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 Russuloid 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 FeSO₄. 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*, molecular phylogeny, Tropical Africa

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 Guinean Sudanian region.

Together with the genera Lactifluus, Russula, Amanita, Tomentella, Cantharellus, Xerocomus, Boletellus, Boletus, Pulveroboletus, Veloporphyrillus, and Tylotus, the genus Lactarius represents the common dominant ectomycorrhizal (ECM) fungal taxa in tropical African vegetation types (Verbeken & Buycx 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, 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 km$^2$), Togo exhibits not only a high ecosystem diversity, but also one of the highest number of plant species per square km in comparison to other West African countries (Akpagan 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 Faza O 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. subballioptaeus 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 & 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 Basidimycota 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 |
|----------------------------------|--------------------|-------------|-----------------------|
| 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 Faso| 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              |
| Lactifluus 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) 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 Pipertes, 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. (UDP013804), Lactarius sp. (UDP016864), 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.
**Lactarius s. str. in Togo**

**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 Verbeke & Walleyn (2010).**

| Features              | L. baliophaeus | L. griseogalus | L. subbaliophaeus |
|-----------------------|----------------|----------------|-------------------|
| **Basidiomata**       |                |                |                   |
| Pileus                | 30–70(–90) mm; greyish yellow to brownish (4A3-5 to 4B3-5), dark brown 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 lamellulæ** | Broadly adnate, to decurrent, crowded L+H = 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 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 FeSO₄** | 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 |
| **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, thinly-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, almostly septate, mostly with tapering apex |
| **Pileipellis**       | Hymeniderm, suprapellis 10–25 × 3–5 µm, thinly-walled | Palisade, suprapellis thick, 25–40 × 3–5 µm, thinly-walled | Palisade, suprapellis thick 20–60 × 3–5 µm, thinly-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. Basidiosomes winged, pleurocystidia inconspicuous, fusiform or tortuous, often with tapering apex marginals, a palisade pileipellis with suprapellis composed of cylindrical cells, thinly-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 bluing.

**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+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. Basidiosomes 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, plagé 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. Pleurophleurocystidia 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. **Stipitpellis** a trichoderm to ixotrichoderm with entangled hyphae at the base.
Fig. 2. Light microscopy of *Lactarius subbaliophaeus* (MD100). A. Pleuroprocystidia. B. Pileipellis. C. Marginal cells. D. Hymenium. E. Pleuropseudocystidia. F. Spores. Bars = 10 µm.
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 subcylindrical, fusiform
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 FeSO₄; 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),

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

| Species                          | Specimens          | Collector/Date | Locality and ecosystem type                                      |
|----------------------------------|--------------------|----------------|----------------------------------------------------------------|
| *Lactarius sp.*                  | MD391              | Dao Maba       | Aledjo woodlands dominated by *Isoberlinia tomentosa* and *Uapaca togoensis*, 09°13'6'' N 01°11.4'' E |
| *Lactarius afroscrobiculatus*    | BR 163477-32; 163418-79; C2268 | Andre De Kesel; Atsu Guelly | 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; Aledjo, gallery forest dominated by *Uapaca guineensis* *Isoberlinia doka* and *Uapaca togoensis*, 09°16'34.0'' N 01°13.32'' E |
| *Lactarius atro-olivinus*        | BR 163674-35; BR163490-45; ADK4538; ADK4813; ADK4836 | Andre De Kesel; 15 May 2010; 17 May 2010; Dao Maba | Fazao, woodland dominated by *Berlinkia grandiflora*, 09°16'46'' N 01°12'24.16'' E; Ola, gallery forest dominated by *Berlinkia grandiflora* and *Uapaca guineensis*, 07°33'12.3'' N 0°52'37.8'' E |
| *Lactarius kabansus*             | MD132              | Dao Maba       | Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°45'24'' N 0°48'08'' E |
| *Lactarius melanogalus*          | ADK4292            | Andre De Kesel | Aledjo, gallery forest dominated by *Berlinkia grandiflora*, 09°16'46'' N 01°12'24.16'' E |
| *Lactarius miniatescens*         | BR 163828-92; MD09; MD151; MD401 | Andre De Kesel; Dao Maba; 27 May 2008; 19 Jun 2011; 18 July 2013 | Tchamba, Gallery forest dominated by *Milletia thonningii*, *Lonchocarpus sericeus*, 08°56.6'9.2'' N 01°31.9'6.6'' E; Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°48'52.35'' N 0°45'29.2'' E; Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°42'21'' N 0°46'18'' E; Aledjo, gallery forest dominated by *Berlinkia grandiflora* and *Uapaca guineensis*, 09°16'28.5'' N 01°13'21.5'' E |
| *Lactarius Subbaliophaeus* sp. nov. | MD100; MD14       | Dao Maba       | Fazao, woodland dominated by *Uapaca togoensis* and *Afzelia africana*, 08°42'21'' N 0°46'22'' E; Aledjo woodlands dominated by *Isoberlinia tomentosa* and *Uapaca togoensis*, 09°13'8'' N 01°11.44'' E |
| *Lactarius saponaceus*           | BR 158418-17; MD390 | Andre De Kesel; Dao Maba | Fazao, woodland dominated by *Berlinkia tomentosa*, 09°13.9'8.1'' N 01°11.4'44.2'' E; Aledjo, woodland dominated by *Isoberlinia tomentosa*, 09°13'27'' N 01°11'53'' E |
| *Lactarius tenellus*             | BR 163798-62; C2142 | Andre De Kesel; Atsu Guelly; Dao Maba | Fazao, woodland dominated by *Uapaca togoensis*, 08°43'14.5'' N 0°46'33.2'' E; Fazao, woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 08°43'19'' N 0°46'31'' E |

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

whilst the sequence of *L. subbaliophaeus* forms a terminal sister clade together with two samples from Zambia (UBD0130804, UBD016864). *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. forminosus*, *L. aff. wenguansensis*, 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. acrisissum) 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. afrorobiculatus, and L. miniatescens have no apparent preference for a special vegetation type, as they were collected from savanna woodlands as well as gallery forests (Table 3). Moreover, whether in a gallery or savanna, L. afrorobiculatus was collected in habitats that harbour Uapaca species and Isoberlinia doka; L. atro-olivinus occurs often in the presence of Berlinha 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 Lactitius (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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