

## ***Lactifluus persicinus* sp. nov. from the gallery forests of West Cameroon**

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**ABSTRACT**—During field work in the Noun division of western Cameroon during 2011, 2012, and 2014, several collections of an unknown *Lactifluus* species were discovered in a gallery forest with *Uapaca guineensis*. Molecular and morphological research shows that these collections represent a new species, *Lactifluus persicinus*, which is described in this paper. *Lactifluus persicinus* belongs to *Lf.* sect. *Xerampelini* in *Lf.* subg. *Pseudogymnocarpi*.

**KEY WORDS**—*Russulaceae*, ectomycorrhizal fungi, tropical Africa, edible mushroom, pseudocryptic species

### **Introduction**

The *Russulaceae* are a family containing important ectomycorrhizal genera. In 2008, molecular research challenged existing generic concepts, resulting in the splitting of *Russula* Pers. and *Lactarius* Pers. into four segregate genera—*Lactarius*, *Russula*, *Multifurca* Buyck & V. Hofst., and *Lactifluus* (Pers.) Roussel (Buyck et al. 2008). The species-rich milkcap genus *Lactifluus* (*Lf.*) comprises about 150 described species worldwide. Furthermore, at least 17 (possibly up to 44) species still await description (De Crop et al. 2017). *Lactifluus* has a mainly tropical distribution, with the highest known diversity in tropical Africa (Bâ et al. 2012, De Crop et al. 2012, Maba 2015, Maba et al. 2015a, Van de Putte et al. 2009, Verbeken & Walley 2010) and tropical Asia (Le 2007, Stubbe et al.

TABLE 1. *Lactifluus* (*Lf.*) specimens and GenBank sequences used in the molecular analyses. Type specimens are annotated as [T].

SPECIES	VOUCHER (HERBARIUM)	COUNTRY	ITS	LSU
<i>Lactifluus</i> sect. <i>Pseudogymnocarpi</i>				
<i>Lf.</i> cf. <i>pseudogymnocarpus</i>	AV 05-085 (GENT)	Malawi	KR364012	KR364139
<i>Lf.</i> cf. <i>pumilus</i>	EDC 12-066 (GENT)	Cameroon	KR364067	KR364196
<i>Lf. gymnocarpoides</i>	JD 885 (BR)	Congo	KR364074	KR364203
	AV 05-184 (GENT)	Malawi North	KR364024	KR364151
<i>Lf. hygrophoroides</i>	AV 05-251 (GENT)		HQ318285	HQ318208
<i>Lf. longisporus</i>	AV 94-557 (GENT) [T]	America Burundi	KR364118	KR364244
<i>Lf. luteopus</i>	AV 94-463 (GENT) [T]	Burundi	KR364119	—
<i>Lf. medusae</i>	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198
<i>Lf. pseudoluteopus</i>	FH 12-026 (GENT)	Thailand	KR364084	KR364214
<i>Lf. sudanicus</i>	AV 11-174 (GENT) [T]	Togo	HG426469	KR364186
<i>Lactifluus</i> sect. <i>Xerampelini</i>				
<i>Lf. persicinus</i>	EDC 14-371 (GENT)	Cameroon	KX499389	—
	EDC 12-002 (GENT)	Cameroon	KX499392	KX622761
	EDC 12-004 (GENT)	Cameroon	KX499390	KX622760
	EDC 12-001 (GENT) [T]	Cameroon	KR364061	KR364190
	NAL 348 (GENT)	Cameroon	KX499391	—
Uncultured ectomycorrhiza	—	Guinea	AM113441	—
<i>Lf. goossensiae</i>	AB 320 (GENT)	Guinea	KR364132	KR364252
<i>Lf.</i> cf. <i>pseudovolemus</i>	ADK 2927 (BR)	Benin	KR364113	KR364243
	ADK 2968 (BR)	Benin	KX499393	—
<i>Lf. kivuensis</i>	JR Z366 (GENT)	Congo	KX499399	—
	JR Z439 (GENT)	Congo	KX499398	—
	JR Z 310 (GENT) [T]	Congo	KR364027	KR364154
<i>Lf. rubiginosus</i>	JD 959 (BR)	Congo	KR364081	KR364210
	BB 3466 (GENT) [T]	Zambia	KR364014	KR364250
<i>Lf. xerampelinu</i>	TS 1116 (GENT) [T]	Tanzania	KR364039	KR364166
	MH 201176 (GENT)	Mozambique	KR364099	KR364231
	CS 2889 (GENT)	Zimbabwe	KX499394	—
<i>Lf.</i> sp.	EDC 12-176 (GENT)	Cameroon	KR364070	KR364199
	EDC 12-071 (GENT)	Cameroon	KX499396	KX622762
	EDC 14-284	Cameroon	KX499395	—
Uncultured ectomycorrhiza	—	Gabon	FR731875	—
<i>Lf.</i> sp.	AV 11-022 (GENT)	Tanzania	KX499397	KX622763
Unnamed clade				
<i>Lf.</i> sp.	JN 2011-012 (GENT)	Vietnam North	KR364045	KR364171
	TENN 065929 (TENN)		KR364102	KR364233
	EDC 14-501 (GENT, MFLU)	America Thailand	KR364127	—
<i>Lf. volemoides</i>	TS 0705 (GENT) [T]	Tanzania	KR364038	KR364165
<i>Lactifluus</i> sect. <i>Aurantiiifolii</i>				
<i>Lf. aurantiifolius</i>	AV 94-063 (GENT) [T]	Burundi	KR364017	KR364144

<b><i>Lactifluus</i> sect. <i>Rubroviolascentini</i></b>				
<i>Lf. aff. rubroviolascens</i>	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195
<i>Lf. carmineus</i>	AV 99-099 (GENT) [T]	Zimbabwe	KR364131	KR364251
<i>Lf. denigricans</i>	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178
<i>Lf. kigomaensis</i>	AV 11-006 (GENT)	Tanzania	KR364052	KR364179
<i>Lf. sp.</i>	EDC 11-159 (GENT)	Tanzania	KR364050	KR364177
<b><i>Lactifluus</i> sect. <i>Polysphaerophori</i></b>				
<i>Lf. pegleri</i>	PAM/Mart 12-091 (LIP)	Martinique	KP691416	KP691425
<i>Lf. sp.</i>	RC/Guy 09-036 (LIP)	French Guiana	KJ786645	KJ786550
	MR/Guy 13-145	French Guiana	KJ786691	KJ786595
	MCA 3937 (GENT)	Guyana	KR364109	KR364240
<i>Lf. veraecrucis</i>	M 8025 (ENCB) [T]	Mexico	KR364112	KR364241
<b>Outgroup (<i>Lf.</i> subg. <i>Lactifluus</i>)</b>				
<i>Lf. corrugis</i> s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143
<i>Lf. crocatus</i>	KVP 08-034 (GENT)	Thailand	HQ318243	HQ318151
<i>Lf. vitellinus</i>	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144
<i>Lf. volemus</i>	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175
<i>Lf. acicularis</i>	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132

2010, Van de Putte 2012, Van de Putte et al. 2010). Species diversity is high in the Guineo-Sudanian domain (Maba 2015; Maba et al. 2014, 2015a, 2015b), with around 38 *Lactifluus* species currently known from the area. However, certain regions within this area remain poorly explored with potentially many new species yet to be discovered and described. In one such region, Cameroon, most studies have been located in the tropical rainforest (Douanla-Meli & Langer 2009, Verbeken et al. 2008), and little is known about milkcaps in the savannas, from which only one *Lactifluus* species, *Lf. albomembranaceus* S. De Wilde & Van de Putte, has been described (De Crop et al. 2016).

Southern Cameroon is dominated by closed evergreen lowland forest, while the centre of the country is dominated by deciduous woodland and shrubland (WRI 2003). The tropical forest does not undergo a gradual change to savanna or woodland; instead the zone between these two habitats consists of habitat patches with abrupt borders. This transition zone is called the 'forest-savanna mosaic' since it contains forest islands and savannas, as well as woodlands (White 1983). In contrast to savannas and woodlands, dense tree cover characterizes the forest islands, with the resulting competition for light leading to an understory with much lower abundances of grasses than in the savannas or woodlands (Azihou et al. 2013, Hoffmann et al. 2009, Natta et al. 2003). Forest islands that occur alongside waterways are known as gallery forests. The plant groups *Caesalpinioideae* (represented by *Berlinia* Sol. ex Hook. f.)

and *Phyllanthaceae* (represented by *Uapaca* Baill.) dominate the Central and West African gallery forests. These trees are ectomycorrhizal hosts, making the humid gallery forests an ideal habitat in which to find ectomycorrhizal fungi.

A recent survey of wild mushrooms in the West region of Cameroon revealed nine edible *Lactifluus* species, all collected in gallery forests (Njouonkou et al. 2016). From the same division, *Lf. albomembranaceus* was described (De Crop et al. 2016). It is from this habitat that the new species described here, *Lactifluus persicinus*, was discovered.

## Materials & methods

### Sampling

During fieldwork in 2011, 2012, and 2014 in gallery forests within the Noun division of western Cameroon, 15 collections of *Lactifluus persicinus* were made.

### Morphological analysis

Macroscopic characters were observed from fresh material, with colour codes referring to Kornerup & Wanscher (1978). Microscopic characters were observed from dried material. Spores were mounted in Melzer's reagent and measured in side view without ornamentation. A total of 186 spores were measured among 14 collections. The measurements are given as  $[Ava-2xSDa]-Ava-Avb-[Avb+2xSDB]$  in which  $Ava$  = lowest mean value for the measured collections,  $Avb$  = greatest mean value and  $SDa/b$  = standard deviation of the lowest and greatest mean value respectively. As an indication for spore shape,  $Q$  was also determined, where  $Q$  is the quotient of length and width and is given as  $MINQ-AvQa-AvQb-MAXQ$ , with  $AvQa$  and  $AvQb$  the lowest and the highest mean quotient, respectively, for the measured specimens.  $MINQ/MAXQ$  stands for the minimum/maximum value over the quotients of all available measured spores. The hymenium, pileipellis and stipitipellis were mounted in Congo red. The measurements of the basidia do not include the sterigmata. Line drawings were made with the aid of a drawing tube at following magnifications: 6000 $\times$  for spores (Zeiss Axio Scope 2 microscope), 1500 $\times$  for other hymenial elements and sections (Olympus cx31 microscope). For the SEM pictures, the spores were coated in a Quorum Q150TS sputter coater with 5 nm thick Pt/Pd (platinum palladium) and studied using a Jeol JSM-7600F Field Emission Scanning Microscope.

### Molecular analysis

DNA from dry collections was extracted using the protocol described by Nuytinck & Verbeken (2003) with modifications described in Van de Putte et al. (2010). DNA was extracted from fresh material using the CTAB extraction described in Nuytinck & Verbeken (2003). Protocols for PCR amplification follow Le et al. (2007). Two nuclear markers previously shown as 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 et al. 1990) and (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000).

PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Life Technology) at MacroGen. Forward and reverse sequences were assembled into contigs and edited where needed with the Sequencher v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.).

From De Crop et al. (2017) we knew that *Lactifluus persicinus* represented *Lactifluus* subg. *Pseudogymnocarpi* (Verbeken) De Crop and *Lf.* sect. *Xerampelini* De Crop. The dataset for phylogenetic analyses contained sequences from *Lactifluus* subg. *Pseudogymnocarpi* (De Crop et al. 2017) and we added more newly generated sequences plus two GenBank sequences within *Lf.* sect. *Xerampelini*. For other sections only one sequence per species was included. Five species representing *Lf.* subg. *Lactifluus* were used as outgroup (TABLE 1).

Sequences were aligned online using the E-INS-I strategy version of the multiple sequence alignment program MAFFT v7 (Kato & Toh 2008). Trailing ends were trimmed and the alignment was manually edited when necessary in Mega 6 (Tamura et al. 2013). The alignment can be obtained from the first author and TreeBASE (Submission ID 19693). The alignment was partitioned into partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Maximum likelihood (ML) analyses were conducted with RAxML v8.0.24 (Stamatakis 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis et al. 2008). All analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

## Results

The molecular results placed the new species in *Lf.* subg. *Pseudogymnocarpi* and *Lf.* sect. *Xerampelini*. Its sister clade contains collections previously identified as *Lf. goossensiae* (Beeli) Verbeken from Guinea and *Lf. cf. pseudovolemus* (R. Heim) Verbeken from Benin. The new species, described below as *Lf. persicinus*, is morphologically very similar to the collections within this sister clade with which it is compared.

### *Lactifluus persicinus* Delgat & De Crop, sp. nov.

FIGS 1–3

MYCOBANK MB 817873

Differs morphologically from its sister clade (collections AB 320, ADK 2927 and ADK 2968) by its shorter basidia.

TYPE: Cameroon, Western region, Noun division, Koutaba subdivision, Mamevouou village, gallery forest in savannah region, *Uapaca guineensis*, 5°41'01"N 10°49'28"E, 1126 m, 8 May 2012, EDC 12-001 (Holotype, GENT)

ETYMOLOGY: Referring to the peach-like color and surface of the basidiocarp.

PILEUS 37–73 mm diam., firm, plano-concave with depressed centre to deeply infundibuliform; margin straight to inflexed, broadly grooved (c. 2–3 mm)

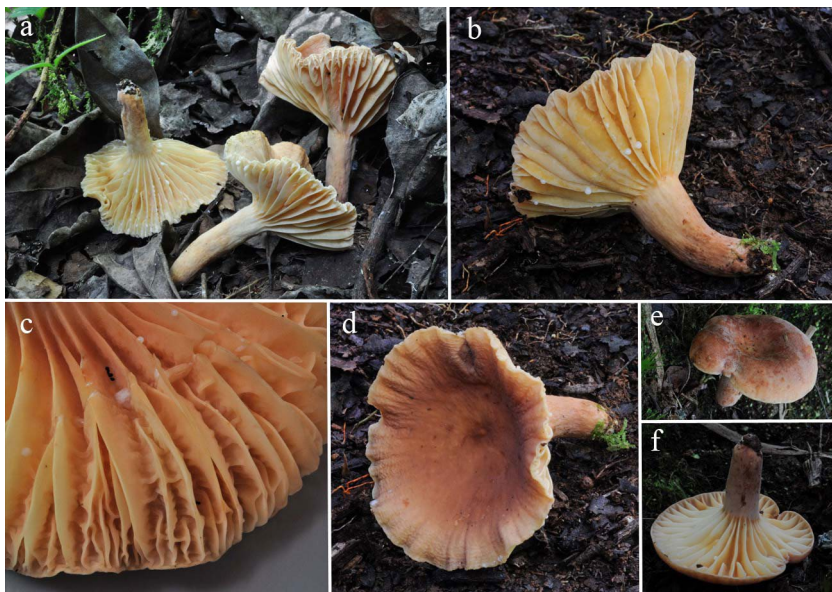


FIG. 1. *Lactifluus persicinus* basidiocarps: a. holotype, EDC 12-001; b. EDC 14-349; c. EDC 12-002; d. EDC 14-349; e. EDC 14-380; f. EDC 14-380. (Photos by E. De Crop).

reaching halfway across the pileus; surface chamois-leather-like, slightly velutinous when young, cream yellow to orange to pink (5A3–6A2), some pilei slightly darker (6B4–7C4), locally more pink or more yellow, concentrically wrinkled near margin. STIPE 32–48 × 8–14 mm, cylindrical, sometimes tapering downwards or curving at the base; surface slightly irregular, almost smooth, very soft (softer than pileus), slightly velutinous or chamois-leather-like, almost concolourous with pileus but slightly paler, cream yellow-orange (5A3, 5A4 to 6A3), sometimes with a pinkish tinge, pale at top, becoming darker orange towards the base (5A5–5B5). LAMELLAE decurrent with rather long teeth, very broad, thick, strongly transvenose and slightly intervenose, sometimes bifurcating towards the margin, brittle, distant (2+3–5 L+ll/cm), paler than pileus, cream yellow with pinkish tinge (4A3–5A2); edge concolourous and entire. CONTEXT white, firm, unchanging, unchanging with guaiac, slightly pink with  $\text{FeSO}_4$ . SMELL not remarkable. TASTE mild. LATEX abundant, watery white, unchanging, taste mild.

BASIDIOSPORES broadly ellipsoid to ellipsoid, rarely slightly elongate, 6.9–9.2–10.9–12.8 × 5.7–6.7–7.7–9.4  $\mu\text{m}$  ( $Q = 1.10–1.33–1.48–1.70$ ); ornamentation amyloid, composed of low (1  $\mu\text{m}$  at most) elongated warts, aligned or connected



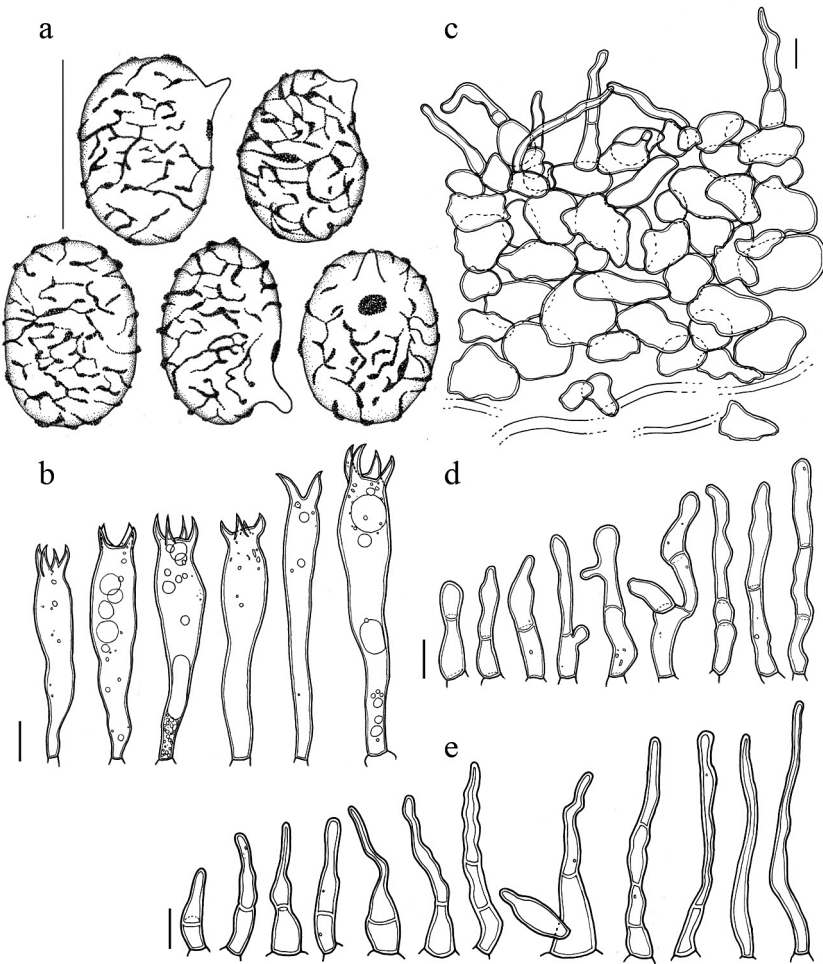


FIG. 2. *Lactifluus persicinus*: a. basidiospores; b. basidia; c. section through the pileipellis; d. sterile elements from the hymenium; e. pileipellis hairs. Scale bar = 10  $\mu$ m. (Line drawings by L. Delgat).

by fine connective lines, forming an incomplete reticulum, with only few isolated warts; plage sometimes distinct and with a central amyloid spot. BASIDIA 45–74(–90)  $\times$  (8–)8.5–11.5(–12.5), cylindrical to subclavate, 4-spored, rarely 2-spored, slightly thick-walled. STERILE ELEMENTS abundant, 21–60  $\times$  3.5–7  $\mu$ m, some emergent, majority not emergent, cylindrical, septate, with rounded apex, wall slightly thickened. PLEUROCYSTIDIA absent. PLEUROPSEUDOCYSTIDIA

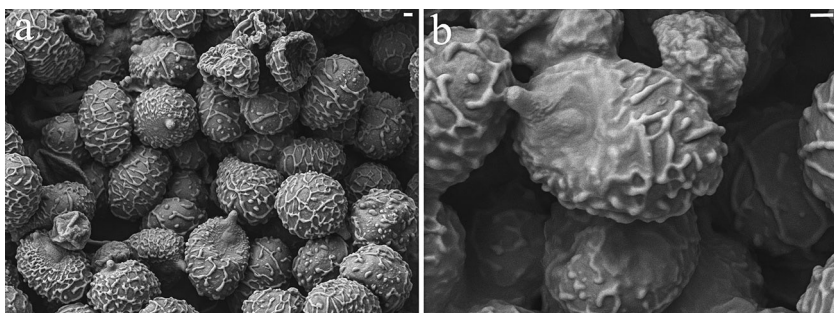


FIG. 3. *Lactifluus persicinus* (holotype, EDC 12-001) basidiospores (SEM): a. overview; b. detail of basidiospores. Scale bars = 1  $\mu$ m.

scarce, 6–10  $\mu$ m diam., cylindrical with rounded apex, often remarkably emergent, sometimes not emergent. LAMELLAR EDGE fertile. HYMENOPHORAL TRAMA cellular, with lactifers and sphaerocytes. PILEIPELLIS a lampropalisade; elements of the suprapellis 19–65  $\times$  3–11  $\mu$ m, cylindrical to cylindrical with broadened base, often septate, thick-walled; subpellis composed of slightly thick-walled globose/isodiametric cells (10–30  $\mu$ m diam.), up to 80  $\mu$ m thick. STIPITPELLIS a lamprotrichoderm to lamprotrichopalisade, with many parallel hyphae, part of them ascending; terminal elements more abundant than in the pileipellis, occurring in tufts, cylindrical, cylindrical with a bulbous base or broadly ellipsoid to globose, often septate, slightly thick-walled.

USE – *Lactifluus persicinus* is used as food by Bamoun people in the Noun division of the west region of Cameroon.

LOCAL NAME – *Lactifluus persicinus* is locally called “Puo’ mbum mon (pwɔ’ mbum mon)” by Bamoun people. “Puo” is a general word for mushroom. “Mbum mon” is the local name of a herbaceous *Euphorbia* species, namely *Euphorbia heterophylla* L., which is an annual plant with white milky latex in all parts of the plant. This name is related to the fact that *Lactifluus persicinus* produces abundant white latex like *Euphorbia* species. However, this name also seems to be used for other milkcap species.

ECOLOGY – found in Cameroon in the ‘forest-savanna mosaic’ zone in gallery forests with *Uapaca guineensis* Müll. Arg.

DISTRIBUTION – known only from Cameroon.

ADDITIONAL SPECIMENS EXAMINED – CAMEROON, WESTERN REGION, Noun division, Koutaba subdivision, Mamevouo village, gallery forest in savannah region, *Uapaca guineensis*, 5°41’01”N 10°49’28”E, 1126 m, 8 May 2012, EDC 12-002, EDC 12-003, EDC 12-004 (GENT); 5°39’12”N 10°50’54”E, 1062 m, 10 May 2012, EDC 12-050 (GENT); 5°40’59”N 10°49’29”E, 1122 m, 27 May 2014, EDC 14-349 (GENT); 5°40’48”N



10°49'28"E, 1139 m, EDC 14-361 (GENT); 5°39'27"N 10°50'57"E, 1063 m, 29 May 2014, EDC 14-371 (GENT); 5°39'26"N 10°50'57"E, 1113 m, EDC 14-374 (GENT); 5°39'10"N 10°51'00"E, 1117 m, EDC 14-376 (GENT); 5°39'10"N 10°51'01"E, 1093 m, EDC 14-378 (GENT); 5°38'60"N 10°51'05"E, 1117 m, EDC 14-380 (GENT); 5°38'58"N 10°51'03"E, 1120 m, EDC 14-385, EDC 14-386 (GENT); 5°39'37"N 10°50'48"E, 1099 m, 22 August 2011, NAL 348 (GENT).

## Discussion

*Lactifluus persicinus* is recognizable in the field as a typical *Lactifluus* because of the chamois-leather-like surface of the cap. This is confirmed under the microscope by the cellular hymenophoral trama and the lampropalisade pileipellis structure. Molecular data also confirm placement within *Lactifluus* (FIG. 4).

Within the genus, *Lactifluus persicinus* belongs to *Lf.* subg. *Pseudogymnocarpi*, which is supported by molecular data (De Crop et al. 2017), as well as by morphological characters, such as the lampropalisade pileipellis structure, yellow/orange cap colour, and absence of colour reactions of the latex as in most species of this subgenus.

*Lactifluus persicinus* is placed within the section *Lf.* sect. *Xerampelini*, a section containing exclusively African species (FIG. 4). It also fits morphologically within this clade, which is characterized by yellowish-orange to reddish-brown cap colours, palisade-like pileipellis structure, absence of clear cystidia, and generally low ornamented spores (usually not higher than 0.2 µm) that are verrucose or form a more or less complete reticulum.

The sister clade of *Lf. persicinus* contains collections previously identified as *Lf. goossensiae* and *Lf. cf. pseudovolemus* (FIG. 4) from Guinea and Benin. However, *Lf. pseudovolemus* is known only from Madagascar, and several macro- and microscopic characters mentioned in the description (Heim 1938) are not concordant with characters of the collections in this clade. In addition, we have studied the type material of *Lf. goossensiae* and this species also seems to differ both macro- and microscopically from the sequenced collections in this clade (e.g. the type of *Lf. goossensiae* has smaller spores). It seems likely that this sister clade represents neither *Lf. pseudovolemus* nor *Lf. goossensiae*. As it was not possible to obtain sequences from the types of *Lf. pseudovolemus* and *Lf. goossensiae* to test the two species molecularly, the identities of the specimens assigned to the sister clade remain unconfirmed.

Although *Lactifluus persicinus* is clearly delimited from its sister clade molecularly, morphologically the two clades are difficult to distinguish from each other and might be considered (pseudo) cryptic species. Macroscopically

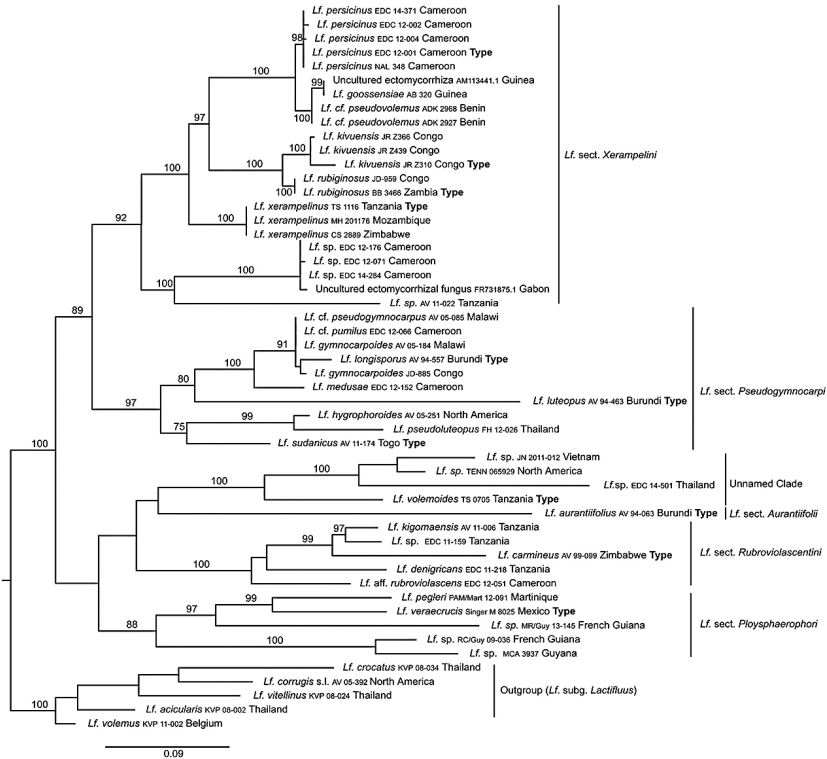


FIG. 4. Overview Maximum Likelihood tree of *Lactifluus* subg. *Pseudogymnocarpi*, based on concatenated ITS and LSU sequence data. ML bootstrap values >70% are shown.

the species look alike; colours and lamellae are similar, as well as the fruit body shape, cap surface, and the latex. Microscopically they are also similar. The spore sizes correspond—7.9–9.3–10.7–13.8 × 5.9–6.8–7.9–9.0 µm (sister clade) vs. 6.9–9.2–10.9–12.8 × 5.7–6.7–7.7–9.4 µm (*Lf. persicinus*)—as do the spore shapes—Q = 1.18–1.35–1.39–1.66 (sister clade) vs. Q = 1.10–1.33–1.48–1.70 (*Lf. persicinus*). Basidia sizes overlap—55–90 × 9.5–11.5 µm (sister clade) vs. 45–74(–90) × (8)–8.5–11.5(–12.5) µm (*Lf. persicinus*). However, the average basidial length differs substantially—74 × 10 µm (sister clade) vs. 56 × 10 µm (*Lf. persicinus*). So the sister clade has on average much longer and more slender basidia than *Lactifluus persicinus*. Collections of the sister clade also have similar sterile elements, which are often septate and slightly thick-walled, and the terminal elements of the pilei- and stipitipellis also look alike. In addition, they were collected in the same kind of habitat, namely in gallery

forests. However, all currently known collections from *Lactifluus persicinus* were found in gallery forests dominated by *Uapaca guineensis*, while the three sister clade collections were found in gallery forests dominated by *Uapaca somon* [= *U. togoensis* Pax].

In summary, while morphologically very similar, these species are genetically distinct and can be morphologically distinguished from each other when the average basidium length is compared. These species could be considered pseudocryptic species; at first they seemed indistinguishable, but after the phylogeny revealed two distinct species, detailed morphological study revealed (a) character(s) that can be used to morphologically distinguish them from each other. This phenomenon is widespread throughout *Lactifluus* and considered typical for this genus with its high molecular diversity and relatively low morphological diversity. Many species complexes occur in *Lf.* subg. *Lactifluus*, *Lf.* subg. *Gymnocarpi*, *Lf.* sect. *Pseudogymnocarpi*, etc. (De Crop 2016, Stubbe 2012, Van de Putte 2012) and as with most of these, the genetic distance between *Lactifluus persicinus* and its sister clade is rather low, as can be seen from the short—although well-supported—branches between them (FIG. 4). This may suggest a relatively recent divergence between species as well as insufficient time for significant morphological divergence to develop.

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