MycoKeys 45: 25–39 (2019)
doi: 10.3897/mycokeys.45.29964
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Lactifluus bicapillus (Russulales, Russulaceae), a new species from the Guineo-Congolian rainforest

Eske De Crop[1], Jonas Lescroart[1], André-Ledoux Njouonkou[2], Ruben De Lange[1], Kobeke Van de Putte[1], Annemieke Verbeken[1]

1 *Research Group Mycology, Department of Biology, Ghent University, Ghent, Belgium* **2** *Department of Biological Sciences, Faculty of Sciences, University of Bamenda, Cameroon*

Corresponding author: *Eske De Crop* (eske.decrop@ugent.be)

Academic editor: *B. Dentinger* | Received 21 September 2018 | Accepted 18 December 2018 | Published 28 January 2019

Citation: De Crop E, Lescroart J, Njouonkou A-L, De Lange R, Van de Putte K, Verbeken A (2019) *Lactifluus bicapillus* (Russulales, Russulaceae), a new species from the Guineo-Congolian rainforest. MycoKeys 45: 25–39. https://doi.org/10.3897/mycokeys.45.29964

Abstract

The milkcap genus *Lactifluus* is one of the most common ectomycorrhizal genera within Central African rainforests. During a field trip to the Dja Biosphere Reserve in Cameroon, a new *Lactifluus* species was found. Molecular and morphological analyses indicate that the species belongs to *Lactifluus* section *Xerampelini* and we formally describe it here as *Lactifluus bicapillus* **sp. nov.**

Keywords

Ectomycorrhizal fungi, *Gilbertiodendron, Lactarius*, phylogeny, taxonomy, tropical Africa, *Uapaca*

Introduction

Rainforests occur in Central Africa and form the main vegetation type in the Guineo-Congolian region (White 1983). Large parts of southern Cameroon and northern Gabon are covered by rainforest, characterised by high humidity, closed canopies, and competition for light in the understory. Common tree species within these rainfor-

ests, such as the Dja Biosphere Reserve, include ectomycorrhizal (ECM) species from the Phyllanthaceae (e.g. *Uapaca* spp. Baill.) and the Fabaceae (i.e. *Gilbertiodendron dewevrei* (De Wild.) J.Léonard) (Sonké and Couvreur 2014). *Uapaca* species mainly occur mixed with other tree species, whereas *G. dewevrei* forms more or less monodominant stands, mixed with an occasional *Uapaca* species. These trees are typical hosts for ECM fungi and Russulaceae have been repeatedly recorded as associated with these trees (Verbeken and Walleyn 2010; De Crop et al. 2016; Delgat et al. 2017; T.W. Henkel pers. comm.).

Within Central African rainforests, the ECM Russulaceae genera *Russula* Pers. and *Lactifluus* (Pers.) Roussel are abundant (Douanla-Meli and Langer 2009; Verbeken and Buyck 2002; Verbeken et al. 2008; Verbeken and Walleyn 2010). The milkcap genus *Lactifluus* is mainly distributed in the tropics (De Crop et al. 2017). It is a species-rich genus with about 160 species distributed worldwide, of which the majority is found in tropical Asia (Le et al. 2007b; Stubbe et al. 2010; Van de Putte et al. 2010), tropical Africa (Van de Putte et al. 2009; Verbeken and Walleyn 2010; De Crop et al. 2012, 2016; Maba et al. 2014, 2015a, b; Delgat et al. 2017; De Lange et al. 2018) and the Neotropics (Henkel et al. 2000; Miller et al. 2002; Smith et al. 2011; Sá et al. 2013; Sá and Wartchow 2013). The genus is relatively understudied and many species remain undescribed due to this mainly tropical distribution. Furthermore, the genus is known for its many species complexes with morphologically cryptic species (Stubbe et al. 2010; Van de Putte et al. 2010, 2012; De Crop et al. 2014; De Crop et al. 2017).

About 20 *Lactifluus* species are known from the rainforests of Central Africa (Verbeken and Walleyn 2010). The actual diversity is expected to be higher for several reasons: (i) the ECM flora is present in most parts of the tropical African rainforest, (ii) most countries in the region are understudied due to difficult political situations or challenging sampling conditions, (iii) seasonality in the rainforest is less pronounced, which makes it difficult to assess the exact fruiting period of these fungi and the fruiting of fungi can be missed during short sampling periods, and (iv) *Lactifluus* is known for its morphologically cryptic diversity with several species complexes occurring. Traditional species descriptions were often based on morphology and this morphologically cryptic diversity makes it difficult to correctly assess the number of species based on morphology alone.

During fieldwork in Cameroon in 2012 and 2014, several *Lactifluus* specimens were found morphologically resembling yet different from the described species within *L.* subg. *Pseudogymnocarpi* (Pacioni & Lalli) De Crop. The phylogenetic results of De Crop et al. (2017), based on four nuclear genes, revealed that this species is new to science. A preliminary microscopic study confirmed the deviating morphology of the Cameroonian collections and a more detailed study of all available material was initiated. In this study, molecular and morphological examinations were performed, the collections were compared with closely related species, and a new species, *Lactifluus bicapillus*, was described based on these results.

Methods

Sampling

Sampling expeditions in Cameroon were carried out in May 2012 and May 2014, in the Guineo-Congolian rainforest of the Dja Biosphere Reserve (East Region of Cameroon), mainly in the vicinity of Somalomo and Lomié. During each expedition, four collections were made of an unknown and putative new milkcap species with characteristics of *L*. subg. *Pseudogymnocarpi*. The collections were found in either monodominant stands of *Gilbertiodendron dewevrei*, or mixed stands with *Uapaca guineensis* Müll. Arg., *U. acuminata* (Hutch.) Pax & K. Hoffm., and *U. paludosa* Aubrév. & Leandri as the main ECM hosts. Specimens were dried using a field drier and candles. The studied collections were deposited in the fungal herbarium of Ghent University (**GENT**).

Morphology

Macroscopic features were all based on fresh material described in the field. Colour codes refer to Kornerup and Wanscher (1978). Microscopic features were studied from dried material. Morphological terminology followed Verbeken and Walleyn (2010). Elements of the pileipellis and hymenium were mounted in Congo Red in L4. Sections of the pileipellis and stipitipellis were first mounted in 10% KOH to enhance cell expansion and then mounted in Congo Red dissolved in water. Basidium length excludes sterigmata length. Measurements are given as MIN–MAX, except for basidiospores. Basidiospores were measured in side view in Melzer's reagent, excluding the ornamentation, and measurements are given as described in Nuytinck and Verbeken (2005): (MIN) [Ava –2 × SDa] – *Ava* – *Avb* – [Avb + 2 × SDb] (MAX), in which Ava/b = lowest/highest mean value for the measured collections, SDa/b = standard deviation of the lowest/highest mean value. MIN/MAX = lowest/highest value measured and only given when they exceed [Ava –2 × SDa] or [Avb + 2 × SDb] respectively. Q stands for 'quotient length/width' and is given as MINQ – *Qa* – *Qb* – MAXQ, in which Qa/b = lowest/highest mean quotient for the measured specimens, MIN/MAXQ = minimum/maximum value over the quotients of all available measured basidiospores. Line drawings were made with the aid of a drawing tube at the original magnifications: 6000 × for basidiospores (Zeiss axioscop 2 microscope), 1000 × for individual elements and sections (Olympus CX31 microscope).

Phylogenetic analysis

DNA was extracted using the CTAB extraction protocol described in Nuytinck and Verbeken (2003). Protocols for PCR amplification follow Le et al. (2007a). Two nuclear markers that were previously shown to be informative within this subgenus (De

Table 1. Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses. The arrangement of the subgenera and sections in the table follows their position in the concatenated phylogeny of the genus *Lactifluus* (Fig. 1).

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
Genus *Lactifluus*				
Lactifluus* subg. *Pseudogymnocarpi				
Lactifluus* sect. *Pseudogymnocarpi				
L. cf. *longisporus*	AV 11-025 (GENT)	Tanzania	KR364054	KR364181
L. cf. *pseudogymnocarpus*	AV 05-085 (GENT)	Malawi	KR364012	KR364139
L. cf. *pumilus*	EDC 12-066 (GENT)	Cameroon	KR364067	KR364196
L. gymnocarpoides	JD 885 (BR)	Congo	KR364074	KR364203
	AV 05-184 (GENT)	Malawi	KR364024	KR364151
L. hygrophoroides	AV 05-251 (GENT)	North America	HQ318285	HQ318208
L. longisporus	AV 94-557 (Isotype, GENT)	Burundi	KR364118	KR364244
L. luteopus	AV 94-463 (Isotype, GENT)	Burundi	KR364119	None
L. medusae	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198
L. pseudoluteopus	FH 12-026 (GENT)	Thailand	KR364084	KR364214
L. rugatus	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235
L. sudanicus	AV 11-174 (Isotype, GENT)	Togo	HG426469	KR364186
Lactifluus* sect. *Xerampelini				
L. bicapillus sp. nov.	EDC 12-176 (GENT)	Cameroon	KR364070	KR364199
	EDC 12-174 (GENT)	Cameroon	MH549201	MH549201
	EDC 14-245 (GENT)	Cameroon	MH549204	MH549204
	EDC 12-169 (GENT)	Cameroon	MH549200	MH549200
	EDC 14-249 (Holotype, GENT)	Cameroon	MH549203	MH549203
	EDC 14-284 (GENT)	Cameroon	KX499395	None
	EDC 14-238 (GENT)	Cameroon	MH549202	MH549202
	EDC 12-071 (GENT)	Cameroon	KX499396	KX622762
	L6470/Gab40 (env. seq.)	Gabon	FR731875	None
L. cf. *pseudovolemus*	ADK 2927 (GENT)	Benin	KR364113	KR364243
L. goossensiae	AB 320 (GENT)	Guinea	KR364132	KR364252
L. kivuensis	JR Z 310 (Holotype, GENT)	Congo	KR364027	KR364154
L. rubiginosus	JD 959 (BR)	Congo	KR364081	KR364210
	BB 3466 (Holotype, BR)	Zambia	KR364014	KR364250
L. persicinus	EDC 12-001 (Holotype, GENT)	Cameroon	KR364061	KR364190
L. xerampelinus	TS 1116 (Isotype, GENT)	Tanzania	KR364039	KR364166
Clade 8				
L. sp.	JN 2011-012 (GENT)	Vietnam	KR364045	KR364171
	TENN 065929 (TENN)	North America	KR364102	KR364233
L. armeniacus	EDC 14-501 (Isotype, GENT)	Thailand	KR364127	None
L. volemoides	TS 0705 (Holotype, H)	Tanzania	KR364038	KR364165
Lactifluus* sect. *Aurantiifolii				
L. aurantiifolius	AV 94-063 (Isotype, GENT)	Burundi	KR364017	KR364144
Lactifluus* sect. *Rubroviolascentini				
L. aff. *rubroviolascens*	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195
L. carmineus	AV 99-099 (Holotype, GENT)	Zimbabwe	KR364131	KR364251
L. denigricans	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178
L. kigomaensis	AV 11-006 (Holotype, GENT)	Tanzania	KR364052	KR364179

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
L. subkigomaensis	EDC 11-159 (GENT)	Tanzania	KR364050	KR364177
Lactifluus* sect. *Polysphaerophori				
L. pegleri	PAM/Mart 12-091 (LIP)	Martinique	KP691416	KP691425
L. sp.	RC/Guy 09-036 (LIP)	French Guiana	KJ786645	KJ786550
	MR/Guy 13-145	French Guiana	KJ786691	KJ786595
	MCA 3937 (GENT)	Guyana	KR364109	KR364240
L. veraecrucis	M 8025 (Holotype, ENCB)	Mexico	KR364112	KR364241
Lactifluus* subg. *Lactifluus				
Lactifluus* sect. *Lactifluus				
L. corrugis s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143
L. versiformis	AV-KD-KVP 09-045 (Holotype, GENT)	India	JN388967	JN389031
L. vitellinus	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144
L. volemus	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175

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 and 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 reads were assembled into contigs and edited where needed with the SEQUENCHER v. 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA).

A dataset was constructed, containing sequences of these recent collections, together with sequences of *L.* subg. *Pseudogymnocarpi* extracted from the dataset of De Crop et al. (2017). Furthermore, sequences were compared to sequences in the Unite database using Blastn (Abarenkov et al. 2010). One environmental sequence was found within the same Species Hypothesis and was added to the dataset. The outgroup consisted of four species of *L.* subg. *Lactifluus* (Table 1).

Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v. 7 (Katoh and Standley 2013), using the E-INS-I strategy. Trailing ends of the alignment were trimmed and sequences were manually edited when necessary in MEGA 6 (Tamura et al. 2013). The alignment can be acquired from the first author and TreeBASE (S22916, http://purl.org/phylo/treebase/phylows/study/TB2:S22916).

Sequence data were divided into the following partitions: partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Maximum likelihood (ML) analyses were conducted with RAxML v. 8.0.24 (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

Our molecular results show that the recently collected specimens form a well-supported monophyletic clade within *Lactifluus* subg. *Pseudogymnocarpi*, *L.* sect. *Xerampelini* (Fig. 1). The species is sister to a well-supported clade of all other species within this section, with *L. xerampelinus* (Karhula & Verbeken) Verbeken being its closest relative. Morphological and ecological data confirm that these collections are different from all other species in *L.* sect. *Xerampelini*, therefore the new species is described here as *Lactifluus bicapillus* sp. nov.

Taxonomy

Lactifluus bicapillus **Lescroart & De Crop**
Mycobank: MB827400
Figs 2–4

Diagnosis. *Lactifluus bicapillus* differs from *L. xerampelinus* by its yellowish-orange to dark red cap, fertile lamella edge, a lampropalisade with two types of terminal elements as pileipellis type, and a distribution in the Guineo-Congolian rainforest.

Holotype. CAMEROON. East Region, Haut-Nyong division, Somalomo subdivision, Dja Biosphere Reserve, alt. ca 640 m, 3°21.83'N, 12°44.18'E, rainforest with *Uapaca paludosa* and *U. guineensis*, 14 May 2014, leg.: De Crop & Verbeken, EDC 14-249 (GENT!).

Basidiocarps medium-sized. **Pileus** 34–79 mm in diameter, firm, infundibuliform to deeply infundibuliform, planoconvex with central depression when younger; margin involute when juvenile, becoming inflexed up to reflexed when older; edge entire, sometimes eroded when older; surface felty to chamois leather-like, often slightly pruinose in the centre, often grooved, concentrically wrinkled, in young specimens completely velutinous and somewhat translucent; rubiginous (7D6–7) in centre, becoming paler and more orange towards the margin (6C5–6 to 5A5–6); young specimens dark reddish or burgundy in centre, to bright orange or yellow at the margin (8F6 to 7B6, to 6A5, 4AG); secondary velum absent. **Stipe** 16–39 × 6–12 mm, cylindrical to slightly tapering downwards, often laterally curved near the base, central to eccentric insertion to pileus, entire or bruised appearance, sometimes with white flocks near the base; surface smooth and felty, sometimes pruinose, yellowish orange (5AB5–6), becoming slightly paler and more yellow near the base and/or lamellae (5A4–5). **Lamellae** intervenose, transvenose, sparingly bifurcating; attachment adnate to decurrent with some lamellae forming a small tooth; juveniles not brittle, rather thin, older specimens brittle to very brittle, thick to very broad; edge entire and concolourous; distant, 3–5 + 6–9 L+l/cm, between 2 lamellae often 3 lamellulae, with regular short long-short pattern; creamy yellow (3A2) to yellowish orange (4A4). **Context** white, with a faint yellow tinge, colour not changing when cut, but in 1 collection (EDC 14-238) becoming brown when damaged, rather solid and full,

Figure 1. Overview Maximum Likelihood tree of *Lactifluus* subg. *Pseudogymnocarpi*, based on concatenated ITS and LSU sequence data. Sequences of the here described species *Lactifluus bicapillus* are written in bold. Maximum Likelihood bootstrap values > 70 are shown. Numbers of undescribed sections refer to De Crop et al. (2017).

smell sweet or not distinct, taste mild. **Latex** white, somewhat astringent, rather abundant, becomes less abundant and more watery with age, mild, colour rarely changing brownish when isolated. **Chemical reactions** unchanging with Fe_2SO_4; context faint blue after 5 sec. with guaiac.

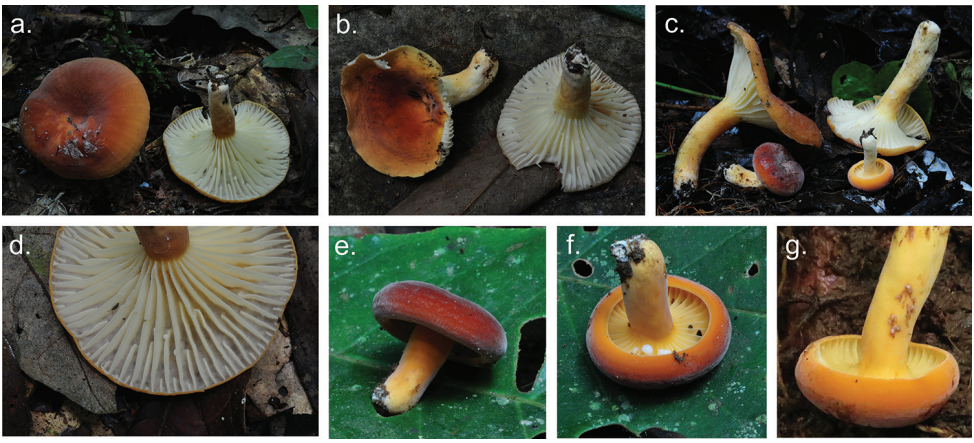

Figure 2. Basidiomata of *Lactifluus bicapillus*. **a–c** Basidiomata of *Lactifluus bicapillus* (EDC 12-176, EDC 12-174, holotype EDC 14-249 resp.) **d** Detail of lamellae (EDC 14-176), e) young specimen (EDC 12-169) **f** Detail of latex (EDC 12-169) **g** Detail of brown colour change of the latex (EDC 14-238) (photographs **a–f** by E. De Crop, **g** by A. Verbeken).

Basidiospores [6.2]–7.3–7.9–[9.6](10.3) × [4.6]–5.5–5.9–[6.8] µm; ellipsoid, with Q = (1.22)1.31–1.39(1.51); ornamentation amyloid, composed of low ridges and warts, up to 0.2 µm high, forming an incomplete to complete reticulum; plage inamyloid or centrally amyloid. **Basidia** 43–62 × 8–12 µm, rather long, narrowly subclavate, 1-, 2- or 4-spored; content oleiferic. **Sterile elements** abundant, 19.5–40 × 3.5–5.5 µm, not emergent, cylindrical, septate with clamp-like bulges under the septum, with rounded apex. **Pleurocystidia** absent. **Pleuropseudocystidia** very scarce in mature specimens, abundant in young specimens, narrowly and irregular cylindrical to flexuose, 3.3–4.6 µm diam., not emerging, apex obtuse, oleiferic content. **Lamellae-edge** fertile, consisting of basidioles with some basidia. Marginal cells absent. **Hymenophoral trama** cellular, with sphaerocytes and abundant lactifers. **Pileipellis** a lampropalisade, up to 275 µm thick; terminal elements of two types, without transitional forms: the first type long and slender, thick-walled and often septate, with a wide base, up to 7 µm, and growing thinner towards the apex, down to 1–2 µm, length 52–92 µm, often narrowing rather abruptly, and twisted; the second type short and broad, also thick-walled and often septate, not specifically narrower towards the apex, often twisted, 20–44 × 5–7 µm; subpellis composed of mostly rounded cells. **Stipitipellis** similar to pileipellis but not as thick; terminal elements of the long type 52–75 × 5–7 µm; terminal elements of the short type 22–29 × 5–7 µm. **Clamp-connections** absent.

Distribution. Known from Cameroon and Gabon.

Ecology. Guineo-Congolian rainforest, scattered on forest floor under *Gilbertiodendron dewevrei*, *Uapaca guineensis*, *U. acuminata*, and *U. paludosa.*

Etymology. A combination of 'bi' and 'capillus', referring to the two types of terminal elements in the pileipellis and stipitipellis.

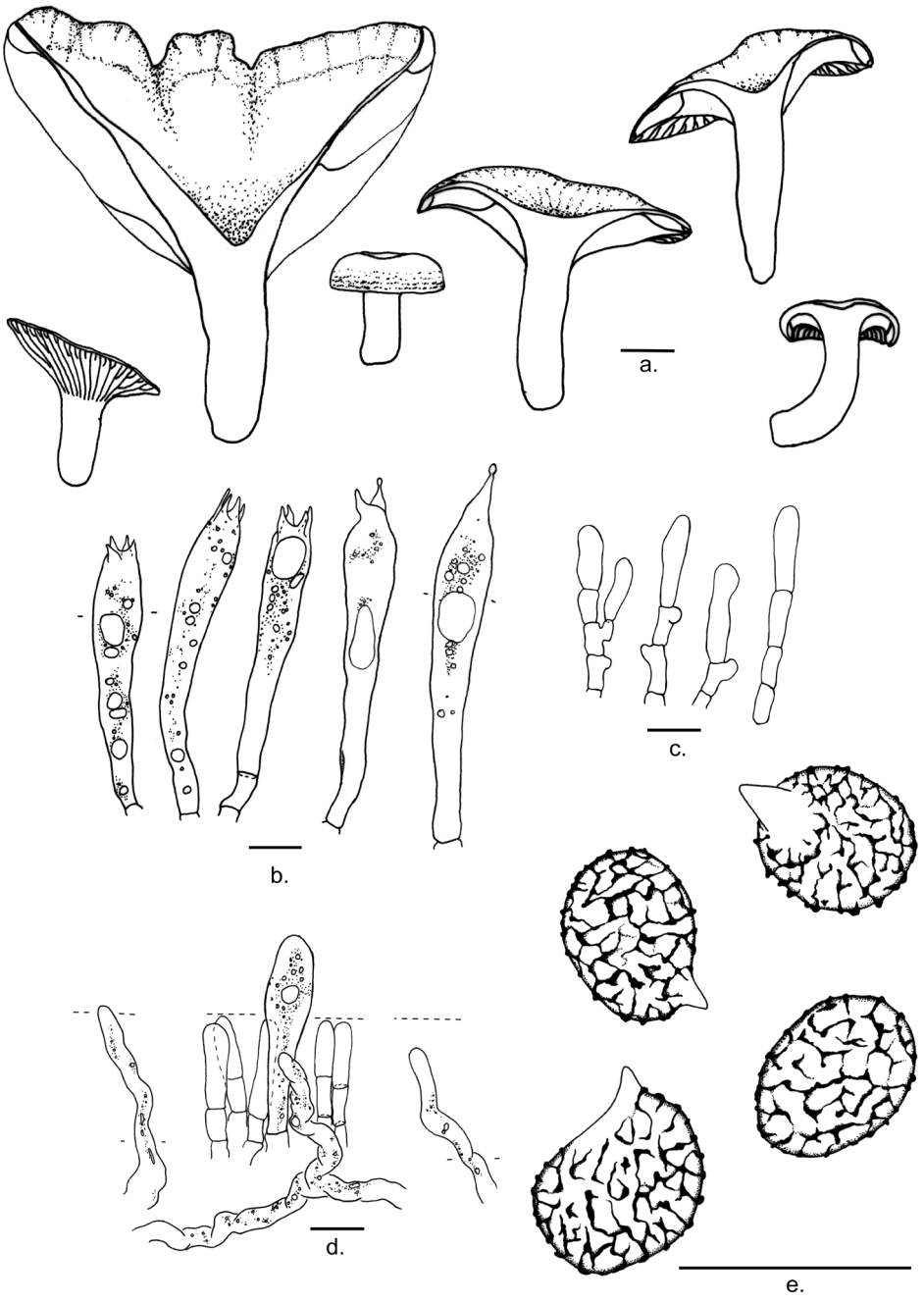

Figure 3. Microscopic features of *Lactifluus bicapillus* **a** Basidiocarps (from EDC 12-071, EDC 12-169, EDC 12-174, EDC 12-176, and EDC 14-249) **b** Basidia (from EDC 12-071, and EDC 14-249) **c** Sterile elements from the hymenium (from EDC 12-169) **d** Pleuropseudocystidia (from EDC 12-169) **e** Basidiospores (from EDC 14-249). Illustrations by E. De Crop, J. Lescroart and A. Verbeken. Scale bar: 10 μm.

Figure 4. Microscopic features of *Lactifluus bicapillus* (continued) **a** Terminal elements of the pileipellis (from EDC 12-071) **b** Terminal elements of the stipitipellis (from EDC 12-176) **c** Section through the pileipellis (from holotype EDC 14-249). Illustrations by E. De Crop and J. Lescroart. Scale bar: 10 µm.

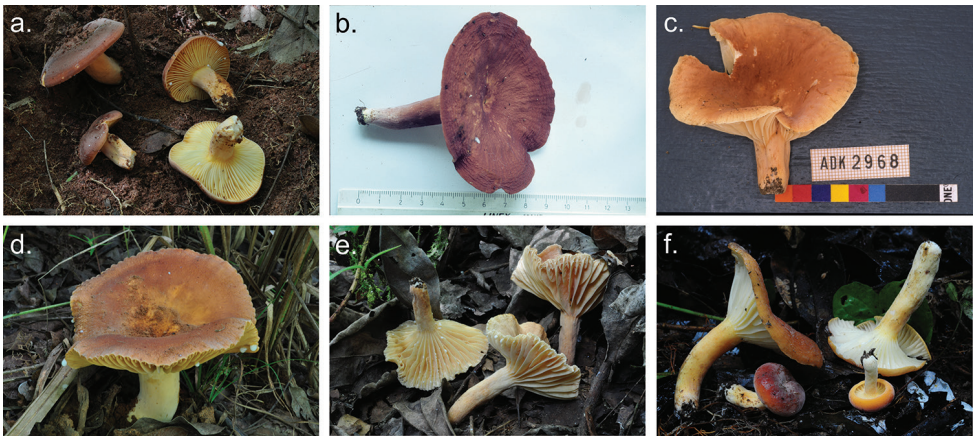

Figure 5. Basidiomata of described species of *Lactifluus* sect. *Xerampelini* **a** *L. xerampelinus* (EDC 11-113) **b** L. *kivuensis* (JR Z 233) **c** L. cf. *pseudovolemus* (ADK 2968) **d** *L. rubiginosus* (EDC 11-120) **e** *L. persicinus* (EDC 12-001, *holotypus*) **f** *L. bicapillus (*EDC 14-249, holotypus) (photographs a, **d–f** by E. De Crop, **b** by J. Rammeloo and **c** by A. De Kesel).

Conservation status. Unknown.

Specimens examined. Cameroon. East Region, Haut-Nyong division, Somalomo subdivision, Koulou village, alt. ca 650 m, 3°23.61'N, 12°44.50'E, rainforest, *Gilbertiodendron dewevrei*, *Uapaca guineensis*, *U. acuminata,* 15 May 2012, De Crop, EDC 12-071 (GENT); East Region, Haut-Nyong division, Lomié subdivision, Bosquet village, alt. ca 610 m, 3°07.82'N, 13°53.36'E, rainforest with many *Uapaca* trees, on a riverbank, *Uapaca guineensis*, 24 May 2012, De Crop, EDC 12-169 (GENT); Ibidem, *Gilbertiodendron dewevrei*, De Crop, EDC 12-174 (GENT); Ibidem, *Uapaca guineensis*, EDC 12-176 (GENT); East Region, Haut-Nyong division, Somalomo subdivision, Dja Biosphere Reserve, alt. ca 650 m, 3°21.90'N, 12°44.15'E, rainforest, *Uapaca paludosa, U. guineensis,* 14 May 2014, De Crop & Verbeken, EDC 14-238 (GENT); Ibidem, alt. ca 640 m, 3°21.83'N, 12°44.18'E, De Crop & Verbeken, EDC 14-249 (GENT); Ibidem, alt. ca 650 m, 3°19.87'N, 12°45.42'E, rainforest, near the river, *Uapaca sp.*, 17/05/2014, De Crop & Verbeken, EDC 14-284 (GENT).

Discussion

Lactifluus bicapillus is recognized in the field by its yellowish-orange to dark-red cap, a concolourous or somewhat paler stipe, yellow lamellae, and unchanging white latex. *L. bicapillus* is placed in *L.* subg. *Pseudogymnocarpi, L.* sect. *Xerampelini*. Species in this section are characterized by yellowish-orange to reddish-brown caps, a palisade-like structure as pileipellis, the absence of true pleurocystidia, and generally low ornamented basidiospores (not higher than 0.2 µm) ranging from verrucose to almost completely reticulate (De Crop et al. 2017). *Lactifluus bicapillus* perfectly concurs with

these morphological characteristics, providing additional support for its placement in *L.* sect. *Xerampelini*.

Lactifluus sect. *Xerampelini* is exclusively known from Africa and contains six described species (Fig. 5): *L. goossensiae* (Beeli) Verbeken, *L. kivuensis* (Verbeken) Verbeken, *L. persicinus* Delgat & De Crop, *L. pseudovolemus* (R. Heim) Verbeken, *L. rubiginosus* (Verbeken) Verbeken, and *L. xerampelinus* (Verbeken and Walleyn 2010; Delgat et al. 2017).

Lactifluus bicapillus differs in ecology from all but one species of *L.* sect. *Xerampelini*. Species from this section occur in woodlands, gallery forests and rainforests (Verbeken and Walleyn 2010). *Lactifluus xerampelinus* and *L. rubiginosus* are found in miombo woodland in East Africa, while *L. goossensiae* is known from both Sudanian woodland and Central African gallery forests. *Lactifluus persicinus* and *L. pseudovolemus* occur in West African gallery forests. Both *L. kivuensis* and *L. bicapillus* are found in the Guineo-Congolian rainforest, associated with *Gilbertiodendron dewevrei* and *Uapaca* species.

Macroscopically, *L. bicapillus* differs from the other species of this section by a combination of bright cap colours, which vary from dark red to bright orange near the edge, cream white lamellae and pale yellow-orange stipe colours in adult basidiocarps (Fig. 5).

All species from *L.* sect. *Xerampelini* have ellipsoid to elongate basidiospores, with amyloid ornamentation composed of very low warts and ridges (up to 0.2 µm high) that are isolated, aligned or forming an incomplete reticulum. All seven species have long and slender basidia, mostly cylindrical and 4-spored. However, 1- and 2-spored basidia are present in *L. bicapillus*, *L. persicinus*, and *L. pseudovolemus.* True cystidia are absent in all species. Pleuropseudocystidia are scarce in *L. bicapillus*, *L. persicinus*, and *L. kivuensis*, abundant in the other species. These pleuropseudocystidia are occasionally emergent in all species; however, emergent pleuropseudocystidia were not observed in *L. bicapillus*. *Lactifluus persicinus* and *L. bicapillus* have a fertile lamellar edge, whilst the others have a sterile lamellar edge (or unknown in *L. pseudovolemus* and *L. goossensiae*).

All species of this section have palisade-like structures as pileipellis. *Lactifluus bicapillus*, *L. persicinus*, and *L. goossensiae* have a lampropalisade with thick-walled terminal elements. *Lactifluus pseudovolemus* has a palisade in which the elements of the pileipellis are slightly thickened. *Lactifluus kivuensis*, *L. xerampelinus*, and *L. rubiginosus* have a palisade to trichopalisade, with only thin-walled elements of the pileipellis. Only *Lactifluus bicapillus*, *L. persicinus*, and *L. goossensiae* have terminal elements that are narrow near the apex. Furthermore, *L. bicapillus* is the only species within this section with two types of terminal elements in the pilei- and stipitipellis.

With the finding of *Lactifluus bicapillus*, *L.* sect. *Xerampelini* now contains seven described species, all from sub-Saharan Africa. Together with the recently described *L. persicinus* (Delgat et al. 2017), *L. bicapillus* was found during two sampling expeditions in Cameroon. Even though those expeditions only covered a small area of the Guineo-Congolian rainforest and gallery forests, we collected at least five species new to science (De Crop et al. 2017). This highlights the large *Lactifluus* diversity in Africa, with many areas still unexplored and probably many new species still to be found.

Acknowledgements

The first author is supported by the "Special Research Fund Ghent University" (BOF, grants B/13485/01 and BOF-PDO-2017-001201). The 2012 survey in Cameroon was financially supported by the Faculty Committee Scientific Research (FCWO) of Ghent University. The 2014 survey in Cameroon was financially supported by the Research Foundation Flanders (FWO, grant V416214N) and by the King Leopold III Fund for Nature Exploration and Conservation. We express our gratitude to all who helped during fieldwork, especially to the conservators and Ecogards in post in the Dja Biosphere Reserve (from 2012 to 2014) and Mr Tchana Tchonkui Merlin. We would like to thank Viki Vandomme for conducting lab work. We thank André De Kesel and Jan Rammeloo for providing pictures of *Lactifluus* species. We thank the reviewers and the editor for their constructive suggestions and detailed comments on the manuscript.

References

Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vralstad T, Liimatainen K, Peintner U, Koljalg U (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytologist 186: 281–285. https://doi.org/10.1111/j.1469-8137.2009.03160.x

De Crop E, Nuytinck J, Van de Putte K, Lecomte M, Eberhardt U, Verbeken A (2014) *Lactifluus piperatus* (Russulales, Basidiomycota) and allied species in Western Europe and a preliminary overview of the group worldwide. Mycological Progress 13: 493–511. https://doi.org/10.1007/s11557-013-0931-5

De Crop E, Nuytinck J, Van de Putte K, Wisitrassameewong K, Hackel J, Stubbe D, Hyde KD, Roy M, Halling RE, Moreau PA, Eberhardt U, Verbeken A (2017) A multi-gene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. Persoonia 38: 58–80. https://doi.org/10.3767/003158517X693255

De Crop E, Tibuhwa D, Baribwegure D, Verbeken A (2012) *Lactifluus kigomaensis* sp. nov. from Kigoma province,Tanzania. Cryptogamie Mycologie 33: 421–426. https://doi.org/10.7872/crym.v33.iss4.2012.421

De Crop E, Van de Putte K, De Wilde S, Njouonkou AL, De Kesel A, Verbeken A (2016) *Lactifluus foetens* and *Lf. albomembranaceus* sp. nov. (Russulaceae): look-alike milkcaps from gallery forests in tropical Africa. Phytotaxa 277: 159–170. https://doi.org/10.11646/phytotaxa.277.2.3

De Lange R, De Crop E, Delgat L, Tibuhwa D, Baribwegure DAV (2018) *Lactifluus kigomaensis* and *L. subkigomaensis*: two look-alikes in Tanzania. Mycoscience 59: 371–378. https://doi.org/10.1016/j.myc.2018.02.004

Delgat L, De Crop E, Njouonkou AL, Verbeken A (2017) Lactifluus persicinus sp. nov. from the gallery forests of West Cameroon. Mycotaxon 132: 471–483. https://doi.org/10.5248/132.471

Douanla-Meli C, Langer E (2009) Fungi of Cameroon II. Two new Russulales species (Basidiomycota). Nova Hedwigia 88: 491–502. https://doi.org/10.1127/0029-5035/2009/0088-0491

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Henkel TW, Aime MC, Miller SL (2000) Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status. Mycologia 92: 1119–1132. https://doi.org/10.2307/3761479

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kornerup A, Wanscher JH (1978) Methuen Handbook of Colour. Methuen, London.

Le HT, Nuytinck J, Verbeken A, Lumyong S, Desjardin DE (2007a) *Lactarius* in northern Thailand: 1. *Lactarius* subgenus *Piperites*. Fungal Diversity 24: 173–224.

Le HT, Verbeken A, Nuytinck J, Lumyong S, Desjardin DE (2007b) *Lactarius* in northern Thailand: 3. *Lactarius* subgenus *Lactoriopsis*. Mycotaxon 102: 281–291.

Maba DL, Guelly AK, Yorou NS, Agerer R (2015a) Diversity of *Lactifluus* (Basidiomycota, Russulales) in West Africa: 5 new species described and some considerations regarding their distribution and ecology. Mycosphere 6: 737–759. https://doi.org/10.5943/mycosphere/6/6/9

Maba DL, Guelly AK, Yorou NS, Verbeken A, Agerer R (2014) Two New *Lactifluus* species (Basidiomycota, Russulales) from Fazao Malfakassa National Park (Togo, West Africa). Mycological Progress 13: 513–524. https://doi.org/10.1007/s11557-013-0932-4

Maba DL, Guelly AK, Yorou NS, Verbeken A, Agerer R (2015b) Phylogenetic and microscopic studies in the genus *Lactifluus* (Basidiomycota, Russulales) in West Africa, including the description of four new species. IMA Fungus 6: 13–24. https://doi.org/10.5598/imafungus.2015.06.01.02

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Proceedings of the Gateway Computing Environments Workshop (GCE): 1–8. https://doi.org/10.1109/GCE.2010.5676129

Miller SL, Aime MC, Henkel TW (2002) Russulaceae of the Pakaraima Mountains of Guyana – I New species of pleurotoid *Lactarius*. Mycologia 94: 545–553. https://doi.org/10.2307/3761789

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic-Biology 49: 278–305. https://doi.org/10.1093/sysbio/49.2.278

Nuytinck J, Verbeken A (2003) *Lactarius sanguifluus* versus *Lactarius vinosus* – molecular and morphological analyses. Mycological Progress 2: 227–234. https://doi.org/10.1007/s11557-006-0060-5

Nuytinck J, Verbeken A (2005) Morphology and taxonomy of the European species in *Lactarius* sect. *Deliciosi* (Russulales). Mycotaxon 92: 125–168.

Sá MCA, Baseia IG, Wartchow F (2013) *Lactifluus dunensis*, a new species from Rio Grande do Norte, Brazil. Mycosphere 4: 261–265. https://doi.org/10.5943/mycosphere/4/2/9

Sá MCA, Wartchow F (2013) *Lactifluus aurantiorugosus* (Russulaceae), a new species from Southern Brazil. Darwiniana (nueva serie) 1: 54–60.

Smith ME, Henkel TW, Aime MC, Fremier AK, Vilgalys R (2011) Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytologist 192: 699–712. https://doi.org/10.1111/j.1469-8137.2011.03844.x

Sonké B, Couvreur TLP (2014) Tree diversity of the Dja Faunal Reserve, southeastern Cameroon. Biodivers Data Journal 2: e1049. https://doi.org/10.3897/BDJ.2.e1049

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771. https://doi.org/10.1080/10635150802429642

Stubbe D, Nuytinck J, Verbeken A (2010) Critical assessment of the *Lactarius gerardii* species complex (Russulales). Fungal Biology 114: 271–283. https://doi.org/10.1016/j.funbio.2010.01.008

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Van de Putte K, De Kesel A, Nuytinck J, Verbeken A (2009) A new *Lactarius* species from Togo with an isolated phylogenetic position. Cryptogamie Mycologie 30: 39–44.

Van de Putte K, Nuytinck J, Das K, Verbeken A (2012) Exposing hidden diversity by concordant genealogies and morphology – a study of the *Lactifluus volemus* (Russulales) species complex in Sikkim Himalaya (India). Fungal Diversity 55: 171–194. https://doi.org/10.1007/s13225-012-0162-0

Van de Putte K, Nuytinck J, Stubbe D, Huyen TL, Verbeken A (2010) *Lactarius volemus* sensu lato (Russulales) from northern Thailand: morphological and phylogenetic species concepts explored. Fungal Diversity 45: 99–130. https://doi.org/10.1007/s13225-010-0070-0

Verbeken A, Buyck B (2002) Diversity and ecology of tropical ectomycorrhizal fungi in Africa. In: Watling R, Frankland JC, Ainsworth AM, Isaac S, Robinson C (Eds) Tropical Mycology. Vol. 1, 11–24.

Verbeken A, Stubbe D, Nuytinck J (2008) Two new *Lactarius* species from Cameroon. Cryptogamie Mycologie 29: 137–143.

Verbeken A, Walleyn R (2010) Monograph of *Lactarius* in Tropical Africa. National Botanic Garden, Belgium, 161 pp. [154 pls]

White JC (1983) The Vegetation of Africa. A Descriptive Memoir to Accompany the UNESCO/AETFAT/UNSO Vegetation Map of Africa. UNESCO, Paris, 356 pp.

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1