



Lactifluus (Russulaceae) diversity in Central America and the Caribbean: melting pot between realms

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Abstract Species of the ectomycorrhizal genus *Lactifluus*, and often entire sections, are typically unique to a single continent. Given these biogeographic patterns, an interesting region to study their diversity is Central America and the Caribbean, since the region is closely connected to and often considered a part of the North American continent, but biogeographically belong to the Neotropical realm, and comprises several regions with different geologic histories. Based on a multi-gene phylogeny and morphological study, this study shows that Central America, Mexico and the Caribbean harbour at least 35 *Lactifluus* species, of which 33 were never reported outside of this region. It was found that species from the Caribbean generally show affinities to South American taxa, while species from the Central American mainland generally show affinities to Northern hemispheric taxa. We hypothesise that host specificity and/or climate play a crucial role in these different origins of diversity. Because of these different affinities, Caribbean islands harbour a completely different *Lactifluus* diversity than the Central American mainland. The majority of species occurring on the islands can be considered endemic to certain islands or island groups. In this paper, detailed morphological descriptions are given, with a focus on the unique diversity of the islands, and identification keys to all hitherto described *Lactifluus* species occurring in Central America and the Caribbean are provided. One new section, *Lactifluus* sect. *Nebulosi*, and three new species, *Lactifluus guadeloupensis*, *Lactifluus lepus* and *Lactifluus marmoratus* are described.

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INTRODUCTION

The *Russulaceae* family represents a dominant group of ectomycorrhizal fungi in almost all forest ecosystems worldwide. In 2008, molecular research showed that the traditionally recognised genera *Russula* and *Lactarius* were not monophyletic, resulting in a rearrangement of the generic landscape into four separate genera; *Lactarius* sensu novo, *Russula*, *Multifurca* and *Lactifluus* (Buyck et al. 2008, 2010). The genus *Lactifluus* comprises around 200 described species worldwide, although its total diversity is estimated to be much higher, and has its highest species richness in the tropics (De Crop 2016). *Lactifluus* species, and often sections, are usually unique to a single continent, so there is little to no overlap in species diversity between continents.

Central America, part of Mexico, and the Caribbean present a geographically interesting area to study, since they are often considered part of the North American continent and are closely connected to it, but biogeographically belong to the Neotropical

realm together with the South American continent. Given the biogeographic patterns in *Lactifluus*, the question arises whether species occurring in Central America and the Caribbean are most closely related to North or to South American species.

Most of Central America and the Caribbean is situated on the Caribbean plate. Cuba is an exception as it originated on the Caribbean plate but became stuck on the North American plate. The northern part of Hispaniola is a piece of Cuba that was sheared off as the Caribbean plate moved eastward. The Central American mainland is located on the western edge of this plate and consists of seven countries: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama. It is a tectonically active region and the landscape of the mainland of Central America is strongly determined by mountain ranges. The region contains diverse forest types: tropical moist broadleaf forests, tropical dry broadleaf forests, tropical coniferous forests and montane forests. The tropical lowlands of Central America are dominated by plants of South American origin, examples of ectomycorrhizal hosts are *Coccoloba*, *Guapira*, *Neea* and *Pisonia*. This lowland vegetation strongly differs from the montane vegetation, which includes mainly species with temperate North American origins, such as *Alnus*, *Pinus* and *Quercus*. In addition, the southern part of Mexico also belongs to the Neotropical realm, and consists of habitats similar to those present in Central America.

Central America is bordered in the east by the Caribbean Sea. The Caribbean islands comprise two main geographical groups: the Greater Antilles and the Lesser Antilles, which have independent origins. The Antilles are mostly volcanic in origin, arisen due to the subduction of the North American plate under the Caribbean plate. The Greater Antilles are geologically the oldest and lie along the Northern edge of the Caribbean plate.

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They comprise the islands of Cuba, Cayman Islands, Jamaica, Hispaniola (i.e., Dominican Republic and Haiti), Puerto Rico and the Virgin Islands. The largest Greater Antillean islands are Cuba, Hispaniola and Jamaica. Several different forest types occur on the Greater Antilles, such as lowland forests (e.g., rainforests with *Carapa guianensis*; semi-deciduous forests with *Coccoloba diversifolia*, *Guapira fragrans*; seasonal evergreen forests with *Ceiba pentandra*), submontane forests (e.g., rainforests with *Cecropia peltata*, *Dacryodes excelsa*, *Mora abbotii*, *Oxandra laurifolia*, *Sloanea berteriana*; pine forests with *Pinus caribaea*, *Pinus cubensis*; sclerophyllous forest with *Coccoloba retusa*, *Quercus oleoides* subsp. *sagraeana*) and montane forests (e.g., rainforests with *Alchornea latifolia*, *Magnolia* spp., *Ocotea ekmanii*, *Solanum acropterum*; (mixed) pine forests with *Pinus cubensis*, *Pinus occidentalis*) (Panagopoulos 1999).

The Lesser Antilles on the other hand are a series of small islands on the eastern edge of the Caribbean plate. The major part of the Lesser Antilles is volcanic in origin, though a small group of islands (i.e., Antigua and Barbuda, Grande-Terre of Guadeloupe, Marie-Galante and Barbados) is of coral (calcareous) origin, building an arc dorsally contiguous to the main volcanic one. This yields a special interest to Guadeloupe, which comprises both ecologies (i.e., volcanic for Basse-Terre and calcareous for Grande-Terre). In contrast, Trinidad and Tobago are continental islands that were cut off by sea level rise from the South American mainland while the other islands emerged from the ocean floor. Trinidad, Guadeloupe and Martinique are the largest Lesser Antillean islands. Vegetation of the Lesser Antilles has been studied in detail, and according to the literature the forests can be classified in four main types:

1. altitudinal, montane or cloud forest;
2. hygrophytic or submontane rain forest;
3. mesophytic or (semi-)evergreen seasonal forest; and
4. xerophytic or tropical semi-deciduous forest.

Montane forests occupy small areas near the highest mountains of Martinique and the vegetation consists of shrubs, most typically *Clusia plukenetii*. In hygrophytic forests, the annual rainfall is over 2700 mm/year and a pronounced dry season is absent. In these forests, epiphytes are abundant and tree species are virtually all evergreen. Dominant tree species include *Dacryodes excelsa*, and the endemic *Licania ternatensis*, *Magnolia dodecapetala* and *Sloanea massonii*. Mesophytic forests have a lower annual rainfall and are characterised by species of *Inga*, *Nectandra*, *Simarauba* and *Tabebuia*, but patches of several ectomycorrhizal *Coccoloba* spp. are also present. In xerophytic forests annual rainfall is lower than 1250 mm/year and there is a distinct dry season. Approximately half of the tree species are deciduous and the canopy commonly consists of species of *Bursera*, *Ceiba*, *Coccoloba*, *Guapira*, *Lonchocarpus*, and *Pisonia* (Stehle 1947, Beard 1948, Pegler & Fiard 1983, Panagopoulos 1999, Joseph 2013). *Coccoloba*, *Guapira* and *Pisonia* are known ectomycorrhizal hosts (e.g., Tedersoo et al. 2010, Alvarez-Manjarrez et al. 2018) and it is in these xerophytic forests that ectomycorrhizal fungi predominate (Pegler & Fiard 1983).

Extensive collections of agaricoid Basidiomycota were made on the Lesser Antilles, making it the best studied region in Central America and the Caribbean, and a total of ten *Lactarius* species were recorded in this area (Pegler & Fiard 1979, 1983). Recently, most of these species have been combined in *Lactifluus* (Montoya et al. 2012, Verbeken et al. 2012). However, these species are in need of a thorough molecular study. Other areas in Central America and the Caribbean have been studied less intensively, yielding only three species of *Lactifluus* described from the Greater Antilles, one *Lactifluus* species from Central America and four species from southern Mexico (Singer 1973,

Montoya et al. 1996, 2012, Miller et al. 2000, Montoya & Bandalá 2004, Crous et al. 2019, Delgat et al. 2019).

In this paper, a phylogeny based on multiple loci, detailed morphological information and identification keys for *Lactifluus* species occurring in the Caribbean, and Central America and Mexico are provided. Three new species are described here: *Lactifluus guadeloupensis*, **L. lepus* and *L. marmoratus*. A new section, *L. sect. Nebulosi* is described, *L. castaneibadius* and *L. murinipes* are synonymised, and *Lactarius coccolobae*, *Lactarius fuscomarginatus*, *Lactarius pegleri* and *Lactarius sect. Panuoidei* are combined in *Lactifluus*.

(* *L.* = *Lactifluus*)

MATERIAL AND METHODS

Sampling

Collections for the Lesser Antilles were made during regular fieldtrips to the islands Martinique and Guadeloupe between 2003 and 2015, organised within the program 'Les champignons des Petites Antilles: diversité, écologie et protection' initiated and conducted by R. Courtecuisse and permitted since 2006 by grants and funding from different organizations (see Acknowledgements). Field work focused on different habitats such as xero-, meso- and hygrophytic forests. The collections from Western Panama were obtained during a field trip to Chiriqui province in 2018, sampling mostly montane forests, but to a lesser extent also lowlands. Herbarium vouchers were gathered for other regions.

Morphological analysis

Macroscopic characters were observed from fresh material, with colour codes referring to Korerup & Wanscher (1978). Microscopic characters were observed from dried material. Basidiospores were mounted in Melzer's reagent and hymenium, pileipellis and stipitipellis were studied in Congo red in L4. The basidiospore measurements (i.e., length, width and $Q = \text{quotient of length and width}$) are given as $[Ava-2xSDa]-Ava-Avb-[Avb+2xSdb]$, in which Ava = lowest mean value for the measured specimens, Avb = greatest mean value, SDa/b = standard deviation of the lowest and greatest mean value, respectively (number of spores measured per specimen = 15–20). If there was only one specimen, measurements are given as $[Av-2xSD]-Av-[Av+2xSD]$. Basidiospores were measured in side view excluding ornamentation. Measurements of basidia, cystidia and pileipellis terminal elements are given as $[Av-2xSD]-Av-[Av+2xSD]$, based on minimum 10 measurements per structure and species. Measurements of basidia do not include 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).

Molecular analysis

DNA was extracted from fresh material preserved in CTAB (Cetyl trimethylammonium bromide) using the CTAB extraction method described in Nuytinck & Verbeken (2003). The protocol described by Dentinger et al. (2010) was used for dried collections from Kew herbarium, a modified CTAB protocol (Tel-Zur et al. 1999; modified by Agentschap Plantentuin Meise) was used for other dried collections. PCR amplification protocols follow Le et al. (2007). Four nuclear markers previously shown as informative within this genus (Stubbe et al. 2010, Van de Putte et al. 2010, Montoya et al. 2012) 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 (White et al. 1990, Gardes & Bruns 1993);

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

Species	Herbariumnumber	Herbarium	GenBank accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
<i>Lactarius fuliginosus</i>	MTB 97-24	GENT	JQ446111	JQ446180	JQ446240	KR364392
<i>Lactarius hatsudake</i>	FH 12-052	GENT	KR364085	KR364215	KR364285	KR364411
<i>Lactarius miniatescens</i>	AV 11-177	GENT	KR364059	KR364187	KR364315	KR364443
<i>Lactarius olympianus</i>	ED 08-018	GENT	KR364089	KR364220	KR364320	KR364448
<i>Lactarius scrobiculatus</i>	JN 2001-058	GENT	KF432968	–	KR364344	KR364474
<i>Lactarius tenellus</i>	ADK 3598	GENT	KF133280	KF133313	KF133345	KR364482
<i>Lactifluus acicularis</i>	KVP 08-002	GENT	HQ318226	HQ318132	HQ328869	JN389131
<i>Lactifluus acrisissimus</i>	EDC 11-112	GENT	KR364041	KR364168	KR364254	KR364366
<i>Lactifluus albomembranaceus</i>	EDC 12-046	GENT	KR364064	KR364193	KR364257	KR364369
<i>Lactifluus allardii</i>	JN 2004-008	GENT	KF220016	KF220125	KF220217	KR364370
<i>Lactifluus ambicystidiatus</i>	HKAS J7008	HKAS	KR364108	KR364239	KR364309	KR364437
<i>Lactifluus annulatoangustifolius</i>	BB 00-1518	GENT, PC	AY606981	KR364253	–	–
<i>Lactifluus annulifer</i>	TH 9014 G4819	BRG, DUKE PC	KC155376 KM073086	KC155376	–	–
<i>Lactifluus armeniacus</i>	EDC 14-501	MFLU, GENT	KR364127	–	–	–
<i>Lactifluus aurantiifolius</i>	AV 94-063	GENT	KR364017	KR364144	–	–
<i>Lactifluus aureifolius</i>	AV 11-074	GENT	KR364056	KR364183	KR364259	KR364371
<i>Lactifluus auriculiformis</i>	AV 12-050	GENT	KR364086	KR364216	KR364260	KR364372
<i>Lactifluus bertillonii</i>	JN 2012-016	GENT	KR364087	KR364217	KR364261	KR364373
<i>Lactifluus bhandaryi</i>	TENN 051830	TENN	KR364111	KR364140	–	–
<i>Lactifluus bicapillus</i>	EDC 12-176	GENT	KR364070	KR364199	KR364300	KR364428
<i>Lactifluus bicolor</i>	DS 06-247	GENT	JN388955	JN388987	JN375590	JN389186
<i>Lactifluus brachystegiae</i>	AV 99-002	GENT	KR364018	KR364145	KR364262	KR364374
<i>Lactifluus brunellus</i>	TH 9130	BRG, DUKE	JN168728	–	–	–
<i>Lactifluus brunneocarpus</i>	AB 305	GENT	KR364035	KR364162	KR364343	KR364473
<i>Lactifluus brunneoviolascens</i>	AV 13-038	GENT	KR364123	KR364246	KR364264	KR364376
<i>Lactifluus brunnescens</i>	AV 05-083	GENT	KR364019	KR364146	KR364263	KR364375
<i>Lactifluus caribaeus</i>	CL/MART06.014 J.P. Fiard 818A J.P. Fiard 827B	LIP K K	MK046841 MK046852 MK046853	MK046791	–	–
<i>Lactifluus carmineus</i>	AV 99-099	GENT	KR364131	KR364251	KR364265	–
<i>Lactifluus castaneibadius</i>	J.P. Fiard 837A	K	MK046854	–	–	–
<i>Lactifluus chamaeleontinus</i>	JD 946	MEISE	KR364079	KR364208	KR364267	KR364377
<i>Lactifluus chiapanensis</i>	Bandala 4374a	GENT	GU258297	GU265580	GU258316	KR364378
<i>Lactifluus chrysocarpus</i>	LE 253907	LE	JX442761	JX442761	–	–
<i>Lactifluus clarkeae</i>	MN 2004002	L	KR364011	HQ318205	KR364268	KR364379
<i>Lactifluus coccolobae</i>	OKM-27240	CFMR	MK774805	–	–	–
<i>Lactifluus cocosmus</i>	ADK 4462	GENT	KR364013	KR364141	KR364269	KR364380
<i>Lactifluus conchatulus</i>	LTH 457	GENT	GU258296	GU265659	GU258399	KR364381
<i>Lactifluus corrugis</i>	AV 05-392 SAA12 L2	GENT GENT	JQ753822 KR364088	KR364143 KR364218	JQ348127 KR364361	– KR364491
<i>Lactifluus crocatus</i>	KVP 08-034	GENT	HQ318243	HQ318151	HQ328888	JN389145
<i>Lactifluus cyanovirescens</i>	JD 988	GENT	KR364082	KR364211	KR364270	KR364382
<i>Lactifluus denigricans</i>	EDC 11-218	GENT	KR364051	KR364178	KR364272	KR364384
<i>Lactifluus densifolius</i>	AV 11-111	GENT	KR364057	KR364184	KR364273	KR364385
<i>Lactifluus dissitus</i>	AV-KD-KVP 09-134	GENT	JN388978	JN389026	JN375628	JN389172
<i>Lactifluus distantifolius</i>	LTH 288	GENT	HQ318274	HQ318193	KR364274	JN389155
<i>Lactifluus domingensis</i>	ANGE1035 ANGE542 ANGE837 ANGE838	JBSD JBSD JBSD JBSD	MK931340 MK931339 MK931342 MK931341	MN128988 MN128987 MN128989 MN128990	MK937131 MK937130 MK937133 MK937132	– – MN128629 –
<i>Lactifluus dwaliensis</i> Asia	LTH 55	GENT	KF220111	KF220204	KF220278	KR364386
<i>Lactifluus edulis</i>	FN 05-628	GENT	KR364020	KR364147	KR364275	KR364387
<i>Lactifluus flammans</i>	JD 941	MEISE	KR364078	KR364207	KR364303	KR364431
<i>Lactifluus flocktonae</i>	JET1006	MEL	JX266621	JX266637	–	–
<i>Lactifluus foetens</i>	ADK 2840	MEISE	KR364023	KR364150	KR364279	KR364391
<i>Lactifluus fuscomarginatus</i>	LM4719 LM 4379	XAL XAL	HQ168368 HQ168367	– HQ168367	– –	– –
<i>Lactifluus genevievae</i>	GG-DK 17-02-05	GENT	GU258294	GU265657	GU258397	KR364401
<i>Lactifluus gerardii</i>	AV 05-375 JN 2007-029	GENT GENT	GU258254 GU258224	GU265616 GU265582	GU258353 GU258318	KR364403 –
<i>Lactifluus glaucescens</i>	LGAM 2010-0132	LGAM-AUA	KR364105	KR364236	KR364280	KR364407
<i>Lactifluus guadeloupensis</i>	RC/Guad11-023	LIP	KP691412	KP691421	KP691430	–
<i>Lactifluus guanensis</i>	GUA-104	CFMR	MK046851	–	–	–
<i>Lactifluus gymnocarpus</i>	EDC 12-047	GENT	KR364065	KR364194	KR364282	KR364408
<i>Lactifluus hallingii</i>	R. E. Halling 4977 R. E. Halling 7993 NVE 520 R. E. Halling 7938 FH 18-077	NY NY NY NY GENT	MK931343 MK931333 KF937338 MK931327 MK931338	– – – MN128984 MN128991	– MK937128 – MK937127 MK937129	– – – – MN128628
<i>Lactifluus hygrophoroides</i>	AV 05-251	GENT	HQ318285	HQ318208	HQ328936	KR364413

Table 1 (cont.)

Species	Herbariumnumber	Herbarium	GenBank accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
<i>Lactifluus igniculus</i>	LE 262983	LE	JX442759	JX442759	–	–
<i>Lactifluus kigomaensis</i>	AV 11-006	GENT	KR364052	KR364179	KR364288	KR364415
<i>Lactifluus kivuensis</i>	JR Z 310	GENT	KR364027	KR364154	–	–
<i>Lactifluus lamprocystidiatus</i>	EH 72-195	GENT	KR364015	–	–	–
<i>Lactifluus leae</i>	FH 12-013	GENT	KF432957	KR364213	KR364292	KR364419
<i>Lactifluus leonardii</i>	GG 07-02-04		GU258308	GU265668	GU258408	KR364495
<i>Lactifluus leoninus</i>	DS 07-454	GENT	KF220055	JN388989	JN375592	JN389188
<i>Lactifluus leptomerus</i>	AV-KD-KVP 09-131	GENT	JN388972	JN389023	JN375625	JN389169
<i>Lactifluus lepus</i>	RC/Guad 08-042	LIP	KP691414	KP691423	KP752179	–
	RC/Guad 05-029	LIP	MK046811	MK046774	–	–
<i>Lactifluus leucophaeus</i>	LTH 182	GENT	KF220059	KF220157	KF220243	KR364420
<i>Lactifluus longipilus</i>	LTH 184	GENT	HQ318256	HQ318169	HQ328905	JN389152
<i>Lactifluus luteolus</i>	AV 05-253	GENT	KR364016	KR364142	KJ210067	KR364440
<i>Lactifluus luteopus</i>	EDC 11-087	GENT	KR364049	KR364176	KR364312	KR364441
<i>Lactifluus madagascariensis</i>	BB 99-409	PC	AY606977	DQ421975	DQ421914	–
<i>Lactifluus marmoratus</i>	LD 15-066	GENT	MK046805	MK046768	MK063907	MK089574
<i>Lactifluus melleus</i>	AV 11-183	GENT	KR364060	KR364189	KR364277	KR364389
<i>Lactifluus multiceps</i>	TH 9154A	BRG, DUKE	JN168731	–	–	–
<i>Lactifluus murinipes</i>	LD 15-018	GENT	MK046814	–	–	–
	LD 15-057	GENT	MK046815	–	–	–
	LD 15-054	GENT	MK046828	MK046778	MK063911	MK089578
	LD 15-015	GENT	MK046832	MK046782	MK063915	MK089580
	LD 15-020	GENT	MK046836	MK046785	MK063918	MK089581
	LD 15-032	GENT	MK046838	MK046787	MK063920	MK089579
	CL/MART06.019	LIP	KP691417	KP691426	–	–
	F.1890	LIP	KP691418	–	–	–
	J.P. Fiard 849A	K	MK046855	–	–	–
<i>Lactifluus nebulosus</i>	LD 15-059	GENT	MK046804	MK046766	MK063905	MK089572
	LD 15-061	GENT	MK046833	MK046783	MK063916	MK089573
	PAM-Mart12-90	LIP	KP691415	KP691424	KP691432	KR364396
	PAM/Mart 05-091	LIP	MK046850	MK046799	–	–
	J.P. Fiard 828A	K	MK046856	–	–	–
<i>Lactifluus neotropicus</i>	R.W.G. Dennis 97	K	MN102680	–	–	–
<i>Lactifluus nonpiscis</i>	AV 11-137	GENT	KR364058	KR364185	KR364317	KR364445
<i>Lactifluus ochrogalactus</i>	AV/KD/KVP 09-120	GENT	KR364130	KR364248	KR364318	KR364446
<i>Lactifluus oedematopus</i>	KVP 12-001	GENT	KR364100	KR364232	KR364319	KR364447
<i>Lactifluus pallidilamellatus</i>	Montoya 4716	GENT	JQ753824	JQ348268	–	MN128632
	AV 17-012	GENT	MN102682	–	–	–
	AV 17-015	GENT	MN102684	–	–	–
<i>Lactifluus panuoides</i>	D.N. Pegler 3133	K	MK046858	–	–	–
	RC/Guy10_024	LIP	KJ786647	KJ786551	KP691428	–
	RC/Mart 03-077	LIP	MK046807	MK046770	MK063908	MN128627
	CL/GUAD06.045	LIP	MK046843	–	–	–
	R.W.G. Dennis 109A	K	MK046859	–	–	–
<i>Lactifluus pegleri</i>	LD 15-042	GENT	MK046831	MK046781	MK063914	MK089582
	LD 15-014	GENT	MK046835	MK046784	MK063917	MK089583
	LD 15-049	GENT	MK046837	MK046786	MK063919	–
	PAM-Mart12-91	LIP	KP691416	KP691425	KP691433	KR364397
	PAM/Mart 05-088	LIP	MK046808	MK046771	–	–
	PAM-Mart12-54	LIP	MK046809	MK046772	–	–
	J.P. Fiard 821B	K	MK046860	–	–	–
<i>Lactifluus pelliculatus</i>	JD 956	MEISE	KR364080	KR364209	KR364321	KR364449
<i>Lactifluus persicinus</i>	EDC 12-001	GENT	KR364061	KR364190	KR364298	KR364426
<i>Lactifluus petersenii</i>	AV 05-300	GENT	GU258281	GU265642	GU258382	KR364450
<i>Lactifluus phlebonemus</i>	EDC 12-023	GENT	KR364062	KR364191	KR364322	KR364451
<i>Lactifluus phlebophyllus</i>	BB 00-1388	PC	AY606974	DQ421979	DQ421918	–
<i>Lactifluus pilosus</i>	LTH 205	GENT	KR364006	KR364134	KR364323	KR364452
<i>Lactifluus pinguis</i>	AV-RW 04-023=LTH117	GENT	HQ318211	HG318111	HQ328858	JN389126
<i>Lactifluus piperatus</i>	2001 08 19 68	GENT	KF220119	KF241840	KF241842	KR364453
<i>Lactifluus pulchellus</i>	KW 304/FH 12-037	GENT	KR364092	KR364223	KR364306	KR364434
<i>Lactifluus pumilus</i>	EDC 12-066	GENT	KR364067	KR364196	KR364332	KR364462
<i>Lactifluus putidus</i>	LD 15-006	GENT	MK046818	–	–	–
	LD 15-017	GENT	MK046819	–	–	–
	LD 15-002	GENT	MK046820	–	–	–
	LD 15-040	GENT	MK046821	–	–	–
	LD 15-041	GENT	MK046822	–	–	–
	LD 15-065	GENT	MK046823	–	–	–
	LD 15-011	GENT	MK046824	–	–	–
	LD 15-033	GENT	MK046825	–	–	–
	LD 15-034	GENT	MK046826	MK046777	MK063910	MK089575
	LD 15-062	GENT	MK046827	–	–	–
	LD 15-004	GENT	MK046829	MK046779	MK063912	MK089577
	LD 15-039	GENT	MK046830	MK046780	MK063913	MK089576
	LD 15-030	GENT	MK046834	–	–	–
	PAM/Mart 11-013	LIP	KP691413	KP691422	KP691431	–
	PAM-Mart12-88	LIP	MK046806	MK046769	–	–
	RC/Mart 05-110	LIP	MK046810	MK046773	–	–

Table 1 (cont.)

Species	Herbariumnumber	Herbarium	GenBank accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
<i>Lactifluus putidus</i> (cont.)	RC/Guad 06-005	LIP	MK046813	MK046776	–	–
	PAM/Mart 05-030	LIP	MK046840	MK046790	–	–
	PAM-Mart12-103	LIP	MK046845	MK046795	–	–
	RC/Mart 03-001	LIP	MK046849	MK046798	–	–
	PAM/Mart 05-085	LIP	MK046839	MK046788	–	–
	RC/Mart10 011	LIP	MK046812	MK046775	MK063909	–
<i>Lactifluus ramipilosus</i>	EDC 14-503	MFLU, GENT	KR364128	–	–	–
<i>Lactifluus reticulatovenosus</i>	EH 6472	GENT	GU258286	GU265649	GU258389	–
<i>Lactifluus robustus</i>	JN 2011-074	GENT	KR364047	KR364173	KR364358	KR364488
<i>Lactifluus roseolus</i>	AV 99-160	GENT	KR364032	KR364159	KR364333	KR364463
<i>Lactifluus roseophyllus</i>	JN 2011-076	GENT	KF220107	KF220202	KF220276	KR364464
<i>Lactifluus rubiginosus</i>	JD 959	MEISE	KR364081	KR364210	KR364304	KR364432
<i>Lactifluus rubrobrunnescens</i>	EH 7194	GENT	KR364115	–	–	–
<i>Lactifluus rubroviolascens</i>	EDC 12-051	GENT	KR364066	KR364195	KR364334	KR364465
<i>Lactifluus rufomarginatus</i>	ADK 3358	MEISE	KR364033	KR364160	KR364335	KR364466
<i>Lactifluus rugatus</i>	EP 1212/7	LGAM-AUA	KR364104	KR364235	KR364337	KR364467
<i>Lactifluus russulisporus</i>	REH 9398	NY	KR364097	KR364229	KR364307	KR364435
<i>Lactifluus ruvubuensis</i>	JD 303	MEISE	KR364009	KR364137	KR364310	KR364438
<i>Lactifluus sainii</i>	PUN 7046	PUN	KM658971	–	–	–
<i>Lactifluus sepiaceus</i>	PL 40509		GU258287	GU265650	GU258390	KR364475
	PL 34204		GU258288	GU265651	GU258391	–
<i>Lactifluus</i> sp.	AV 05-325	GENT	MK931329	MN128985	–	–
	TH7460	BRG, DUKE	KT339233	KT339233	–	–
	TH7880	BRG, DUKE	KT339212	KT339212	–	–
	F1068593	F	MN102703	–	–	–
	MAN_MAN919	FLOR	KY936896	–	–	–
	AV 04-195	GENT	KF220045	KF220146	KF220232	KR364404
	AV 05-374	GENT	KF220049	KF220150	KF220236	KR364405
	AV 07-056	GENT	KR364008	KR364136	KR364293	KR364421
	AV 99-012	GENT	KR364021	KR364148	KR364276	KR364388
	AV 05-249	GENT	MK931325	–	MK937125	–
	AV-KD-KVP 09-121	GENT	JN388979	JN389014	JN375616	JN389160
	DS 06-003	GENT	GU258231	GU265588	GU258325	JN389185
	AV 04-181	GENT	MK931328	–	DQ421935	–
	EDC 12-068	GENT	KR364068	KR364197	KR364299	KR364427
	EDC 12-195	GENT	KR364071	KR364200	KR364301	KR364429
	JD 907	GENT	KR364076	KR364205	KR364302	KR364430
	JN 2011-014	GENT	KF220104	KF220199	KF220273	KR364406
	JN 2011-036	GENT	KF220105	KF220200	KF220274	KR364454
	JN 2011-071	GENT	KR364043	KR364169	KR364255	KR364367
	JN 2011-072	GENT	KF220106	KF220201	KF220275	KR364455
	JN 2011-077	GENT	KR364044	KR364170	KR364256	KR364368
	AV 05-283	GENT	GU258259	GU265621	GU258358	–
	KVP 08-011	GENT	HQ318232	HQ318139	HQ328876	JN389135
	JN 2011-012	GENT	KR364045	KR364171	KR364294	KR364422
	KVP 08-031	GENT	HQ318240	HQ318148	HQ328885	JN389142
	AV 05-330	GENT	JQ753828	HQ318129	JQ348140	–
	AV 05-275	GENT	MK931336	–	–	–
	LTH 270	GENT	EF560685	GU265598	GU258335	KR364402
	LTH 274	GENT	KR364107	KR364238	KR364325	KR364457
	AV 05-227	GENT	JQ753832	JQ348284	JQ348150	–
	AV 05-293	GENT	JQ753834	JQ348287	JQ348153	–
	AV 17-011	GENT	MN102681	–	–	–
	AV 17-014	GENT	MN102683	–	–	–
	AV 17-016	GENT	MN102685	–	–	–
	FH 18-054	GENT	MN102686	MN101703	MN120442	MN120459
	FH 18-060	GENT	MN102687	MN101704	MN120454	MN120471
	FH 18-061	GENT	MN102688	MN101705	MN120456	MN120467
	FH 18-062	GENT	MN102689	MN101706	MN120450	MN120472
	FH 18-063	GENT	MN102690	MN101707	MN120458	MN120469
	FH 18-067	GENT	MN102691	MN101708	MN120443	MN120460
	FH 18-068	GENT	MN102692	MN101709	MN120447	MN120464
	FH 18-069	GENT	MN102693	MN101710	MN120452	MN120474
	FH 18-124	GENT	MN102694	MN101711	MN120451	MN120473
	FH 18-128	GENT	MN102695	MN101712	MN120457	MN120468
	FH 18-129	GENT	MN102696	MN101713	MN120455	MN120470
	FH 18-131	GENT	MN102697	MN101714	MN120445	MN120461
	FH 18-132	GENT	MN102698	MN101715	MN120448	MN120465
	FH 18-133	GENT	MN102699	MN101716	MN120446	MN120462
	FH 18-134	GENT	MN102700	MN101717	MN120449	MN120466
	FH 18-135	GENT	MN102701	MN101718	MN120444	MN120463
	FH 18-141	GENT	MN102702	MN101719	MN120453	MN120475
	MCA 3937	GENT	KR364109	KR364240	KR364350	–
	RC/Guy 09-004bis	LIP	KJ786643	KP691419	KP691427	–
	RC/Guy 09-036	LIP	KJ786645	KJ786550	KP752178	–
	RC/Guy 12-007	LIP	KJ786668	MK046789	–	–
	Halling 8262	NY	GU258235	GU265593	GU258330	–
	MR/Guy 13-145	PC	KJ786691	KJ786595	MK063906	–
G3185	PC	KJ786694	KJ786603	KP691434	KR364399	
MR/Guy 13-032	PC	KJ786686	KJ786581	–	–	
G3264	PC	KJ786706	KJ786620	KP691435	KR364400	
MR-GUY-14-195	PC	KM073084	–	–	–	

Table 1 (cont.)

Species	Herbariumnumber	Herbarium	GenBank accession numbers				
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>	
<i>Lactifluus</i> sp. (cont.)	G4727	PC	KM073087	–	–	–	
	G4836	PC	KM073083	–	–	–	
	G4797		KM073085	–	–	–	
	JLC 060310-01		KJ786714	–	–	–	
	TENN 064342	TENN	KR364103	KR364234	KR364324	KR364456	
	TENN 065854	TENN	KR364101	MN128986	KR364271	KR364383	
	TENN 065929	TENN	KR364102	KR364233	KR364308	KR364436	
	LM4640	XAL	HQ168369	–	–	–	
	LM-UNAH 0072	XAL	HM639277	–	–	–	
	LM-UNAH 0073	XAL	HM639278	–	–	–	
	PGK13-130		KP691436	KR605507	–	–	
	ASM 12,075		MN102679	–	–	–	
	<i>Lactifluus subclarkeae</i>	REH 9231	NY	KR364095	KR364227	KR364346	KR364477
	<i>Lactifluus subgerardii</i>	AV 05-269	GENT	GU258263	GU265625	GU258362	KR364478
<i>Lactifluus subiculatus</i>	SLM 10114	BRG, RMS	JQ405654	–	–	–	
<i>Lactifluus subkigomaensis</i>	EDC 11-159	GENT	KR364050	KR364177	KR364295	KR364423	
<i>Lactifluus subpruinus</i>	JN 2011-061	GENT	KR364046	KR364172	KR364357	KR364487	
<i>Lactifluus subvellereus</i>	AV 05-210	GENT	KR364010	KR364138	KR364347	KR364479	
<i>Lactifluus subvolemus</i>	KVP 08-048	GENT	JQ753927	–	KR364356	KR364486	
<i>Lactifluus sudanicus</i>	AV 11-174	GENT	HG426469	KR364186	KR364348	KR364480	
<i>Lactifluus tanzanicus</i>	AV 11-017	GENT	KR364053	KR364180	KR364296	KR364424	
<i>Lactifluus tenuicystidiatus</i>	JN 2011-080	GENT	KR364048	KR364174	KR364359	KR364489	
<i>Lactifluus uapacae</i>	AV 07-048	GENT	KR364007	KR364135	KR364352	KR364483	
<i>Lactifluus urens</i>	EDC 14-032	GENT	KR364124	KR364247	KR364353	–	
<i>Lactifluus vellereus</i>	ATHU-M 8077	ATHU-M	KR364106	KR364237	KR364354	KR364484	
<i>Lactifluus velutissimus</i>	JD 886	MEISE	KR364075	KR364204	KR364355	KR364485	
<i>Lactifluus venezuelanus</i>	RC/Mart 03-120	LIP	MK046842	MK046792	–	–	
	RC/Guad 03-048	LIP	MK046847	MK046797	–	–	
	RC/Guad11-017	LIP	KP691411	KP691420	KP691429	–	
	PAM/Guad 10-036	LIP	MK046844	MK046794	MK063922	MN128630	
	PAM/Guad 10-037	LIP	MK046846	MK046796	MK063923	MN128631	
	F.1980		MK046848	–	–	–	
<i>Lactifluus veraecrucis</i>	M 8025	ENCB	KR364112	KR364241	–	–	
<i>Lactifluus versiformis</i>	AV-KD-KVP 09-045	GENT	JN388967	JN389031	JN375632	JN389177	
<i>Lactifluus vitellinus</i>	KVP 08-024	GENT	HQ318236	HQ318144	HQ328881	JN389138	
<i>Lactifluus volemoides</i>	TS 0705	GENT	KR364038	KR364165	–	–	
<i>Lactifluus volemus</i>	KVP 11-002	GENT	JQ753948	KR364175	KR364360	KR364490	
	REH 9320	NY	KR364096	KR364228	KR364362	KR364492	
<i>Lactifluus wirrabara</i>	GG 24-01-04		GU258307	GU265667	GU258407	KR364494	
<i>Lactifluus xerampelinus</i>	MH 201176	GENT	KR364099	KR364231	KR364364	KR364496	

- a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000);
- 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 et al. 1999, Matheny 2005); and
- 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 (Stiller & Hall 1997, Matheny et al. 2002).

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 with Sequencher v. 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA) or BioLMICS (BioAware SA NV). The dataset was supplemented with closely related sequences from GenBank and worldwide reference sequences from De Crop et al. (2017) (Table 1). Sequences were aligned online in the multiple sequence alignment program MAFFT v. 7 (Katoh & Toh 2008), using the E-INS-I strategy. Trailing ends were trimmed and the alignment was manually edited where needed in Mega 6 (Tamura et al. 2013). The alignment is deposited in TreeBASE (Submission ID 24693). First, the ITS+LSU alignment was partitioned into partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Both *RPB1* and *RPB2* alignments were partitioned into the intron(s) and the first, second and third codon positions of

the exon. PartitionFinder was used to find the appropriate partitioning scheme (Lanfear et al. 2017). Maximum likelihood (ML) analyses, using RAXML v. 8.0.24 (Stamatakis 2014), were combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis et al. 2008). There was no supported conflict between the separate gene trees, so they were concatenated. ML analyses were conducted on the CIPRES Science Gateway (Miller et al. 2010).

Distribution of closest relative(s)

To investigate whether species occurring in Central America and the Caribbean are more closely related to North or to South American species, the following steps were undertaken. For each largest possible clade consisting only of species occurring in Central America and/or the Caribbean (i.e., a total of 22 clades), the closest relative(s) was/were determined by considering the closest node with a bootstrap support value of at least 70. If there was only one most closely related clade, or if those most closely related clades originated from the same continent, a value of one was added towards the count for that continent. If the closest relatives of a clade were originating from multiple continents, a value of one was added for each of these continents. For each Central American or Caribbean clade, hosts and altitude were noted when possible, and counts of the closest relatives of different plant families and altitudes were performed in the same way. These counts were visualised in a bar plot using R (R Core Team 2018).

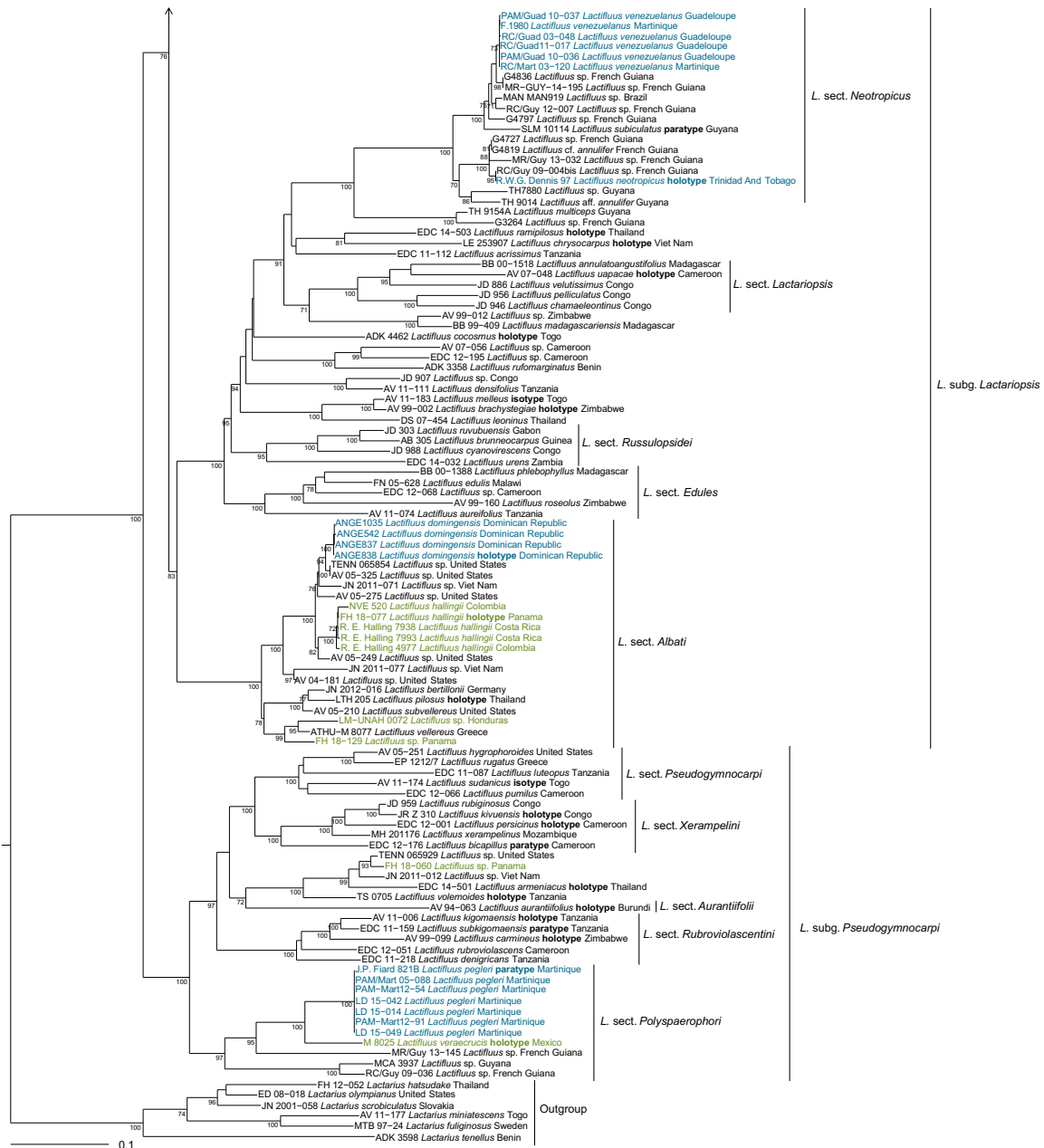


Fig. 1 (cont.)

RESULTS

Taxonomy

Lactifluus subg. *Lactifluus*

Lactifluus sect. *Lactifluus*

Lactifluus pallidilamellatus (Montoya & Bandala) Van de Putte, Mycotaxon 120: 444. 2012

≡ *Lactarius pallidilamellatus* Montoya & Bandala, Cryptog. Mycol. 25 (1): 16. 2004.

Ecology — Found in mesophytic (subtropical) forest, with *Carpinus caroliniana*.

Distribution — Known from Veracruz, Mexico.

Notes — *Lactifluus pallidilamellatus* is characterised by its slender habit, orange pileus and stipe, crowded, cream to

yellowish lamellae, brown discolouring context, copious white latex, prominent lamprocystidia, basidiospores measuring $(6.4\text{--}7.2\text{--}8.8 \times (5.6\text{--}6.4\text{--}7.5\text{--}8)) \mu\text{m}$, with a heavy reticulum and relatively short terminal elements in the pileipellis, measuring $16\text{--}40.8 \times 2.4\text{--}12.8 \mu\text{m}$ (Montoya & Bandala 2004). The most closely related described species is *L. oedematopus* from Europe (Fig. 1), which is morphologically very similar, and has also been reported from forests with *Carpinus* (Van de Putte et al. 2016), but their different distribution easily distinguishes them from each other.

No other species in *L. sect. Lactifluus* have been described from Central America, nor the Caribbean or South America. However, our phylogeny reveals four undescribed species from this area in this section, three from Panama and one from Mexico (Fig. 1). Since this section represents a difficult species complex (Van de Putte 2012), in which morphological differences are often very subtle, a detailed study on this complex will be needed to further unravel these species.

Lactifluus* sect. *Gerardii (A.H. Sm. & Hesler) Stubbe, Persoonia 38: 76. 2016

Lactifluus fuscomarginatus (Montoya, Bandala & I. Haug) Delgat, *comb. nov.* — MycoBank MB831635

≡ *Lactarius fuscomarginatus* Montoya, Bandala & I. Haug, Mycologia 104 (1): 176. 2012.

Ecology — Found in *Fagus grandifolia* var. *mexicana* forest.
Distribution — Known from Veracruz, Mexico.

Notes — *Lactifluus fuscomarginatus* is characterised by the dark pileus and stipe, whitish lamellae with blackish brown lamellae edges, pigmented, projecting and subcylindrical cheilo-leptocystidia and basidiospores measuring $(8-9-10-10.2-11 \times (7-8-9-9.2-10.5(-11) \mu\text{m})$ ($Q = 1.08-1.14$), with reticulate ornamentation, with rounded to somewhat sharp ridges up to $0.5 \mu\text{m}$ high. *Lactifluus fuscomarginatus* is situated in a clade together with three undescribed species from Mexico and Central America, and the closest described relatives to this clade are *L. reticulatovenosus* from Indonesia and *L. subgerardii* from North America (Fig. 1). *Lactifluus fuscomarginatus* thus belongs to *L.* sect. *Gerardii* (Fig. 1), and not to *Lactarius* subg. *Plinthogalus*, and is here combined in *Lactifluus*.

Lactifluus* subg. *Gymnocarpi (R. Heim ex Verbeken) De Crop, Persoonia 38: 75. 2016

Lactifluus* sect. *Nebulosi Delgat, *sect. nov.* — MycoBank MB828339

Typus. *Lactarius nebulosus* Pegler, Kew Bull. 33: 610. 1979 (≡ *Lactifluus nebulosus* (Pegler) De Crop).

Pileus medium to large sized, firm, convex with central depression to infundibuliform, with inflexed margin when young; surface slightly velutinous, often slightly wrinkled, sometimes smooth, irregular or strongly wrinkled, white-grey with brown spots to brown. *Stipe* central, cylindrical to tapering downwards, firm to stuffed, whitish, staining brown. *Lamellae* subdecurrent to deeply decurrent, often rather distant, white to cream; edge concolourous. *Context* white, in most cases turning brown. *Odour* in most cases unpleasant, fishy. *Latex* watery white, in most cases staining brown and taste mild, in the case of *L. murinipes* unchanging and acid.

Basidiospores subglobose to ellipsoid; ornamentation composed of isolated, rounded warts up to $1 \mu\text{m}$ high. True *pleuro-macrocytidia* present in most species, up to $140-185 \mu\text{m}$ long, cylindrical to subfusiform with rounded, mucronate, rostrate or slightly moniliform apex. *Pileipellis* a trichoderm to palisade; terminal elements in most cases thin-walled.

Notes — *Lactifluus* sect. *Nebulosi* corresponds to clade 9 in De Crop et al. (2017) and belongs in *L.* subg. *Gymnocarpi*. In contrast to the other described sections in this subgenus that completely lack true pleurocytidia, most species of *L.* sect. *Nebulosi* have conspicuous pleuromacrocytidia. The section contains only Neotropical collections and is characterised by dull fruiting body colours (a brown-grey pileus and a white-grey



Fig. 2 Basidiocarps. a. *Lactifluus putidus*, LD 15-002 (*L. Delgat*); b. *Lactifluus nebulosus*, LD 15-061, inset LD 15-059 (*L. Delgat*); c. *Lactifluus guadeloupensis*, RC/Guad11-023 (*R. Courtecuisse*); d. *Lactifluus marmoratus*, LD 15-066 (*L. Delgat*); e. *Lactifluus murinipes*, LD 15-020 (*L. Delgat*); f. *Lactifluus lepus*, RC/Guad 05-029 (*R. Courtecuisse*); g. *Lactifluus panuoides*, RC/Mart 03-077 (*R. Courtecuisse*); h. *Lactifluus venezuelanus*, PAM/Guad 10-036 (*P.-A. Moreau*); i. *Lactifluus pegleri*, LD 15-014 (*L. Delgat*).

stipe), an unpleasant fishy odour in all species except *L. murinipes*, and broadly ellipsoid spores with isolated, rounded warts up to 1 µm high.

Lactifluus putidus (Pegler) Verbeken, Mycotaxon 120: 446, 2012 — Fig. 2a, 3

≡ *Lactarius putidus* Pegler, Kew Bull. 33 (4): 620. 1979.

Pileus 14–66 mm diam, plano-convex with central depression and inflexed margin when young, infundibuliform with straight to deflexed margin when adult; surface somewhat to very wrinkled, brown (6E3–4, 6F3–5) with yellowish light brown areas, often in the centre (4A3–5). **Stipe** 8–30 × 4–14 mm, cylindrical, white to yellowish white (4A1–4), with brown stains (5D5, 6E3–5). **Lamellae** decurrent, medium spaced to distant, sometimes slightly intervenose, white to cream (4A2–3), staining brown (6E5, 6F4–7); edge entire and concolourous. **Context** white, firm, turning dark brown, often first turning violaceous brown, dark green blue with guaiac, greyish blue-green with FeSO₄. **Odour** very unpleasant, like rotten fish or urine. **Taste** mild. **Latex** very abundant, watery white, staining brown, mild.

Basidiospores broadly ellipsoid to ellipsoid, 6.5–7.7–9.3–10.6 × 5.3–6.2–6.9–7.7 µm (Q = 1.04–1.23–1.36–1.53); ornamentation amyloid, composed of isolated rounded warts up to 1 µm high, rarely connected by fine connective lines; plage inamyloid. **Basidia** 51–67.5–84.5(–88) × 9–11–13.5 µm, subclavate, 4-spored. **Pleuromacrocystidia** 72.5–121.5–170.5 × 5.5–8–10.5 µm, cylindrical to subfusiform with rounded, mucronate or tapering apex, rarely branching, thin-walled. **Pleuropseudocystidia** scarce, 4 µm diam, not or slightly emergent. **Lamellar edge** fertile. **Hymenophoral trama** mixed, with hyphae,

lactifers and sphaerocytes. **Pileipellis** a palisade; elements of the suprapellis 29–41.5–53.5(–55) × 2.5–3.5–4 µm, cylindrical to capitate up to 10.5 µm; subpellis composed of isodiametric cells. **Stipitipellis** resembling pileipellis.

Ecology — Found in xerophytic and mesophytic forests, with *Bursera simaruba*, *Coccoloba diversifolia*, *Coccoloba pubescens*, *Coccoloba swartzii*, *Guapira fragrans*, *Inga laurina*, *Lonchocarpus roseus* and *Pisonia subcordata*.

Distribution — Known from the islands of Martinique and Guadeloupe.

Specimens examined. MARTINIQUE, Réserve naturelle La Caravelle, Commune de Trinité, Bois de Pointe rouge, soil with *Guapira fragrans*, *Coccoloba pubescens*, 03 Nov. 2015, Lynn Delgat, LD 15-004 (GENT); *ibid.*, soil with *Guapira fragrans*, *Coccoloba pubescens*, *Coccoloba swartzii*, 03 Nov. 2015, Lynn Delgat, LD 15-011 (GENT); Réserve naturelle La Caravelle, Trinité, Tartane, vicinity of ruins of Chateau Dubuc (14.769397°, -60.889991°, alt. 30.7 m), soil with *Guapira fragrans*, *Coccoloba swartzii*, 08 Nov. 2015, Lynn Delgat, LD 15-030 (GENT); Réserve naturelle La Caravelle, Commune de Trinité, Bois de Pointe rouge (14.757943°, -60.932939°, alt. 47.8 m), soil with *Coccoloba pubescens*, *Guapira fragrans*, 10 Nov. 2015, Lynn Delgat, LD 15-039 (GENT); Commune du Prêcheur, Anse Lévrier (14.844459°, -61.218130°, alt. 64.1 m), soil with *Guapira fragrans*, 14 Nov. 2015, Lynn Delgat, LD 15-062 (GENT); *ibid.*, (14.844562°, -61.217664°, alt. 78.3 m), soil with *Guapira fragrans*, 14 Nov. 2015, Lynn Delgat, LD 15-065 (GENT).

Notes — *Lactifluus putidus* is a commonly encountered species and is probably the most frequent *Lactifluus* species in the area (numerous specimens from various collectors have been accumulated through the years and are mainly deposited in K and LIP). It can easily be recognised in the field by the strongly wrinkled cap in mature specimens, the strong, unpleasant odour and the very abundant latex. When the fruiting body is cut, the latex often first turns violaceous brown before turning dark brown. Microscopically, the presence of capitate terminal elements in the pileipellis and stipitipellis is a distinctive character, which was not observed in any of the other species from the Antilles. Molecularly, *L. putidus* is closely related to *L. coccolobae* (Fig. 1), but can be distinguished from it by the strongly wrinkled pileus, the capitate elements in the pileipellis and the absence of gelatinised hyphae in the pileipellis, in addition to *L. coccolobae* being associated with *Coccoloba uvifera* in dune habitats in the Greater Antilles.

Lactifluus coccolobae (O.K. Mill. & Lodge) Delgat, *comb. nov.* — MycoBank MB828348

≡ *Lactarius coccolobae* O.K. Mill. & Lodge, Mycologia 92 (3): 564. 2000.

Ecology — Found in sand on dunes under *Coccoloba uvifera*.

Distribution — Known from Puerto Rico and Guana Island, British Virgin Isles.

Notes — Based on the original description this species fits morphologically in *L.* sect. *Nebulosi*, notably because of the dull basidiocarp colours, the brown staining of the latex and context, the strong fishy odour and the spore ornamentation with isolated warts (Miller et al. 2000). The placement in this section is confirmed molecularly by the position of the type sequence in the phylogeny (Fig. 1). A similar species is *L. guanensis*, which also occurs in the Greater Antilles with *Coccoloba uvifera*, but *L. coccolobae* can be distinguished from this species by its narrower basidia (8–9.5 µm wide), slightly shorter spores (7.2–9(–10.8) µm long), lower spore ornamentation (up to 0.3 µm) and a gelatinised pileipellis (Miller et al. 2000). *Lactifluus coccolobae* is closely related to the Lesser Antillean *L. putidus* (Fig. 1), but can easily be distinguished from it by the absence of a strongly wrinkled pileus, the absence of capitate elements in the pileipellis and the presence of gelatinised hyphae in the pileipellis.

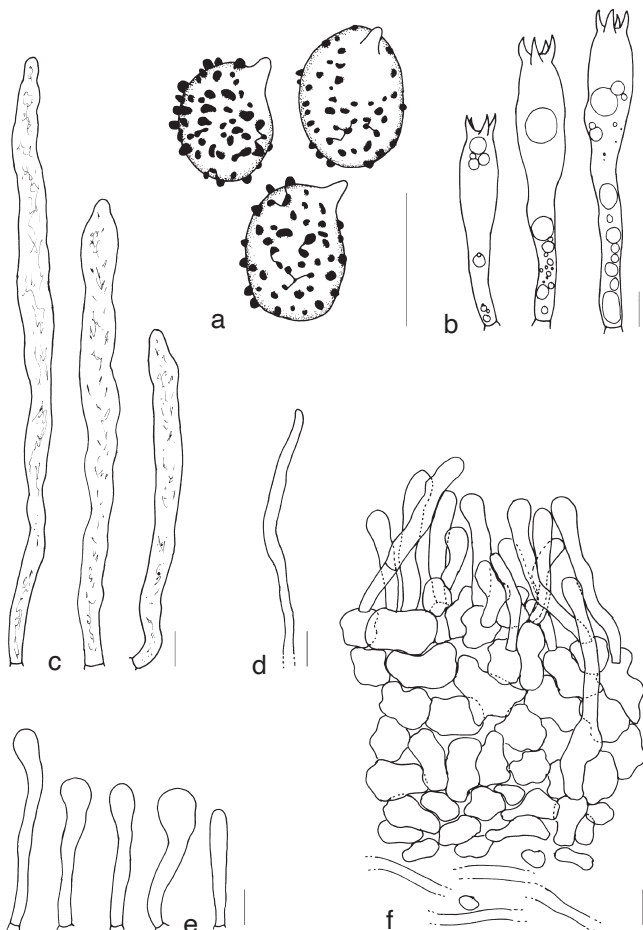


Fig. 3 *Lactifluus putidus* (LD 15-004, LD 15-011, LD 15-030, LD 15-065). a. Basidiospores; b. basidia; c. macrocystidia; d. pseudocystidium; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

Lactifluus guadeloupensis Delgat & Courtec., *sp. nov.* — MycoBank MB828343; Fig. 2c, 4

Diagnosis: Differs from *L. nebulosus* and *L. marmoratus* by the irregular pileus surface, the pruinose lamellae and the presence of many 2-spored basidia.

Typus. GUADELOUPE, Grande-Terre, Commune de Saint François, Baie Olive (16.249905°, -61.283412°, alt. 2.9 m), with *Guapira* and *Fabaceae*, 06 Aug. 2011, *R. Courtecuisse*, RC/Guad11-023 (LIP).

Etymology. Refers to the island where the species was found.

Pileus up to 40 mm diam, convex with slight central depression; young basidiocarps sometimes umbilicate; surface irregularly bumpy, finely pubescent to velvety, marmorate greyish brown mixed with yellowish to yellowish ochraceous, becoming dull rusty brown. *Stipe* 20 × 6–10 mm, short, slightly tapering downwards; surface pruinose, with rather variable colours, dirty cream when young to yellowish brown or marmorate when adult. *Lamellae* adnate to subdecurrent, distant, thick, more or less intervenose through irregular ridges or bumps, pruinose, yellowish cream, turning rusty brownish. *Context* cream, turning brown, slightly stuffed. *Odour* unpleasant, reminding of fish or rotting material, then becoming a more classical lactarioid odour while drying or reminding the odour of *L. volemus* (i.e., agreeable shellfish-like, Jerusalem artichokes). *Taste* not observed. *Latex* watery white, colour change and taste not observed. *Basidiospores* mostly broadly ellipsoid, some subglobose or ellipsoid; size very variable, 7.1–9.4–9.6–11.8(–11.9) × 5.8–7.5–7.9–9.4(–9.5) (Q = 1.08–1.23–1.26–1.41); ornamentation amyloid, composed of rounded warts, up to 1 µm high, isolated; plage inamyloid. *Basidia* (54–)56.5–75.5–94.5 × 8–10.5–13(–14) µm, cylindrical to subclavate, majority 4-spored but many 2-spored and some 1- and 3-spored, sometimes with

wall locally thickened. *Pleuromacrocyttidia* 53–104.5–155.5 × 6.5–8–10 µm, abundant, cylindrical with moniliform apex, thin-walled. *Pleuropseudocystidia* absent. *Lamellar edge* fertile. *Hymenophoral trama* mixed, with hyphae, lactifers and sphaerocytes. *Pileipellis* a palisade to trichopalisade; elements of the suprapellis 29–60–91.5 × 2.5–4.5–6 µm, cylindrical, some septate, some branching; subpellis composed of isodiametric cells and more elongated cells. *Stipitipellis* resembling pileipellis, terminal elements slightly shorter, 29.5–42.5–56 × 3–5–6.5(–7) µm.

Ecology — Found in xerophytic forests with *Guapira* and *Fabaceae*.

Distribution — Only known from type locality.

Notes — *Lactifluus guadeloupensis* is morphologically similar to *L. nebulosus* and *L. marmoratus*. However, it can be macroscopically distinguished from these two species by an irregular pileus surface and pruinose lamellae. Microscopically it can be distinguished from *L. nebulosus* by longer basidia, longer terminal elements in the pileipellis and potentially by the presence of many 2-spored and some 1- and 3-spored basidia. For microscopic differences with *L. marmoratus*, see the notes on that species. The relationships of *L. guadeloupensis* are not completely resolved, and it is closely related to *L. coccolobae*, *L. guanensis*, *L. marmoratus*, *L. nebulosus* and *L. putidus* (Fig. 1). However, *Lactifluus guadeloupensis* is recorded from Grande-Terre of Guadeloupe and it is the only hitherto known Lesser Antillean *Lactifluus* species found on calcareous soil of coral origin, in contrast to the volcanic origin of the localities of the other *Lactifluus* species.

Lactifluus marmoratus Delgat, *sp. nov.* — MycoBank MB828344; Fig. 2d, 5

Diagnosis. Differs from *L. guadeloupensis* and *L. nebulosus* by the slightly larger spores, the larger and especially wider basidia, the presence of branching pleuromacrocyttidia and the large isodiametric cells in the pileipellis and stipitipellis.

Typus. MARTINIQUE, Commune du Prêcheur, Anse Lévrier (14.845595°, -61.216511°, alt. 56 m), soil with *Guapira fragrans*, 14 Nov. 2015, *Lynn Delgat*, LD 15-066 (GENT).

Etymology. Refers to the marbled colour of the pileus.

Pileus 39 mm diam, convex with slight central depression and deflexed margin; surface chamois-leather like, grey (8E2), with some white, and greyish orange (5B4) and light brown (6D4) spots. *Stipe* 27 × 6 mm, cylindrical, somewhat tapering downwards; surface chamois-leather like, greyish brown (6E2) to light brownish grey (6C1, 6D2), marbled with light brown (6D5) spots. *Lamellae* decurrent, rather distant, with some bifurcations near the margin, white, staining brown; edge even and concolourous. *Context* white, slightly stuffed, unchanging, dark green blue with guaiac, slowly dark greenish grey with FeSO₄. *Odour* fishy, like fresh fish, shrimps or crayfish. *Taste* insignificant. *Latex* rather scarce, watery white, staining brown; taste mild.

Basidiospores broadly ellipsoid to ellipsoid, rarely subglobose, 8.9–10.5–12.1 × 7.3–8.3–9.3 µm (Q = (1.11–)1.13–1.26–1.40); ornamentation amyloid, composed of rounded warts, up to 1 µm high, mostly isolated, sometimes connected by fine connective lines; plage inamyloid. *Basidia* 68–85–102 × 12.5–14–16 µm, subclavate, 4-spored. *Pleuromacrocyttidia* 84–134–184 × 7–9–11 µm, medium abundant, cylindrical with rounded, mucronate or slightly moniliform apex, some branching in two, thin-walled. *Pleuropseudocystidia* 4.5–10 µm diam, not emergent; content oleiferic. *Lamellar edge* fertile. *Hymenophoral trama* mixed, with hyphae, lactifers and sphaerocytes. *Pileipellis* a palisade; elements of the suprapellis 22–50–78 × 3–5–8 µm, cylindrical to lageniform, with brown intracellular

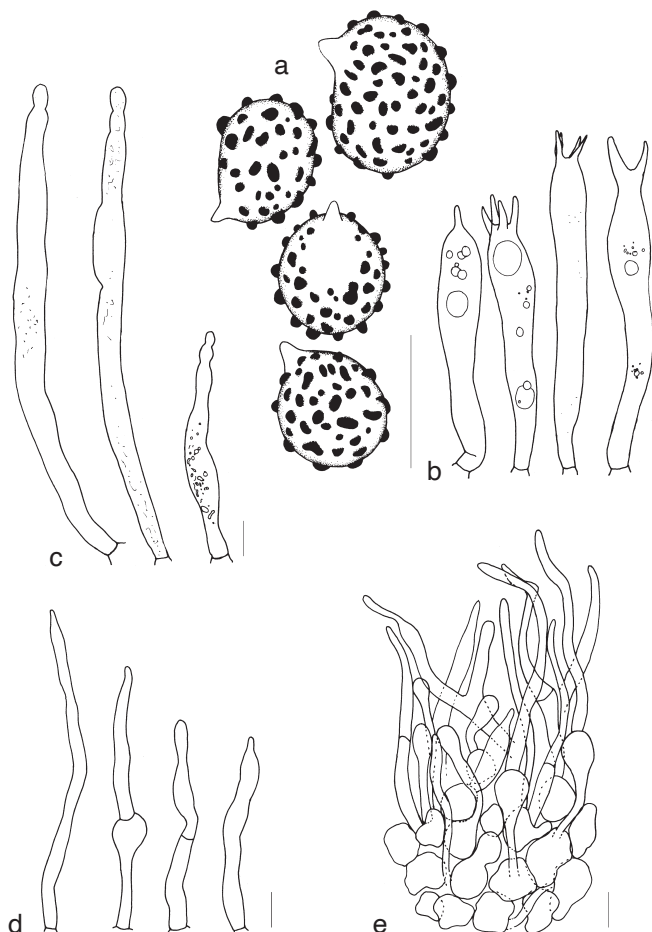


Fig. 4 *Lactifluus guadeloupensis* (RC/Guad11-023). a. Basidiospores; b. basidia; c. macrocyttidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 µm.

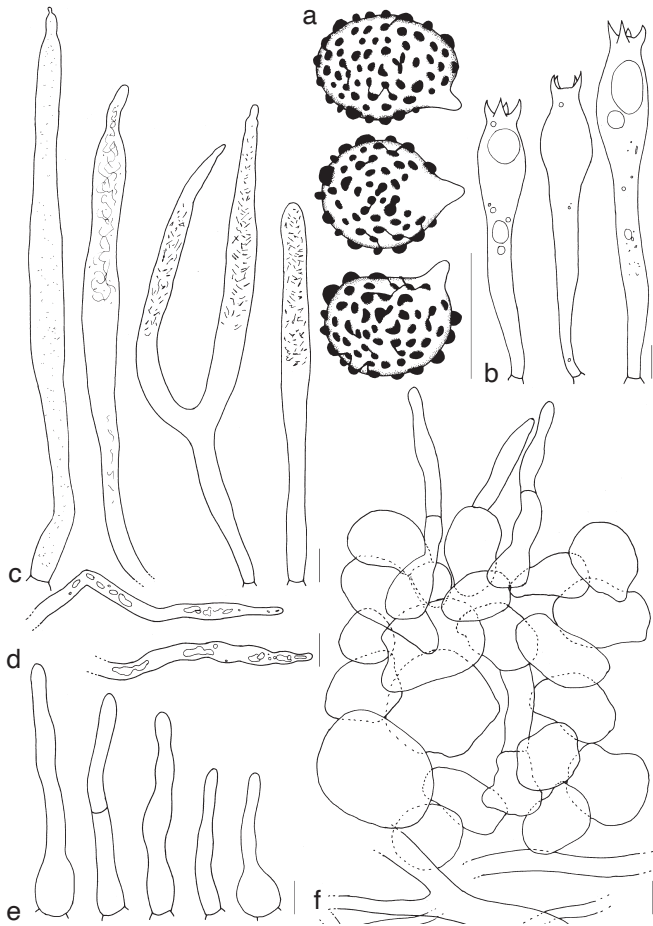


Fig. 5 *Lactifluus marmoratus* (LD 15-066). a. Basidiospores; b. basidia; c. macrocystidia; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 μ m.

pigmentation, often septate; subpellis composed of very large isodiametric cells, 15–45 μ m diam. *Stipitipellis* resembling pileipellis, but without brown intracellular pigment in the terminal cells.

Ecology — Found in mesophytic forests with *Guapira fragrans*.

Distribution — Only known from type locality.

Notes — *Lactifluus marmoratus* macroscopically resembles *L. guadeloupensis* and *L. nebulosus*. However, there are some subtle macroscopic differences: *L. nebulosus* differs by the lack of brown tinges in the pileus and the white stipe, and *L. guadeloupensis* differs by the irregular pileus surface and the pruinose lamellae. In contrast to the other species, the context of *L. marmoratus* does not change colour when cut, although this could be caused by the scarcity of the latex in the studied specimen. Microscopically, *L. marmoratus* differs from both species by the slightly larger spores, the larger and especially wider basidia, the presence of branching pleuromacrocystidia and the large isodiametric cells in the pileipellis and stipitipellis. Molecularly, it is not clear what the most closely related species is to *L. marmoratus*, but it is closely related to *L. coccolobae*, *L. guanensis*, *L. nebulosus* and *L. putidus* (Fig. 1).

Lactifluus nebulosus (Pegler) De Crop, Persoonia 38: 76. 2016 — Fig. 2b, 6

≡ *Lactarius nebulosus* Pegler, Kew Bull. 33 (4): 610. 1979.

Pileus 7–83 mm diam, hemispherical with depressed centre and inflexed margin when young, infundibuliform with straight to deflexed margin when adult; surface chamois-leather like,

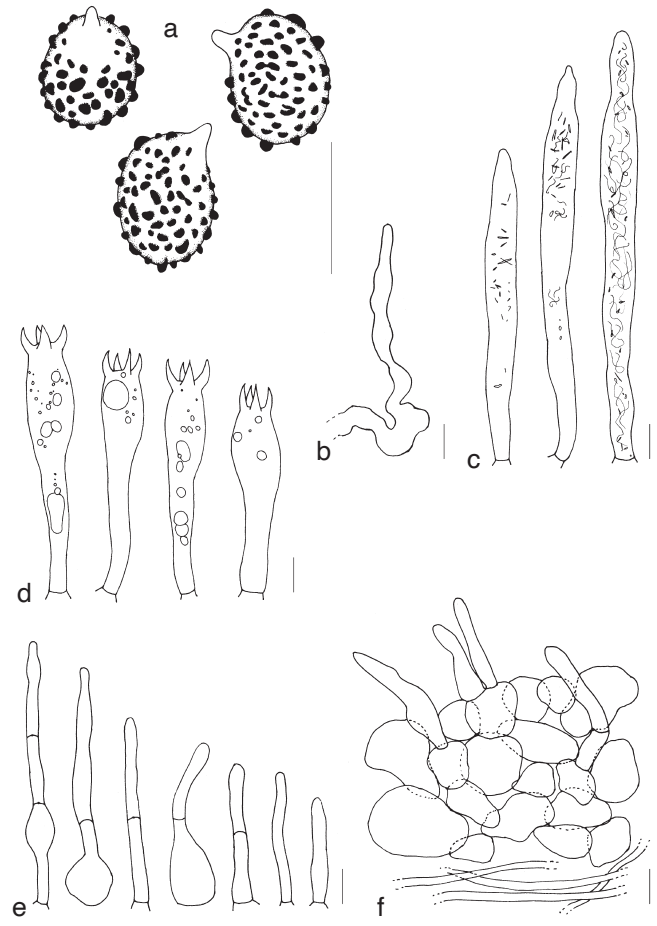


Fig. 6 *Lactifluus nebulosus* (LD 15-036, LD 15-059, LD 15-061). a. Basidiospores; b. pseudocystidium; c. macrocystidia; d. basidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 μ m.

often slightly wrinkled, grey (4C2, 5B-C2, 5D3, 6B1) and white, with some yellow (4A2–4) areas, and brown spots (5D4). *Stipe* 5–30 \times 3–15 mm, cylindrical; surface irregular, white, with brown (6D4, 6E5) stains. *Lamellae* decurrent to deeply decurrent, distant, often bifurcating near the margin, white to cream (4A2), staining brown (5C4, 6E5); edge even and concolourous. *Context* white, stuffed, turning brown when cut (7D3), with the middle part of the stipe remaining unchanged in adult specimens, dark green-blue with guaiac, dark greenish grey with FeSO₄. *Odour* like *Russula xerampelina*; shellfish. *Taste* insignificant, mild. *Latex* abundant to very abundant in young specimens, watery white, staining brown; taste mild.

Basidiospores broadly ellipsoid to ellipsoid, 7.3–9–9.5–11.3 (–12.8) \times 5.9–6.9–7.2–8.4(–9.7) μ m (Q = 1.09–1.30–1.32–1.46); ornamentation amyloid, composed of rounded warts, up to 1 μ m high, isolated, rarely connected by fine connective lines; plage inamyloid. **Basidia** 47.5–59.5–72 \times 10–11.5–12.5(–13) μ m, subclavate, 4-spored. **Pleuromacrocystidia** (54)–59.5–112.5–166 \times 6–9.5–13 μ m, very abundant, cylindrical with rounded, mucronate or slightly moniliform apex, thin-walled. **Pleuropseudocystidia** very scarce, not emergent, content oleiferic. **Lamellar edge** fertile. **Hymenophoral trama** mixed, with hyphae, abundant lactifers and abundant sphaerocytes. **Pileipellis** a palisade; elements of the suprapellis 18–43–68(–72) \times 2.5–4–5 μ m, cylindrical to lageniform, often septate; subpellis composed of isodiametric cells. *Stipitipellis* resembling pileipellis.

Ecology — Found in xerophytic forests with *Coccoloba pubescens*, *Coccoloba swartzii* and *Guapira fragrans*.

Distribution — Only known from the island of Martinique.

Specimens examined. MARTINIQUE, Réserve naturelle La Caravelle, Commune de Trinité, Tartane, vicinity of ruins of Chateau Dubuc (14.769464°, -60.890001°, alt. 31.2 m), soil with *Guapira fragrans*, *Coccoloba pubescens*, 08 Nov. 2015, Lynn Delgat, LD 15-031 (GENT); *ibid.*, (14.769232°, -60.887195°, alt. 24.3 m), soil with *Coccoloba pubescens*, 08 Nov. 2015, Lynn Delgat, LD 15-036 (GENT); Réserve naturelle La Caravelle, Commune de Trinité, Tartane, close to bar 'Le Phare', above the mangrove, Anse Four à Chaux (14.765185°, -60.897090°, alt. 20.1 m), soil with *Coccoloba swartzii*, *Guapira fragrans*, 12 Nov. 2015, Lynn Delgat, LD 15-059 (GENT); *ibid.*, (14.764985°, -60.897280°, alt. 18.8 m), soil with *Coccoloba pubescens*, *Guapira fragrans*, 12 Nov. 2015, Lynn Delgat, LD 15-061 (GENT).

Notes — *Lactifluus nebulosus* is sometimes confused with *L. caribaeus*, only differing by the absence of grey tinges in the pileus of *L. caribaeus* and its stronger unpleasant odour. However, microscopic differences are clear: *L. nebulosus* has larger spores, a palisade structure of the pileipellis, and conspicuous macrocystidia, while *L. caribaeus* has a trichoderm and no macrocystidia. Concordant with these clear microscopic differences, *L. nebulosus* and *L. caribaeus* are molecularly not sister species. In contrast, *L. nebulosus* is probably most closely related (although unsupported: bootstrap = 69) to the recently described *L. guanensis* from the British Virgin Isles (Fig. 1). However, this species can easily be distinguished from *L. nebulosus* by the presence of an amyloid plage, the absence of pleurocystidia and the lamprotrichoderm structure of the pileipellis (Crous et al. 2019). *Lactifluus nebulosus* is also macro- and microscopically similar to *L. guadeloupensis* sp. nov. and *L. marmoratus* sp. nov.; for comparison see the notes on those respective species.

Lactifluus guanensis Delgat & Lodge, Persoonia 43: 375. 2019

Ecology — Found on sandy soil under *Coccoloba uvifera*.

Distribution — Only known from Guana Island, British Virgin Isles.

Notes — This recently described species fits morphologically in *L.* sect. *Nebulosi*, notably because of the dull basidiocarp colours, the brown staining of the latex and context, the unpleasant odour and the spore ornamentation with isolated warts. The placement in this section is confirmed molecularly by the position of the type sequence in the phylogeny (Fig. 1). Compared to *L. coccolobae*, which also occurs on Guana Island, *L. guanensis* has broader basidia (9.5–12–14(–14.5) µm), slightly longer spores ((7.3–)7.5–9.5–11.4(–11.7) µm), higher spore ornamentation (up to 1 µm high) and non-gelatinised pileipellis (Crous et al. 2019). Compared to *Lactifluus* species from the Lesser Antilles, *L. guanensis* is easily distinguishable, notably due to the often amyloid plage, the absence of macrocystidia and the lamprotrichoderm structure of the pileipellis consisting of thick-walled elements. In addition, only *L. coccolobae* was also found in association with *Coccoloba uvifera*.

Lactifluus murinipes (Pegler) De Crop, Persoonia 38: 76. 2016 — Fig. 2e, 7

≡ *Lactarius murinipes* Pegler, Kew Bull. 33 (4): 623. 1979.

≡ *Lactifluus castaneibadius* (Pegler) De Crop, Persoonia 38: 76. 2016.

≡ *Lactarius castaneibadius* Pegler, Kew Bull. 33 (4): 622. 1979.

Pileus 8–75 mm diam, plano-convex with inflexed margin when young, infundibuliform with straight to deflexed margin when adult; surface chamois-leather like, sometimes slightly wrinkled, dark brown (6F4–6, 7F4, 8F4–5), sometimes with some lighter areas (5B4, 5D4, 6D4). **Stipe** 11–30 × 5–14 mm, cylindrical to slightly tapering downwards, white to yellowish white (4A2), turning light brown (5B3, 5C3, 5D4–5, 6D4), sometimes with orange stains (6C6). **Lamellae** subdecurrent to decurrent, medium to distant, often bifurcating near the margin, slightly

transverse, white to cream (4A2), staining brown (5B3–4, 6C6); edge crenulate and concolourous. **Context** white, firm, slowly turning brown, dark green blue with guaiac, no reaction with FeSO₄. **Odour** weakly sweet. **Taste** first mild, then acrid to very acrid, sometimes mild when latex is scarce or absent. **Latex** scarce to abundant, watery white, not changing colour; taste acrid to very acrid or first mild, then acrid to very acrid. **Basidiospores** subglobose to broadly ellipsoid, some slightly ellipsoid, 6.1–8–8.2–9.8(–10.1) × 5.5–6.5–6.8–8 µm (Q = 1.04–1.19–1.23–1.36), in some collections rare large spores are present (11.3–14.5 × 8.6–12.9 µm); ornamentation amyloid, composed of rounded warts, up to 0.5–1 µm high, mostly isolated, sometimes connected by fine connective lines; plage inamyloid. **Basidia** 50.5–65.5–81 × (8–)8.5–10.5–12 µm, subclavate, mostly 4-spored, sometimes 1- or 2-spored. **Pleuromacrocystidia** 62–102–142 × 7–9–11(–11.5) µm, very abundant, cylindrical with rounded, mucronate or rostrate apex, thin-walled. **Pleuropseudocystidia** very abundant, 4–7 µm diam, not or slightly emergent; positive reaction with sulphovanillin. **Lamellar edge** fertile. **Hymenophoral trama** filamentous, with hyphae and abundant lactifers. **Pileipellis** a trichoderm; elements of the suprapellis 16–61.5–106.5(–150) × (2–)2.5–4–5 µm, cylindrical, some septate; subpellis composed of hyphae; with emerging dermatocystidia 41–55.5–70 × 4–5.5–7 µm and dermatopseudocystidia 5.5–7 µm diam. **Stipitipellis** resembling pileipellis, terminal elements slightly shorter than in the pileipellis, 22–45.5–68.5 × 3–4–5 µm.

Ecology — Found in xerophytic forests with *Coccoloba diversifolia*, *Coccoloba pubescens*, *Coccoloba swartzii*, *Guapira fragrans*, *Lonchocarpus* sp. and *Tabebuia* sp.

Distribution — Only known from the island of Martinique.

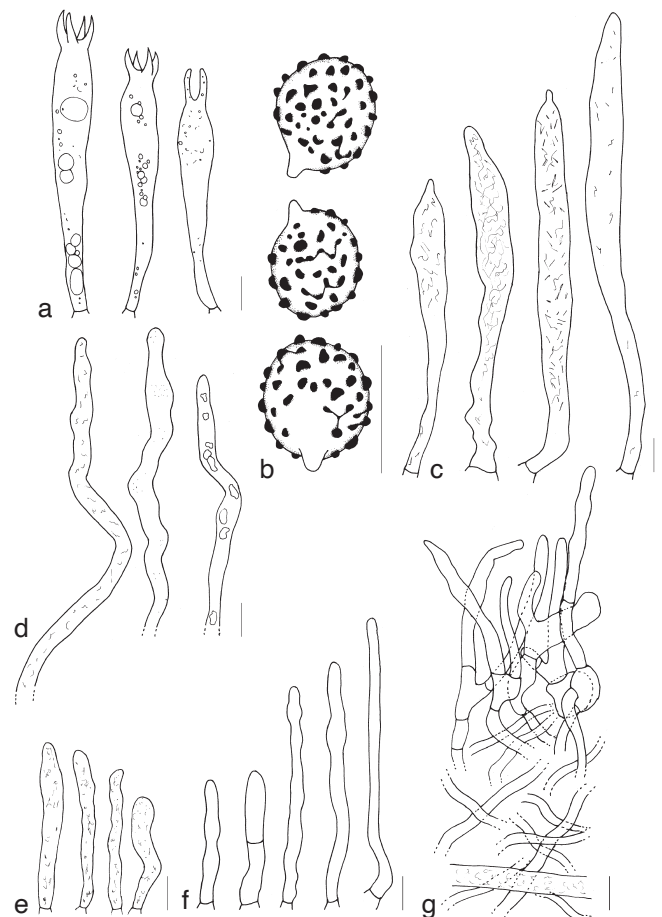


Fig. 7 *Lactifluus murinipes* (LD 15-015, LD15-018, LD15-020, LD 15-054, LD 15-057). a. Basidia; b. basidiospores; c. macrocystidia; d. pseudocystidia; e. dermatocystidia; f. pileipellis terminal elements; g. section through the pileipellis. — Scale bars = 10 µm.

Specimens examined. MARTINIQUE, Réserve naturelle La Caravelle, Commune de Trinité, Tartane, close to bar 'Le Phare', above the mangrove, Anse Four à Chaux (14.761724°, -60.925873°, alt. 16.9 m), soil with *Guapira fragrans*, 04 Nov. 2015, Lynn Delgat, LD 15-015 (GENT); *ibid.*, (14.767778°, -60.888451°, alt. 13.3 m), soil with *Guapira fragrans*, 04 Nov. 2015, Lynn Delgat, LD 15-018 (GENT); *ibid.*, (14.769612°, -60.890165°, alt. 33.7 m), soil with *Guapira fragrans*, *Coccoloba pubescens*, 04 Nov. 2015, Lynn Delgat, LD 15-020 (GENT); *ibid.*, (14.765021°, -60.897273°, alt. 18.9 m), soil with *Coccoloba swartzii*, *Guapira fragrans*, 12 Nov. 2015, Lynn Delgat, LD 15-054 (GENT); *ibid.*, (14.764985°, -60.897280°, alt. 18.8 m), soil with *Coccoloba swartzii*, *Guapira fragrans*, 12 Nov. 2015, Lynn Delgat, LD 15-057 (GENT); Réserve naturelle La Caravelle, Commune de Trinité, Tartane, vicinity of ruins of Chateau Dubuc (14.768835°, -60.889188°, alt. 23.9 m), soil with *Coccoloba swartzii*, 08 Nov. 2015, Lynn Delgat, LD 15-032 (GENT).

Notes — *Lactifluus murinipes* is easily distinguishable from the other Antillean species in *L.* sect. *Nebulosi*. Macroscopically, it differs by the dark brown pileus, by the latex that tastes acrid and does not turn brown, by the absence of an unpleasant odour and by the absence of a reaction with FeSO_4 .

Microscopically, the trichodermial pileipellis with abundant conspicuous dermatomyositis helps distinguish *L. murinipes* from most other similar species.

It was previously already suspected that *L. castaneibadius* and *L. murinipes* were synonyms (De Crop et al. 2017). Our phylogeny (Fig. 1) confirms the synonymy of these species molecularly, since sequences of the holotypes of both species are part of the same well-supported clade (p -distance = 0.002). They were previously morphologically distinguished from each other by the colour of the stipe, the sulphovanillin reaction of the pseudocystidia and the spore size and shape. However, stipe colour appears quite variable because of the colour-changing

context. Additionally, it was observed that all recent collections belonging to this clade have a positive sulphovanillin reaction, but the reaction time differs between collections, which could explain the perceived absence of the reaction in collections that react slower. The different spore sizes mentioned in the original description ($7.5\text{--}8\text{--}9 \times 5.5\text{--}7\text{--}8 \mu\text{m}$ in *L. castaneibadius* and $9\text{--}9.5\text{--}10.5 \times 6.5\text{--}7.5\text{--}8.5 \mu\text{m}$ in *L. murinipes*) can be considered intraspecific variation. The difference in spore shape ($Q = 1.16$ in *L. castaneibadius* and $Q = 1.28$ in *L. murinipes*) could be explained by the difference in spore size, since the measurements of the recent collections of this species show that larger spores generally have slightly bigger Q values. In conclusion, morphological differences between these species were subtle and are now known to be intraspecific variation.

Lactifluus caribaeus (Pegler) Verbeke, Mycotaxon 120: 446. 2012 — Fig. 8

≡ *Lactarius caribaeus* Pegler, Kew Bull. 33 (4): 617. 1979.

Pileus 40–75 mm diam, plano-convex or depressed to almost infundibuliform, with involute margin when young; surface slightly velutinous, slightly wrinkled, ivory yellow, bruising with cinnamon to clay spots. **Stipe** 20–40 × 10–25 mm, tapering downwards, very thick and robust, white, turning cinnamon. **Lamellae** deeply decurrent, slightly distant, thick, occasionally anastomosing, cream; edge entire and concolourous. **Context** whitish, firm, turning light cinnamon, light green blue with guaiac, rapidly dark green with FeSO_4 . **Odour** strong, unpleasant, recalling urine. **Taste** mild. **Latex** white, staining cinnamon.

Basidiospores subglobose to broadly ellipsoid, rarely slightly ellipsoid, $6.6\text{--}7.6\text{--}8.5 \times 5.8\text{--}6.3\text{--}6.8 \mu\text{m}$ ($Q = 1.06\text{--}1.20\text{--}1.35$); ornamentation amyloid, composed of rounded warts, up to 0.5 μm high, isolated, sometimes connected by fine connective lines; plage inamyloid. **Basidia** 47.5–59–71 × 8–9–10.5 μm , cylindrical to subclavate, 4-spored. **Pleuromacrocytidia** absent. **Pleuro-pseudocystidia** 3–9 μm diam, not emergent to emergent; apex rounded or mucronate; content oleiferic. **Lamellar edge** fertile. **Hymenophoral trama** mixed, with hyphae, lactifers and sphaerocytes. **Pileipellis** a trichoderm; elements of the suprapellis 26.5–49–72 × 2.5–3.5–4.5 μm , cylindrical, some septate; subpellis composed of hyphae. **Stipitipellis** resembling pileipellis.

Ecology — Found in xerophytic forests with *Coccoloba pubescens*.

Distribution — Only known from the island of Martinique.

Specimens examined. MARTINIQUE, Sainte-Luce, Trois Rivières, Ravine Saint-Pierre, towards Ravin Gros Vaisseaux (north of the nationale 5) (14.467552°, -60.926485°, alt. 22.1 m), soil, 28 Nov. 2006, J.P. Fiard, CL/MART06.014 (LIP); Réserve naturelle La Caravelle (alt. 5 m), soil with *Coccoloba pubescens*, *Myrica coriacea* and *Zanthoxylon caribaeum*, 26 Oct. 1976, J.P. Fiard, J.P. Fiard 818A, holotype (K).

Notes — Only one recent collection of this species was found, which lacks a field description and picture, so the macroscopic part of the description is based on the original description and picture (Pegler & Fiard 1979, 1983). *Lactifluus caribaeus* can be recognised macroscopically by the combination of the ivory yellow pileus and the strong, unpleasant odour. Molecularly, *L. caribaeus* is most closely related to *L. chiapanensis* from Mexico (Fig. 1), and microscopically, they share the trichodermial structure of the pileipellis. The only other Lesser Antillean species with this type of pileipellis structure is *L. murinipes*. However, *L. murinipes* is macroscopically clearly distinct from *L. caribaeus* because of the dark brown pileus, the acrid unchanging latex and the absence of an unpleasant odour. In contrast, *L. nebulosus* does look macroscopically similar to *L. caribaeus* and is sometimes confused with this species, as already discussed in the notes on *L. nebulosus*. Another morphologically similar species is *L. coccolobae*, known from

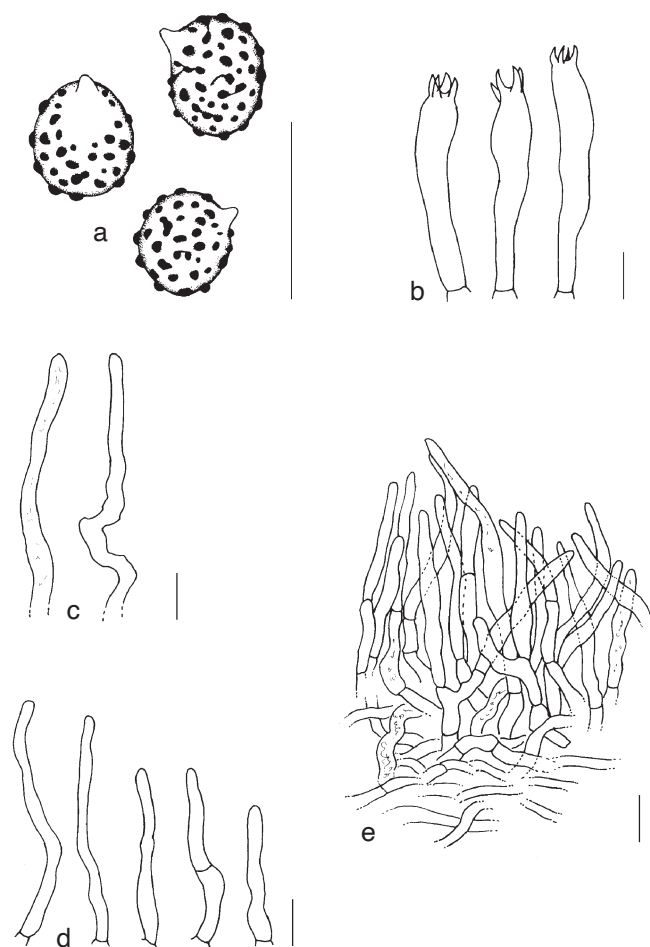


Fig. 8 *Lactifluus caribaeus* (CL/MART06.014). a. Basidiospores; b. basidia; c. pseudocystidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 μm .

Puerto Rico and Guana Island (BVI). This species differs from *L. caribaeus*, notably by the gelatinised pileipellis, the adnate and crowded lamellae and its dune habitat in association with *Coccoloba uvifera*.

Lactifluus chiapanensis (Montoya, Bandala & Guzmán) De Crop, *Persoonia* 38: 76. 2016

≡ *Lactarius chiapanensis* Montoya, Bandala & Guzmán, *Mycotaxon* 57: 412. 1996.

Ecology — Found in tropical forest with *Gymnopodium anti-gonoides*.

Distribution — Known from Chiapas, Mexico.

Notes — *Lactifluus chiapanensis* fits morphologically in *L.* sect. *Nebulosi*, notably because of the brown staining of the latex and context, and the spore ornamentation with isolated warts. It is most closely related to *L. caribaeus* (Fig. 1), from which it can be distinguished by the dark to greyish brown pileus colours and the presence of macrocystidia, measuring $92\text{--}110.4 \times 10.4\text{--}13.6 \mu\text{m}$ (Montoya et al. 1996).

Lactifluus unnamed clade 1

Lactifluus lepus Delgat & Courtec., *sp. nov.* — MycoBank MB828346; Fig. 2f, 9

Diagnosis. Differs from species of *L.* sect. *Nebulosi* by the striate pectinate margin, the widely spaced lamellae, the considerably smaller spores, the thick-walled terminal elements of the pileipellis and the presence of many 1- and 2-spored basidia.

Typus. GUADELOUPE, Petit-Bourg, Tambour, valley of the Tambour river (16.189618° , -61.595192° , alt. 22.7 m), 03 Sept. 2005, *R. Courtecuisse* et *F. Lurel*, RC/Guad 05-029 (LIP).

Etymology. Referring to the many 2-spored basidia with rather long sterigmata, reminding of the silhouette of a hair (*lepus*).

Pileus 35 mm diam, convex with slight central depression, margin pectinate-striate, slightly bumpy on the striae but not strongly lumpy; surface clearly rugose to wrinkled, more or less radially in the middle and more concentrically towards the margin in some places, pale yellow with some patches more brownish or rusty, in the centre a bit darker (reminding of the colours of *Russula fellea*). *Stipe* 20×7 mm, cylindrical, slightly curved, surface finely pruinose, smooth or slightly wrinkled, cream with pale orange hue. *Lamellae* decurrent, very distant, somewhat thick, pale cream, edge entire and concolourous. *Context* very pale cream, staining brown. *Odour* fruity. *Taste* not observed. *Latex* white; taste extremely acrid.

Basidiospores subglobose to broadly ellipsoid, rarely globose, $5.6\text{--}6.1\text{--}6.6 \times 4.8\text{--}5.4\text{--}5.9$ (–6) μm ($Q = 1.01\text{--}1.14\text{--}1.27$ (–1.28)); ornamentation amyloid, composed of isolated rounded warts, up to 1 μm high; plage inamyloid. *Basidia* $53\text{--}65.5\text{--}77.5$ (–79) $\times 7.5\text{--}8\text{--}9 \mu\text{m}$, subclavate, many 1-, 2- and 4-spored basidia, rarely 3-spored. *Pleuromacrocystidia* $49.5\text{--}85.5\text{--}121.5$ (–136) $\times 5\text{--}7\text{--}8.5 \mu\text{m}$, abundant, cylindrical to slightly fusiform, sometimes branching, thin-walled, arising from deep in the hymenium. *Pleuroseudocystidia* very abundant, $3\text{--}4 \mu\text{m}$ diam, sometimes branching, not or slightly emergent. *Lamellar edge* sterile; marginal cells $20\text{--}40 \times 2.5\text{--}4 \mu\text{m}$. *Subhymenium* cellular. *Hymenophoral trama* filamentous, with hyphae and abundant lactifers. *Pileipellis* a lampropalisade; elements of the suprapellis $28\text{--}78.5\text{--}129.5 \times 4\text{--}5\text{--}6 \mu\text{m}$, cylindrical, often with subcapitate apex, thick-walled; subpellis composed of isodiametric cells. *Stipitipellis* a trichoderm; terminal elements as in pileipellis.

Ecology — Found in hygrophytic forests.

Distribution — Only known from the island of Guadeloupe.

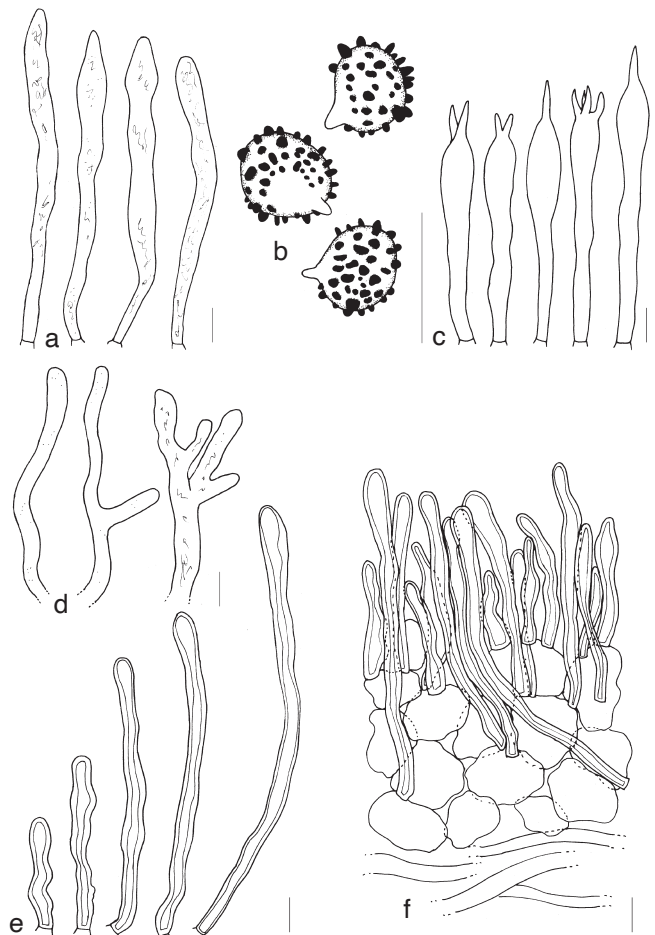


Fig. 9 *Lactifluus lepus* (RC/Guad 05-029). a. Macroscystidia; b. basidiospores; c. basidia; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 μm .

Notes — In our phylogeny, as well as in the worldwide molecular analysis of *Lactifluus* by De Crop et al. (2017), *L. lepus* is a rather isolated species in *L.* subg. *Gymnocarpi*. So far only one closely related species is known: an undescribed species from French Guiana (Fig. 1). *Lactifluus lepus* can easily be distinguished from the species of *L.* sect. *Nebulosi* by the striate pectinate margin, the widely spaced lamellae, the considerably smaller spores and the thick-walled terminal elements of the pileipellis.

Lactifluus* sect. *Panuoidei (Singer) Delgat, *comb. nov.* — MycoBank MB831636

≡ *Lactarius* sect. *Panuoidei* Singer, *Kew Bull.* 7 (3): 301. 1952. — *Typus.* *Lactifluus panuoides* (Singer) De Crop, *Persoonia* 38: 76. 2016.

≡ *Lactarius panuoides* Singer, *Kew Bull.* 7 (3): 300. 1952.

≡ *Pleurogala panuoides* (Singer) Redhead & Norvell, *Mycotaxon* 48: 377. 1993.

Lactifluus panuoides (Singer) De Crop, *Persoonia* 38: 76. 2016 — Fig. 2g, 10

≡ *Lactarius panuoides* Singer, *Kew Bull.* 7 (3): 300. 1952.

≡ *Pleurogala panuoides* (Singer) Redhead & Norvell, *Mycotaxon* 48: 377. 1993.

Basidiocarp pleurotoid. *Pileus* $10\text{--}23 \times 8\text{--}24$ mm diam, spatuliform, irregularly lobed; surface finely pubescent, slightly furfuraceous, wrinkled at the insertion, yellowish cream, staining pale rosy rusty brown, sometimes almost completely purplish brown when old. *Stipe* excentric and reduced. *Lamellae* converging

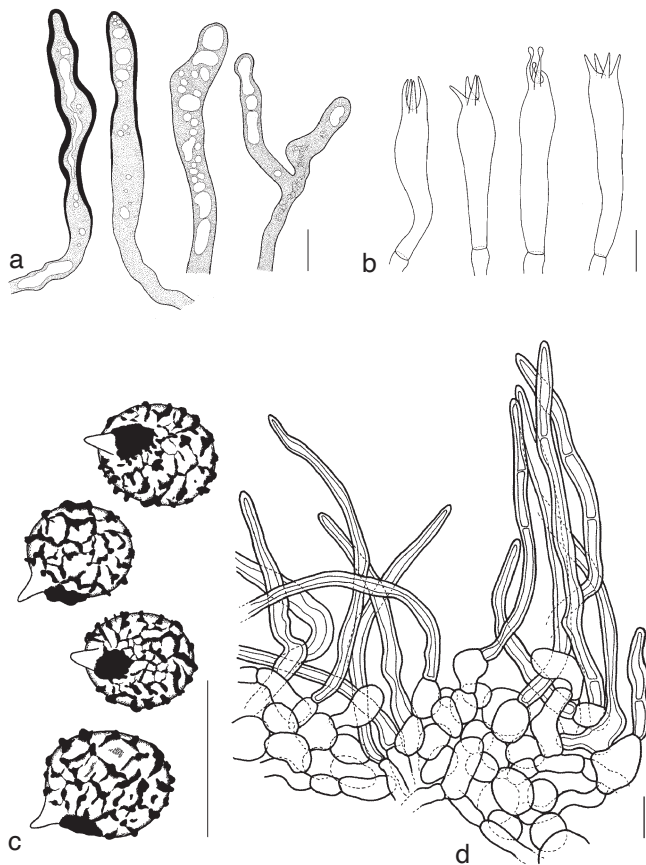


Fig. 10 *Lactifluus panuoides* (Pegler 3133). a. Pseudocystidia; b. basidia; c. basidiospores; d. section through the pileipellis. — Scale bars = 10 μ m.

to the stipe, not crowded, cream, staining pale rusty brown. *Context* thin. *Odour* not remarkable. *Taste* mild. *Latex* watery white, changing to rosy-brownish. *Subiculum* tomentose, white to cinnamon.

Basidiospores subglobose to broadly ellipsoid, 5.7–6.8–7.9 (–8.7) \times 5–5.8–6.7 (–6.9) μ m (Q = 1.05–1.17–1.29), ornamentation amyloid, composed of rounded to irregular warts, up to 1 μ m high, connected by thick connectives, subreticulate, plage completely amyloid. *Basidia* 27–37.5–47.5 (–48) \times 8–9–10 μ m, subclavate, 4-spored. *Pleuromacrocytidia* absent. *Pleuropseudocystidia* 7.5–9.5 μ m diam, thick-walled, content granular and very refringent, slightly emergent. *Lamellar edge* fertile. *Hymenophoral trama* filamentous, with hyphae and abundant lactifers. *Pileipellis* a palisade; elements of the suprapellis 18.5–88–158 \times 4.5–5–6 μ m, cylindrical with obtuse apex, thick-walled, often septate; subpellis composed of isodiametric cells.

Ecology — Found on wood in hygrophytic forests.

Distribution — Known from the Lesser Antilles and French Guiana.

Specimens examined. FRENCH GUIANA, Commune de Regina, Savane-Roche Virginie trail, PK 122,7 (4.309524°, –52.133775°, alt. 2.8 m), slope forest, 26 Apr. 2010, R. Courtecuisse, RC/Guy 10-024 (LIP). — GUADELOUPE, Basse-Terre, Sofaia trail, on fallen twigs and debris in hygrophytic forest, 25 Oct. 1977, D.N. Pegler, Pegler 3133 (K). — MARTINIQUE, Fort-de-France, Les Nuages, la Médaille, Rivière Blanche forest, on trunk, 04 Sept. 2003, R. Courtecuisse, RC/Mart 03-077 (LIP). — TRINIDAD, Naranja, 02 Oct. 1949, Dennis 109 (K), holotype.

Notes — *Lactifluus panuoides* is the only pleurotoid *Lactifluus* species known to occur in the Lesser Antilles. This species has some striking characters: the thick-walled pseudocystidia and the completely amyloid plage, which is common in *Russula*,

but rare in *Lactifluus*. Other Neotropical pleurotoid species are *L. brunellus* and *L. multiceps*, both described from Guyana. *Lactifluus panuoides* can be easily distinguished from them. *Lactifluus brunellus* also belongs in *L.* subg. *Gymnocarpi*, but it differs from *L. panuoides* by the brown basidiomes with white margin, the shaggy fasciculate subiculum, the slightly larger basidia (43–53 \times 9–10.5 μ m) and spores (6.8–8.4 \times (5.6–)6–7.2 μ m), the inamyloid plage, the rarely connected echinulate-spinuose basidiospore ornamentation and the pileipellis hairs which frequently have swollen bases. *Lactifluus multiceps* on the other hand belongs to *L.* subg. *Lactariopsis*. It can be distinguished from *L. panuoides* by the golden yellow to brownish orange basidiomes, the well-developed stipe, the strong acrid taste, the larger spores (7.2–9.2 \times 6.4–8 μ m), the plage without a large amyloid spot and the longer pileipellis hairs (40–220 \times 4–7 μ m) which frequently have swollen bases. Additionally, both *L. brunellus* and *L. multiceps* are found in forests dominated by *Dicymbe corymbosa* (Miller et al. 2002). Molecularly, *L. panuoides* represents an isolated clade (Fig. 1; De Crop et al. 2017). The collection from Guyana represents a second species in this clade (Delgat, unpubl. results). Given that *L. panuoides* is the type of *Lactarius* sect. *Panuoides*, this clade represents *L.* sect. *Panuoides*, which is recombined in this paper.

Lactifluus subg. *Lactariopsis* (Henn.) Verbeken

Lactifluus sect. *Neotropicus* J. Duque, Delgat, Verbeken, M.A. Neves & A.A. Carvalho, Syst. Biodivers. 18: in press. 2020.

Lactifluus venezuelanus (Dennis) De Crop, Persoonia 38: 77. 2016 — Fig. 2h, 11

\equiv *Lactarius venezuelanus* Dennis, Kew Bull. Add. Ser. 3: 467. 1970.

Pileus 28–80 mm diam, convex with deep central depression, striate for a 1/3 to a 1/2 of the radius; surface smooth to slightly bumpy, pale apricot orange to bright brownish orange, hygrophorous. *Stipe* 15–35 \times 4–10 mm, tapering downwards, fistulose; surface apically with small ridges which are continuing from the decurrent teeth of the gills, pale apricot, rustier towards the base. *Lamellae* subdecurrent to decurrent, not crowded, slightly pruinose, yellowish cream to pale rusty ochre; edge entire and concolourous. *Context* white to very pale apricot. *Odour* weak, more or less fruity or like rubber. *Taste* insignificant or slightly acrid, very astringent in young specimens. *Latex* white, quickly disappearing, hence absent in most specimens, not changing colour.

Basidiospores subglobose to broadly ellipsoid, 5.9–6.6–6.8–7.7 \times 5.2–5.6–5.8–6.5 μ m (Q = 1.06–1.14–1.17–1.34 (–1.39)); ornamentation amyloid, composed of rounded to irregular warts, up to 1 μ m high, often connected by lower connective lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 34.5–43–52 \times 8–10–11.5 (–12) μ m, subclavate, mostly 4-spored, some 1- and 2-spored present. *Pleurocystidia* absent. *Pleuropseudocystidia* abundant, 6–10 μ m diam, not or slightly emergent. *Lamellar edge* sterile; marginal cells 15.5–25–34.5 \times 2.5–3.5–4.5 μ m, narrowly cylindrical to subfusiform, often tapering near apex. *Hymenophoral trama* mixed, with hyphae, lactifers and sphaerocytes. *Pileipellis* a palisade; elements of the suprapellis 8–65–122.5 \times 2.5–3–3.5 (–4) μ m, cylindrical with obtuse apex, sometimes septate; subpellis composed of isodiametric cells. *Stipitipellis* resembling pileipellis.

Ecology — Found in hygrophytic or mesophytic forests with *Guapira fragrans*.

Distribution — Known from the islands of Martinique and Guadeloupe.

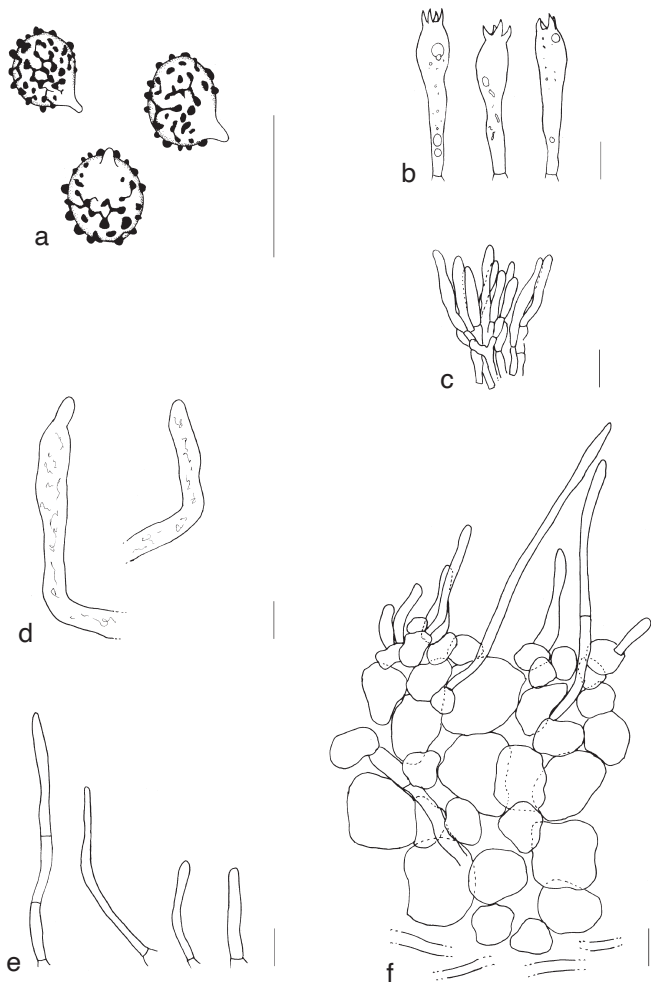


Fig. 11 *Lactifluus venezuelanus* (RC/Guad 03-048, RC/Guad11-017, RC/Mart 03-120). a. Basidiospores; b. basidia; c. marginal cells; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 μ m.

Specimens examined. GUADELOUPE, Basse-Terre, commune de Saint-Claude, Beausoleil, Trace du plateau Dimba (16.026758°, -61.702051°, alt. 440.6 m), 09 Sept. 2003, R. Courtecuisse, RC/Guad 03-048 (LIP); Basse-Terre, Petit-Bourg, Route de la Traversée, near the national park of Guadeloupe, 14 Aug. 2010, P.-A. Moreau, PAM/GUAD 10.037 (LIP); Basse-Terre, Goyave, Chutes de Moreau, 05 Aug. 2011, R. Courtecuisse, RC/Guad11-017 (LIP). — MARTINIQUE, Fort-de-France, Plateau Perdrix, 05 Sept. 2003, R. Courtecuisse, RC/Mart 03-120 (LIP).

Notes — *Lactifluus venezuelanus* is easily distinguishable from other Antillean *Lactifluus* species due to the orange striate pileus, the apical ridges on the stipe and the absence of latex in most fruiting bodies. This species is placed in *L.* subg. *Lactariopsis*, which is confirmed morphologically by the absence of true pleurocystidia and the absence of a colour change of the context and/or latex as in most species of this subgenus. The most closely related described species is *L. subiculatus* from Guyana (Fig. 1), which shares the striate margin of the pileus and the absent or scarce latex. This species can hardly be confused with *L. venezuelanus* because of the smaller fruiting bodies (9–30 mm), the presence of a well-developed subiculum, the larger and much wider basidia (45–60 \times 15–18 μ m wide) and the larger spores (7.6–9.2(–9.6) \times 6.4–7.6 μ m) (Miller et al. 2012). The most closely related Antillean species is *L. neotropicus* (Fig. 1), which can easily be distinguished from *L. venezuelanus* because of the presence of an annulus, the absence of a striate margin and the presence of thick-walled terminal elements in the pileipellis.

Lactifluus neotropicus (Singer) Nuytinck, Mycotaxon 118: 450, 2011 — Fig. 12

≡ *Lactarius neotropicus* Singer, Kew Bull. 7 (3): 299. 1952.

Pileus 30–40 mm diam, convex with central depression; surface smooth at the centre, slightly velutinous, with indistinct squamules towards the margin, brown to umber, with a paler ochraceous zone at the margin. **Stipe** 10–15 \times 7–8 mm, cylindrical or tapering downwards, solid, pale buff, with apical annulus just below lamellae insertion; annulus narrow, membranous with a fibrillose margin, concolourous with the stipe surface. **Lamellae** adnexed to subdecurrent, crowded, thin, pale buff; edge concolourous. **Context** pale ochraceous buff to pale vinaceous cinnamon, unchanging. **Odour** none. **Taste** very acrid. **Latex** watery white, scarce.

Basidiospores subglobose to broadly ellipsoid, 6.2–7.7.9(–8) \times 5.3–6.1–6.9(–7) μ m (Q = 1.03–1.16–1.30(–1.36)); ornamentation amyloid, composed of rounded warts, up to 0.5 μ m high, often connected by lower connective lines, forming an incomplete reticulum; plage inamyloid. **Basidia** 30–37–43.5 \times 8.5–9.5–11 μ m, subclavate, 4-spored. **Pleurocystidia** absent. **Pleuropseudocystidia** not observed. **Hymenophoral trama** mixed, with hyphae, lactifers and sphaerocytes. **Pileipellis** a palisade; elements of the suprapellis 18–54.5–117.5 \times 3.5–6–8.5, cylindrical with obtuse apex, sometimes tapering near the top, thick-walled; subpellis composed of isodiametric cells.

Ecology — Found in hygrophytic forests.

Distribution — Only known from type locality.

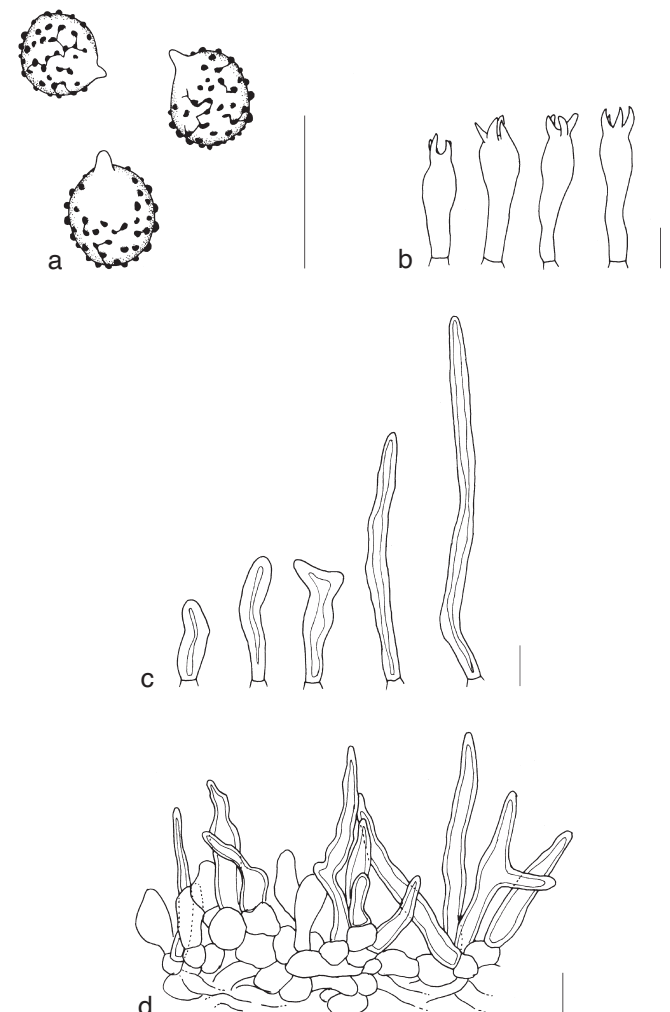


Fig. 12 *Lactifluus neotropicus* (R.W.G. Dennis 97). a. Basidiospores; b. basidia; c. pileipellis terminal elements; d. section through the pileipellis. — Scale bars = 10 μ m.

Specimens examined. TRINIDAD, Diego Martin, between Maqueripe trail and North Post, 30 Sept. 1949, R.W.G. Dennis, R.W.G. Dennis 97 (K), holotype.

Notes — The holotype of *L. neotropicus* clusters in the phylogeny together with a collection from French Guiana (Fig. 1), but since only ITS1 is available for the holotype and ITS variability is relatively low in *L. sect. Neotropicus*, further research is necessary to determine whether this collection could represent *L. neotropicus*. Since no certain recent collections were found of *L. neotropicus*, the macroscopic part of the description is based on the original description (Singer 1952). Besides the holotype, two other collections identified as *L. neotropicus* exist, collected in 1977 by Fiard. However, one of them was not available for loan due to the scarcity of the material. Microscopical study of the second collection, i.e., J.P. Fiard 1043A from Guadeloupe, showed that, compared to the holotype, this collection has much longer basidia ($55\text{--}64.5\text{--}80 \times 7\text{--}8.5\text{--}9.5 \mu\text{m}$) and subcapitate pileipellis hairs, which suggest it could represent a new species. Aside from this putative new species, *L. neotropicus* is the only species in the Antilles with velum. This velum, together with the absence of a striate margin and the presence of thick-walled terminal elements in the pileipellis, makes *L. neotropicus* easily distinguishable from *L. venezuelanus*. Both species belong in *L. subg. Lactariopsis*, which is the only subgenus in which secondary velum is observed (Montoya et al. 2012). Another Neotropical *Lactifluus* species which has velum is *L. annulifer*, which is described from the Brazilian Amazon. *Lactifluus annulifer* is quite similar to *L. neotropicus* but differs notably by the mild taste of the context, the much larger spores ($8.8\text{--}13 \times 8\text{--}10 \mu\text{m}$) and the presence of macrocystidia (Singer et al. 1983).

Lactifluus sect. Albati (Bataille) Verbeken, Mycotaxon 118: 451. 2011

Lactifluus domingensis Delgat & Angelini, IMA Fungus 10 (14): 7. 2019

Ecology — Found in montane forests with *Pinus occidentalis*.

Distribution — Only known from Dominican Republic, on the island of Hispaniola.

Notes — This recently described species fits morphologically in *L. sect. Albati*, because of the large white basidiomes, the acrid taste and the velutinous cap. The placement in this section is supported molecularly by the position of the type sequence in the phylogeny (Fig. 1). Compared to *L. hallingii*, which also belongs in *L. sect. Albati*, but occurs in Central America, *L. domingensis* has slightly higher spore ornamentation (up to $1.7 \mu\text{m}$), somewhat shorter basidia ($(42\text{--})43\text{--}53\text{--}63.5 \times 9.5\text{--}12.5\text{--}15.5 \mu\text{m}$), somewhat differently shaped macrocystidia (i.e., more often a mucronate or moniliform apex, more rarely with a rounded or tapering apex) and a loosely arranged cutis to trichoderm as a pileipellis. In addition, *L. hallingii* is associated with *Quercus*, while *L. domingensis* is associated with *Pinus occidentalis* (Delgat et al. 2019).

Lactifluus hallingii Delgat & De Wilde, IMA Fungus 10 (14): 10. 2019

Ecology — Found in montane forests with *Quercus humboldtii*, *Quercus seemanii*, *Quercus copeyensis*, *Quercus* sp.

Distribution — Known from Costa Rica, Panama and Colombia.

Notes — This recently described species fits morphologically in *L. sect. Albati*, because of the large white basidiomes, the acrid taste and the velutinous cap. The placement in this section is confirmed molecularly by the position of the type sequence in

the phylogeny (Fig. 1). For a comparison with *L. domingensis*, see the notes on *L. domingensis*.

Lactifluus subg. Pseudogymnocarpi (Verbeken) De Crop, Persoonia 38: 75. 2016

Lactifluus sect. Polysphaerophori (Singer) Verbeken, Mycotaxon 120: 445. 2012

Lactifluus pegleri (Pacioni & Lalli) Delgat, *comb. nov.* — MycoBank MB826723; Fig. 2i, 13

≡ *Lactarius pegleri* Pacioni & Lalli, Mycotaxon 44 (1): 182. 1992.

Pileus 13–90 mm diam, hemispherical with involute margin and central depression when young, infundibuliform with straight margin when adult; surface slightly velutinous, wrinkled near the margin, burgundy red (9D7–9E4–10D4) with some paler, more orange areas (5A4–6, 5B5, 6B3, 7D3), especially near the margin. **Stipe** 11–50 × 6–20 mm, cylindrical; surface slightly velutinous, reddish yellow-orange (4A4, 5A3–4, 6A2, 6B3, 7C3, 8C4, 8D5). **Lamellae** subdecurrent to decurrent, rather distant, some intervenose, some bifurcating near the margin, cream (3A2, 4A2); edge even and concolourous. **Context** white, firm, slowly dark green blue with guaiac, slowly darkening with FeSO_4 . **Odour** insignificant. **Taste** mild. **Latex** scarce to abundant, watery white, not changing colour, taste mild.

Basidiospores broadly ellipsoid, some subglobose or ellipsoid, $6.8\text{--}7.4\text{--}8.2\text{--}9.3 \times 5.5\text{--}6.1\text{--}6.7\text{--}7.5 \mu\text{m}$ ($Q = 1.06\text{--}1.22\text{--}1.38$); ornamentation amyloid, composed of rounded warts,

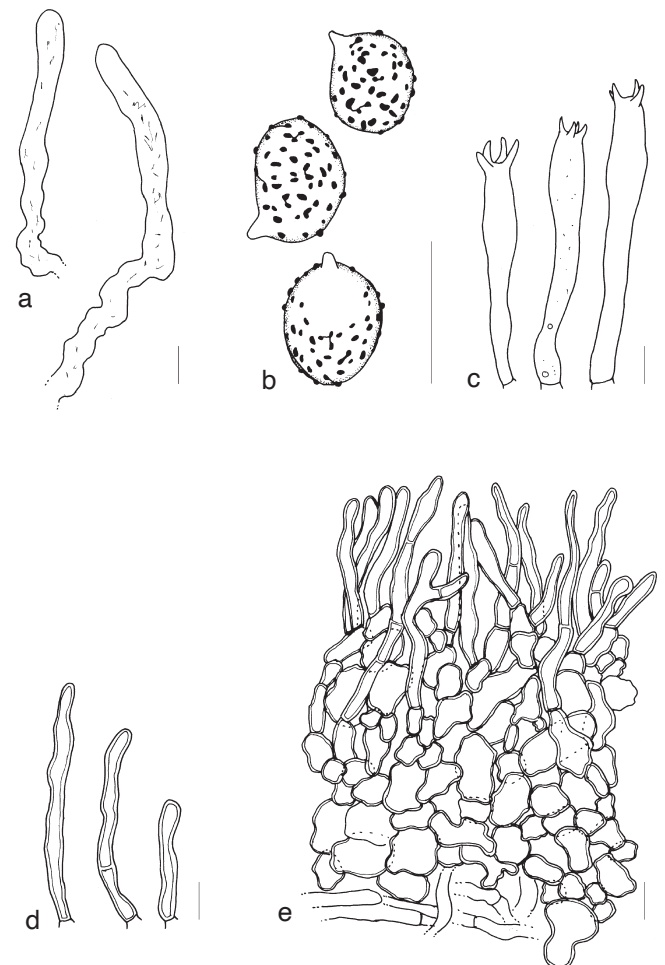


Fig. 13 *Lactifluus pegleri* (LD 15-042, LD15-049). a. Pseudocystidia; b. basidiospores; c. basidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 μm .

up to 0.2–0.5 µm high, mostly isolated, sometimes connected by fine connective lines; plage inamyloid. *Basidia* (51–)53–68.5–84.5 × 7–8.5–10(–10.5) µm, cylindrical to subclavate, slightly refringent, 4-spored, often arising deep in the hymenium. *Pleurocystidia* absent. *Pleuropseudocystidia* 6–7.5 µm diam, not emergent. *Lamellar edge* fertile. *Hymenophoral trama* cellular, with sphaerocytes and broad lactifers. *Pileipellis* a palisade; elements of the suprapellis 16.5–52–87 × 3.5–4–4.5, cylindrical with obtuse apex, slightly thick-walled, some septate; subpellis composed of rather small, slightly thick-walled isodiametric cells. *Stipitipellis* resembling pileipellis.

Ecology — Found in xerophytic forests with *Coccoloba pubescens* and *Guapira fragrans*.

Distribution — Only known from the island of Martinique.

Specimens examined. MARTINIQUE, Réserve naturelle La Caravelle, Commune de Trinité, Tartane, close to bar 'Le Phare', above the mangrove, Anse Four à Chaux (14.761724°, -60.925873°, alt. 16.9 m), soil with *Guapira fragrans*, 04 Nov. 2015, Lynn Delgat, LD 15-014 (GENT); *ibid.*, (14.762887°, -60.897124°, alt. 10.6 m), soil with *Coccoloba pubescens*, *Guapira fragrans*, 12 Nov. 2015, Lynn Delgat, LD 15-049 (GENT); Réserve naturelle La Caravelle, Commune de Trinité, Bois de Pointe Rouge, soil with *Coccoloba pubescens*, *Guapira fragrans*, 10 Nov. 2015, Lynn Delgat, LD 15-042 (GENT).

Notes — *Lactifluus pegleri* is an easily recognizable species because of its fleshy habitus and notable burgundy red to orange pileus colour. It is known from several localities in Martinique and additional specimens collected during the 2003–2015 field trips (coll. R. Courtecuisse) are deposited in LIP. This species was first identified by Pegler as *L. hygrophoroides*, and only later proposed as a new species (Lalli & Pacioni 1992). Indeed, macroscopically, *L. pegleri* strongly reminds of *L. hygrophoroides*. However microscopically, *L. hygrophoroides* has more elongated spores (Q = 1.35) and a more reticulate spore ornamentation (Lalli & Pacioni 1992). Additionally, the ecology of these two species is very different, with *L. hygrophoroides* occurring with temperate broad-leaved species (e.g., oaks). An important distinguishing feature reported in the original description, was the trichodermial structure of the pileipellis (Lalli & Pacioni 1992), however, our microscopical study of the collections shows that *L. pegleri* has a lampropalisade structure as a pileipellis, similar to *L. hygrophoroides*. Despite the similarity between these two species, the phylogeny confirms that they are different species and shows that they are not even closely related, although they both belong to *L.* subg. *Pseudogymnocarpi* (Fig. 1). *Lactifluus pegleri* is the only Antillean species in this subgenus and like most species in this subgenus, *L. pegleri* has a lampropalisade, unchanging latex and an orange to reddish brown pileus. The most closely related species is *L. veraecrucis* from Mexico (Fig. 1), which strongly resembles *L. pegleri* according to the original description (Singer 1973) and a microscopic study of the isotype. Recent collections of *L. veraecrucis* are needed to find characters, besides their different distribution, to distinguish them. Given that *L. veraecrucis* has been designated as the type of *L.* sect. *Polysphaerophori*, *L. pegleri* belongs to this section as well. Next to these two Central American species, this section also contains at least three unnamed South American species. Species previously classified in this section based on morphology, such as *L. venezuelanus* and *L. nebulosus*, do not belong in this section and the section is in need of an updated morphological description.

Lactifluus veraecrucis (Singer) Verbeken, Mycotaxon 120: 445. 2012

≡ *Lactarius verae-crucis* Singer, Beih. Sydowia 7: 104. 1973.
 ≡ *Lactarius veraecrucis* Singer. 1973.

Ecology — Found in tropical rain forest.

Distribution — Known from Chiapas and Veracruz, Mexico.

Notes — *Lactifluus veraecrucis* represents the type species of *L.* sect. *Polysphaerophori*. Its lamellar trama is completely cellular, and the presence of sphaerocytes in the trama was one of the original characters used to define this section, which caused numerous species to be included in this section. Currently only two described species are confirmed to belong in this section: *L. veraecrucis* and *L. pegleri*. *Lactifluus veraecrucis* is closely related to *L. pegleri* (Fig. 1) from the Lesser Antilles, and morphologically closely resembles it. The spores of *L. veraecrucis* are slightly more subglobose (7–8.3 × 6.5–7 µm) than those of *L. pegleri*, but the easiest way to distinguish them is their different distribution. Recent collections of *L. veraecrucis* could help to find additional characters to distinguish between these species.

KEY TO CARIBBEAN SPECIES

1. Context and latex not changing colour 2
1. Context, pileus, lamellae, stipe and/or latex changing to brown 4
2. Basidiospore ornamentation mostly composed of isolated warts; basidia 51–84.5 µm long; pileus colour burgundy red with orange; context mild. *L. pegleri*
2. Basidiospore ornamentation with many connectives between the warts; basidia shorter, 30–52 µm long; pileus colour orange to brown; context slightly to very acrid (subg. *Lactariopsis*) 3
3. Annulus or velar remnants present on the stipe; pileus not striate; terminal elements of the pileipellis thick-walled *L. neotropicus*
3. Annulus or velar remnants absent; pileus rather thin and striate; terminal elements of the pileipellis thin-walled. *L. venezuelanus*
4. Basidiocarp large and white; pileus velutinous; context acrid; associated with *Pinus occidentalis* *L. domingensis*
4. Not this combination of characters (subg. *Gymnocarpi*) 5
5. Habitus pleurotoid *L. panuoides*
5. Habitus agaricoid; basidiospores with isolated verrucae 6
6. Pileipellis structure a trichoderm; pleurocystidia absent 7
6. Pileipellis structure a trichopalissade to palissade; pleuro-macrocytidia 49.5–184 × 5–13 µm 9
7. Terminal elements of the pileipellis thick-walled
- *L. guanensis*
7. Terminal elements of the pileipellis thin-walled 8
8. Pileipellis gelatinised, found in dunes with *Coccoloba uvifera*. *L. coccolobae*
8. Pileipellis not gelatinised, found in forests with other *Coccoloba* species. *L. caribaeus*
9. Pileus surface very irregular with a striate pectinate margin *L. lepus*
9. Pileus surface rather smooth or wrinkled, margin not striate 10
10. Taste of latex and context acrid; latex not changing colour; no reaction with FeSO₄; odour not distinctly unpleasant *L. murinipes*
10. Taste of latex and context mild; latex changing colour to brown; context reacting green-grey with FeSO₄; odour distinctly unpleasant 11
11. Pileus colour brown, often light brown in the centre; pileus surface often strongly wrinkled; odour characteristically very strongly unpleasant; terminal elements up to 55 µm *L. putidus*
11. Pileus colour marbled; pileus surface at most slightly wrinkled, surface chamois-leather like; terminal elements up to 68–91.5 µm 12

- 12. Brown tinges present in pileus; stipe colour variable (grey-dirty cream); basidiospores broadly ellipsoid (average $Q = 1.24-1.26$) 13
- 12. No brown tinges in pileus; stipe white; basidiospores ellipsoid (average $Q = 1.30-1.32$) *L. nebulosus*
- 13. Basidia rather broad (average width 14 μm); large sphaerocytes (15–45 μm) in the pileipellis *L. marmoratus*
- 13. Basidia rather slender (average width 10.5 μm); small sphaerocytes (up to 15 μm) in the pileipellis *L. guadeloupensis*

KEY TO CENTRAL AMERICAN AND MEXICAN SPECIES

- 1. Basidiocarps large and white; pileus velutinous; taste acrid *L. hallingii*
- 1. Basidiocarps not white; pileus not velutinous 2
- 2. Pileus colour orange 3
- 2. Pileus colour greyish brown to blackish brown 4
- 3. Latex unchanging; pleurocystidia absent; spore ornamentation up to 0.5 μm high, not forming a reticulum. *L. veraecrucis*
- 3. Latex staining brown; conspicuous pleurolamprocystidia; spore ornamentation up to 1–2 μm high, forming a complete reticulum *L. pallidilamellatus*
- 4. Latex unchanging; pleuromacrocytidia present; spore ornamentation not forming a reticulum *L. chiapanensis*
- 4. Latex staining brown; pleurocystidia absent; spore ornamentation reticulate *L. fuscomarginatus*

Distribution of closest relative(s)

Figure 14 shows the distribution of the closest relative(s):

- i. for taxa originating from Central America or the Caribbean;
- ii. according to host association of Central American and Caribbean taxa; and
- iii. by altitude of those taxa.

The majority of Caribbean clades have a South American closest relative, while Central American clades show a more mixed result. Clades found associated with *Fabaceae*, *Polygonaceae* or *Nyctaginaceae* have closest relatives in South America, while clades found associated with *Betulaceae*, *Fagaceae* or

Pinaceae have closest relatives from North America, Asia and/or Europe. Clades reported from altitudes lower than 500 m were found to have South American relatives, while clades reported from higher altitudes, mainly over 1000 m, were found to have North American, Asian and/or European relatives.

DISCUSSION

Diversity of *Lactifluus* spp. in the Antilles

Studying *Lactifluus* diversity in the Antilles has not only revealed new species, but also a new section. The newly described section *Lactifluus* sect. *Nebulosi* contains six Lesser Antillean species, as well as *L. chiapanensis* from Mexico, and *L. coccolobae* and *L. guanensis* from the Greater Antilles (Fig. 1). The section belongs in *L.* subg. *Gymnocarpi*. In contrast to the other described sections in this subgenus that completely lack true pleurocystidia, most species of *L.* sect. *Nebulosi* have conspicuous pleuromacrocytidia. The section contains only Neotropical collections and is characterised by dull fruiting body colours (a brown-grey pileus and a white-grey stipe), an unpleasant fishy odour in all species except *L. murinipes*, and broadly ellipsoid spores with isolated, rounded warts up to 1 μm high. This type of isolated spore ornamentation is relatively rare in the genus and is reminiscent of the African *L.* sect. *Phlebonemi* in its traditional morphological delineation, which contains the species *L. angustus*, *L. arsenei*, *L. nonpiscis*, *L. phlebonemus* and *L. pisciodorus*. It is noteworthy that all these species also exhibit an unpleasant and often fishy odour (Verbeke & Walley 2010). Interestingly, two other Antillean species, *L. lepus* and *L. pegleri*, have isolated spore ornamentation. Both species do not belong in *L.* sect. *Nebulosi*, but in different sections in *L.* subg. *Gymnocarpi* and *L.* subg. *Pseudogymnocarpi*, respectively. The high proportion of this type of ornamentation in the Lesser Antilles, which occurs in eight out of eleven species with three independent origins, combined with the relatively rare occurrence globally, raises the question whether this type of spore ornamentation could represent an evolutionary advantage in the habitats of the Lesser Antilles and if it could be correlated to certain environmental variables.

Besides the six species of *L.* sect. *Nebulosi*, five other species were recorded, bringing the total known diversity of the Lesser Antilles to eleven *Lactifluus* species, although some species

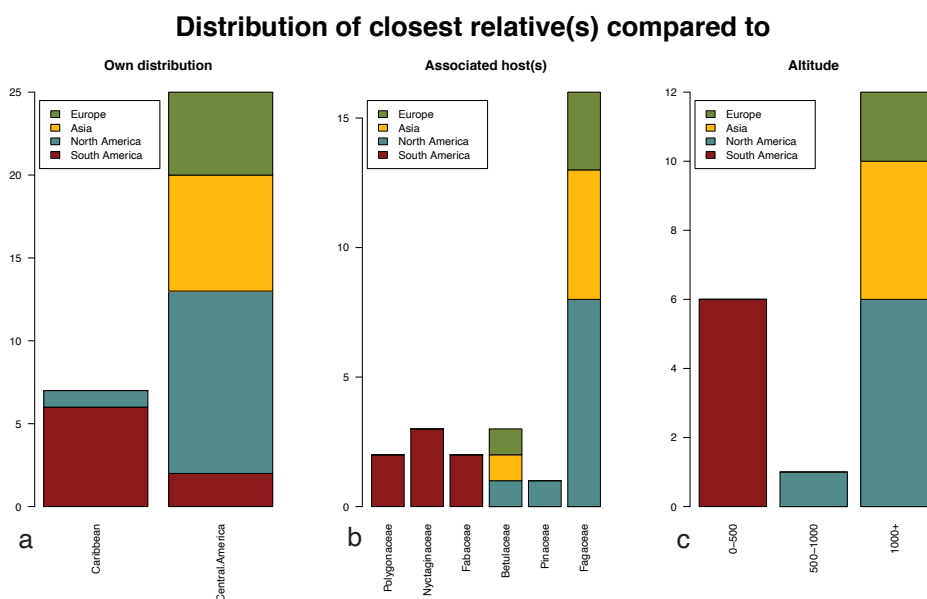


Fig. 14 Number of taxa with closest relative(s) from Europe, Asia, North America or South America compared to: a. Central American or Caribbean distribution; b. different host families of Central American and Caribbean taxa; c. different altitudes of Central American and Caribbean taxa.

remain rare (i.e., known from only one or two collections). Even though intraspecific variation is poorly known or unknown for these rare species, we are convinced it is appropriate to describe them, given the extensive sampling and the clear morphological and molecular differences. Of the eleven species occurring in the Lesser Antilles, eight or nine have not been reported from other areas and can be classified as endemic to the Lesser Antilles. Only *L. panuoides* and *L. venezuelanus* have been reported from the mainland, from French Guiana (Fig. 1) and Venezuela (Dennis 1970), respectively. *Lactifluus neotropicus* might also occur in French Guiana (Fig. 1), but more research is needed to confirm this record. *Lactifluus nebulosus* was previously reported from the British Virgin Islands (Miller et al. 2000), but this collection (GUA-104) was shown to represent a different species: *L. guanensis* (Crous et al. 2019), so no overlap in *Lactifluus* species between the Greater Antilles and the Lesser Antilles has been found thus far. The Greater Antilles are considerably less diverse in *Lactifluus* species than the Lesser Antilles, with only three known species, despite the much larger surface area. Considering the small surface area of the Lesser Antilles, less than 15 000 km², these islands can be considered highly diverse in *Lactifluus* species. This result contrasts with an earlier observed pattern of ectomycorrhizal communities being less diverse in smaller areas (Peay et al. 2007). However, the distribution of these species across the different islands of the Lesser Antilles is still poorly studied. Given the small distances between the islands, it could be expected that most species occur on multiple islands, although it could be possible that some species are endemic to a single island. To investigate this, more sampling is needed across the archipelago.

The case of the Lesser Antilles demonstrates that sampling different forest types is important, even though in tropical climate ectomycorrhizal fungi are considered to be more abundant in xerophytic forests (Pegler & Fiard 1983). Our results confirm the predominance of *Lactifluus* at the xerophytic forests of the Lesser Antilles, with about 75 % of the collections originating from this type of forests. Species occurring in these forests are occasionally also found in hygrophytic or mesophytic forests, often in association with *Guapira fragrans*, a common host tree in xerophytic forests that can be present in mesophytic or hygrophytic forests as well. Despite their association with a host tree that is abundant in xerophytic forests, some species, such as *L. venezuelanus*, show a clear preference for mesophytic or hygrophytic forests. Other species, such as *L. lepus*, *L. marmoratus* and *L. panuoides*, are also exclusively known from these forest types. Because of the presence of these unique species, mesophytic and hygrophytic forests should not be overlooked when studying the diversity of ectomycorrhizal fungi, even though collections can be less abundant and/or less conspicuous, and therefore harder to find.

Diversity of *Lactifluus* spp. in Central America and Mexico

In Central America and Mexico, our phylogeny revealed the presence of at least 21 species of *Lactifluus*, of which only five species have been described. Many of these species occur in species complexes, such as *L. sect. Gerardii*, *L. sect. Lactifluus* and *L. sect. Piperati*. These sections belong to *L. subg. Lactifluus*, and no Neotropical representatives other than *L. fusco-marginatus* were reported for this subgenus before (De Crop et al. 2017). Therefore, it seems likely that these 16 unnamed clades represent new undescribed species. However, given the difficulty of delimiting species in complexes such as these, with morphological differences often being very subtle, these complexes will need detailed study to allow describing these species. Despite the limited number of described species, our phylogeny shows that there is a high diversity of *Lactifluus* spp.

in Central America and Mexico. In addition, many species are known from only one or two collections and are originating from a limited number of regions, so it can be expected that the number of species will rise even more.

Contrasting diversity patterns of *Lactifluus* spp. between Central America/Mexico and the Antilles

No overlap has been found between species from Central America and Mexico and species from the Caribbean, contrary to what was detected for several other ectomycorrhizal fungi associated with *Coccoloba uvifera* or *Pinus* for example (Ortiz-Santana et al. 2007, Pölme et al. 2017). Moreover, some contrasting biogeographical patterns between the two regions can be observed, such as how the species are distributed. In the Antilles, 14 species were found, distributed across seven clades, while in Central America and Mexico, 20 species were found, distributed across 17 clades. This shows that there were a relatively limited number of colonisations in the Antilles, after which some clades radiated, especially *L. sect. Nebulosi*, while in Central America there were many repeated colonisations, with only limited radiation. In addition, the majority of Central American species occur in *L. subg. Lactifluus*, and the majority of Caribbean species in *L. subg. Gymnocarpi*. Another contrasting pattern is their different affinities, with Antillean taxa mainly showing South American affinities, and Central American and Mexican taxa mainly showing North American, Asian and European affinities (Fig. 14). Previously, the Lesser Antilles were suspected to contain *Lactifluus* species of both North and South American origin (Pegler & Fiard 1983, Hackel 2014). However, the Antillean *L. pegleri* turns out to be unrelated to the North American *L. hygrophoroides*. In fact, all Lesser Antillean *Lactifluus* species are embedded within South American clades in the phylogeny (Fig. 1), so they all appear to have more recent common ancestors with South American species. Lesser Antillean species were mainly found associated with *Coccoloba* (*Polygonaceae*) and *Guapira* (*Nyctaginaceae*), both important ectomycorrhizal hosts in South America, showing that host specificity could be responsible for the South American origin of these taxa. In contrast, the three species from the Greater Antilles have mixed origins, with *L. coccolobae* and *L. guanensis* belonging to *L. sect. Nebulosi*, which consists mainly of Lesser Antillean species and has South American affinities, and *L. domingensis* having North American affinities (Fig. 1). *Lactifluus coccolobae* and *L. guanensis* were found with *Coccoloba uvifera* (*Polygonaceae*), while *L. domingensis* was found in montane forests with *Pinus occidentalis* (*Pinaceae*).

In Central America and Mexico on the other hand, the majority of the species were found to have North American, Asian or European affinities, and only few species were found to have South American affinities, despite being connected to this continent. Species with South American affinities are *L. veraecrucis*, of which the host relationship is unknown, and *L. chiapanensis*, which was found associated with *Gymnopodium floribundum*, a species found at low altitudes in Mexico, Guatemala and Belize. The genus *Gymnopodium* is only known from Central America, but belongs to the *Polygonaceae* family, to which also the ectomycorrhizal *Coccoloba* belongs. *Coccoloba* is distributed across Mexico, Central America, the Caribbean and South America, and was reported as a host for most other species in the same section as *L. chiapanensis*. In contrast, other Central American species were found with *Carpinus* (*Betulaceae*), *Fagus* or *Quercus* (*Fagaceae*), important ectomycorrhizal host genera in North America, Europe and Asia. These plant families can, however, also be found in the western mountain ranges in South America, and *L. hallingii* for example, which is associated with *Quercus*, was also reported from localities at high altitudes in Colombia.

Affinities of *Lactifluus* spp. with North and South American taxa in the light of tectonics

The land masses of Central America arose as a result of the subduction of the Cocos plate under the Caribbean plate, with Costa Rica and Panama forming a separate block neighbouring the Nasca plate in the south and the South American plate in the southeast (Kellogg et al. 1995). The land masses emerged first in the northern part of Central America during Oligocene and Miocene, and finally the isthmus was closed probably in the area of Panama approximately until 4 million years ago (e.g., Haug et al. 2001). This process allowed plants and their fungi to move southwards from North America and to colonise Central America before the isthmus was closed, while northwards dispersal from South America was at first impeded by a broad water passage and later only possible by island hopping over an island archipelago that later fused into the land bridge of Panama.

The Antilles are located at the northern and eastern border of the Caribbean plate and are mostly the result of complex subduction events of the Atlantic plate underneath the Caribbean plate. The Caribbean plate originated from the Pacific on the western side of Northern South America and moved first northwards and later eastwards resulting in shear zones with the South American plate until it attained its modern aspect by the end of the Middle Eocene (Freeland & Dietz 1971). Therefore, there were possibly more opportunities for dispersal from South America onto the Antilles than from North America. In the middle Oligocene connections between North temperate flora and at least Puerto Rico existed, as pollen records showed that for example *Fagus* occurred on the island (Graham & Jarzen 1969). However, suitable habitats for the temperate flora were located at high elevation, and due to the intense erosion on Caribbean islands, those habitats have disappeared. The lower elevation of islands today could act as an additional barrier to the dispersion of North American taxa or their Central American relatives.

CONCLUSIONS

In summary, we hypothesise that host specificity, possibly at host family level, is a crucial factor causing the observed biogeographical patterns. Taxa associated with *Fabaceae*, *Polygonaceae* and *Nyctaginaceae* show South American affinities and taxa associated with *Betulaceae*, *Fagaceae* and *Pinaceae* show North American, Asian and European affinities (Fig. 14). Sampling ectomycorrhizae may help to further determine to which degree host specificity shapes *Lactifluus* diversity. However, since different plant taxa exhibit different distributions, the effects will also be strongly associated with abiotic factors, such as climate and altitude. We found that species occurring at low altitudes show affinities to South American taxa, and species occurring at high altitudes, in montane forests, show affinities to North American, Asian and European taxa (Fig. 14). These high altitudes have a similar climate and vegetation to temperate regions, while at low altitudes climate and vegetation are similar to tropical regions. Therefore, it is not possible to separate the effects of climate and host association, and both could be considered fundamental explanatory factors of the ability of a taxon to colonise a certain region. In addition, the different geological histories of Central America/Mexico and the Antilles may also have contributed to the contrasting diversity patterns between the regions.

These results suggest that host specificity and climate could shape phylogenetic patterns in ectomycorrhizal fungi, and encourage studying the effects of host specificity and climate on *Lactifluus* diversity patterns on a global scale.

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