of selected species, the name of the countries of selected species are given in parentheses. *New species

partially connected, forming a complete reticulum, plage distinct and not amyloid. **Basidia** 4-spored, 40–55×9–10(10) µm, subclavate, with sterigmata 4–5.5×2–2.5. **True pleurocystidia** absent. **Pseudopleurocystidia** 4–8 µm diam., abundant, not always emergent, cylindrical, fusiform with upwards tapering apex, tortuous, sometimes mucronate or dichotomous and with slightly granular content. Lamellae edge sterile. **Marginal cells** 35–46×3–5 7(8) µm, cylindrical, septate, sometimes fusiform and slightly thick-walled. **Hymenophoral trama** composed of isodiametrical cells of

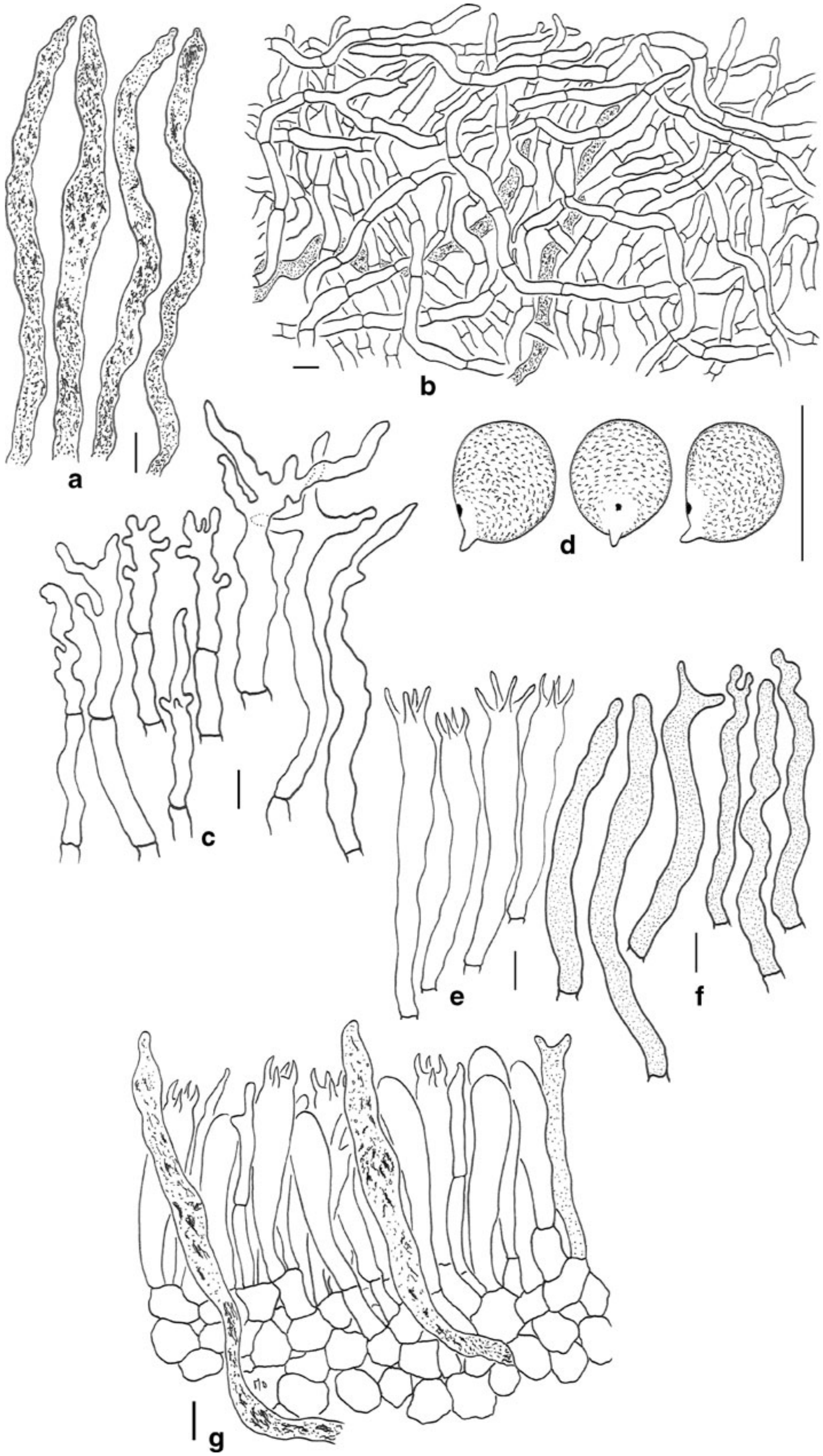


Fig. 2 Drawings *Lactifluus fazaoensis*. **a** Pleuropseudocystidia. **b** Pileipellis. **c** Cheilocystidia. **d** Spores. **e** Basidia. **f** Pleurocystidia. **g** Hymenium. *Bar* 10 μm. Section line drawing

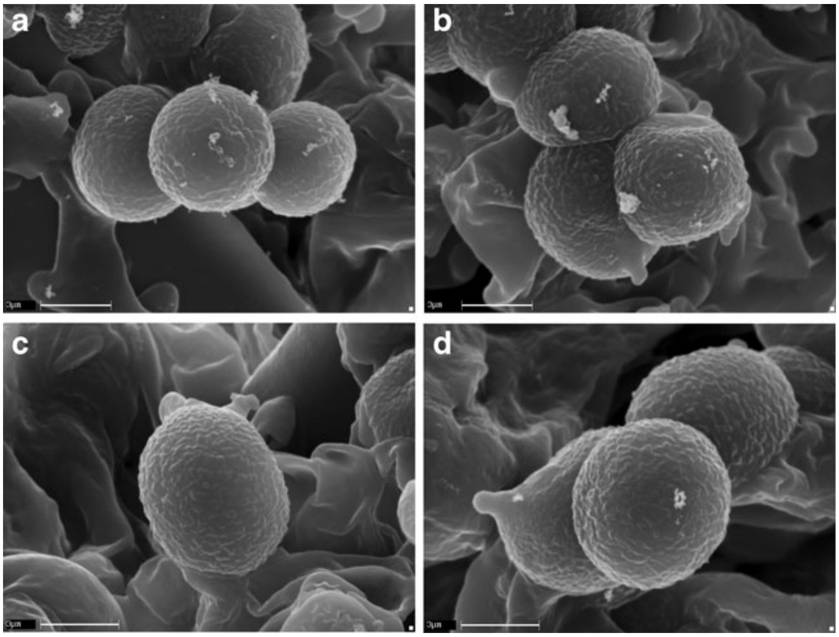


Fig. 3 SEM (spores observed). *Lactifluus fazaoensis*. **a** General and dorsal view. **b** Profile and facial view. **c** Dorsal lateral view. **d** Dorsal and facial view showing the amyloid spot

12–35 µm diam. and laticiferous hyphae. **Pileipellis** a lampropalisade with very high suprapellis elements of 23–210(230)×2–3–4(5) µm, hair-shaped, irregular, cylindrical, fusiform, often septate and thick-walled; subpellis composed of laticiferous hyphae mixed with isodiametrical cells of 15–25 µm diam. **Stipitipellis** a lampropalisade; terminal elements of suprapellis 20–80×3–5(6) µm, cylindrical to irregular, subpellis as in pileipellis. **Clamp**s absent.

Materiel studied

Togo, Central region, Prefecture of Tchaoudjo, Fazao Malfakassa National Park, 08°42′21″N 00°46′18″E, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, leg. & det. Maba Dao, 19 June 2011 herb. Maba Dao 105; Holotype (TOGO), Isotype Munich (M) and GENT (Verbeken 11–178), GenBank **acc. no** HG426469, Mycobank **MB 805562.**

Further material studied Togo, Central region, Prefecture of Tchaoudjo, Fazao Malfakassa National Park, 08°42′58″N 00°46′22″E, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*. Leg. det. Maba Dao, 19 June 2011, herb. Maba Dao 148; (TOGO, M and GENT). GenBank **acc. no** HG426476.

Togo, Central region, Prefecture of Assoli, Aledjo Protected Area, 09°20′21″N, 01°14′18.9″E, woodland dominated by *Isoberlinia doka* and *I. tomentosa* and *Uapaca togoensis*, Pax. leg. & det. Maba Dao, 31.May.2008, herb. Maba Dao 15; (TOGO, M). - Togo, Central region, Prefecture of Assoli, Alédjo Protected Area, 09°16′12′5″N 01°12′20′3″ E, gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis*, leg. Guelly, det. Maba Dao, 26 June 2008, herb. C2158; (TOGO, M).

Etymology

Referring to the type origin that is included in the Sudanian domain.

Discussion

Based on both morphology and molecular phylogeny, *L. fazaoensis* fits well in *L.* subg. *Edules*, a subgenus with species characterized by rather firm and yellowish to greyish orange basidiocarps, weakly ornamented spores often with a central amyloid spot, the absence of thick-walled elements in the hymenium and a trichoderm to trichopalisade as pileipellis stucture (Verbeken 1998). In this study, this subgenus is represented by five species notably *L. inversus*, *L. phlebophyllus*, *L. densifolius*, *L. edulis* and *L. nodosicystidiosus*. The subgenus contains very tasty and popular edible species such as *L. edulis*, as well as burning acrid species such as *L. aureifolius*. Future records of *L. fazaoensis* are needed to check on the taste of this species.

Morphologically, *Lactifluus fazaoensis* resembles *Lactifluus edulis* and *Lactifluus aureifolius* by its stature and the mat and dry pileus with pale greyish orange tinges. *Lactifluus fazaoensis* differs by lamellae which are broader and very distant (L+l=3–4/cm) and less brightly coloured than

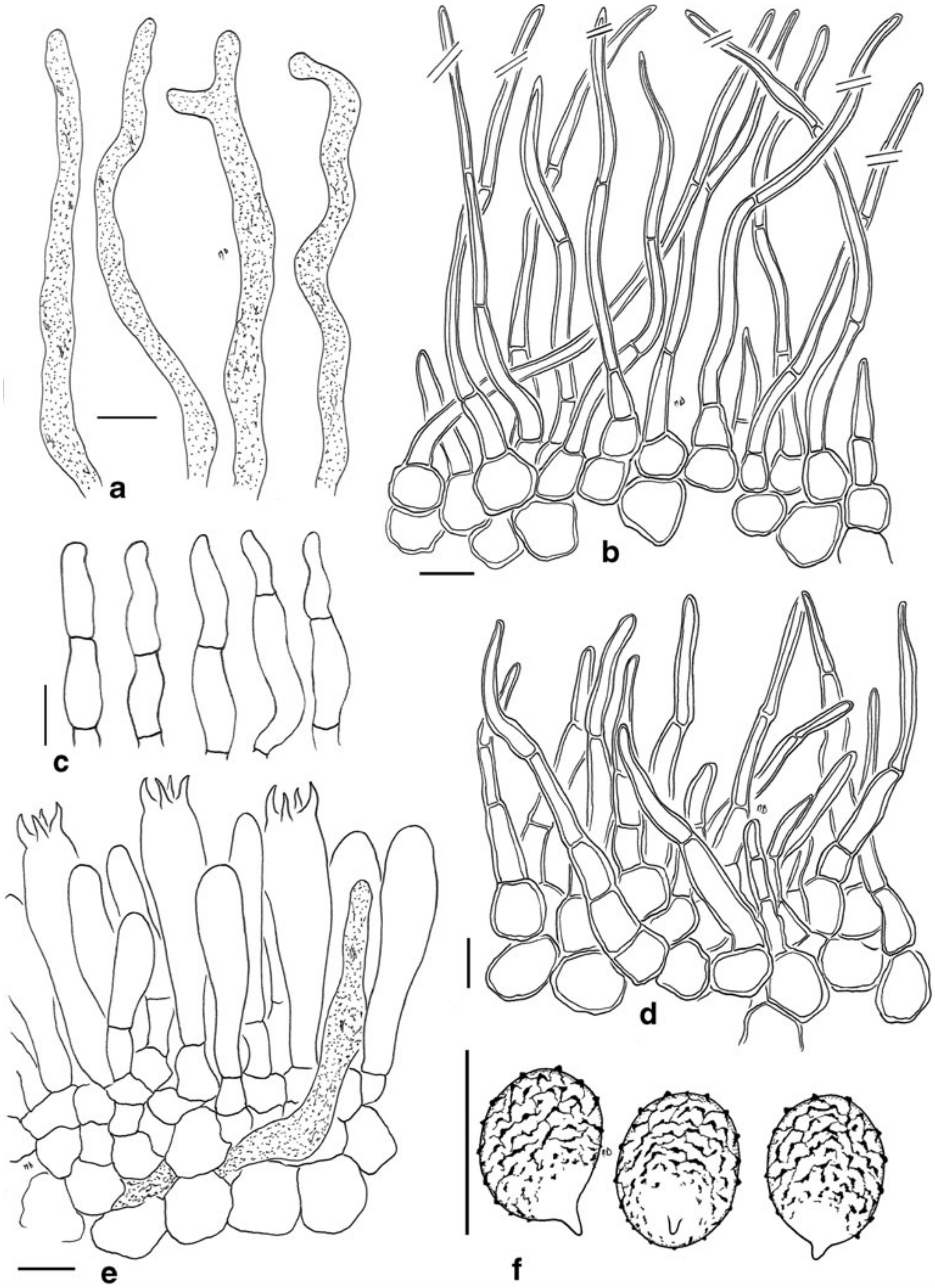
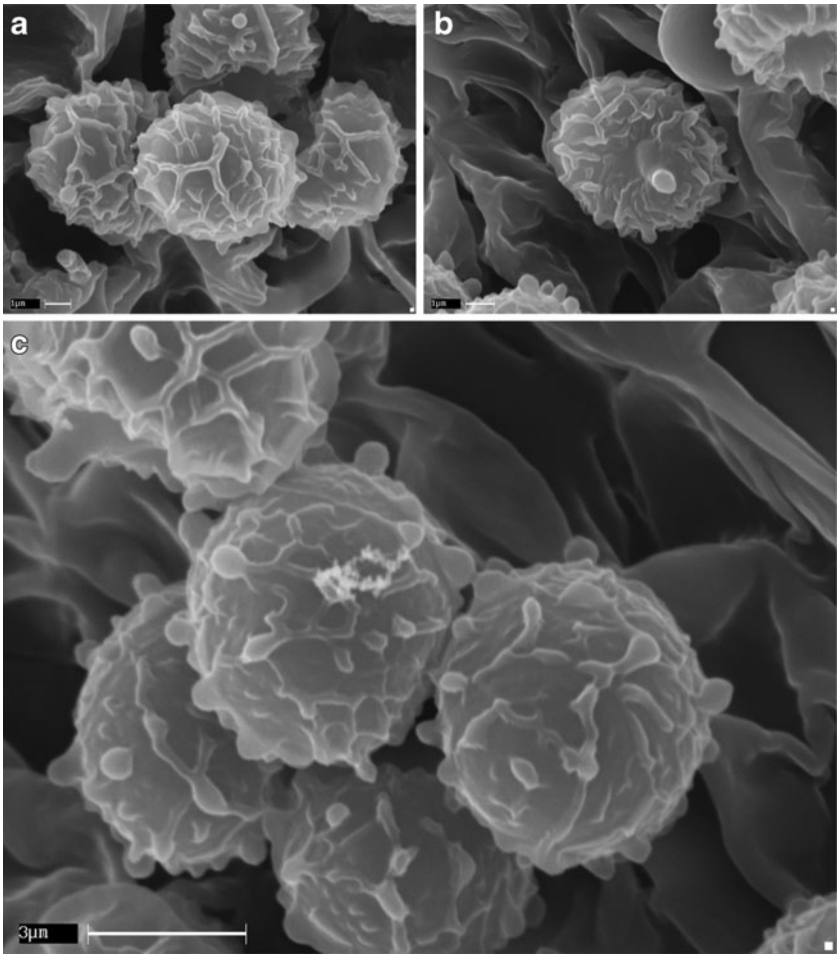


Fig. 4 Drawings *Lactifluus sudanicus*. **a** Pleuropseudocystidia. **b** Pileipellis. **c** Marginal cells. **d** Stipitipellis. **e** Hymenium. **f** Spores (from MD105). *Bar* 10 μm. Section line drawing

in *L. aureifolius*. Microscopically, *L. fazaoensis* has features completely different from *L. edulis*. Like *L. aureifolius*, this new species has a trichoderm with transition to a cutis as a pileipellis and an ixocutis to cutis as stipitipellis, while *L. edulis* has a trichopalisade pileipellis and a trichopalisade stipitipellis. Contrary to *L. edulis* and *L. aureifolius* that do not have pleurocystidia, *L. fazaoensis* has abundant, cylindrical, tortuous and irregular to branched pleurocystidia and especially the cheilocystidia are tortuous to very irregularly branched and commonly diverticulate. In addition, *L. fazaoensis* shows basidiospores with very weakly developed amyloid warts ornamentation and mainly a distinct plage with amyloid spot, which is absent in *L. edulis*. Especially, the diverticulate cheilocystidia are a very striking character which is very rare in the genus. It is also observed in *Lactifluus ruvubuensis* (Verbeken) Verbeken, a representative of *L.* subg. *Russulopsis* (Verbeken and Walleyn 2010). In *L.* subg. *Edules*, some species show a few of these irregular elements in the hymenium (*L. nodosicystidiosus*; Buyck et al. 2007), but in this new species, the whole lamellae edge is covered by these branching cells and some of them are also present in the hymenium as pleurocystidia.

Fig. 5 SEM (spores observed). *Lactifluus sudanicus*. **a** Lateral and dorsal view. **b** Front view. **c** General view



As to the other new species, *L. sudanicus*, molecular data show that *Lactifluus longisporus*, *L. volemoides* and *L. flammans* are, respectively, the closest relatives. These species belong to *L.* subg. *Lactifluus* that encompasses species with

Fig. 6 Photographs of basidiocarps. *Lactifluus fazaoensis*. **a** General view. **b** Pileus view. **c** General view of lamellae. **d** Detailed view of Lamellae

Fig. 7 Photographs of basidiocarps. *Lactifluus sudanicus*. **a**, **b** Top views of pileus. **c** Lamellae and stipe view. **d** Detailed view of Lamellae

firm and fleshy, yellowish to greyish orange basidiocarps, typically low ornamented spores and a palisade or lampropalisade as pileipellis structure (Verbeken 1998). Morphologically, *L. sudanicus* more resembles *L. flammans* than *L. longisporus* and *L. volemoides*, but while *L. sudanicus* has a tomentose to slightly pruinose pellis and pale orange to yellowish (5A4–5 to 4A4–5) pileus, *L. flammans* has a concentrically winkled pellis and deep orange (5A7–8, 6A7–8 to 6B78) colour that becomes paler in the centre when older (Verbeken and Walleyn 2010). *L. sudanicus* has 3 or 7 lamellulae between two lamellae and that is not the case in any one of the three species mentioned above.

The four species have a lampropalisade pileipellis, which differs by the size of the suprapellis elements. *L. sudanicus* has suprapellis element up to 210(230) µm high, while these elements are shorter in the other three species (35–130 µm). *L. flammans* also differs by the lack of pleurolamprocystidia and by a plage with sometimes a faintly amyloid spot (Verbeken and Walleyn 2010). One species of the subg. *Lactifluus* that has pileipellis mostly close to *L. sudanicus*, is *L. medusae*. However, the suprapellis elements of *L medusae* are up to 300 µm and this species differs considerably from *Lactifluus sudanicus* by the presence of pleurolamprocystidia with thickened wall (Verbeken and Walleyn 2010).

L. sudanicus sequence is phylogenetically nested within the *L.* subg. *Lactifluus* clade. Currently, this subgenus forms six sections and contains 46 known species (Verbeken et al. 2012) and has its major distribution in tropical Africa with sections endemic to tropical Africa (*Rubroviolascentini* (Singer) Verbeken) to almost completely African (*Pseudogymnocarpi* Verbeken, *Polysphaerophori* (Singer) Verbeken) (Verbeken et al. 2011, 2012). The subdivision of the large subgenus and the exact placement of *L. sudanicus* to one of the sections within this subgenus is still subjective because of the high genetic diversity observed in this subgenus (Buyck et al. 2010; Verbeken et al. 2011, 2012).

The present study corroborate with the numerous previous studies, (Buyck et al. 2008, 2010; Verbeken et al. 2011, 2012) that suggests a high genetic diversity within the genus *Lactifluus*. It confirms the hypothesis of the high diversity of sudanian domain that has been observed in Benin (Van Rooij et al. 2003) and the probability of discovering new species belonging to both the genera *Lactarius* and *Lactifluus*.

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Chapter 3

The genus *Lactarius s. str.* (*Basidiomycota, Russulales*) in Togo (West Africa): phylogeny and a new species described.

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The genus *Lactarius s. str.* (*Basidiomycota, Russulales*) in Togo (West Africa): phylogeny and a new species described

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Abstract: *Lactarius s. str.* represents a monophyletic group of about 40 species in tropical Africa, although the delimitation of the genus from *Lactifluus* is still in progress. Recent molecular phylogenetic and taxonomic revisions have led to numerous changes in names of tropical species formerly referred to *Lactarius*. To better circumscribe the genus *Lactarius* in Togo, we combined morphological data with sequence analyses and phylogeny inference of rDNA ITS sequences. Morphological and molecular data were generated from specimens sampled in various native woodlands and riverside forests; Lactarioid- and *Russula* sequences from public GenBank NCBI, and UNITE are included for phylogenetic analysis. The Maximum likelihood phylogeny tree inferred from aligned sequences supports the phylogenetic position of the studied samples from Togo within the subgenera *Piperites*, and *Plinthogali*.

Lactarius s. str. includes about 13 species described from West Africa, of which eight were not previously known from Togo, including one new species: *Lactarius subbaliophaeus* identifiable by the presence of winged basidiospores, a pallisadic pileipellis with a uprapellis composed of cylindrical cells, inconspicuous pleurocystidia, and fusiform or tortuous, often tapering apex marginal cells. It can also be recognised by a transparent white latex that turns pinkish and then blackish, and a bluish reaction of the flesh context with $FeSO_4$. These features mentioned do not match any of the morpho-anatomically most similar species, notably *L. baliophaeus* and *L. griseogalus*.

Key words:
distribution
ecology
ectomycorrhizas
Lactifluus
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Tropical Africa

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INTRODUCTION

The diversity of biological organisms in a site can be assessed and quantified only when the underlying species richness has been comprehensively investigated. In the meantime, it has been shown that extensive species inventories of vulnerable ecosystems are urgently needed to monitor these changes in the future (Raxworthy *et al.* 2008). A combination of morphological and anatomical studies with molecular tools in the assessment of fungal diversity, the delimitation of taxa, and identification of new species, provides fresh possibilities (Nilsson *et al*. 2006, Begerow *et al*. 2010). Furthermore, different studies have demonstrated that cryptic species are common throughout the fungi (Wubet *et al*. 2004, Savolainen *et al*. 2005), and consequently this requires the use of modern methods (molecular tools) such as those based on the extraction of ribosomal DNA (DNA barcoding) and phylogenetic studies to establish the distinction between taxa, mainly at species level, by highlighting the interspecific as well as the intraspecific variability (Nilsson *et al*. 2006, Lumbsch & Huhndorf 2007, Begerow *et al*. 2010). Although anatomical characters are still the only unequivocal systematic and taxonomic characters of value in routine fieldwork and identifications, the use of molecular tools in species inventories and so species biodiversity assessment is inevitable. Reliance on morpho-anatomological characters in the identification process can be problematic due to the plasticity of these characters in some cases (Begerow *et al*. 2010). Thus, DNA barcoding is currently and commonly used in various domains of biology including mycology, although new fungus species are still described with no molecular information.

Molecular investigations in *Russulales* have led to the splitting of *Lactarius s. lat.* into three separate genera, and the newly circumscribed genus *Lactarius s. str.* is now a distinct monophyletic group, separated from the closely related *Multifurca* and *Lactifluus* (Buyck *et al.* 2008, 2010, Stubbe *et al.* 2010, Verbeken *et al.* 2011). *Lactarius s. str.* represents the largest clade, but has a predominantly temperate distribution; it includes about 80 % of the milkcap species (Verbeken *et al.* 2011) and encompasses the subgenera *Piperites*, *Russularia*, and *Plinthogali*. *Lactifluus*, in contrast, has a mainly southern distribution and in Africa makes up about 20 % of the milkcaps, making *Lactarius s.str.* now a rather limited group in tropical Africa with about 40 species (van Rooij *et al*. 2003, Verbeken & Walleyn 2010) of which about 13 species are known from the Guineo Sudanian region.

Together with the genera *Lactifluus, Russula*, *Amanita*, *Tomentella*, *Cantharellus*, *Xerocomus*, *Boletellus*, *Boletus*, *Pulveroboletus*, *Veloporphyrellus,* and *Tylopilus*, the genus *Lactarius* represents the common dominant ectomycorrhizal (ECM) fungal taxa in tropical African vegetation types (Verbeken & Buyck 2001, De Kesel & Guelly 2007, Rivière *et al.* 2007, Diédhiou *et al.* 2010, Bâ *et al*. 2012, Maba *et al.* 2013).

In West Africa, *Lactarius* and *Lactifluus* species occur predominantly in ceasalpinioid- and phyllantioid-dominated woodlands, savannas, and riverside forests (De Kesel *et al.* 2002, Ducousso *et al.* 2002, Maba 2010, Verbeken & Walleyn 2010, Bâ *et al*. 2012). Nevertheless, numerous ECM root tips formed by species of *Lactifluus* and *Lactarius* have been reported from tropical African dense rain forests (Rivière *et al.* 2007, Diédhiou *et al.* 2010, Bâ *et al.* 2012).

For such a small territory (56 600 km^2), Togo exhibits not only a high ecosystem diversity, but also one of the highest number of plant species per square km^2 in comparison to other West African countries (Akpagana 1989). The country harbours many natural caesalpinioid- and ECM-rich forests in the south-western highland region, but also in the central and northern parts (Afidégnon *et al.* 2002). The Fazao Malfakassa National Park in the central western part of the country at the border with Ghana, and the Aledjo Forest Reserve located in the central part, are two of such ECM-rich forests. Since 2007, numerous mycological collecting trips have been undertaken intensively within both forests. The sampled material comprises many ectomycorrhizal fungal taxa including *Lactarius s. str.* specimens for which morphological and anatomical descriptions have been prepaeed and compared with known species.

Before this study, five *Lactarius s. str.* species had been recorded for Togo, *L. afroscrobiculatus*, *L. atro-olivinus*, *L. miniatescens*, *L. saponaceus,* and *L. tenellus* (De Kesel & Guelly 2007, Maba 2010, Verbeken & Walleyn 2010). The main goal of this paper is to assess the ITS (ITS1 and ITS2) nucleotide-based phylogenetic affinity of *Lactarius s. str*. species now known from Togo, combining sequence analyses with maximum likelihood phylogenetic trees and morpho-anatomical diagnoses. This led us to describe *L. subbaliophaeus* as new species, and also indicates that the Togo *Lactarius* species match genetically both tropical and temperate species.

MATERIAL AND METHODS

Specimen sampling and loan of material

This study is based mainly on collections sampled from Togo, and sampling and conservation was as described in Maba *et al.* (2013). For demarcation between the new species presented here and previously described similar species, we examined specimens of the following species *Lactarius tenellus* (ADK3975, from BR, paratype), *L. kabansus* (AV99-179, from GENT, paratype) and *L. griseogalus* (*R. Nicholson 179*, from K(M), paratype). The anatomy of those three species was studied and and molecular data were obtained from two (*L. tenellus*, *L. kabansus*) were made. Colour terminology follows Kornerup & Wanscher (1978).

Microscopy

For microscopic studies we followed the protocol of Verbeken & Walleyn (2010) as applied in Maba *et al.* (2013), and for SEM micrographs Maba *et al.* (2013). Preliminary identifications were made using the *Lactarius s. lat.* monograph based on material collected in similar ecosystems in the neighbouring country Benin (van Rooij *et al*. 2003). Additionally, we used the monograph of Verbeken & Walleyn (2010) on tropical African *Lactarius s.lat.*species.

DNA extraction, sequencing, and PCR amplification

Ribosomal DNA (rDNA) was retrieved from our dried samples and specimens ADK3975 and AV99-179 (see above) following the protocol used by Maba *et al.* (2013). The ITS of the rDNA, comprising ITS1, ITS2 and 5.8S, was amplified using the fungi specific primer ITS1F in combination with the *Basidiomycota* specific primer ITS4B (Gardes & Bruns 1993). We obtained 19 ITS sequences, *Lactarius s. str.* (10 sequences), *Lactifluus* (7), *Russula* (1), and *Termitomyces* (1; Fig. 1, Table 1). All the sequences have been deposited in the European Nucleotide Archive (ENA).

Sequence analyses and molecular phylogenetic inference

From the best matches generated by BlastN (Altschul *et al.* 1997) searches of each of our sequences, the sequences of named species and unidentified ones but close to our sequences were considered and downloaded. In order to obtain relevant sequences to use in a multiple alignment, a BlastN search was performed against the International Nucleotides Sequences Database (INSD; Benson *et al.* 2008), ENA (http://www.ebi.ac.uk/ena/home), and the UNITE database (Kõljalg *et al.* 2005, Abarenkov *et al.* 2010) focusing on tropical Africa sequences for determining the taxonomic affinity of the samples studied and those of closely related species. The consensus sequences were edited and assembled using BioEdit v. 7.2.5 (last update 24 Sept. 2013; Hall 2005). Our ITS sequence dataset comprised 39 in-group taxa (species and genus level) sequences and five out-group sequences. We consider as in-group, the genera *Lactarius* (24 samples), *Lactifluus* (12 samples), and *Russula* (3), which are all *Russulaceae,* and as out-group, *Termitomyces* (2 taxa), *Agaricus* (2), and *Hymenagaricus* (1; Fig. 1).

The Full Multiple alignment was performed automatically (L-INS-i) using the latest available online version of MAFFT v. 7.130b (Katoh & Toh 2008; update of 27 Sept. 2013), by applying the best accurate option for the alignment. After the online multiple alignment, the resultant sequence dataset was corrected manually by removing ambiguously aligned regions as well as mismatched and empty common columns.

Fig. 1. Best Maximum Likelihood phylogenetic tree showing the placement of *Lactarius* samples from Togo including the newly described species and other species from tropical Africa. Bootstrap values higher than 80 % are shown above the branches. GenBank (UNITE, NCBI and ENA) sequences accession numbers are shown preceding species names and followed by country of origin of selected species. (*) indicates the new species.

Table 1. List of the newly generated ITS rDNA sequences.

Species	Collection numbers	Country	ENA accession numbers
Lactarius afroscrobiculatus	ADK4599	Togo	HG917377
Lactarius kabansus	MD132	Togo	HG917376
Lactarius kabansus	AV99-179	Zimbabwe	HG917390
Lactarius saponaceus	MD390	Togo	HG917378
Lactarius subbaliophaeus	MD100	Togo	HG917372
Lactarius tenellus	MD149	Togo	HG917373
Lactarius tenellus	ADK397	Kenya	HG917389
Lactarius sp.	MD391	Togo	HG917380
Lactarius miniatescens	MD151	Togo	HG917374
Lactarius aff. *miniatescens*	MD177	Burkina Fasso	HG917375
Lactifluus edulis	C2168	Togo	HG917384
Lactifluus densiofolius	C2362	Togo	HG917385
Lactifluus longipes	ADK4315	Togo	HG917383
Lactifluus longipes	C2445	Togo	HG917391
Lactifluus foetens	C1822	Togo	HG917382
Lactilfuus foetens	MD150	Togo	HG917381
Lactifluus rubiginosus	MD389	Togo	HG917386
Termitomyces sp.	MD388	Togo	HG917388
Russula congoana	MD129	Togo	HG917387

Our final sequence dataset was composed of 44 ITS rDNA sequences (including those newly generated and those from GenBank) for a length of 700 bp. The most Maximum Likelihood (ML) bootstrap tree was inferred in MEGA 5.2 (Tamura *et al*. 2011, update June 2013) by applying the General Time Reversible nucleotide substitution model (GTR + G +I). Gamma Distribution (G) was set as the rates among sites in the Rates and Patterns parameters (Shape parameters = 4). 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 (TIO). The phylogeny tree was obtained with the bootstrap method of analysis of 1000 replicate trees.

RESULTS

ITS rDNA sequence and phylogenetic analyses

The phylogenetic analysis of all 44 sequences is presented in Fig. 1, four well-supported clades were obtained (Groups I to IV). The first (clade I) was larger and well supported (100 %) clade and constitutes *Lactarius s. str* (clade I), and included 24 sequences, including 10 of those newly generated (Table 3), notably *Lactarius afroscrobiculatus* ADK4599 (1 sample), *L. tenellus* (2), *L. kabansus* (2), *L. miniatescens* (1), *L. saponaceus* (1), *Lactarius* sp. MD391(1 sample), *Lactarius* aff. *miniatescens* MD177 (1), and the specimen MD100 (1). The second (clade II) encompased sequences of *Lactifluus*. The third (clade III) represented the genus *Russula* with three species, and the last (clade IV) the out-group with five taxa.

The sequences of *Lactarius* we investigated formed a monophyletic group and were well supported within the larger monophyletic group. In this *Lactarius* clade, sequences of *L. torminosus* from Belgium, *L.* aff. *wenquanensis* from Thailand, and *L. purpureus* from Papua New Guinea, all belonging to the subgenus *Piperites*, are well supported in this genus with tropical Africa taxa sequences included.

Specimen MD100 nested within the *Lactarius* clade, suggesting it was a member of *Lactarius*. Morphological and molecular arguments/dissimilarities with the closest species provided below support our decision to describe specimen MD100 as a new species, namely *L. subbaliophaeus*.

BlastN search suggests the unidentified sequence of *Lactarius* sp. (UDB013804), *Lactarius* sp. (UDB016864), and of *L. baliophaeus* (GU258277), all from Zambia, as closest to that of the newly proposed species, with identity rates of 94 %, 92 %, and 90 % respectively. As the clade containing *L. baliophaeus* (sect. *Nigrescentes*), is strongly supported as close to the new species, there is no doubt that *L. subbaliophaeus* is a member of this section according to our phylogenetic inference results (Fig. 1), and so agreed with its morpho-anatomical affiliation (Table 2). Sequences of the loaned material of *L. tenellus* and *L. kabansus* fell into a strongly supported internal clade (99 %) of *Lactarius s. str.* representing a portion of *L.* sect. *Plinthogali*, and support the placement of the sampled specimenss from Togo (100 %). These latter above two species represent *Lactarius* subg. *Plinthogali*, sect. *Plinthogali*.

TAXONOMY

Lactarius subbaliophaeus Maba & Yorou, **sp. nov.**

MycoBank MB807658
(Figs 2–4)

Etymology: The epithet refers to the morphological and anatomical similarity with *L. baliophaeus*.

Table 2. Summary of the distinctive features of the most similar species, *Lactarius baliophaeus*, and *L. griseogalus,* and those of *L. subbaliophaeus*, using data from Verbeken & Walleyn (2010).

Features	***L. baliopheus***	***L. griseogalus***	***L. subbaliophaeus***
Basidiomata	Pileus 30–70(–90) mm; greyish yellow to brownish (4A3-5 to 4B3-5), dark blond to yellowish brown (5D4-5 to 5E4-5)	Pileus to 36 mm, very dark brown, almost black	Pileus 40–65 mm; greyish brown to beige- brown (5CD3 to 6DE3)
Lamellae and lamellulae	Broadly adnate, to decurrent, crowded L+l = 4+12 to 3+6/cm	Broadly adnate, not decurrent, distant (total 28)	Broadly adnate, to slightly subdecurrent, distant L+l = 4–5/cm
Context	Firm, white to cream then orange-red, greyish red, finally black	Very thin and transparent, turning red then red- orange, finally black	Firm, first whitish becoming blackish finally black
Latex	Water-like, then successively brownish, blood-red, buff, cream	Hyaline turning grey then dark brown	Transparent white, becoming pinkish grey (7A2) then blackish
Reaction of context to FeSO$_4$	Unchanging	Weakly greyish green	Bluing
Taste	Mild, then acrid	Mild, slightly astringent	Bitter and acrid
Basidiospores	Globose to subglobose	Usually ellipsoid, rarely subglobose	Globose, subglobose rarely ellipsoid
	Q = 1.01–1.07–1.09–1.15	Q = 1.07–1.21–1.36–1.55	Q = 1.04–1.11–1.27
	Plage distally amyloid	Plage not, or slightly amyloid	Plage not amyloid
Cystidia	Pleurocystidia 40–55 × 9–11 µm scarce to abundant, often arising deep in the hymenium, slightly thick-walled	Pleurolamprocystidia 50–65 × 7–10 µm abundant fusiform or irregular, thick-walled	Pleurocystidia 35–56 × 6–9 µm, scarce, inconspicuous subcylindrical, subclavate, thin-walled
Marginal cells	21–40 × (2–)3–5 µm, cylindrical, tortuous or fusiform, rounded, mucronate, with tapering apex	20–35 × 4–7 µm, rarely clavate, mostly fusiform	23–72 × 3–6 µm, subcylindical, fusiform or tortuous, almostly septate, mostly with tapering apex
Pileipellis	Hymeniderm, suprapellis 10–25 × 3–5 µm, thin-walled	Palisade, suprapellis thick, 25–40 × 3–5 µm, thin-walled	Palisade, suprapellis thin 20–60 × 3–5 µm, thin-walled

Diagnosis: Pileus locally smooth, mostly veined in the centre, striate at the margin, with greyish brown to beige brown colour. Lamellae are distant, adnate and slightly subdecurrent. Basidiospores winged, pleurocystidia inconspicuous, fusiform or tortuous, often with tapering apex marginal cells, a palisadic pileipellis with suprapellis composed of cylindrical cells, thin-walled. Marginal cells of lamellae subcylindrical, fusiform or tortuous, mostly septate and mostly with tapering apex. *Lactarius subbaliophaeus* is recognized by the transparent white latex turning first pinkish, then blackish; context with FeSO$_4$ bluish; taste bitter and acrid.

Type: **Togo**: *Central region*: Prefecture of Tchaoudjo, National Park of Fazao-Malfakassa, 08°42'11'' N 0°46'25'' E, on soil in gallery forest dominated by *Uapaca guineensis* and *Afzelia africana*, 16 June 2011, *Dao Maba MD100* (TOGO – **holotype**; GENT – isotype). GenBank accession no. HG917372.

Description: *Pileus* 40–65 mm diam, sometimes asymmetric, plano-convex, depressed in the centre, becoming sub-infundibuliform when old; slightly umbonated, dry, matt, locally smooth, veined in the centre, striate at the margin, greyish brown to beige-brown (5CD3 to 6DE3), locally pale at the margin. *Margin* incurved, edge crenulated, sometimes slightly striate when old. *Lamellae* spaced or distant, adnate, slightly subdecurrent, unequal, irregular (L+l = 4-5/cm), becoming blackening when injured. *Stipe* 30–45 × 8–13 mm, rigid, irregular, dry, central, clavate to subclavate at the base, yellowish grey (4B3), becoming darkish when bruised. *Context* first whitish becoming blackish, thinner at the margin and thick in the centre of pileus. *Latex* very abundant, transparent white, becoming pinkish grey (7A2) then blackish; *taste* bitter and acrid, smell not observed. *Chemical reaction*: context bluing with FeSO$_4$. *Basidiospores* globose, subglobose rarely ellipsoid, 8–*8.5*–9 × 7–*7.5*–8(–8.5) µm (Q = 1.04–*1.11*–1.27; *n* = 75), amyloid ornamentation composed of ridges up to 0.5–1 µm, sometimes more and forming almost a complete reticulum, plage mostly inamyloid. Basidia 4-spored, 20–66 × 11.5–14 µm, clavate, with a granule-like or guttule-like content, sterigmata 4–*8*–10 × 1–*2*–3 µm. *Pleurocystidia*, 35–56 × 6–9 µm, scarce, inconspicuous, subcylindrical to subclavate, rarely projecting, thin-walled. *Pleuropseudocytidia* 4–*5*–6 µm diam, abundant, cylindrical, sometimes tortuous, with brown contents. Lamellar edge sterile. *Marginal cells of lamellae* 23–72 × 3–6 µm, subcylindical, fusiform or tortuous, mostly septate and mostly with tapering apex. *Hymenophoral trama* composed of a mixture of abundant laticiferous hyphae and sphaerocytes at the base. *Pileipellis* a palisade, suprapellis composed of dense cylindrical elements of 22–35 × 3–5 µm, thin-walled and with isodiametric cells at the base. *Stipitipellis* a trichoderm to ixotrichoderm with entangled hyphae at the

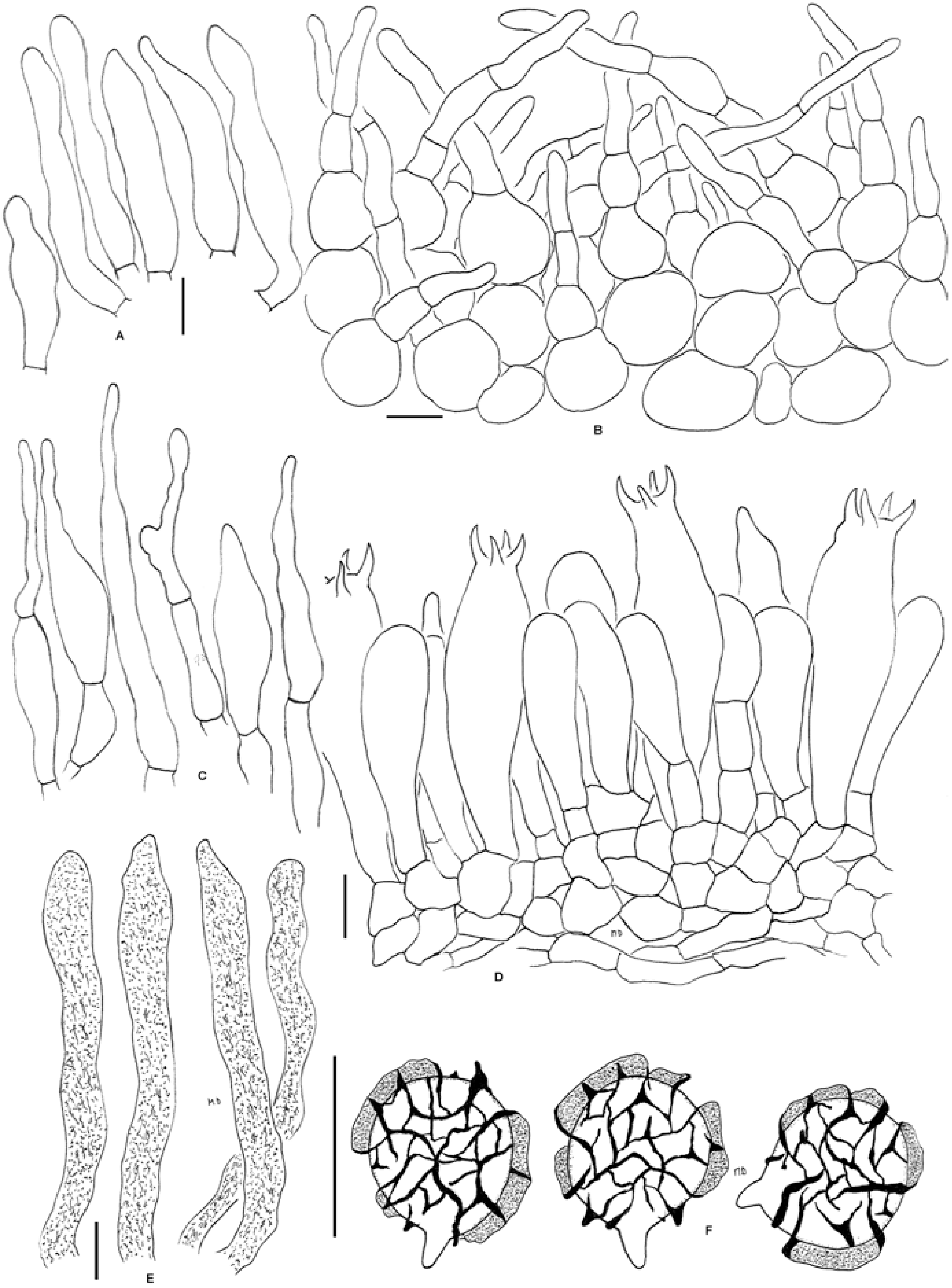

Fig. 2. Light microscopy of *Lactarius subbaliophaeus* (MD100). **A.** Pleuroprocystidia. **B.** Pileipellis. **C.** Marginal cells. **D.** Hymenium. **E.** Pleuropseudocystidia. **F.** Spores. Bars = 10 µm.

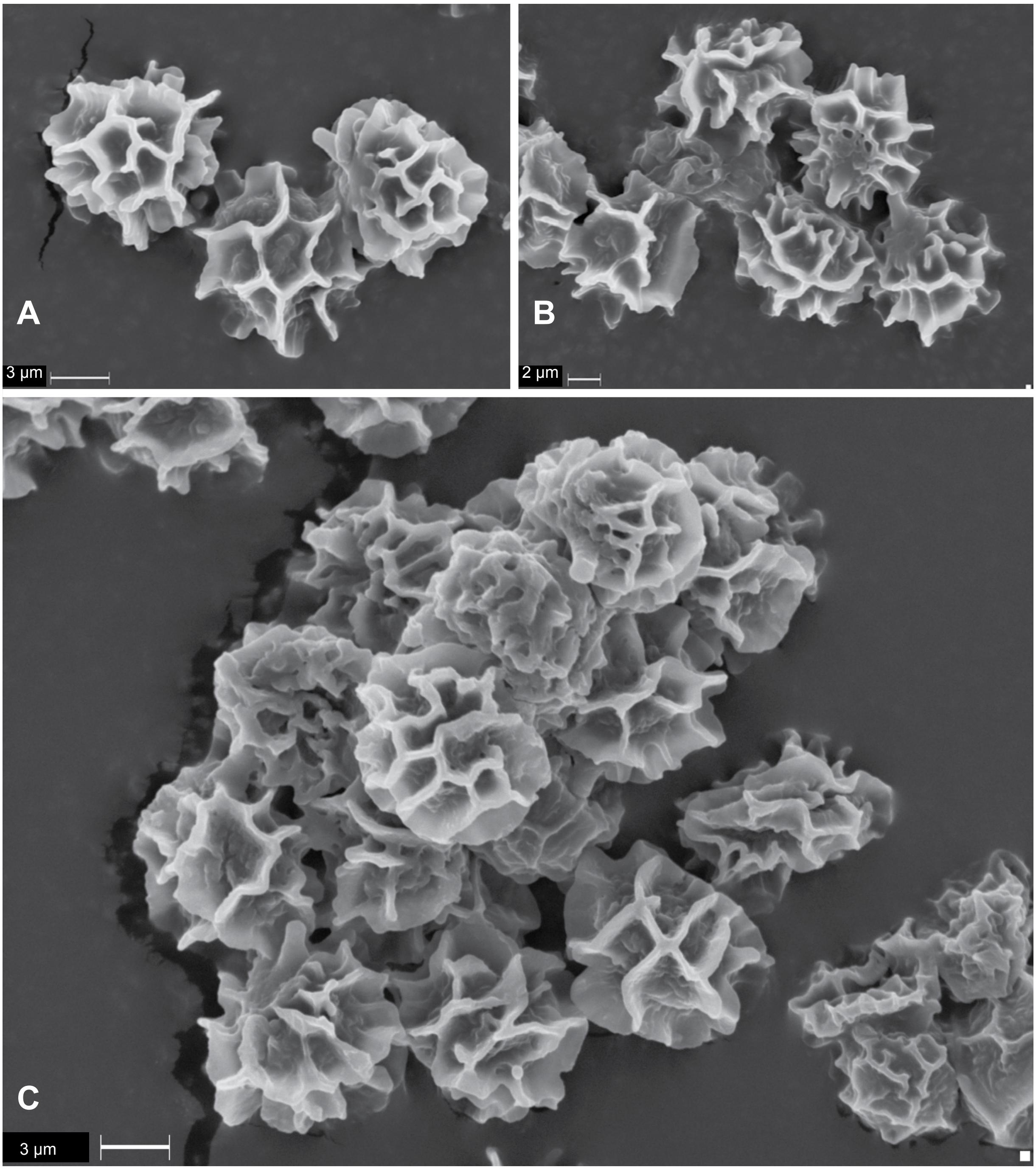

Fig. 3. SEM of *Lactarius subbaliophaeus* (MD100). **A–B.** Basidiospores in differents views. **C.** General view.

base and cylindrical elements in the suprapellis. *Clamps* absent.

Additional specimen examined: **Togo**: *Central region*: Prefecture of Assoli, Forest Reserve of Aledjo, 09°13.9’8.1’’ N 01°11.4’42’’ E, inwoodlands dominated by *Isoberlinia tomentosa* and *Uapaca togoensis,* 26 May 2008, *Dao Maba MD14* (TOGO).

DISCUSSION

Lactarius subbaliophaeus differs from *L. baliophaeus* and *L. griseogalus* in the greyish brown to beige-brown pileus and distant, adnate, slightly subdecurrent lamellae. Microscopically, it has inconspicuous pleurocystidia that are fusiform or tortuous, often tapering at the apex; a palisadic pileipellis with a suprapellis composed of cylindrical cells. The marginal cells of the lamellae are subcylindical, fusiform

Fig. 4. Basidiome of *Lactarius subbaliophaeus* (MD100). **A.** General view (shape and spaced lamellae). **B.** Pileus view. **C.** Lamellae and exuding latex. Bars = 10 mm.

or tortuous, mostly septate, and with a tapering apex. It is easily identifiable by the transparent white latex that turns first pinkish and then blackish, a bluish reaction of the flesh context to $FeSO_4$; and a bitter and acrid taste (Table 2).

Considering the morphological and anatomical features summarized in Table 2, it is clear that *L. subbaliophaeus* differs most from *L. griseogalus* and is closest to *L. baliophaeus*, but differs from the latter.

Detailed analyses of the ITS rDNA sequences revealed that *L. subbaliophaeus* deviates from *L. baliophaeus* by 12 % (sequence length 700 bp). Sequences of both species fall within two different terminal clades, each well supported by a bootstrap of 100 % and 99 %, respectively. The sequence of *L. baliophaeus* clusters as sister species with the sequences of two unidentified collections from Zambia (*Lactarius* sp., UDB013899) and from Cameroon (*Lactarius sp* UDB013969),

Table 3. *Lactarius* species recorded in Togo 2007–2013.

Species	Specimens	Collector/Date	Locality and ecosystem type
Lactarius sp.	MD391[1]	*Dao Maba* 16 July 2013	Aledjo woodlands dominated by *Isoberlinia tomentosa* and *Uapaca togoensis*, 09°13'6" N 01°11.4'38" E
Lactarius afroscrobiculatus	BR 163477-32; 163418-79	*Andre De Kesel* 16 July 2007	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis* and/or *Afzelia africana*, 08°43.9'6.3" N 0°47.6'7.4" E
	C2268[2]	*Atsu Guelly* 31 May 2008	Aledjo, gallery forest dominated by *Uapaca guinensis Isoberlinia doka* and *Uapaca togoensis*, 09°16'34.0" N 01°13'32.4" E
Lactarius atro-olivinus	BR 163674-35	*Andre De Kesel* 11 July 2007	Aledjo, gallery forest dominated by *Berlinia grandiflora*, 09°16'46" N 01°12"41.6' E
	BR163490-45	23 May 2008	Ilè Forêt claire à *Uapaca togoensis*, 07°36'7.4" N 0°51'10.5" E
	ADK4538[3]; ADK4813; ADK4836	15 May 2010 17 May 2010	Ola, gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis*, 07°33'12.3" N 0°52'37.8" E
	MD07	*Dao Maba* 9 Sept. 2008	Aledjo, gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis*, 09°16'45" N 01°11'47" E
Lactarius kabansus	MD132	*Dao Maba* 19 June 2011	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°45'24" N 00°48'08" E
Lactarius melanogalus	ADK4292	*Andre De Kesel* 11 July 2007	Aledjo, gallery forest dominated by *Berlinia grandiflora*, 09°16'46" N 01°12'41.6" E
Lactarius miniatescens	BR 163828-92	*Andre De Kesel* 13 July 2007	Tchamba, Gallery forest dominated by *Milletia thonningii*, *Lonchocarpus sericeus*, 08°56.6'9.2 N " 01°31.9'6.6 E "
	MD09	*Dao Maba* 27 May 2008	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°48'52.35" N 0°45'29.2" E
	MD151	19 Jun 2011	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°42'21" N 00°46'18" E
	MD401	18 July 2013	Aledjo, gallery forest dominated by *Berlinia grandiflora* and *Uapaca guineensis*, 09°16'28.5" N 01°13'21.5" E
Lactarius Subbaliophaeus sp. nov.	MD100	*Dao Maba* 16 June 2011	Fazao, woodland dominated by *Uapaca togoensis and Afzelia africana*, 08°42'21" N 0°46'22" E
	MD14	*Dao Maba* 26 May 2008	Aledjo woodlands dominated by *Isoberlinia tomentosa and Uapaca togoensis*, 09°13'8" N 01°11.4'42" E
Lactarius saponaceus	BR 158418-17	*Andre De Kesel* 11 July 2007	Aledjo, woodland dominated by *Isoberlinia tomentosa*, 09°13.9'8.1" N 01°11'44.2" E
	MD390	*Dao Maba* 18 July 2013	Aledjo, woodland dominated by *Isoberlinia tomentosa*, 09°13'27" N 01°11'53" E
Lactarius tenellus	BR 163798-62	*Andre De Kesel* 20 July 2007	Fazao, woodland dominated by *Uapaca togoensis*, 08°43'14.5" N 0°46'33.2" E
	C2142	*Atsu Guelly* 3 June 2008	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°43'19" N 00°46'31" E
	MD149; 126; 159	*Dao Maba* 18–19 June 2011	Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°45'24" N 00°48'08" E; 08°42'25" N 00°46'24" E

[1]MD = collection of *D. Maba*; [2]C= collection of *A. Guelly*; [3]ADK= collection of *A. De Kesel.*

whilst the sequence of *L. subbaliophaeus* forms a terminal sister clade together with two samples from Zambia (UDB0130804, UDB016864). *Lactarius subbaliophaeus* and *L. baliophaeus* belong both to *Lactarius* sect. *Nigrescentes* (Fig. 1), what is corroborated by morpho-anatomical features (see Table 1).

With respect to other species found in Togo (Table 3), the sequence of *L. afroscrobiculatus* of *Lactarius* subg. *Piperites* is sister to the clade that includes the Togoan species, *L. torminosus*, *L.* aff. *wenquanensis*, and *L. purpureus*. This tropical species is known for its typical morphological characters (sticky cap and scrobiculate stipe) and relate it to the temperate species of *Lactarius* subgen. *Piperites* (Heilmann-Clausen *et al.* 1998, Verbeken & Walleyn 2010). Another unidentified sample from Togo (*Lactarius* sp., MD391) clusters with species of *L.* subgen. *Piperites* with an 87 % of bootstrap value. As *Lactarius* subgen. *Piperites* has additional representative species (*L. barbatus* and *L. acrissimus*) in tropical Africa, it is likely that *Lactarius* sp. MD391 constitutes an additional member within this group. Morpho-antomical studies of this collection are still required.

The remaining taxa studied (*L. tenellus*, *L. kabansus*, *L. miniatescens,* and *L. saponaceus* from Togo and *L. angiocarpus* from Zambia) are well supported as members of *L.* sect. *Plinthogali*, revealing *L.* sect. *Plinthogali* as polyphyletic (Fig. 1), as *L. saponaceus, L. angiocarpus,* and *L. miniatescens* form a clade sister to *L.* sect. *Nigrescentes*, whereas *L. kabansus* and *L. tenellus* belong to a different clade with 100 % bootstrap support (Fig. 1, Sect. *Plinthogali* 1 and 2).

Contrary to *L.* sect. *Plinthogali* with more taxa found in Togo from *L.* sect. *Nigrescentes,* only two well characterized species have been found. This section appears actually monophyletic, but morpholo-anatomical studies on the more closely related samples are in this section needed. Generally, additional sequences of particularly these two sections as well as from other species are necessary for a better understanding of the phylogenetic tendency/relationship between species of tropical African *Lactarius s. str*. species. Nevertheless, our study reconfirms the monophyly of *Lactarius s. str.* as elegantly demonstrated by Buyck *et al*. (2008, 2010).

Ecologically, species including *Lactarius atro-olivinus*, *L. afroscrobiculatus,* and *L. miniatescens* have no apparent preference for a special vegetation type, as they were sampled from savanna woodlands as well as gallery forests (Table 3). Moreover, whether in a gallery or savanna, *L. afroscrobiculatus* was collected in habitats that harbour *Uapaca* species and *Isoberlinia doka*; *L. atro-olivinus* occurs often in the presence of *Berlinia grandiflora* and *Uapaca guineensis*; and *L.miniatescens* with *Uapaca* species. In contrast, *L. tenellus* is almost always sampled from savanna woodlands that harbour *U. togoensis* and *I. doka* as native ectomycorrhizal trees, with a tendency to prefer *U. togoensis*. *Lactarius saponaceus* seems to preferably occur in presence of *I. tomentosa*. *Lactarius kabansus* is widely distributed in the Congo-Zambezian domain and constitutes the first record from the Guineo-Sudanian domain, and was sampled from savanna woodlands dominated by *I. doka* and *U. togoensis*. *Lactarius subbaliophaeus* was sampled twice in savanna woodlands dominated by *U. togoensis,* and *I. tomentosa* or *Afzelia africana*. Future investigations will reveal more details regarding their distribution and ecological preferences.

During several consecutive collection trips, *Lactarius s. str.* appeared to be relatively poorly represented in Togo as compared with *Lactifluus* (Maba *et al.* 2013), both genera being collected in the same habitats. *Lactarius s. str.* was represented by eight taxa with six known at species level, and two still unidentified. Of the seven sections including *Chromospermi*, *Piperites*, *Amari*, *Russularia*, *Nigrescentes*, *Plinthogali*, and *Pseudofuliginosi* reported for tropical Africa, four are represented in the vegetation types of Togo (*Piperites*, *Nigrescentes*, *Plinthogali,* and *Pseudofuliginosi*). However, considering that 75 % of the species of *Lactarius* harvested in West Africa are found in the ecosystems of Togo, and mainly as not all parts of the vegetation types have been investigated, it can be expected that additional new species are still to be collected and described.

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Chapter 4

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

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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 (Rivière *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 caesalpinioid- 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 specimens was made using the results of investigations in similar ecosystems (van Rooij *et al.* 2003). Additionally, we used the monograph of Verbeken & Walleyn (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 nurDNA 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* (untill 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 % boostrap support. The sister clade (composed of a *Gloeocystidielium* 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 subclade 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 fazaoensis	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 heimii	C2018	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
	MD132	Togo	HG917376
Lactarius miniatescens	MD151	Togo	HG917374
Lactarius subbalophaeus	MD100	Togo	HG917372
Lactarius tenellus	MD149	Togo	HG917373

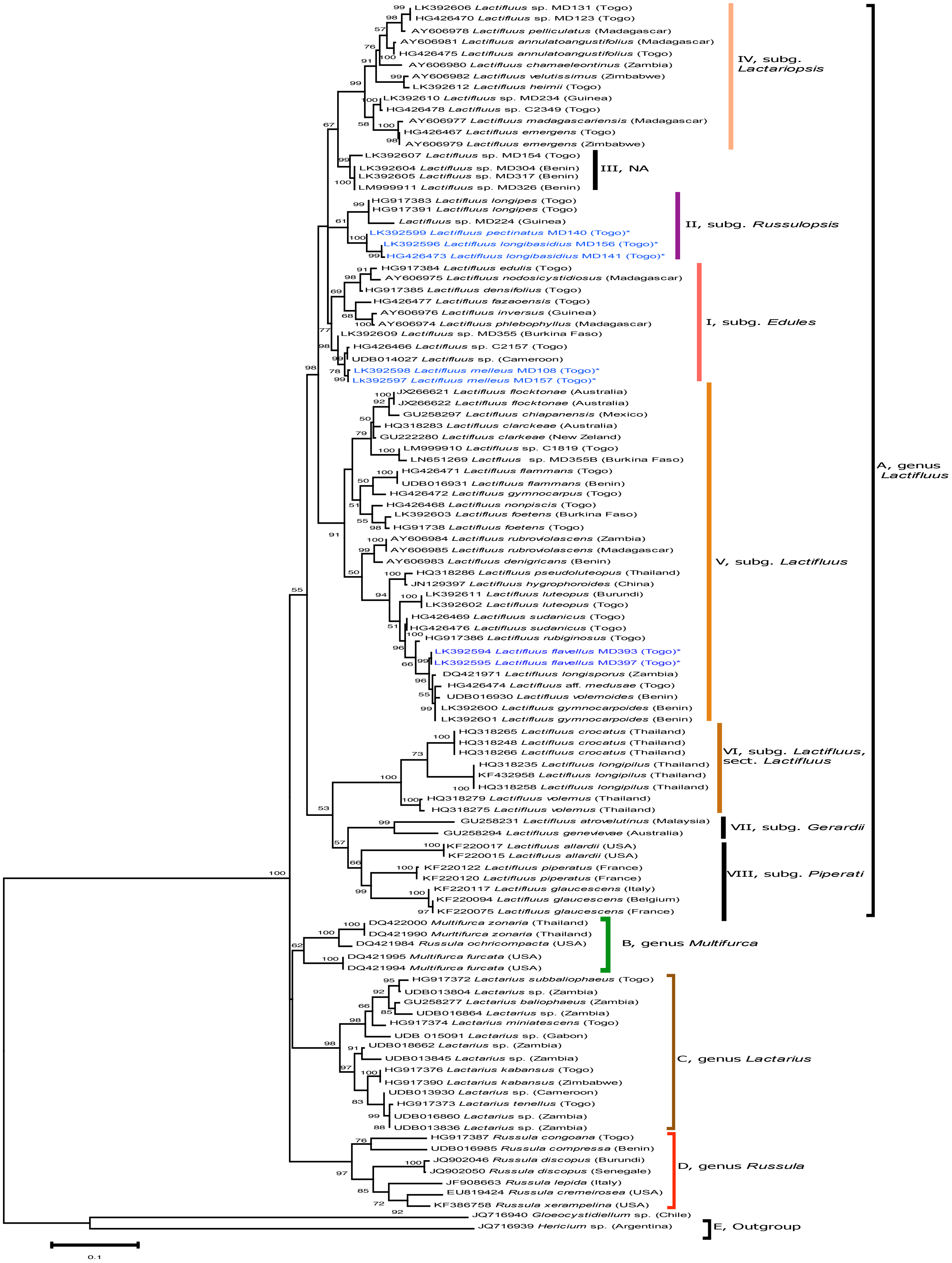

Fig. 1. Maximum Likelihood (ML) tree showing the placement of the four newly described species (*L. flavellus*, *L. longibasidius*, *L. melleus* and *L. pectinatus*) within the *Russulaceae*. Bootstrap values higher than 50 % are shown above/below the branches.

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, Bèna 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.

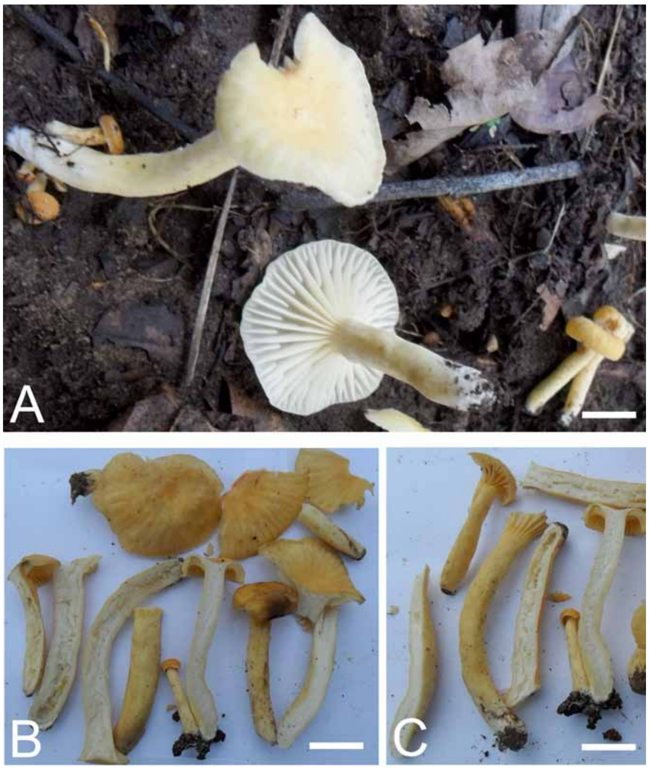

Fig. 2. *Lactifluus flavellus* (MD393) basidiome. **A.** Lamellae and the pileus. **B.** Pileus and stipe detail. C. Stipe. Bars = 10 mm.

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

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

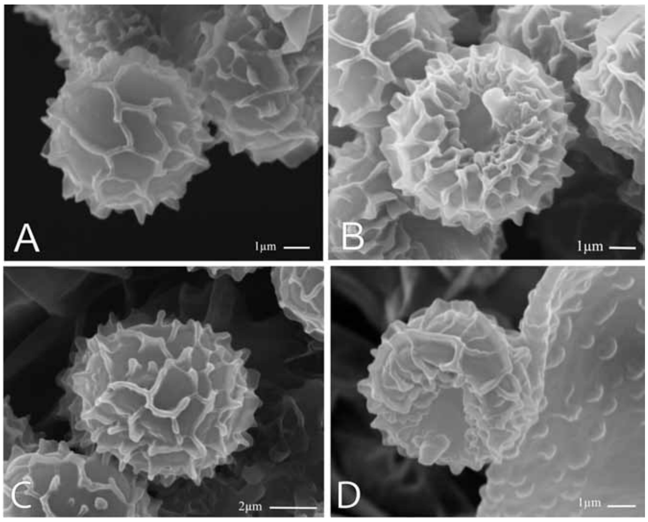

Fig. 4. *Lactifluus flavellus* (MD393) SEM of basidiospores.**A**, **C.** Dorsal view. **B**, **D.** Lateral and detail showing the plage.

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+l = 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) µm (Q = (1.08–)1.15–*1.25*–1.30(–1.35); *n* = 75), amyloid ornamentation of ridges to 0.5 µm in height, mostly connected, forming almost a complete reticulum; amyloid 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, scare 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 Eglekoutsè, 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 & Walleyn 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 Eglekoutsè, 7°31'6.18'' N 0°54'7'' 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.

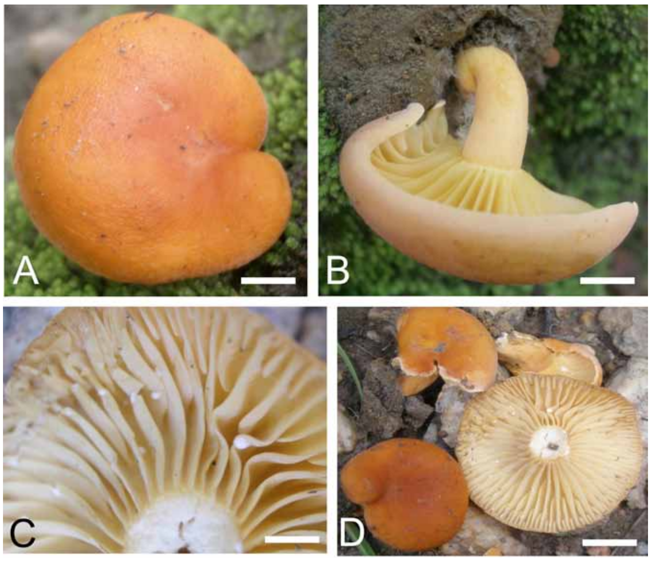

Fig. 5. *Lactifluus longibasidius* (MD156) basidiome. **A**. Pileus. **B.** Margin and stipe. **C.** Lamellae. **D.** General view (pileus, lamellae, and stipe). Bars = 10 mm.

Diagnosis: *Pileus* irregular, asymmetric, plano-concave to plano-convex, eccentric, even reniform; pellis dry, shiny, wrinkled, strongly rugulose, slightly velvety; reddish orange, darkest in the centre, light orange to dark orange. *Lamellae* adanate, decurrent to subdecurrent, moderately distant, irregular and inequal. Recognized by the particularly long basidia, sometimes tortuous, abundant and long pleurocystidia which are emergent, subcylindrical to cylindrical, sometimes tapering upwards, capitate or conical, thin-walled; marginal cells of lamellae subcylindrical to cylindrical, sometimes branched and septate; pileipellis lamprotrichoderm-like, with a suprapellis composed of subcylidrical to fusiform cells, with a distinctly tapering apex, septate and rather bifurcate, mixed with very slender and thick-walled cells; stipitipellis a trichoderm, composed of wide, irregular, septate, bifurcate, and very slender cells.

Type: **Togo**: Central region: Prefecture of Tchaoudjo, 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* MD156 (TOGO – holotype; GENT– isotype). ENA accession no. LK392596.

Description: *Pileus* 25–55 mm diam, very irregular in shape, sometimes asymmetric, concave when young, plano-concave to plano-convex and depressed when old, eccentric and even reniform; pellis dry, shiny, wrinkled, strongly rugulose and slightly velvety, slightly striate near the margin when older, reddish orange (7B7-8) in the centre, darkest in the centre on young basidiomes, light orange to dark orange (5A5-8) near the margin, old specimens paler near the margin. *Margin* incurved to enrolled. *Lamellae* adnate, decurrent to subdecurrent, moderately distant, irregular, unequal (L+l = 5–7/cm), pastel yellow, light yellow (3A4-5) to orange-yellow (4A4-5). *Stipe* 18–35 × 10–15 mm, central to eccentric, cylindrical, sometimes tapering downwards, dry, firm, smooth, light yellow (4A4-6). *Context* of pileus orange

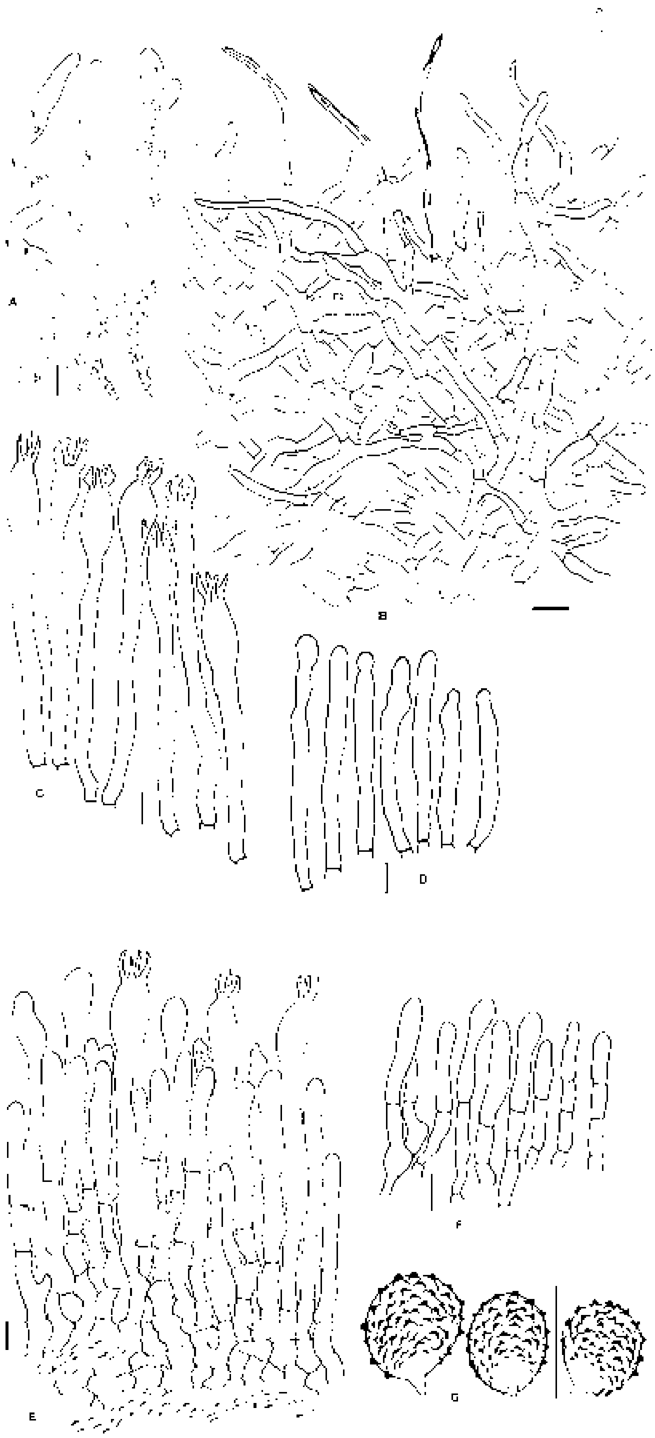

Fig. 6. *Lactifluus longibasidius* (MD156) light microscopy. **A.** Pleuropseudocystidia. **B.** Pileipellis. **C.** Basidia. **D.** Pleurocystidia. **E.** Hymenium. **F.** Marginal cells. **G.** Basidiospores. Bars = 10 µm.

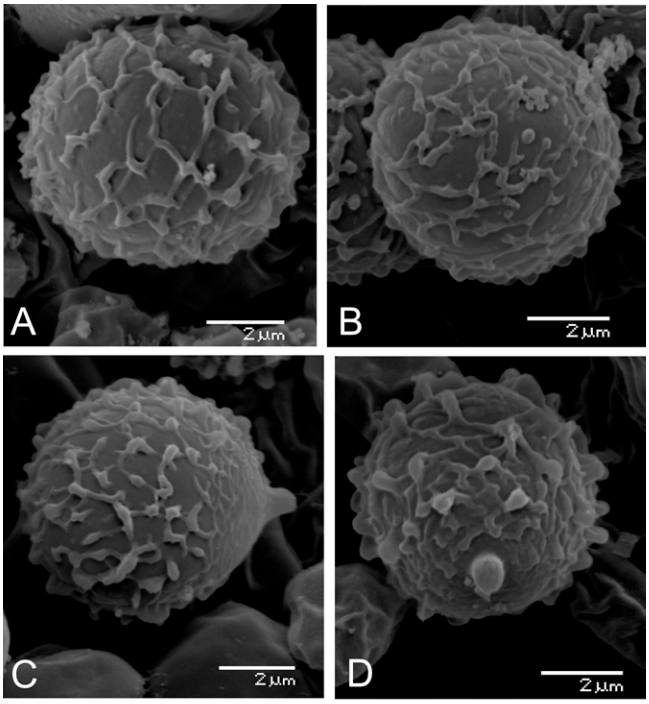

Fig. 7. *Lactifluus longibasidius* (MD156) SEM of basidiospores. **A.** Dorsal to lateral view. **B.** Proximal profile view. **C.** Lateral front view. **D.** Proximal to dorsal view.

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. *Pileipellis* (Fig. 6B) lamprotrichoderm-like, terminal elements 2–3(–5) µm wide, subcylidrical 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 Tchaoudjo, 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.

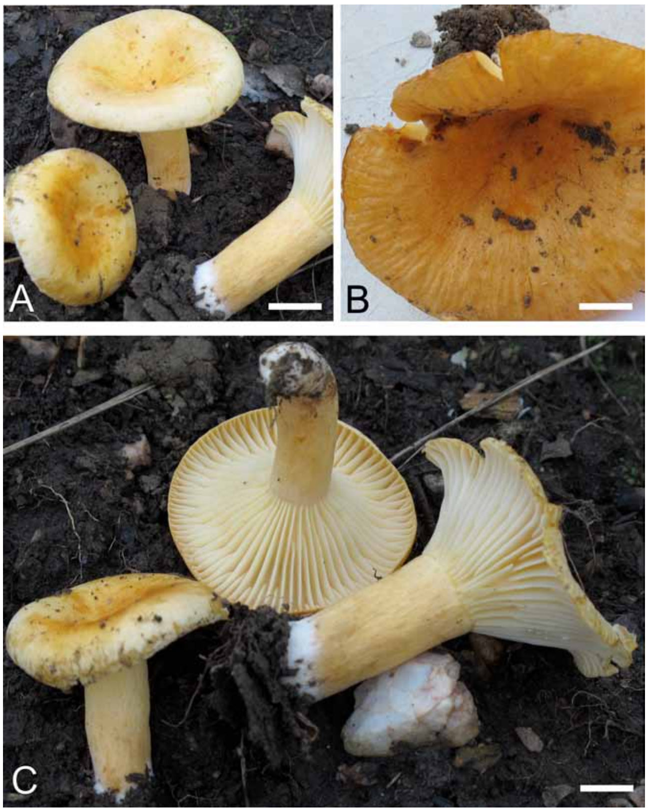

Fig. 8. *Lactifluus melleus* (MD157) basidiome. **A.** Pileus and stipe. **B.** Pileus. **C.** Lamellae and stipe. Bars = 10 mm.

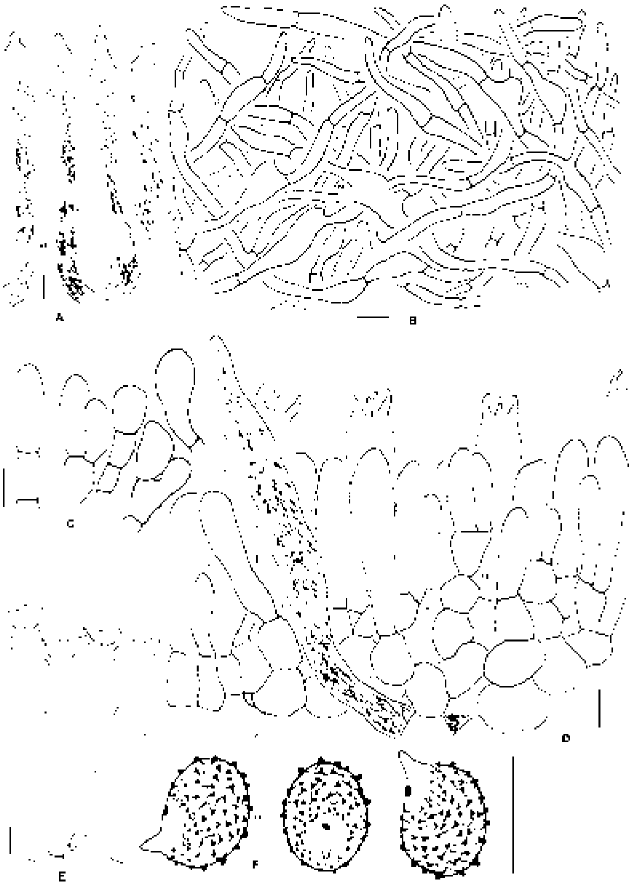

Fig. 9. *Lactifluus melleus* (MD157) light microscopy. **A.** Pleuropseudocystidia. **B.** Pileipellis. **C.** Marginal cells. **D.** Hymenium. **E.** Basidia. **F.** Basidiospores. Bars = 10 µm.

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 – holotyp**e**; GENT –isotype). ENA accession no. LK392597.

Description: *Pileus* 40–60 mm diam, concave when young then plano-concave, strongly depressed in the centre,

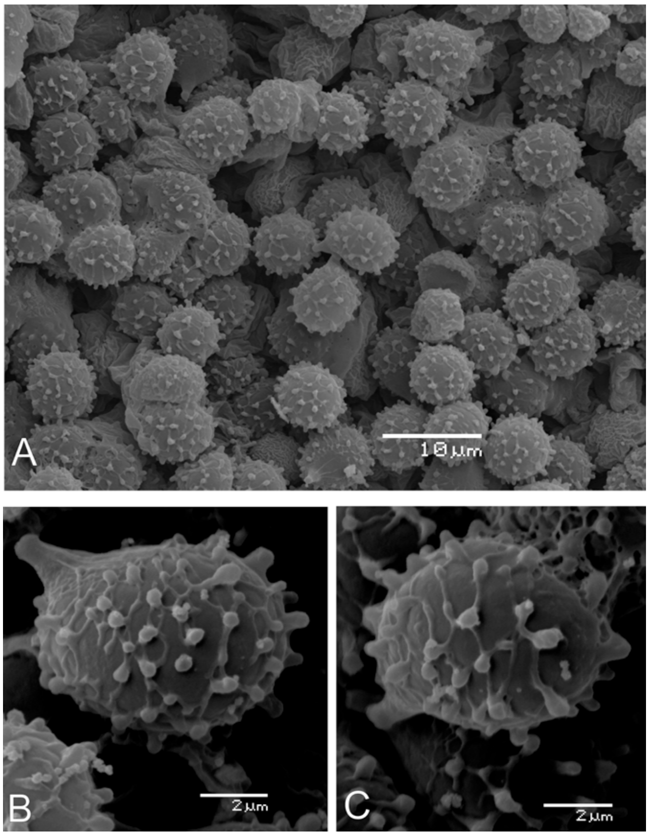

Fig. 10. *Lactifluus melleus* (MD157) SEM of basidiospores. **A.** Overview. **B.** Lateral view. **C.** Lateral/dorsal view.

sometimes subinfundibuliform when old, pellis velvety and soft, appearing smooth in freshly collected specimens, but in dehydrated samples striate to 1/3 from the margin; dry, honey-coloured, light yellow to deep yellow or warm yellow, slightly more pronounced in the centre (4A4-8). *Margin* straight in young specimens,grooved and striate in old specimens to 1/3 from the margin to the cente, slightly thick, striate and involuted. *Lamellae* adnate, broadly subdecurrent to decurrent, moderately spaced, unequal, irregular with 1(--3) lamellulae between two lamellae (L+l = 4–6/cm), very brittle; yellowish white to light yellow (4A2-4). *Stipe* 30–35 × 12–15 mm, smooth, soft, central, cylindrical and tapering downwards, firm, solid, concolorous with the pileus. *Context* of pileus brittle, slightly thickened in the centre, fleshy, white in the pileus as well as in the stipe. *Latex* not abundant, white and unchanging, taste not observed.

Basidiospores (Figs 9E, 10A–C) subglobose to ellipsoid, rarely elongate (8.0–)8.5–*9.0*–9.5(–10.0) × 7.0–*7.5*–8.0 µm (Q = (1.08–)1.15–*1.25*–1.30(–1.34); *n* = 80), ornamentation of well developed amyloid warts, to 0.5 µm high, connected by fine lines, sometimes almost with a complete reticulum; amyloid spot present in a distinct plage. *Basidia* (Fig. 9E) 4-spored, 50–65(–70) × 10–12 µm, subclavate, sterigmata 4–7 × 2 µm. *Lamella edge* sterile. *Marginal cells* (Fig. 9C) 32–50 × 5–6(–7) µm, irregular, cylindrical to subclavate, thin-walled. *Hymenophoral trama* mostly cellular, composed of sphaerocytes and laticiferous hyphae. *Pleurocystidia* absent. *Pleuropseudocystidia* (Fig. 9A) very abundant, 11–16(–20) µm diam, cylindrical, rarely tortuous, projecting to 40–50 µm above the hymenium, sometimes tapering upwards, moniliform to mucronate, thin-walled, contents brown. *Pileipellis* (Fig. 9B) an ixocutis to a trichoderm, suprapellis of irregular, cylindrical to subclavate cells, septate, interwoven; subpellis of irregular sphaerical, isodiametric cells, 5–15 µm diam; dermatocystidia not very abundant, sometimes with a moniliform to mucronate apex. *Stipitipellis* an ixocutis to a cutis, suprapellis composed of irregular, thin-walled cells, septate and sometimes branched, densely interwoven; subpellis composed of a mixture of isodiametric cells and laticiferous hyphae. *Clamps* absent.

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 & Walleyn 2010).

Additional specimen examined: **Togo**: Central region: Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, 08°42'23'' N 046'27'', 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; pellis 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. *Pleurocystidia* 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, septate.

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.

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+1 = 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, 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) × (6.0–)6.5–*7*–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, no amyloid spot present in the plage. *Basidia* (Fig. 12F) 54–62 × 8–10 µm, subclavate to clavate, 4-spored, sterigmata 8–11 × 2–3 µm. *Pleurocystidia* (Fig. 12E) rather abundant, 55–65 × 6–8 µm, emergent, cylindrical to subcylindrical, slightly fusiform, sometimes septate, thin-walled. *Pseudopleurocystidia* **(**Fig. 12B) abundant, 3–8 µm diam, very irregular, fusiform to tortuous, moniliform, capitate, with irregular, brown contents. *Lamella edge* sterile. *Marginal cells* (Fig. 12D) 20–45 × 3–5 µm, cylindrical to fusiform, septate. *Hymenophoral trama* composed of numerous sphaerocytes mixed with laticifers. *Pileipellis* (Fig. 12B) ixotrichopalisade-like, terminal elements

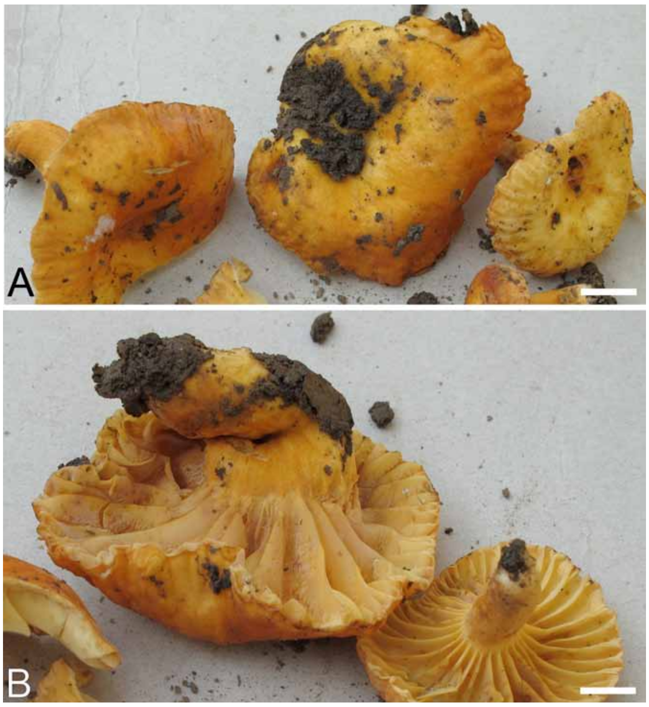

Fig. 11. *Lactifluus pectinatus* (MD140) basidiome. **A.** Pileus. **B.** Lamellae and stipe. Bars = 10 mm.

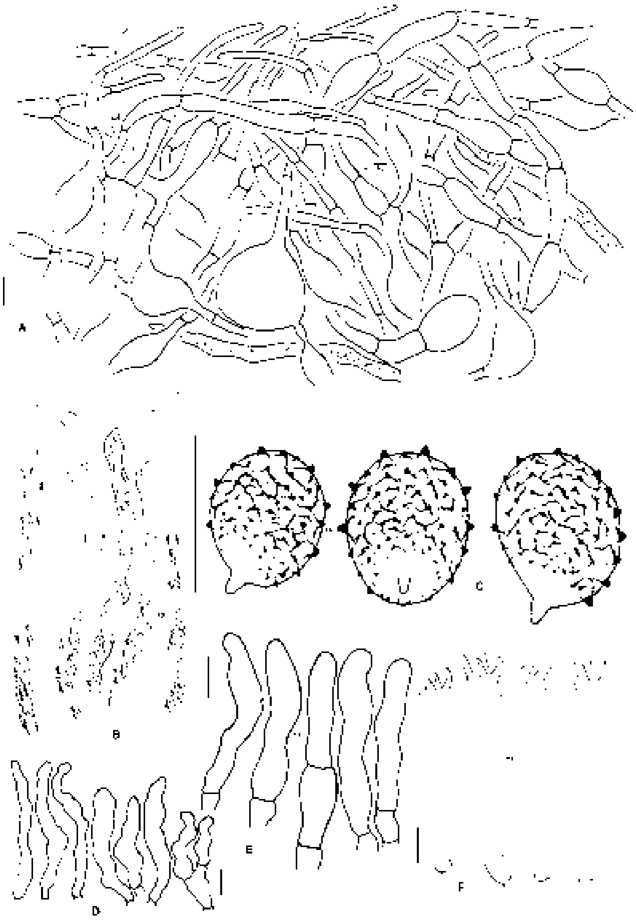

Fig. 12. *Lactifluus pectinatus* (MD140) light microscopy. **A.** Pileipellis. **B.** Pleuropseudocystidia. **C.** Basidiospores. **D.** Marginal cells. **E.** Pleurocystidia. **F.** Basidia. Bars = 10 µm.

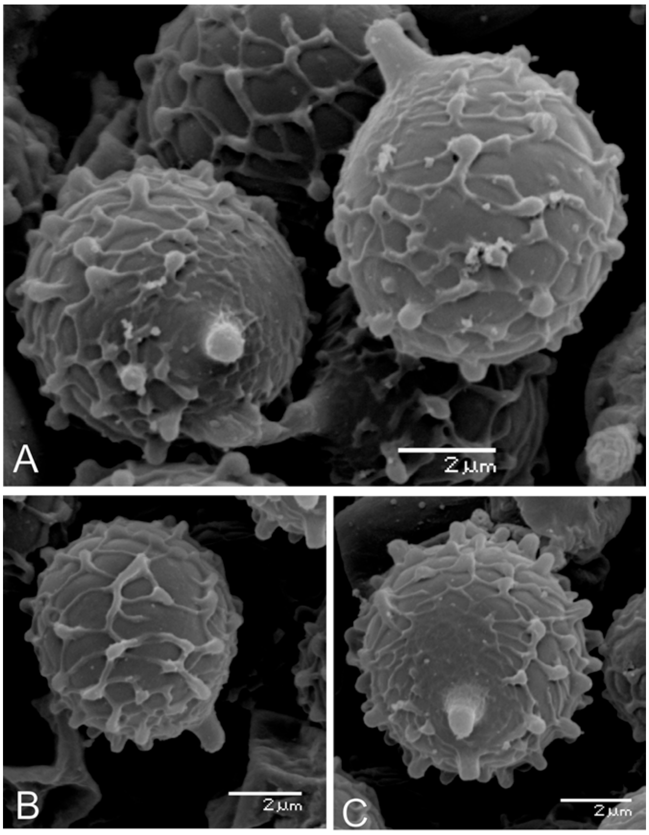

Fig. 13. *Lactifluus pectinatus* (MD140) SEM of basidiospores. **A.** Proximal view (left), lateral view (right). **B.** Dorsal view. **C.** Proximal view.

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 plage. 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 *Lactifluus* species from tropical Africa (van Rooij *et al.* 2003, Buyck *et al.* 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. *Edules*. 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 pileipellis, composed of cylindrical to subclavate cells. These characters separate it from the previously named *L. edules*, *L. aureifolius,* and *L. densifolius. Lactifluus melleus* has basidiospore ornamentation and a pileipellis that fits within *Lactifluus* subgen. *Lactariopsis* sect. *Chamaeleontini.* It has thick-walled hair-shaped elements in the pileipellis, pleurocystidia are absent, and the basidiospore ornamentation is to 0.5 µm. It also agrees with *Lactifluus* subgen. *Russulopsis* in having basidiospores with obtuse amyloid warts to 0.5 µm, the presence of dermatocystidia, and a pileipellis with diverticulate and subclavate cells (Verbeken & Walleyn 2010).

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 lactarioid species, and a strongly striate pileus, even in young basidiomes. In addition, *L. flavellus* has some microscopic features, including pleurocystidia, that are not present in *L. uapacae*. *Lactifluus flavellus* has a palisade-like pileipellis and a hymenoderm-like stipitipellis while these are, respectively, a lampropalisade and a lamprotrichopalisade in *L. uapacae.* The hymenium of *L. flavellus* is crowded with distinctly emergent pleurocystidia to 85 µm, whereas these are absent from *L. uapacae*, and incrustations are present in the hymenium as well as in the pileipellis of *L. flavellus*, but absent in *L. uapacae*.

Considering the morpho-anatomical analyses, *L. flavellus* has a combination of features (*see above*) that fit in *Lactifluus* subgen. *Lactifluus,* a placement confirmed by the phylogenetic analysis. *Lactifluus melleus* is supported within the *Lactifluus* subgen. *Edules* clade, while *L. longibasidius* and *L. pectinatus* are supported phylogenetically within the *Lactifluus* subgen. *Russulopsis* clade. However, within the genus *Lactifluus*, the species including *L. rufomarginatus*, previously classified in *Lactifluus* sect. *Russulopsidei*, *L. cocosmus* and *L. aurantiifolius*, remained unclassified in any of the accepted subgenera (Verbeken *et al.* 2011, 2012, Stubbe *et al.* 2012). Similarly, *L. brachystegiae*, previously classified in sect. *Chamaeleontini*, is currently placed in subgen. *Russulopsis* sect. *Russulopsidei*.

This study confirms the high species diversity of *Lactifluus* in tropical West Africa and points to the patchy and/or paucity, of information on lactarioids from West African ecosytems. Apart from the species descibed in this paper and some other previous publications (van Rooij *et al.* 2003, van de Putte *et al.* 2009, Maba *et al*. 2013, 2014), numerous samples from the same area are still waiting to be assessed. Our results support the monophyly of *Lactifluus* within *Russulaceae*. From the present study and from Maba *et al*. (2013, 2014), we also conclude that a new delimitation at subgenus and section level is needed within *Lactifluus*, to take into account the combinations of morphological and microscopical characters displayed in the recently described species. We consider that mycological inventories throughout tropical African ecosystems, and particularly in West Africa, remain crucial for a real assessment of the extent of tropical African mycodiversity, and will thereby help to highlight the evolutionary traits within milkcaps.

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Chapter 5

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 ectomycorrizal 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 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 public GenBanks. A Maximum Likelihood phylogenetic tree was inferred from 111 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, Guineo-Sudanian and Congo-Zambezian domain occur many common species. Patterns of occurrence of the recorded *Lactifluus* species from Guineo-Sudanian ecozones are also highlighted.

Key word Ecology, Guineo-Sudanian, *Lactarius*, *Lactifluus*, Molecular phylogeny, Taxonomy.

Introduction

Although there has been remarkable progress in tropical mycological investigations for the last twenty years, tropical Africa ecozones and particularly West Africa, remain very poorly explored (Rivière et al. 2007; Diédhiou et al. 2013; Maba et al. 2013, 2014, 2015). Forest ecosystems of tropical Africa encompass various vegetation types with high diversity of native ectomycorrhizal (ECM) trees (Verbeken & Buyck 2001; Rivière et al. 2007; Rinaldi et al 2008; Bâ et al. 2012; Diédhiou et al. 2013; Sanon et al. 2013). Although in general *Lactarius* and *Lactifluus*, together the milkcaps, are among the best studied macrofungi in the continent with many species discovered and described the last decade, numerous studies continuously argued on the importance and the real need of intensifying mycological investigations of African forest ecosystems, particularly the Sudanian domain, for their better knowledge (Verbeken & Buyck 2001; van Rooij et al. 2003; Rivière et al. 2007; Van de Putte et al. 2009; Verbeken et al. 2011, 2013; Maba et al. 2013-2015), the current species richness, the distribution and niche range/differentiation of tropical *Lactifluus* and *Lactarius* species from Guineo-Sudanian domain is not adequately assessed yet. Available data are mostly focused on systematics and taxonomy (van Rooij et al. 2003; Rivière et al. 2007; Maba et al. 2013-2015), which also highlighted some patterns of chorology of core species and their putative partner forest trees (Verbeken & Buyck 2001; Rivière et al. 2007; Sanon et al. 2013; Maba et al. 2014, 2015).

Ecologically, the Guineo-Sudanian domain harbors ceasalpinioid- and phyllantoid-dominated savannas, woodlands, open forests and riverside forests, but also rainforests, with various native ECM trees (Diédhiou et al. 2013; Sanon et al. 2013), which are widespread and grow preferentially in mixed patches (Sanon et al. 2013). In riverside forests and rainforests, the most common ECM- trees include *Anthonotha fragrans*, *A. macrophylla*, *Afzelia bella*, *Berlinia grandiflora, Cryptosepalum tetraphyllum*, *Gilbertiodendron limba* and, *Uapaca guineensis*, *U. heudelotii*, *U. esculenta*, and *U. chevalieri*. On the contrary, *Afzelia africana*, *Albizia chevalieri*, *Burkea africana*, *Detarium microcarpum, Isoberlinia doka*, *I. tomentosa*, *Monotes kerstingii, Pterocarpus erinaceus*, *Uapaca togoensis* and *U. somon* occur preferentially in open forests, woodlands, and savannas (White 1983).

Unfortunately, these forest ecosystems are under alarming destruction, a constantly growing threat as reported in FAO (2010), characterized by forest degradation and deforestation by clear cutting of forest trees including the aforementioned symbiotic tree species. With no doubt, threats on forest ECM trees, the increasing rate of destruction and disturbance of natural ecosystems are precipitating catastrophic extinction of species as supposed by Bickford et al. (2007), and thereby disturb the ecological balance of forest ecosystems at different levels. In Guineo-Sudanian forest ecosystems, the genera *Scleroderma*, *Tomentella*, *Russula, Lactarius* and *Lactifluus* are among the most studied ECM fungi (van Rooij et al. 2003; Yorou et al. 2011, 2012; Verbeken & Walleyn 2010; Sanon et al. 2013; Maba et al. 2013- 2015; Sanon et al. 2014). Recent progress in molecular DNA phylogenetic analyses and morpho-anatomical investigations within lactarioid taxa (Buyck et al. 2008; Verbeken & Walleyn 2010; van de Putte et al. 2010; Verbeken et al. 2011; De Crop et al. 2013; Maba et al. 2013, 2014, 2015) highlighted the high genetic diversity of the genus *Lactifluus*, including numerous cryptic species. Such high diversity raises numerous questions related either to host tree specificity and/or preference/selectivity (Zhou and Hide 2001), or endemism patterns specific to the Sudanian domain.

Although it is the phylogenetic largest clade within milkcaps (Buyck et al. 2008; Verbeken et al. 2011; Verbeken & Nuytinck 2013), the genus *Lactarius* shows low genetic variability and has a predominantly temperate distribution, whereas *Lactifluus* has its major distribution in the tropics with numerous endemic species in tropical Africa (Verbeken et al. 2011; Verbeken & Nuytinck 2013). With more than 500 species of milkcaps known worldwide, for about 650 expected (Verbeken & Nuytinck 2013), *Lactifluus* currently encompasses about 74 tropical African species, of which about 38 are from the Guineo-Sudanian domain, including the five new species hereby described. However, Verbeken et al. (2011) and Maba et al. (2014, 2015) suggested to intensify studies on *Lactifluus* in order to better elucidate the edibility, conservation status, host specificity/preference and phenology/chorology of West African species. Indeed, previous studies (Maba et al. 2013-2015) that combined traditional morpho-anatomical and DNA-based techniques allow detecting and documenting numerous species, both taxonomically and ecologically. In addition, as has already been mentioned by Bickford et al. (2007), cryptic species identification and description have important implications for conservation and natural resource protection and management, and the biodiversity estimate as well.

In order to better circumscribe species limits and the ecological plasticity of *Lactifluus* species, mycological prospections have been undertaken in West African forest ecosystems including inter alia the northern Guinean seasonal, dry, dense, riparian and open forests, woodlands and savannas in various countries (Benin, Togo, Burkina Faso, Mali, and Guinea) from 2007 to 2013. From numerous specimens we sampled, 5 new *Lactifluus* species are described hereby as new to science. Chorological patters of recorded *Lactifluus* species and their putative partner forest trees are discussed.

Material and Methods

Specimens were sampled between 2007 and 2013 in various West African forest ecosystems, including inter alia the northern Guinean seasonal, dry, dense, riparian and open forests, woodlands and savannas following a Megatransect 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 specimen 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). Holotypes of the new species are conserved in TOGO herbarium and isotypes in GENT and M (Thiers 2012).

Light and Scanning Electron Microscopy

Microscopic studies were performed focusing on the lactarioid anatomical diagnose features, as undertaken by Verbeken & Walleyn (2010), and Maba et al. (2013-2015). Measurements are given referring to Buyck (1991), as 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 & Walleyn (2010) about tropical African *Lactarius* s. l.

DNA Extraction, sequencing and PCR amplification

Genomic DNA was extracted from dried sampled specimens following the protocol used by Maba et al. (2013). The Internal Transcribed Spacer regions (ITS) of the nuclear ribosomal DNA including ITS1, ITS2 and 5.8S regions were amplified using the fungi specific primer

ITS1F in combination with the basidiomycetes specific primer ITS4B (Gardes & Bruns 1993). A total of 51 ITS sequences were obtained (Tab. 1, Fig. 1) and the sequences of the newly described species have been deposited at European Nucleotide Archive/ENA (Table 1 below).

Sequence editing, analyses and molecular phylogenetic inference

We first checked and downloaded the fully identified sequences (up to species level) in addition to unidentified (up to genus level), but closely related to our generated sequence by Blastn search (Altschul et al. 1997). In order to obtain relevant sequences to use in our multiple alignment, and to optimize the alignment, a Blastn earch was performed following Maba et al. (2013). Thus, generated dataset already compiled by Maba et al. (2015), in which preference was given to tropical African sequences available in public GenBanks, was considered. However, worldwide sequences with 85% minimum of similarity, and about 90% minimum of query cover, to each of our new generated sequences, were considered close and useful for a better phylogenetic support, and taxonomic resoluteness of the newly recorded species.

The new generated dataset sequences were edited and assembled using the program BioEdit v7.2.3, (Hall 2005, update 12. Nov. 2013). Our final dataset was composed of 111 in-group taxa (species and genus level) and 2 out-group taxa. We consider as in-group, the genera *Lactifluus* (81 samples), *Lactarius* (17 samples), *Multifurca* (5 samples) and *Russula* (8 samples), which represent *Russulaceae,* and the out-group include one *Gloeocystidiellum* sequence and one *Hericium* sequence (Fig. 1).

The Full Multiple alignment was performed automatically using the online version (last update 03/2014) of the program MAFFT v7.130b (Katoh and Toh 2008), by applying the best accurate option for the alignment. After the online multiple alignment, the dataset was slightly corrected manually by removing the ambiguously aligned regions as well as the mismatched and empty common columns. Our final sequence dataset was composed of 113 rDNA ITS sequences (including our generated ones and those obtained from GenBanks) for a total length of 658 bp. The final alignment dataset matrix is submitted to TreeBASE (S17549), but can be also provided by the first author.

The evolutionary history (Relationship of taxa, Fig. 1) was inferred using the Neighbor- Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 2.10956206 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 4). The analysis involved 113 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 658 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

Results

Lactifluus versus *Lactarius* in tropical Africa

The genus *Lactifluus* is mainly well distributed in tropics but has also representatives in North Temperate Zone, Australia and New Zeland (Verbeken et al. 2011, 2012; Verbeken & Nuytinck 2013). Although synapomorphic characters for the genus *Lactifluus* and its sister genus *Lactarius* still not highlighted (Verbeken & Nuytinck 2013), species of both closely related genera, in tropical Africa, can be distinguish by the major morpho-anatomical features presented in the table 2 (above), as have highlighted previous and current studies (van Rooij et al 2003; Verbeken & Walleyn 2010; Verbeken & Nuytinck 2013; Maba et al. 2013-2015).

ITS sequence analyses

The Maximum Composite Likelihood tree obtained from the phylogenetic analysis conducted from a total of 113 sequences is shown in Fig. 1. The *Russulaceae* sequences included in the analysis are monophyletic (100 %) supported, and are split into nine (9) subclades that are grouped I to IX. Sequences of *Hericium erinaceum* (EI784265) and *Gloeocystidiellum* sp. (KJ140715) deviate and form the out-group subclade. In this present phylogenetic analysis, representative sequences of the genus *Lactifluus* (clade Lf, the largest clade, representing the studied one) displays six (6) supported subclades that represent the traditional delimited *L.* subgenera- *Lactariopsis* (Henn.) Verbeken (clade I), *Edules* (Verbeken) Verbeken (clade III), *Russulopsis* (Verbeken) Verbeken (clade IV), *Lactifluus* (Pers.) Roussel (clade V, VI-A), *Gerardii* (A.H. Sm. & Hesler) Stubbe (clade VI-B), and *Piperati* Verbeken (clade VI-C). The subclade II encompasses sequences of unidentified specimens from Togo (MD154) and Benin (MD304, MD317, and MD326). Of genus *Lactifluus* clade, *L.* subgenera- *Gerardii* (clade VI-B), *Piperati* (clade VI-C), and a subclade formed by sequences of known species of *L.* subg. *Lactifluus* (*L. longipilus*, *L. crocatus*, and *L. volemus*) form a complex clade (VI), with 78% of bootstrap support. Clade VII represents the genus *Multifurca,* and is supported as monophylum (92 %). This clade is nested between *Lactifluus* clade (Lf) and the monophyletic clade *Lactarius* (VIII). This latter-mentioned clade (VIII) encompasses 17 sequences, and is monophyletic supported by 99% of bootstrap. At the same time, sequences (8, worldwide) of genus *Russula* included in the analysis deviate as external monophyletic clade (IX) by 97% bootstrap support.

The sequences of two newly described species (MD123 and MD131, C2349 and MD234) clustered within the *L.* subg. *Lactariopsis* clade, with 98% and 57% of bootstrap support respectively, with already known species. At the same time the sequences of the specimens C2157 and MD355 (two newly described species), fit in subg. *Edules*, with respectively 99% of bootstrap support with already knew species (Fig. 2). In *L.* subg. *Russulopsis* subclade, nested the sequence of one new described species (MD224), supported by 99% of bootstrap value with *L. 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 accommodated them into five new species, notably: *Lactifluus annulatolongisporus* (specimens MD123 and MD131) and *Lactifluus membranaceus* (C2349, DPM05 and MD234) within *L.* subg. *Lactariopsis*; *Lactifluus brunneocarpus* (MD219B and MD224) in *L.* subg. Russulopsis, and *Lactifluus burkinabei* (MD355) and *Lactfluus guellii* (C2157 and C2163), both within *L.* subg. *Edules*.

Taxonomy

1-***Lactifluus annulatolongisporus*** Maba, sp. nov.

(Figs. 3, 4, 5) Genbank ENA, **accession number HG426470**,
Mycobank **MB811601**

Diagnosis: Pileus 40-65 mm diam., plano-convex, depressed to umbilicate, slightly subinfundibuliform; partial velum remnants and forming an evanescent annulus; pellis dry, pruinose, whitish, orange white at the margin, pale orange in the center, appearing zonate-like. Lamellae are slightly crowded, broadly adnate to subdecurent. Context whitish to fleshy, firm; thick in the center of the pileus and thin near margin. Latex scarce, whitish and unchanging. Basidiospores 10-10.5-11 × 6.0-7.0-7.5 µm, strongly elongate, with very short amyloid warty ornamentation composed fine lines, thicker in their middle part, not clearly distinguishable under light microscope; amyloid spot in the plage distinctly present. Basidia 40-65 × 10-12 µm, four- spored. Pleurocystidia absent. Pleuropseudocystidia 6-30 µm diam. Marginal cells of lamellae 25- 60 × 4-11 µm, utriform, tortuous, dichotomously branched. Pileipellis a lamprotrichopalisade with suprapellis compose of very slender, thick-walled, septate, and sometimes forked or branched cells.

Pileus (Fig. 3A-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. 4C, 5A-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. 4C). **Basidia** (Fig. 4B) 40-65 × 10-12 µm, subcylindrical and four-spored. **Pleurocystidia** absent. **Pleuropseudocystidia** (Fig. 4D) 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. 4E) 25-60 × 4-11 µm, utriform, tortuous, dichotomously branched. **Pileipellis** (Fig. 4B) 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 studied

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, leg. Dao Maba, herb. MD123; TOGO (Holotype), Isotype GENT (AV11-147) - 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, leg. Dao Maba, herb. MD131 (TOGO), ENA acc. no. LK392606.

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

2- *Lactifluus brunneocarpus* Maba, sp. nov.

(Figs. 6,7, 8) GenBank ENA, **accession number LK392608,**
Mycobank MB811602

Diagnosis: Pileus 60-100 mm diam., very thin, plano-convex, depressed then infundibuliform;

pellis wet, strongly striate near margin; brownish orange to brownish yellow, darker in the center. Lamellae are adnate to subdecurrent, distinctly distant or spaced, rarely forked at the margin, very fragile and very brittle, regular. Context of pileus very thin, slightly thick in the center, very fragile and brittle, stipe firm; whitish to pale orange. Latex abundant, whitish, changing slightly to green. Basidiospores 7.0-8.5-9.5 × 6.0-6.5-7.0 µm, broadly ellipsoid with irregular, conical or rounded, and isolated amyloid warts ornamentation. Basidia 50-75 × 9-10 µm, four-spored. Pleurocystidia 45-80 × 7-10 µm; very abundant, irregularly shaped, subcylindrical, tortuous, much branched, and commonly diverticulate, thin-walled, septate, apex tapering. Pleuropseudocystidia 6-10 µm diam., not abundant, emergent, irregularly subcylindrical, apex tapering, sometimes inflated. Marginal cells 40-60 × 2-5 µm, subcylindrical to cylindrical, sometimes septate sometimes frocked at apex. Pileipellis 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, cylindrical to subcylindrical.

Pileus (Fig. 6A-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. 7E, 8A-B) broadly ellipsoid, 7.0-8.5-9.5 × 6.0-6.5-7.0 µm (Q=1.17-1.2- 1.25-1.3-1.35; n=75), ornamentation amyloid, composed of well developed irregular, conical or rounded, and isolated warts; amyloid spot in the plage absent. **Basidia** (Fig. 7C) 50-75 ×9-10 µm; 4-spored; subcylindrical to subclavate, tapering downwards; sterigmata 3-5 × 1.5-2.5 µm, well developed. **Pleurocystidia** (Fig. 7D) very abundant, 45-80 × 7-10 µm; irregularly shaped, subcylindrical, tortuous, much branched and commonly diverticulate, thin-walled, septate, apex tapering. **Pleuropseudocystidia** (Fig. 7B) not abundant, 6-10 µm diam.; emergent, irregularly 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. 7F) 40-60 × 2-5 µm, subcylindrical to cylindrical, sometimes septate sometimes frocked at apex. **Pileipellis** (Fig. 7A) 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 3-5 µm diam., cylindrical to subcylindrical. **Stipitipellis** identical to pileipellis. **Clamps** absent.

Material studied

Guinea, Malouwaita, N10°32'7.7" W09°22'8.6", on soil in rainforest dominated by *Uapaca heudelotii*, 18 July 2011 leg. Dao Maba, herb. 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, leg. Dao Maba, herb. MD219B; (TOGO).

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

3- *Lactifluus burkinabei* Maba, sp. nov.

(Figs. 9, 10, 11) Genbank ENA, accession **number LK392609**,

Mycobank MB811603

Diagnosis: Pileus 60-105 mm diam., firm, fleshy and thick, plano-convex and depressed, to subinfundibuliform; pellis wet, smooth, sticky; orange to deep orange. Lamellae are broadly, decurrent, irregular, unequal, very commonly forked, strongly anastomosing at the insertion of the stipe, widely spaced; taste bitter and spicy. Context fleshy and firm, pale yellow to butter yellow, very thick in the center, and slightly thinner at the margin, stipe fleshy and firm; changing to brown with FeSO4 . Latex slightly abundant, whitish, unchanging. Basidiospores 8.0-9.0-10 × 7.0-7.5-8.0 µm subglobose to ellipsoid, with amyloid warts ornamentation almost interconnected forming reticulum, and seldom isolated. Basidia 55-75 × 10-12 µm, two- and four-spored, subcylindrical to subclavate. Pleuroleptocystidia 60-75 × 10-13 µm, very abundant, emergent, subcylindrical to subclavate, thin-walled, apex tapering upwards, mucronate to rostrate. Pleuropseudocystidia 8-25 µm diam., subcylindrical, mostly inflated, apex capitate to mucronate. Marginal cells 15-35 × 5-6 µm, cylindrical to subcylindrical, sometimes fusiform, septate; apex distinctly mucronate or flared. Pileipellis an ixotrichopalisade to trichopalisade, with suprapellis cells subcylindrical, fusiform, clavate to subglobose, irregularly branched, septate. Dermatocystidia abundant, apex mucronate to subcapitate, contents needle-like. Stipitipellis a trichopalisade to lamprotrichopalisade.

Pileus (Fig. 9A-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= 8-9/cm), very commonly forked, strongly anastomosing at the insertion of the stipe, widely spaced, light yellow to orange yellow (4A6-8). Stipe 15-40 × 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 FeSO4 on the context.

Basidiospores (Fig. 10G, Fig. 11B-C) subglobose to ellipsoid, 8.0-9.0-10 × 7.0-7.5-8.0 µm (Q=1.06-1.15-1.25; n=75); ornamentation amyloid, composed of irregularly shaped warts, almost interconnected forming reticulum, and seldom isolated; plage with a strong amyloid spot. **Basidia** (Fig. 10C) 55-75 × 10-12 (13) µm; variable, two- and four-spored; subcylindrical to subclavate; sterigmata 6-12 ×2-3 µm, sometimes with irregular shape, apex sometimes bulging or appearing swollen. **Pleuroleptocystidia** (Fig. 10E) very abundant, 60-75 × 10-13 µm; subcylindrical to subclavate, thin-walled, apex often tapering upwards, almost mucronate to rostrate, emergent. **Pleuropseudocystidia** (Fig. 10B) very abundant, 8-25 µm diam.; irregularly subcylindrical, mostly inflated, apex capitate to mucronate; very emergent and projecting up to 40 µm 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. 10D) 15-35 × 5-6(8) µm, very variable in shape, cylindrical to subcylindrical, sometimes fusiform, sometimes septate; apex distinctly mucronate or flared. **Pileipellis** (Fig. 10A) an ixotrichopalisade to trichopalisade, with abundant clavate to subglobose cells (up to 50 µm diam.), suprapellis elements subcylindrical sometimes fusiform, irregularly branched, septate. **Dermatocystidia** (Fig. 10F) abundant, 40-65 × 4-7 µm, 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 studied

Burkina Faso, Bobodiolasso, Orodara, DAN, N10°53'6.9" E04°50'27.9", on soil in gallery forest dominated by *Berlinia grandifolia* and *Uapaca guineensis,* 12 July 2013, leg. Dao Maba, herb. MD355; TOGO (Holotype), Isotype Munich (M).

Etymology: Referring to the country of the sampled specimen.

4- *Lactifluus guellii* Maba, sp. nov.
(Figs. 12, 13, 14) Genbank ENA, **accession number HG426466**,
Mycobank MB811604

Diagnosis: 35-60 µm diam., plano-convex, depressed to subinfundibuliform; margin first incurved, then straight and slightly crenulate; pellis dehiscent, sticky, faintly striate when young, strongly near margin when older; orange white, pale orange to light orange darker in center. Lamellae adnate to subdecurrent, spaced, unequal, irregular, sometimes bifurcate or forked at the margin. Context thin near margin, fleshy and firm in the center of the pileus and the stipe; whitish. Latex scarce, whitish and unchanging. Basidiospores 7.5-9.5-10.5 × 6.5-7.5-8.0 µm, broadly ellipsoid to elongate, well- developed amyloid blunt warts ornamentation connected by fine lines. Basidia 45-70 × 10-12 µm, four-spored. Pleurocystidia 45-75 × 5-8 µm, abundant, subcylindrical, mostly tortuous to fusiform, thin-walled. Pleuropseudocystidia very abundant, 4-15 µm diam. Marginal cells of lamellae 10-35 × 4-8 µm, subcylindrical, clavate to fusiform, thin-walled, bifurcate and septate. Pileipellis a lamprotrichoderm-like with abundant swollen hyphae; terminal hyphae sometimes thick-walled. Stipitipellis mixed ixocutis-like.

Pileus 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/cm), sometimes bifurcate or forked at the margin (up to 1⁄3 from margin), orange white. **Stipe** 25-30 × 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. 13B, 14A-D) broadly ellipsoid to elongate, 7.5-9.5-10.5(11.5) × 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 (>0.5µm high) connected by fine lines; plage with amyloid spot (Fig. 13, 14). **Basidia** (Fig. 13E) 45-70 × 10-12 µm, subcylindrical, four-spored. **Pleurocystidia** (Fig. 13F) rather abundant, 45-75 × 5-8 µm, irregularly shaped, subcylindrical, mostly tortuous to fusiform, thin-walled. **Pleuropseudocystidia** (Fig. 13D) 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. 13C) 10-35 × 4-8 µm, broadly clavate, subcylindrical to fusiform, thin-walled, bifurcate and septate. **Pileipellis** (Fig. 13A) 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 studied

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. Atsu Guelly, det. Dao Maba, herb. 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. Atsu Guelly, det. Dao Maba, herb. C2163; (TOGO).

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

5- *Lactifluus membranaceus* Maba, sp. nov.
(Figs. 15, 16, 17) Genbank ENA, **accession number** HG426478,
Mycobank **MB811605**

Diagnosis: Pileus 50-65 mm diam., convex then plano-convex to depressed, universal velum membranous; pellis dry, velvety, uniform, smooth; beige, orange white to yellow orange; remnants of secondary velum forming, thin, and an evanescent annulus. Lamellae are very thin, broadly, decurrent, irregular, unequal, very commonly forked, slightly dense. Context of pileus pale orange, to orange white, thick in the center, slightly thinner near margin, solid in the stipe. Latex scarce, whitish and unchanging. Basidiospores 7.0-7.5-8.0 × 6.0-7.0-7.5 µm, globose to subglobose, sometimes ellipsoid, with weak amyloid ornamentation composed of very short, fine lines-like, not well distinctive under light microscope; very low developed warts slightly connected under SEM. Basidia 35-57 × 8-10 µm, four-spored. Pleurocystidia absent. Pleuropseudocystidia 6-15 µm diam., subcylindrical, slightly inflated, apex tapering, mucronate, slightly inflated, very rarely tortuous. Marginal cells of lamellae 20-35 × 5-6 µm, cylindrical to subcylindrical, slightly fusiform, septate. Pileipellis and stipitipellis a lamprotrichopalisade, suprapellis composed of slender cells, irregularly branched to diverticulate, very tortuous to fusiform, tapering upwards, septate.

Pileus (Fig. 15A-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+l = 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. 16E, 17A-D) globose to subglobose, sometimes ellipsoid, 7.0-7.5-8.0(8.5) × (5.5)6.0-7.0-7.5 µm, (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. 16D) 35-57 × 8-10 µm, four-spored, subcylindrical to subclavate; sterigmata 7-8 × 1.5-2 µm. **Pleurocystidia** absent. **Pleuropseudocystidia** (Fig. 16C) 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. 16A) 20-35 × 5-6 µm, distinctly cylindrical to subcylindrical, slightly fusiform, septate. **Pileipellis** (Fig. 16B) 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.

Material studied

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, leg. & det. Dao Maba, herb. C2349; TOGO (Holotype) - Guinea, Malouwaita, N10°32'5.7" W9°22'8.6", on soil in rainforest dominated by *Uapaca heudelotii*, 18 July 2011, leg. Dao Maba, herb. 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, leg. Dao Maba, herb. DPM05 (TOGO).

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

Discussion

Species *Lactifluus annulatolongisporus* and *L. membranaceus* described here fit the traditionally delimited subg. *Lactariopsis* (Fig. 1 and 2) that encompasses all tropical African annulate lactarioids taxa, but also those without annulate. Both species present the following morpho-anatomical characters that support their phylogenetic placement within *L.* 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 scare and unchanging, lack of pleurocystidia, and presence of a lamprotrichopalisade as pileipellis and stipitipellis (Verbeken & Walleyn 2010). *Lactifluus annulatolongisporus* is in some respect close to *Lactifluus zenkeri* due to its marginal cells that are branched, dichotomously bifurcate and tortuous (Fig. 4E); and to *L. heimii* (Verbeken) Verbeken, which has ellipsoid to elongate basidiospores up to 11.6 µm long, and 1.64 for ratio (Verbeken & Walleyn 2010). *L. annulatolongisporus* differs considerably from all hitherto examined members of *L.* subg. *Lactariopsis* possessing an annulus (Verbeken & Walleyn 2010), by the presence of amyloid spot in the plage of its basidiospores; unlike *L. heimii* and *L. zenkeri*. The specimens examined (MD123 and MD131), present strongly bulbous to clavate pleuropseudocystidia, distinctly bulged in their middle, tapering up- and downward, sometimes branched or bifurcate, and are therefore different from those of *L. zenkeri* and *L. heimii.* Although morphologically different to *L. zenkeri,* also by the basidiospores (size, ornementation and presence of amyloid spot), *Lactifluus annulatolongisporus* and *L. zenkeri* have branched, dichotomously bifurcate and tortuous marginal cells, unlike *L. heimii*, which has the same elements that are shortly cylindrical to clavate (Verbeken & Walleyn 2010). In addition, species including *Lactifluus annulatoangustifolius* (Verbeken) Verbeken, *L. heimii*, *L. velutissimus* (Verbeken) Verbeken, *L. zenkeri* and the newly described *L. annulatolongisporus,* have lamprotrichopalisade to lampropalissade as peleipellis structure, which differs only by the sizes of terminal elements (Verbeken & Walleyn 2010). Thus, the combination of morpho-anatomical characters of *L. annulatolongisporus* distinguishes it from the other annulate species.

Lactifluus zenkeri is the one, in some respect morpho-anatomically close to the newly described *Lactifluus membranaceus* (specimens C2349, MD234 and DPM05). Both species have a velvety, indehiscent, and dry pellis; remnants of secondary velum, 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 & Walleyn 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 *L. annulatolongisporus*, scarce pleuropseudocystidia, basidiospores mostly ellipsoid, amyloid spot mostly absent in plage (Verbeken & Walleyn 2010). On the contrary, *L. membranaceus* has distinctly cylindrical to subcylindrical, septate marginal cells (Fig. 16A), rarely fusiform, very abundant pleuropseudocystidia (Fig. 16C), and basidiospores (Fig. 16E, 17A-D) mostly globose to subglobose, with distinctly amyloid spot present in plage.

Both, *L. annulatolongisporus* and *L. membranaceus* fit *L.* subg. *Lactariopsis,* and their sequences are well supported in this subclade.

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, basidiospores ornamentation composed of irregular, rounded, and obtuse warts isolated, and fits therefore *L.* subg. *Russulopsis* that encompasses some known species including *L. ruvubuensis* (Verbeken) Verbeken and *L. longipes* (Verbeken) Verbeken (Verbeken & Walleyn 2010), with closely related characters. Morphologically *L. 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, *L. 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 & Walleyn 2010), while *L. ruvubuensis* has a thick basidiome with dehiscent, tomentose and finely fibrose towards the margin pellis, and unequal, strongly decurrent lamellae, according to Verbeken & Walleyn (2010). Moreover, unlike *L. longipes,* which has long stipe (up to 80 mm high), *L. brunneocarpus* has a stipes with measurements comprised between 35 and 50 mm high (for 11 different stipes sized). Microscopically, *L. brunneocarpus* differs from the two above mentioned closets species by its strongly diverticulate, branched and tortuous pleurocystidia (Fig. 7D) that are absent in *L. longipes*. But, in addition, pleuropseudocystidia are much branched and tortuous in both, *L. ruvubuensis* and *L. longipes* (Verbeken & Walleyn 2010), while they are subcylindrical and slightly tapering upwards in *L. brunneocarpus* (Fig. 7B). The marginal cells of *L. brunneocarpus* (Fig. 7F) are subcylindrical and septate, and the basidiospores (Fig. 7E) present well-developed irregular, rounded to conical, and isolated amyloid warts as ornamentation unlike *L. ruvubuensis*. *L. brunneocarpus* even morpho-microscopically different from *L. ruvubuensis* and *L. longipes* as mentioned above, fits *L.* subg. *Russulopsis* and its sequence forms a subclade with two sequences of *L longipes*, supported by 99%.

The sequences of the newly described species *L. burkinabei* (specimen MD355), and *L. guellii* (specimen C2157), cluster within *L.* subg. *Edules*. Morphologically, *L. 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 & Walleyn 2010). Microscopically, *L. burkinabei* has subglobose to ellipsoid basidiospores (Fig. 10G, 11A-C), with irregularly shaped amyloid warts, interconnected and forming a reticulum, seldom isolated. It has additionally, two type of basidia (Fig. 10C), two-spored (about 1⁄4 to 1⁄3 of the basidia) as those observed for *L. inversus* (Verbeken & Walleyn 2010), and four-spored ones that both, are subcylindrical to subclavate; its pleuroleptocystidia (Fig. 9E) present almost mucronate to rostrate, often upwards tapering apices, closely related to those observed for *Lactifluus indusiatus* Verbeken (Verbeken & Walleyn 2010) of subg. *Lactariopsis* sect. *Chamaeleontini* Verbeken. *L. burkinabei* presents an ixotrichopalisade to trichopalisade (Fig. 10A) pileipellis, composed of very abundant clavate to subglobose cells, in mixture with mucronate to subcapitate dermatocystidia (Fig. 10F); this pileipellis feature has never been observed for any known African lactarioids (van Rooij et al. 2003; Verbeken & Walleyn 2010).

Conversely, *L. 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. 13B, 14A-D), with strong, well-developed blunt amyloid warty ornamentation (>0.5µm high), finely interconnected at the base, closely related to those observed in *Lactifluus melleus* Maba (Maba et al. 2015). The pleurocystidia of *L. guellii* (Fig. 13F) are irregularly shaped, mostly tortuous to fusiform in contrast to the pleuroleptocystidia of *L. burkinabei* (Fig. 10E) that are almost mucronate to rostate with often upwards tapering apices. These features are unlike that of *L. melleus*. Pleuropseudocystidia of *L. guellii* (Fig. 13D) are emergent, fusiform, tortuous and mucronate, closely related to those of *L. 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 plage (Verbeken & Walleyn 2010). *L. 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 plage. In the phylogeny analyses, *L. guellii* is supported (97%) as sister species with one unidentified from Cameroon (UDB014027), with that it forms a terminal clade; together with *L. melleus* a clade with 89 % of bootstrap support. At the same time the sequence of *L. burkinabei* is well supported by 98 % as a subclade of subgenus *Edules*, the subgenus itself is supported by 74% of bootstrap support value.

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

Species of the genus *Lactifluus* are widespread in Guineo-Sudanian ecosystems and occur preferentially earliest between end of May to July and latest between end of August and September (Verbeken and Buyck 2001 van Rooij et al. 2003; Verbeken & Walleyn 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 *L. annulatoangustifolius, L. edulis, L. foetens, L. gymnocarpus, L. luteopus, L. nonpiscis* and *L. sudanicus* have shown no preference regarding vegetation type, as they had been collected in both woodlands and riverside/rain forests (supplement). *Lactifluus brunneocarpus, L. chamaeleontinus, L. densifolius, L. guellii, L. rubiginosus, L. longipes,* and *L. flavellus* were collected only in riverside forests. Whereas, *L. annulatolongisporus, L. emergens, L. gymnocarpoides, L. medusae, L. membranaceus, and L. melleus* were collected only in woodlands (supplement), *L. flammans* is collected mostly in woodlands (five times), but also in riverside forest (twice). *Lactifluus burkinabei, L. fazaoensis, L. heimii, L. inversus, L. 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, and will also highlight whether any species are endemic in the Sudanian domain.

In contrast, some species including *L. medusae*, *L. densifolius*, *L. edules*, *L. heimii*, *L. velutissimus* are not restricted to Zambezian 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-Zambezian domain, in woodland or in riverside forests, of numerous *Lactifluus* species including *L. annulatoangustifolius, L. aurantiifolius*, *L. chamaeleontinus, L. carmineus*, *L. densifolius, L. edulis, L. emergens, L. flammans, L. gymnocarpus, L.*

gymnocarpoides, L. heimii, L. inversus, L. luteopus, L. medusae L. nonpiscis, L. longipes, L. longisporus, L. pelliculatus, *L.* pumilus, *L. rubiginosus, L. ruvubuensis*, *L. sesemotani, L. velutissimus, L. volemoides, L. zenkeri,* confirm that in both, Guineo- Sudanian and Congo-Zambezian domain, occur several common species 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* from tropical Africa. The study in some respect confirms those of Van de Putte et al. (2012), and De Corp et al. (2013), which have suggested that the genus *Lactifluus* might contain cryptic and/or semi-cryptic species, based respectively on investigations undertaken within *L.* subg. *Lactifluus* section-*Lactifluus,* and *Piperati*. Evidently, a combination of anatomical and molecular analyses is the best way for interspecific discrimination, as well as species richness assessment. In addition, West African forests ecosystems remain very poorly investigated. Thus, continuous specimen sampling/collecting as well as accelerated rDNA sequencing and anatomical characterization and sequences analyses of ectomycorrhizae, will also contribute to a better understanding of ecological process within this genus.

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Figure legends

Figure 1. Best Maximum Composite Likelihood (MCL) phylogeny tree showing the placement of the sequences of the five newly described species (*L. annulatolongisporus*, *L.*

brunneocarpus, *L. burkinabei* and *L. guellii* and *L. membranaceus*) within our generated sequences and those obtained from public GenBanks. Bootstrap values higher than 50% are shown next to the branches. GenBanks (ENA, UNITE and NCBI) sequences accession numbers and taxa names are mentioned in front of the branches.

Figure 2. *Lactifluus* (subgenera *Lactariopsis*, *Edules* and *Russulopsis*) subtree clade separated from the original tree. The five newly described species: *L. annulatolongisporus*, *L. brunneocarpus*, *L. burkinabei* and *L. guellii* and *L. membranaceus* are shown.

Figure 3. Basidiome of *Lactifluus annulatolongisporus* (MD123). A. Detailed view. B. Lamellae and stipe detailed. C. Pileus view, pellis detailed. – Scale Bars = 10 mm.

Figure 4. 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 µm. Section line drawing

Figure 5. SEM of *Lactifluus annulatolongisporus* (MD123). Basidiospores: A-B. Dorsal view. C. Lateral and detailed view showing the plage.

Figure 6. 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.

Figure 7. Light microscopy of *Lactifluus brunneocarpus* (MD224). A. Pileipellis. B. Pleuropseudocystidia. C. Basidia. D. Pleurocystidia. E. Basidiospores. F. Marginal cells. – Scale Bars = 10 µm. Section line drawing

Figure 8. SEM of *Lactifluus brunneocarpus* (MD224). Basidiospores: A. Lateral front view. B. Proximal profile view showing the plage.

Figure 9. Basidiome of *Lactifluus burkinabei* (MD355). A. Pileus detailed. B. View of lamellae and stipe, detailed. C. View of context change with FeSO4 reagent. – Scale Bars = 10 mm.

Figure 10. 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 µm. Section line drawing

Figure 11. SEM of *Lactifluus burkinabei* (MD355). Basidiospores: A. Overview and detailed view showing the plage with amyloid spot. B. Lateral/dorsal view. C. Dorsal view.

Figure 12. 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.

Figure 13. Light microscopy of *Lactifluus guellii* (C2157). A. Pileipellis B. Basidiospores. C. Marginal cells. D. Pleuropseudocystidia. E. Basidia. F. Pleurocystidia. – Scale Bars = 10 mm. Section line drawing

Figure 14. SEM of *Lactifluus guellii* (C2157). Basidiospores: A. Proximal view (left), lateral view (right). B. Dorsal view. C. Proximal view.

Figure 15. 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.

Figure 16. Light microscopy of *Lactifluus membranaceus* (C2349). A. Marginal cells. B. Pileipellis. C. Pleuropseudocystidia. D. Hymenium. E. Basidiospores. – Scale Bars = 10 µm. Section line drawing

Figure 17. SEM of *Lactifluus membranaceus* (C2349). Basidiospores: A. Overview B-C-D Front.

Fig. 1.

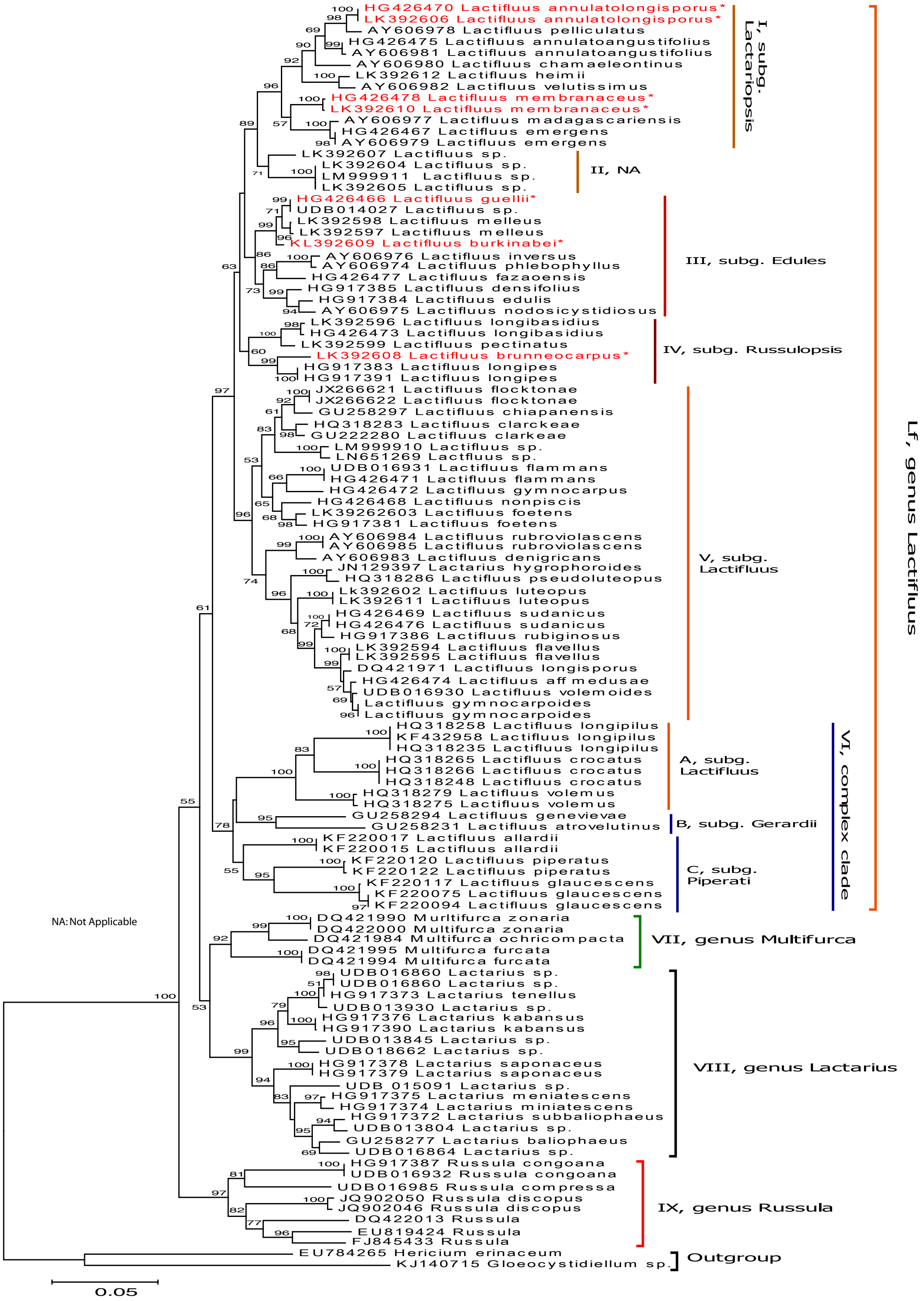
HG426470 Lactifluus annulatolongisporus*
LK392606 Lactifluus annulatolongisporus*
AY606978 Lactifluus pelliculatus
HG426475 Lactifluus annulatoangustifolius
AY606981 Lactifluus annulatoangustifolius
AY606980 Lactifluus chamaeleontinus
LK392612 Lactifluus heimii
AY606982 Lactifluus velutissimus
HG426478 Lactifluus membranaceus*
LK392610 Lactifluus membranaceus*
AY606977 Lactifluus madagascariensis
HG426467 Lactifluus emergens
AY606979 Lactifluus emergens
LK392607 Lactifluus sp.
LK392604 Lactifluus sp.
LM999911 Lactifluus sp.
LK392605 Lactifluus sp.
HG426466 Lactifluus guellii*
UDB014027 Lactifluus sp.
LK392598 Lactifluus melleus
LK392597 Lactifluus melleus
KL392609 Lactifluus burkinabei*
AY606976 Lactifluus inversus
AY606974 Lactifluus phlebophyllus
HG426477 Lactifluus fazaoensis
HG917385 Lactifluus densifolius
HG917384 Lactifluus edulis
AY606975 Lactifluus nodosicystidiosus
LK392596 Lactifluus longibasidius
HG426473 Lactifluus longibasidius
LK392599 Lactifluus pectinatus
LK392608 Lactifluus brunneocarpus*
HG917383 Lactifluus longipes
HG917391 Lactifluus longipes
JX266621 Lactifluus flocktonae
JX266622 Lactifluus flocktonae
GU258297 Lactifluus chiapanensis
HQ318283 Lactifluus clarckeae
GU222280 Lactifluus clarkeae
LM999910 Lactifluus sp.
LN651269 Lactfluus sp.
UDB016931 Lactifluus flammans
HG426471 Lactifluus flammans
HG426472 Lactifluus gymnocarpus
HG426468 Lactifluus nonpiscis
LK39262603 Lactifluus foetens
HG917381 Lactifluus foetens
AY606984 Lactifluus rubroviolascens
AY606985 Lactifluus rubroviolascens
AY606983 Lactifluus denigricans
JN129397 Lactarius hygrophoroides
HQ318286 Lactifluus pseudoluteopus
LK392602 Lactifluus luteopus
LK392611 Lactifluus luteopus
HG426469 Lactifluus sudanicus
HG426476 Lactifluus sudanicus
HG917386 Lactifluus rubiginosus
LK392594 Lactifluus flavellus
LK392595 Lactifluus flavellus
DQ421971 Lactifluus longisporus
HG426474 Lactifluus aff medusae
UDB016930 Lactifluus volemoides
Lactifluus gymnocarpoides
Lactifluus gymnocarpoides
HQ318258 Lactifluus longipilus
KF432958 Lactifluus longipilus
HQ318235 Lactifluus longipilus
HQ318265 Lactifluus crocatus
HQ318266 Lactifluus crocatus
HQ318248 Lactifluus crocatus
HQ318279 Lactifluus volemus
HQ318275 Lactifluus volemus
GU258294 Lactifluus genevievae
GU258231 Lactifluus atrovelutinus
KF220017 Lactifluus allardii
KF220015 Lactifluus allardii
KF220120 Lactifluus piperatus
KF220122 Lactifluus piperatus
KF220117 Lactifluus glaucescens
KF220075 Lactifluus glaucescens
KF220094 Lactifluus glaucescens
DQ421990 Murltifurca zonaria
DQ422000 Multifurca zonaria
DQ421984 Multifurca ochricompacta
DQ421995 Multifurca furcata
DQ421994 Multifurca furcata
UDB016860 Lactarius sp.
UDB016860 Lactarius sp.
HG917373 Lactarius tenellus
UDB013930 Lactarius sp.
HG917376 Lactarius kabansus
HG917390 Lactarius kabansus
UDB013845 Lactarius sp.
UDB018662 Lactarius sp.
HG917378 Lactarius saponaceus
HG917379 Lactarius saponaceus
UDB 015091 Lactarius sp.
HG917375 Lactarius meniatescens
HG917374 Lactarius miniatescens
HG917372 Lactarius subbaliophaeus
UDB013804 Lactarius sp.
GU258277 Lactarius baliophaeus
UDB016864 Lactarius sp.
HG917387 Russula congoana
UDB016932 Russula congoana
UDB016985 Russula compressa
JQ902050 Russula discopus
JQ902046 Russula discopus
DQ422013 Russula
EU819424 Russula
FJ845433 Russula
EU784265 Hericium erinaceum
KJ140715 Gloeocystidiellum sp.
I, subg. Lactariopsis
II, NA
III, subg. Edules
IV, subg. Russulopsis
V, subg. Lactifluus
A, subg. Lactifluus
B, subg. Gerardii
C, subg. Piperati
VI, complex clade
Lf, genus Lactifluus
VII, genus Multifurca
VIII, genus Lactarius
IX, genus Russula
Outgroup
NA: Not Applicable
0.05

Fig. 2.

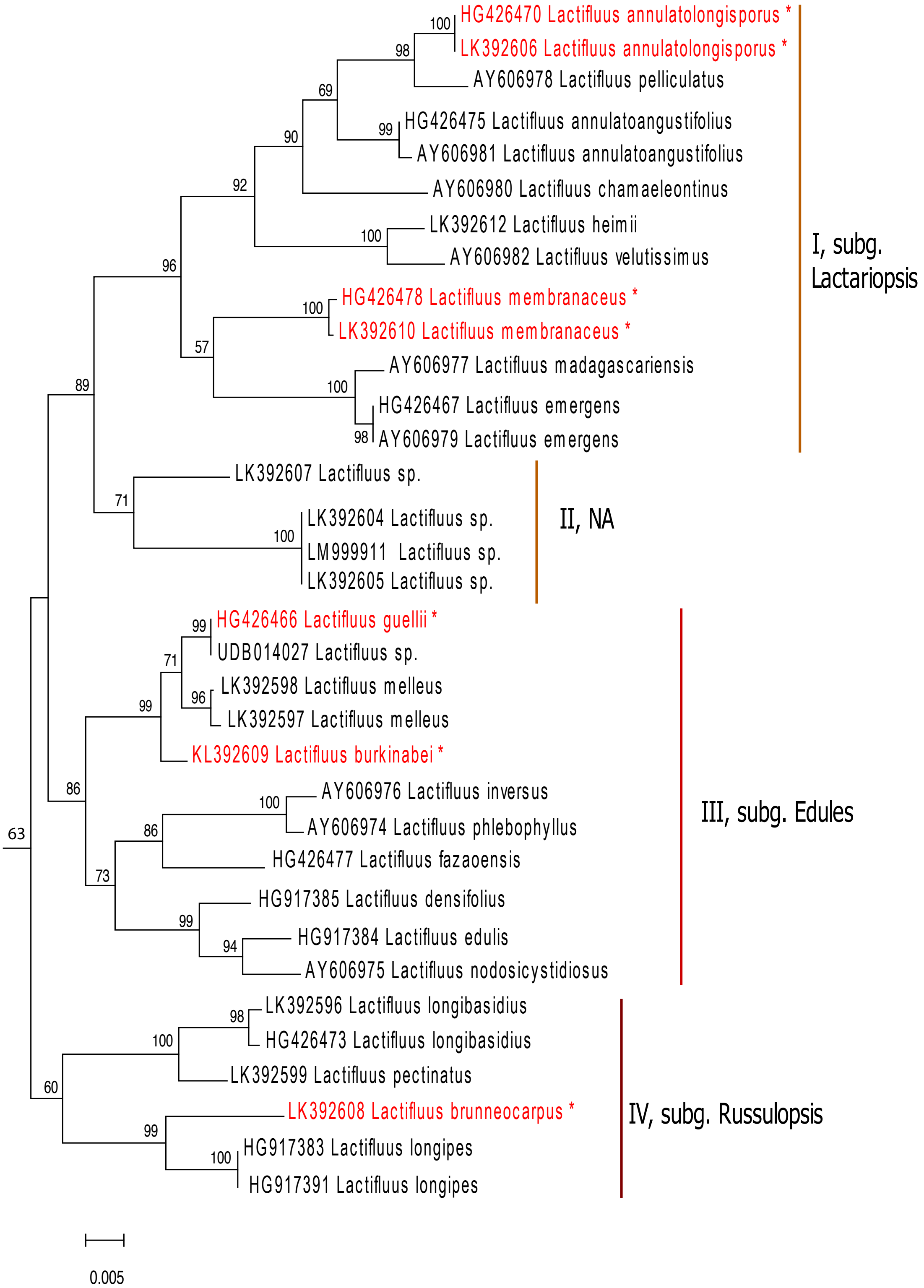
HG426470 Lactifluus annulatolongisporus *
LK392606 Lactifluus annulatolongisporus *
AY606978 Lactifluus pelliculatus
HG426475 Lactifluus annulatoangustifolius
AY606981 Lactifluus annulatoangustifolius
AY606980 Lactifluus chamaeleontinus
LK392612 Lactifluus heimii
AY606982 Lactifluus velutissimus
HG426478 Lactifluus membranaceus *
LK392610 Lactifluus membranaceus *
AY606977 Lactifluus madagascariensis
HG426467 Lactifluus emergens
AY606979 Lactifluus emergens
LK392607 Lactifluus sp.
LK392604 Lactifluus sp.
LM999911 Lactifluus sp.
LK392605 Lactifluus sp.
HG426466 Lactifluus guellii *
UDB014027 Lactifluus sp.
LK392598 Lactifluus melleus
LK392597 Lactifluus melleus
KL392609 Lactifluus burkinabei *
AY606976 Lactifluus inversus
AY606974 Lactifluus phlebophyllus
HG426477 Lactifluus fazaoensis
HG917385 Lactifluus densifolius
HG917384 Lactifluus edulis
AY606975 Lactifluus nodosicystidiosus
LK392596 Lactifluus longibasidius
HG426473 Lactifluus longibasidius
LK392599 Lactifluus pectinatus
LK392608 Lactifluus brunneocarpus *
HG917383 Lactifluus longipes
HG917391 Lactifluus longipes
100
98
69
99
90
92
100
96
100
57
100
98
89
71
100
99
71
96
99
86
100
86
73
99
94
63
98
100
60
99
100
I, subg. Lactariopsis
II, NA
III, subg. Edules
IV, subg. Russulopsis
0.005

Fig. 3. Basidiome *Lactifluus annulatolongisporus*

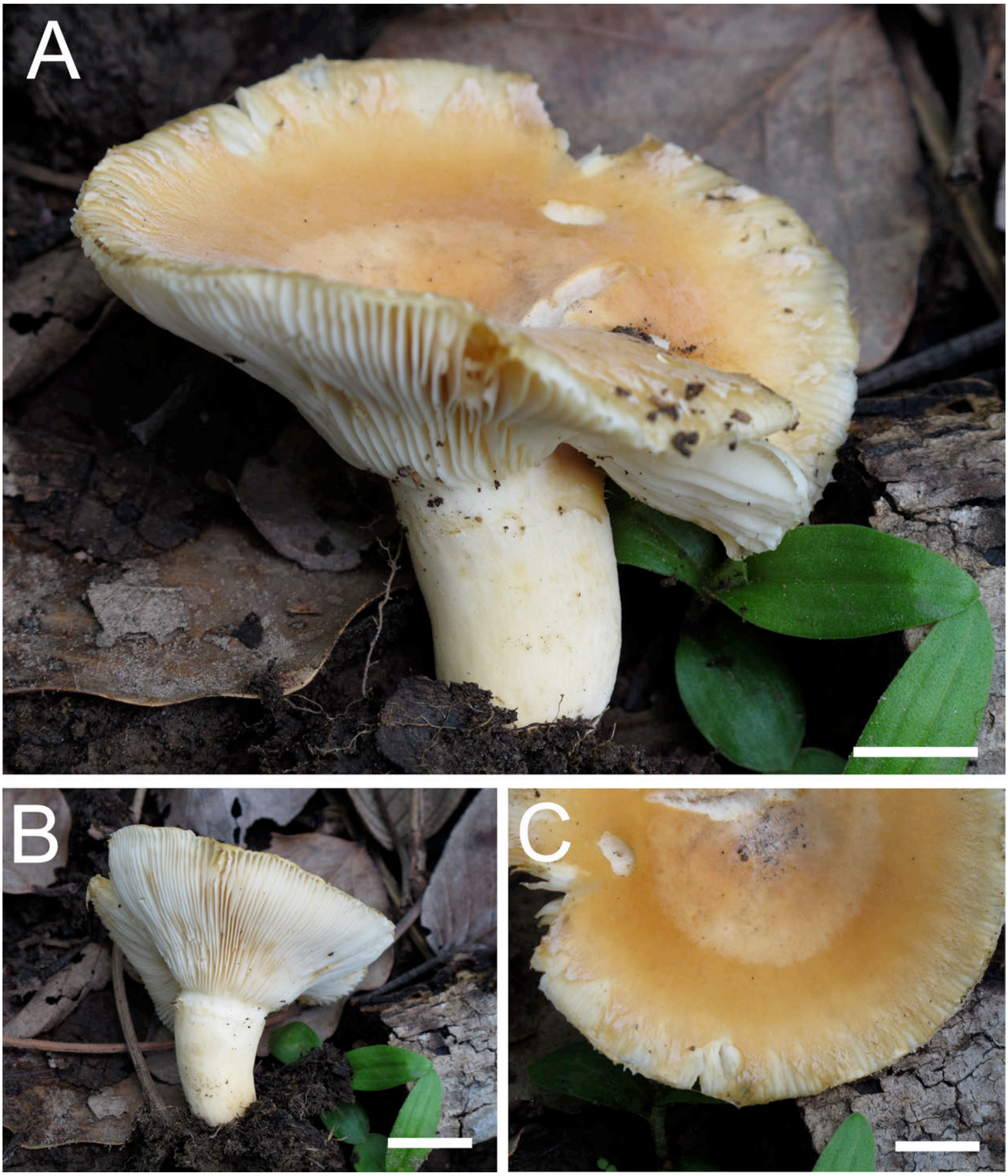

Fig. 4a. *Lactifluus annulatolongisporus*

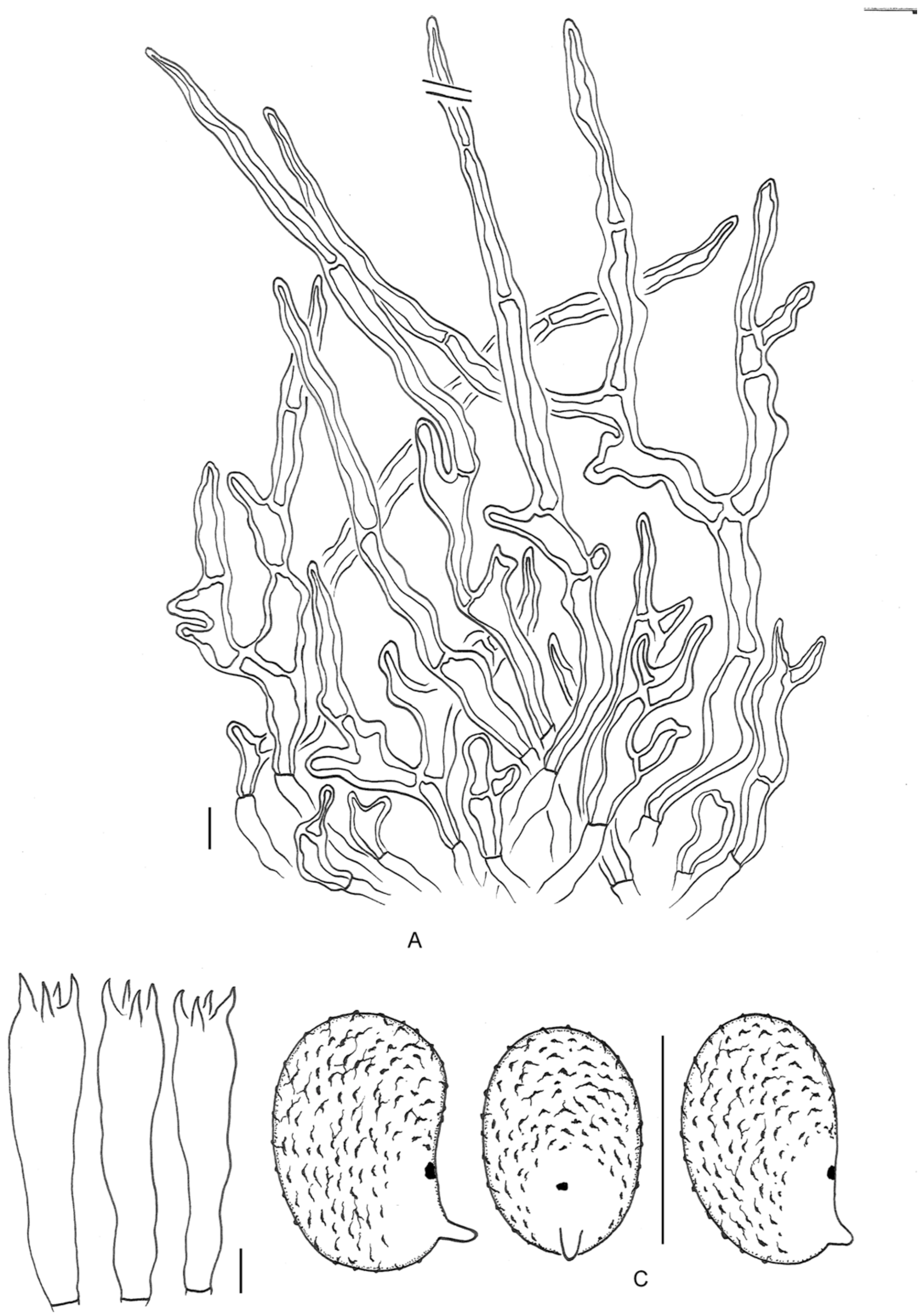

Fig. 4b. *Lactifluus annulatolongisporus*

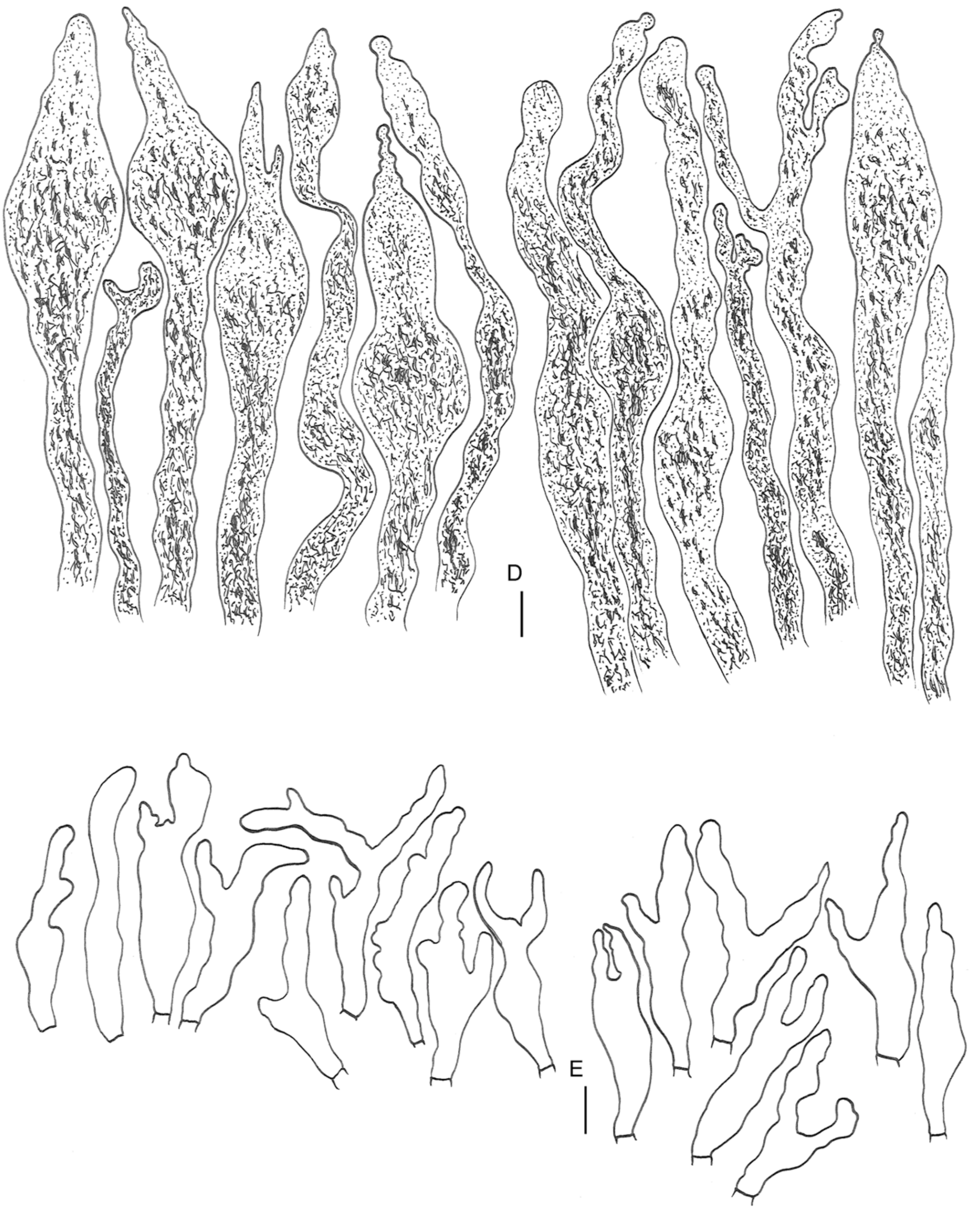

Fig. 5. SEM *Lactifluus annulatolongisporus*

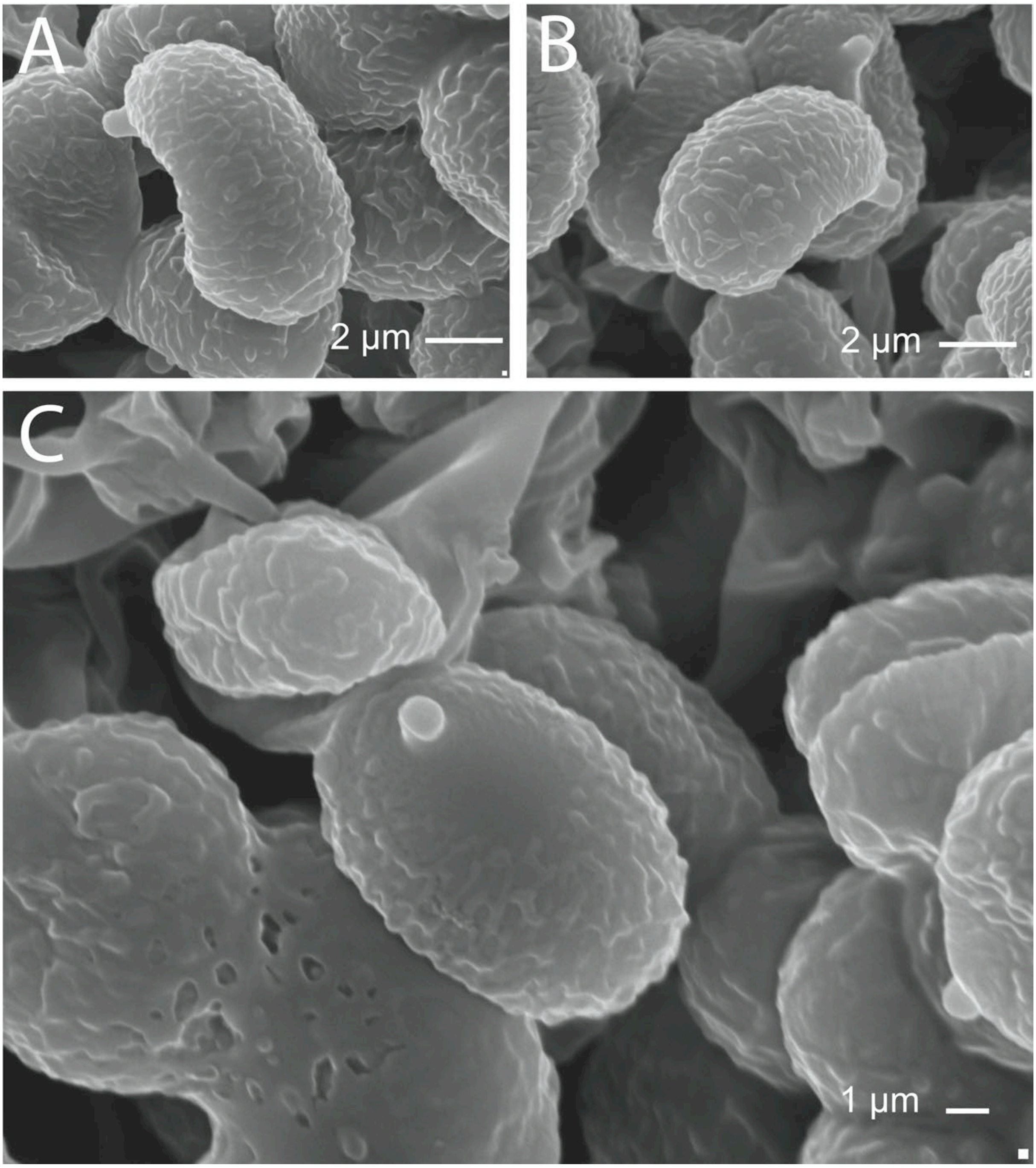

Fig. 6. Basidiome *Lactifluus brunneocarpus*

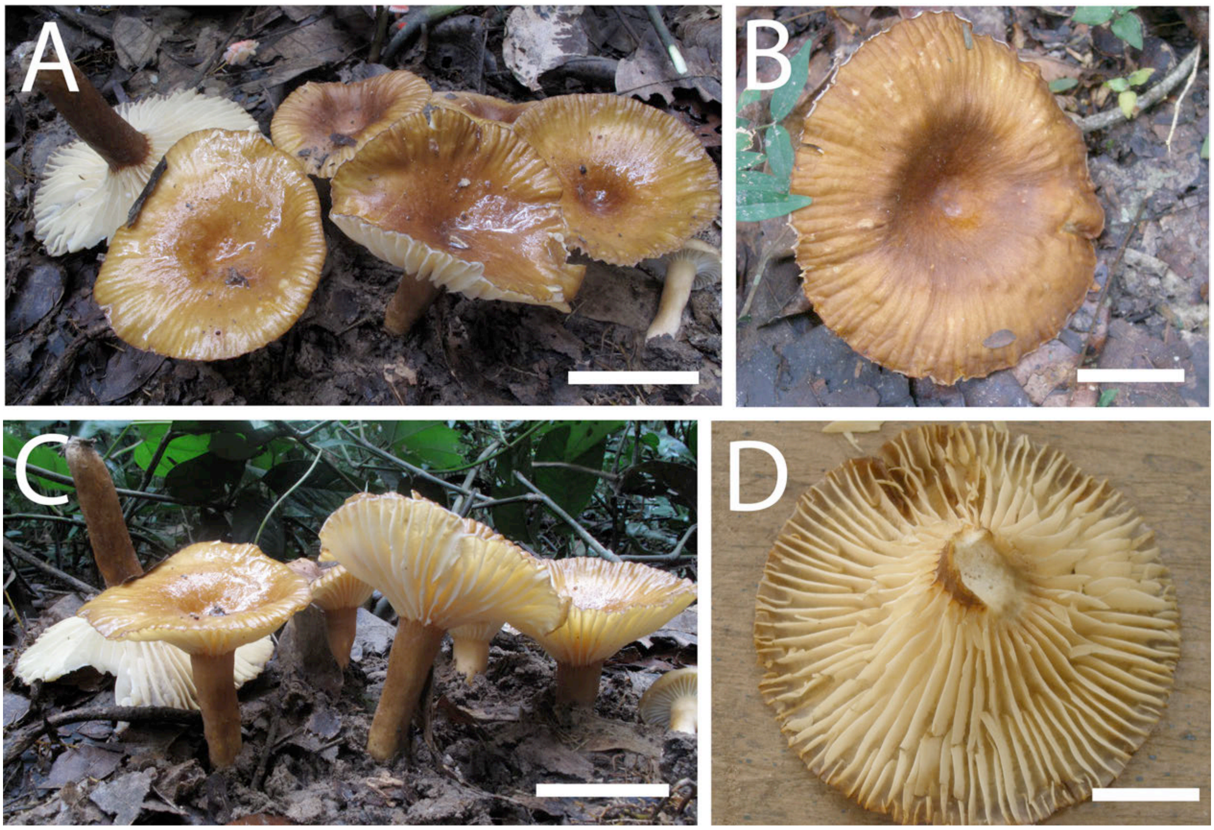

Fig. 7. Light microscopy *Lactifluus brunneocarpus*

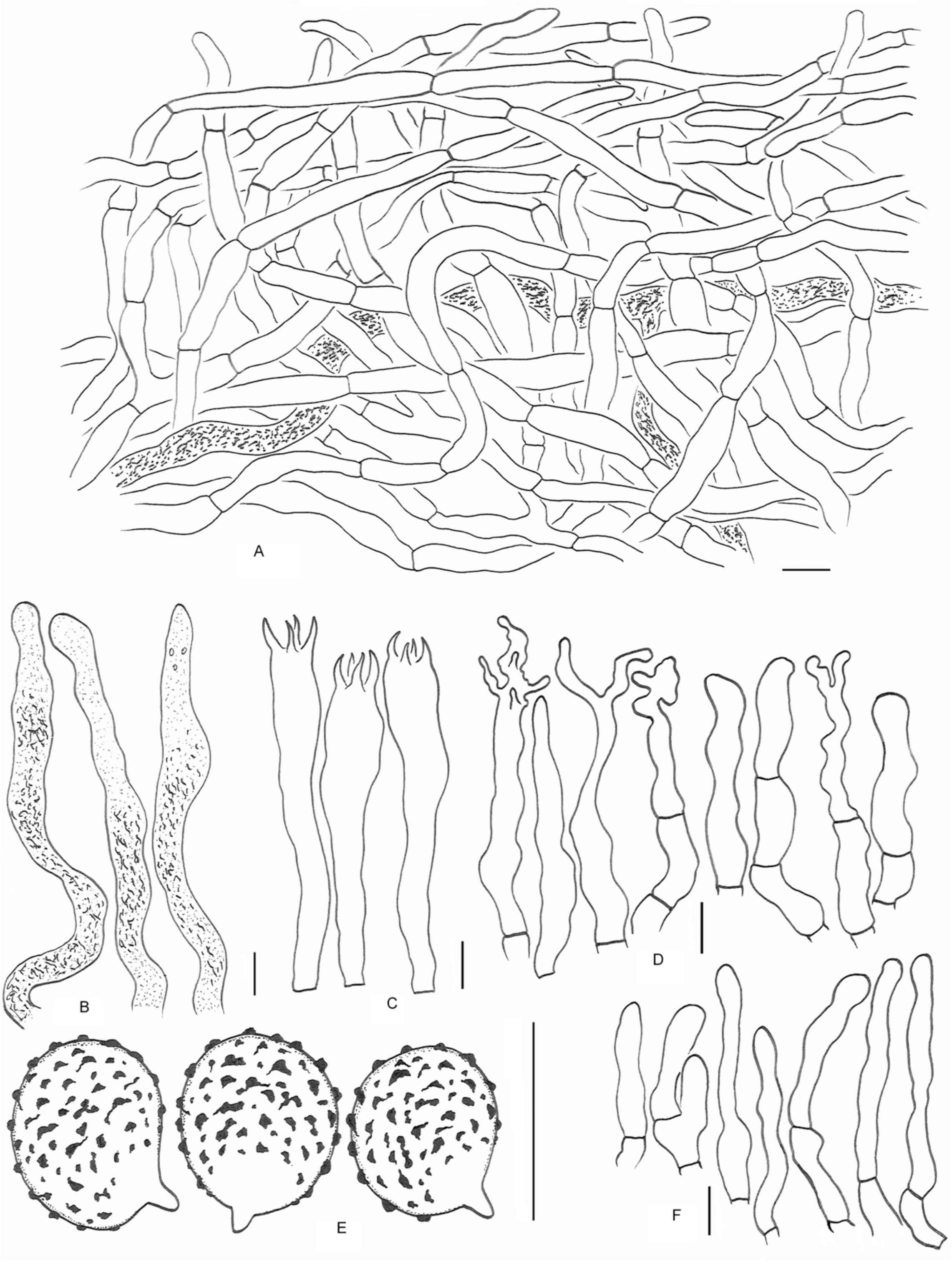

Fig. 8. SEM *Lactifluus brunneocarpus*

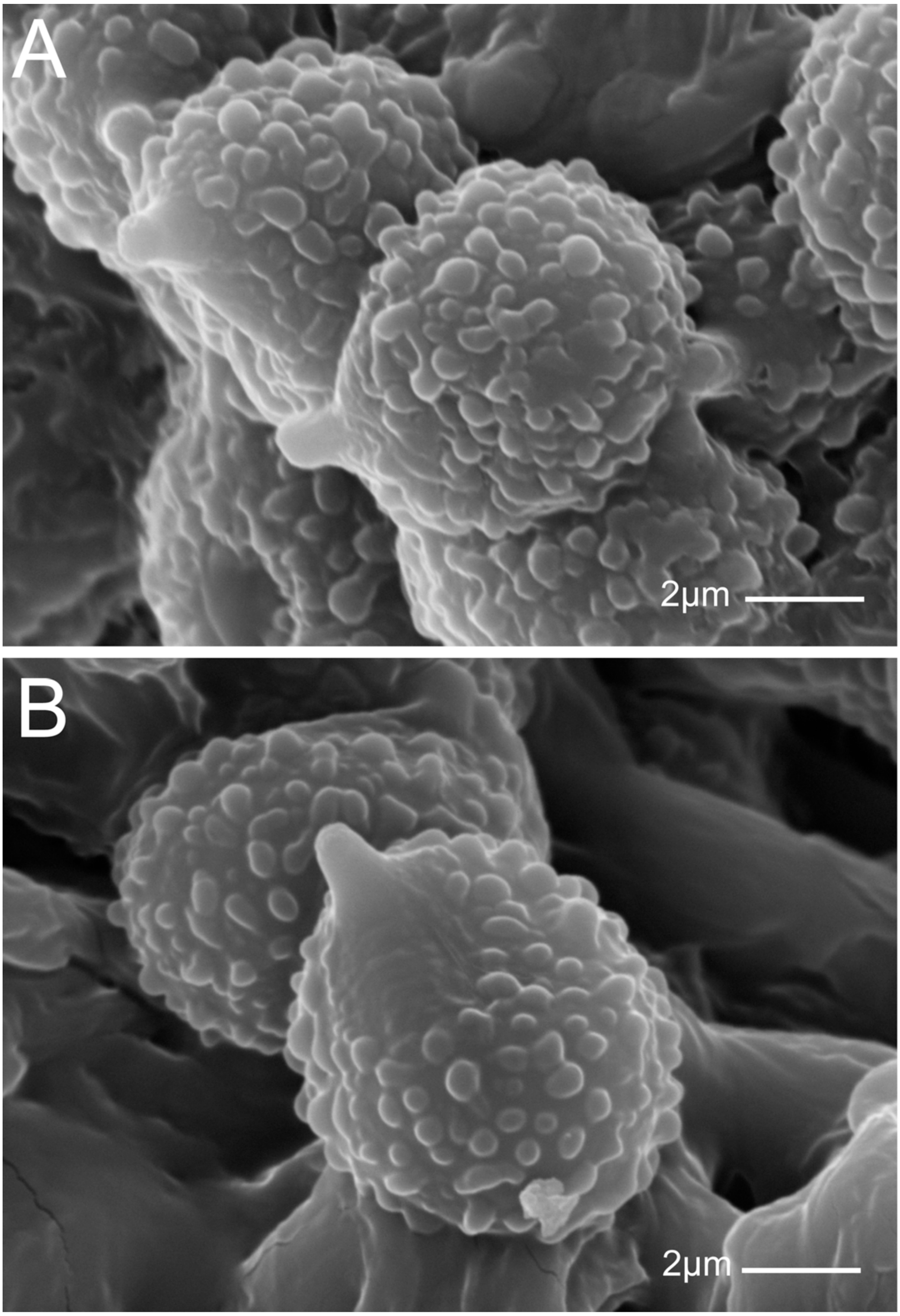

Fig. 9. Basidiome *Lactifluus burkinabei*

Fig. 10a. Light microscopy *Lactifluus burkinabei*

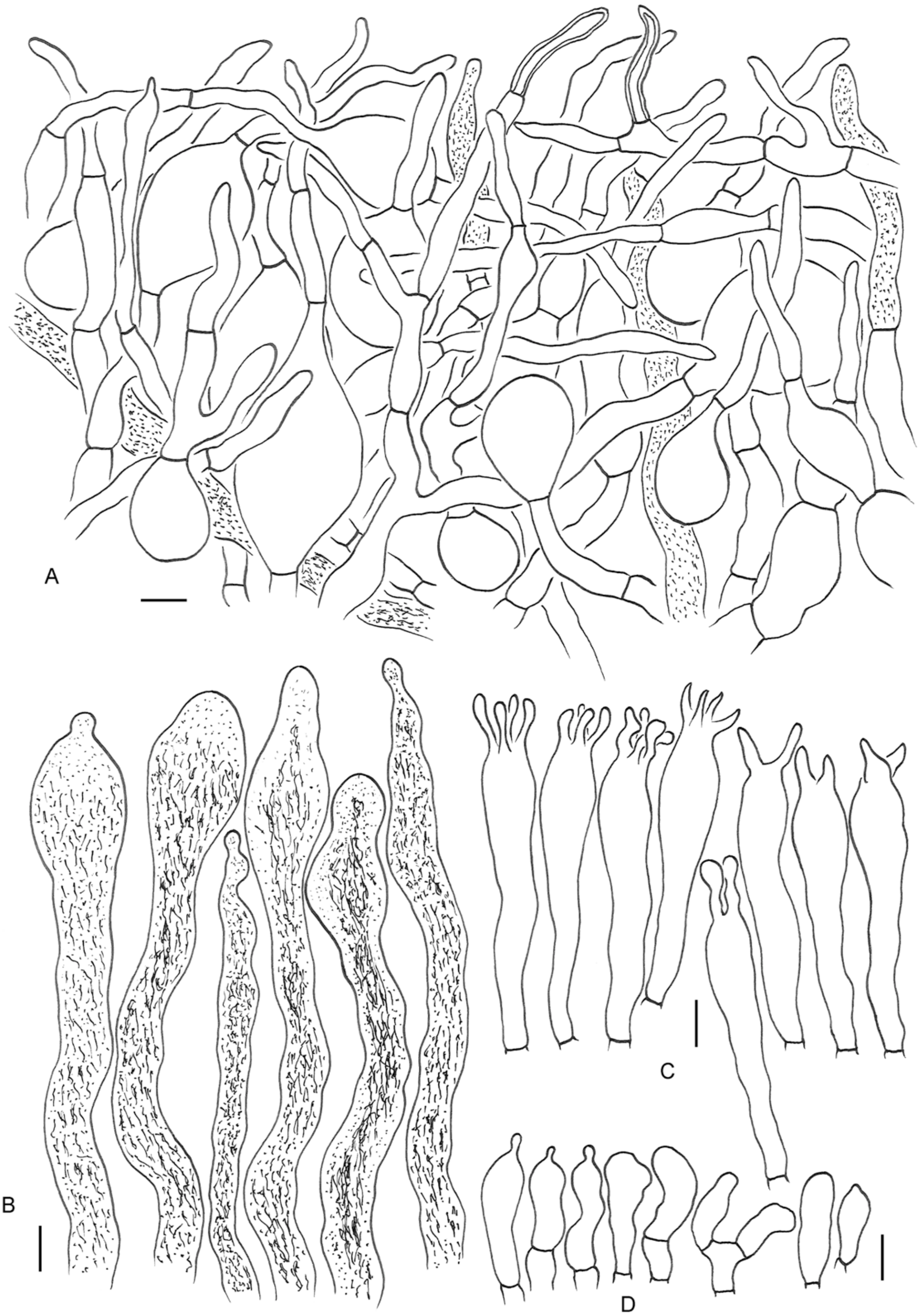

Fig. 10b. Light microscopy *Lactifluus burkinabei*

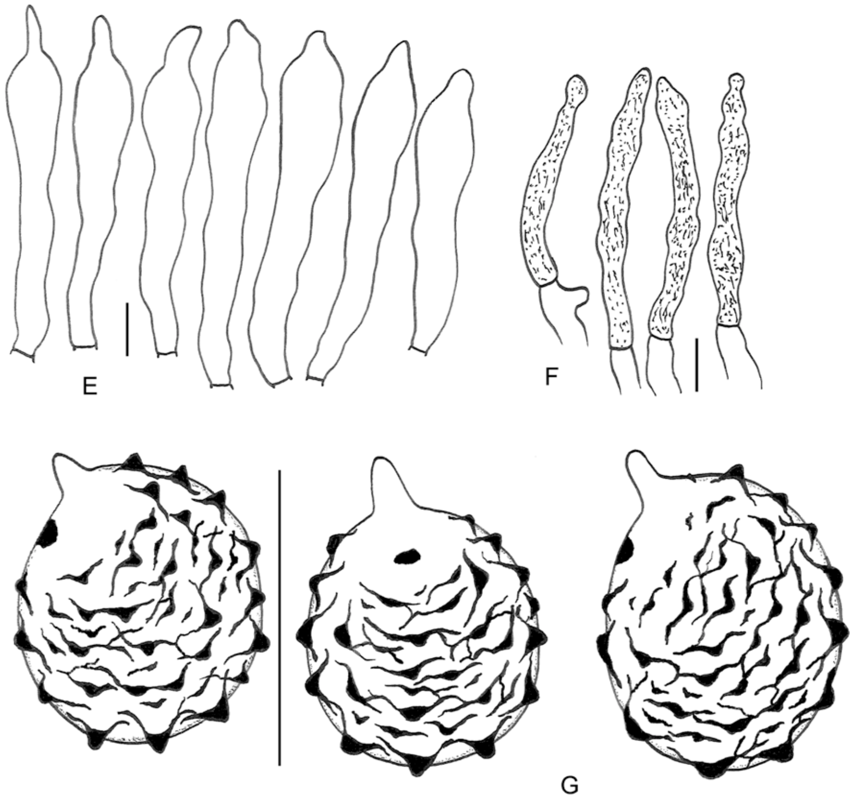

Fig. 11. SEM *Lactifluus burkinabei*

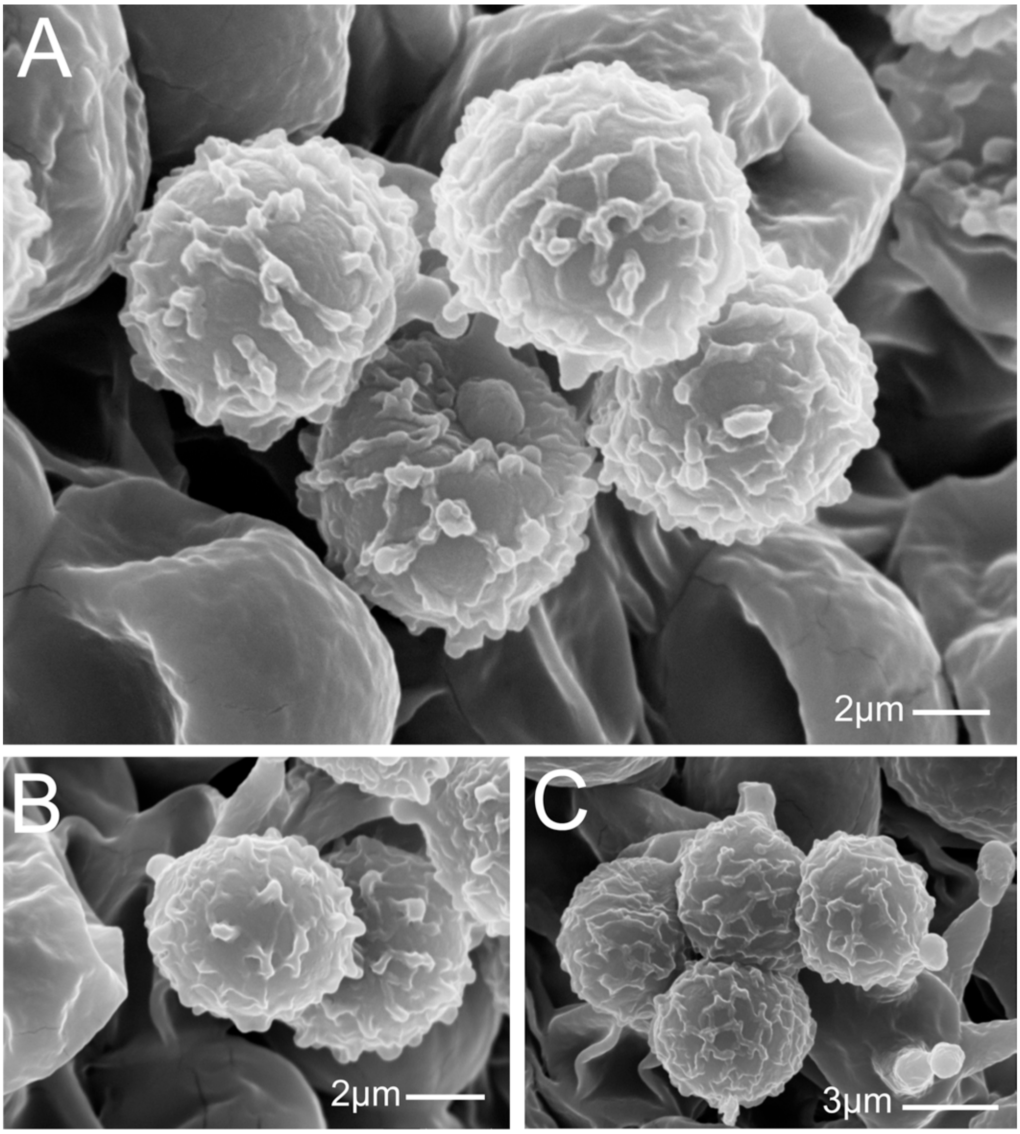

Fig. 12. Basidiome *Lactifluus guellii*

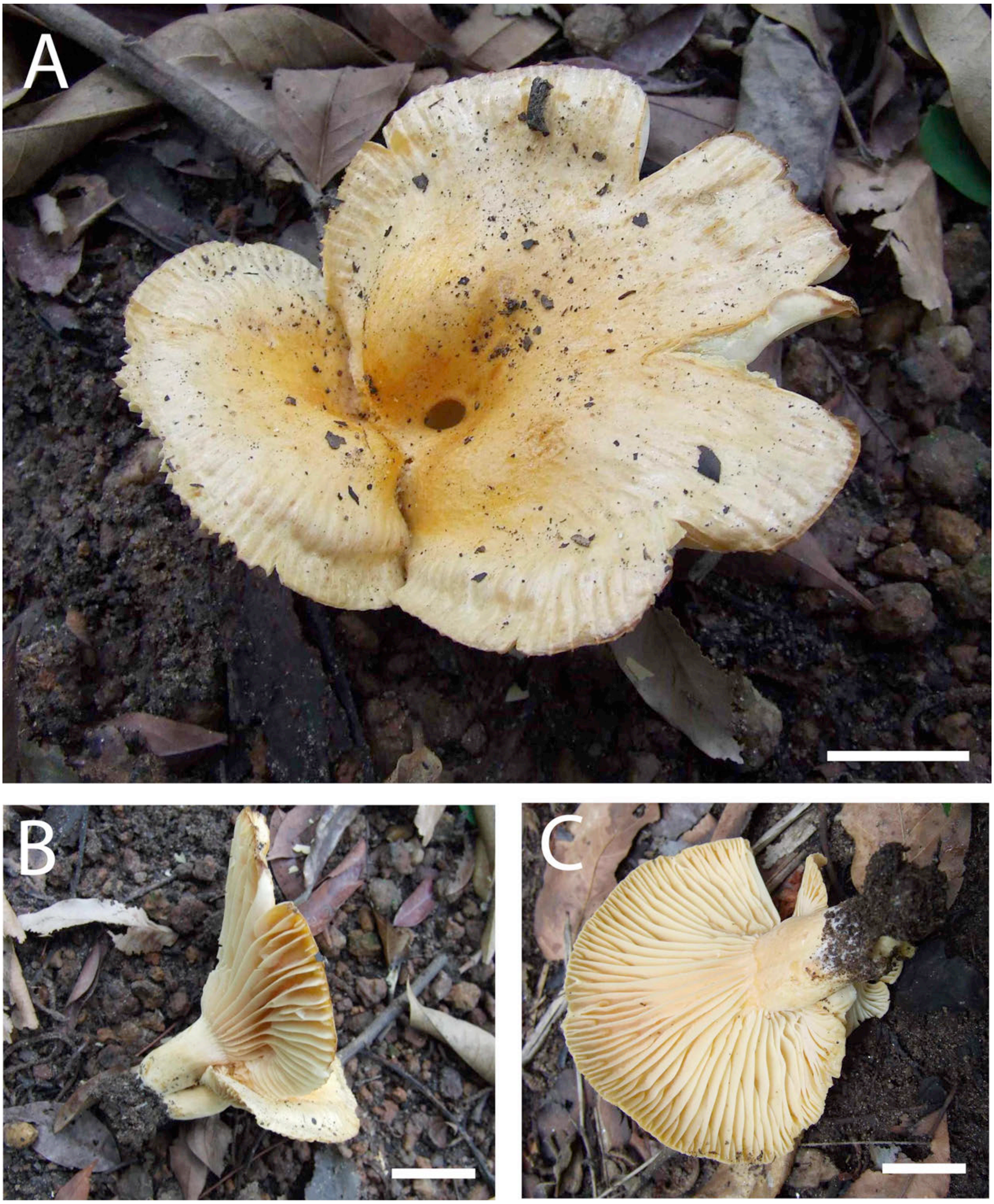

Fig. 13a. Light microscopy *Lactifluus guellii*

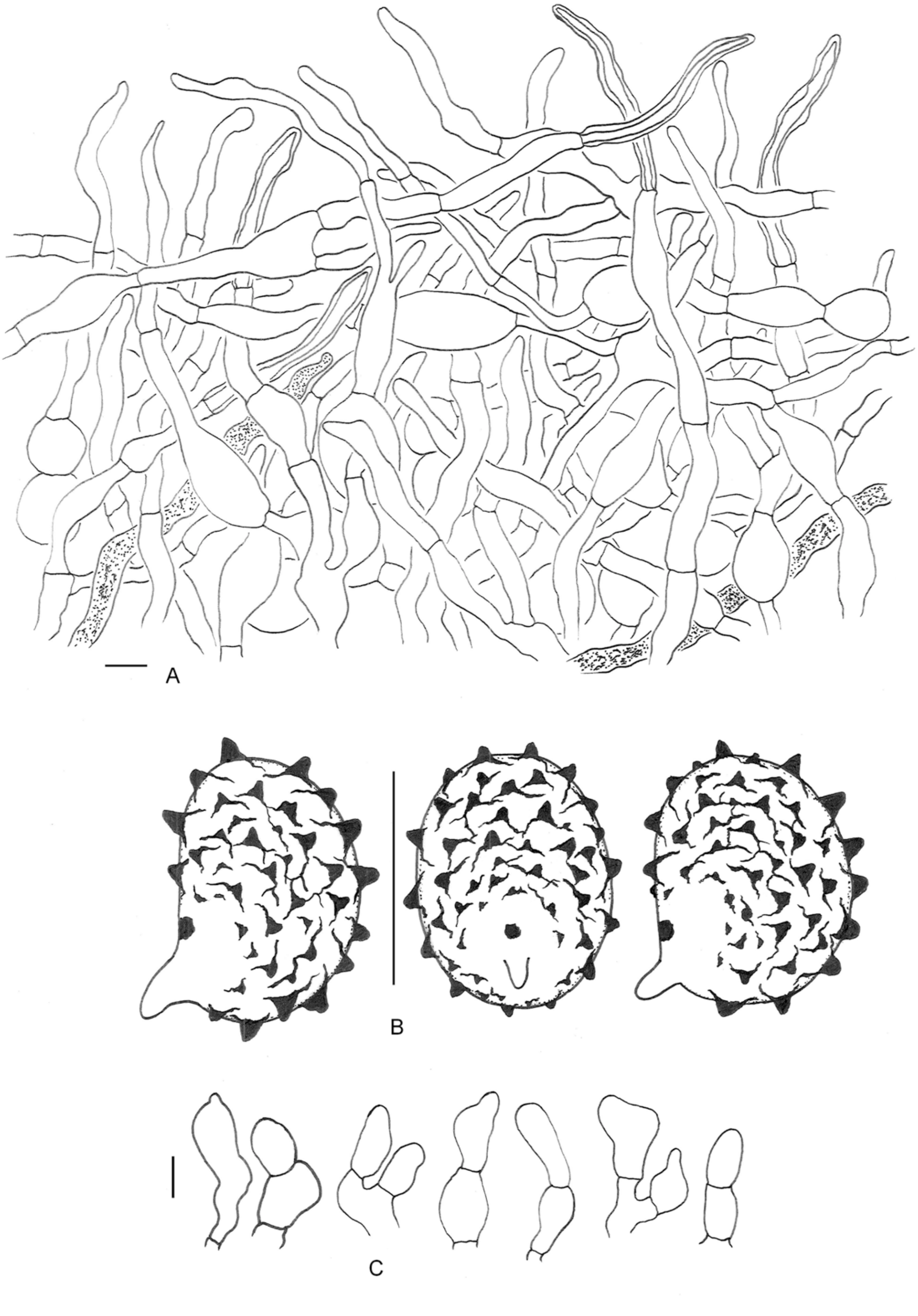

Fig. 13b. Light microscopy *Lactifluus guellii*

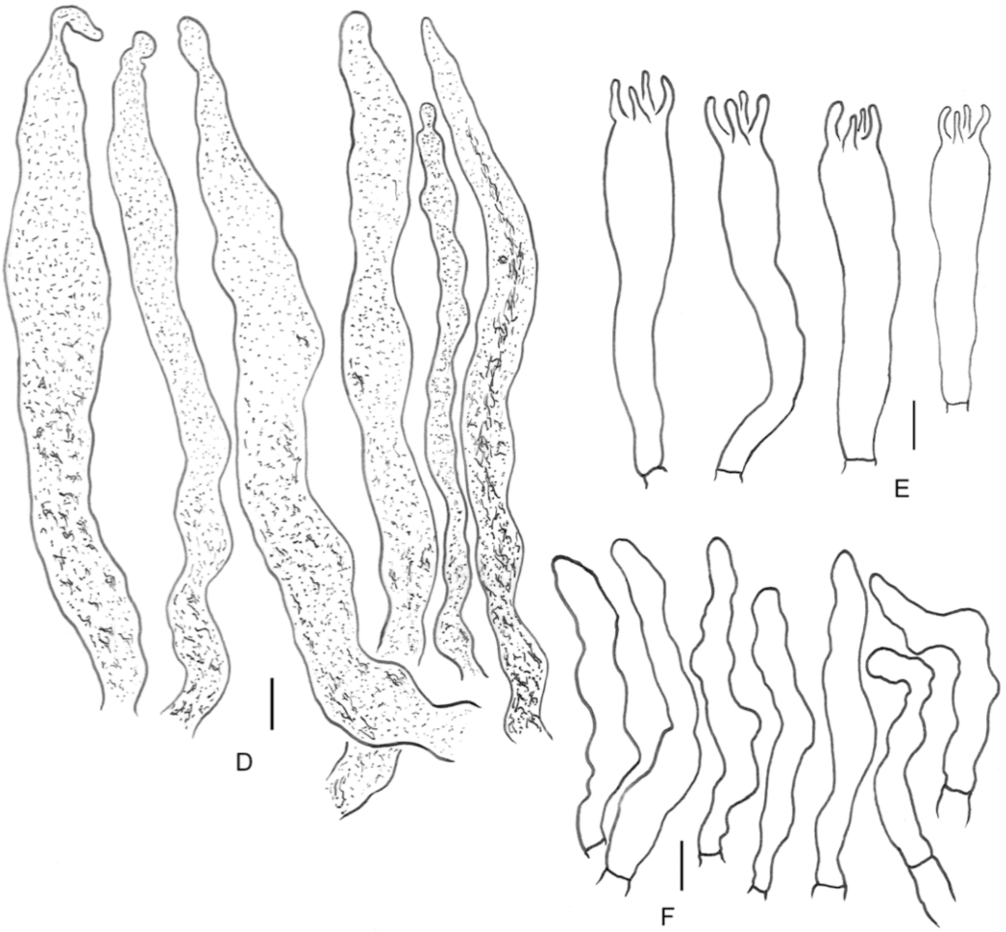

Fig. 14. SEM *Lactifluus guellii*

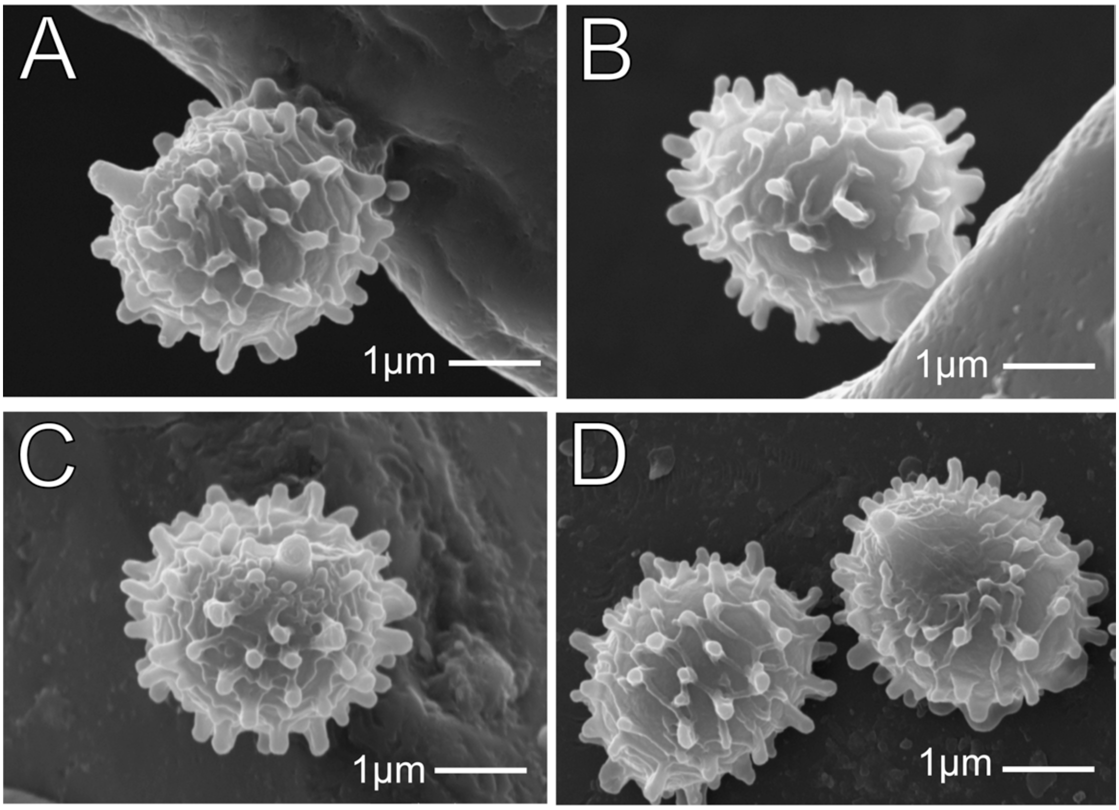

Fig. 15. Basidiome *Lactifluus membranaceus*

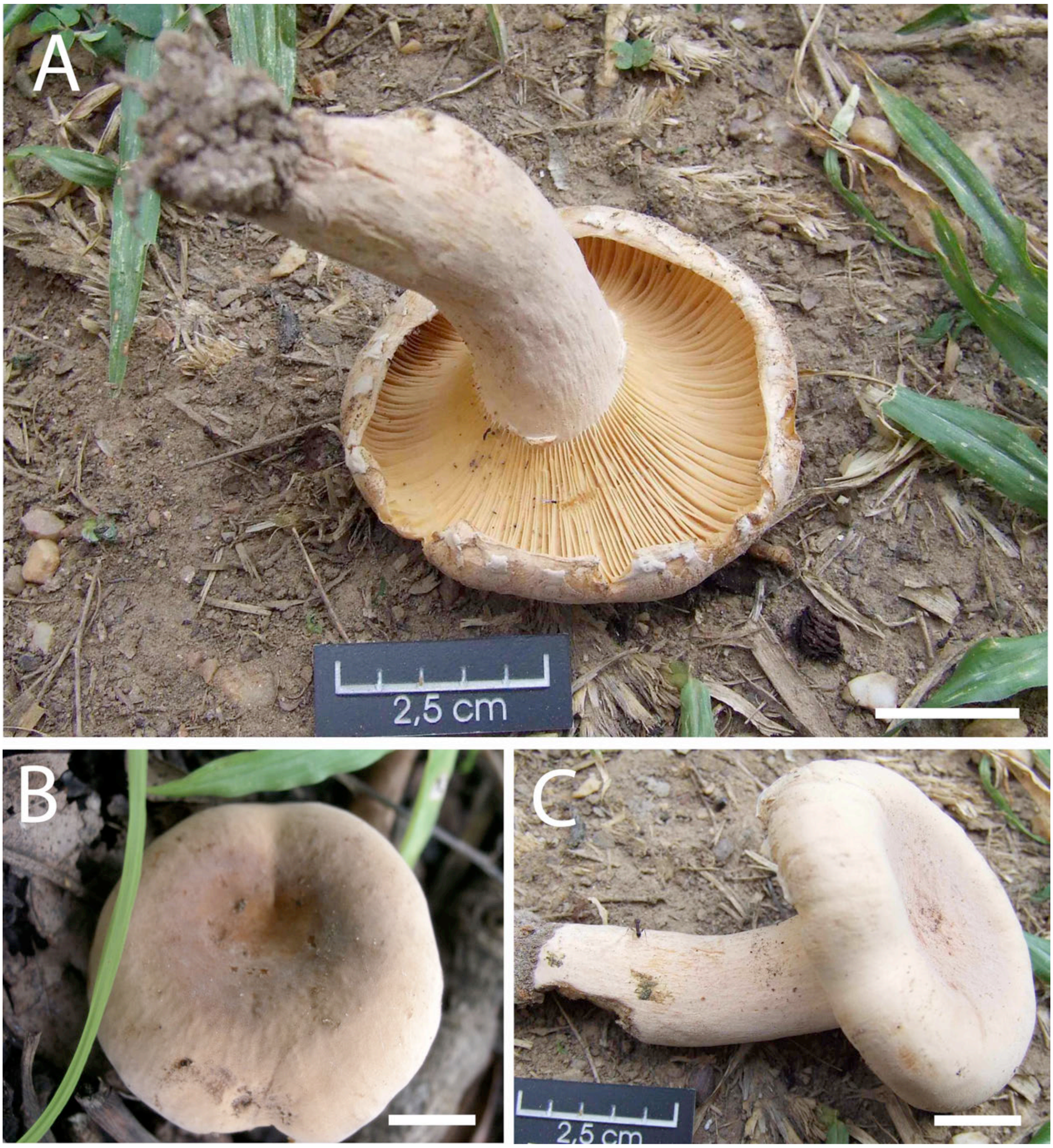

Fig. 16. Light microscopy *Lactifluus membranaceus*

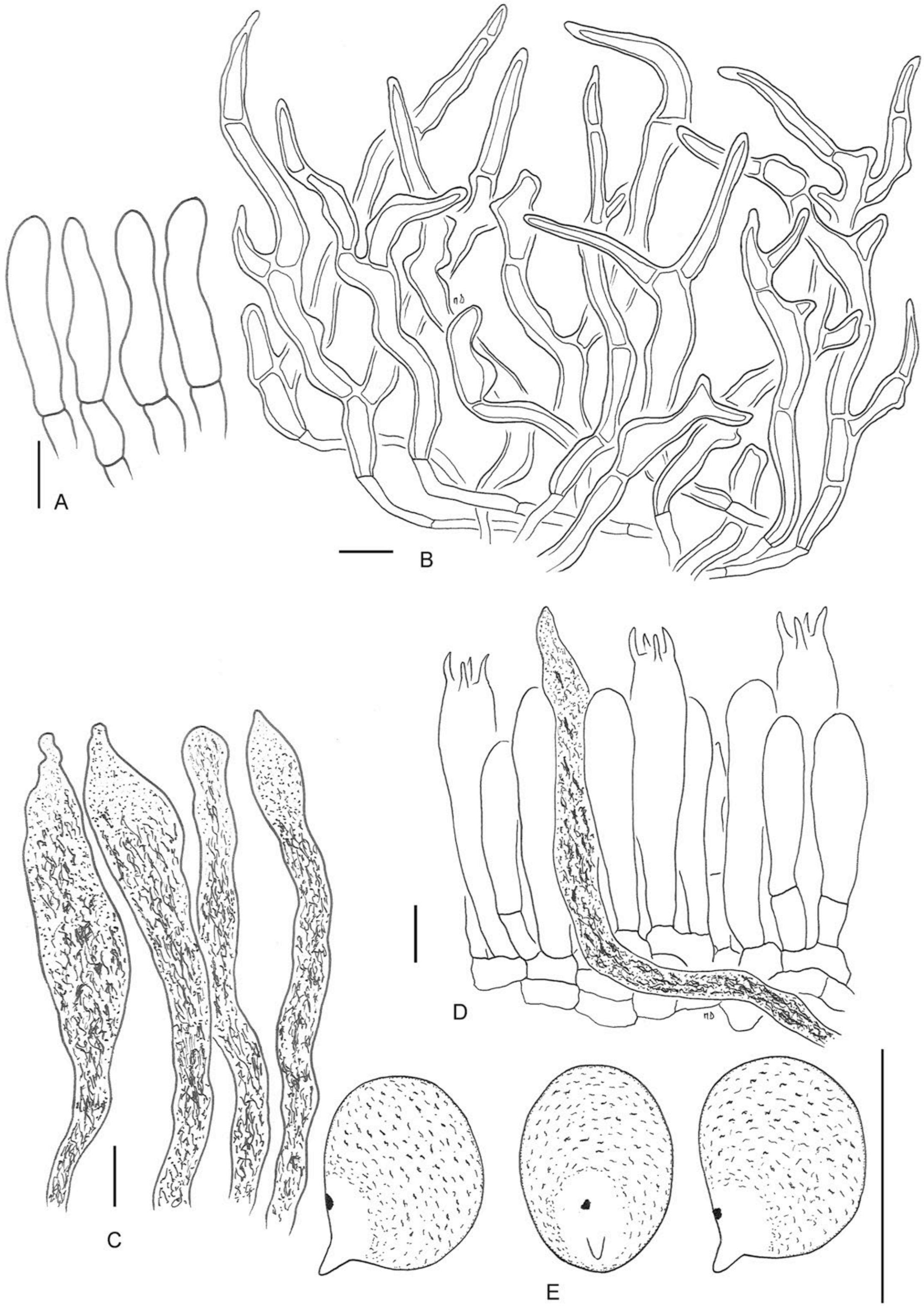

Fig. 17. SEM *Lactifluus membranaceus*

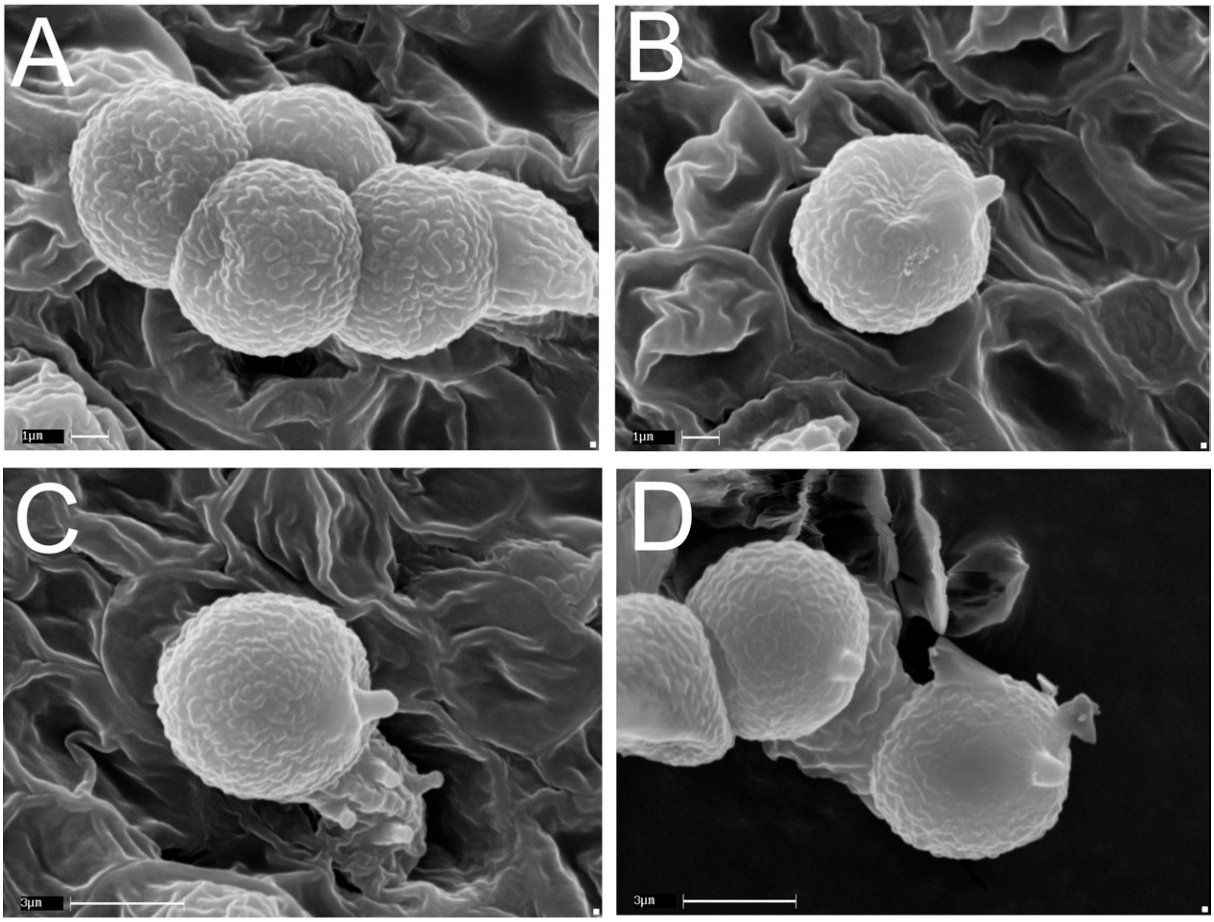

Table 1: List of our generated and public Genbank sequences included in phylogeny analyses

Species	**Genbank ENA, accession numbers**	**Localities**
***Lactifluus* (81 sequences)**		
Lactifluus allardii (Coker) De Crop	KF220017, KF220015	USA
Lactifluus annulatoangustifolius (Beeli) Buyck	HG426475	Togo
Lactifluus annulatoangustifolius (Beeli) Buyck	AY606981	Madagascar
Lactifluus annulatolongisporus sp. nov.*	HG426470, LK392606	Togo
Lactifluus atrovelutinus (J.Z. Ying) X.H. Wang	GU258231	Malaysia
Lactifluus burkinabei sp. nov.*	LK392609	Burkina Faso
Lactifluus brunneocarpus sp. nov.*	LK392608	Guinea
Lactifluus chamaeleontinus (R. Heim) Verbeken	AY606980	Zambia
Lactifluus chiapanensis Montoya, Band.-Muñoz & Guzmán	GU258297	Mexico
Lactifluus clarckeae (Cleland) Verbeken	HQ318283	Australia
Lactifluus clarckeae	GU222280	New Zealand
Lactifluus crocatus (Van de Putte & Verbeken) Van de Putte	HQ318265, Q318248, HQ318266	Thailand
Lactifluus denigricans (Verbeken & Karhula) Verbeken	AY606983	Benin
Lactifluus densifolius (Verbeken & Karhula) Verbeken	HG917385	Togo
Lactifluus edulis (Verbeken & Buyck) Buyck	HG917384	Togo
Lactifluus emergens (Verbeken) Verbeken	HG426467	Togo
Lactifluus emergens (Verbeken) Verbeken	AY606979	Zimbabwe
Lactifluus fazaoensis Maba, Yorou & Guelly	HG426477	Togo
Lactifluus flammans (Verbeken) Verbeken	HG426471	Togo
Lactifluus flammans	UDB016931	Benin
Lactifluus flavellus Maba & Guelly	LK392594, LK392595	Togo
Lactifluus flocktonae Cleland & Cheel	JX2666621, JX266622	Australia

Lactifluus foetens (Verbeken & Van Rooij) Verbeken	HG917381	Togo
Lactifluus foetens	LK392603	Burkina Faso
Lactifluus genevievae (Stubbe & Vebeken) Stubbe	GU258294	Australia
Lactifluus glaucescens (Crossl.) Verbeken	KF220117	Italy
Lactifluus glaucescens	KF220094	Belgium
Lactifluus glaucescens	KF220075	France
Lactifluus guellii sp. nov.*	HG426466	Togo
Lactifluus gymnocarpoides (Verbeken) Verbeken	LK392601	Benin
Lactifluus gymnocarpoides	LK392600	Benin
Lactifluus gymnocarpus (R. Heim ex Singer) Verbeken	HG426472	Togo
Lactifluus heimii (Verbeken) Verbeken	LK392612	Togo
Lactifluus hygrophoroides (Berk. & M.A. Curtis) Kuntze	JN129397	China
Lactifluus inversus (Gooss.-Font. & R. Heim) Verbeken	AY606976	Guinea
Lactifluus longibasidius Maba & Verbeken	LK392596, HG426473	Togo
Lactifluus longipes (Verbeken) Verbeken	HG917391, HG917383	Togo
Lactifluus longipilus (Van de Putte H.T. Le & Verbeken) Van de Putte	HQ318235, HQ318258, KF432958	Thailand
Lactifluus longisporus (Verbeken) Verbeken	DQ421971	Zambia
Lactifluus luteopus (Verbeken) Verbeken	LK392602	Togo
Lactifluus luteopus	LK392611	Burundi
Lactifluus medusae (Verbeken) Verbeken	HG426474	Togo
Lactifluus madagascariensis (Verbeken & Buyck) Buyck	AY606977	Madagascar
Lactifluus melleus Maba	LK392598, LK392597	Togo
Lactifluus membranaceus sp. nov.*	LK392610	Guinea
Lactifluus membranaceus sp. nov.*	HG426478	Togo
Lactifluus nodosicystidiosus (Verbeken & Buyck)	AY606975	Madagascar

Buyck		
Lactifluus nonpiscis (Verbeken) Verbeken	HG426468	Togo
Lactifluus pectinatus Maba & Yorou	LK392599	Togo
Lactifluus piperatus (L.: Fr.) Kuntze	KF220122, KF220120	France
Lactifluus pelliculatus (Beeli) Buyck	AY606978	Madagascar
Lactifluus phlebophyllus (R. Heim) Buyck	AY606074	Madagascar
*Lactifluus pseudoluteopu*s (X.H. Wang & Verbeken) X.H. Wang	HQ318286	Thailand
Lactifluus rubroviolascens (R. Heim) Verbeken	AY606984	Zambia
Lactifluus rubroviolascens	AY606985	Madagascar
Lactifluus rubiginosus (Verbeken) Verbeken	HG917386	Togo
Lactifluus sudanicus Maba, Yorou & Guelly	HG426469, HG426476	Togo
Lactifluus velutissimus (Verbeken) Verbeken	AY606982	Zimbabwe
Lactiflus volemus (Fr.: Fr.) Kuntze	HQ318279, HQ318275	Thailand
Lactifluus volemoides (Karhula) Verbeken	UDB016930	Benin
Lactifluus sp.	LK392607	Togo
Lactifluus sp.	LK931501	Togo
Lactifluus sp.	LK392604	Benin
Lactifluus sp.	LK392605	Benin
Lactifluus sp.	LM999911	Benin
Lactifluus sp.	LN651269	Burkina Faso
Lactifluus sp.	LM999910	Togo
Lactifluus sp.	UDB014027	Cameroon
***Lactarius* (17 sequences)**		
Lactarius baliophaeus Pegler	GU258277	Zambia
Lactarius kabansus Pegler & Piearce	HG917376	Togo
Lactarius kabansus	HG917390	Zimbabwe
Lactarius miniatescens Verbeken Van Rooij	HG917375	Burkina Faso
Lactarius miniatescens	HG917374	Togo
Lactarius tenellus Verbeken & Walleyn	HG917373	Togo

Lactarius saponaceus Verbeken	HG917379	Guinea
Lactarius saponaceus	HG917378	Togo
Lactarius subbaliophaeus Maba & Yorou	HG917372	Togo
Lactarius sp.	UDB013804	Zambia
Lactarius sp.	UDB015091	Gabon
Lactarius sp.	UDB018664	Zambia
Lactarius sp.	UDB018662	Zambia
Lactarius sp.	UDB013845	Zambia
Lactarius sp.	UDB013930	Cameroon
Lactarius sp.	UDB016860	Zambia
Lactarius sp.	UDB013836	Zambia
***Multifurca* (5 sequences)**		
Multifurca zonaria (Buyck & Desjardin) Buyck & V. Hofst.	DQ422000, DQ421990	Thailand
Multifurca furcata (Coker) Buyck & V. Hofst.	DQ421995, DQ421994	USA
Multifurca ochricompacta (Bills & O.K. Mill.) Buyck & V. Hofst.	DQ421984	USA
***Russula* (8 sequences)**		
Russula cremeirosea Murrill	EU819424	USA
Russula congoana Pat.	HG917387	Togo
Russula congoana	UDB016932	Benin
Russula compressa Buyck	UDB016985	Benin
Russula discopus R. Heim	JQ902046	Burundi
Russula discopus	JQ902050	Senegal
Russula lipida (Fr.: Fr.) Fr	JF908663	Italy
Russula xerampilina (Schaeff.) Fr.	KF386758	USA
Out group (2 sequences)		
Gloeocystidiellum sp.	KJ140715	USA
Hericium erinaceum	EU784265	K(M)62494)

(*) Newly described species

Table 2: Major morpho-anatomical features of the genus *Lactifluus* versus *Lactarius* from tropical Africa (van Rooij et al 2003; Verbeken & Walleyn 2010; Verbeken & Nuytinck 2013; Maba et al. 2013, 2014, 2015)

Characters	***Lactifluus***	***Lactarius***
Basidiome	Includes pleurotoid and annulate taxa	Includes gasteroid and epigeous taxa, present Scrobicules on the cap and/or stipe.
Coloration	Mostly (pale-, whitish-) yellow, orange, yellow-orange, orange-brown, yellow-brown, orange-greyish	Mostly olivaceous, ochraceous, greyish, brownish, yellowish brown, cream, dark orange, darkish
Latex	Very scare to abundant, white to watery, greyish orange, transparent, watery to waxy, fluid	Very scare to abundant, opaque, opalescent, transparent, white, cream, brownish, hyaline, fluid
Chemical reactions	Context changes or not with gaiac, Fe_2SO_4, NaOH, KOH, NH_4OH, HCl, phenol, phenolaniline, pyramidon, aniline.	
Basidiopores	Very variable, often weakly to well ornamented, reticulate and never winged	Strongly ornamented, often developed ridges (up to 1.5 µm high) reticulate-like and winged reticulum
Hymenophoral trama	Sphaerocytes almost always present, hyaline hyphae often lacking or very scare, laticiferous hyphae	Sphaerocytes often lacking, subhymenium rarely cellular, mostly composed of hyaline hyphae in mixture with laticifers
Pleurocystidia	(Pleuro)-lamprocystidia, leptocystidia, subclavate, moniliform to rostrate, sometimes forked or diverticulate to tortuous, sometimes septate, emergent	(Pleuro)-lamprocystidia macrocystidia, lampromacrocystidia, emergent, sometimes apex tapering upwards, mucronate
Pseudocystidia	Often emergent, cylindrical to	Often emergent, cylindrical to

	subcylindrical, fusiform to tortuous, inflated, branched, often tapering upwards, apex mucronate to capitate, mostly needle-like to granular, and oleiferic contents	subcylindrical, fusiform to tortuous, rarely branched, tapering upwards, apex mucronate, mostly granular to slightly needle-like, and oleiferic contents
Marginal Cells	Often cylindrical to subcylindrical, fusiform, tortuous, branched, very diverticulate, strongly inflated, soften septate	Subcylindrical, fusiform, tortuous, branched, subclavate to inflated, rarely rounded to mucronate, sometimes thickened, sometimes septate
Pileipellis (Stipitipellis)	Mostly lampro/tricho-palisadic, palisadic, ixocutis, trichoderm or a mixture, interwoven cutis transgrading to trichoderm; suprapellis with fusiform and clavate to subglobose cells.	Mostly palisadic, rarely ixocutis to trichoderm, and hymeniderm
Dermatocystidia	Subcylindrical, capitate to subcapitate with needle-like or dark brown guttules	Lacking
Genetic variability:	Highly diversified	Less diversified

Supplement: Occurrence and frequency of collected *Lactifluus* species in Guineo-Sudanian ecosystems (2007-2013)

Species	**Colloctor/Date**	**Locality and ecosystem type**
Lactifluus annulatoangustifolius	André De Kesel, 07/2007, ADK4318 Atsu Guelly, 07/2007, C2184 A. De Kesel, 05/2010, ADK4804 Dao Maba, 05/ 2008 Dao Maba, 06/2011, MD145	(1) Aledjo, gallery forest, N09°16'537" E01°13.4'12" (1) Aledjo, gallery forest, N09°16'53.7" E01°13.4'12" (1) Kougnohou, gallery forest N07°40'29.3" E0°47'35.7" (1) Bafilo, Woodland, N09°20'21" E01°14'18.9" (1) Fazao, Woodland, N08°42'21" E00°46'22"
*Lactifluus annulatolongisporus**	Dao Maba, 06/2011, MD123 Dao Maba, 06/2011, MD131	(1) Fazao, Woodland, N08°42'58" E00°46'22" (1) Fazao Woodland, N08°42'32" E00°45'13"
*Lactifluus brunneocarpus**	Dao Maba, 07/2011, MD224 Dao Maba, 07/2011, MD219B	(4) Malouwaita, rainforest N08°32'7.7" W09°22'8.6" (4) Malouwaita, rainforest N08°19'7.3" W09°13'20.1"
*Lactifluus burkinabei**	Dao Maba, 07/2013, MD355	(3) Orodara, DAN, gallery forest N10°53'6.9" E04°50'27.9"
Lactifluus chamaeleontinus	A. De Kesel, 07/2007, ADK4304 Dao Maba, 09/2007, DPM15	(1) Aledjo, gallery forest N09°16'46" E01°12.4'16" (1) Aledjo, gallery forest N09°16'34", E01°13'33"
Lactifluus densifolius	A. De Kesel, 07/2007, ADK4285 Atsu Guelly, 05/2008, C2167 Atsu Guelly, 06/2008, C2362 A. De Kesel, 05/2010, ADK4808	(1) Aledjo, gallery forest, N09°16'46" E01°12.4'16" (1) Aledjo, gallery forest, N09°16'12.5" E01°12'20.3" (1) Fazao, gallery forest, N08°42'58" E00°46'22"

		(1) Kougnohou, gallery forest N07°40'29.3" E00°47'35"
Lactifluus edulis	A. De Kesel, 07/2007, ADK4373 Atsu Guelly, 05/2008, C2168	(1) Kparatao, Woodland, N09°11.6’30" E00°59'13.4" (1) Aledjo, gallery forest, N09°16'12.5" E01°12'20.3"
Lactifluus emergens	Dao Maba, 05/2008, DPM04 Dao Maba, 06/2011, MD143	(1) Aledjo, Woodland, N09°13.9'8.1" E01°11.4'42" (1) Fazao, Woodland, N08°42'59" E00°46'35"
Lactifluus flavellus	Dao Maba, 07/2013, MD393, MD397	(1) Bena, gallery forest N07°31'6.6" E00°54'7.41"
Lactifluus fazaoensis	Dao Maba, 06/2011, MD152	(1) Fazao, Woodland, N08°43'08" E00°46.5'6.8"
Lactifluus flammans	A. De Kesel, 07/2007, ADK4303, A. De Kesel, 07/2007, ADK4420 Atsu Guelly, 05/2007, C2271 Dao Maba, 06/2011, MD124 Dao Maba, 07/2013, MD382 Dao Maba, 07/2013, MD302 Dao Maba, 07/2013, MD321, MD323, MD331	(1) Aledjo, Woodland, N09°13.9'8.1" E01°11.4'42" (1) Fazao, Woodland, N08°43.3'8.5" E00°46.9'8" (1) Bafilo, Woodland, N09°20'21" E01°14'18.9" (1) Fazao, gallery forest, N08°40'8.1" E00°45'50" (1) Aledjo, gallery forest N09°16'28" E01°13'21" (2) N'Dali, Woodland, N09°45'52.3" N02°35'8.2" (2) Gando, Woodland, N09°45'45.3" E02°19'55.9"
Lactifluus foetens	A. De Kesel, 07/2007, ADK4283 A. De Kesel, 07/2007, ADK4411 Atsu Guelly, 05/2007, C1873 Dao Maba, 06/2011, MD150	(1) Aledjo, gallery forest, N09°16'46" E01°12'41.6" (1) Aledjo, Woodland, N08°42'49" E00°51'7.30" (1) Aledjo, Woodland, N09°13'9.8" E01°11'44.2"

	Dao Maba, 07/2013, MD359	(1) Fazao, Woodland, N0º8.42'12" E0º46' 32" (3) Kou, gallery forest, N11°11'38" E04°26'57"
*Lactifluus guellii**	Atsu Guelly, 07/2007, C2157 Atsu Guelly, 07/2007, C2163	(1) Aledjo, gallery forest, N09°16'53.7" E01°13'41.2" (1) Aledjo, gallery forest, N09°16'12." E01°12'20.3"
Lactifluus gymnocarpus	A. De Kesel, 07/2007, ADK4442 A De Kesel, 07/2007, ADK4471 A. De Kesel, 05/2010, ADK4830 Dao Maba, 06/2011, MD125	(1) Aledjo, Woodland, N08°41'13.3" E00°45.6'23" (1) Aledjo, Woodland, N08°43'14.5" E00°46'33.2" (1) Bena-Ola gallery forest N07°32'44.3" E00°55'37" (1) Fazao, gallery, forest N08°40'8.1" E00°45'50"
Lactifluus gymnocarpoides	A. De Kesel, 07/2007, ADK4302 A. De Kesel, 07/2007, ADK4386 A. De Kesel, 07/2007, ADK4421 Nadjombe P. 09/2007 NPR 025 Dao Maba, 08/2008, DPM20 Dao Maba, 07/2013, MD301 Dao Maba, 07/2013, MD318 Dao Maba, 07/2011, MD236	(1) Fazao, Woodland, N09°13.9'8.1" E01°11'44.2" (1) Fazao, Woodland, N08°43.9'6.3" E00°47.6'7.4" (1) Fazao, Woodland, N08°43.3'8.5" E00°46.9'8.0" (1) Kparatao, Woodland, N09°11'45.9" E00°59'07.7" (1) Aledjo, Woodland, N09°13.9'8.1" E01°11.4'42" (2) N'Dali, Woodland, N09°45'52" N02°35'8.2" (2) Gando, Woodland, N09°45'45.3" E02°19'55.9" (4) Kouroussa Woodland, N10°32'9" W09°37'37.9"
Lactifluus heimii	A. De Kesel, 07/2007, ADK4327	(1) Aledjo, gallery forest, N09°16.5'37" E01°13.4'12"

Lactifluus inversus	A. De Kesel, 07/2007, ADK4316	(1) Aledjo, gallery forest, N09°16.5'37" E01°13.4'12"
Lactifluus longibasidius	Dao Maba, 06/2011, MD156 Dao Maba, 06/2011, MD141	(1) Fazao, Woodland, 8°42'21" N 0°46'22" (1) Fazao, Woodland, N08°42'21" E00°46'18"
Lactifluus longipes	A. De Kesel, 05/2010, ADK4837 A. De Kesel, 05/2010, ADK4854 Atsu Guelly, 05/2007, C1828 A. De Kesel, 07/2007, ADK4315	(1) Bena-Ola gallery forest, N07°33'12.3" E00°52'37.8" (1) Bena-Ola gallery forest, N07°21'43.4" E0°55'3.5" (1) Aledjo, gallery forest, N09°16'52.5" E01°13'42.7" (1) Aledjo, gallery forest, N09°16'53.7" E01°13'41.2"
Lactifluus luteopus	A. De Kesel, 07/2007, ADK4325 A. De Kesel, 07/2007, ADK4422 Nadjombe P. 09/2007 NPR 024 Dao Maba, 06/2011, MD102 Dao Maba, 06/2011, MD122 Dao Maba, 07/2011, MD172 Dao Maba, 07/2011, MD 240 Dao Maba, 07/2011, MD212, MD213 Dao Maba, 07/2013 MD338	(1) Aledjo, gallery forest, N09°16'53.7" E01°13'41.2" (1) Fazao, Woodland, N08°43'38.5" E00°46.9'8.0" (1) Kparatao, Woodland, N09°11'52,9" E00°59’13" (1) Fazao, gallery forest, N08°42'11" E00°46'24" (1) Fazao, Woodland, N08°43.9'6.3" E0°47.6'7.4" (3) Mouhoun, gallery forest, N10°53'58" E04°50'48" (4) Kouroussa Woodland, N10°32'9" W09°37'37.9" (4) Moussaya, Woodland, N10°41'27" W09°58'40" (2) Atakora, Kpota, gallery forest, N10°12'44.9" E01°26'46.2"
Lactifluus medusae	Dao Maba, 06/2011, MD142 Dao Maba, 07/2013 MD305,	(1) Fazao, Woodland, N08°42'21" E00°46'22" (2) N'Dali, Woodland, N09°45'52"

	MD306	E02°35'8.2"
Lactifluus melleus	Dao Maba, 06/2011, MD108 Dao Maba, 06/2011, MD157	(1) Fazao, Woodland, N8°30'56" E00°54'44.1" (1) Fazao, Woodland, N08°42'23" E0º46'27"
*Lactifluus membranaceus**	Atsu Guelly, 06/2008, C2349 Dao Maba, 05/2008, MD05 Dao Maba, 07/2011, MD234	(1) Fazao, Woodland, N08°45'24" E00°48'08" (1) Aledjo, Woodland, N09°13.9'8.1" E01°11.4'42" (4) Kouroussa Woodland, N10°32'9" W09°37'37.9"
Lactifluus nonpiscis	Dao Maba, 06/2011, MD100 Dao Maba, 07/2011, MD170 Dao Maba, 07/2011, MD178	(1) Fazao, Woodland, N08°42'27" E00°40'35" (3) Mouhoun, gallery forest, N10°35'36" E04°50'22" (3) Kou, gallery forest, N11°53'58" E04°50'48"
Lactifluus pectinatus	Dao Maba, 06/2011, MD140	(1) Fazao gallery forest, N08°16'12" E00°46'18"
Lactifluus rubiginosus	Atsu Guelly, 05/2008, C2183 A. De Kesel, 05/2010, ADK4810 Dao Maba, 07/2013, MD370 Dao Maba, 07/2013, MD389	(1) Aledjo, gallery forest, N09°40'12.5" E01°12'20" (1) Kougnohou, gallery forest, N07°40'29.3" E00°47'35.7" (1) Bafilo, gallery forest, N09°20'25" E01°14'28" (1) Aledjo, gallery forest, N09°16'28" E01°13'21"
Lactifluus sudanicus	Atsu Guelly, 05/2008, C2137 Atsu Guelly, 05/2008, C2158 Dao Maba, 08/2008, MD15 Dao Maba, 06/2011, MD105 Dao Maba, 06/2011, MD148 Dao Maba, 07/2011, MD162 Dao Maba, 07/2013, MD360	(1) Aledjo, gallery forest, N09°16'12.5" E01°12'20" (1) Aledjo, gallery forest, N09°16'53.7" E01°13'41.2" (1) Aledjo, Woodland, N09°20'21" E01°14'18.9" (1) Aledjo, Woodland, N09°20'21", E01°14'18.9" (1) Fazao, Woodland, N08°42'21" E00°46'18"

		(3) Kou, deciduous rainforest, N11°11'17.5" E04°26'53.5" (3) Kou, deciduous rainforest, N09°16'28" E01°13'21"

Chapter 6

The genus *Lactifluus* (*Basidiomycota Russulales*) from West Africa: What do we know? Diversity, morpho-anatomy, molecular phylogeny and new species described presenting thromboplera and knobs.

Maba DL, Sanon E, Verbeken A, Kamou H, Agerer R (Mycological Progress. Under review)

The genus *Lactifluus* (*Basidiomycota Russulales*) from West Africa: What do we know? Diversity, morpho-anatomy, molecular phylogeny and new species presenting thromboplerous hyphae with knobs.

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Abstract

Mycological investigations of milkcaps are subject of ongoing research (world wide) since many samples are still waiting to be analyzed, and many vegetation types are in need for mycological inventories. This study want to give an update of the actual state of knowledge of *Lactifluus* in West Africa. About 44 *Lactifluus* species are presently known from the Guineo-Sudanian domain, 17 species (39.5%) are actually known only from this domain. Recent morpho-anatomical investigations based on specimens sampled from 2007 to 2013 in West African forest ecosystems, have enabled to assess species richness and distribution, of this ectomycorrhizal fungal group in the Guineo-Sudanian domain. This study includes new *Lactifluus* species with interesting anatomical features: thromboplera, commonly known as oleiferous hyphae, with knobs appearing attached to them. We generated 54 ITS sequences, aligned them against milkcap taxa from other tropical African ecozones (Guineo-Congolean evergreen forests, Zambezian miombo), and to worldwide representatives. A Maximum Likelihood phylogenetic tree was inferred from a dataset of 121 sequences. The phylogenetic placement of the specimens, combined with morpho-anatomical data, supports the description of three new *Lactifluus* species that are accommodated within *Lactifluus* subg. *Lactariopsis.* A new section within this subgenus is proposed to accommodate this group of species.

Key word: *Lactifluus, Lactarius*, molecular phylogeny, systematic, taxonomy, tropical Africa.

Introduction

Many tropical African vegetation types, including forests and woodlands, are dominated by numerous ectomycorrhizal fungi, with the genus *Lactifluus* (Pers.) Roussel as one of the best represented groups. Various investigations are being undertaken for a better knowledge of current species richness, distribution, ecology and molecular phylogeny of *Lactifluus* species (Stubbe et al. 2012; De Crop et al. 2013; Maba et al. 2013, 2014, 2015a, b). Moreover, previous studies, including those of Stubbe et al. (2010) and Van de Putte et al. (2010), have focused at *Lactifluus* species, and have highlighted their taxonomy, their systematic arrangement; and provided evidence of considerable species richness, and species complexity within this genus.

After a first monograph by Heim in (1955), Verbeken & Walleyn (2010) provided a more up-to-date monograph giving an overview of tropical African *Lactarius* s. l. taxa, including numerous species predominantly from the Congo-Zambian domain. Van Rooij et al. (2003) investigated *Lactarius* s. l. from Benin (West Africa), providing 22 species of which five were described as new. In addition, van de Putte al. (2009) described one new species from Togo, currently affiliated to subgenus *Lactifluus,* but it remains, according to Verbeken et al. (2012), with an uncertain position. Recent investigations by Maba et al. (2013, 2014, 2015a, b), based on morpho-anatomy, supported by molecular phylogenetic analyses, have repeatedly shown that species richness of *Lactifluus* could be high in tropical African ecosystems and particularly in the Guineo-Sudanian domain, where numerous new species have been discovered.

Moreover, Guineo-Sudanian forest ecosystems harbor various native ectomycorrhizal trees occurring in woodlands, open forests and riverside forests, as well as in rainforests (Rivière et al. 2007; Rinaldi et al. 2008; Bâ et al. 2012; Diédhiou et al. 2013, Maba et al. 2015a, b). Such ecosystems constitute a privileged domain where different ectomycorrhizal fungi, including *Agaricales, Boletales*, *Cantharellales, Russulales,* and *Thelephorales,* can be observed (Verbeken & Buyck 2001; Rivière et al. 2007; Yorou et al. 2011, 2012; Rinaldi et al. 2008; Sanon et al. 2013; Maba et al. 2015b) and new species, even in the well-studied genus *Lactifluus* remain to be discovered. Recent inventories and newly described species from Guineo-Sudanian forest ecosystems (Maba et al. 2013, 2015a, b) also provide striking new morphological characters.

To better understand the diversity and evolutionary history of species occurring in the Guineo-Sudanian domain, mycological inventories have been undertaken in various forest ecosystems of the above-mentioned domain from 2007 to 2013. This paper highlights the current state-of-the-art of the genus *Lactifluus* from West Africa. Using morpho-anatomy, coupled with molecular phylogenetic analysis of generated Internal transcribed Spacer (ITS) sequences, we propose three *Lactifluus* species as new to science, and a new section within *Lactifluus* subg. *Lactariopsis*.

Material and Methods

All specimens of the generated dataset were sampled in various riverside and open forests, and woodlands from Benin, Togo, Burkina Faso, and Guinea, from 2007 to 2013 following a Megatransect. The specimens described here were sampled in Mouhoun riverside forest (specimen MD166), Burkina Faso; in N'Dali (Parakou) woodlands (specimens MD304, MD307, MD308, MD309), in Gando woodlands (specimens MD317, MD320, MD326, MD329, MD333), Benin; in Moussaha woodlands (specimen MD211), Guinea; while specimen MD154 was sampled in Fazao Malfakassa National Park, and MD366 in Aledjo Reserve, Togo. Sampling methods, and preliminary morphological data recording, as well as specimen preparation for conservation follows Maba et al. (2013). Colours were recorded following Kornerup & Wanscher (1978). Collections and holotypes are deposited in TOGO herbarium (Thiers 2012).

Microscopy and scanning electron microscopy (SEM)

Microscopic studies focus on anatomical diagnostic features of lactarioids (Maba et al. 2015b). The borrowed species used by Maba et al. (2015a) were considered in the comparative microscopic studies. In addition, hymenophoral tramata were examined in ammonia Congo red (Horak 2005), in cresyl blue, in KOH and sulphoaldehyde referring to Clémençon (2012), and *Russulales* characters (http://www2.muse.it/russulales-news/in_characteristics.asp). SEM micrographs were obtained using the procedures applied by Maba et al. (2013). Preliminary identification of specimens were made using the monograph of tropical African lactarioids of Verbeken & Walleyn (2010), and the study of van Rooij et al. (2003), based on collections from the neighboring country, Benin.

DNA Extraction, sequencing and PCR amplification

Desoxyribonucleic acid (DNA) was extracted from dried specimens following the protocol used by Maba et al. (2013). The Internal Transcribed Spacer regions (ITS) of the nuclear ribosomal DNA, including ITS1, ITS2 and 5.8S regions, were amplified using the fungi specific primer ITS1F in combination with the basidiomycetes specific primer ITS4B (Gardes & Bruns 1993). In addition to the generated dataset by Maba et al (2015a), 12 ITS sequences of which 6 are newly generated were added. Sequences of new species are deposited at the European Nucleotide Archive (ENA), and species are registered at MycoBank.

Sequence editing, analyses and molecular phylogenetic inference

For getting a consensus sequence that could be included in our phylogenetic analysis, and to optimize the alignment, the generated dataset already compiled by Maba et al. (2015a) was considered, in which preference was given to tropical African sequences available in public GenBanks. Moreover, worldwide, closely related sequences with 90% minimum of similarity, and 95 % of Query cover, to our newly generated ones were downloaded and included in the new dataset, for molecular support of the newly recorded species.

The new 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 119 *Russulaceae* sequences including 93 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 09. 2014), as applied by Maba et al. (2015a). 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 121 rnDNA ITS sequences, and had a total length of 668 bp.

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (-8127.1935) is obtained and shown in figure 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search (Subtree-Pruning-Regrafting Extensive, level 5) were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach (Tamura et al. 2004). A discrete Gamma distribution (G) was used to model evolutionary rate differences among sites (6 categories (+G, parameter

= 0.2670)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0000% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 121 nucleotide sequences. There were a total of 668 positions in the final dataset. The phylogeny test was obtained by applying 1000 replicates (Felsenstein 1985). Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013)

Results

ITS rnDNA sequence analyses

The phylogenetic analysis conducted from a total of 121 sequences, resulted in the Maximum Likelihood tree shown in Fig. 1. All 119, *Russulaceae* sequences included in the analysis are monophyletic (100%). The inferred phylogenetic tree shows nine supported (60 to 100%) clades, that were annotated I to IX. In this phylogenetic analysis, each genus including *Lactifluus*, *Lactarius*, *Multifurca* and *Russula*, is monophyletic.

Clade I (Fig. 1 & 2), represents *Lactifluus* subg. *Lactariopsis,* and encompasses 23 sequences which correspond to 13 species, supported by 69% of bootstrap. The sequence of the newly described species (specimen MD166 from Burkina Faso) is nested within this clade, with 66% of support (Fig. 2). In addition, seven sequences form a monophyletic subclade of the subgenus *Lactariopsis* clade, with 70% bootstrap support. Of this subclade, five sequences from Benin (specimens MD 304, MD317, and MD320), Guinea (specimen MD211), and Togo (specimen MD366), form a subclade supported by 99%. The remaining two (MD154 from Togo, and MD307 from Benin) forming an own clade are supported by 100%. Both groups are sisters and represent two different species.

Clade II (Fig. 1) encompasses six sequences of four known species, and represents *L*. subg. *Russulopsis*. This clade is monophyletically nested within the *Lactifluus* clade and is supported as monophyletic by 60%.

A total of 14 sequences of nine known species and one unidentified sample from Cameroon, are representatives of *L*. subg. *Edules.* This subgenus forms clade III (Fig. 1), and is monophyletic (84%) within *Lactifluus*.

The large clade IV (Fig. 1) is complex, and encompasses 33 sequences of worldwide known species and represents *L.* subg. *Lactifluus*. However, the clade of two sequences of *Lactifluus aurantiifolius* (AY606987 and AY606986) is nested as a clade of a trichotomy within this complex clade, it forms its own section *Aurantiifolii* (Verbeken) Verbeken, and remains therefore with uncertain systematic position within *Lactifluus* subg. *Lactifluus* (Verbeken et al. 2011, 2012).

Clade (V), supported as a monophylum (79%), contains 17 sequences of known species; none of them is of African origin. This clade encompasses sequences of species that belong to well-delimited taxa: *Lactifluus* subgen. *Gerardii*, (subclade V-C), *Piperati* (subclade V-D), and *Lactifluus* subg. *Lactifluus* sect. *Lactifluus* (subclade V-B).

Genus *Multifurca* (clade VI) is nested between *Lactifluus* clade (i) and the monophyletic clade *Lactarius* (VII). This clade (VI) encompasses five representative sequences (none of them is of African origin) that represent three known species, and is monophyletic (83%) within *Russulaceae*. *Lactarius* clade (VII) encompasses 14 sequences, which correspond to 12 taxa, and is also monophyletic (96%) within *Russulaceae*. *Russula* sequences (seven worldwide) deviate as external monophyletic clade (VIII) by 99% bootstrap support.

Considering the distinctly separate position of collection MD166, collections MD154 and MD307, and collections MD366, MD308, MD320, MD317, MD2011, within *Lactifluus* subg. *Lactariopsis*, they are described as new species as *L. longicystidiatus*, *L. togoensis*, and *L. knobsoides*, respectively. Morpho-anatomical dissimilarities and in comparison to known lactarioid species (see below) support this conclusion.

Taxonomy

Lactifluus togoensis Maba, sp. nov. (Fig. 3-5)
MycoBank MB 813555

Etymology: Referring to the origin country of the type material

Type: **Togo**, Central region, Prefecture of Tchaoudjo, Fazao-Malfakassa National Park, N08°42'21" E00°46'22", in woodland dominated by *Isoberlinia doka*, *Isoberlinia tomentosa* and *Uapaca togoensis*, 19 June 2011, Dao Maba MD154 (Holotype, TOGO), Verbeken 11-180 (Isotype, GENT), ENA acc. Nº LK392607

Diagnosis:

Pileus 40-60 mm diam., thin to slightly thick-fleshed in the center; convex to plano-convex, depressed, then umbilicate; pellis dry, indehiscent, smooth, slightly glabrous, rather sticky; yellowish white to light yellow, much darker in the center. Lamellae broadly adnate to subdecurrent, very spaced, brittle, never forked. Context of pileus fleshy and firm in center, thin near margin, whitish; stipe stuffed, cavernous, white. Latex scarce, white, unchanging. Stipe 15-25 × 8-15 mm. Basidiospores 7.0-8.5-9.5 × 6.0-7.0-7.5 µm, with amyloid warty ornamentation, sometimes forming an incomplete reticulum; amyloid spot in the plage absent. Basidia 40-55 × 9-12 µm. Pleurocystidia absent. Pleuropseudocystidia 8-13 µm diam., abundant, irregularly subcylindrical, with apex sometimes tapering, capitate to mucronate, sometimes flared, emergent. Pileipellis ixocutis-like, composed of very densely interwoven, slightly ascending hyphae, irregularly shaped, subcylindrical, slightly subclavate, thin-walled, frequently septate, branched. Hymenophoral trama composed of a mixture of sphaerocytes, thromboplera irregularly shaped, thin-walled, with pale colored and homogeneous contents, rarely strongly septate, with knobs frequently attached on hyphae.

Pileus (Fig. 3A-C) 40-60 mm diam., thin near margin and slightly thick-fleshed in the center, initially convex to plano-convex, depressed, then umbilicate; pellis dry, indehiscent, smooth, slightly glabrous, rather sticky, striate up to 1/3 from the margin when dehydrated; yellowish white to light yellow (4A2-4), much darker in the center (4A5-7). **Margin** first smooth then slightly striate**,** inflected to incurved, downrolled in young basidiome, straight to slightly uprolled when older, striated. **Lamellae** broadly adnate to subdecurrent, irregular, unequal (L+l = 4-5/cm), very spaced, brittle, never forked; light yellow to pale orange (4A3-4). **Stipe** 15-25 × 8-15 mm, cylindrical, central, tapering downwards, fleshy and firm, concolorous to lamellae. **Context** of pileus fleshy and firm in the 2/3 from the center, thin near margin, whitish; stipe stuffed, cavernous; white. **Latex** scarce, whitish, unchanging; taste and smell not special.

Basidiospores (Fig. 4A & 5A-E) globose, subglobose to ellipsoid, 7.0-8.5-9.5 × 6.0-7.0-7.5 µm (Q=1.05-1.5-1.35; n=70), ornamentation amyloid; composed of warts (up to 0.5µm high) sometimes forming a partial or incomplete reticulum; amyloid spot in the plage absent. **Basidia** (Fig. 4D) (35)40-55 × 9-12 µm; 4-spored; subcylindrical to subclavate; sterigmata 3-6 ×1-2 µm. **Pleurocystidia** absent. **Pleuropseudocystidia** (Fig. 4C) very abundant, 8-13 µm diam.; irregularly subcylindrical, apex sometimes tapering, capitate to mucronate, sometimes flared; emergent and projecting above the hymenium; with needle-like brown contents in

Congo-red. **Hymenophoral trama** (Fig. 4D, subhymenium) mostly cellular, composed of a mixture of sphaerocytes, filamentous to irregularly shaped thromboplera, with thin walls, pale yellowish, homogeneous contents, rarely septate in the trama, but more in the subhymenium, with frequent, attached knobs; laticifers tortuous, some of them with knobs; knobs (Fig. 4E) 2-6 µm diam., abundant, spherical to ovoid, sometimes dome-like, appearing attached on the thromboplera, as well as on lactifers. **Lamellae edge** sterile. **Marginal cells** (Fig. 4F) 15-35 × 5-10 µm, variably shaped, subcylindrical, subclavate, apex sometimes tapering, mucronate, septate. **Pileipellis** (Fig. 4B) ixocutis-like, composed of very densely interwoven, slightly ascending hyphae of 3-6 µm diam., irregularly shaped, subcylindrical, slightly subclavate, thin-walled, frequently septate, branched, in mixture with some laticifers. **Stipitipellis** identical to pileipellis. **Clamp**s absent.

***Additional material examined*:** **Benin,** Borgou, Gando, N09°45'45.3’’ E02°19'55.9’’, deciduous woodlands dominated by *Isoberlinia tomentosa, I. doka*, and *Uapaca togoensis,* 17 July 2013, Dao
Maba MD307 (TOGO)

Lactifluus knobsoides Maba sp. nov. (Fig. 6-8)
MycoBank MB 813558

Etymology: Referring to the presence of numerous knobs attached to thromboplera and laticifers.

Type: **Benin,** Borgou, Gando, N09°45'45.3’’ E02°19'55.9’’, deciduous woodlands dominated by *Isoberlinia tomentosa, I. doka*, and *Uapaca togoensis,* 17 July 2013, Dao Maba MD320, (Holotype, TOGO), ENA acc. Nº LN849742
Diagnosis:
Pileus 40-90 mm diam., very thin, convex, plano-convex to depressed, finally umbilicate to infundibuliform; pellis indehiscent, dry, glabrous, rather sticky, cracked, veined to grooved, subfibrillose, striate near the margin; pale orange to light orange towards margin, darker in the center. Lamellae broadly adnate, moderately spaced. Context very thin-fleshed in the pileus, brittle, slightly thick in the center, in stipe firm; whitish to pastel yellow. Latex very scarce, white, unchanging. Stipe 15-35 × 10-15 mm. Basidiospores 8-9.5-10 × 7.0-7.5-8.5 µm, with well developed to irregular amyloid warts, ornamentation often interconnected, plage

sometimes with an amyloid spot. Basidia 50-60 × 10-13 µm. Pleurocystidia present as pleuroleptocystidia 45-60 × 9-11 µm, abundant, moniliform to rostrate-like, thin-walled. Pileipellis a cutis to ixocutis, composed of very densely interwoven, slightly ascending hyphae, irregularly shaped, subcylindrical, subclavate, thin-walled, septate, and often branched. Hymenophoral trama composed of a mixture of sphaerocytes and thromboplera, irregularly shaped, thin-walled, with pale colored, homogeneous contents, rarely strongly septate, with frequent knobs attached to the hyphae.

Description: **Pileus** (Fig. 6A-F) 40 - 90 mm diam., very thin, at first convex then plano-convex, depressed, finally umbilicate to infundibuliform; pellis dry, indehiscent, glabrous, sometimes rather sticky, cracked, veined to grooved, sometimes subfibrillose, striate near margin; pale orange (5A3) to light orange (5A5-6) towards margin, darker in center (5A7). **Margin** inflected to incurved when young then straight to slightly uprolled, sometimes striate in old specimens. **Lamellae** broadly adnate, distinctly distant or moderately spaced (L+l = 4-5-6/cm), often bifurcate near the insertion to the stipe, unequal, irregular, with two different size types of lamellulae, yellowish white to pastel yellow (4A2-4). **Stipe** concolorous to pileus, 15-35 × 10-15 mm, short and relatively stout, smooth in young basidiome, fibrillose when old, cylindrical, central and tapering downwards, dry, fleshy and firm; an evanescent whitish annulus remaining on the stipe. **Context** very thin-fleshed in the pileus, brittle, slightly thick in the center, in stipe firm; whitish to pastel yellow (4A2-3). **Latex** very scarce, unchanging; taste and smell not special.

Basidiospores (Fig. 7D, 8A-C) broadly ellipsoid to sometimes elongate, rarely subglobose, (7.5)8-<u>9.5</u>-10(11) × 7.0-<u>7.5</u>-8.5 µm (Q=1.15-<u>1.25</u>-1.3(1.40); n=65); ornamentation amyloid, composed of well-developed irregular warts, often interconnected and forming sometimes a reticulum; plage with faintly to strong amyloid spot. **Basidia** (Fig. 7C) 50-60 × 10-13 µm; 4-spored, 2-spored, and 1-spored, with predominance of 4-spored basidia; subcylindrical to subclavate, tapering downwards. **Pleurocystidia** present as **pleuroleptocystidia** 45-60 × 9-11 µm, abundant, moniliform to rostrate-like, thin-walled. **Pleuropseudocystidia** (Fig. 7B) 8-15 µm diam.; abundant, emergent, irregularly shaped, fusiform to tortuous, subcylindrical, apex tapering, mucronate, sometimes inflated, sometimes forked, often bulged, sometimes with knobs, contents brown, needle-like in Congo-red. **Hymenophoral trama** mostly cellular composed of a mixture of sphaerocytes and thromboplera (Fig. 7E) that are filamentous to irregularly shaped, thin-walled, with pale and homogeneous contents; rarely septate in the trama, but strongly in the subhymenium, with frequent knobs attached to hyphae; some tortuous laticifers (Fig. 7G) also with knobs. Knobs (Fig. 7E) 2-9 µm diam., abundant, spherical to ovoid, sometimes dome-like. **Lamellae edge** sterile. **Marginal cells** (Fig. 7F) 15-40(50) × 3-5 µm, irregularly shaped, fusiform, tortuous, sometimes forked or bifurcate, rarely subcylindrical, septate. **Pileipellis** (Fig. 7A) a cutis to ixocutis, composed of very densely interwoven, slightly ascending hyphae of 3-10 µm diam., hyphae irregularly shaped, subcylindrical, subclavate, thin-walled, septate, often branched, in mixture with numerous laticifers. **Stipitipellis** identical to pileipellis. **Clamp**s absent.

***Additional material examined*:** **Benin,** Parakou, N'Dali, N09º45'52.3'' E02º35'8.2'', in woodland dominated by *Isoberlinia doka* and *I. tomentosa*, 7 July 2013, Dao Maba MD304, MD307, MD308, MD309, (TOGO). - Borgou, Gando, N09°45'45.3'' E02°19'55.9'', in deciduous woodlands dominated by *Isoberlinia tomentosa*, *I. doka*, and *Uapaca togoensis,* 17 July 2013, Dao Maba MD317, MD320, MD329, MD333, (TOGO). - **Guinea,** Moussaha, in woodlands dominated by Uapaca esculenta and *Anthonotha fragrans*, N10º41'27'' W09º58'40'', 13 July 2011, Dao Maba MD211, (TOGO). - **Togo**, Central region, Bafilo, N09º20'25.4'' E01º14'27.7'', in woodland dominated by *Isoberlinia tomentosa* and *Uapaca togoensis,* 17 July 2013, Dao Maba MD366, (TOGO)

Lactifluus longicystidiosus Maba sp. nov. (Fig. 9-11)
MycoBank MB813561

Etymology: Referring to the distinctly emerging cystidia (pleuropseudocystidia) above the hymenium

Type: **Burkina Faso**, Bobo Dioulasso, N10º53'36'' W04º50'22.9'', in gallery forest dominated by *Berlinia grandiflora,* 8 July 2011, Dao Maba MD166, (Holotype, TOGO), ENA acc. Nº LN849748

Diagnosis:

Pileus 45-85 mm diam., convex, plano-convex to depressed, infundibuliform; pellis indehiscent, wet, slightly fibrillose, striate, smooth in the center; pale orange to light orange. Lamellae broad, subdecurrent to decurrent, wide spaced. Context very thin near the margin, fleshy, firm in the center of the pileus; stipe stuffed, medullary, whitish; taste slightly acrid. Latex not abundant, white, unchanging. Stipe 25-35 × 10-15 mm. Basidiospores 6.5-8.5-10.5 × 5.5-7.5-9 µm, with amyloid warty ornamentation, sometimes interconnected, plage with amyloid spot. Basidia 45-70 × 10-12 µm, four-spored. Pleurocystidia present as pleuroleptocystidia 45-65 × 5-8 µm, abundant, moniliform to rostrate-like, thin-walled. Pleuropseudocystidia 4-15 µm diam., scarce to abundant. Pileipellis a cutis to trichoderm-like, with a mixture of abundant subcylindrical to irregularly shaped, thin-walled hyphae with slender, hair-shaped terminal, thick-walled elements, which are oriented in all directions. Marginal cells of the lamellae 35-75 × 4-8 µm, highly tortuous, fusiform, forked to very diverticulate, thin-walled, septate.

Description: Basidiome (Fig. 9) uniformly colored. **Pileus** 45-85 mm diam., thin-fleshed, at first convex to plano-convex, slightly depressed, finally infundibuliform; margin striate, at first incurved, then straight; pellis indehiscent, wet, slightly fibrillose, striate up to 1/3 from the margin, smooth in the center; pale orange (5A3) to light orange (5A4). **Lamellae** broad (up to 8 mm), subdecurrent to decurrent, wide spaced, slightly thick, brittle, unequal, irregular, with two different size types of lamellulae, with one lamellula between 2 lamellae (L+l=3-4/cm), never forked; light orange (5A4). **Stipe** 25-35 × 10-15 mm, cylindrical, tapering downwards, smooth; pale orange (5A3). **Context** very thin up to 2/3 from the margin, fleshy, firm in the center of the pileus; stipe stuffed, medullary, whitish; taste slightly acrid. **Latex** white, not abundant, and unchanging. **Chemical reaction**: context unchanging with KOH and $FeSO_4$.

Basidiospores (Fig. 10F, 11A-D) globose, subglobose to ellipsoid, 6.5-8.5-10.5(11) × 5.5-7.5-9 µm (Q=1.00-1.5-1.35-; n=65); amyloid ornamentation composed of warts, sometimes interconnected; plage with distinct, amyloid spot. **Basidia** (Fig. 10A, E) 45-70 × 10-12 µm, subcylindrical and tetra-spored. Pleurocystidia present as pleuroleptocystidia (Fig. 10C) 45-65 × 5-8 µm, abundant, moniliform to rostrate-like, thin-walled. **Pleuropseudocystidia** (Fig. 10A, B) scarce to abundant, 4-15 µm diam., tortuous to fusiform, tapering upwards, mucronate, distinctly emerging, sometimes up to 130 µm above the hymenium, content needle-like and granular in Congo red. **Lamellar edge** sterile. **Hymenophoral trama** (Fig. 10A, subhymenium) cellular, composed of a mixture of sphaerocytes and laticifers. **Marginal cells** (Fig. 10D) 35-75 × 4-8 µm, irregularly shaped, highly tortuous, fusiform, forked to very diverticulate, rarely subcylindrical, septate, thin-walled. **Pileipellis** (Fig. 10E) an interwoven cutis transgrading to trichoderm-like, a mixture of abundant subcylindrical to irregularly shaped, thin-walled hyphae, 2-4 µm diam., with slender hair-shaped terminal (up to 205 µm long), thick-walled elements, which are oriented in all directions; sometimes septate and branched. **Stipitipellis**, similar to pileipellis, thick-walled hyphae less abundant than in pileipellis, but strongly diverticulating, bifurcate, or tortuous. **Clamp**s absent.

Discussion

Our three proposed new species belong to *L.* subg. *Lactariopsis*, a subgenus which is containing species with a distinct veil and a stipe presenting a distinct, more or less fugacious annulus, as well as species without an annulus but sometimes with velar remnants at the pileus' margin (Verbeken & Walleyn 2010). The annulate species of this subgenus are accommodated in *L.* sect. *Lactariopsis* Verbeken (Verbeken), while species without annulus form *L.* sect. *Chamaeleontini* (Verbeken) Verbeken. But this division is purely practical and not supported by molecular data as already mentioned in Verbeken & Walleyn (2010). Microscopically the subgenus is characterized by thick-walled elements in the pileipellis, abundant sphaerocytes in the hymenophoral trama, very scarce pleurocystidia, and abundant, emergent and broad pleuropseudocystidia. A lamprotrichopalisade pileipellis structure and absence of pleurocystidia are common for *L.* sect. *Lactariopsis*, while a cutis or an ixocutis-like structure transgrading to a trichoderm, a trichopalisade pileipellis, and the presence of pleurocystidia is characteristic for *L.* sect. *Chamaeleontini.*

Morphologically *Lactifluus longicystidiosus* is rather close to *Lactifluus sesemotani* (Beeli) Buyck, and *Lactifluus laevigatus* (Verbeken) Verbeken. These species do not have an annulus, and have the same habitus as *L. longicystidiosus*. But *Lactifluus sesemotani* is thick-fleshed, with dehiscent and smooth pellis, yellowish ochre then ochraceous (4A4-7 to 5AB4), while *Lactifluus longicystidiosus* is thin-fleshed, with an indehiscent, up to 1/3 from the margin striated pellis, with pale orange (5A3) to light orange (5A4) coloration. The also thick-fleshed *L. laevigatus* with light orange to greyish orange (4A5 to 5AB5) color is morphologically closer to *L. longicystidiosus* by an indehiscent pellis, which is slightly fibrous, and a striated margin. Both have one lamellula between two lamellae, while *L. sesemotani* has zero to one in between as described in Verbeken & Walleyn (2010).

Microscopically, *Lactifluus longicystidiosus*, and *L. sesemotani* have similar basidiospores, globose to subglobose and rarely ellipsoid, and subglobose to ellipsoid, respectively. However, unlike *L. sesemotani*, *L. longicystidiosus* has a plage with a distinct amyloid spot. Both species differ distinctly by the shape of their marginal cells, which are very tortuous, very diverticulate, and mostly bifurcate in *Lactifluus longicystidiosus*, while fusiform to slightly inflated in *L. sesemotani* (Verbeken & Walleyn 2010). In addition, *L. sesemotani* has a mixed trichopalisade pileipellis structure that presents isodiametric to fusiform suprapellis elements (Verbeken & Walleyn 2010). On the contrary, *L. longicystidiosus* shows an interwoven cutis transgrading to a trichoderm-like pileipellis, composed of a mixture of subcylindrical, thin-walled hyphae, and very slender, hair-shaped, thick-walled elements oriented in all directions, that are similar to those of *Lactifluus madagascariensis* (Verbeken & Buyck) Buyck, another representative of *L.* sect. *Chamaeleontini* (Verbeken & Walleyn 2010). However, unlike *L. longicystidiosus, Lactifluus madagascariensis* has a pale to straw yellow pellis, darker in the center, pale near margin; latex absent, whereas it is well observed in *L. longicystidiosus*; pleuroleptocystidia are absent, its marginal cells are mostly cylindrical, and basidiospores broadly ellipsoid (Verbeken & Walleyn 2010). This is confirmed by their genetic divergence by regarding their placement in the phylogenetic analysis. *Lactifluus laevigatus* differs considerably from *L. longicystidiosus* by its broadly ellipsoid basidiospores, an ixocutis as peleipellis with recumbent and ascending chains of short cylindrical elements (Verbeken & Walleyn 2010).

Microscopically some species of *L.* subg. *Lactariopsis* sect. *Chamaeleontini* present leptocytidia, and a pileipellis a structure, including a trichoderm and ixocutis to cutis-like structure, dissimilar to the species of sect. *Lactariopsis* (Verbeken & Walleyn 2010). *Lactifluus longicystidiosus* differs considerably to all above-mentioned species by its very tortuous and diverticulate marginal cells, thick-walled, hair-like, very slender, up to 205 µm long elements of pileipellis. In addition, it has very abundant and striking pleuropseudocystidia, emerging up to 130 µm above the hymenium (although the feature of emergent and large pseudocystidia is typical for the subgenus, these dimensions have never been observed in any known species), and the presence of abundant pleuroleptocystidia. Similar pleuroleptocystidia of 65-80 × 8-10 µm, occur in *Lactifluus indusiatus* (Verbeken) Verbeken, but are unknown in other species.

Compared to the already known tropical African *Lactifluus* species, *Lactifluus emergens* (Verbeken) Verbeken is in some respect morphologically close to the newly described *Lactifluus togoensis*. Both species have a dry, smooth, glabrous, and rather sticky pellis, that is yellowish white to light yellow, but darker in the center in *L. togoensis* (4A5-7). In addition, both have very spaced and brittle lamellae, that are however thick and anastomosing at some places in *L. emergens*, while these are thin, and never forked in *L. togoensis*. They differ distinctly by their pileus margin. It is at first smooth to strongly striate and grooved in *Lactifluus emergens* (Verbeken & Walleyn 2010), while it is first smooth then slightly striate, inflected to incurved, bent downwards when young, then straight to slightly uprolled when older in *Lactifluus togoensis*.

Both latter species differ considerably by their microscopic features. *Lactifluus emergens* has basidiospores with amyloid ornamentation composed of very weakly developed (less than 0.2µm high) warts, while the ones of *Lactifluus togoensis* are composed of distinct warts (up to 0.5µm high) forming sometimes an incomplete reticulum. In addition, *L. emergens* has a trichopalisade-like pileipellis, with abundant thick-walled elements, whereas *Lactifluus togoensis* has an ixocutis-like pileipellis, composed of very dense, interwoven irregularly shaped hyphae, thick-walled elements are absent. Above all, *Lactifluus togoensis* has many thromboplera, which have knobs as described above, and pleuropseudocystidia with knobs, unknown for *L. emergens*. Due to this peculiar structure *L. togoensis* is certainly closely related to *Lactifluus knobsoides*, as confirmed by their molecular-phylogenetic affinity.

The newly described *L. knobsoides* is characterized by a thin flesh, a dry, indehiscent, glabrous, cracked, veined, subfibrillose, rather sticky pellis, sometimes striated near the margin; and by a pale orange to light orange colour at the margin, darker in the centre. This combination of morphological features does not fit to any known *Lactifluus* species. Nonetheless, *L. knobsoides* is somewhat close to *L. emergens* by a glabrous, veined and subfibrillose pellis, which is rather sticky, too. But both species differ by the basidiome colour, which is yellowish white to light yellow at the margin in *L. emergens*, as compared to *L. knobsoides* that is pale orange to light orange there. In addition, unlike *L. emergens,* some specimens of *L. knobsoides* have a distinct evanescent whitish annulus on the stipe. Moreover, *L. knobsoides* has in contrast broadly adnate lamellae that are unequal, irregular, and often bifurcate near the insertion to the stipe. Microscopically *L. knobsoides* has a cutis to ixocutis pileipellis, composed of very dense, interwoven, thin-walled, irregularly shaped, subcylindrical, subclavate, often branched and septate hyphae, similar to those of *L. cyanovirescens* (Verbeken) Verbeken, as described in Verbeken & Walleyn (2010). *Lactifluus emergens*, however, has a trichopalisade-like pileipellis (Verbeken & Walleyn 2010). Moreover, marginal lamellae cells of *L. knobsoides* are irregularly shaped, fusiform, tortuous, sometimes forked or bifurcate, rarely subcylindrical, unlike those of *L. emergens* that are mostly subcylindrical. This combination of morpho-anatomical features makes *L. knobsoides* distinctly different from known annulate species, of *Lactifluus* subg. *Lactariopsis*, as well as from *L. togoensis*, its most closely related species. Both have a pileipellis structure without thick-walled elements, recalling that of *L. laevigatus* of *L.* subg. *Lactariopsis* sect. *Chamaeleontini*. In addition, the pileipellis structures of these two new species are slightly similar to those of *L.* subg. *Russulopsis* sect. *Russulopsidei* (Verbeken) Verbeken, including *Lactifluus cyanovirescens* and *L. urens* (Verbeken) Verbeken (see Verbeken & Walleyn 2010).

Lactifluus togoensis and *Lactifluus knobsoides* have abundant knobs and thromboplera as described above. These latter mentioned microscopic characters, even distinctly different, recall the clamp-like structures mentioned by Verbeken & Walleyn (2010) for some tropical African milkcaps including *Lactarius amarus* R. Heim, *Lactifluus brunnescens* (Verbeken) Verbeken, and *Lactifluus aurantiifolius* Verbeken, but the identity of these structures of the latter species' is unclear, and only short descriptions are given to these clamp-like forms observed in the stipitipellis of *L. aurantiifolius,* as not being more frequent towards the base but best observed halfway the stipe (Verbeken & Walleyn 2010). These features differ considerably to the thromboplera and knobs observed in the new species.

L. togoensis and *L. knobsoides* not only share this striking character, being numerous thromboplera and knobs, which was formerly never observed in the milkcaps, but they also form a distinct separate clade within the subgenus based on our ITS analysis.

This justifies the creation of a new section, which is here proposed to accommodate *Lactifluus togoensis* and *L. knobsoides*: *Lactifluus* subg. *Lactariopsis* sect. *Thrombopleri.*

Repetitive observations of the hymenophoral trama of 12 specimens sampled in three countries, led to identify two particular anatomical features: secretory hyphae, called thromboplera (Clémençon 2012), formerly and commonly known as oleiferous hyphae (Fayo 1889) that bear characteristic knob-like extensions. Thromboplera are according to Clémençon (2012), very long, very irregularly shaped, and thin-walled hyphae with pale coloured, homogeneous contents, orthochromatic in cresyl blue, rarely septate in the trama, but more frequent in the subhymenium of the lamellae. They are denser there, even interwoven and terminally bifurcate, where they form a continuum with generative hyphae from which they derive. Knobs are frequently attached to the hyphae, are subspherical to ovoid. They are more frequently attached to the thromboplera, but also to certain laticifers, where they show the same needle-like contents. They are present in most *Russulales* (http://www2.muse.it/russulales-news/in_characteristics.asp; Clémençon 2012), but these secretory hyphae with their knobs have never been described for tropical lactarioids. Both structures are thus identified for the first time in tropical African *Lactifluus* species. Future investigations will lead to a better knowledge of these distinctive characters.

Lactifluus subg. Lactariopsis* sect. *Thrombopleri Maba & Agerer, sect. nov.

MycoBank MB813565

Etymology: Referring to the presence of numerous thromboplera (oleiferous hyphae) in both newly described species.

Type species: ***Lactifluus knobsoides*** Maba

Lactifluus subg. *Lactariopsis* sect. ***Thrombopleri*** is characterized by basidiomes with an evanescent whitish annulus remaining on the stipe, the presence of thromboplera with frequent knobs attached to the hyphae, and tortuous laticifers, some of which with knobs. Presence of pleuroleptocystidia, a cutis to ixocutis-like pileipellis, composed of very dense, interwoven and thin-walled, irregularly shaped, subcylindrical, slightly subclavate, septate, and often branched hyphae.

Differs from *Lactifluus* (Pers.) Roussel subg. *Lactariopsis* (Verbeken) Verbeken sect. *Lactariopsis* (Verbeken) Verbeken by the presence of pleuroleptocystidia and a pileipellis/stipitipellis with entirely absence of thick-walled and hair-shaped elements; from sect. *Chamaeleontini* (Verbeken) Verbeken by an evanescent annulus remaining on the stipe, and fusiform, tortuous, sometimes forked or bifurcate marginal cells; from sect. *Albati* (Bataille) Verbeken by emergent pleuropseudocystidia, presence of leptocystidia, and the absence of macrocystidia (Heilmann-Clausen et al. 1998). Section *Thrombopleri* differs from all above-mentioned sections by a cutis to ixocutis-like pileipellis structure, presence of thromboplera, with frequent knobs attached to the hyphae, and tortuous lactifers, some of which bearing knobs, too.

Identification key

Pileipellis a cutis to ixocutis, composed of very dense, interwoven and thin-walled, irregularly shaped, subcylindrical, slightly subclavate, septate, and often branched hyphae. Hymenophoral trama mostly cellular, composed of a mixture of sphaerocytes, thromboplera, with frequent knobs; laticifers tortuous, some of them with knobs as those of thromboplera. Hitherto known from Benin, Guinea, and Togo……………………………………………………………………***L.* sect. *Thrombopleri***

1. Basidiome without an annulus; pileus margin later straight, striated; lamellae very spaced, never forked; pleurocystidia absent; basidia 4-spored; basidiospores globose, subglobose to ellipsoid, 7.0-8.5-9.5 × 6.0-7.0-7.5 µm;....………………........................***Lactifluus togoensis***

2. Basidiome with an evanescent whitish annulus; pileus margin slightly downrolled, then straight to slightly uprolled, striate; lamellae broadly adnate to subdecurrent moderately spaced, strongly bifurcate near the insertion to the stipe; pleurocystidia present, formed as pleuroleptocystidia; basidia 4-spored, 2-spored, and 1-spored; basidiospores, broadly ellipsoid to somewhat elongate, rarely subglobose, 8-9.5-10 × 7.0-7.5-8.5 µm. ………………………………………………………………...…***Lactifluus knobsoides***

West African *Lactifluus* and future perspectives

The genus *Lactifluus* totalizes actually about 141 species worldwide, about 136 of them are affiliated to six subgenera. Five have a still unresolved position, thereunder the tropical African species *L. aurantiifolius* Verbeken, *L. cocosmus* van de Putte & De Kesel, *L. kigomensis* De Crop & Verbeken, and *L. rufomarginatus* Verbeken & van Rooij. The actual number of Lactifluus species includes recent investigations by De Crop et al. (2012), Miller et al. (2012), Morozova et al. (2013), Sá & Wartchow (2013), Sá et al. (2013), Maba et al. (2013, 2015a, b; and above described species).

The present study confirms the high species richness of the genus *Lactifluus* in tropical African forest ecosystems, in comparison to its sister genus *Lactarius* which totalized 40 known species (Maba et al. 2014). About 60% of accepted tropical African *Lactifluus* species (about 73) occur in West African vegetation types, accommodated systematically in four subgenera (Fig. 12), including subg. *Edules* (7species), *Lactariopsis* (12 species), *Lactifluus* (16), *Russulopsis* (6 species), and three species remain unclassified regarding section affiliation. Representative species of *Lactifluus* subgenera *Gerardii* (A.H. Sm. & Hesler) Stubbe and *Lf.* subg. *Piperati* Verbeken have not been harvested in Africa yet. Although many samples are still awaiting analysis, and many West African vegetation types remain unexplored regarding mycological inventories, 43 *Lactifluus* species (table 2) are known from West Africa forest ecosystems, of which 17 are actually only known from GS domain. With about 28 species generally known until now of *L.* subg. *Lactariopsis* (Verbeken et al. 2011;

Miller et al. 2012; Morozova et al. 2013; Sá & Wartchow 2013; Sá et al. 2013; Maba et al. 2013, 2015a, b), including the three newly described in this study, 17 are thus occurring in tropical Africa, but very probably many more remain to be described.

Although species richness of ectomycorrhizal trees in GS domain could be smaller as compared to the Congo-Zambezien (CZ) domain (Verbeken & Buyck 2001), ectomycorrhizal fungal species richness in general and particularly of lactarioids within GS forest ecosystems may approach those of the CZ domain. Many collections from GS forest ecosystems remain unidentified, and many vegetation types that harbour native ECM trees are so far very poorly or never investigated, in term of mycology. Probably, co-occurrence of host tree species in GS could promote lactarioids diversity at local scale by providing unique habitats for host-specific taxa, as suggested by Tedersoo et al. (2012) for ECM fungal richness. Interestingly, although the CZ domain has been investigated more intensively than the GS regarding *Russulaceae*, six (about 14%) of known *Lactifluus* species (table 2) seem to be restricted to GS. These species are common and have been frequently harvested in vegetation types of this latter mentioned domain. In particular, species including *Lactifluus sudanicus* Maba et al., *L. membranaceus* Maba, *L. foetens* (Verbeken & van Rooij) Verbeken, *L. rufomarginatus* Verbeken & van Rooij, *Lactifluus togoensis* and *L. knobsoides* have been sampled in at least two countries of WA (table 2), and some even repeatedly (e.g. *Lactifluus sudanicus* and *L. foetens*). Ecologically, the newly described *Lactifluus longicystidiosus* is known only from one locality (Burkina Faso), from riverside forest, while *Lactifluus togoensis* was sampled twice in woodlands from Benin and Togo. Thus, future investigations/inventories will help to a better knowledge of the distribution/niche differentiation of both species. On contrary, *Lactifluus knobsoides* has been sampled in four different woodlands that harbor mostly *Isoberlina* spp., and *Uapaca* spp., from three countries (Benin, Guinea and Togo), suggesting thereby its preference to this vegetation type.

Evidently, mycological investigations within *Russulaceae* and particularly milkcaps are subject of ongoing research. Recent investigations within *Lactifluus* from Westafrica, continue to provide morpho-anatomical features of taxonomic relevance. Moreover, endemism status of some lactarioids in tropical Africa has been suggested by previous studies including those of van Rooij et al. (2003), Verbeken &Walleyn (2010), Verbeken et al. (2011), Verbeken & Nuytinck (2013). Phylogenetically, although distant clades fall sometimes apart due to the high genetic variability (Verbeken et al. 2011; Verbeken & Nuytinck 2013), all *Lactifluus* representative sequences integrated in this phylogenetic analysis are monophyletically supported (81%), confirming thereby the previous studies of

Verbeken et al. (2011, 2012). The present investigation, although supporting the monophyly of the genus *Lactifluus* clade, highlighted the paraphyly/polyphyly of *Lactifluus* subg. *Lactifluus* clade (IV + V-B), as well as *L.* subg. *Lactariopsis* clade (I).

Current progress in molecular tools including metabarcoding, appears to be a suitable tool in these mycological investigations (Epp et al. 2012; Riaz 2011; Tedersoo et al. 2012). It will allow to access the fungal mycobiota, including species richness, ecological status of species and their habitats, and to elucidate the phylogenetic kinship/relationship, and evolutionary tendencies. It appears therefore urgently necessary to intensify mycological inventories particularly in tropical Africa, where threats on forest ecosystems by human actions are still increasing.

Acknowledgment

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Figure legends

Figure 1. Best Maximum Likelihood (ML) molecular phylogenetic tree showing the placement of our generated sequences, including the three newly described species (*L. knobsoides,* and *L. longicystidiosus, L. togoensis*) of worldwide sequences obtained from public GenBanks. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 121 nucleotide sequences.

Figure 2. *Lactifluus* subg. *Lactariopsis* subtree clade separated from the original tree. The four representative sections, including the newly delimited section *Thrombopleri,* are shown.

Figure 3. Basidiome of *Lactifluus togoensis*: A, C. General view (MD154). B. Pileus view (MD307). – Scale Bars = 10 mm.

Figure 4. Light microscopy of *Lactifluus togoensis* (MD154). A. Basidiospores. B. Pileipellis. C. Pleuropseudocystidia. D. Hymenium and subhymenium. E. Some thromboplera with knobs. F. Marginal cells. – Scale Bars = 10 µm.

Figure 5. SEM of *Lactifluus togoensis* basidiospores (MD154): A. General view. B. Lateral to ventral view. C. Dorsal to basal view. D. Lateral view. E. Apical to ventral view.

Figure 6. Basidiome of *Lactifluus knobsoides* A-C. Pileus surface (A: MD320; B: MD317; C: MD329). D-F. Lamellae and stipe. (D: MD320; E: MD329; F: MD317). – Scale Bars = 10 mm.

Figure 7. Light microscopy of *Lactifluus knobsoides* (MD320). A. Pileipellis. B. Pleuropseudocystidia. C. Basidia. D. Basidiospores. E. Portions of thromboplera with knobs. F. Marginal cells. G. Portions of laticifers with knobs – Scale Bars = 10 µm.

Figure 8. SEM of *Lactifluus knobsoides* (MD320). Basidiospores: A, C. Lateral to dorsal view. B. Lateral view.

Figure 9. Basidiome of *Lactifluus longicystidiosus* (MD166). Pileus, lamellae and stipe. – Scale Bar = 10 mm.

Figure 10. Light microscopy of *Lactifluus longicystidiosus* (MD166). A. Subhymenium. B. Pleuropseudocystidia. C. Marginal cells. D. Basidia. E. Pileipellis. F. Basidiospores. – Scale Bars = 10 µm.

Figure 11. SEM of *Lactifluus longicystidiosus* (MD166). Basidiospores: A. Dorsal to ventral view. B. ventral view. C. Apical to ventral view. D. Dorsal view.

Figure 12. Species richness of *Lactifluus* subgenera worldwide, in tropical Africa, and in West Africa.

Fig. 1. Phylogenetic tree

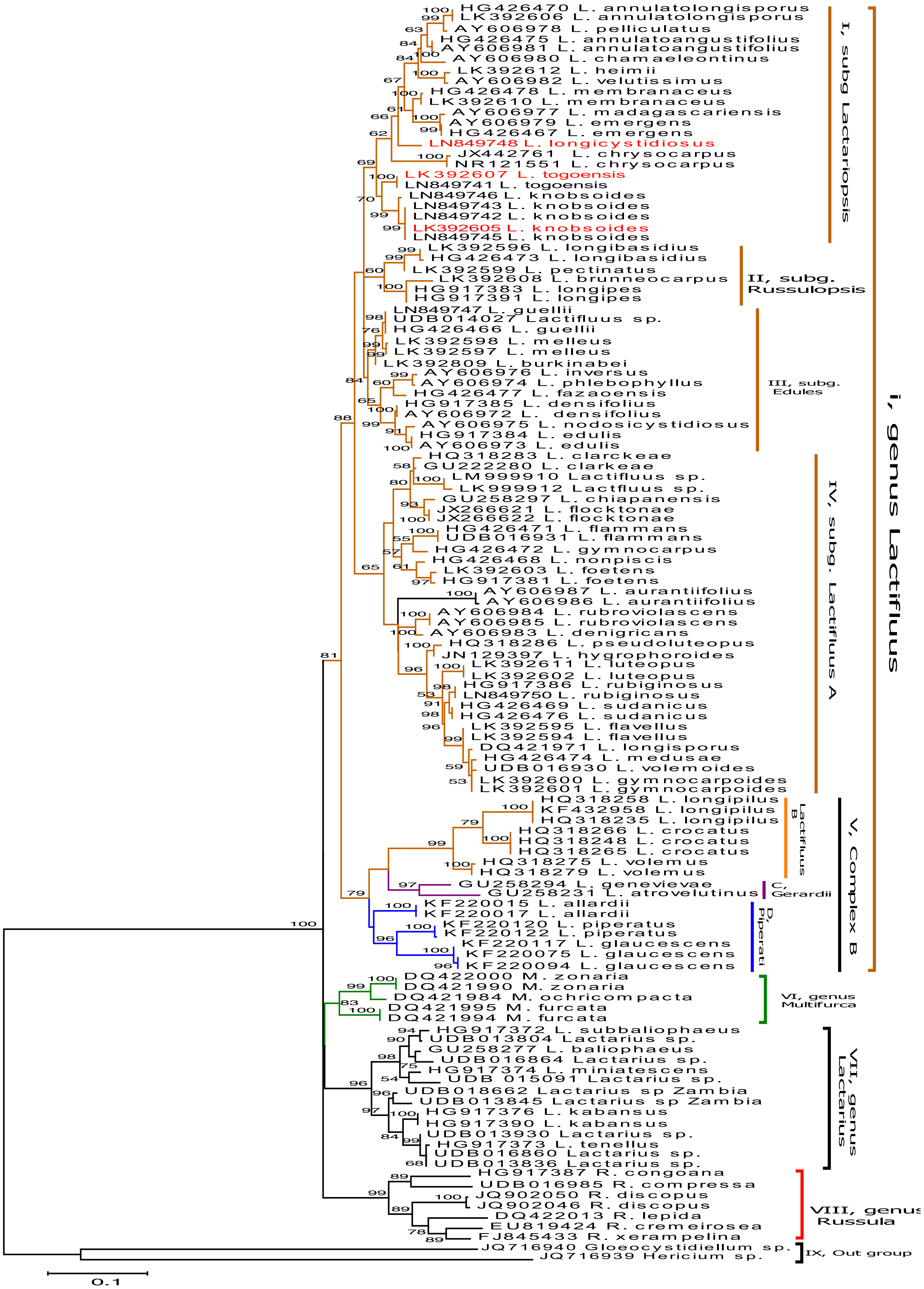

Fig. 2. Subtree Subgenus *Lactariopsis*

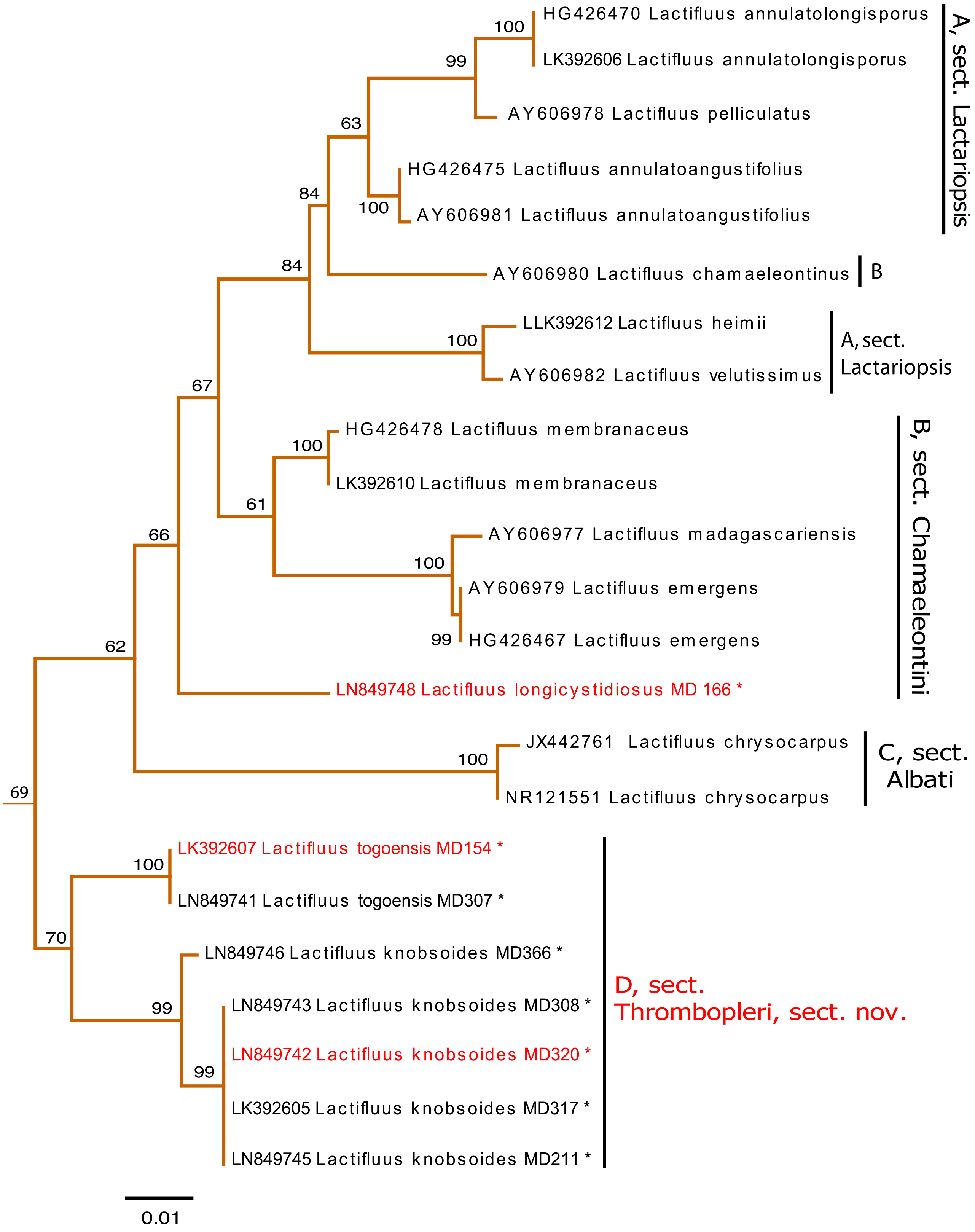

Fig. 3. Basidiome *Lactifluus togoensis*

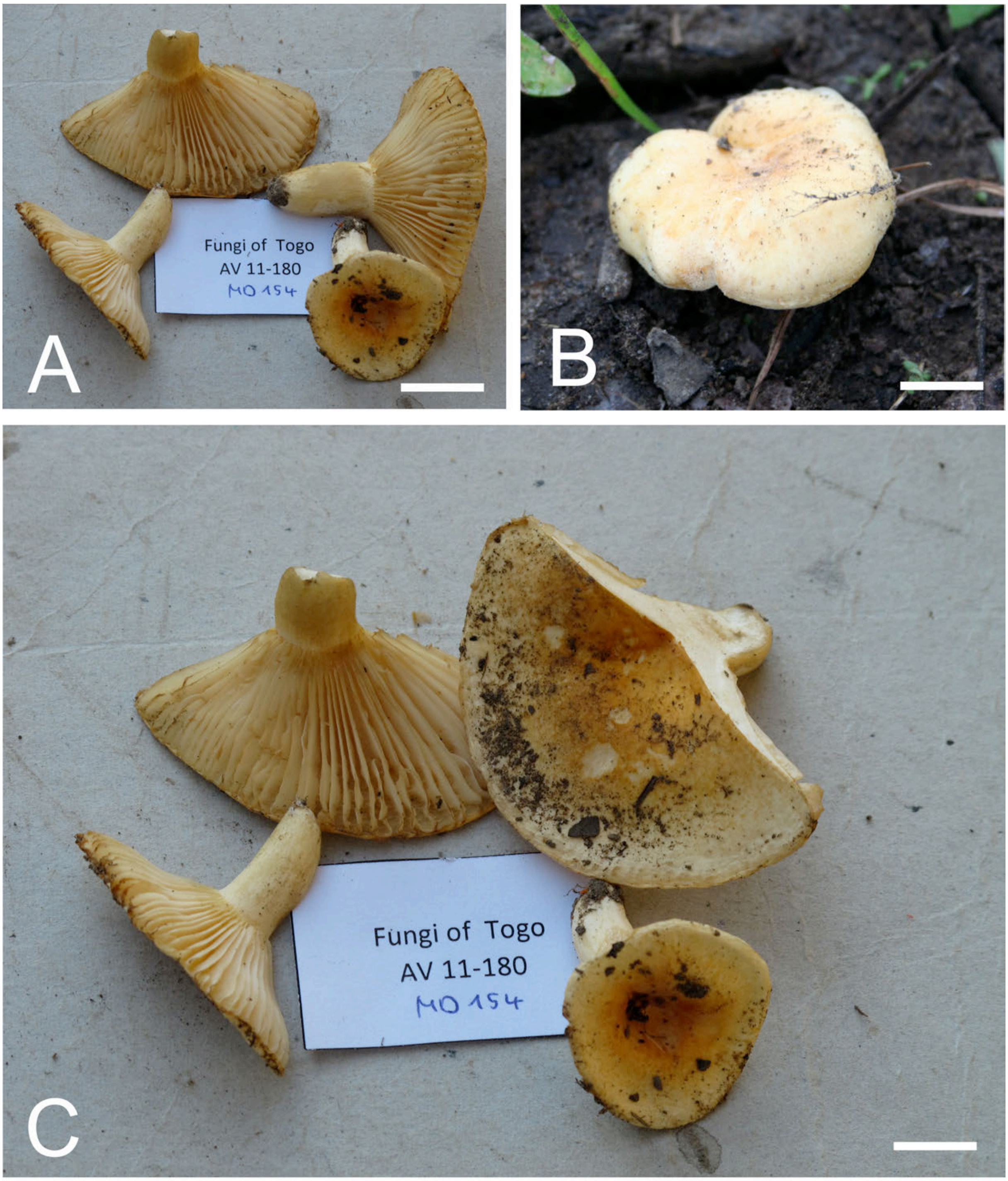

Fig. 4a. Light microscopy *Lactifluus togoensis*

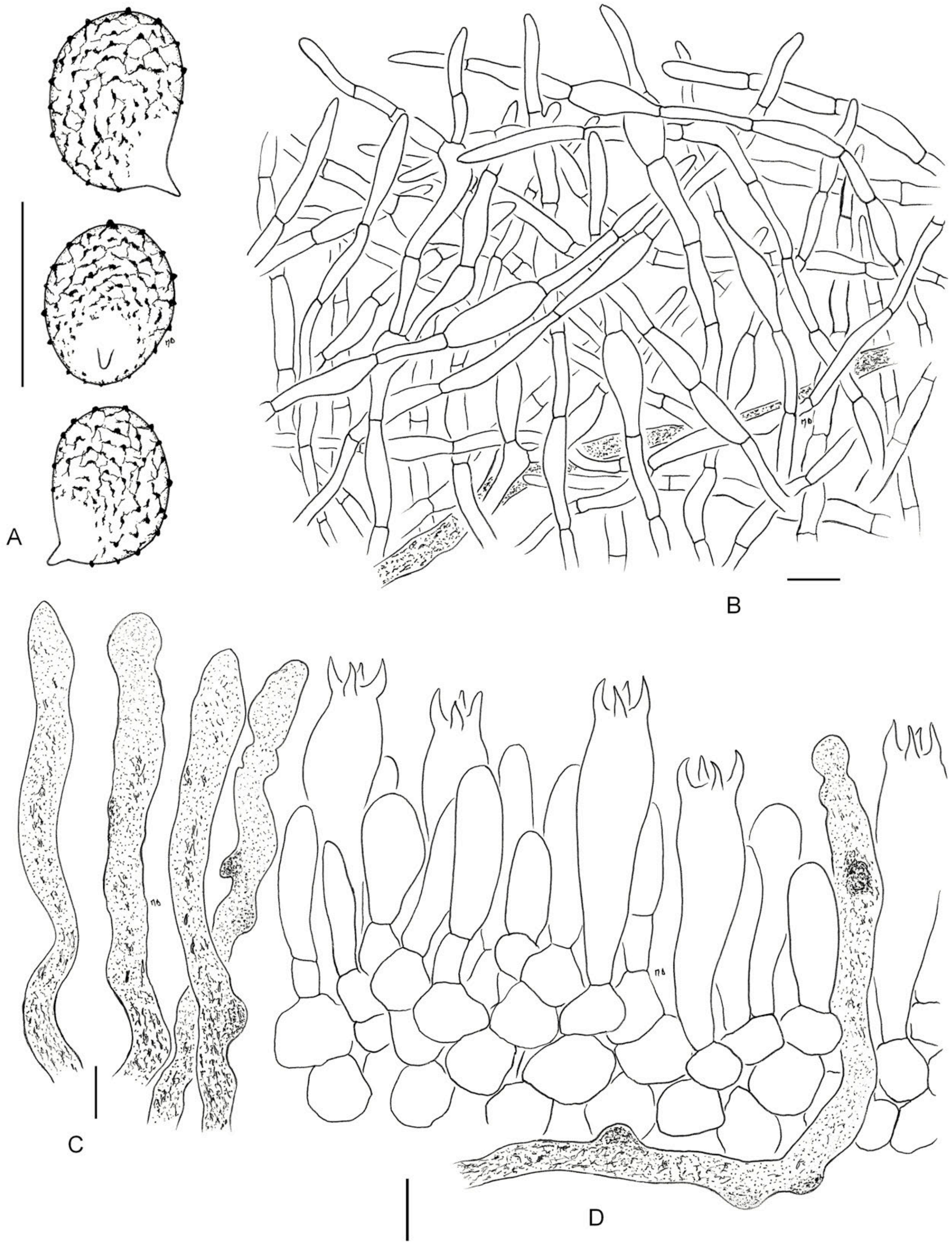

Fig. 4b. Light microscopy *Lactifluus togoensis*

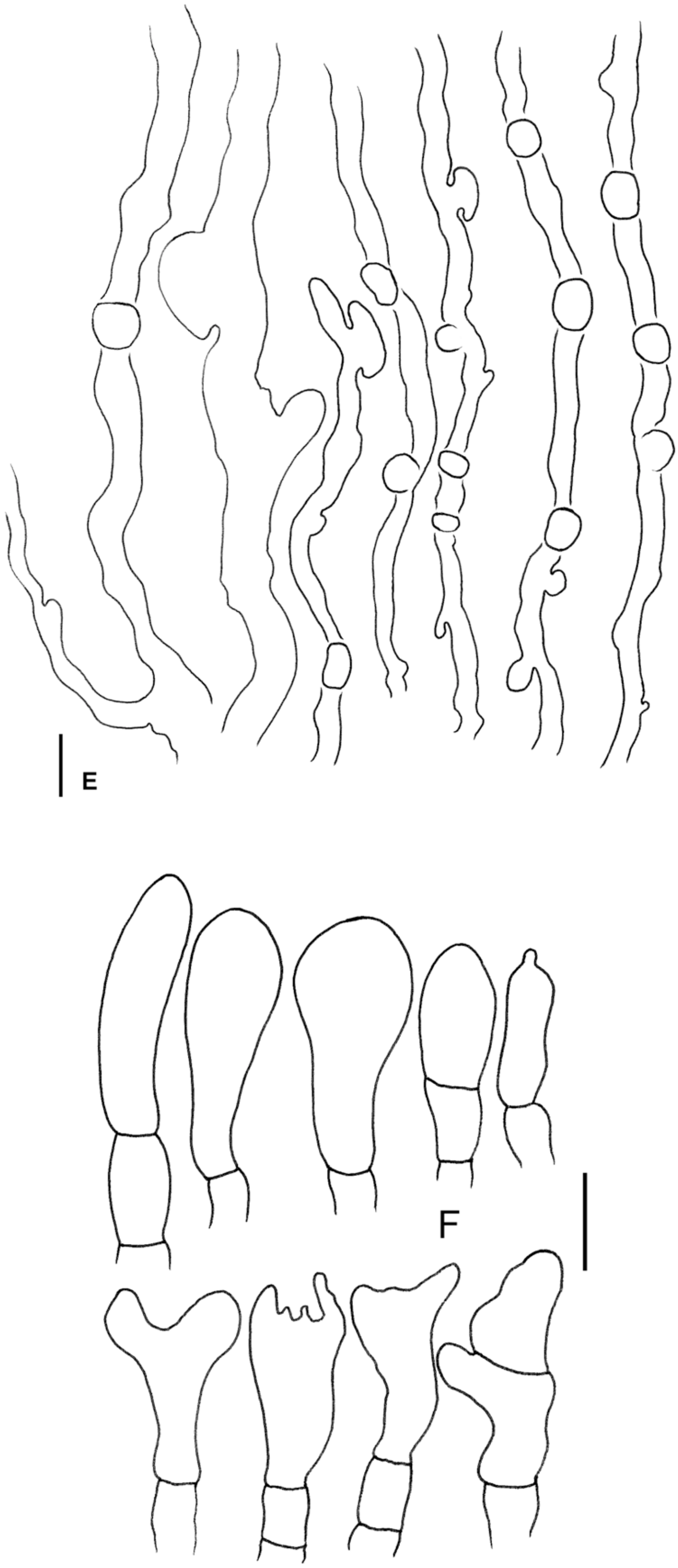

Fig. 5. SEM *Lactifluus togoensis*

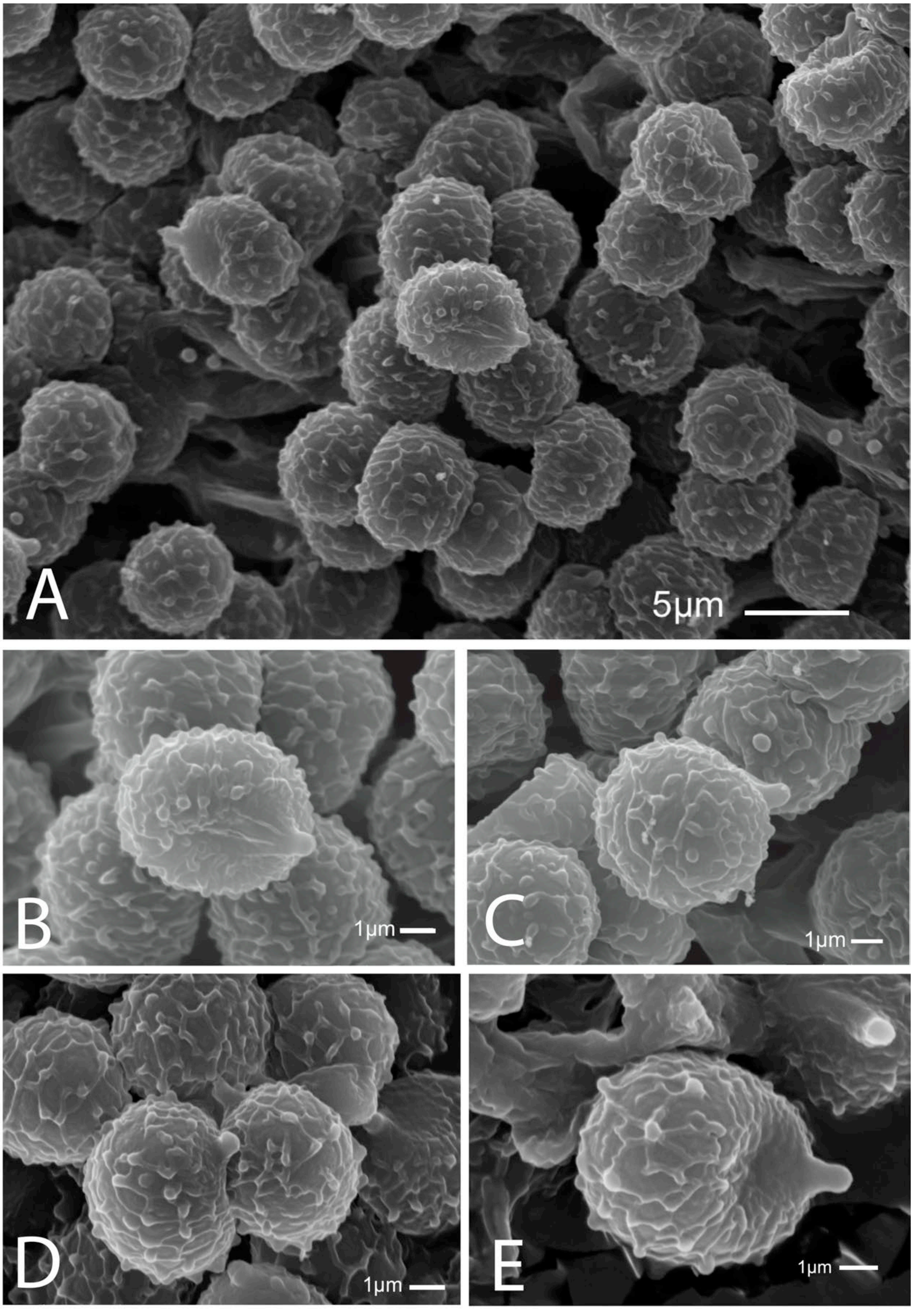

Fig. 6. Basidiome *Lactifluus knobsoides*

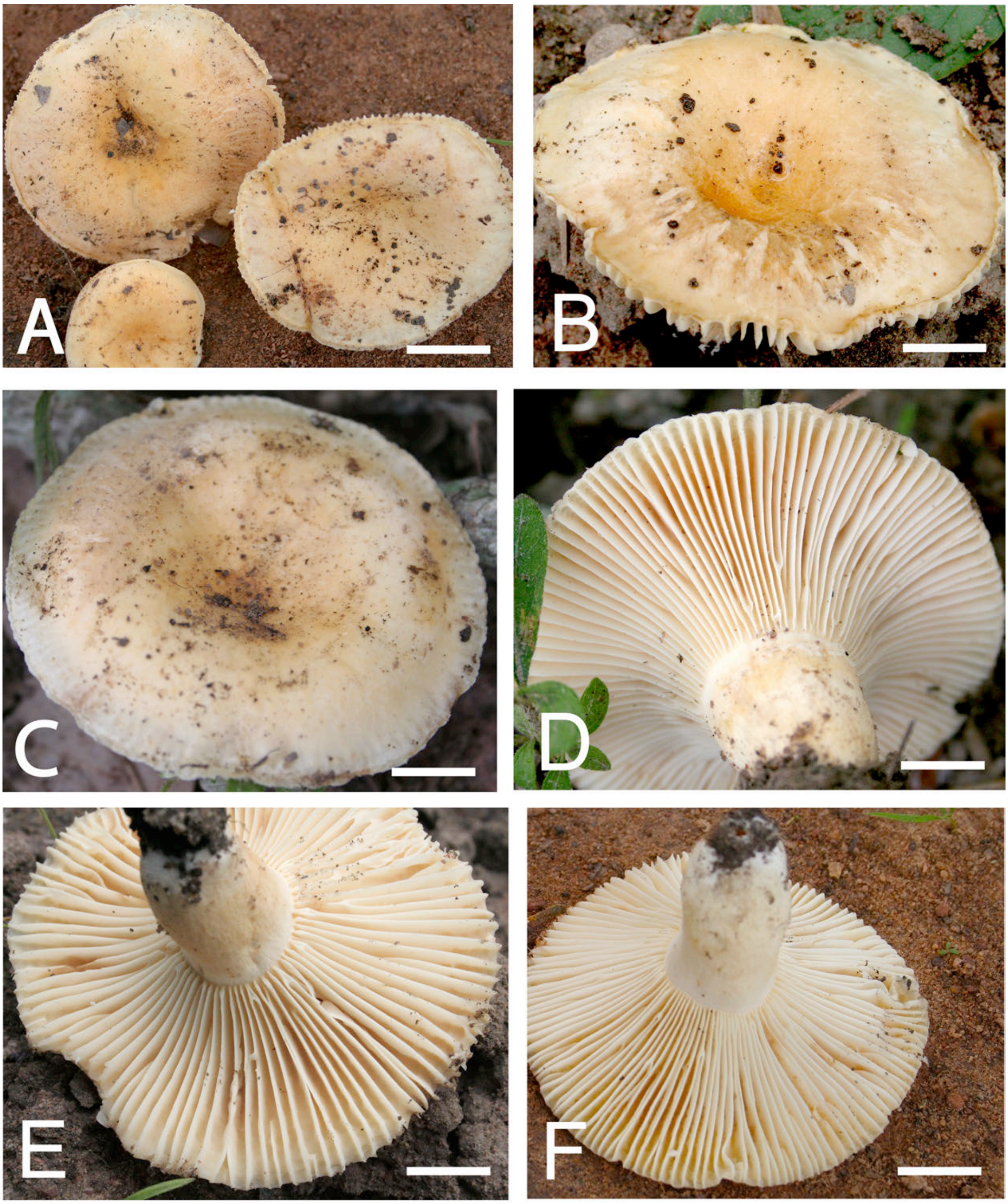

Fig. 7a. Light microscopy *Lactifluus knobsoides*

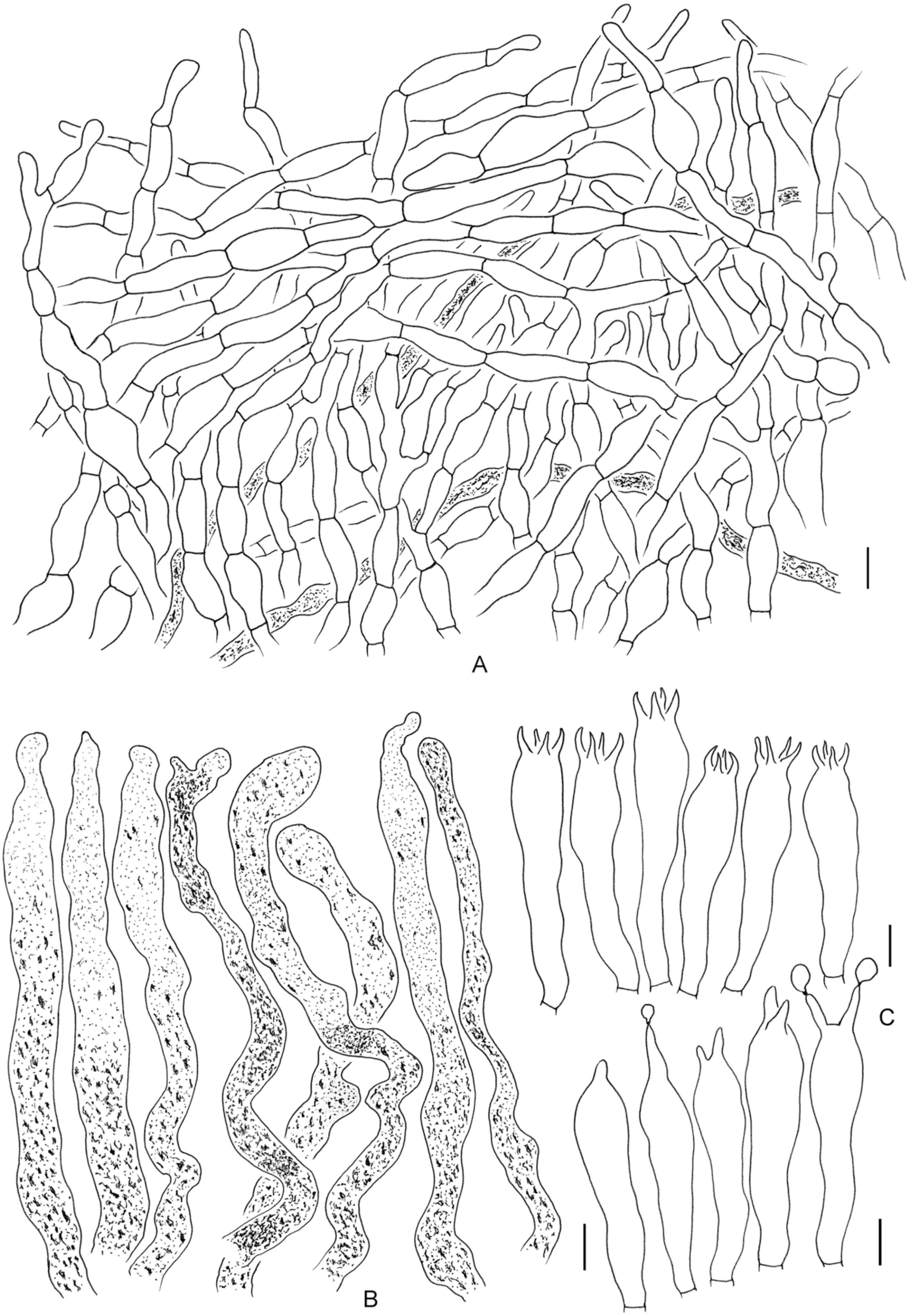

Fig. 7b. Light microscopy *Lactifluus knobsoides*

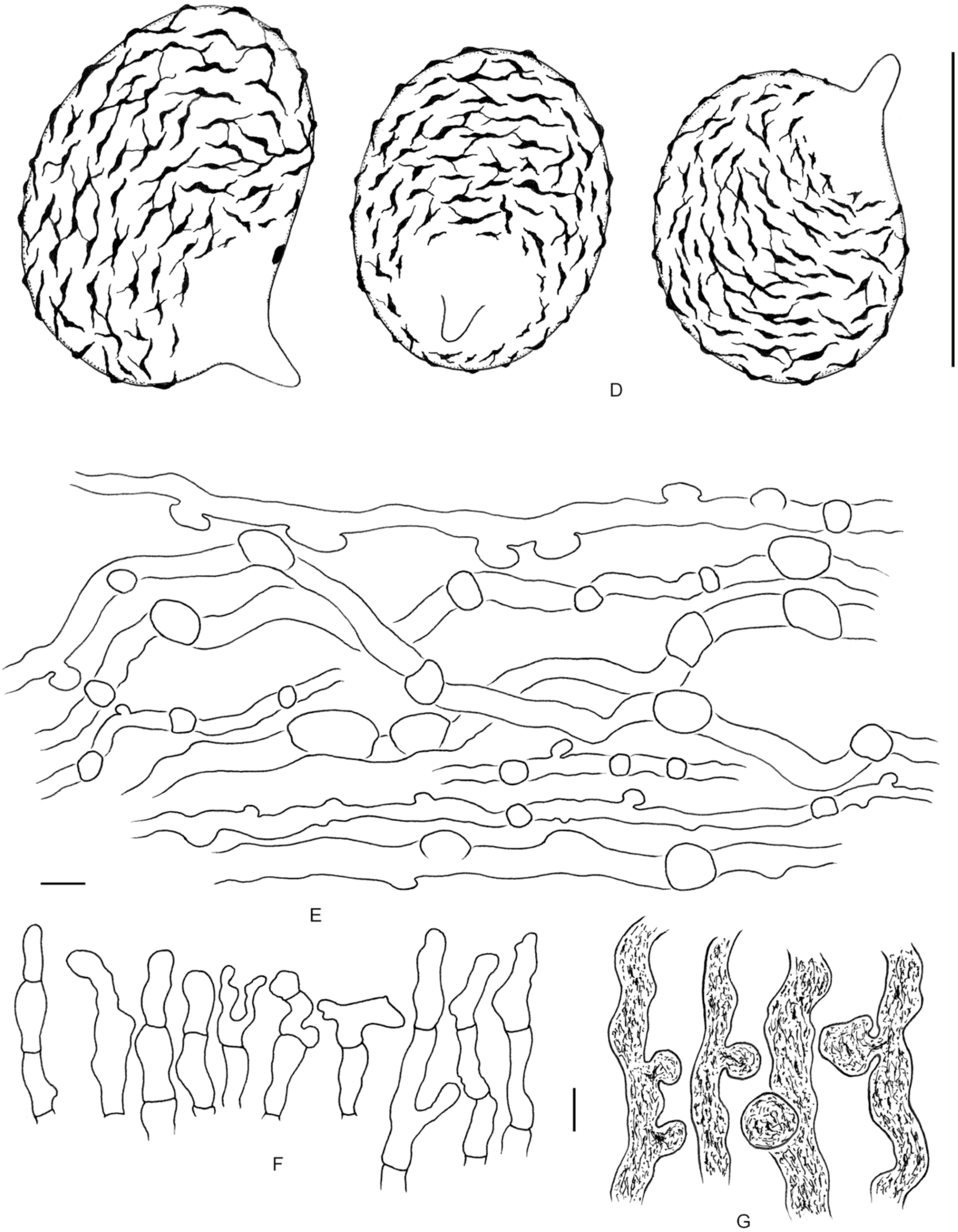

Fig. 8. SEM *Lactifluus knobsoides*

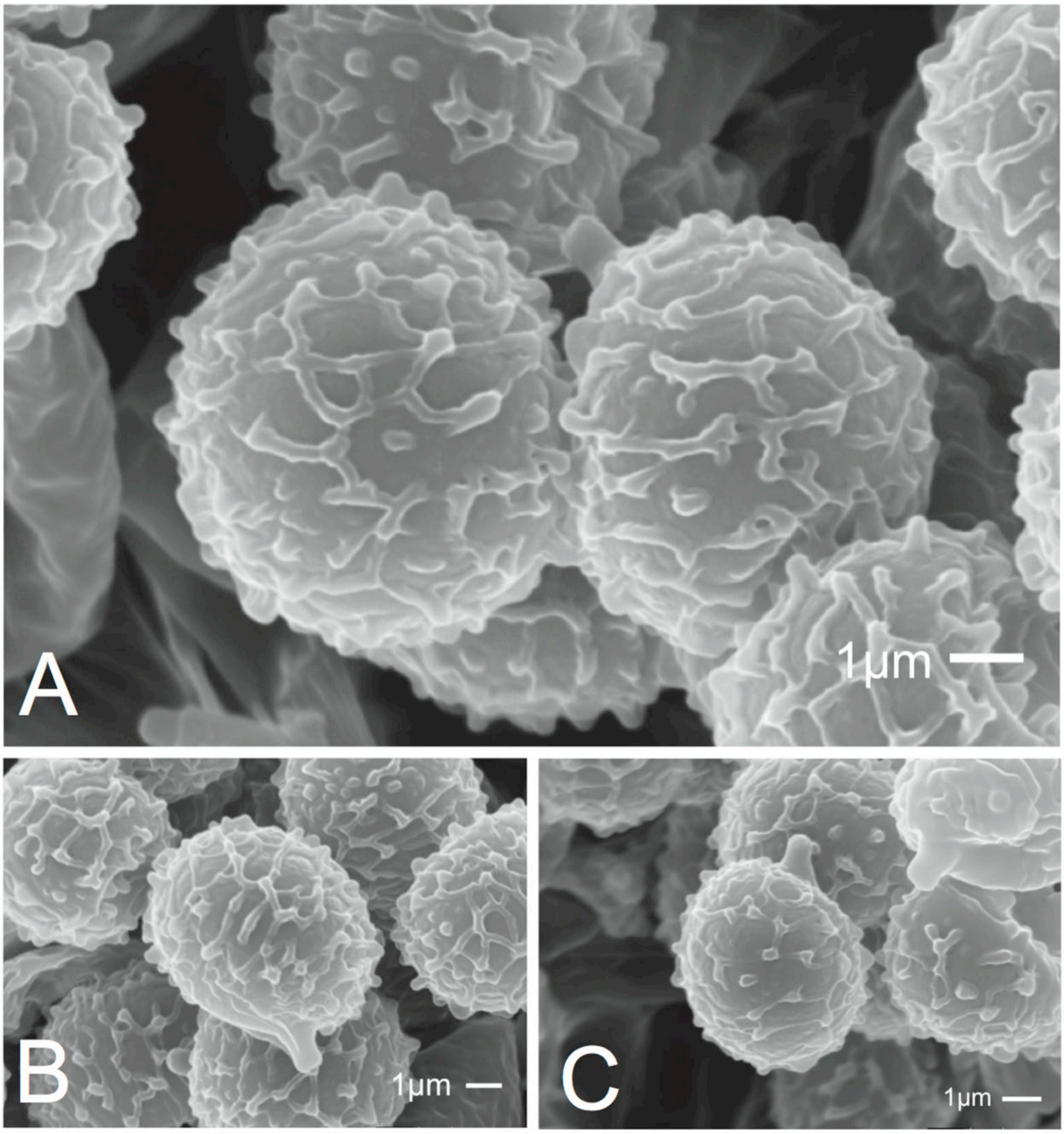

Fig. 9. Basidiome *Lactifluus longicystidiosus*

Fig. 10a. Light microscopy *Lactifluus longicystidiosus*

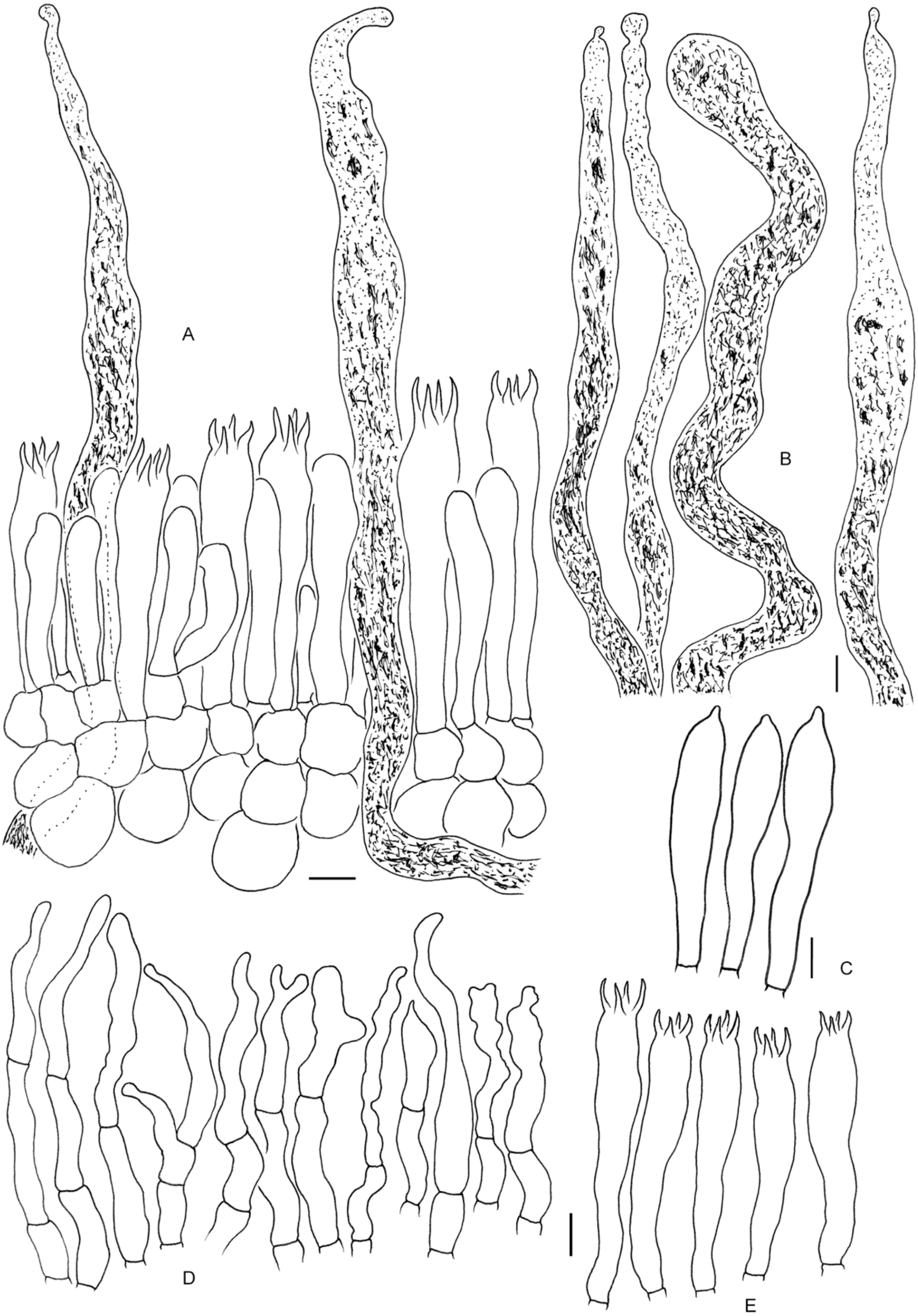

Fig. 10b. Light microscopy *Lactifluus longicystidiosus*

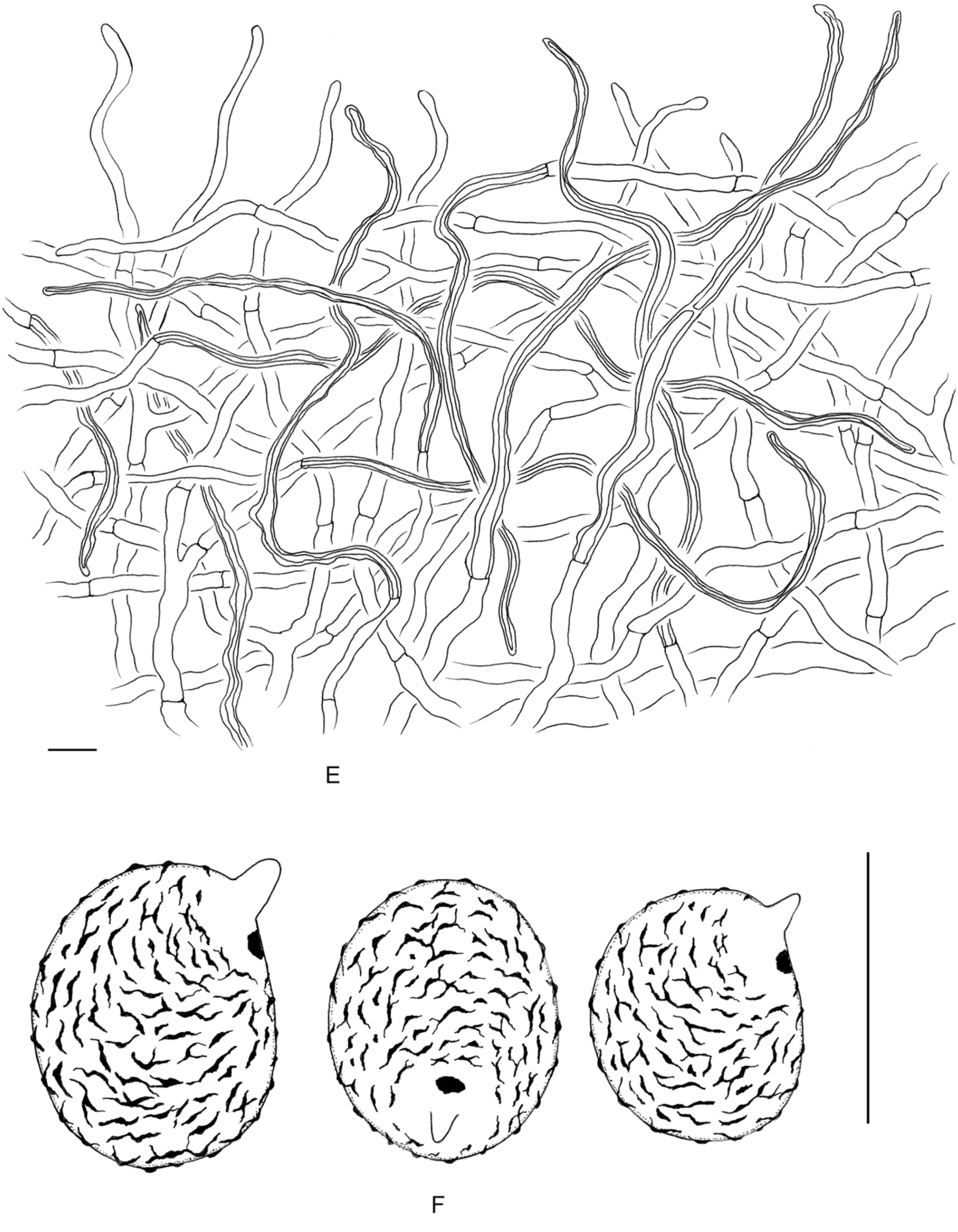

Fig. 11. SEM *Lactifluus longicystidiosus*

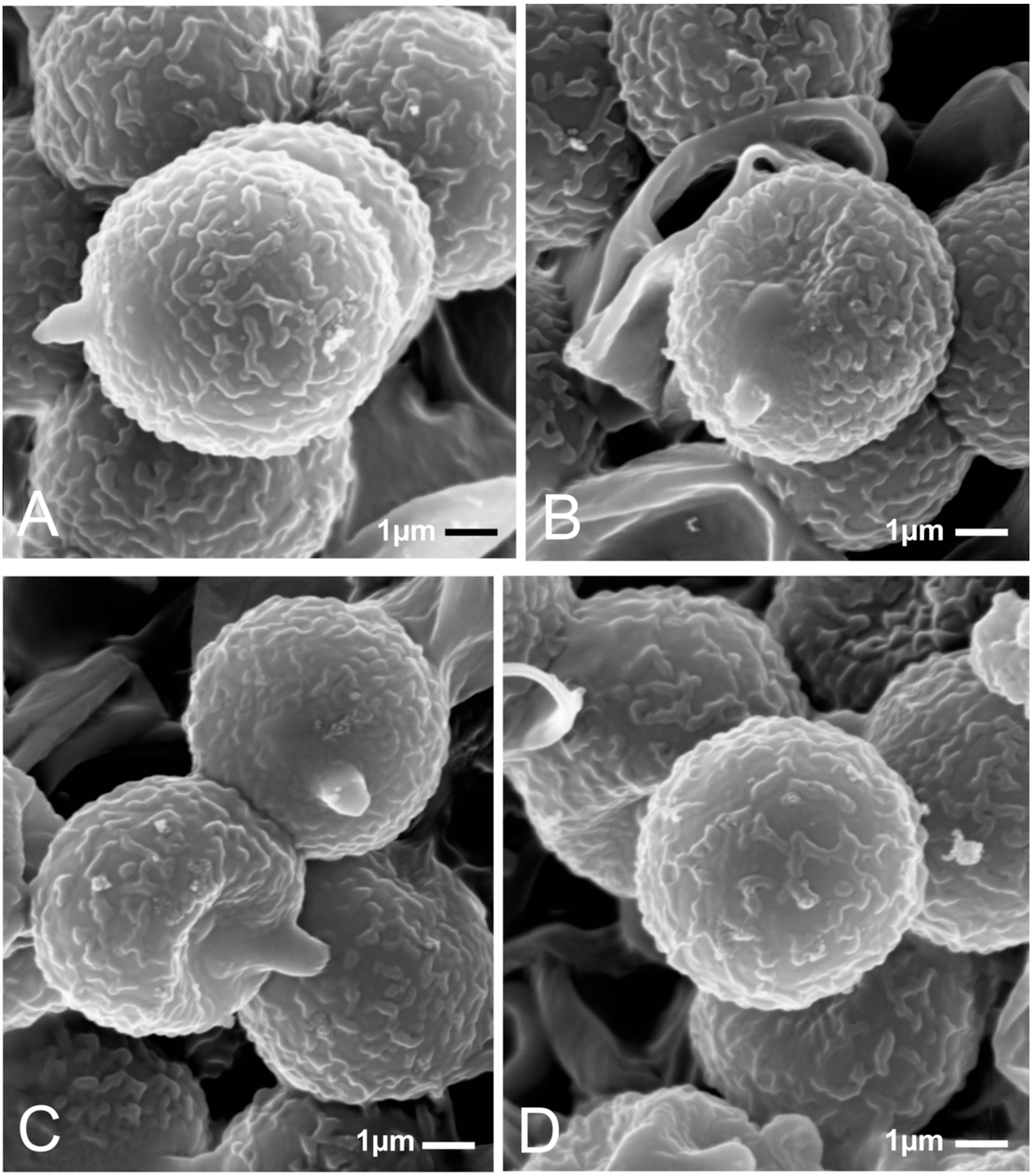

Fig. 12: Species richness of *Lactifluus* subgenera worldwide, intropical Africa, and in West Africa

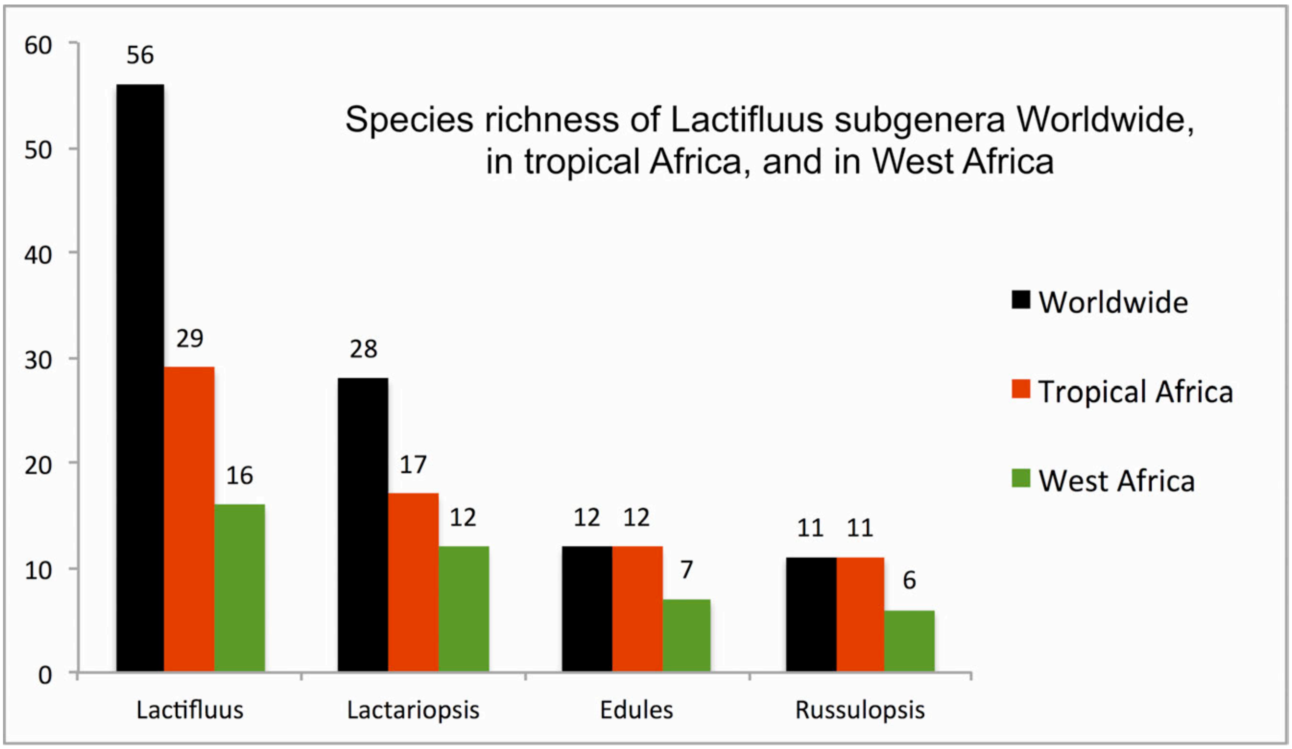

Table 1: Sequences used in molecular phylogenetic analyses (species, accession number, and country of origin)

Species	**GenBanks accession numbers**	**Samples origin**
Lactifluus (93 sequences)		
Lactifluus allardii	KF220017, KF220015	USA
Lactifluus annulatoangustifolius	HG426475	Togo
Lactifluus annulatoangustifolius	AY606981	Madagascar
Lactifluus anulatolongisporus	HG426470, LK392606	Togo
Lactifluus aurantiifolius	AY606087	Zimbabwe
Lactifluus aurantiifolius	AY606986	Madagascar
Lactifluus atrovelutinus	GU258231	Malaysia
Lactifluus burkinabei	LK392609	Burkina Faso
Lactifluus brunneocarpus	LK392608	Guinea
Lactifluus chamaeleontinus	AY606980	Zambia
Lactifluus chiapanensis	GU258297	Mexico
Lactifluus chrysocarpus	JX442761, NR121551	Vietnam
Lactifluus clarckeae	HQ318283	Australia
Lactifluus clarckeae	GU222280	New Zealand
Lactifluus crocatus	HQ318265, Q318248, HQ318266	Thailand
Lactifluus denigricans	AY606983	Benin
Lactifluus densifolius	HG917385	Togo
Lactifluus densifolius	AY606972	Burundi
Lactifluus edulis	HG917384	Togo
Lactifluus edulis	AY606973	Zimbabwe
Lactifluus emergens	HG426467	Togo
Lactifluus emergens	AY606979	Zimbabwe
Lactifluus fazaoensis	HG426477	Togo
Lactifluus flammans	HG426471	Togo
Lactifluus flammans	UDB016931	Benin
Lactifluus flavellus	LK392594, LK392595	Togo
Lactifluus flocktonae	JX2666621, JX266622	Australia
Lactifluus foetens	HG917381	Togo
Lactifluus foetens	LK392603	Burkina Faso
Lactifluus genevievae	GU258294	Australia
Lactifluus glaucescens	KF220117	Italy
Lactifluus glaucescens	KF220094	Belgium
Lactifluus glaucescens	KF220075	France
Lactifluus guellii	HG426466	Togo
Lactifluus guellii	LN849747	Togo
Lactifluus gymnocarpoides	LK392601, LK392600	Benin

Lactifluus gymnocarpus	HG426472	Togo
Lactifluus heimii	LK392612	Togo
Lactifluus hygrophoroides	JN129397	China
Lactifluus inversus	AY606976	Guinea
Lacifluus knobsoides	LK392605	Benin
Lacifluus knobsoides	LN849746	Togo
Lacifluus knobsoides	LM999911	Benin
Lacifluus knobsoides	LN849745	Guinea
Lacifluus knobsoides	LN849742	Benin
Lacifluus knobsoides	LN849743	Benin
Lactifluus longibasidius	LK392596, HG426473	Togo
Lactifluus longicystidiosus	LN849748	Burkina Faso
Lactifluus longipes	HG917391, HG917383	Togo
Lactifluus longipilus	HQ318235, HQ318258, KF432958	Thailand
Lactifluus longisporus	DQ421971	Zambia
Lactifluus luteopus	LK392602	Togo
Lactifluus luteopus	LK392611	Burundi
Lactifluus madagascariensis	AY606977	Madagascar
Lactifluus medusae	HG426474	Togo
Lactifluus melleus	LK392598, LK392597	Togo
Lactifluus membranaceus	LK392610	Guinea
Lactifluus membranaceus	HG426478	Togo
Lactifluus nodosicystidiosus	AY606975	Madagascar
Lactifluus nonpiscis	HG426468	Togo
Lactifluus pectinatus	LK392599	Togo
Lactifluus pelliculatus	AY606978	Madagascar
Lactifluus phlebophyllus	AY606074	Madagascar
Lactifluus piperatus	KF220122, KF220120	France
Lactifluus pseudoluteopus	HQ318286	Thailand
Lactifluus rubroviolascens	AY606984	Zambia
Lactifluus rubroviolascens	AY606985	Madagascar
Lactifluus rubiginosus	HG917386	Togo
Lactifluus rubiginosus	LN849750	Togo
Lactifluus sudanicus	HG426469, HG426476	Togo
Lactifluus togoensis	LK392607	Togo
Lactifluus togoensis	LN849741	Benin
Lactifluus velutissimus	AY606982	Zimbabwe
Lactiflus volemus	HQ318279, HQ318275	Thailand
Lactifluus volemoides	UDB016930	Benin

Lactifluus sp.	LN651269	Burkina Faso
Lactifluus sp.	LM999910	Togo
Lactifluus sp.	UDB014027	Cameroon
Lactarius (14 sequences)		
Lactarius baliophaeus	GU258277	Zambia
Lactarius miniatescens	HG917374	Togo
Lactarius kabansus	HG917376	Togo
Lactarius kabansus	HG917390	Zimbabwe
Lactarius subbaliophaeus	HG917372	Togo
Lactarius tenellus	HG917373	Togo
Lactarius sp.	UDB013804	Zambia
Lactarius sp.	UDB015091	Gabon
Lactarius sp.	UDB018664	Zambia
Lactarius sp.	UDB018662	Zambia
Lactarius sp.	UDB013845	Zambia
Lactarius sp.	UDB013930	Cameroon
Lactarius sp.	UDB016860	Zambia
Lactarius sp.	UDB013836	Zambia
Multifurca (5 sequences)		
Multifurca zonaria	DQ422000, DQ421990	Thailand
Multifurca furcata	DQ421995, DQ421994	USA
Multifurca ochricompacta	DQ421984	USA
Russula (7 sequences)		
Russula cremeirosea	EU819424	USA
Russula congoana	HG917387	Togo
Russula compressa	UDB016985	Benin
Russula discopus	JQ902046	Burundi
Russula discopus	JQ902050	Senegal
Russula lipida	JF908663	Italy
Russula xerampilina	KF386758	USA
Out group (2 sequences)		
Gloeocystidiellum sp.	JQ716940	Chile
Hericium sp.	JQ716939	Argentina

Table 2: Checklist of currently known *Lactifluus* species in West Africa, Guineo-Sudanian domain (Verbeken & Walleyn 2010; Maba et al. 2013, 2015a, b)

Species	Distribution
L. annulatoangustifolius	Guinea, Liberia, Togo: rainforest / gallery forest
L. anulatolongisporus *	Togo: woodland
L. aurantiifolius	Benin: woodland
L. brunneocarpus *	Guinea: rainforest
L. burkinabei *	Burkina Faso: gallery forest
L. chamaeleontinus	Togo, Benin: gallery forest
L. cocosmus *	Togo: woodland
L. denigricans	Benin: gallery forest
L. densifolius	Togo: gallery forest
L. edules	Benin, Togo: gallery forest and woodland
L. emergens	Benin, Togo: woodland
L. fazaoensis *	Togo: woodland
L. flammans	Benin, Guinea, Togo: gallery forest and woodland
L. flavellus *	Togo: gallery forest
L. foetens *	Benin, Burkina Faso, Togo: gallery forest and woodland
L. guellii *	Togo: gallery forest
L. goossensiae	Guinea: woodland
L. gymnocarpoides	Benin, Guinea, Togo: woodland
L. gymnocarpus	Guinea, Ivory Coast, Liberia, Togo: gallery forest and woodland
L. heimii	Togo: gallery forest
L. inversus	Guinea, Togo: rainforest/ gallery forest
L. knobsoides *	Benin, Guinea, Togo: woodland
L. longibasidius *	Togo: woodland
L. longicystidiosus *	Burkina Faso: gallery forest
L. longisporus	Benin: woodland
L. longipes	Togo: rainforest
L. luteopus	Benin, Burkina Faso, Guinea, Togo: gallery forest and woodland
L. medusae	Benin, Togo: woodland
L. melleus *	Togo: woodland
L. membranaceus *	Guinea, Togo: woodland
L. nonpiscis	Burkina Faso, Togo: gallery forest and woodland
L. pectinatus *	Togo: gallery forest
L. pelliculatus	Guinea, Togo: rainforest/ gallery forest
L. pseudogymnocarpus	Benin: woodland
L. pumilus	Benin, Senegal, Togo: woodland

L. rubiginosus	Togo: gallery forest
L. ruvubuensis	Guinea: rainforest
L. rufomarginatus *	Benin, Togo: gallery forest
L. sesemotani	Ivory coast: rainforest
L. sudanicus *	Burkina Faso, Togo: gallery forest and woodland
*L. togoensis**	Benin, Togo: woodland
L. volemoides	Benin: gallery forest
L. zenkeri	Senegal, Togo: woodland

(*) Actually known exclusively from West Africa.

Chapter 7

General Discussion

Specimens sampled

Specimens used in this study include samples collected from three collection events undertaken in West Africa vegetation in 2011, 2012 and 2013, respectively. However, specimens formerly collected in Togo from 2007 to 2010 were included, too. Thus, about 940 lactarioid specimens (758 *Lactifluus* and 182 *Lactarius* s. str.) of which 692 from Togo, 102 from Burkina Faso, 79 from Guinea and 65 from Benin (Table 1) were collected. About 730 (610 *Lactifluus* and 120 *Lactarius* s. str.) samples were microscopically examined for assembling them according to their morpho-anatomical resemblance. A total of 140 (115 *Lactifluus* and 25 *Lactarius* s. str.) collections were thereby accurately illustrated through line drawings. SEM pictures have been taken from 40 (30 *Lactifluus* and 10 *Lactarius* s. str.) specimens at the Department Biology I (LMU), at Ghent University (Belgium), and Natural Museum of Paris (France).

Table 1: Sampled lactarioid specimens in West Africa countries (2007 - 2013)

Countries	*Lactifluus*	*Lactarius*
Benin	47	18
Burkina Faso	78	26
Guinea	64	15
Togo	569	123
Total	758	182

Species richness and morpho-anatomical characters of West African lactarioids

Species of the genera *Lactarius* and *Lactifluus* are commonly widespread in West African forest ecosystems. Both genera occur in the same vegetation types and species are being sampled under the same ectomycorrhizal trees.

However, *Lactarius* s. str., even being worldwide the largest clade with high species richness, has in general less representatives in the tropics, and particularly in tropical Africa and West Africa (Maba et al. 2014). Before the present study, *Lactarius* s. l. from tropical Africa including West Africa counted approximately 100 described species (van Rooij et al. 2003; Douanla-Meli & Langer 2009; van de Putte et al. 2009; Verbeken & Walleyn 2010; De Crop et al. 2012). Species richness was estimated even at about 150 species for tropical Africa

(Verbeken 2001), of which 60 species might be common to the Sudano-Zambezian domains (Karhula & Härkönen 1998; Verbeken 1995, 1996a-c, 1997, 1998a, b; Verbeken 2000; Verbeken & Walleyn 2010).

At current state of knowledge of tropical African lactarioids, *Lactarius* s. str. totalizes 37 accepted species (**Paper II**) that belong to three subgenera and seven sections (Table 2). Seven species are still with uncertain taxonomic position. Of these accepted *Lactarius* s. str. species, 13 are currently known from West Africa (Verbeken & Walleyn 2010; Maba et al. 2014).

Table 2: Currently known *Lactarius* s. str. representatives in tropical Africa (Verbeken & Walleyn 2010; Maba et al. 2014)

Subgenera	Sections	Number of species	
		Tropical Africa	West Africa
Piperites	*Piperites* (Fr.) Burl.	3	2
Not assigned	*Amari* Verbeken	2	-
Russularia	*Russularia* Fr. ex Burl.	1	-
Plinthogali	*Nigrescentes* Verbeken	5	4
	Plinthogali (Burl.) Singer	23	6
	Pseudofuliginosi Verbeken	2	1
Not assigned	*Chromospermi* Verbeken	1	-

On the Contrary, *Lactifluus,* with about 141 described species worldwide (Verbeken et al. 2012; De Crop et al. 2012; Miller et al. 2012; Morozova et al. 2013; Sá & Wartchow 2013; Sá et al. 2013; Maba et al. 2013, 2015a, b, c), has its main distribution in the Southern hemisphere, and comprises therefore more representatives in tropical Africa, including West Africa than in temperate regions. This latter genus encompasses about 73 accepted tropical African species (**Paper V**) of which 41 are known from West Africa (Verbeken & Walleyn 2010; Maba et al. 2013; 2015a, b, c), and accommodated into four subgenera, and 10 sections (table 3). No representatives of the subgenera *Gerardii* and *Piperati* have yet been inventoried from tropical Africa. However, many vegetation types of African forest ecosystems are still waiting for being investigated.

Table 3: Currently known *Lactifluus* representatives in tropical Africa and West Africa (Verbeken et al. 2011, 2012; Maba et al. 2013, 2015a, b, c)

Genus	Sections	Number of species	
		Tropical Africa	West Africa
Edules	No section delimited	12	7
Lactariopsis	*Lactariopsis* (Henn.) Verbeken	6	5
	Chamaeleontini (Verbeken) Verbeken	9	5
	Thrombopleri Maba & Agerer	2	2
Lactifluus	*Polysphaerophori* (Singer) Verbeken	8	4
	Phlebonemi (R. Heim ex Verbeken) Verbeken	5	1
	Pseudogymnocarpi (Verbeken) Verbeken	9	8
	Rubroviolascentini (Singer) Verbeken	2	1
	Tomentosi (McNabb) Verbeken	5	2
Russulopsis	*Russulopsidei* (Verbeken) Verbeken	11	6

Lactifluus sect. *Aurantiifolii* (Verbeken) Verbeken, with only one species described from tropical Africa, has not yet been assigned to any subgenus (Verbeken et al. 2012)

Morphologically, tropical African *Lactarius* and *Lactifluus* present **basidiomes** that vary from whitish yellow, yellowish white, cream, yellow-orange, orange, yellowish brown, orange-brown, orange-greyish, greyish, brownish, ochraceous, dark orange, to darkish brown (van Rooij et al. 2003; Verbeken et & Walleyn 2010; Maba et al. 2013, 2014, 2015a, b, c) (Appendix 1). The **lamellae** are commonly adnate, decurrent to subdecurrent, strongly spaced to very dense, irregular to regular (pattern of insertion), and unequal (presence of lamellulae in mixture with lamellae) to rarely equal (absence of lamellulae) in both genera (van Rooij et al. 2003; Verbeken & Walleyn 2010 and personal observations) (Appendix 1**). Context** as well as chemical reactions used for context and latex test are also almost similar for *Lactarius* and *Lactifluus* (Verbeken et & Walleyn 2010; Maba et al. 2015b). Colour changes after application of chemical reactions depend on the type of reagents, and vary from one species to another within both genera. The commonly used chemical reactions include, guaiac, $FeSO_4$, NaOH, KOH, NH_4OH, HCl, phenol, phenolaniline, pyramidon, aniline.

However, apart from agaricoid form of fruitbodies, gasteroid (with closed or partially closed fruitbodies) and scrobiculate (with scrobicules on pileus or stipe, or on both) species are until now known only in *Lactarius*, while pleurotoid (caps laterally attached, with no stem or eccentric, and gills decurrent along it) and annulate (with partial velum remnants and forming an annulus) species are known only in *Lactifluus.* Most dissimilarities between *Lactarius* s. str. and *Lactifluus* taxa refer rather to microscopical than to morphological characters.

Microscopically *Lactarius* s. str. has in general **basidiospores** with mostly very high amyloid ridges of up to 2 µm high, sometimes qualified as zebroid (Verbeken & Walleyn 2010) (Appendice 5 I-J). In addition, **macrocystidia**, cystidia with needle-like contents, are observed in more *Lactarius* s. str. species in comparison to *Lactifluus*. **Sphaerocytes** are often lacking in *Lactarius* s. str. species, and the **subhymenia** are rarely cellular in comparison to *Lactifluus*. However, both genera have representatives that show **basidiospores** with amyloid ornamentations composed of well developed (up to 1µm high) irregular, conical or rounded, and isolated warts (Appendix 5 F-H). On the contrary, weakly developed amyloid ornamentations (very short, fine, barely visible or not well distinctive under light microscope, less than 0,2 µm high) of basidiospores are until now only known for *Lactifluus* species (Appendix 5 A-C), unlike to *Lactarius* s. str. Moreover, 2-spored and 1-spored **Basidia**, **pleuroleptocystidia**, deformed basidia, and **dermatocystidia** are observed in *Lactifluus*, and not yet identified in *Lactarius* s. str. **Pileipellis** and **stipitipellis** structures are mostly lampro/tricho-palisadic, palisadic, an ixocutis, trichoderm or a mixture for *Lactifluus* species, but mostly palisadic, rarely an ixocutis, a trichoderm, or hymeniderm for *Lactarius* s. str. species. (van Rooij et al. 2003; Verbeken et & Walleyn 2010; Maba et al. 2015b) (Appendix 3). **Marginal cells** are cylindrical to subcylindrical, fusiform and septate in both genera (Verbeken & Walleyn 2010; Maba et al. 2013, 2014, 2015a, b, c). Unlike in *Lactarius*, very diverticulate and strongly bifurcates marginal cells are found in *Lactifluus* (Appendix 2).

In this study, morpho-anatomy of West African *Lactifluus* (**paper I, III, IV, and V)** and *Lactarius* (**paper, II, IV)** are addressed with anatomical illustrations. Apart from microscopic studies in order to diagnose the existing or already known lactarioids, and to confirm their occurrence in West African forest ecosystems, particular attention was given to 60 specimens that were in great detail anatomically studied and illustrated. They have shown particular morpho-anatomical characters. This investigation provided 21 lactarioid species new to sciences, of which 15 (14 *Lactifluus* and 1 *Lactarius* s. str.) are published (**paper I-V**), and six (4 *Lactifluus* and 2 *Lactarius*) are still waiting for publication. The boundary/distinctive morpho-anatomical characters of *Lactarius* versus *Lactifluus* from West Africa are highlighted along this study. The total number of known lactarioid species from tropical Africa increased thus from about 100 to more than 115. This is addressed in **paper V**, with emphasis on many new species that are still waiting to be published, as well as many on vegetation types that await to be investigated. These studies highlighted also the high species richness of *Lactifluus* compared to *Lactarius* in African forests ecosystems (**paper IV-V)**.

Morphologically, no significant particular characters were observed for West African *Lactifluus,* in comparison with those of Congo-Zambezian domain. The variability of basidiome coloration of specimens from Guineo-Sudanian and Congo-Zambezian domain remains almost identical or follows the same gradient. Nevertheless, phenotypic plasticity within some *Lactifluus* species from West Africa is mentioned in **paper III and IV**. Most dissimilarities or demarcations between *Lactifluus* and *Lactarius* have been observed mainly with microscopic characters. Verbeken & Walleyn (2010) provide illustrations of the major anatomic characters of tropical African lactarioids based on species originating mostly from Congo-Zambezian domain and Benin in the presented studies. The present study provides anatomical features of specimens originating from Guineo-Sudanian domain. Interestingly, a great shape diversity of anatomical elements has been observed for *Lactifluus* and *Lactarius* s. str. specimens from the Guineo-Sudanian domain, showing additional anatomical characters, and new combinations of shapes (**Paper I-V**).

Pileipellis structures with often subclavate to subglobose hyphal cells (**paper III and IV**), and very slender and hair-shaped hyphal ends, and longer thick-walled terminal elements have been observed (**paper I and V**). Strongly diverticulate marginal cells and/or true cheilocystidia (**paper I, IV and V**), longer basidia, as well as extremely long pleuropseudocystidia (**paper V**) have been found (**paper V**) in specimens from Guineo-Sudanian domain. Moreover, and interestingly, the occurrence of thromboplera (also known as oleiferic hyphae), and knobs attached to thromboplera and lactifers have been observed for the first time from specimens in Guineo-Sudanian domain (**paper V**). In contrary, of 120 *Lactarius* s. str. examined specimens, no particular or striking anatomical character, in comparison to the known ones (Verbeken & Walleyn 2010) was found. The description of new species in *Lactarius* s. str. (**paper II**) was based on the combination of existing characters, in comparison to combinations presented by already known species, like response to chemical reagents applied on the context, taste, and molecular phylogenetic deviations.

Investigations undertaken in **paper II** laid also emphasis on cryptic species within *Lactarius* s. str. Interestingly, the latter study has shown the unequivocal importance of morpho-anatomical characters for the characterization of fungal diversity and species identification.

Ecology and distribution of tropical African and West African lactarioids

Ecologically, *Russulaceae* in general and the genera *Lactarius* s. str. and *Lactifluus* in particular, play a major role as prevalent ectomycorrhizal fungal partners of forest trees in tropical African ecosystems (Verbeken & Buyck 2001; Rivière et al. 2007; Diédhiou et al. 2013). Species of the genera *Lactifluus* and *Lactarius* are widespread in Guineo-Sudanian ecosystems. Both genera occur predominantly in woodlands, riverside forests, and rainforests dominated by *Caesalpiniaceae* and *Phyllantaceae* (De Kesel et al. 2002; Ducousso et al. 2002; Verbeken & Buyck 2001). In these northern Guinean seasonal forests, ectomycorrhizal fungi, including *Lactarius* s. str. and *Lactifluus* species, play an essential role for the growth, regeneration of forest trees, and in the ecosystem functioning (Rivière et al. 2007; Diédhiou et al. 2010, 2013). Moreover ectomycorrhizae formed by species of the genera *Lactarius* s. str. and *Lactifluus* with native forest trees in tropical Africa has been repeatedly reported (Verbeken & Buyck 2001; Bâ et al. 2011; Diédhiou et al. 2010, 2013).

In West African forest ecosystems, the high diversity of *Lactarius* and *Lactifluus* species undergoes big threats through a continuous regression of their habitats. The Sudanian woodlands disappear at an alarming rate of about 4,5 % each year according to the FAO report (2010). Numerous species occur in highly fragmented habitats and/or in ecological islands, suggesting that they are highly endangered. In Benin for example, numerous species of *Lactarius* s. str. and *Lactifluus* species are either critically endangered of extinction or vulnerable (Yorou & De Kesel 2011). Countless *Lactarius* s. str. and *Lactifluus* species are more likely vulnerable, as the Sudanian woodlands of whole West Africa are subjected to the same threats and human pressure.

In this study, the preferential habitats, tree partners, distribution, and the status of conservation of the principal lactarioid species from Guineo-Sudanian domain are highlighted (**papers II, IV, V**). The occurrence and distribution/niche differentiation of *Lactifluus* in tropical Africa and West Africa are specified in **paper IV** and **V**, while those of *Lactarius* are given in the **paper II**. The co-occurrence of some *Lactarius* and *Lactifluus* species in Congo-Zambezian and Guineo-Sudanian domains is highlighted in **paper II** and **IV**, with emphasis on species restricted to the Guineo-Sudanian domain (case of *Lactifluus*). At the same time the distribution at national level (by countries) of *Lactifluus* in West African forest ecosystems is provided in the **paper V**. Additionally, the selective tendency of occurring in woodland or in riverside/rain forests, or preference regarding vegetation type of some tropical African *Lactarius* and *Lactifluus* is shown in **paper II** for *Lactarius* and **IV** for *Lactifluus*. This study contradicted the assumption that some species recorded formerly only from Congo-Zambezian, are restricted to this domain (Verbeken & Buyck 2001). Indeed, **paper IV** emphasis on the occurrence of these species in vegetation types of Guineo-Sudanian domain.

Molecular investigations and phylogenetic position of West African *Lactarius* s. str. and *Lactifluus*

Although the genera *Lactarius* s. str. and *Lactifluus* appear as the most taxonomically best documented fungal group in tropical Africa (Karhula & Härkönen 1998; Verbeken 1995, 1996a-c, 1997, 1998a, b; Verbeken 2000; van Rooij et al. 2003; Verbeken & Walleyn 2010), it is worth mentioning that only very few molecular phylogenetic data are available. The first molecular investigation within lactarioids from tropical Africa at regional or national level has been that of Buyck et al. (2007). This investigation combined the molecular phylogenetic approach and morpho-anatomical studies to provide the first modern record of Madagascar lactarioid taxa, of which two species including *Lactifluus madagascariensis* and *L. nodosicystidiosus* were assumed to be possibly endemic species of the country. The most recent and advanced monograph of lactarioids in tropical Africa (Verbeken & Walleyn 2010) failed to integrate molecular data. There are very few papers in which molecular data of West African *Lactarius* s. str. and *Lactifluus* species have been addressed (Rivière et al. 2007; Diédhiou et al. 2010). In spite of the reliability and consistency of the investigations by Buyck et al. (2008), which constitute the restructuring within lactarioids, this work did integrate only one sequence of specimens originating from West Africa (one of 67). Obviously, this is due to the lack of sequences in the public GenBanks, and to the scarcity of mycological inventories undertaken in this area. The scarcity of DNA sequences of tropical African and particularly West African *Lactarius* s. str. and *Lactifluus* species in public GenBanks, limits not only phylogenetic studies, but also the possibility of assessing the contribution of tropical African specimens to evolutionary hypotheses. This is particularly disadvantageous, as Alexander (2006) stipulates that ectomycorrhizal fungi, including the genus *Lactarius* s. l., would have an African origin. Moreover, investigation of van de Putte et al. (2009) on specimens from Togo provided evidence that one species presents some morphological and molecular affinities with European taxa. It is very likely that specimens of tropical Africa will supply very interesting molecular and anatomical information to the understanding of the evolution of the genera *Lactarius* s. str. and *Lactifluus.*

For the present thesis, nuclear ribosomal DNA (nrDNA) was successfully extracted and amplified from 184 specimens, of which 108 purified products were sent to the sequencing service of the Institute for Genetics, Department Biology I (LMU). A total of 69 good ITS sequences including *Lactifluus* (50), *Lactarius* (15), *Russula* (3) and one Termitomyces were obtained from the samples, and deposited at European Nucleotide Archive (ENA) with accession numbers (Appendix 7).

The phylogenetic positions/placement of West African lactarioids is treated during this thesis. The analysis has supported the demarcation between *Lactarius* and *Lactifluus* genera, as highlighted in the **paper I to V.** The relationship of West African *Lactifluus*, in comparison to those from Congo-Zambezian domain and worldwide is shown. The high nrDNA variability of the genus *Lactifluus*, falling apart into distant clades is highlighted in this thesis (**paper I to V**). Sequences of representatives of each of the genera *Lactarius* and *Lactifluus* are supported as monophylum. However, *Lactarius* subg. *Plinthogali* showed a polyphyly (**paper II)**. At the same time, while *Lactifluus* subg. *Edules*, subg. *Russulopsis* has been showen as monophyletic, *Lactifluus* subg. *Lactifluus* and subg. *Lactariopsis* were a paraphyletc/polyphyletic, with a high species complexity (**paper IV and V**). The molecular phylogenetic analyses undertaken so long, including numerous tropical African lactarioid, as well as worldwide sequences, supported until now the absence of representative species of the genus *Multifurca* in West African forest ecosystems. However, it would be premature to conclude on the total absence of *Multifurca* representatives in tropical African forest ecosystems, given that many vegetation types are still without any mycological investigation.

This thesis supports the worldwide accepted and commonly used nrDNA ITS (ITS1 and ITS2) region phylogenetic studies. Although the alignment is always complex, it is accepted as fungal metabarcoding marker to access the diversity and composition of fungal communities, as well for inter-and intraspecific characterization (Blaalid et al 2013; Schoch et al. 2012). Indeed, it has been shown that, while the 5.8S gene is highly conserved, the ITS1 and ITS2 spacers normally provide resolution at a within-genus and often within-species level (Nilsson et al. 2008), and it has already been debated that ITS1 or ITS2 provides the best taxonomic resolution at the species level (Bellemain et al. 2010; Mello et al. 2011). The various molecular phylogenetic analyses conducted in this thesis using nrDNA ITS (ITS1 and ITS2 and 5.8 5 region) have supported the usefulness of sequences obtained from West

African specimens and those obtained from worldwide specimens including Congo-Zambezian domain. Thus, some sequences of specimens originating from West Africa are supported in subclades that contain sequences of species originating from temperate and tropical regions **(paper II-V)**. Therefore, neither a higher degree of discrepancy was observed between sequences originating from West Africa and of worldwide ones, nor the generated sequences originating from West Africa have deviated as separate clade from those of the worldwide (**paper I-V**). The thesis supports the presence of high interspecific genetic variability within the entire genus *Lactifluus* (**paper III, IV and V**) as suggested by Verbeken at al. (2011). This is confirmed by the high anatomical differences observed during microscopical examinations, and the occurrence of some particular characters mentioned above. Nevertheless, *Lactifluus* subg. *Gerardii* and subg. *Piperati,* that have no representatives in tropical Africa, although well supported by molecular phylogeny within *Lactifluus*, are separate from the other subgenera (**paper III, IV and V**). In light of the results presented here, it appears that morpho-anatomical similarities do not always follow molecular phylogenetic results. This is at least true for tropical lactarioid taxa (**paper II, III, IV and V**).

Ethnomycology: Used *Lactifluus* and *Lactarius* s. str. species in West Africa.

Various investigations have revealed the uses of wild edible fungi by local inhabitants in Africa (Rammeloo and Walleyn 1993; Walleyn and Rammeloo 1994; De Kesel et al. 2002; 2008; De Kesel and Yorou 2002; Boa 2006; Guissou et al. 2005; Maba 2010; Eyi-Ndong et al. 2011; Hama et al. 2012; Yorou et al. 2014). Unfortunately, investigations focusing particularly on the use of tropical African and West African lactarioids are lacking. Only Maba (2010), focused on the genus *Lactarius* s. l., and has provided detailed ethnomycological information on *Lactarius* s. l. species from Aledjo reserve forest from Togo. However, local populations from West Africa have identified many lactarioid taxa as food, (Table 4). De Kesel et al. (2002), Boa (2006), and Maba (2010), have pointed out that, wild edible fungi (including lactarioids), are considered as food, only when these wild fungi are really consumed, and confirmed by investigations made from local populations, to reveal their uses. Thus, 11 edible *Lactifluus* species from West Africa have been identified as food, used as food additives (De Kesel et al. 2002; Ducousso et al. 2002; Boa 2006; Maba 2010; Yorou et al. 2014), and three as edible, identified as food in Eastern, Central and Southern Africa, but not used in West Africa (Table 4). At the same time, two *Lactarius* s. str. species, *Lactarius kabansus* and *L. tenellus,* are recognized as food. Moreover, Maba (2010) revealed that some species, including *Lactifluus gymnocarpoides*, *L. sudanicus,* and *L. edulis* are much appreciated by local inhabitants. In addition, some tropical African *Lactifluus* species, unknown in West Africa, including *Lactifluus latifolius L. pseudovolemus L. rubroviolascens* are identified as edible, while *Lactifluus laevigatus L. phlebophyllus*, and *L. tanzanicus* are recognized as food (Boa 2006).

Table 4: List of West African used *Lactifluus* and *Lactarius* species ("Food" signifies confirmed use of species; "edible" is a noted property without confirmed consumption. De Kesel et al. 2002; Boa 2006; Maba 2010; Yorou et al. 2014)

Species	Uses
Lactifluus annulatoangustifolius	Food
Lactifluus edulis	Edible
Lactifluus denigricans	Food
Lactifluus densifolius	Food
Lactifluus gymnocarpoides	Food
Lactifluus gymnocarpus	Food
Lactifluus heimii	Food
Lactifluus luteopus	Food
Lactifluus inversus	Edible
Lactifluus medusae	Food
Lactifluus pelliculatus	Edible
Lactifluus sesemotani	Edible
Lactifluus sudanicus	Food
Lactifluus volemoides	Food
Lactarius kabansus	Food
Lactarius tenellus	Food

Investigations of De Kesel et al. (2002) and Maba (2010) revealed that edible fungi are sometimes boiled and/or bleached before use to prepare the meal. This practice has been known as effective to remove any labile toxins as well as chemical components responsible for some undesirable tastes. Thus, some species with slightly bitter or astringent taste, such as *Lactifluus gymnocarpoides*, or hard flesh, as *Lactarius tenellus,* are often bleached in potassium or ashy boiling water that is discarded and renewed once, before adding the mushrooms to a sauce (Maba 2010). De Kesel et al. (2002) emphasized the fact that, in terms of wild edible fungal biomass, the genus *Lactarius* s. l. is the most productive (particularly *Lactifluus gymnocarpoides*), in comparison to the genera *Amanita*, *Cantharellus*, *Russula,* and *Termitomyces* that are all common in African ecosystems.

Furthermore, no toxicity of lactarioid taxa from West Africa is so far reported. Also, although some West African wild fungi are used in traditional medicine (Guissou 2005; Boa 2006; Hama et al. 2012), these available data have not revealed/indicated the particular use of lactarioids in West Africa for medical purpose.

Taxonomic novelties in this study

Table 5: Newly described species

Species	Paper
Lactifluus fazaoensis Maba et al. *Lactifluus sudanicus* Maba et al.	Paper I
Lactarius subbaliophaeus Maba & Yorou	Paper II
Lactifluus flavellus Maba & Guelly *Lactifluus longibasidius* Maba & Verbeken *Lactifluus melleus* Maba *Lactifluus pectinatus* Maba & Yorou	Paper III
Lactifluus guellii Maba *Lactifluus burkinabei* Maba *Lactifluus brunneocarpus* Maba *Lactifluus annulatolongisporus* Maba *Lactifluus membranaceus* Maba	Paper IV
Lactifluus togoensis Maba *Lactifluus knobsoides* Maba *Lactifluus longicystidiosus* Maba	Paper V

General conclusion

The present thesis combined detailed morpho-anatomical examinations and molecular approaches to highlight species richness, distribution, taxonomy, nomenclature and phylogenetic relationship of West African *Lactifluus* and *Lactarius* s. str. species. The genus *Lactifluus* is so far predominant in tropical Africa and West African forest ecosystems, compared to that of *Lactarius* s. str. A total of 758 *Lactifluus* specimens, and 182 of *Lactarius* s. str. have been collected in Guineo-Sudanian domain of West Africa (Benin, Burkina Faso, Guinea, Togo). A total of 44 *Lactifluus* and 13 *Lactarius* s. str. species are thus known for West Africa, 73 and 40, respectively are accepted for tropical Africa. A total of 14 *Lactifluus* and one *Lactarius* s. str. are published as new for science, while four *Lactifluus* and two *Lactarius* s. str. need additional study before final conclusion and publication and are therefore not included in this dissertation. This thesis supports the importance of morpho-anatomical characters as indispensable and irreplaceable for the delimitation of taxa, including species and even cryptic species identification. In light of the results, more inventories/collections, and basic taxonomic works remain to be done on the underexplored tropical forest ecosystems, as well as on West Africa ones, where several vegetation types are still without any mycological investigation. As these areas are critically endangered of extinction due to human pressure and exacerbated by climate change effects, further studies are urgently needed.

The anatomical exanimations conducted for this dissertation have revealed several striking characters that are useful for identification and of taxonomic relevance. They might explain/support the high genetic variability within the genus *Lactifluus* (Verbeken et al. 2011; Verbeken & Nuytinck 2013), as compared to its sister genus *Lactarius*. These characters might contain information on the natural kinship/relationships between the taxa. An example for that is the presence of thromboplera (Clémençon 2012) and laticifers, both with attached knobs in *Lactifluus togoensis* Maba, and *L. knobsoides* Maba of *Lactifluus* subg. *Lactariopsis* sect. *Thrombopleri* Maba & Agerer. Additional striking anatomical characters are the pileipellis structures observed for some species that contain globose to subglobose, and subclavate cells as in *Lactifluus pectinatus* Maba & Yorou, *L. burkinabei* Maba, that recall those of *Lactarius* subgenus *Russularia* (Verbeken & Nuytinck 2013), and also an interwoven cutis transgrading to a trichoderm in *L. longicystidiosus* Maba, and a trichoderm with transition to a cutis in *L. fazaoensis* Maba et al. In contrary, no high discrepancy of anatomical characters was observed for West African *Lactarius* s. str. species.

Recent molecular approaches (Buyck et al. 2008, 2010) have highlighted kinship/relationship of lactarioids, leading to the acceptance of the two separate genera

Lactifluus and *Lactarius* s. str. Molecular phylogenetic analyses undertaken for this thesis are very comprehensive. Worldwide ITS sequences of *Lactifluus* and *Lactarius* s. str. were included in these analyses for a better taxonomic resolution. Moreover, neither pleurotoid forms of *Lactifluus* nor angiocarp fruitbodies of *Lactarius* are collected in West African vegetation types. The study supports the assumption that the diversity of lactarioids might be still higher in tropical Africa, with predominance of *Lactifluus*.

Although lactarioids in particular, and *Russulaceae* in general, are currently undergoing studies, some issues remain to be considered for future researches. Do lactarioids have temperate or tropical origin? What are the diversification rates of tropical lactarioid clades, in comparison of temperate clades? Where is the geographic ancestral state of lactarioid taxa? The global diversity and host relation in the clades of lactarioid fungi is still unclear and is not adequately accessed yet. Thus, continuous sampling/collecting, accelerated nrDNA sequencing of fruitbodies, as well as ectomycorrhizal sequencing and analysis are still needed. Socio-economically, some lactarioid species contribute to the survival of local populations in tropical Africa and West Africa (De Kesel et al. 2002; Boa 2006; Maba 2010; Yorou et al. 2014). But we still do not know what are the nutritional values of edible *Lactifluus* and *Lactarius* species for local populations; also what are the endangered lactarioid species, due to climate change effects, exacerbated by human degradation of their habitats.

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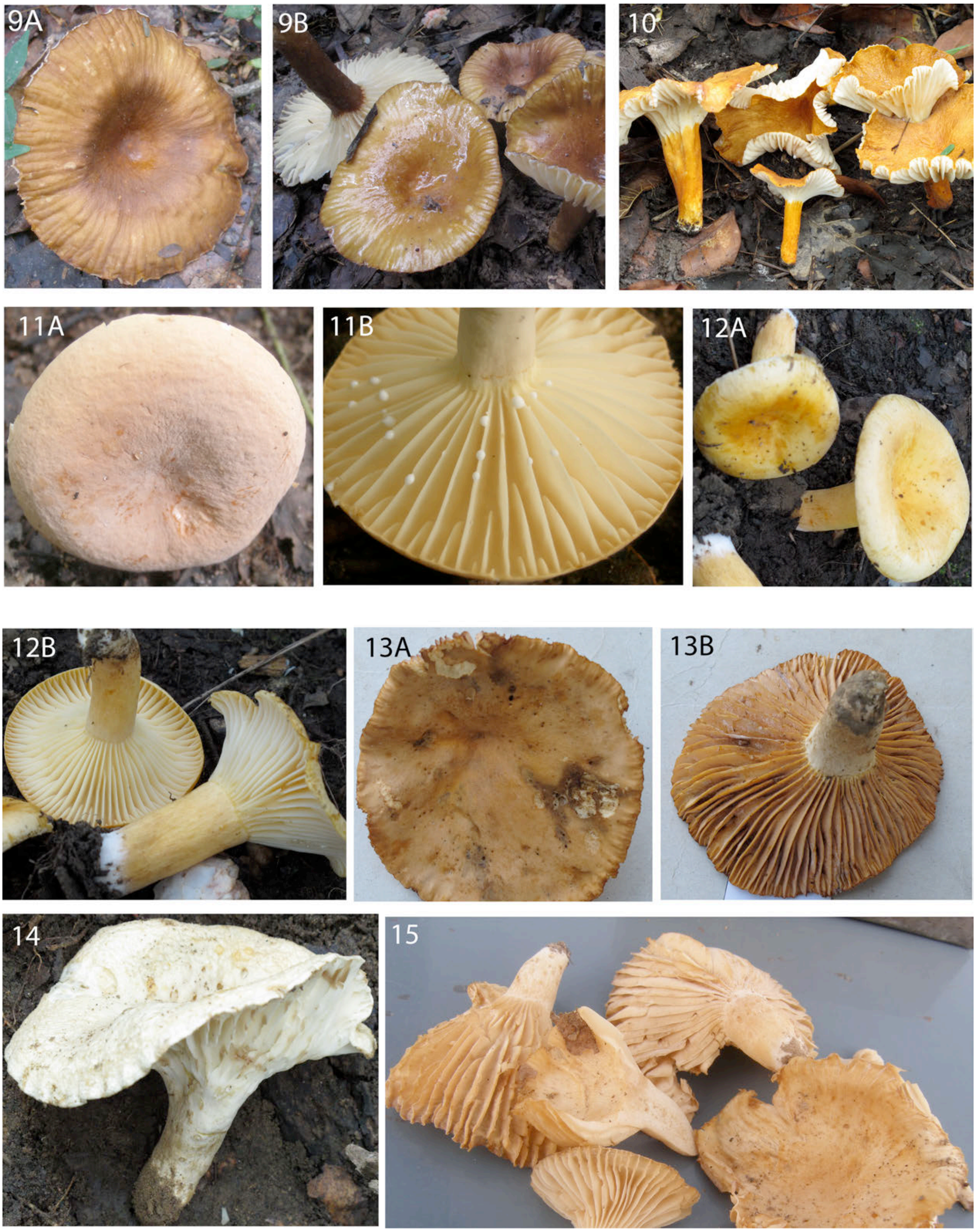
Appendix

Appendix 1: Diversity of basidiomes of West African lactarioids

A: ***Lactifluus***

1. A-B – *Lactifluus guellii*, 2. A-B – *Lf. sudanicus*, 3. A-B – *Lf. emergens*, 4. A-D – *Lf. rubiginosus*, 5. A-B – *Lf. nonpiscis*, 6. A-B – *Lf. flammans*, 7. – *Lf.* aff. *cocosmus*, 8. A-B – *Lf. luteopus*



9. A-B – *Lactifluus brunneocapus*, 10. – *Lactifluus* sp., 11. A-B – *Lf.* sp., 12. A-B – *Lf. melleus*, 13. A-B – *Lf. fazaoensis*, 14. A-B – *Lf. foetens*, 15. – *Lf. longicystidiosus*

16. A-B – *Lactifluus longibasidius*, 17. A-B – *Lf. pectinatus,* 18. A-B – *Lactifluus* sp., 19. A-B – *Lf. membranaceus*, 20. A-C – *Lf. knobsoides*, 21. A-B – *Lf. burkinabei*, 22. A-B – *Lf.* aff. *foetens*

23. A-B – *Lactifluus flavellus*, 24. A-B – *Lf. togoensis,* 25. A-B – *Lactifluus* sp., 26. A-B – *Lf. annulatoangustifolius*, 27. A-B – *Lf. annulatolongisporus*

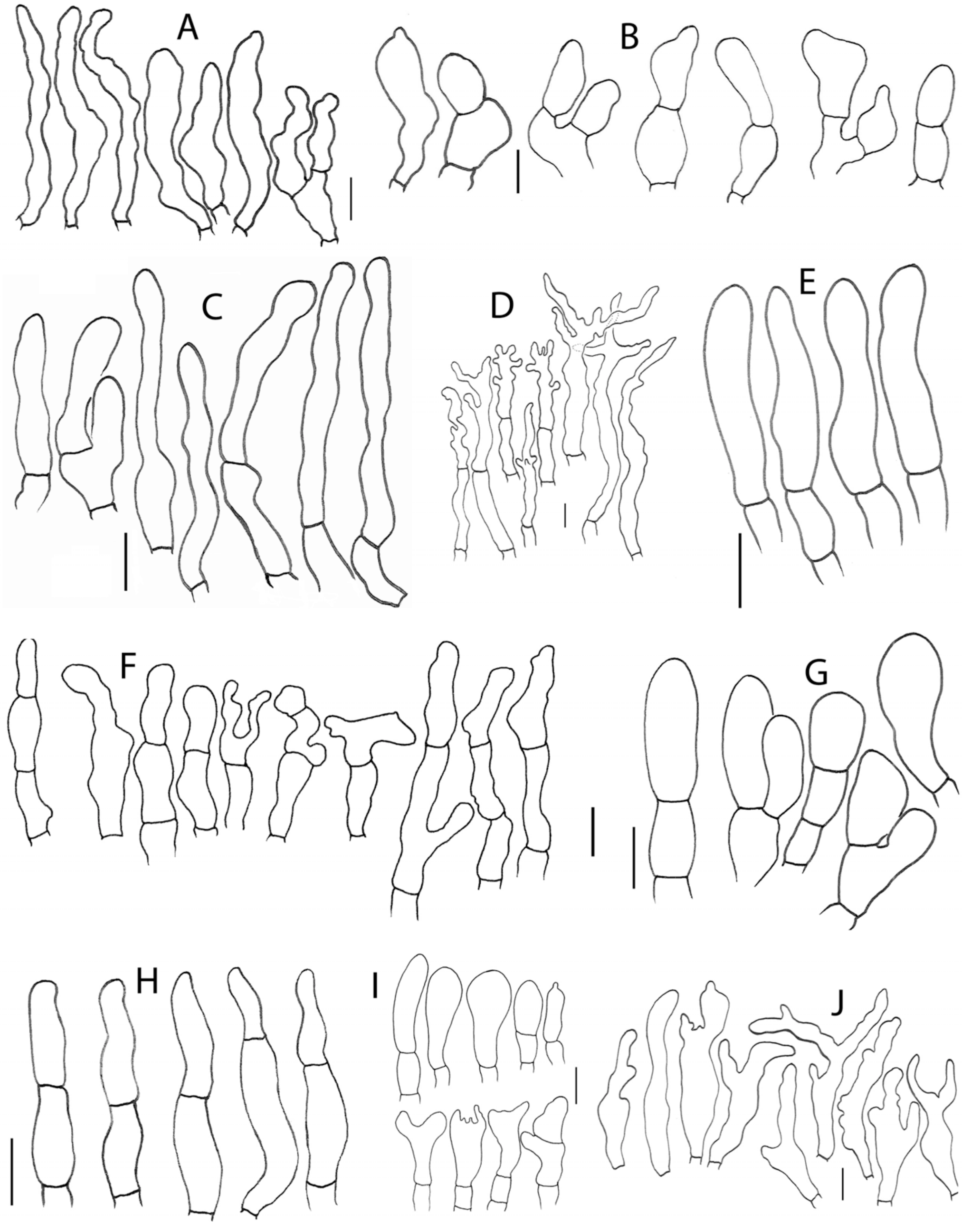
28. A-C – *Lactifluus* sp., 29. A-C – *Lf. medusae*

30. – *Lactifluus longisporus*, 31. A-C – *Lf. pimulus,* 32. A-B – *Lactifluus* sp., 33. A-B – *Lf.* sp., 34. A-B – *Lf. densifolius*, 35. – *Lf. longipes*

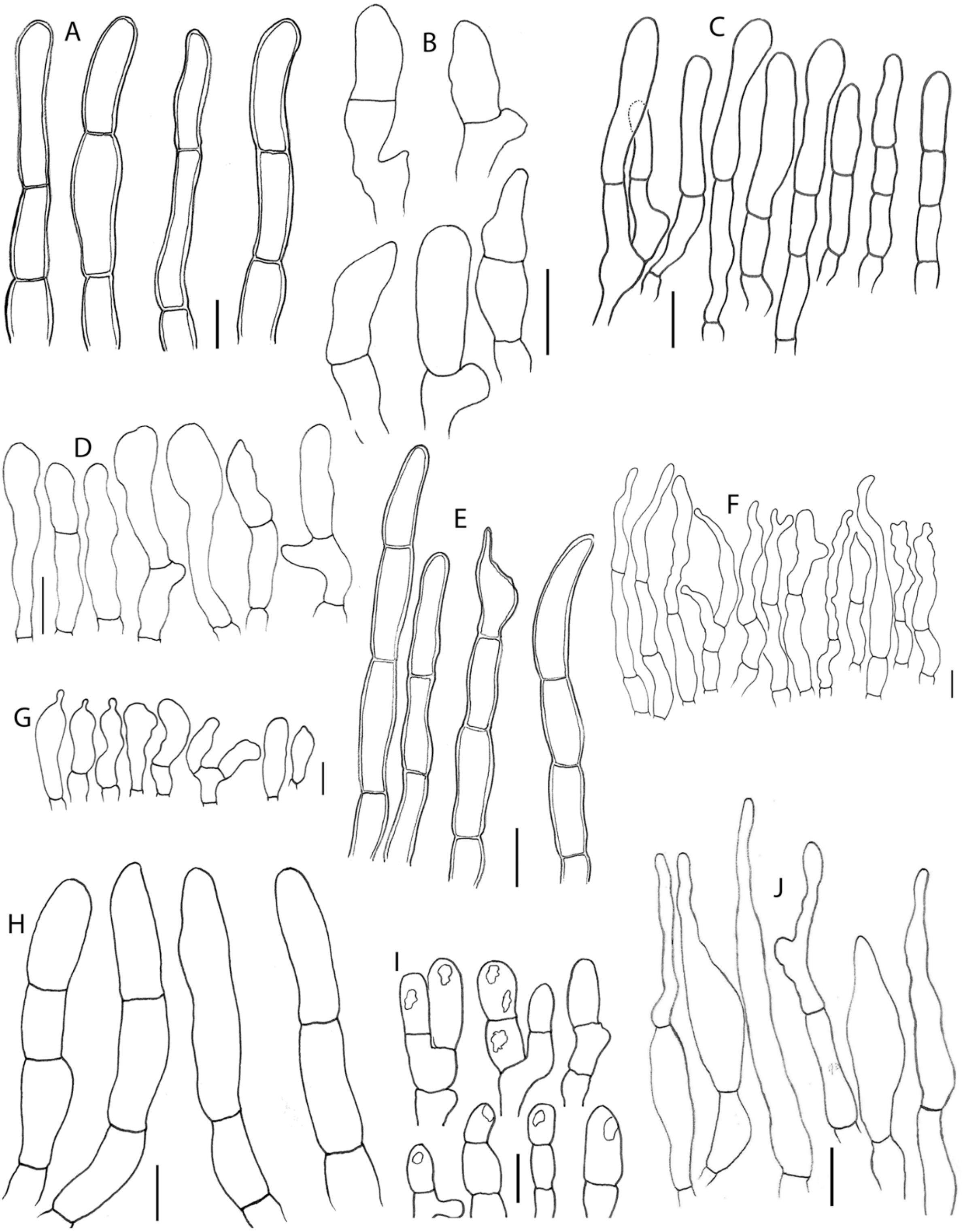
B: *Lactarius*

1. A-B – *Lactarius atro-olivinus*, 2. A-B – *L. afroscrobiculatus,* 3. A-B – *L. meniatescens*, 4. A-B – *L. kabansus*, 5. A-C –*L. saponaceus*, 6. A-B – *L. tenellus*, 7. A-C – *L. subbaliophaeus*

Appendix 2: Diversity of marginal cells of West African lactarioids

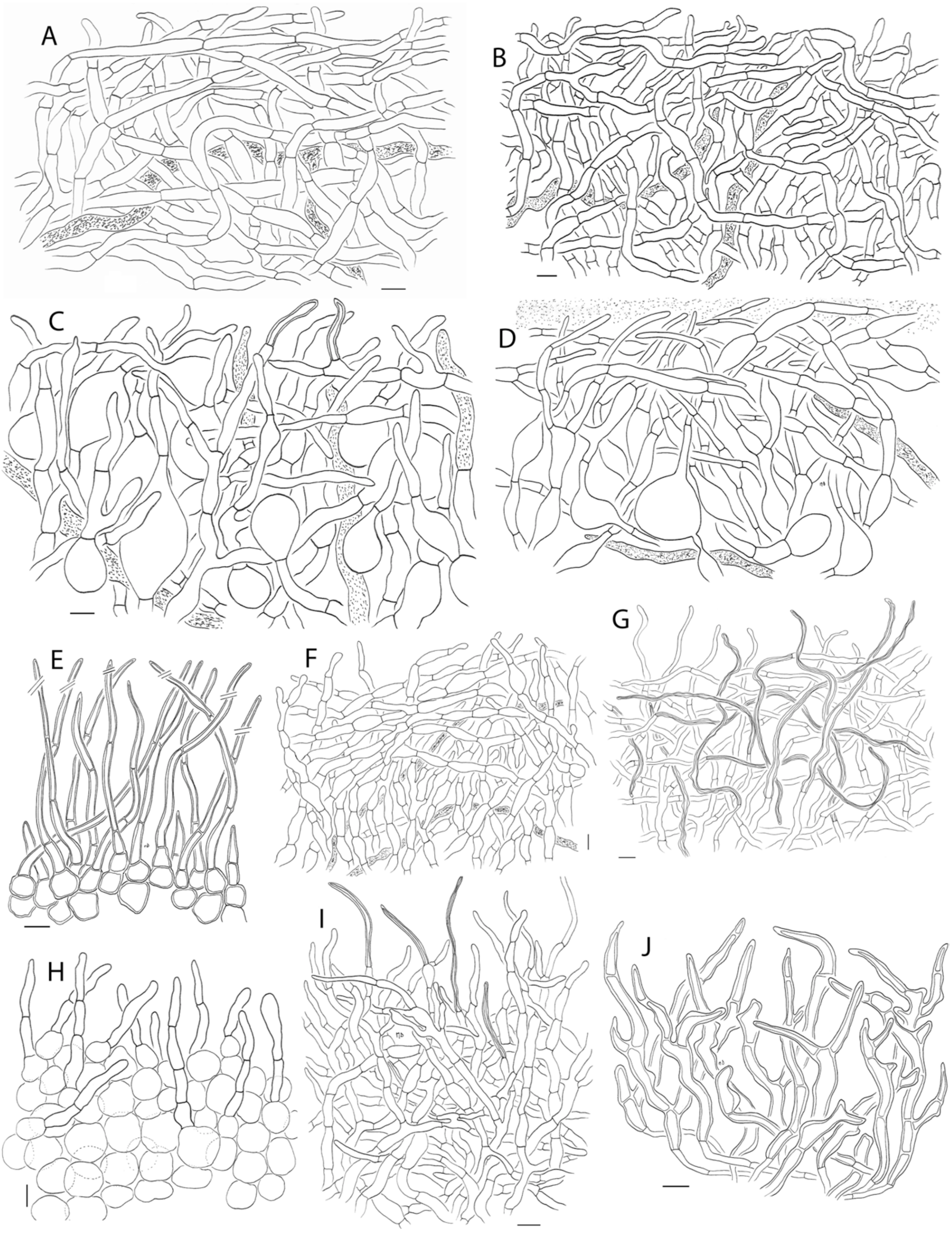


A – *Lactifluus pectinatus*, B – *Lf. guellii*, C – *Lf. brunneocarpus*, D – *Lf. fazaoensis*, E – *Lf. membranaceus*, F – *Lf. knobsoides*, G – *Lf. melleus,* H – *Lf. sudanicus*, I – *Lf. togoensis*, J – *Lf. annulatolongisporus*.

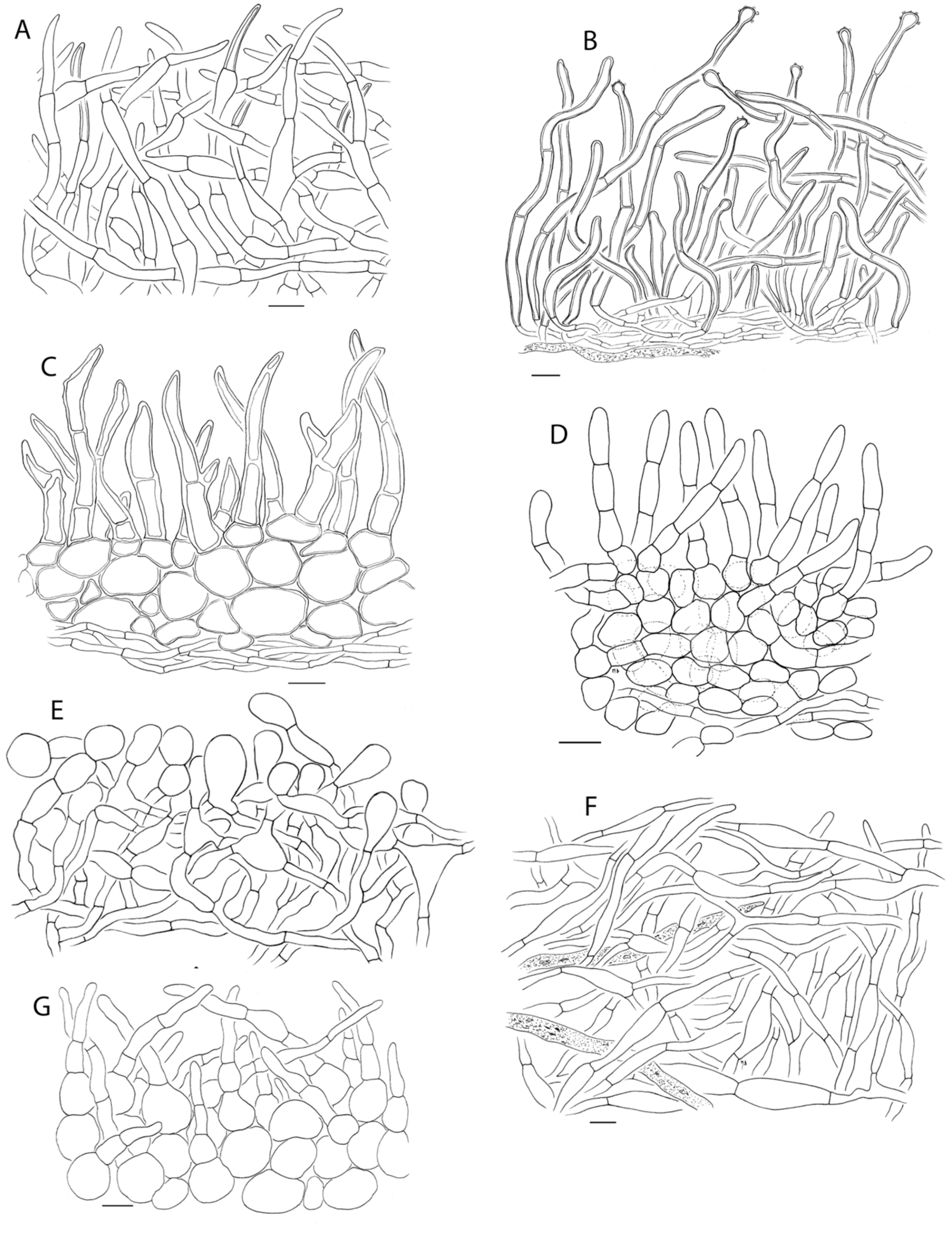


A – *Lactifluus nonpiscis*, B – *Lf. emergens*, C – *Lf. longibasidius*, D – *Lf. flavellus*, E – *Lf. gymnocarpus*, F – *Lf. longicystidiosus*, G – *Lf. burkinabei,* H – *Lactarius* sp., I – *L. kabansus*, J – *L. subbaliophaeus*.

Appendix 3: Diversity of pileipellis structure of West African lactarioids

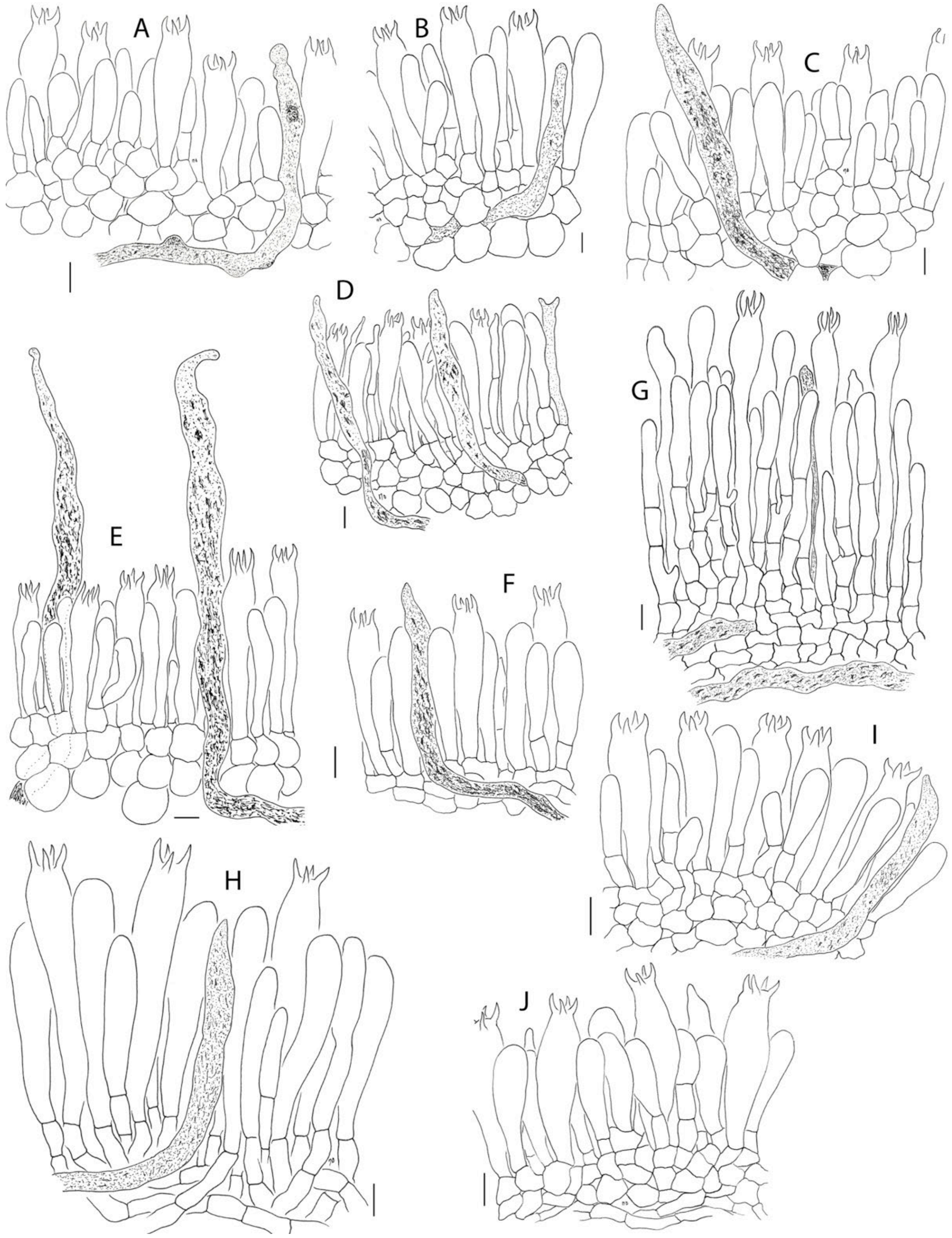


A – *Lactifluus brunneocarpus*, B – *Lf. fazaoensis*, C – *Lf. burkinabei*, D – *Lf. pectinatus*, E – *Lf. sudanicus*, F – *Lf. knobsoides*, G – *Lf. longicystidiosus,* H – *Lf. flavellus*, I – *Lf. longibasidius*, J – *Lf. membranaceus*.



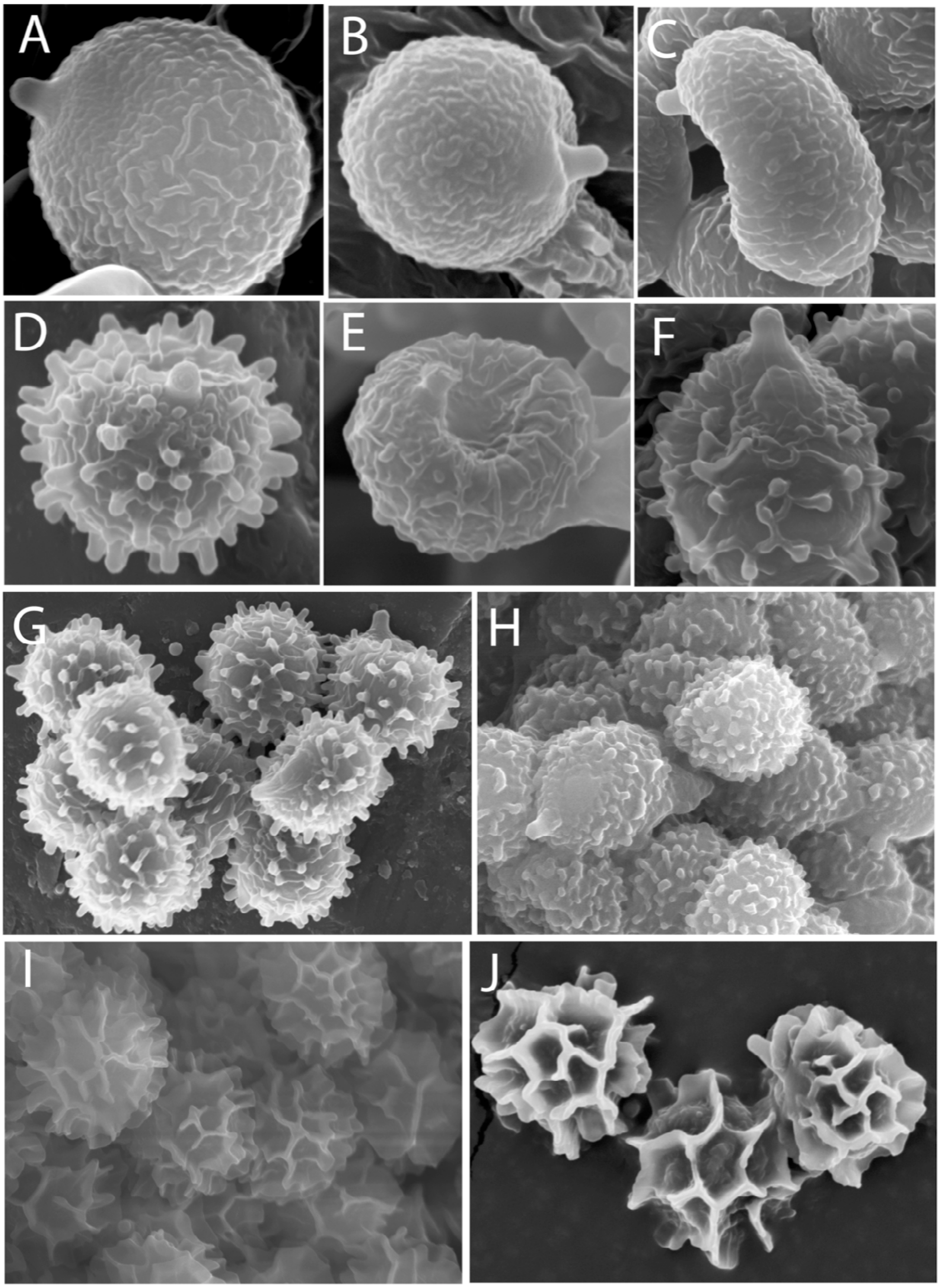
A – *Lactifluus emergens*, B – *Lf. nonpiscis*, C – *Lf. gymnocarpus*, D – *Lf. rubiginosus*, E – *Lactarius kabansus,* F – *L.* sp., G – *L. subbaliophaeus*

Appendix 4: Diversity of hymenia and subhymenia of West African lactarioids, showing pleuropseudocystidia with needle like contents.



A – *Lactifluus togoensis*, B – *Lf. sudanicus*, C – *Lf. melleus*, D – *Lf. fazaoensis*, E – *Lf. longicystidiosus*, F – *Lf. membranaceus*, G – *Lf. longibasidius*, H – *Lf. emergens*, I – *Lf. rubiginosus*, J – *Lactarius subbaliophaeus*.

Appendix 5: Diversity of basidiospores of West African lactarioids

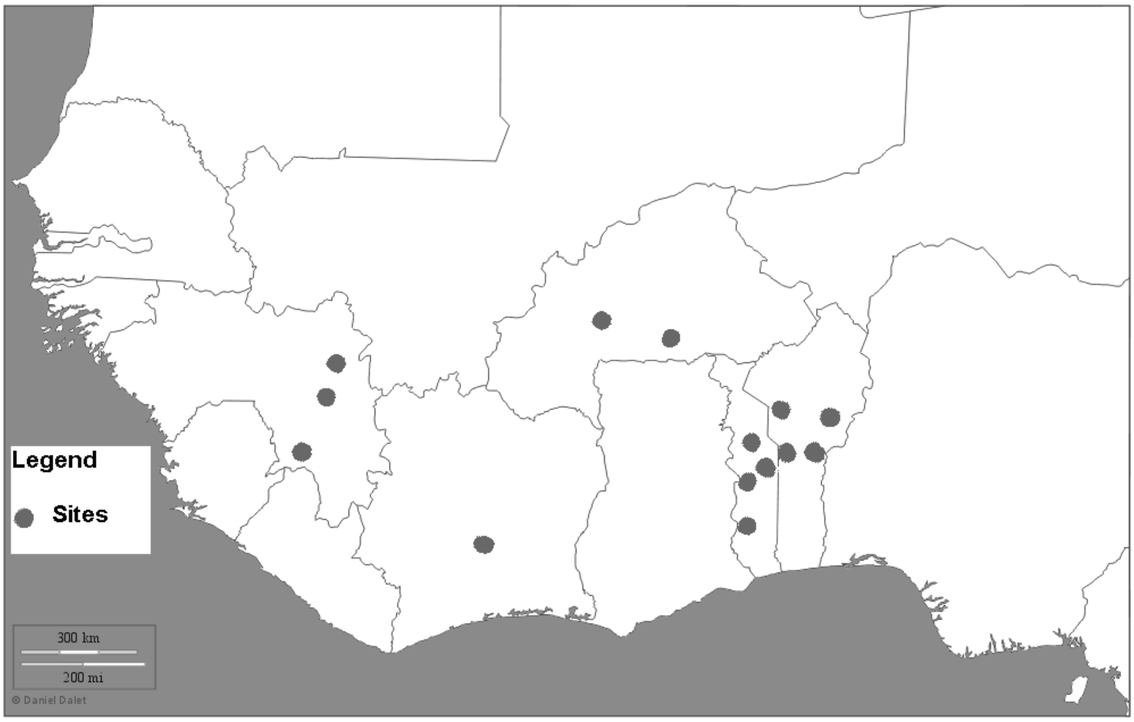


A – *Lactifluus heimii*, B – *Lf. membranaceus*, C – *Lf. annulatolongisporus*, D and G – *Lf. guellii*, E – *Lf. flavellus*, F – *Lf. melleus*, G – *Lf. longicystidiosus,* H – *Lactarius* sp., I – *Lactarius* sp., J – *L. subbaliophaeus*.

Appendix 6: Sampling of lactarioids in West Africa

A- Megatransect through various vegetation types in 5 West African countries

B- Collection sites of lactarioids in West Africa



Appendix 7: Voucher specimens and DNA sequences of West African *Lactifluus*, *Lactarius*, and allied genera deposited ENA GenBank

Species	**GenBanks accession numbers**	**Samples origin**
Lactifluus (50 sequences)		
Lactifluus annulatoangustifolius	HG426475	Togo
Lactifluus annulatolongisporus	HG426470, LK392606	Togo
Lactifluus burkinabei	LK392609	Burkina Faso
Lactifluus brunneocarpus	LK392608	Guinea
Lactifluus densifolius	HG917385	Togo
Lactifluus edulis	HG917384	Togo
Lactifluus emergens	HG426467	Togo
Lactifluus fazaoensis	HG426477	Togo
Lactifluus flammans	HG426471	Togo
Lactifluus flavellus	LK392594, LK392595	Togo
Lactifluus foetens	HG917381	Togo
Lactifluus foetens	LK392603	Burkina Faso
Lactifluus guellii	HG426466	Togo
Lactifluus guellii	LN849747	Togo
Lactifluus gymnocarpoides	LK392601, LK392600	Benin
Lactifluus gymnocarpus	HG426472	Togo
Lactifluus heimii	LK392612	Togo
Lacifluus knobsoides	LK392605	Benin
Lacifluus knobsoides	LN849746	Togo
Lacifluus knobsoides	LN849745	Guinea
Lacifluus knobsoides	LN849743	Benin
Lacifluus knobsoides	LN849742	Benin
Lactifluus longibasidius	LK392596, HG426473	Togo
Lacifluus aff. *knobsoides*	LK392604, LM999911	Benin
Lactifluus longicystidiosus	LN849748	Burkina Faso
Lactifluus longipes	HG917391, HG917383	Togo
Lactifluus luteopus	LK392602	Togo

Lactifluus luteopus	LK392611	Burundi
Lactifluus medusae	HG426474	Togo
Lactifluus melleus	LK392598, LK392597	Togo
Lactifluus membranaceus	LK392610	Guinea
Lactifluus membranaceus	HG426478	Togo
Lactifluus nonpiscis	HG426468	Togo
Lactifluus pectinatus	LK392599	Togo
Lactifluus rubiginosus	HG917386	Togo
Lactifluus rubiginosus	LN849750	Togo
Lactifluus sudanicus	HG426469, HG426476	Togo
Lactifluus togoensis	LK392607	Togo
Lactifluus togoensis	LN849741	Benin
Lactifluus sp.	LK931501	Togo
Lactifluus sp.	LN651269	Burkina Faso
Lactifluus sp.	LM999910	Togo
Lactarius (15 sequences)		
Lactarius miniatescens	HG917374	Togo
Lactarius miniatescens	HG917375	Burkina Faso
Lactarius kabansus	HG917376	Togo
Lactarius kabansus	HG917390	Zimbabwe
Lactarius subbaliophaeus	HG917372	Togo
Lactarius tenellus	HG917373	Togo
Lactarius tenellus	LN849752	Burkina Faso
Lactarius tenellus	HG917389	Kenya
Lactarius saponaceus	HG917379	Guinea
Lactarius saponaceus	HG917378	Togo
Lactarius saponaceus	LN849751	Benin
Lactarius afroscrobiculatus	HG917377	Togo
Lactarius sp.	HG917380	Togo
Lactarius sp.	Submitted	Benin
Lactarius sp.	Submitted	Burkina Faso
Russula (3 sequences)		

Russula congoana	HG917387	Togo
Russula sp.1	LN849754	Benin
Russula sp.1	LN849753	Guinea
Termitomyces		
Termitomyces sp.	HG917388	Togo

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The DAAD scholarship was offered to me on an annual basis. In this context, progress reports, coupled with references regarding my previous performance, ability and skills, were prerequisites for renewing the scholarship. PD. Dr. Peter Döbbeler and Prof. Dr. Günther Heubl have not hesitated to write reference letters respectively in 2013 and 2014 for this purpose. In parallel, PD. Dr. Marc Gottschling, PD. Dr. Peter Döbbeler, and Prof. Dr. Günther Heubl always showed interest in my achievements and often inquired about my scientific progress. For this friendly and collegial attention and support, I extend my sincere gratitude to them.

I would like to thank Prof. Dr. Susanne Renner, Chair of Systematic Botany and Mycology, and for the particular interest she showed for my achievements.

I conducted my first mycological investigation as bachelor-student 2007 in Togo, and have never imagined that I would continue working on tropical fungi during following years. The great interest in mycology has been developed in me by the encouragement of Prof. Atsu Kudzo Guelly from my home University. Prof. Guelly teaches Botany and tropical ecology at the University of Lomé in Togo (West Africa), and although his personal area of expertise is not fungal, he was able to give lecture of general information on macrofungi. In 2008 Prof. Guelly put me in touch with his collaborator, a Belgian mycologist Dr. De Kesel, with whom he co-supervised my Master studies in 2009-2010. The present thesis is the result of this initiative. Prof. Guelly has being my moral mentor since my bachelor study. He and his family are my second family in Togo. I would like to express my gratitude and thanks to him for the basic crucial role he played, and for his commitment to the promotion of mycology in Togo.

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Earlier in 2008, I met an irreplaceable friend and colleague: Dr. Souleman N. Yorou. He was in Togo for his first visit for mycological investigation. He immediately selected me, trusted me, and encouraged me for completing my Master study in Mycology, on the genus *Lactarius* s. l. With no reserve he involved me in his project that brought me for the first time to Germany and Belgium. He encouraged me to submit a proposal to DAAD for my PhD thesis in 2012. For this friendly support and collegial and very particular attention, I would like to express my deepest thanks to him, also for his commitment to further me to a young West African mycologist in general.

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Curriculum Vitae

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Education

10/2012-05/2015	Doctoral thesis: Diversity, Molecular phylogeny, Ecology and Distribution of the genera *Lactifluus* and *Lactarius* (Russulales, Basidiomycota) in West Africa. Advisor: Prof. Dr. Reinhard Agerer, Ludwig-Maximilians-Universität, München (Germany)
2009-2010	Master in Developmental Biology, option: Mycology at Faculty of Sciences, University of Lomé-Togo
2006-2007	"Maîtrise" in Botany, option: Plant Biology. Faculty of Science, University of Lomé-Togo.
2003-2006	Bachelor of Natural Sciences, Faculty of Sciences, University of Lomé-Togo.
2002	Baccalaureate, specializing in Biology

Internships

09/2007-03/2008	Voluntary training at Environmental Ministry and Logging (ODEF-Togo): Inventories, Statistics and Management.
July (02-26) 2007	Attending to training course: Building of individual and institutional capacities in taxonomy and collection management: West African mycology. Organized by the Botanic Garden of Belgium, and the Department of Botany and Plant Ecology of University of Lomé

March-May 2011	Laboratory work at Ludwig-Maximilians-Universität München (Germany), hosted by Professor Reinhard Agerer: Microscopic identification and illustration of fungi

Workshop

September 2014	International *Russulales* Workshop, Areál Zdravia, Jedľové Kostoľany (Slovakia)
June 2011	Committee member for organization of a seminar and intensive training on tropical mycology researches, organized by the Department of Botany, University of Lomé (Togo) and the Department of Biology I, Ludwig-Maximilians-Universität München: Field trip and microscopic characterization.
November 2010	Committee member for organization of a seminar on West African mycology in Burkina Faso: Capacity building and exchange in West Africa

Publications and Contributions

Maba DL, Sanon E, Verbeken A, Kamou H and Agerer R (2015) The genus *Lactifluus* (*Basidiomycota Russulales*) from West Africa: What do we know? Diversity, morpho-anatomy, molecular phylogeny and new species described presenting thromboplera and knobs (In review).

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Oral presentations to international Conferences

Maba DL, Pondika N, Agbaleynon K, Kamou H, Abalo-Loko S, Yorou SN, Guelly AK, Akpagana K (2014) Diversity and Molecular phylogeny of Togoan *Lactifluus* & *Lactarius* species. Journées Scientifiques Internationales de Lomé XVI^{ème} édition (JSIL 2014).

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Guelly KA, **Maba DL**, De Kesel A, Yorou NS (2011) Les Cantharellus spp. du Togo. Atelier « Les Mycorhizes: Un outil biologique pour le développement en Afrique » 21 au 23 février, Pôle de Recherche ISRA-Hann, Dakar (Sénégal)

Posters to international Conferences

Maba DL, Guelly AK, Yorou NS, Agerer R (2014) Morpho-anatomical and molecular phylogenetic study within *Lactifluus* subgenera *Edules* and *Russulopsis* from tropical Africa. The 10th International Mycological Congress, Queen Sirikit National Convention Center (QSNCC), Bangkok, Thailand, 03-08 August 2014

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Professional experience

2008-2012	Teacher in Natural Sciences in High School (Atakpamé-Togo), employed by the national ministry of education, Togo.
2010-2012	Responsible of practical courses in Mycology, Faculty of Sciences, University of Lomé, Department of Botany and Plant Ecology.