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Lactifluus kigomaensis and *L. subkigomaensis*: Two look-alikes in Tanzania

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ABSTRACT

A look-alike of *Lactifluus kigomaensis*, described in 2012 from primary miombo woodlands in the Kigoma Province of northwestern Tanzania, is proposed here as *L. subkigomaensis*. The phylogeny based on the molecular markers ITS, LSU, *RPB1* and *RPB2* shows that *L. subkigomaensis* is a sister species to *L. kigomaensis*. Detailed descriptions of both species are given here, aiming at finding good characters to unravel these look-alikes. Both species are consumed and offered for sale on local markets.

1. Introduction

Miombo woodland is one of the most important and richest ectomycorrhizal vegetation types of Africa. In Tanzania, the Kigoma province is the region containing the largest untouched miombo zones in the country. Edible mushrooms within miombo woodland are well-explored in Tanzania, however, not in Kigoma (Härkönen, Saarimäki, & Mwasumbi, 1994, 1995, 1998, 2003, 1993; Saarimäki, Härkönen, & Mwasumbi, 1994; Calonge, Härkönen, Saarimäki, & Mwasumbi, 1997; Karhula, HÄrkönen, Saarimäki, Verbeken, & Mwasumbi, 1998; Tibuhwa, Buyck, Kivaisi, & Tibell, 2008, 2012; Buyck, Kauff, Couloux, & Hofstetter, 2012).

This paper reveals two species behind the formerly described *Lactifluus kigomaensis* De Crop & Verbeken. In its original publication, *L. kigomaensis* was mentioned to share quite some characters with species of *L.* sect. *Pseudogymnocarpi* (Verbeken) Verbeken (thick-walled hairs in the pileipellis and lamprocystidia), but because of the trichodermic structure of the pileipellis it was deviating from other representatives in this section. The preliminary phylogenetic results at that time suggested that *L. kigomaensis* had an isolated position within the phylogeny of *Lactifluus* (Pers.) Roussel. In the meantime new phylogenetic results show that *L. kigomaensis* is actually two species and is placed within *L.* sect. *Rubroviolascentini* (Singer) Verbeken, an exclusively African clade within *L.* subg. *Pseudogymnocarpi* (Verbeken) De Crop, of which all species are characterised by pleurolamprocystidia (De Crop et al., 2017). Other representatives of *L.* sect. *Rubroviolascentini* are L. *carmineus* (Verbeken & Walleyn) Verbeken, *L. rubroviolascens* (R. Heim) Verbeken and L. *denigricans* (Verbeken & Karhula) Verbeken.

Since the publication of *Lactifluus kigomaensis* in 2012 (De Crop, Tibuhwa, Baribwegure, & Verbeken, 2012), our knowledge on the diversity of *Lactarius* Pers. and *Lactifluus* in tropical Africa has increased fairly, bringing the total number to 41 (instead of 39) and 75 (instead of 59) species respectively (Maba, Guelly, Yorou, & Agerer, 2015a, 2014a, b, b; Beenken, Sainge, & Kocyan, 2016; De Crop, 2016; De Crop et al., 2016; Delgat, De Crop, Njouonkou, & Verbeken, 2017; Maba, 2015). But even more important, our insight in the genus *Lactifluus* has changed considerably and led to a new classification (De Crop, 2016; De Crop et al., 2017). In this new classification, *Lactifluus* is divided into four subgenera and 19 sections. *Lactifluus* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop

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consists of 4 sections, *L.* subg. *Lactariopsis* (Henn.) Verbeken consists of 4 sections, *L.* subg. *Lactifluus* consists of 6 sections and *L.* subg. *Pseudogymnocarpi* consists of 5 sections. All subgenera, except *L.* subg. *Lactifluus*, contain even more unknown clades that probably represent new sections (De Crop, 2016; De Crop et al., 2017).

In De Crop et al. (2012), collection AV 11-006 was chosen as holotype of *L. kigomaensis* but the microscopic description and line drawings were based on collection AV 11-066. Molecular analysis revealed that collections AV 11-006 and AV 11-066 represent two different species. Consequently, the original description of *Lactifluus kigomaensis* is actually a mixture, with a macroscopic description based on the holotype collection and a microscopic description based on a macroscopically almost identical sister species of *L. kigomaensis.* This work rectifies the description of *Lactifluus kigomaensis* and describes a second new *Lactifluus* species from the Kigoma province in Tanzania, *Lactifluus subkigomaensis* De Lange & De Crop sp. nov. An additional analysis also showed that collection EDC 11-013 was wrongly mentioned in the studied material of De Crop et al. (2012). The collection does not represent *L. kigomaensis* nor *L. subkigomaensis,* nor a closely related species.

2. Materials and methods

2.1. Sampling

During field work in 2011 from March until April in miombo woodlands in the Kigoma province (Northwestern Tanzania), two collections of *Lactifluus kigomaensis* and four collections of *L. subkigomaensis* were found (from collection EDC 11-018 there is no dried specimen available, only a CTAB sample). The studied collections are deposited in herbarium Universitatis Gandavensis (GENT).

2.2. Morphological analysis

The macroscopic description was based on fresh material. Microscopic characters were studied from dried material in Congo-red in SDS (Sodium dodecyl sulfate). Spore ornamentation was observed in Melzer's reagent. For the terminology used we refer to Verbeken (1998) and Verbeken and Walleyn (2010). Line drawings were made with the aid of a drawing tube (Zeiss camera lucida on a Zeiss Axioskop 2 microscope equipped with a magnification changer of 2.5× for spores, without magnification changer for sections and an Olympus U-DA on an Olympus CX21 microscope for individual elements) at original magnifications of 6000× for spores and 1000× for sections and 1500× for individual elements. Pleurolamprocystidia were measured at original magnification of 400× . Basidia length excludes sterigmata length. Spores were measured in side view in Melzer's reagent, excluding the ornamentation. Spore measurements are given as (MINa) [AVa-2*SD]–AVa–AVb–[AVb+2*SD] (MAXb), with AVa = lowest mean value for the measured collections and AVb = greatest mean value for the measured collections, SD = standard deviation, MINa = lowest extreme value of collection “a” and MAXb = greatest extreme value of collection “b”. The Q-value (quotient length/width) is given as (MIN Qa) Qa–Qb (MAX Qb), with Qa = lowest mean ratio for the measured collections and Qb = greatest mean ratio for the measured collections, MIN Qa = lowest extreme ratio of collection “a” and MAX Qb = greatest extreme ratio of collection “b”. For the color codes, we refer to Kornerup and Wanscher (1978).

2.3. Molecular analysis

DNA from dry collections was extracted using the protocol described by Nuytinck and Verbeken (2003), with modifications described in Van de Putte, Nuytinck, Stubbe, Le, and Verbeken (2010). DNA from fresh material was extracted using the CTAB extraction described in Nuytinck and Verbeken (2003). Protocols for PCR amplification follow Le, Nuytinck, Verbeken, Lumyong, and Desjardin (2007). Four nuclear markers that were previously shown informative within this subgenus (De Crop et al., 2017) were used: (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, using primers ITS-1F and ITS4 (Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990), (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo, Lutzoni, Rehner, Johnson, & Vilgalys, 2000), (3) the region between the conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (*RPB2*), using primers bRPB2-6F and fRPB2-7cR (Liu, Whelen, & Benjamin, 1999; Matheny, 2005) and (4) the region between domains A and C of nuclear gene encoding the largest subunit of RNA polymerase II (*RPB1*), using primers RPB1-Ac and RPB1-Cr (Matheny, Liu, Ammirati, & Hall, 2002; Stiller & Hall, 1997). When necessary, internal primers RPB1- F3 and RPB1-R4 were used (De Crop et al., 2017). PCR products were sequenced using an automated ABI 3730 XL capillary sequencer at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with the SequencherTM v5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA).

We know from De Crop et al. (2017) that *Lactifluus kigomaensis* and *L. subkigomaensis* belong to *L.* subg. *Pseudogymnocarpi*, *L.* sect. *Rubroviolascentini*. Our dataset contains the *L.* subg. *Pseudogymnocarpi* sequences from De Crop et al. (2017), including all known representatives *of L.* sect. *Rubroviolascentini*. One sequence per species was used, except for *L. kigomaensis* and *L. subkigomaensis,* of which more sequences were added. Five species of *L.* subg. *Lactifluus* were used as outgroup (Table 1).

Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v7 (Katoh & Toh, 2008), using the E-INS-I strategy. Trailing ends of the alignments were trimmed and the alignments were manually edited when necessary in Mega 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The alignments can be obtained from the first author and TreeBASE (Submission ID 20557). The alignments were partitioned into following partitions: ITS-LSU-alignment: partial 18S, ITS1, 5.8S, ITS2, LSU; *RPB2*-alignment: the *RPB*2 intron and the first, second and third codon positions of the exon, and *RPB1*-alignment: the different *RPB*1 introns and the first, second and third codon positions of the exons. Maximum likelihood (ML) analyses were conducted with RAxML v8.2.10 (Stamatakis, 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis, Hoover, & Rougemont, 2008). Analyses were performed on each alignment separately. The resulting gene trees did not show any supported conflicts, therefore all gene trees could be concatenated. The concatenated tree was used in the Results. All analyses were performed on the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010).

3. Results

Our molecular results clearly show that collections AV 11-006 and AV 11-066 represent two different species (Fig. 1). The new species is a sister species of *Lactifluus kigomaensis* (Fig. 1). This is supported by morphological differences (see Discussion). Based on these morphological and molecular differences, the new species is here described as *Lactifluus subkigomaensis* sp. nov. A revised description of *L. kigomaensis* is provided (see Fig. 2).

Lactifluus kigomaensis De Crop & Verbeken, *Cryptogamie*

Table 1
Specimens and GenBank accession numbers of DNA sequences used in the molecular analysis.

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus subg. *Pseudogymnocarpi*						
Lactifluus cf. *longisporus*	AV 11-025 (GENT)	Tanzania	KR364054	KR364181	KR364311	KR364439
Lactifluus cf. *pseudogymnocarpus*	AV 05-085 (GENT)	Malawi	KR364012	KR364139	KR364329	KR364459
Lactifluus cf. *pumilus*	EDC 12-066 (GENT)	Cameroon	KR364067	KR364196	KR364332	KR364462
Lactifluus gymnocarpoides	JD 885 (MEISE)	Congo	KR364074	KR364203	KR364283	KR364409
Lactifluus hygrophoroides	AV 05-251 (GENT)	North America	HQ318285	HQ318208	HQ328936	KR364413
Lactifluus luteopus Type	AV 94-463 (GENT)	Burundi	KR364119	None	KR364313	None
Lactifluus medusae	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198	KR364314	KR364442
Lactifluus pseudoluteopus	FH 12-026 (GENT)	Thailand	KR364084	KR364214	KR364331	KR364460
Lactifluus rugatus	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235	KR364337	KR364467
Lactifluus sudanicus Type	AV 11-174 (GENT)	Togo	HG426469	KR364186	KR364348	KR364480
Lactifluus cf. *pseudovolemus*	ADK 2927 (GENT)	Benin	KR364113	KR364243	KR364330	KR364461
Lactifluus goossensiae	AB 320 (GENT)	Guinea	KR364132	KR364252	KR364281	None
Lactifluus rubiginosus	JD 959 (MEISE)	Congo	KR364081	KR364210	KR364304	KR364432
Lactifluus xerampelinus	MH 201176 (GENT)	Mozambique	KR364099	KR364231	KR364364	KR364496
Lactifluus volemoides	MH 201187 (GENT)	Mozambique	KR364098	KR364230	KR364363	KR364493
Lactifluus pegleri	PAM/Mart 12-091 (LIP)	Martinique	KP691416	KP691425	KP691433	KR364397
Lactifluus sulcatipes	MCA 3937 (GENT)	Guyana	KR364109	KR364240	KR364350	None
Lactifluus sect. *Rubroviolascentini*						
Lactifluus aff. *rubroviolascens*	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195	KR364334	KR364465
Lactifluus carmineus Type	AV 99-099 (GENT)	Zimbabwe	KR364131	KR364251	KR364265	None
Lactifluus denigricans	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178	KR364272	KR364384
Lactifluus subkigomaensis Type	AV 11-066 (GENT)	Tanzania	KY562728	None	KY562734	MG779280
Lactifluus subkigomaensis	AV 11-029 (GENT)	Tanzania	KY562730	MG779278	KY562733	None
Lactifluus subkigomaensis	EDC 11-159 (GENT)	Tanzania	KR364050	KR364177	KR364295	KR364423
Lactifluus subkigomaensis	EDC 11-018 (GENT)	Tanzania	KY562729	MG779277	KY562735	MG779279
Lactifluus kigomaensis Type	AV 11-006 (GENT)	Tanzania	KR364052	KR364179	KR364288	KR364415
Lactifluus kigomaensis	EDC 11-012 (GENT)	Tanzania	KY562731	None	KY562732	None
Outgroup						
Lactifluus corrugis s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143	JQ348127	None
Lactifluus crocatus	KVP 08-034 (GENT)	Thailand	HQ318243	HQ318151	HQ328888	JN389145
Lactifluus vitellinus	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144	HQ328881	JN389138
Lactifluus acicularis	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132	HQ328869	JN389131

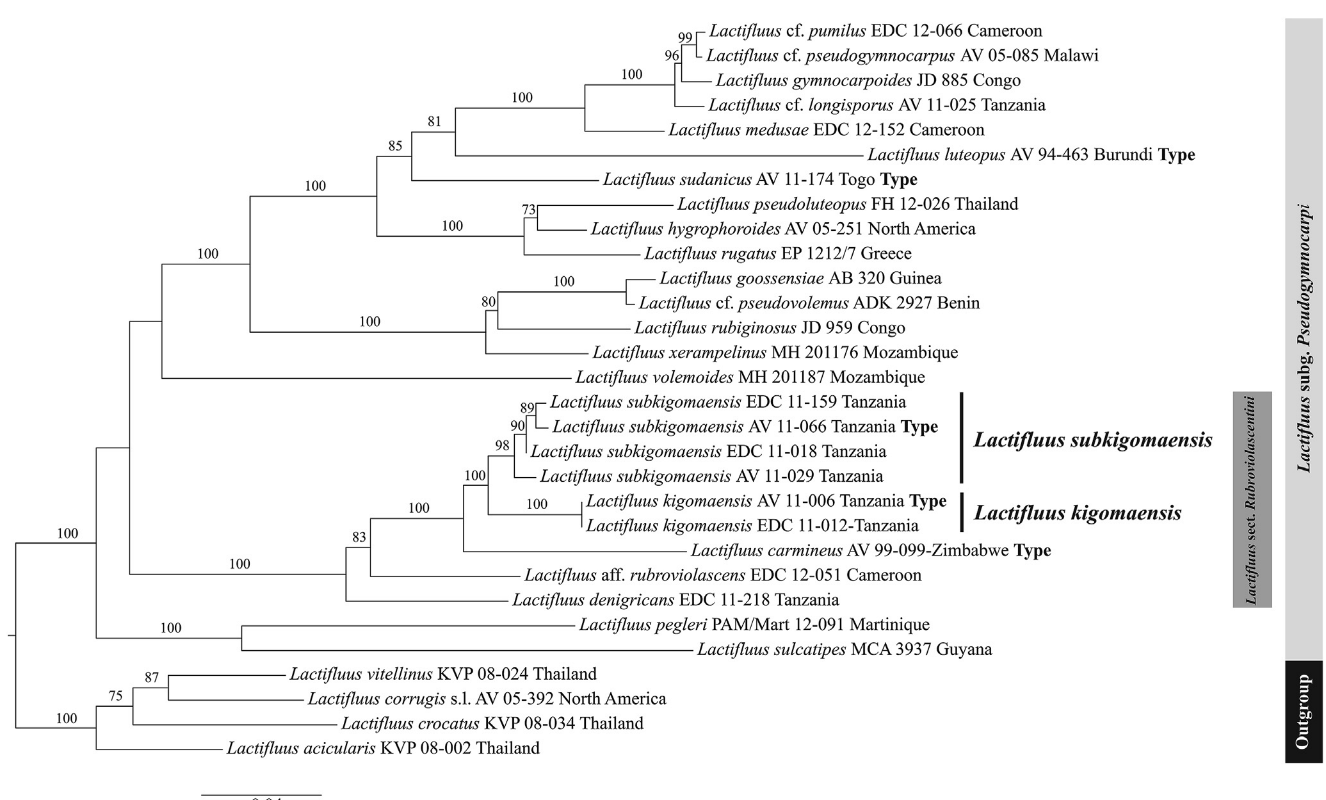

Fig. 1. Maximum Likelihood (ML) tree of *Lactifluus* subg. *Pseudogymnocarpi*, based on concatenated ITS, LSU, *RPB1* and *RPB2* sequence data. ML bootstrap values > 70% are shown.

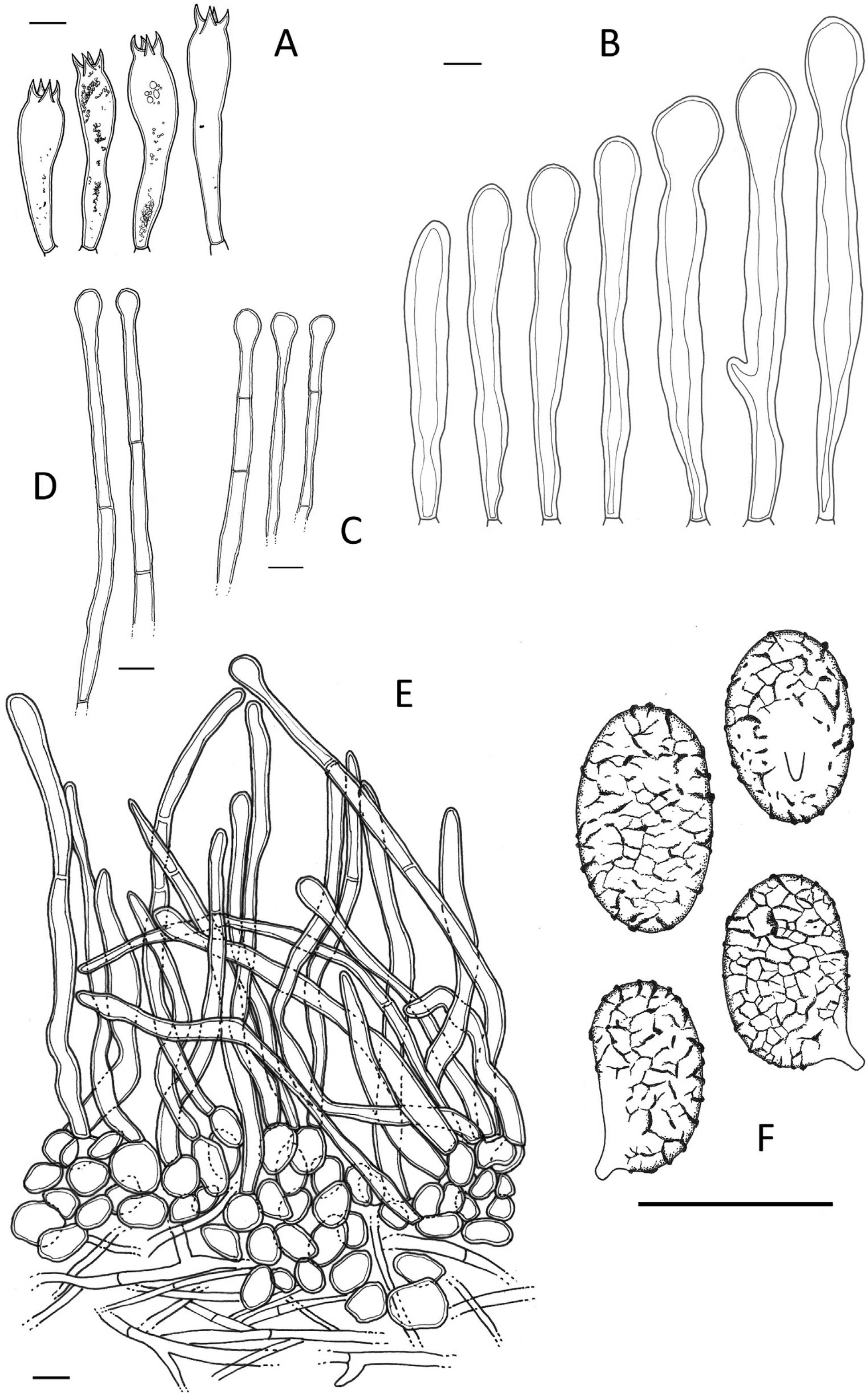

Fig. 2. *Lactifluus kigomaensis* (EDC 11-012 and AV 11-006). **A:** Basidia. **B:** Pleurolamprocystidia. **C:** Capitate elements of the stipitipellis. **D:** Capitate elements of the pileipellis. **E:** Section through the pileipellis. **F:** Basidiospores (*Bars* = 10 μm).

Mycologie 33(4): 422 (2012).

Emendavit: description based on collections AV 11-006 and EDC 11-012 instead of the original description based on mixed material of *L. kigomaensis* and *L. subkigomaensis*.

Pileus 63–65 mm diam, firm, moderately thick, planoconvex, irregularly shaped, somewhat knotty; surface dry, somewhat felty or chamois-leather-like, strongly and irregularly cracking, with concentrical wrinkles at the extreme margin, almost unicolorous, only paler in the cracks, pale brown, brownish orange or brown (7CD6–7, 7DE7), slightly paler towards margin. **Stipe** 45 × 17 mm, irregularly cylindrical, with some folds and ridges, curved, centrally attached; surface smooth, dry, pale reddish orange, 6AB4–5. **Lamellae** decurrent with teeth, moderately distant (9 L + l/cm), with abundant lamellulae of different lengths, pale yellow (4A4–5A4), staining purplish-brown by the latex (pale, not dark). **Context** white, very solid and firm in stipe and in pileus, slightly changing flesh-colored to pale orange when cut, dirty salmon to grey with Fe_2SO_4, unchanging with guaiac; smell very much like *Lactifluus volemus*, agreeable, lobster-like; taste agreeable, nut-like. **Latex** rather abundant, semitransparent, between watery and white, staining the lamellae pale purplish brown to grey; taste mild.

Basidiospores ellipsoid to elongate, 7.8–*8.8–8.9*–10.0 (10.3) × 5.0–*5.9–6.2*–7.0 μm (Q = (1.29) 1.45–1.50 (1.71)), n = 60; ornamentation amyloid, composed of low, up to 0.3 μm high, fine and regular ridges forming an almost complete reticulum with very dense meshes; many isolated warts and short ridges present; plage inamyloid. **Basidia** 46–69 × 10–15 μm, narrowly clavate to clavate, 4-spored, with refringent to slightly thickened walls. **Pleurolamprocystidia** abundant, strongly emergent and arising deep in the hymenium; 75–145 × 7–13 μm and up to 18 μm wide at the top; strongly thick-walled, cylindrical with a subcapitate to capitate top; sometimes with small lateral branch. **Pleuropseudocystidia** rare, 4 μm diam, not emergent, tortuose or irregularly cylindrical. **Lamellae-edge** fertile with basidia and basidioles; cheilolamprocystidia absent. **Hymenophoral trama** composed of sphaerocytes and lactifers. **Pileipellis** a lampropalisade, up to 210 μm thick; terminal elements cylindrical, thick-walled, sometimes distinctly capitate, also shorter and thicker fusiform elements, mostly irregular, sometimes with a small lateral branch, 25–175 × 5–8 μm and up to 15 μm wide at capitate top; subpellis composed of irregularly shaped, rounded to isodiametric cells which are slightly thick-walled. **Stipitipellis** a lampropalisade, also with cylindrical, distinctly capitate elements and fusiform elements.

Specimens examined: Tanzania. Kigoma Province, Mboyogo Kigoma, Kitwe, alt. 780 m, S04°54.96′ E29°36.51', purchased from Katonga market, sold in a mixture with *Cantharellus* spp. *Amanita loosii* Beeli, *Russula* spp. 15 Mar 2011, Verbeken, AV 11-006 (Holotypus, GENT); near Kigoma, Msitwa Katara, alt. 816 m, S04°54.52′ E29°36.06′, young and managed miombo forest with *Brachystegia* sp. 16 Mar 2011, De Crop, EDC 11-012 (GENT).

Lactifluus subkigomaensis De Lange & De Crop **sp. nov.** Fig. 3.
Mycobank: MB 819976.

Diagnosis: Very similar to *Lactifluus kigomaensis* in macroscopical and microscopical characters, but differs by the lamprotrichodermic structure of the pilei- and stipitipellis, and the less reticulate and more unevenly high ornamentation of the spores.

Holotype: Tanzania. Kigoma Province, Ilunde Forest reserve, near Uvinza, alt. 1082 m, S05°05.29′ E30°24.02', primary miombo forest, 20 Mar 2011, Verbeken, AV 11-066 (Holotypus, GENT).

Etymology: Look-alike of *Lactifluus kigomaensis*.

Pileus 43–85 mm diam, planoconvex with central depression to V-shaped (infundibuliform but with curved margin); surface very smooth and felty, a bit rugulose, concentrically wrinkled at the edge (especially in older specimens), locally with fine to strong and deep cracks; color brownish, reddish or brick-colored (7BC4–5, to 7DE6–7), a bit paler to more yellowish at the margin, unchanging with age. **Stipe** 22–31 × 11–23 mm, cylindrical to slightly tapering downwards, regular, straight to slightly curved, centrally attached; surface smooth and felty, a bit wrinkled in older specimens; color yellow to orange (5A3 to 5AB4), locally more yellowish (4A6 but more orange and 5A6 but more yellow), paler at the top, with no distinct or only narrow (<1 mm) white zone at the top, becoming darker with age. **Lamellae** adnate to decurrent with teeth, locally anastomosing, slightly transvenated, sometimes (rarely) bifurcating, broad, thick, very brittle, rather distant (8 L + l/cm), with abundant lamellulae of different lengths, white to cream (3A2). **Context** white, rather pinkish in cavities, solid, firm; slightly salmon pink with Fe_2SO_4, unchanging with guaiac; smell not remarkable, agreeable; taste mild, agreeable. **Latex** watery white like milk, changing pale pinkish or orange brown, later grey brown, staining the lamellae (pale) grey brown; taste mild.

Basidiospores broadly ellipsoid to ellipsoid, 7.5–*8.4–9.1*–9.9 × 5.2–*6.1–6.2*–6.9 μm (Q = (1.13) 1.37–1.49 (1.66)); ornamentation amyloid, composed of low, up to 0.3 μm high, ridges forming an incomplete reticulum; many isolated warts and short ridges present; plage inamyloid. **Basidia** 45–50 × 8–11 μm, cylindric to narrowly clavate, 4-spored, with refringent to slightly thickened walls. **Pleurolamprocystidia** very abundant, very emergent and arising deep in the hymenium; 90–120 × 7–11 μm and up to 15 μm wide at the top; cylindrical and typically capitate, distinctly swollen at the top; very thick-walled. **Pleuropseudocystidia** rare, usually not emergent, 3–5 μm diam, slightly tortuose. **Lamellae-edge** fertile, composed of basidia and occasionally a cheilocystidium. **Hymenophoral trama** cellular, with lactifers and sphaerocytes. **Pileipellis** a lamprotrichoderm, up to 220 μm thick; terminal elements cylindric to distinctly capitate, 50–170 × 4–6 μm and up to 13 μm wide at capitate top, thick-walled; subpellis composed of intricate, hyaline hyphae. **Stipitipellis** a lamprotrichoderm, also with distinctly capitate terminal elements present.

Specimens examined: Tanzania. Kigoma Province, Ilunde Forest reserve, near Uvinza, alt. 1082 m, S05°05.29′ E30°24.02′, primary miombo forest, 20 Mar 2011, Verbeken, AV 11-066 (Holotypus, GENT); Kigoma Province, Ilunde Forest reserve, near Uvinza, alt. 1033 m, S05°05.24′ E30°24.05′, primary miombo forest, 31 Mar 2011, De Crop, EDC 11-159 (GENT); Kigoma Province, Ilunde Forest reserve, near Uvinza, alt. 1082 m, S05°05.29′ E30°24.02', primary miombo forest, 17 Mar 2011, Verbeken, AV 11-029 (GENT); Kigoma Province, Ilunde Forest reserve, near Uvinza, alt. 1082 m, S5°05.29′ E30°24.02′, primary miombo forest, 17 Mar 2011, De Crop, EDC 11-018 (only CTAB sample).

4. Discussion

The striking features of both *Lactifluus kigomaensis* and *L. subkigomaensis* in the field are the strongly cracking pileus, the lamellae that are staining purplish brown to grey brown by the latex and their agreeable smell (fishy, lobster-like as in *L. volemus*). Microscopically the capitate elements are very striking for both species, both in the hymenium as pleurocystidia, and in the pilei- and stipitipellis as terminal elements. As stated in De Crop et al. (2012) these capitate elements are rather rare in African *Lactifluus* species and only observed in *Lactifluus nonpiscis* (Verbeken) Verbeken and *Lactifluus rubroviolascens* (R. Heim) Verbeken, where they occur as terminal elements in the pilei- and stipitipellis, but never as pleurocystidia.

Macroscopically, *Lactifluus kigomaensis* and *L. subkigomaensis* are very alike and cannot be distinguished unambiguously based on our current data (Fig. 4); the color of the lamellae, which is darker in *L. kigomaensis*, could be an indication but more collections should be examined to confirm this statement. Microscopically both species have similar spores and hymenial structures, although the spore ornamentation of *L. kigomaensis* is more reticulate and has more evenly high ornamentation than *L. subkigomaensis*. The most remarkable difference is found in the pellis structures: the pilei- and stipitipellis structure of *L. kigomaensis* is a

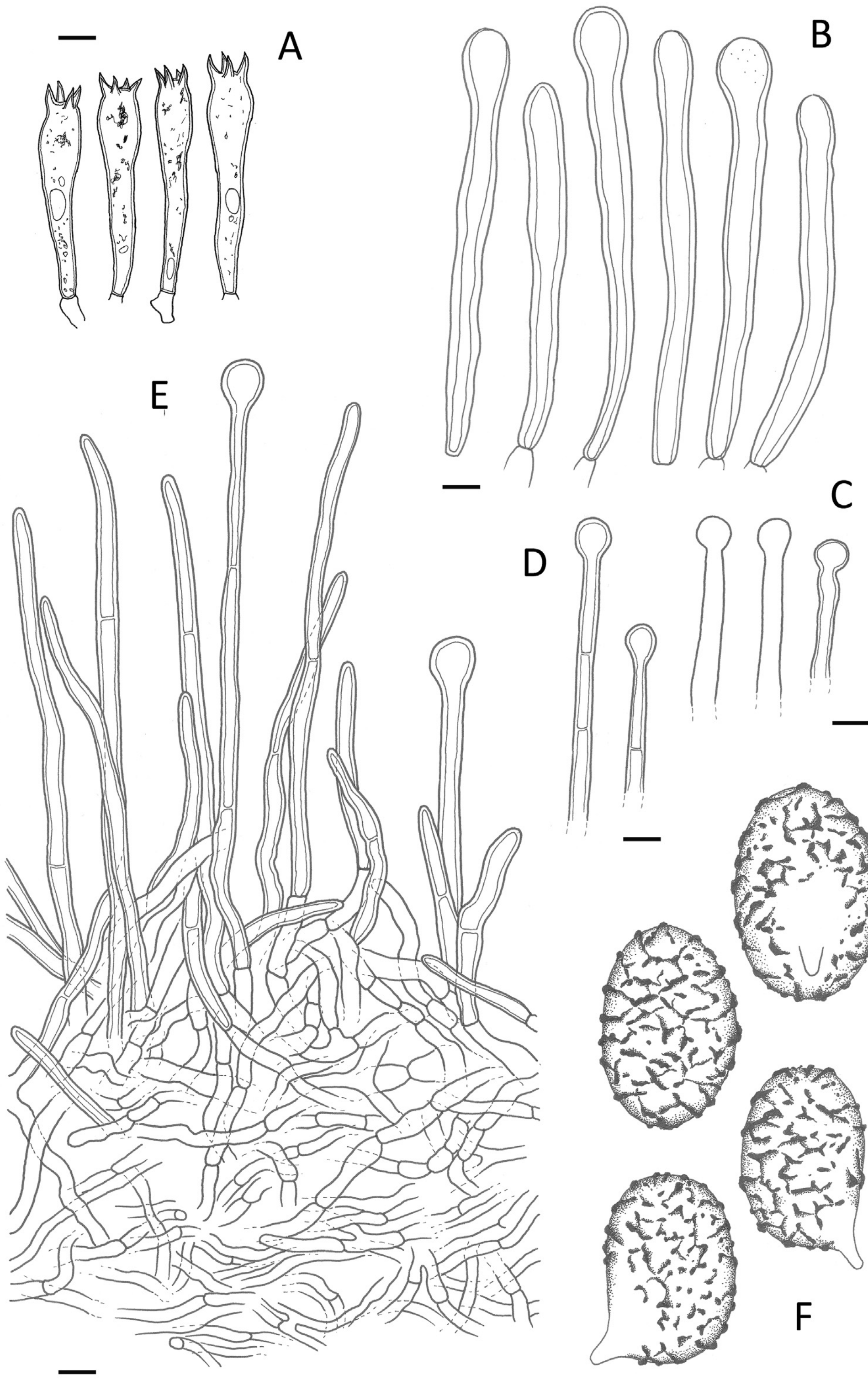

Fig. 3. *Lactifluus subkigomaensis* (holotype). **A:** Basidia. **B:** Pleurolamprocystidia. **C:** Capitate elements of the stipitipellis. **D:** Capitate elements of the pileipellis. **E:** Section through the pileipellis. **F:** Basidiospores (*Bars* = 10 μm).

lampropalisade, whereas *L. subkigomaensis* has a lamprotrichoderm.

Within the genus *Lactifluus*, both *L. kigomaensis* and *L. subkigomaensis* belong to *L.* subg. *Pseudogymnocarpi*, as shown in De Crop et al. (2017) and supported by morphological characters like the orange to reddish brown cap colors, the presence of pleurolamprocystidia and ellipsoid to elongate spores with low ornamentation. Within L. subg. *Pseudogymnocarpi* both L. *kigomaensis* and *L. subkigomaensis* belong to *L.* sect. *Rubroviolascentini* (Fig. 1). *Lactifluus subkigomaensis* is an exceptional species for this section

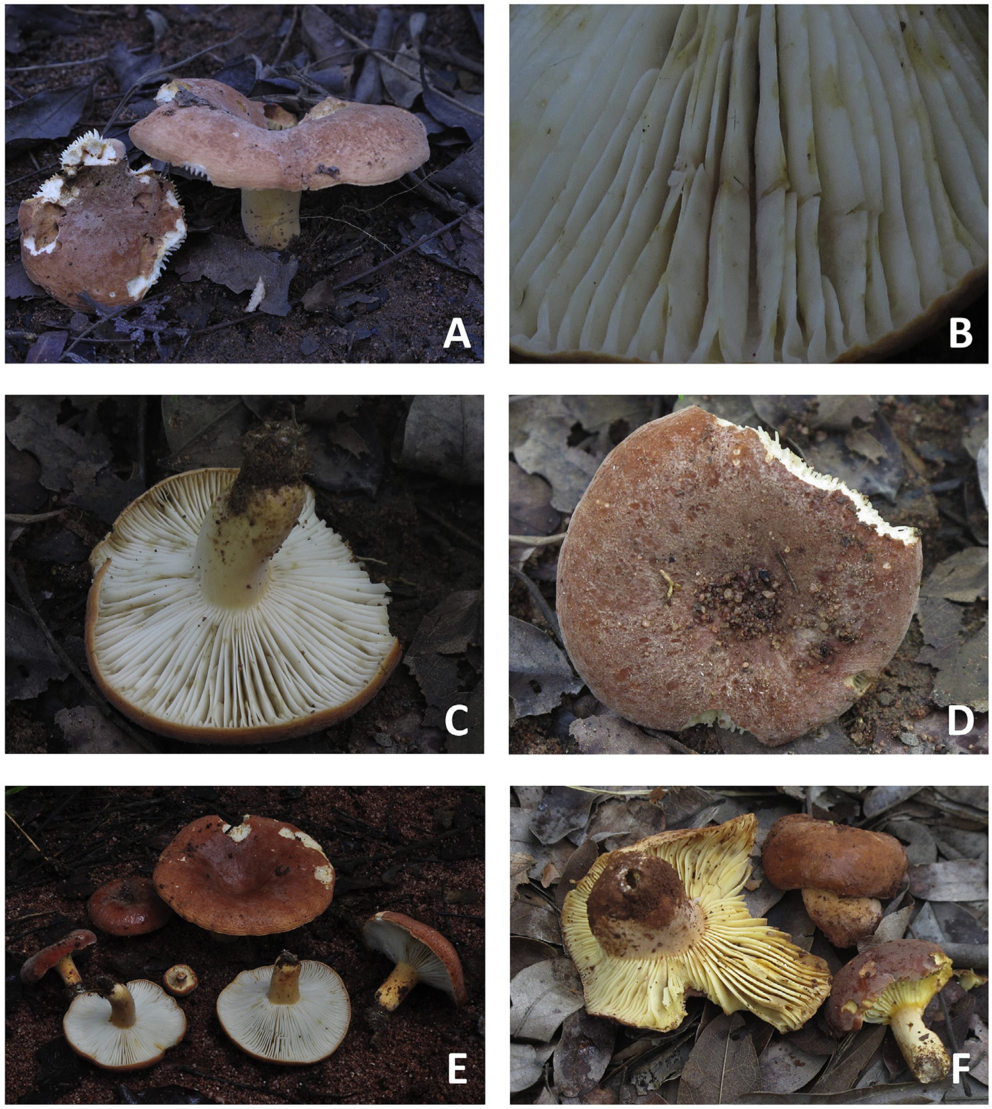

Fig. 4. **A**–**E** *Lactifluus subkigomaensis*. **A**, **B:** Collection AV 11-029. **C**, **D:** Collection AV 11-066. **E:** Collection EDC 11-159. **F:** *Lactifluus kigomaensis* (collection EDC 11-012).

because of the trichodermic structure of the pileipellis, while all other representatives have a palisadic structure of the pileipellis. *Lactifluus carmineus* is the sister species of the clade *L. kigomaensis*/*L. subkigomaensis*, which is remarkable because of the totally different morphology of this species. *Lactifluus carmineus* has much smaller (pileus 13–30 mm diam) and more delicate fruitbodies, with very distant gills and a more reddish cap color (10F8 in young specimens, 9E8 to 8D8 in older specimens). Microscopically, *L. carmineus* lacks capitate elements and has slightly larger spores with an ornamentation composed of elongated warts forming a very incomplete but extremely dense reticulum (Verbeken & Walleyn, 2010).

Disclosure

The authors declare no conflicts of interest.

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