

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 subballiophaeus* 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 FeSO₄. These features mentioned do not match any of the morpho-anatomically most similar species, notably *L. balliophaeus* and *L. griseogalus*.

Key words:

distribution
ecology
ectomycorrhizas
Lactifluus
molecular phylogeny
Tropical Africa

Article info: Submitted: 7 February 2014; Accepted: 28 April 2014; Published: 8 May 2014.

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

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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 & Walley 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 ceasalpinoid- and phyllantoid-dominated woodlands, savannas, and riverside forests (De Kesel *et al.* 2002, Ducouso *et al.* 2002, Maba 2010, Verbeken & Walley 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 km²), Togo exhibits not only a high ecosystem diversity, but also one of the highest number of plant species per square km² in comparison to other West African countries (Akpagana 1989). The country harbours many natural caesalpinoid- 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 prepared 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 & Walley 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. subballiophaeus* 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 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 & Walley (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 & Walley (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 Nucleotide 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.

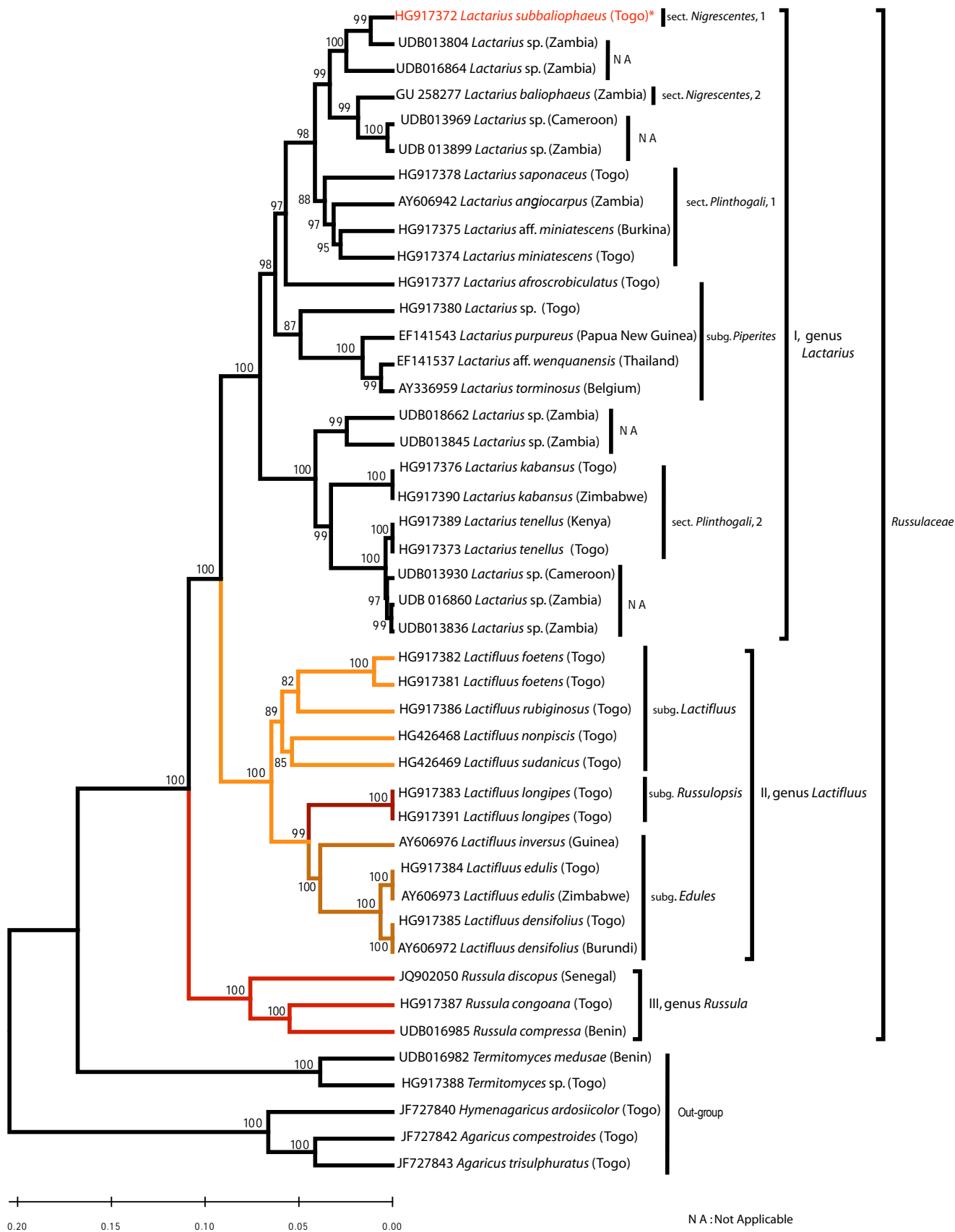


Fig. 1. Best Maximum Likelihood phylogenetic tree showing the placement of *Lactarius* samples from Togo including the newly described species and other species from tropical Africa. Bootstrap values higher than 80 % are shown above the branches. GenBank (UNITE, NCBI and ENA) sequences accession numbers 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
<i>Lactarius afroscrobiculatus</i>	ADK4599	Togo	HG917377
<i>Lactarius kabansus</i>	MD132	Togo	HG917376
<i>Lactarius kabansus</i>	AV99-179	Zimbabwe	HG917390
<i>Lactarius saponaceus</i>	MD390	Togo	HG917378
<i>Lactarius subbaliophaeus</i>	MD100	Togo	HG917372
<i>Lactarius tenellus</i>	MD149	Togo	HG917373
<i>Lactarius tenellus</i>	ADK397	Kenya	HG917389
<i>Lactarius</i> sp.	MD391	Togo	HG917380
<i>Lactarius miniatescens</i>	MD151	Togo	HG917374
<i>Lactarius</i> aff. <i>miniatescens</i>	MD177	Burkina Fasso	HG917375
<i>Lactifluus edulis</i>	C2168	Togo	HG917384
<i>Lactifluus densiofolius</i>	C2362	Togo	HG917385
<i>Lactifluus longipes</i>	ADK4315	Togo	HG917383
<i>Lactifluus longipes</i>	C2445	Togo	HG917391
<i>Lactifluus foetens</i>	C1822	Togo	HG917382
<i>Lactifluus foetens</i>	MD150	Togo	HG917381
<i>Lactifluus rubiginosus</i>	MD389	Togo	HG917386
<i>Termitomyces</i> sp.	MD388	Togo	HG917388
<i>Russula congoana</i>	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) encompassed 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 specimens 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 & Walley (2010).

Features	<i>L. baliophaeus</i>	<i>L. griseogalus</i>	<i>L. subbaliophaeus</i>
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+I = 4+12 to 3+6/cm	Broadly adnate, not decurrent, distant (total 28)	Broadly adnate, to slightly subdecurrent, distant L+I = 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 Q = 1.01–1.07–1.09–1.15 Plage distally amyloid	Usually ellipsoid, rarely subglobose Q = 1.07–1.21–1.36–1.55 Plage not, or slightly amyloid	Globose, subglobose rarely ellipsoid Q = 1.04–1.11–1.27 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, subcylindrical, fusiform or tortuous, almost 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 FeSO₄ bluish; taste bitter and acrid.

Type: Togo: Central region: Prefecture of Tchoudjo, National Park of Fazao-Malfakassa, 08°42'11" N 0°46'25" E, on soil in gallery forest dominated by *Uapaca guineensis* and *Azelia 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 subinfundibuliform 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+I = 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 FeSO₄. *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. *Pleuroseudocystidia* 4–5–6 µm diam, abundant, cylindrical, sometimes tortuous, with brown contents. Lamellar edge sterile. *Marginal cells of lamellae* 23–72 × 3–6 µm, subcylindrical, 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

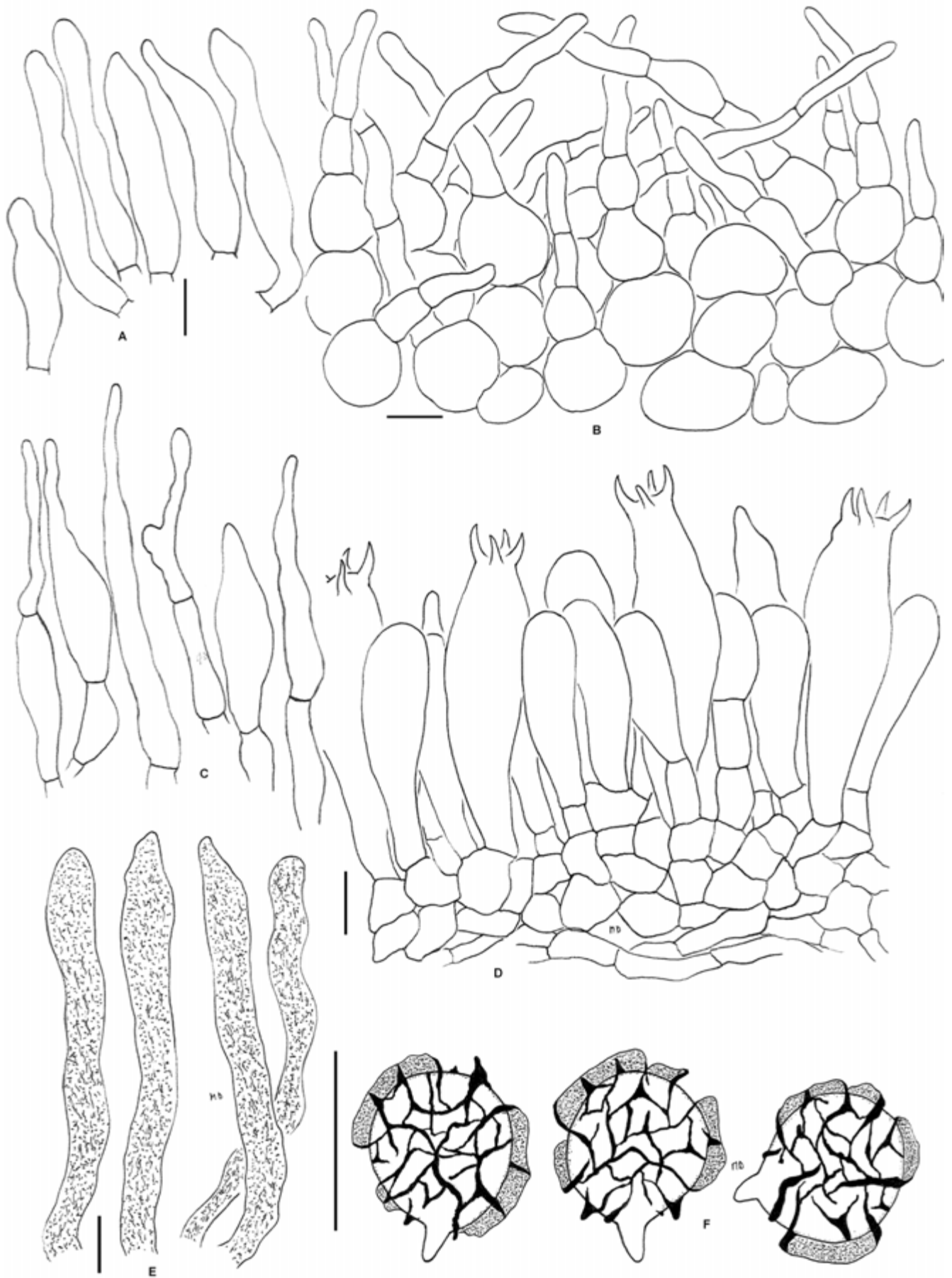


Fig. 2. Light microscopy of *Lactarius subbaliophaeus* (MD100). **A.** Pleuroprocystidia. **B.** Pileipellis. **C.** Marginal cells. **D.** Hymenium. **E.** Pleuropseudocystidia. **F.** Spores. Bars = 10 μ m.

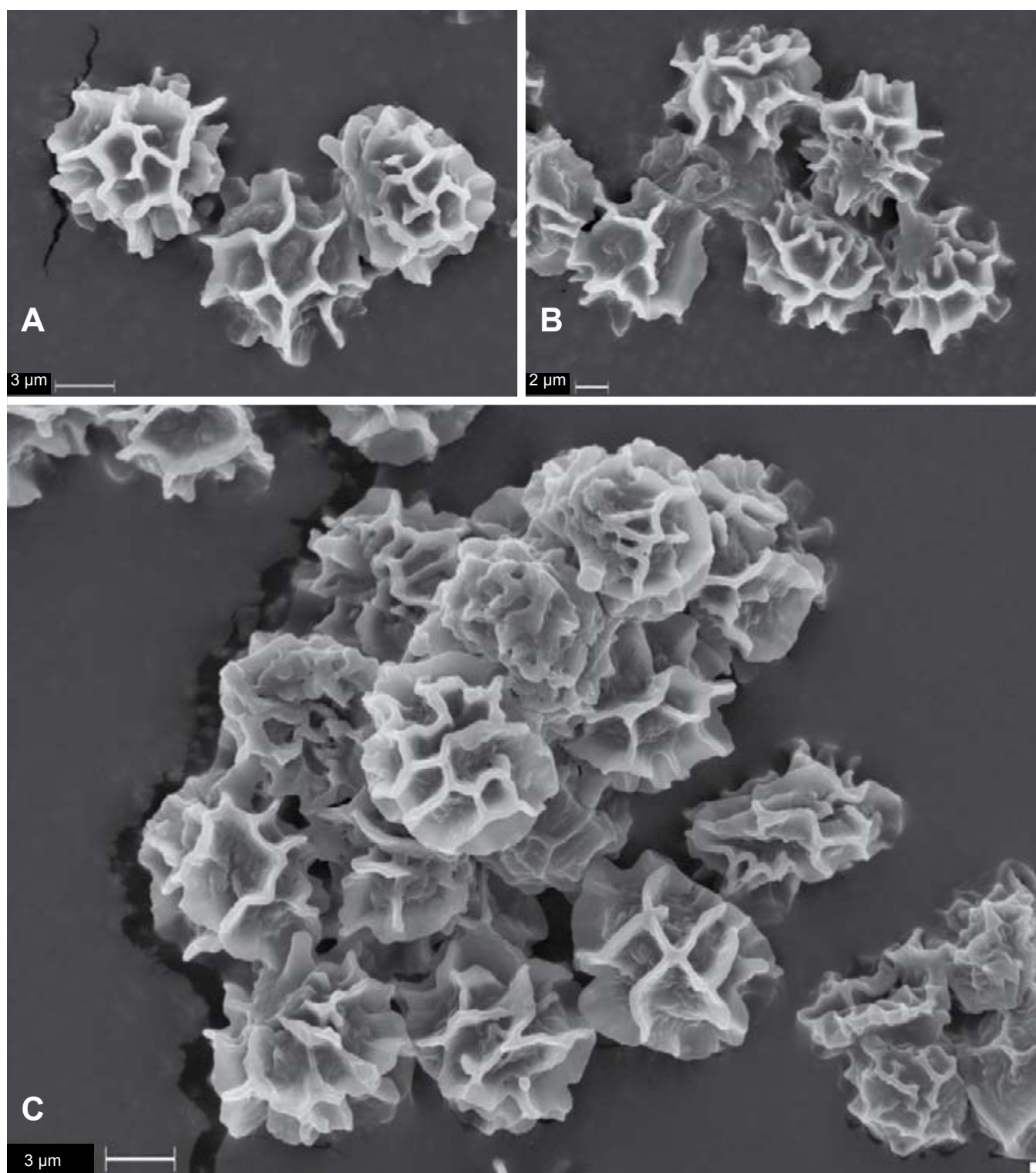


Fig. 3. SEM of *Lactarius subbaliophaeus* (MD100). A–B. Basidiospores in different views. C. General view.

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

¹MD = collection of *D. Maba*; ²C= collection of *A. Guelly*; ³ADK= 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* subgen. *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 & Walley 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. acrisimus*) in tropical Africa, it is likely that *Lactarius* sp. MD391 constitutes an additional member within this group. Morpho-anatomical 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 morpho-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 *Isobertinia 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-Zambesian 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.

ACKNOWLEDGMENTS

We are much indebted to the International Foundation for Sciences (IFS, grant D/5178-1), the German Academic Exchange Service (DAAD, grant A/11/72562) and the German Research Foundation (DFG GZ-Yo174/2-1) for financial support.

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