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

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*Basidiomycota*
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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 abbottii*, *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 & Bandala 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 Chiriquí 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 Kornerup & 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 = 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× for spores (Zeiss Axio Scope 2 microscope), 1500× 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.

| | | | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|
| Species | Herbariumnumber | Herbarium | ITS | LSU | *RPB2* | *RPB1* |
| *Lactarius fuliginosus* | MTB 97-24 | GENT | JQ446111 | JQ446180 | JQ446240 | KR364392 |
| *Lactarius hatsudake* | FH 12-052 | GENT | KR364085 | KR364215 | KR364285 | KR364411 |
| *Lactarius miniatescens* | AV 11-177 | GENT | KR364059 | KR364187 | KR364315 | KR364443 |
| *Lactarius olympianus* | ED 08-018 | GENT | KR364089 | KR364220 | KR364320 | KR364448 |
| *Lactarius scrobiculatus* | JN 2001-058 | GENT | KF432968 | – | KR364344 | KR364474 |
| *Lactarius tenellus* | ADK 3598 | GENT | KF133280 | KF133313 | KF133345 | KR364482 |
| *Lactifluus acicularis* | KVP 08-002 | GENT | HQ318226 | HQ318132 | HQ328869 | JN389131 |
| *Lactifluus acrissimus* | EDC 11-112 | GENT | KR364041 | KR364168 | KR364254 | KR364366 |
| *Lactifluus albomembranaceus* | EDC 12-046 | GENT | KR364064 | KR364193 | KR364257 | KR364369 |
| *Lactifluus allardii* | JN 2004-008 | GENT | KF220016 | KF220125 | KF220217 | KR364370 |
| *Lactifluus ambicystidiatus* | HKAS J7008 | HKAS | KR364108 | KR364239 | KR364309 | KR364437 |
| *Lactifluus annulatoangustifolius* | BB 00-1518 | GENT, PC | AY606981 | KR364253 | – | – |
| *Lactifluus annulifer* | TH 9014 | BRG, DUKE | KC155376 | KC155376 | – | – |
| | G4819 | PC | KM073086 | – | – | – |
| *Lactifluus armeniacus* | EDC 14-501 | MFLU, GENT | KR364127 | – | – | – |
| *Lactifluus aurantiifolius* | AV 94-063 | GENT | KR364017 | KR364144 | – | – |
| *Lactifluus aureifolius* | AV 11-074 | GENT | KR364056 | KR364183 | KR364259 | KR364371 |
| *Lactifluus auriculiformis* | AV 12-050 | GENT | KR364086 | KR364216 | KR364260 | KR364372 |
| *Lactifluus bertillonii* | JN 2012-016 | GENT | KR364087 | KR364217 | KR364261 | KR364373 |
| *Lactifluus bhandaryi* | TENN 051830 | TENN | KR364111 | KR364140 | – | – |
| *Lactifluus bicapillus* | EDC 12-176 | GENT | KR364070 | KR364199 | KR364300 | KR364428 |
| *Lactifluus bicolor* | DS 06-247 | GENT | JN388955 | JN388987 | JN375590 | JN389186 |
| *Lactifluus brachystegiae* | AV 99-002 | GENT | KR364018 | KR364145 | KR364262 | KR364374 |
| *Lactifluus brunellus* | TH 9130 | BRG, DUKE | JN168728 | – | – | – |
| *Lactifluus brunneocarpus* | AB 305 | GENT | KR364035 | KR364162 | KR364343 | KR364473 |
| *Lactifluus brunneoviolascens* | AV 13-038 | GENT | KR364123 | KR364246 | KR364264 | KR364376 |
| *Lactifluus brunnescens* | AV 05-083 | GENT | KR364019 | KR364146 | KR364263 | KR364375 |
| *Lactifluus caribaeus* | CL/MART06.014 | LIP | MK046841 | MK046791 | – | – |
| | J.P. Fiard 818A | K | MK046852 | – | – | – |
| | J.P. Fiard 827B | K | MK046853 | – | – | – |
| *Lactifluus carmineus* | AV 99-099 | GENT | KR364131 | KR364251 | KR364265 | – |
| *Lactifluus castaneibadius* | J.P. Fiard 837A | K | MK046854 | – | – | – |
| *Lactifluus chamaeleontinus* | JD 946 | MEISE | KR364079 | KR364208 | KR364267 | KR364377 |
| *Lactifluus chiapanensis* | Bandala 4374a | GENT | GU258297 | GU265580 | GU258316 | KR364378 |
| *Lactifluus chrysocarpus* | LE 253907 | LE | JX442761 | JX442761 | – | – |
| *Lactifluus clarkeae* | MN 2004002 | L | KR364011 | HQ318205 | KR364268 | KR364379 |
| *Lactifluus coccolobae* | OKM-27240 | CFMR | MK774805 | – | – | – |
| *Lactifluus cocosmus* | ADK 4462 | GENT | KR364013 | KR364141 | KR364269 | KR364380 |
| *Lactifluus conchatulus* | LTH 457 | GENT | GU258296 | GU265659 | GU258399 | KR364381 |
| *Lactifluus corrugis* | AV 05-392 | GENT | JQ753822 | KR364143 | JQ348127 | – |
| | SA A12 L2 | GENT | KR364088 | KR364218 | KR364361 | KR364491 |
| *Lactifluus crocatus* | KVP 08-034 | GENT | HQ318243 | HQ318151 | HQ328888 | JN389145 |
| *Lactifluus cyanovirescens* | JD 988 | GENT | KR364082 | KR364211 | KR364270 | KR364382 |
| *Lactifluus denigricans* | EDC 11-218 | GENT | KR364051 | KR364178 | KR364272 | KR364384 |
| *Lactifluus densifolius* | AV 11-111 | GENT | KR364057 | KR364184 | KR364273 | KR364385 |
| *Lactifluus dissitus* | AV-KD-KVP 09-134 | GENT | JN388978 | JN389026 | JN375628 | JN389172 |
| *Lactifluus distantifolius* | LTH 288 | GENT | HQ318274 | HQ318193 | KR364274 | JN389155 |
| *Lactifluus domingensis* | ANGE1035 | JBSD | MK931340 | MN128988 | MK937131 | – |
| | ANGE542 | JBSD | MK931339 | MN128987 | MK937130 | – |
| | ANGE837 | JBSD | MK931342 | MN128989 | MK937133 | MN128629 |
| | ANGE838 | JBSD | MK931341 | MN128990 | MK937132 | – |
| *Lactifluus dwaliensis Asia* | LTH 55 | GENT | KF220111 | KF220204 | KF220278 | KR364386 |
| *Lactifluus edulis* | FN 05-628 | GENT | KR364020 | KR364147 | KR364275 | KR364387 |
| *Lactifluus flammans* | JD 941 | MEISE | KR364078 | KR364207 | KR364303 | KR364431 |
| *Lactifluus flocktonae* | JET1006 | MEL | JX266621 | JX266637 | – | – |
| *Lactifluus foetens* | ADK 2840 | MEISE | KR364023 | KR364150 | KR364279 | KR364391 |
| *Lactifluus fuscomarginatus* | LM4719 | XAL | HQ168368 | – | – | – |
| | LM 4379 | XAL | HQ168367 | HQ168367 | – | – |
| *Lactifluus genevievae* | GG-DK 17-02-05 | GENT | GU258294 | GU265657 | GU258397 | KR364401 |
| *Lactifluus gerardii* | AV 05-375 | GENT | GU258254 | GU265616 | GU258353 | KR364403 |
| | JN 2007-029 | GENT | GU258224 | GU265582 | GU258318 | – |
| *Lactifluus glaucescens* | LGAM 2010-0132 | LGAM-AUA | KR364105 | KR364236 | KR364280 | KR364407 |
| *Lactifluus guadeloupensis* | RC/Guad11-023 | LIP | KP691412 | KP691421 | KP691430 | – |
| *Lactifluus guanensis* | GUA-104 | CFMR | MK046851 | – | – | – |
| *Lactifluus gymnocarpus* | EDC 12-047 | GENT | KR364065 | KR364194 | KR364282 | KR364408 |
| *Lactifluus hallingii* | R. E. Halling 4977 | NY | MK931343 | – | – | – |
| | R. E. Halling 7993 | NY | MK931333 | – | MK937128 | – |
| | NVE 520 | | KF937338 | – | – | – |
| | R. E. Halling 7938 | NY | MK931327 | MN128984 | MK937127 | – |
| | FH 18-077 | GENT | MK931338 | MN128991 | MK937129 | MN128628 |
| *Lactifluus hygrophoroides* | AV 05-251 | GENT | HQ318285 | HQ318208 | HQ328936 | KR364413 |

**Table 1** (cont.)

| | | | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|
| Species | Herbariumnumber | Herbarium | ITS | LSU | *RPB2* | *RPB1* |
| *Lactifluus igniculus* | LE 262983 | LE | JX442759 | JX442759 | – | – |
| *Lactifluus kigomaensis* | AV 11-006 | GENT | KR364052 | KR364179 | KR364288 | KR364415 |
| *Lactifluus kivuensis* | JR Z 310 | GENT | KR364027 | KR364154 | – | – |
| *Lactifluus lamprocystidiatus* | EH 72-195 | GENT | KR364015 | – | – | – |
| *Lactifluus leae* | FH 12-013 | GENT | KF432957 | KR364213 | KR364292 | KR364419 |
| *Lactifluus leonardii* | GG 07-02-04 | | GU258308 | GU265668 | GU258408 | KR364495 |
| *Lactifluus leoninus* | DS 07-454 | GENT | KF220055 | JN388989 | JN375592 | JN389188 |
| *Lactifluus leptomerus* | AV-KD-KVP 09-131 | GENT | JN388972 | JN389023 | JN375625 | JN389169 |
| *Lactifluus lepus* | RC/Guad 08-042 | LIP | KP691414 | KP691423 | KP752179 | – |
| | RC/Guad 05-029 | LIP | MK046811 | MK046774 | – | – |
| *Lactifluus leucophaeus* | LTH 182 | GENT | KF220059 | KF220157 | KF220243 | KR364420 |
| *Lactifluus longipilus* | LTH 184 | GENT | HQ318256 | HQ318169 | HQ328905 | JN389152 |
| *Lactifluus luteolus* | AV 05-253 | GENT | KR364016 | KR364142 | KJ210067 | KR364440 |
| *Lactifluus luteopus* | EDC 11-087 | GENT | KR364049 | KR364176 | KR364312 | KR364441 |
| *Lactifluus madagascariensis* | BB 99-409 | PC | AY606977 | DQ421975 | DQ421914 | – |
| *Lactifluus marmoratus* | LD 15-066 | GENT | MK046805 | MK046768 | MK063907 | MK089574 |
| *Lactifluus melleus* | AV 11-183 | GENT | KR364060 | KR364189 | KR364277 | KR364389 |
| *Lactifluus multiceps* | TH 9154A | BRG, DUKE | JN168731 | – | – | – |
| *Lactifluus murinipes* | 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 | – | – | – |
| *Lactifluus nebulosus* | 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 | – | – | – |
| *Lactifluus neotropicus* | R.W.G. Dennis 97 | K | MN102680 | – | – | – |
| *Lactifluus nonpiscis* | AV 11-137 | GENT | KR364058 | KR364185 | KR364317 | KR364445 |
| *Lactifluus ochrogalactus* | AV/KD/KVP 09-120 | GENT | KR364130 | KR364248 | KR364318 | KR364446 |
| *Lactifluus oedematopus* | KVP 12-001 | GENT | KR364100 | KR364232 | KR364319 | KR364447 |
| *Lactifluus pallidilamellatus* | Montoya 4716 | GENT | JQ753824 | JQ348268 | – | MN128632 |
| | AV 17-012 | GENT | MN102682 | – | – | – |
| | AV 17-015 | GENT | MN102684 | – | – | – |
| *Lactifluus panuoides* | 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 | – | – | – |
| *Lactifluus pegleri* | 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 | – | – | – |
| *Lactifluus pelliculatus* | JD 956 | MEISE | KR364080 | KR364209 | KR364321 | KR364449 |
| *Lactifluus persicinus* | EDC 12-001 | GENT | KR364061 | KR364190 | KR364298 | KR364426 |
| *Lactifluus petersenii* | AV 05-300 | GENT | GU258281 | GU265642 | GU258382 | KR364450 |
| *Lactifluus phlebonemus* | EDC 12-023 | GENT | KR364062 | KR364191 | KR364322 | KR364451 |
| *Lactifluus phlebophyllus* | BB 00-1388 | PC | AY606974 | DQ421979 | DQ421918 | – |
| *Lactifluus pilosus* | LTH 205 | GENT | KR364006 | KR364134 | KR364323 | KR364452 |
| *Lactifluus pinguis* | AV-RW 04-023=LTH117 | GENT | HQ318211 | HG318111 | HQ328858 | JN389126 |
| *Lactifluus piperatus* | 2001 08 19 68 | GENT | KF220119 | KF241840 | KF241842 | KR364453 |
| *Lactifluus pulchellus* | KW 304/FH 12-037 | GENT | KR364092 | KR364223 | KR364306 | KR364434 |
| *Lactifluus pumilus* | EDC 12-066 | GENT | KR364067 | KR364196 | KR364332 | KR364462 |
| *Lactifluus putidus* | 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.)

| | | | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|
| Species | Herbariumnumber | Herbarium | ITS | LSU | *RPB2* | *RPB1* |
| *Lactifluus putidus* (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 | – |
| *Lactifluus ramipilosus* | EDC 14-503 | MFLU, GENT | KR364128 | – | – | – |
| *Lactifluus reticulatovenosus* | EH 6472 | GENT | GU258286 | GU265649 | GU258389 | – |
| *Lactifluus robustus* | JN 2011-074 | GENT | KR364047 | KR364173 | KR364358 | KR364488 |
| *Lactifluus roseolus* | AV 99-160 | GENT | KR364032 | KR364159 | KR364333 | KR364463 |
| *Lactifluus roseophyllus* | JN 2011-076 | GENT | KF220107 | KF220202 | KF220276 | KR364464 |
| *Lactifluus rubiginosus* | JD 959 | MEISE | KR364081 | KR364210 | KR364304 | KR364432 |
| *Lactifluus rubrobrunnescens* | EH 7194 | GENT | KR364115 | – | – | – |
| *Lactifluus rubroviolascens* | EDC 12-051 | GENT | KR364066 | KR364195 | KR364334 | KR364465 |
| *Lactifluus rufomarginatus* | ADK 3358 | MEISE | KR364033 | KR364160 | KR364335 | KR364466 |
| *Lactifluus rugatus* | EP 1212/7 | LGAM-AUA | KR364104 | KR364235 | KR364337 | KR364467 |
| *Lactifluus russulisporus* | REH 9398 | NY | KR364097 | KR364229 | KR364307 | KR364435 |
| *Lactifluus ruvubuensis* | JD 303 | MEISE | KR364009 | KR364137 | KR364310 | KR364438 |
| *Lactifluus sainii* | PUN 7046 | PUN | KM658971 | – | – | – |
| *Lactifluus sepiaceus* | PL 40509 | | GU258287 | GU265650 | GU258390 | KR364475 |
| | PL 34204 | | GU258288 | GU265651 | GU258391 | – |
| *Lactifluus* 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.)

|  |  |  | GenBank accession numbers |  |  |  |
|---|---|---|---|---|---|---|
| Species | Herbariumnumber | Herbarium | ITS | LSU | *RPB2* | *RPB1* |
| *Lactifluus* 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 | – | – | – |
| *Lactifluus subclarkeae* | REH 9231 | NY | KR364095 | KR364227 | KR364346 | KR364477 |
| *Lactifluus subgerardii* | AV 05-269 | GENT | GU258263 | GU265625 | GU258362 | KR364478 |
| *Lactifluus subiculatus* | SLM 10114 | BRG, RMS | JQ405654 | – | – | – |
| *Lactifluus subkigomaensis* | EDC 11-159 | GENT | KR364050 | KR364177 | KR364295 | KR364423 |
| *Lactifluus subpruinosus* | JN 2011-061 | GENT | KR364046 | KR364172 | KR364357 | KR364487 |
| *Lactifluus subvellereus* | AV 05-210 | GENT | KR364010 | KR364138 | KR364347 | KR364479 |
| *Lactifluus subvolemus* | KVP 08-048 | GENT | JQ753927 | – | KR364356 | KR364486 |
| *Lactifluus sudanicus* | AV 11-174 | GENT | HG426469 | KR364186 | KR364348 | KR364480 |
| *Lactifluus tanzanicus* | AV 11-017 | GENT | KR364053 | KR364180 | KR364296 | KR364424 |
| *Lactifluus tenuicystidiatus* | JN 2011-080 | GENT | KR364048 | KR364174 | KR364359 | KR364489 |
| *Lactifluus uapacae* | AV 07-048 | GENT | KR364007 | KR364135 | KR364352 | KR364483 |
| *Lactifluus urens* | EDC 14-032 | GENT | KR364124 | KR364247 | KR364353 | – |
| *Lactifluus vellereus* | ATHU-M 8077 | ATHU-M | KR364106 | KR364237 | KR364354 | KR364484 |
| *Lactifluus velutissimus* | JD 886 | MEISE | KR364075 | KR364204 | KR364355 | KR364485 |
| *Lactifluus venezuelanus* | 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 | – | – | – |
| *Lactifluus veraecrucis* | M 8025 | ENCB | KR364112 | KR364241 | – | – |
| *Lactifluus versiformis* | AV-KD-KVP 09-045 | GENT | JN388967 | JN389031 | JN375632 | JN389177 |
| *Lactifluus vitellinus* | KVP 08-024 | GENT | HQ318236 | HQ318144 | HQ328881 | JN389138 |
| *Lactifluus volemoides* | TS 0705 | GENT | KR364038 | KR364165 | – | – |
| *Lactifluus volemus* | KVP 11-002 | GENT | JQ753948 | KR364175 | KR364360 | KR364490 |
|  | REH 9320 | NY | KR364096 | KR364228 | KR364362 | KR364492 |
| *Lactifluus wirrabara* | GG 24-01-04 |  | GU258307 | GU265667 | GU258407 | KR364494 |
| *Lactifluus xerampelinus* | MH 201176 | GENT | KR364099 | KR364231 | KR364364 | KR364496 |

2. a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000);
3. the region between the conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (*RPB2*), using primers bRPB2-6F and fRPB2-7cR (Liu et al. 1999, Matheny 2005); and
4. the region between domains A and C of nuclear gene encoding the largest subunit of RNA polymerase II (*RPB1*), using primers RPB1-Ac and RPB1-Cr (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 BioloMICS (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 1 000 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).

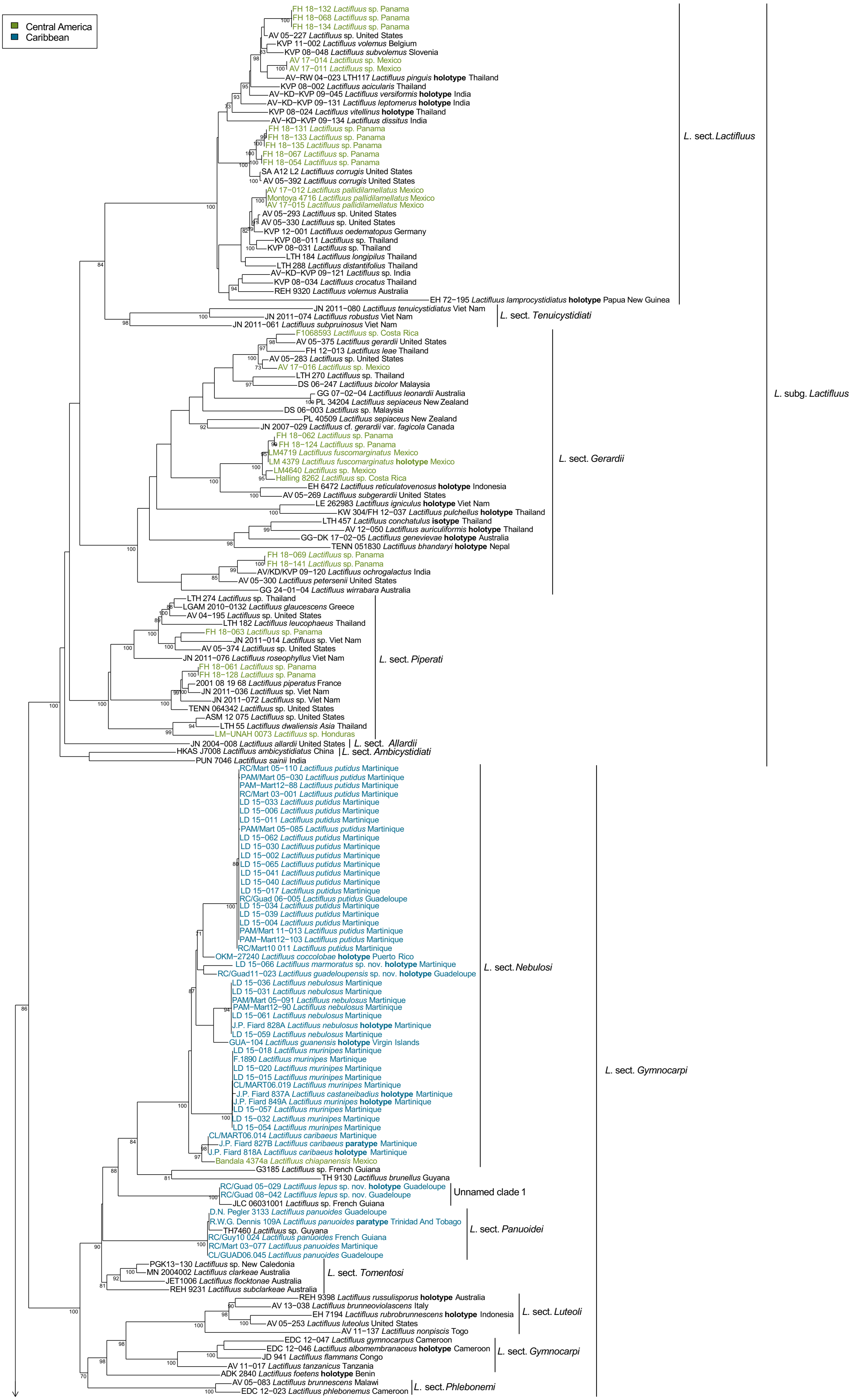

**Fig. 1** Maximum Likelihood tree based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values > 70 are shown.

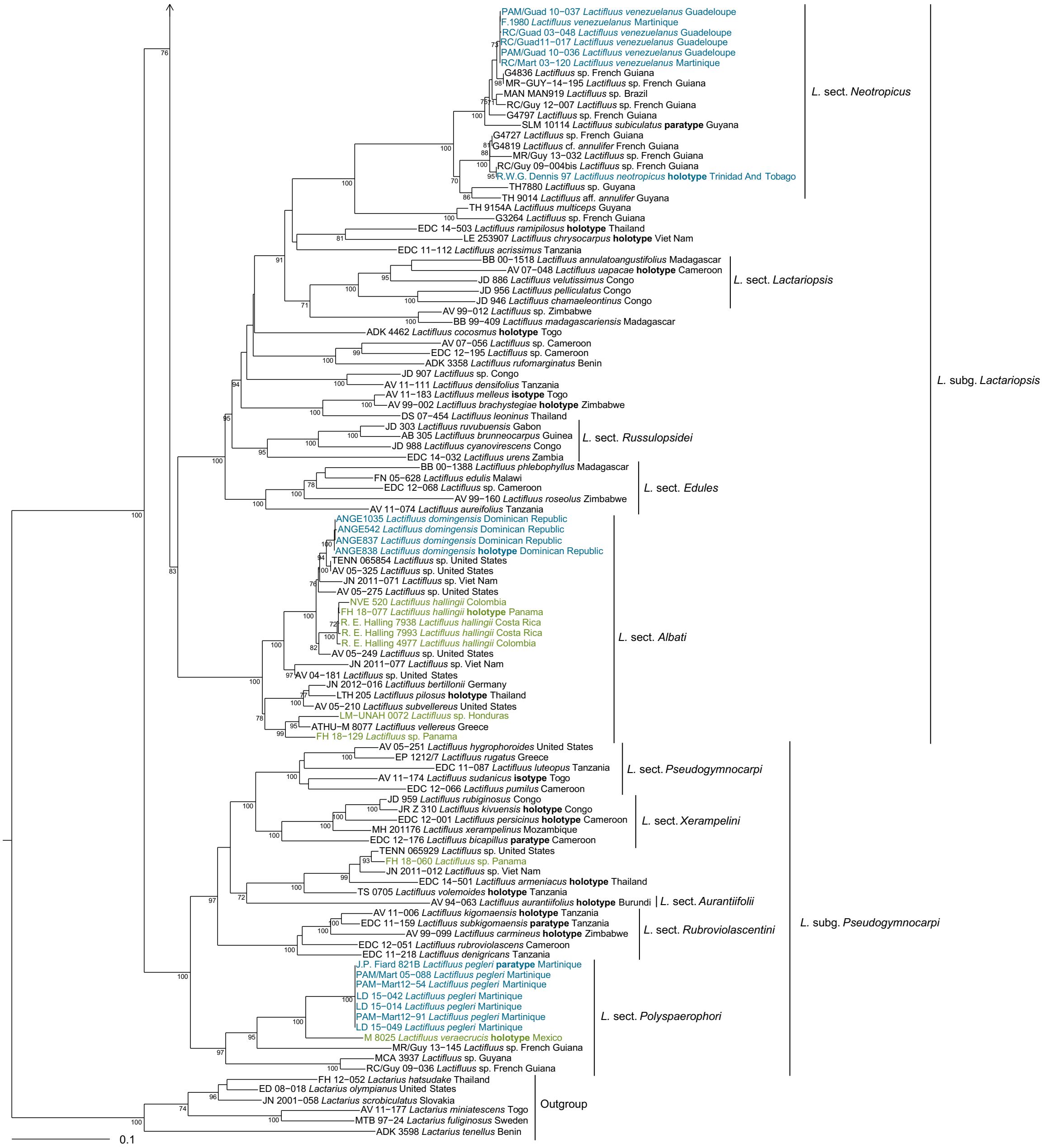

**Fig. 1** (cont.)

## 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–)7.2–8.8 × (5.6–)6.4–7.5(–8) µm, with a heavy reticulum and relatively short terminal elements in the pileipellis, measuring 16–40.8 × 2.4–12.8 µm (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 × (7–)8–*9–9.2*–10.5(–11) µm (Q = 1.08–1.14), with reticulate ornamentation, with rounded to somewhat sharp ridges up to 0.5 µm 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 acrid.
*Basidiospores* subglobose to ellipsoid; ornamentation composed of isolated, rounded warts up to 1 µm high. True *pleuromacrocystidia* present in most species, up to 140–185 µm 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 pleurocystidia, most species of *L.* sect. *Nebulosi* have conspicuous pleuromacrocystidia. 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 $FeSO_4$. *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.

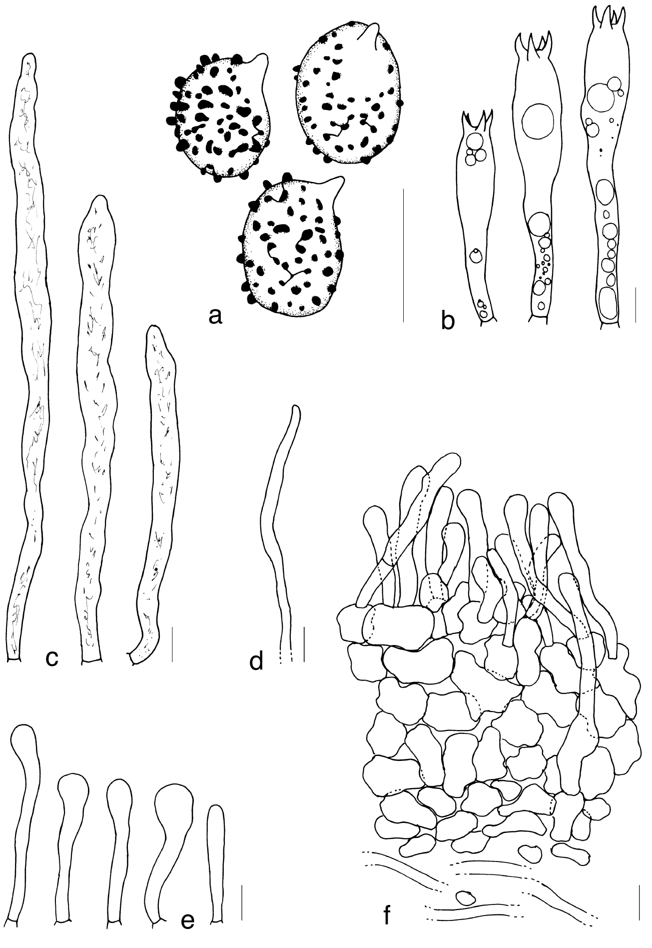

**Fig. 3** *Lactifluus putidus* (LD 15-004, LD 15-011, LD 15-030, LD15-065). a. Basidiospores; b. basidia; c. macrocystidia; d. pseudocystidium; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

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 swarzii*, 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.

### ***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. *Pleuromacrocystidia* 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.

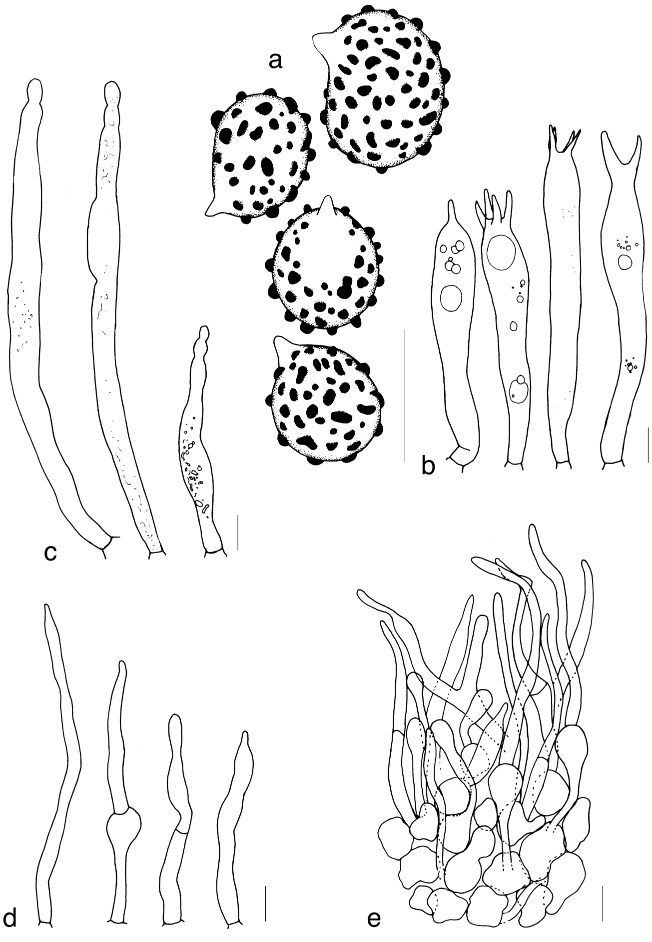

**Fig. 4** *Lactifluus guadeloupensis* (RC/Guad11-023). a. Basidiospores; b. basidia; c. macrocystidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 µm.

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 pleuromacrocystidia 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 $FeSO_4$. *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. *Pleuromacrocystidia* 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

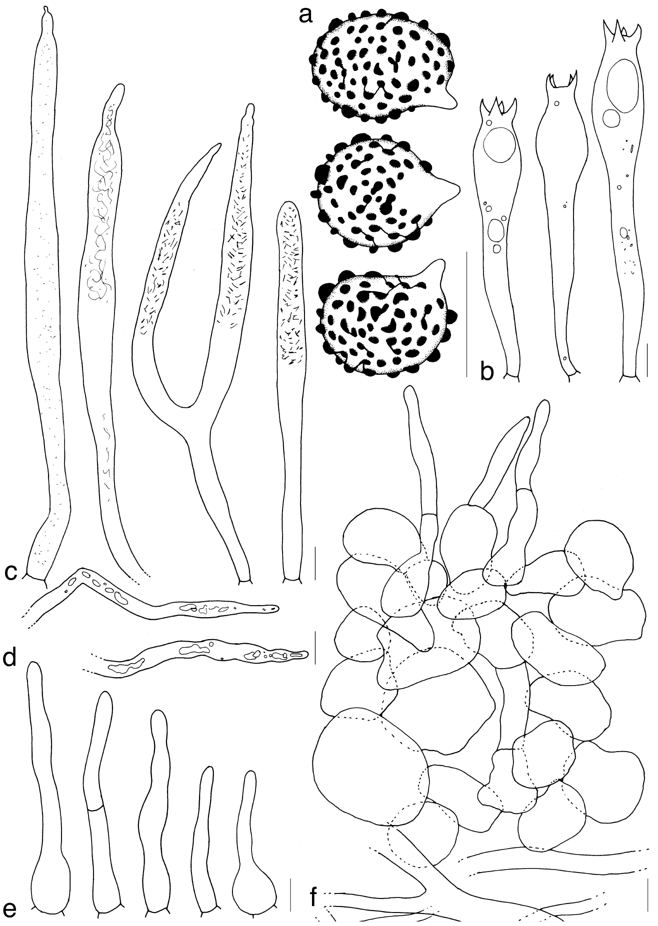

**Fig. 5** *Lactifluus marmoratus* (LD 15-066). a. Basidiospores; b. basidia; c. macrocystidia; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

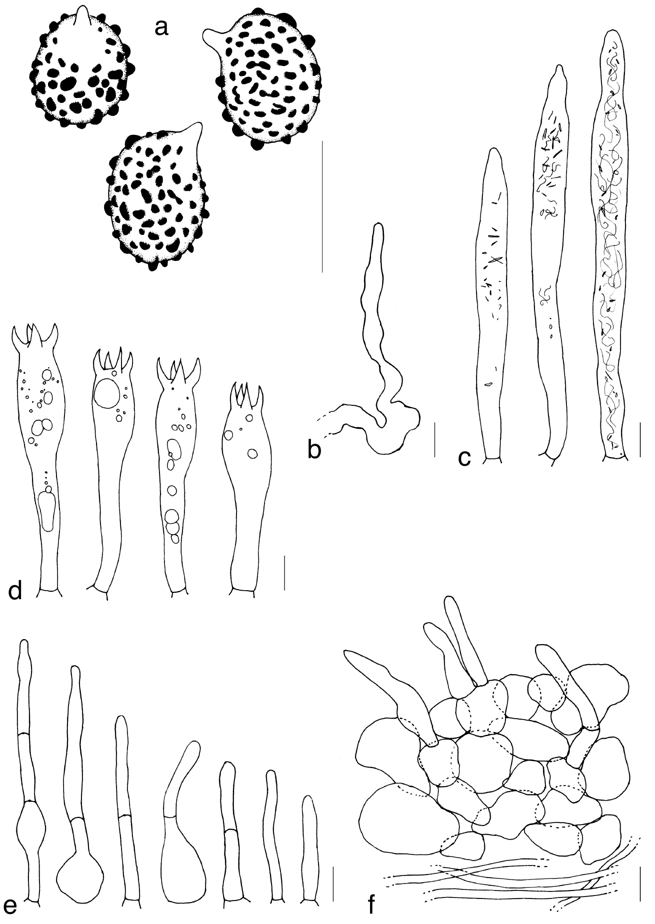

**Fig. 6** *Lactifluus nebulosus* (LD 15-036, LD 15-059, LD 15-061). a. Basidiospores; b. pseudocystidium; c. macrocystidia; d. basidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

pigmentation, often septate; subpellis composed of very large isodiametric cells, 15–45 µm 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, often slightly wrinkled, grey (4C2, 5B-C2, 5D3, 6B1) and white, with some yellow (4A2–4) areas, and brown spots (5D4). *Stipe* 5–30 × 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 $FeSO_4$. *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) × 5.9–*6.9–7.2*–8.4(–9.7) µm (Q = 1.09–*1.30–1.32*–1.46); ornamentation amyloid, composed of rounded warts, up to 1 µm high, isolated, rarely connected by fine connective lines; plage inamyloid. *Basidia* 47.5–*59.5*–72 × 10–*11.5*–12.5(–13) µm, subclavate, 4-spored. *Pleuromacrocystidia* (54–)59.5–*112.5*–166 × 6–*9.5*–13 µm, 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) × 2.5–*4*–5 µm, 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 transvenose, 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 $FeSO_4$. *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.

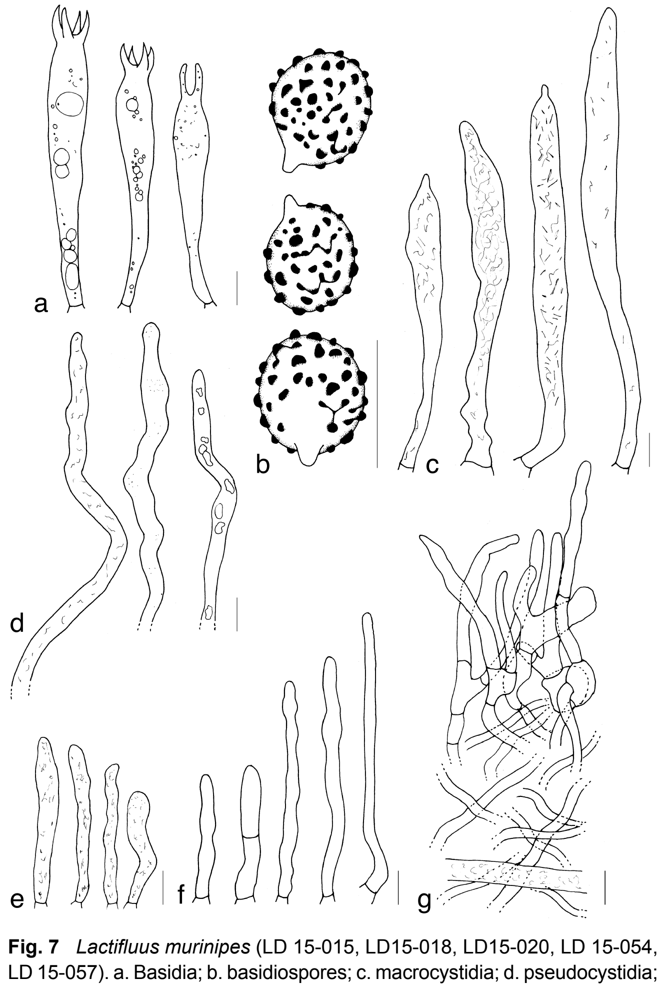

**Fig. 7** *Lactifluus murinipes* (LD 15-015, LD15-018, LD15-020, LD 15-054, LD 15-057). a. Basidia; b. basidiospores; c. macrocystidia; d. pseudocystidia; e. dermatocystidia; f. pileipellis terminal elements; g. section through the pileipellis. — Scale bars = 10 µm.

*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–*8*–9 × 5.5–*7*–8 µm in *L. castaneibadius* and 9–*9.5*–10.5 × 6.5–*7.5*–8.5 µm 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.

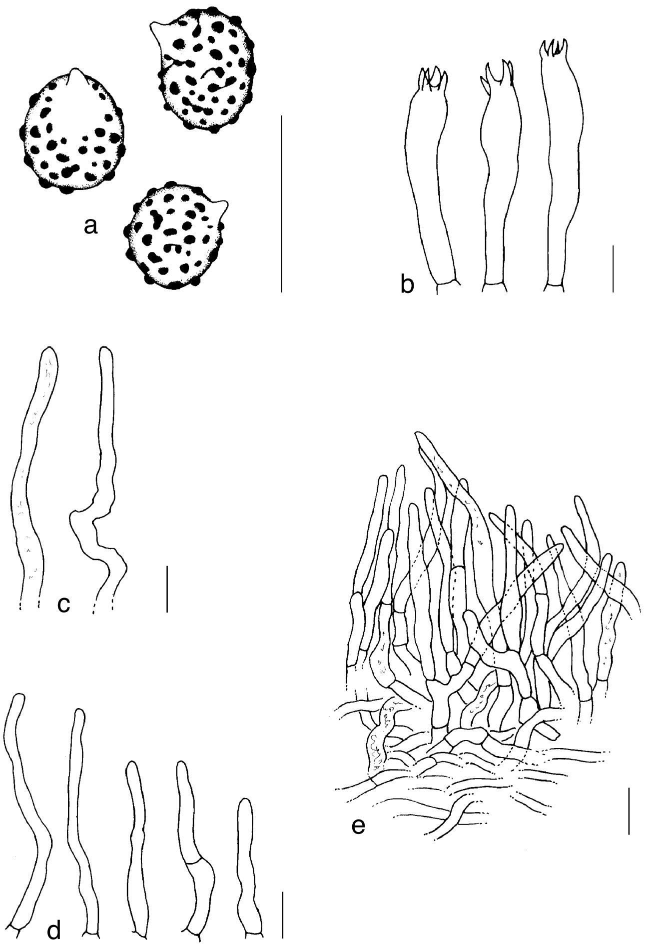

**Fig. 8** *Lactifluus caribaeus* (CL/MART06.014). a. Basidiospores; b. basidia; c. pseudocystidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 µm.

***Lactifluus caribaeus*** (Pegler) Verbeken, 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–*7.6*–8.5 × 5.8–*6.3*–6.8 µm (Q = 1.06–*1.20*–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, cylindric to subclavate, 4-spored. *Pleuromacrocystidia* absent. *Pleuropseudocystidia* 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

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 antigonoides*.

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 92–110.4 × 10.4–13.6 µm (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–*6.1*–6.6 × 4.8–*5.4*–5.9(–6) µm (Q= 1.01–*1.14*–1.27(–1.28)); ornamentation amyloid, composed of isolated rounded warts, up to 1 µm high; plage inamyloid. *Basidia* 53–*65.5*–77.5(–79) × 7.5–*8*–9 µm, subclavate, many 1-, 2- and 4-spored basidia, rarely 3-spored. *Pleuromacrocystidia* 49.5–*85.5*–121.5(–136) × 5–*7*–8.5 µm, abundant, cylindrical to slightly fusiform, sometimes branching, thin-walled, arising from deep in the hymenium. *Pleuropseudocystidia* very abundant, 3–4 µm diam, sometimes branching, not or slightly emergent. *Lamellar edge* sterile; marginal cells 20–40 × 2.5–4 µm. *Subhymenium* cellular. *Hymenophoral trama* filamentous, with hyphae and abundant lactifers. *Pileipellis* a lampropalisade; elements of the suprapellis 28–*78.5*–129.5 × 4–*5*–6 µm, 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.

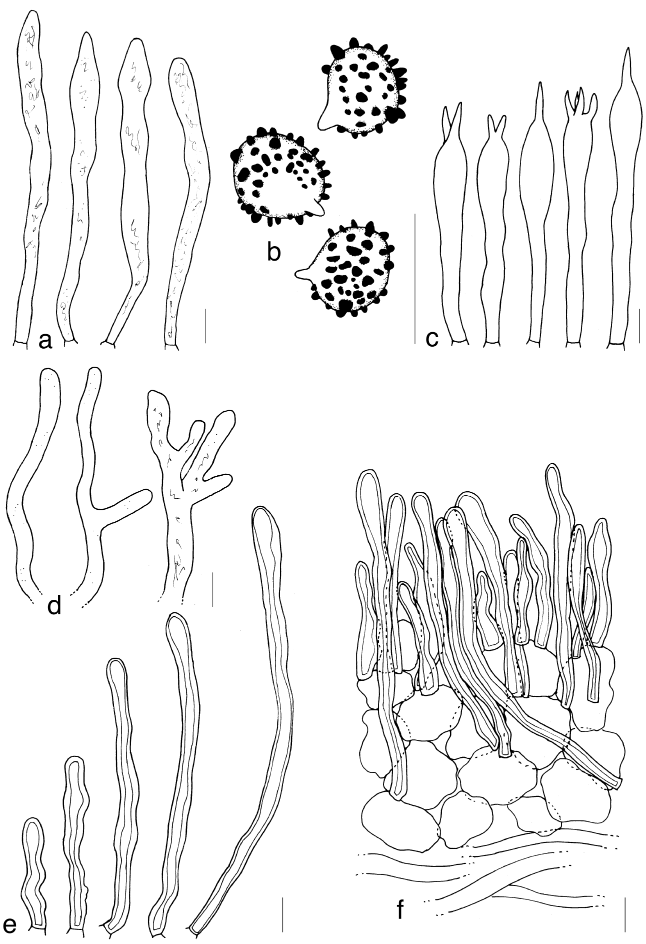

**Fig. 9** *Lactifluus lepus* (RC/Guad 05-029). a. Macrocystidia; b. basidiospores; c. basidia; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

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* 10–23 × 8–24 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

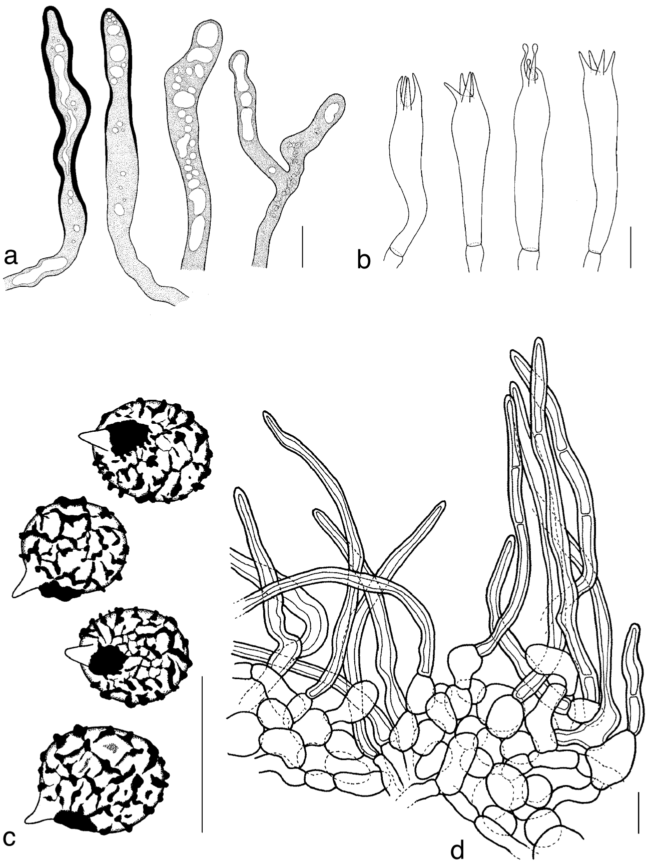

**Fig. 10** *Lactifluus panuoides* (Pegler 3133). a. Pseudocystidia; b. basidia; c. basidiospores; d. section through the pileipellis. — Scale bars = 10 µm.

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) × 5–*5.8*–6.7(–6.9) µm (Q = 1.05–*1.17*–1.29), ornamentation amyloid, composed of rounded to irregular warts, up to 1 µm high, connected by thick connectives, subreticulate, plage completely amyloid. *Basidia* 27–*37.5*–47.5(–48) × 8–*9*–10 µm, subclavate, 4-spored. *Pleuromacrocystidia* absent. *Pleuropseudocystidia* 7.5–9.5 µm 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 × 4.5–*5*–6 µm, 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 × 9–10.5 µm) and spores (6.8–8.4 × (5.6–)6–7.2 µm), 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 × 6.4–8 µm), the plage without a large amyloid spot and the longer pileipellis hairs (40–220 × 4–7 µm) 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. *Panuoidei*, this clade represents *L.* sect. *Panuoidei*, 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

≡ *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, hygrophanous. *Stipe* 15–35 × 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 × 5.2–*5.6*–*5.8*–6.5 µm (Q = 1.06–*1.14*–*1.17*–1.34(–1.39)); ornamentation amyloid, composed of rounded to irregular warts, up to 1 µm high, often connected by lower connective lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 34.5–*43*–52 × 8–*10*–11.5(–12) µm, subclavate, mostly 4-spored, some 1- and 2-spored present. *Pleurocystidia* absent. *Pleuropseudocystidia* abundant, 6–10 µm diam, not or slightly emergent. *Lamellar edge* sterile; marginal cells 15.5–*25*–34.5 × 2.5–*3.5*–4.5 µm, narrowly cylindric 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 × 2.5–*3*–3.5(–4) µm, 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.

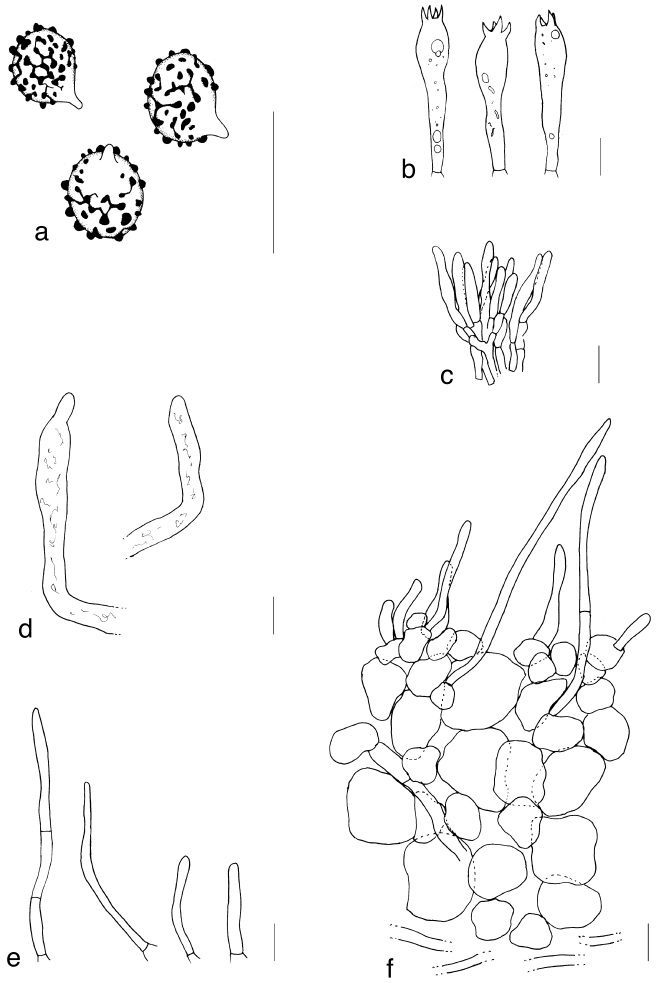

**Fig. 11** *Lactifluus venezuelanus* (RC/Guad 03-048, RC/Guad11-017, RC/Mart 03-120). a. Basidiospores; b. basidia; c. marginal cells; d. pseudocystidia; e. pileipellis terminal elements; f. section through the pileipellis. — Scale bars = 10 µm.

*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 × 15–18 µm wide) and the larger spores (7.6–9.2(–9.6) × 6.4–7.6 µm) (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 × 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) × 5.3–*6.1*–6.9(–7) µm (Q = 1.03–*1.16*–1.30(–1.36)); ornamentation amyloid, composed of rounded warts, up to 0.5 µm high, often connected by lower connective lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 30–*37*–43.5 × 8.5–*9.5*–11 µm, 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 × 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.

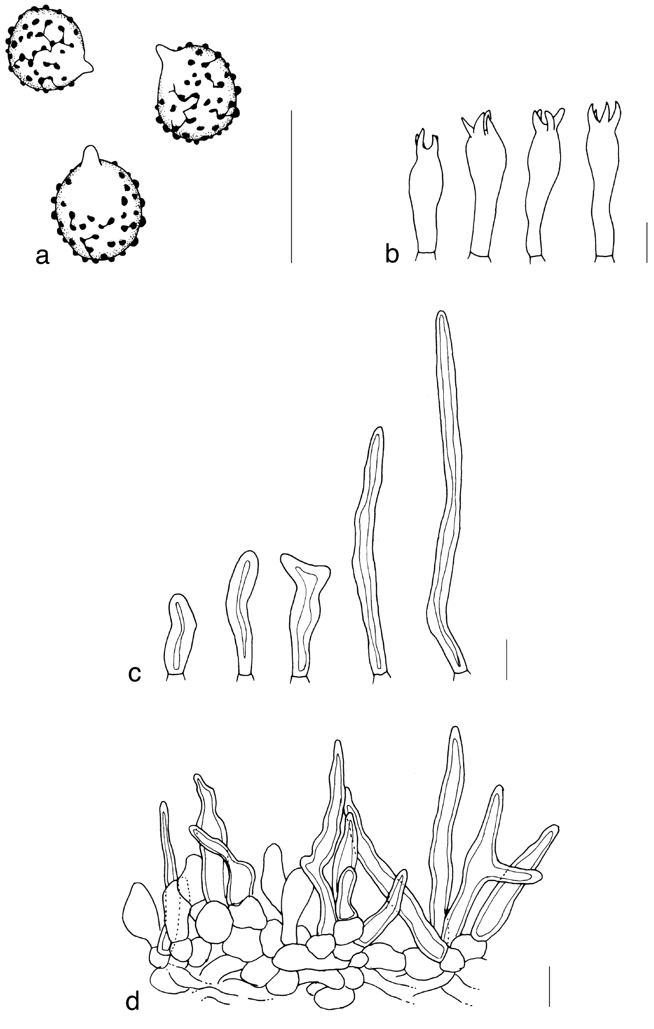

**Fig. 12** *Lactifluus neotropicus* (R.W.G. Dennis 97). a. Basidiospores; b. basidia; c. pileipellis terminal elements; d. section through the pileipellis. — Scale bars = 10 µm.

*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–*64.5*–80 × 7–*8.5*–9.5 µm) 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 (8.8–13 × 8–10 µm) 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 µm), somewhat shorter basidia ((42–)43–*53*–63.5 × 9.5–*12.5*–15.5 µm), 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–*7.4*–*8.2*–9.3 × 5.5–*6.1*–*6.7*–7.5 µm (Q = 1.06–*1.22*–1.38); ornamentation amyloid, composed of rounded warts,

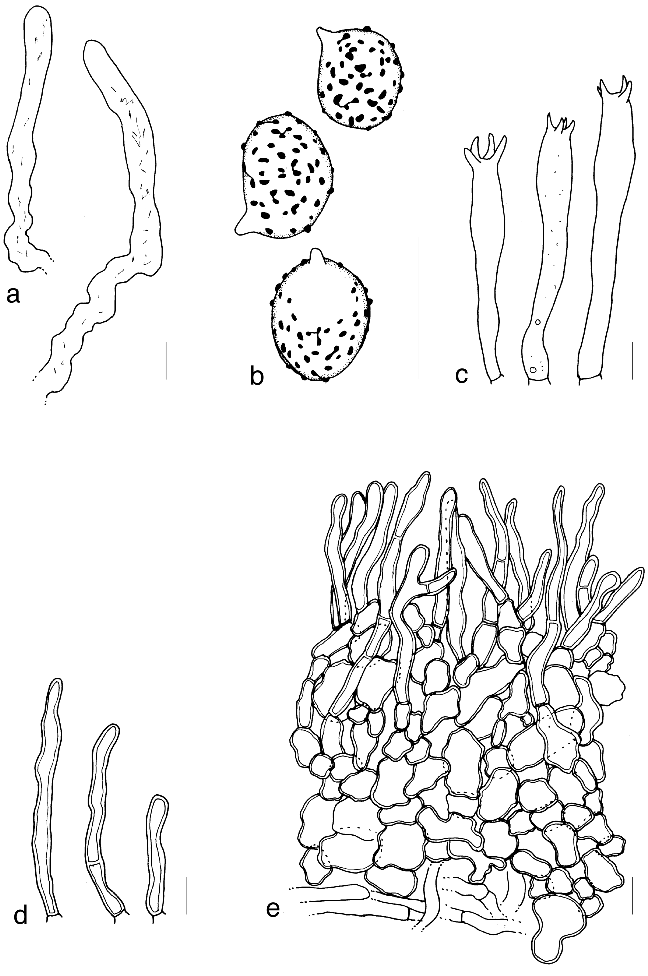

**Fig. 13** *Lactifluus pegleri* (LD 15-042, LD15-049). a. Pseudocystidia; b. basidiospores; c. basidia; d. pileipellis terminal elements; e. section through the pileipellis. — Scale bars = 10 µm.

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 trichopalisade to palisade; pleuromacrocystidia 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 $FeSO_4$; odour not distinctly unpleasant . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . *L. murinipes*
10. Taste of latex and context mild; latex changing colour to brown; context reacting green-grey with $FeSO_4$; 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; pleuromacrocystidia 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 1 000 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 pleuromacrocystidia. 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 (Verbeken & Walleyn 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

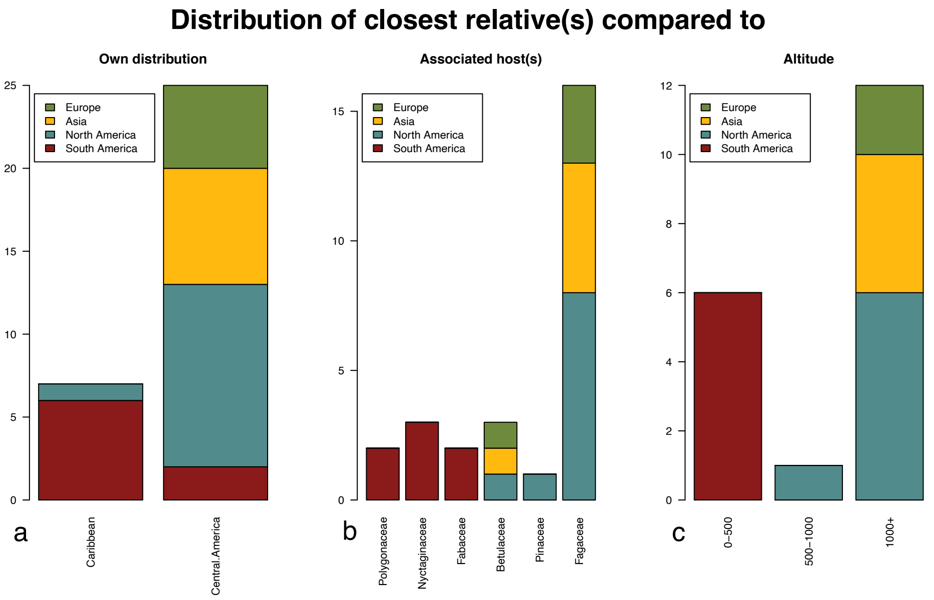

**Fig. 14** Number of taxa with closest relative(s) from Europe, Asia, North America or South America compared to: a. Central American or Caribbean distribution; b. different host families of Central American and Caribbean taxa; c. different altitudes of Central American and Caribbean taxa.

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 km$^2$, 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. fuscomarginatus* 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, Põlme 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 boarder 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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## REFERENCES

Alvarez-Manjarrez J, Garibay-Orijel R, Smith ME. 2018. Caryophyllales are the main hosts of a unique set of ectomycorrhizal fungi in a Neotropical dry forest. Mycorrhiza 28: 103–115.

Beard JS. 1948. The natural vegetation of the Windward and Leeward Island. Ox For Mem.

Buyck B, Hofstetter V, Eberhardt U, et al. 2008. Walking the thin line between Russula and Lactarius: the dilemma of Russula subsect. Ochricompactae. Fungal Diversity 28: 15–40.

Buyck B, Hofstetter V, Verbeken A, et al. 2010. Proposal 1919: To conserve Lactarius nom. cons. (Basidiomycota) with a conserved type. Mycotaxon 111: 504–508.

Crous P, Wingfield M, Lombard L, et al. 2019. Fungal Planet description sheets: 951–1041. Persoonia 44: 223–425.

De Crop E. 2016. Global phylogeny and evolutionary history of the genus Lactifluus. PhD, Biology, Ghent University, Ghent.

De Crop E, Nuytinck J, Van de Putte K, et al. 2017. A multi-gene phylogeny of Lactifluus (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. Persoonia 38: 58–80.

Delgat L, Dierickx G, De Wilde S, et al. 2019. Looks can be deceiving: the deceptive milkcaps (Lactifluus, Russulaceae) exhibit low morphological variance but harbour high genetic diversity. IMA Fungus 10: 14.

Dennis RWG. 1970. Fungus Flora of Venezuela and adjacent countries. Kew Bulletin Additional Series 111: 1–531.

Dentinger B, Margaritescu S, Moncalvo J. 2010. Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. Molecular Ecology Resources 10: 628–633.

Freeland GL, Dietz RS. 1971. Plate tectonic evolution of Caribbean – Gulf of Mexico region. Nature 232: 20–23.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Graham A, Jarzen DM. 1969. Studies in neotropical paleobotany. 1. The Oligocene communities of Puerto Rico. Annals of the Missouri Botanical Garden 56: 308–357.

Hackel J. 2014. Origins and diversification of neotropical taxa in a cosmopolitan lineage of ectomycorrhizal fungi (Basidiomycota: Russulaceae). Master, Université Toulouse III – Paul Sabatier.

Haug GH, Tiedemann R, Zahn R, et al. 2001. Role of Panama uplift on oceanic freshwater balance. Geology 29: 207–210.

Joseph P. 2013. How should the forest types of the Lesser Antilles be described in the intertropical area. Earth Resources 1: 78–102.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298.

Kellogg JN, Vega V, Stallings T, et al. 1995. Tectonic development of Panama, Costa Rica, and the Colombian Andes: constraints from global positioning system geodetic studies and gravity. Special paper of the Geological Society of America 295: 75–90.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. Methuen, London.

Lalli G, Pacioni G. 1992. Lactarius sect. Lactifluus and allied species. Mycotaxon 44: 155–195.

Lanfear R, Frandsen PB, Wright AM, et al. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773.

Le HT, Nuytinck J, Verbeken A, et al. 2007. Lactarius in Northern Thailand: 1. Lactarius subgenus Piperites. Fungal Diversity 24: 173–224.

Liu YJJ, Whelen S, Benjamin DH. 1999. Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Molecular Phylogenetics and Evolution 35: 1–20.

Matheny PB, Liu YJJ, Ammirati JF, et al. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). American Journal of Botany 89: 688–698.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE): 1–8.

Miller OK, Lodge DJ, Baroni TJ. 2000. New and interesting ectomycorrhizal fungi from Puerto Rico, Mona, and Guana Islands. Mycologia 92: 558–570.

Miller SL, Aime MC, Henkel TW. 2002. Russulaceae of the Pakaraima Mountains of Guyana 1. New species of pleurotoid Lactarius. Mycologia 94: 545–553.

Miller SL, Aime MC, Henkel TW. 2012. Russulaceae of the Pakaraima Mountains of Guyana 2. New species of Russula and Lactifluus. Mycotaxon 121: 233–253.

Moncalvo JM, Lutzoni FM, Rehner SA, et al. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic Biology 49: 278–305.

Montoya L, Bandala VM. 2004. Studies on Lactarius: a new species from the Gulf of Mexico area. Cryptogamie Mycologie 25: 15–21.

Montoya L, Bandala VM, Guzmán G. 1996. New and interesting species of Lactarius from Mexico including scanning electron microscope observations. Mycotaxon 57: 411–424.

Montoya L, Bandala VM, Haug I, et al. 2012. A new species of Lactarius (subgenus Gerardii) from two relict Fagus grandifolia var. mexicana populations in Mexican montane cloud forests. Mycologia 104: 175–181.

Nuytinck J, Verbeken A. 2003. Lactarius sanguifluus versus Lactarius vinosus – molecular and morphological analyses. Mycological Progress 2: 227–234.

Ortiz-Santana B, Lodge DJ, Baroni TJ, et al. 2007. Boletes from Belize and the Dominican Republic. Fungal Diversity 27: 247–416.

Panagopoulos N. 1999. A guide to Caribbean vegetation types: Preliminary classification systems and descriptions. Nature Conservancy.

Peay KG, Bruns TD, Kennedy PG, et al. 2007. A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. Ecology Letters 10: 470–480.

Pegler DN, Fiard JP. 1979. Taxonomy and ecology of Lactarius (Agaricales) in the Lesser Antilles. Kew Bulletin 33: 601–628.

Pegler DN, Fiard JP. 1983. Agaric Flora of the Lesser Antilles. HMSO, London.

Põlme S, Bahram M, Kõljalg U, et al. 2017. Biogeography and specificity of ectomycorrhizal fungi of Coccoloba uvifera. Biogeography of mycorrhizal symbiosis: 345–359. Springer.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ [accessed 14 June 2019].

Singer R. 1952. Russulaceae of Trinidad and Venezuela. Kew Bulletin 7: 295–301.

Singer R. 1973. Diagnoses Fungorum Novorum Agaricalium III. Beihefte Sydowia 7: 1–106.

Singer R, Araujo I, Ivory MH. 1983. The ectotropically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. (Litter decomposition and ectomycorrhiza in Amazonian forests 2). Beihefte zur Nova Hedwigia 77: 1–352.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771.

Stehle H. 1947. La végétation sylvatique de l'Archipel Caraïbe.

Stiller JW, Hall BD. 1997. The origin of red algae: Implications for plasmid evolution. Proceedings of the National Academy of Sciences of the United States of America 94: 4520–4525.

Stubbe D, Nuytinck J, Verbeken A. 2010. Critical assessment of the Lactarius gerardii species complex (Russulales). Fungal Biology 114: 271–283.

Tamura K, Stecher G, Peterson D, et al. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Tedersoo L, Sadam A, Zambrano M, et al. 2010. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. Isme Journal 4: 465–471.

Tel-Zur N, Abbo S, Myslabodski D, et al. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera Hylocereus and Selenicereus (Cactaceae). Plant Molecular Biology Reporter 17: 249–254.

Van de Putte K. 2012. Hidden diversity exposed: A case study of Lactifluus volemus sensu lato. PhD, Biology, Ghent University, Ghent.

Van de Putte K, Nuytinck J, De Crop E, et al. 2016. Lactifluus volemus in Europe: three species in one – revealed by a multilocus genealogical approach, Bayesian species delimitation and morphology. Fungal Biology 120: 1–25.

Van de Putte K, Nuytinck J, Stubbe D, et al. 2010. Lactarius volemus sensu lato (Russulales) from northern Thailand: morphological and phylogenetic species concepts explored. Fungal Diversity 45: 99–130.

Verbeken A, Van de Putte K, De Crop E. 2012. New combinations in Lactifluus. 3. L. subgenera Lactifluus and Piperati. Mycotaxon 120: 443–450.

Verbeken A, Walleyn R. 2010. Monograph of Lactarius in tropical Africa. Fungus Flora of Tropical Africa, National Botanic Garden, Belgium: 161 + 154 plates.

White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al. (eds), PCR protocols: a guide to methods and applications: 315–322. Academic Press, New York.