



A multi-gene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus

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Abstract Infrageneric relations of the genetically diverse milkcap genus *Lactifluus* (Russulales, Basidiomycota) are poorly known. Currently used classification systems still largely reflect the traditional, mainly morphological, characters used for infrageneric delimitations of milkcaps. Increased sampling, combined with small-scale molecular studies, show that this genus is underexplored and in need of revision. For this study, we assembled an extensive dataset of the genus *Lactifluus*, comprising 80 % of all known species and 30 % of the type collections. To unravel the infrageneric relationships within this genus, we combined a multi-gene molecular phylogeny, based on nuclear ITS, LSU, *RPB2* and *RPB1*, with a morphological study, focussing on five important characteristics (fruit body type, presence of a secondary velum, colour reaction of the latex/context, pileipellis type and presence of true cystidia). *Lactifluus* comprises four supported subgenera, each containing several supported clades. With extensive sampling, ten new clades and at least 17 new species were discovered, which highlight the high diversity in this genus. The traditional infrageneric classification is only partly maintained and nomenclatural changes are proposed. Our morphological study shows that the five featured characteristics are important at different evolutionary levels, but further characteristics need to be studied to find morphological support for each clade. This study paves the way for a more detailed investigation of biogeographical history and character evolution within *Lactifluus*.

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INTRODUCTION

Russulales

Over the last two decades, molecular research strongly influenced and innovated our traditional view of the order *Russulales* (Larsson & Larsson 2003, Miller et al. 2006, Buyck et al. 2008). It soon became obvious that Friesian and other traditional classification systems overemphasised the phylogenetic importance of basidiocarp shape and hymenophore type. The genera *Russula* and *Lactarius* are different from other agaricoid mushrooms and hence were classified in their own order *Russulales* (Kreisel 1969, Oberwinkler 1977), among others supported by microscopic features such as sphaerocytes in the trama, amyloid spore ornamentation and a gloeoplerous hyphal system. As predicted, taxa with other basidiocarp types had to

be included in this order (Romagnesi 1948, Donk 1971, Oberwinkler 1977, Larsson & Larsson 2003). Molecular data reveal strong support for a russuloid clade with corticioid, resupinate, discoid, effused-reflexed, clavarioid, pileate and sequestrate taxa with smooth, poroid, hydroid, lamellate or labyrinthoid hymenophores, not all of them sharing sphaerocytes and amyloid spore ornamentation. There is morphological support for this *Russulales* clade in the presence of gloeocystidia or a gloeoplerous hyphal system (Larsson & Larsson 2003, Miller et al. 2006). *Russula*, *Lactarius* and some pleurotoid and sequestrate genera form an important group within this clade and are considered the *Russulaceae* Lotsy (Redhead & Norvell 1993, Miller et al. 2001, Larsson & Larsson 2003, Nuytinck et al. 2003, Eberhardt & Verbeke 2004).

Russulaceae

Generic concepts in the mushroom-forming *Russulaceae* changed when it became clear that pleurotoid, sequestrate and veiled forms originated several times, both in *Lactarius* and *Russula*. Morphological and molecular studies of pleurotoid *Russulaceae* species (Verbeke 1998b, Buyck & Horak 1999, Henkel et al. 2000), indicated that those species were placed within either *Russula* or *Lactarius*. Hence, the genus *Pleurogala*, which was erected to accommodate pleurotoid species formerly included in *Lactarius* sect. *Panuoidei* (Redhead & Norvell 1993), was abandoned. Sequestrate species also occur both in *Lactarius* (formerly placed in *Arcangeliiella*, *Gastrolactarius* and *Zelleromyces*) and *Russula* (formerly placed in *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Martellia* and *Macowanites*) (Calonge & Martín 2000, Miller et al. 2001, Binder & Bresinsky 2002, Desjardin 2003, Nuytinck et al. 2003, Eberhardt & Verbeke 2004, Lebel & Tonkin 2007, Verbeke et al. 2014). Species with

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a secondary velum occur both in *Lactarius* and *Russula* and were placed in separate genera (Hennings 1902, Heim 1937, Redhead & Norvell 1993), which was not accepted by Verbeken (1998b). Later, molecular analyses indicated that the *Russulaceae* family also contains several corticioid taxa from three genera: *Boidinia*, *Gloeopeniophorella* and *Pseudoxenasma* (Larsson & Larsson 2003, Miller et al. 2006). *Lactarius* and *Russula* species are ectomycorrhizal, the corticioid taxa are reported to be saprotrophic (Larsson & Larsson 2003, Miller et al. 2006, Tedersoo et al. 2010). However, this is questioned by Miller et al. (2006), who suggest that these corticioid taxa might also be ectomycorrhizal symbionts.

With the inclusion of more tropical taxa, phylogenetic data showed that *Lactarius* and *Russula* are not two well-defined and separate clades. *Russula* appears to be monophyletic only if a small group of species is excluded. This small group forms a clade where *Lactarius* and *Russula* are mixed and it was described as the new genus *Multifurca* (Buyck et al. 2008). The former *Russula* subsect. *Ochricompectae*, the Asian *Russula zonaria* and the American *Lactarius furcatus* were included in this genus. *Multifurca* species are characterised by furcate lamellae, dark yellowish lamellae and spore-prints, a strong zonation of pileus and context and the absence or presence of latex. The remainder of *Lactarius* falls in two different clades (Buyck et al. 2008). The proposal to conserve *Lactarius* (hereafter abbreviated as *L.*) with a conserved type *L. torminosus* (Buyck et al. 2010) was accepted by the 2011 International Botanical Congress (McNeill et al. 2011). The name *Lactarius* is therefore retained for the larger, mainly temperate clade. The subgenera *L. subg. Lactarius* (the former *L. subg. Piperites*), *L. subg. Russularia* and *L. subg. Plinthogalus* now constitute the larger genus *Lactarius* sensu novo. The smaller, mainly tropical clade, with approximately 150 described species (25 % of the known milkcap species), belongs to the genus *Lactifluus* (hereafter abbreviated as *Lf.*) and is typified by *Agaricus lactifluus*, currently known as *Lf. volemus* (Buyck et al. 2010). New combinations were made in a series of three papers for the subgenera *Lf. subg. Lactariopsis*, *Lf. subg. Russulopsis*, *Lf. subg. Edules*, *Lf. subg. Gerardii*, *Lf. subg. Lactifluus* and *Lf. subg. Piperati* (Verbeken et al. 2011, 2012, Stubbe et al. 2012b). No synapomorphic characteristics have been found to consistently separate the genera *Lactarius* and *Lactifluus* and the morphological distinction between the genera is thus far based on several trends. The genus *Lactifluus* is generally characterised by the complete absence of zonate and viscose to glutinose caps. It contains many species with veiled and velvety caps, as well as all known pleurotoid milkcap species (Buyck et al. 2008, Verbeken & Nuytinck 2013). So far, no sequestrate species are known within the genus *Lactifluus*.

Lactifluus

The milkcap genus *Lactifluus* is predominantly represented in the tropics. The highest diversity of the genus is known from Africa (Verbeken & Walley 2010) and Asia (Le et al. 2007b, Stubbe et al. 2010, Van de Putte et al. 2010), but recent studies indicate that the genus is also well-represented in South America (Henkel et al. 2000, Miller et al. 2002, Smith et al. 2011, Sá et al. 2013, Sá & Wartchow 2013). Typical host plants are leguminous trees (*Fabaceae*), members of the *Dipterocarpaceae* and the *Fagaceae*, and of the genera *Uapaca* (*Phyllanthaceae*), *Eucalyptus* and *Leptospermum* (*Myrtaceae*). Due to its mainly tropical distribution, the genus is rather understudied, but more and more species are recognised and described (Wang & Verbeken 2006, Van de Putte et al. 2010, 2012, De Crop et al. 2012, Miller et al. 2012, Stubbe et al. 2012a, Wang et al. 2012, Morozova et al. 2013, Sá et al. 2013, Sá & Wartchow 2013, Maba et al. 2014).

Lactifluus is known for its molecular diversity, with several species complexes (Stubbe et al. 2010, 2012a, Van de Putte et al. 2010, 2012, 2016, De Crop et al. 2014) and species on long and isolated branches (Buyck et al. 2007, Van de Putte et al. 2009, Morozova et al. 2013, Wang et al. 2015). Previous studies questioned the traditional subgenera and sections (Buyck et al. 2008) or even indicated that *Lactifluus* might be paraphyletic (Verbeken et al. 2014). These confusing results emphasize the need for a thorough study, since a genus-wide analysis of *Lactifluus* has never been published.

Current classification of Lactifluus

During the last decade, important changes were published regarding the infrageneric classification of the genus *Lactifluus*. The genus presently contains six subgenera and one unclassified section. A summarizing overview of the situation prior to our global phylogenetic analysis is given here.

Lactifluus subg. Lactariopsis

Lactifluus subg. *Lactariopsis* was traditionally divided into three sections: *Lf. sect. Lactariopsis*, *Lf. sect. Chamaeleontini* and *Lf. sect. Albati* (Verbeken 1998b, Verbeken et al. 2011). These sections were placed together especially based on similarities in pileipellis structure, such as the lack of a pseudoparenchymatous layer in combination with the presence of thick-walled hairs. In the phylogeny of Buyck et al. (2008), *Lf. subg. Lactariopsis* appears to be paraphyletic, with the temperate *Lf. sect. Albati* splitting off from the remaining, predominantly African part of the subgenus. Even though this was noticed, *Lf. sect. Albati* is still considered a section within *Lf. subg. Lactariopsis* by Verbeken et al. (2011) pending a more complete phylogenetic analysis. *Lactifluus* sect. *Lactariopsis* and *Lf. sect. Chamaeleontini* were originally separated based on the presence or absence of a secondary velum and the pileipellis structure (Verbeken 2001, Verbeken et al. 2012). However, the presence of a secondary velum seems to be of limited taxonomic value at this level, as molecular data show that species of both sections intermix in the phylogeny and the monophyly of neither section is supported (Buyck et al. 2007, 2008, Wang et al. 2015).

- *Lactifluus* sect. *Albati* occurs in temperate regions and consists of six known species with firm and white basidiocarps, a velutinous cap and acrid milk. Microscopically they can be recognised by a (lampro) trichoderm as pileipellis, pseudocystidia that are not emergent and the presence of macrocystidia (Heilmann-Clausen et al. 1998, Verbeken 1998b).
- *Lactifluus* sect. *Chamaeleontini* and *Lf. sect. Lactariopsis* mainly occur in tropical Africa, with some exceptions in South-East Asia and South America (Singer 1952, Verbeken & Horak 1999, Miller et al. 2012, Morozova et al. 2013). Species of *Lf. sect. Chamaeleontini* can be recognised by a pileipellis with scattered or absent thick-walled elements, the absence of secondary velum and emergent to highly emergent pseudocystidia. Species of *Lf. sect. Lactariopsis* are characterised by a pileipellis entirely composed of thick-walled elements, emergent to highly emergent pseudocystidia and the presence of a secondary velum, forming a clear annulus (Verbeken 1996a, 1998b, Verbeken & Walley 2010). *Lactifluus* sect. *Lactariopsis* also contains several pleurotoid species from South America and Southeast Asia (Verbeken 1998b, Miller et al. 2012, Morozova et al. 2013).

Lactifluus subg. Edules

This subgenus exclusively consists of African species, which are generally characterised by firm basidiocarps with yellowish to greyish orange to pinkish colours and a cap that is dry and often

cracked, a trichoderm or trichopalisade as pileipellis and a spore ornamentation lower than 0.3 μm (Verbeken 1996a, Verbeken & Walley 1999, 2010). When it was described, the position of *Lf. sect. Edules* within the genus was uncertain (Verbeken 1995, 1996a) and later the section remained unclassified (Buyck et al. 2008). When recombining this section into *Lactifluus*, Verbeken et al. (2011) decided to treat this section on subgenus rank, as *Lf. subg. Edules*.

Lactifluus subg. *Russulopsis*

Verbeken (2001) and Verbeken et al. (2011) proposed this subgenus which includes only one section, *Lf. sect. Russulopsidei*, comprising eight species endemic to tropical Africa. Species are characterised by a dry to viscid pileus, reddish colours in pileus and stipe, and several striking microscopic features such as diverticulate and frequently branched pseudocystidia and a cutis-like pileipellis with distinct dermatocystidia, a character common in *Russula* but rarely observed in milkcaps (Verbeken 1996a, Verbeken & Walley 2010).

Lactifluus subg. *Lactifluus*

Lactifluus subg. *Lactifluus* is the largest subgenus and contains eight sections. The main characteristic of this subgenus is a palisade or palisade-like structure in the pileipellis.

- *Lactifluus* sect. *Lactifluus* contains species occurring throughout Europe, North America and Asia. Its members can be distinguished from species of other sections by a combination of several distinctive microscopic and macroscopic characteristics. Microscopically, they have a lampropalisade as pileipellis, hymenial lamprocystidia and reticulate spore ornamentation. Macroscopically, they can be recognised by clay-buff to orange-brown or reddish brown velutinous caps, abundant white latex that turns brownish when in contact with the flesh and a fish-like odour. Van de Putte et al. (2010, 2012, 2016) discovered a large diversity of cryptic to semi-cryptic species within this section.
- *Lactifluus* sect. *Polysphaerophori* is a predominantly African section, with only one South American representative, *Lf. veraecrucis*. Verbeken & Walley (2010) synonymised *L. sect. Gymnocarpi* with this section, as was also suggested by Montoya et al. (2007). The main characteristics are a strongly wrinkled pileus, a lampropalisade as pileipellis with a suprapellis thicker than the subpellis, the absence of true pleurocystidia, a more or less reticulate spore ornamentation, a hymenophoral trama mainly composed of sphaerocytes and a context that often changes green with FeSO_4 (Verbeken 1996a, Verbeken & Walley 2010).
- *Lactifluus* sect. *Phlebonemi* is mainly represented by African species, although it contains some Asian and European representatives. It is characterised by spores with almost isolated rounded warts with some very fine connective lines and little to no reaction of the context with FeSO_4 (Verbeken 1996a, Verbeken & Walley 2010). Similar to *Lf. sect. Lactifluus* they have latex that immediately changes brown and a fish-like odour, but they differ from that section by their hymenophoral trama mainly composed of narrow hyphae. The distinction between this section and *Lf. sect. Polysphaerophori* is mainly based on differences in spore ornamentation, but Verbeken & Walley (2010) state that this division might be artificial and was only conserved for practical reasons.
- *Lactifluus* sect. *Pseudogymnocarpi* contains seven species, which are all endemic to tropical Africa. The section is characterised by a lampropalisade as pileipellis, the presence of conspicuous lamprocystidia, elongate spores with a low incomplete to complete reticulum and often a central amyloid spot at the plage and a salmon pink reaction of the context with FeSO_4 (Verbeken 1996a, Verbeken & Walley 2010).
- *Lactifluus* sect. *Rubroviolascetini* is a tropical African section containing two species characterised by a palisade as pileipellis, the presence of lamprocystidia, an extremely low spore ornamentation, an inamyloid plage and latex changing from white-buff, to red and finally black when exposed to air (Verbeken 1996a, Verbeken & Walley 2010). The section was distinguished from *Lf. sect. Pseudogymnocarpi* based on the blackening context. However, Verbeken & Walley (2010) note that this distinction is artificial and was only maintained for practical reasons.
- *Lactifluus* sect. *Tomentosi* contains species from Europe, Asia and Oceania, as Verbeken et al. (2012) synonymised *L. sect. Rugati* with this section. It can be recognised by a combination of characters: a dry and cracked pileus with yellow-orange to reddish brown colours, a palisade as pileipellis, a subpellis thicker than the suprapellis, the absence of true pleurocystidia, a more or less reticulate spore ornamentation, a hymenophoral trama mainly composed of sphaerocytes and a context that stains pink with FeSO_4 (Verbeken 1996a, Verbeken & Walley 2010).
- *Lactifluus* sect. *Tenuicystidiati* is an Asian section, recently proposed by Wang et al. (2015). The type of this section was originally placed in *L. sect. Pseudogymnocarpi*, by Wang & Verbeken (2006) due to the morphological similarity to some species of that section. However, this was not supported by molecular results, which suggested a closer affinity with *Lf. sect. Lactifluus*. Because of the clear morphological delimitation between *Lf. sect. Tenuicystidiati* and *Lf. sect. Lactifluus*, this group is now treated as a new section, sister to *Lf. sect. Lactifluus* (Wang et al. 2015). It is characterised by a combination of characteristics: a lampropalisade as pileipellis with slightly thick-walled terminal cells, thin-walled and slender macrocystidia and ellipsoid spores with low and more or less connected ornamentation.
- *Lactifluus* sect. *Ambicystidiati* currently contains only one species known from Asia, *Lf. ambicystidiatus*. This species shows a combination of striking characteristics: an undeveloped lactiferous system and the presence of both macro- and lamprocystidia. Wang et al. (2015) treated *Lf. sect. Ambicystidiati* as an independent section within the genus *Lactifluus*, as this species shows no morphological similarity with any other taxon within the subgenus.

Lactifluus subg. *Gerardii*

Due to striking morphological similarities, *Lf. gerardii* and allies were long considered to belong to *L. subg. Plinthogalus* (Hesler & Smith 1979). Using a combination of molecular and morphological data, Stubbe et al. (2010) found that they form a separate group and actually belong to the genus *Lactifluus* instead of *Lactarius*. These species were transferred to *Lf. subg. Gerardii*, which contains up to 30 described species. The subgenus is distributed in Asia, North and Central America and Australasia. In most cases species in *Lf. subg. Gerardii* can be recognised by a combination of five characteristics: a white spore print, reticulate spore ornamentation not higher than 2 μm , a palisade structure in the pileipellis with globose cells in the subpellis, the lack of macrocystidia and a general habitus of a brown pileus and stipe with contrasting white and mostly distant lamellae (Stubbe et al. 2010). This subgenus also contains several pleurotoid species that are morphologically different, because they lack the general habitus and the striking dark pigmentation of this subgenus and have macrocystidia in their hymenium.

Lactifluus subg. *Piperati*

This subgenus with a Northern hemispherical distribution contains two sections: *Lf.* sect. *Piperati* and *Lf.* sect. *Allardii*. *Lactifluus* sect. *Piperati* contains at least 10 different species distributed over three groups (De Crop et al. 2014) and all of them are characterised by firm, whitish basidiocarps and a hyphoepithelium as pileipellis type with dermatocystidia (Heilmann-Clausen et al. 1998). *Lactifluus* sect. *Allardii* contains only one North American species and can be recognised by a lamprotrichoderm as pileipellis and a vinaceous-cinnamon coloured pileus (Hesler & Smith 1979).

Unclassified section

Lactifluus sect. *Aurantiifolii* has not been placed in a subgenus. The section contains only one African representative, *Lf. aurantiifolius*, that deviates morphologically from all other milkcap species and is characterised by a slightly velutinous to pruinose, vividly coloured and concentrically zonate pileus, brightly coloured lamellae with a paler and fimbriate margin, irregularly verrucose to incompletely reticulate spores, clavate pleuromacrocystidia with slightly thickened walls and a trichoderm pileipellis structure (Verbeken 1996b, Buyck et al. 2007). In previous studies, the classification of this section was uncertain (Buyck et al. 2007, Verbeken et al. 2012).

Unclassified species

Some *Lactifluus* species have unclear taxonomic positions, such as the agaricoid *Lf. caperatus* and *Lf. cocosmus* from Africa and the Australian *Lf. subclarkeae*; and the pleurotoid Neotropical *Lf. multiceps*, *Lf. brunellus* and *Lf. panuoides*.

This study is the first worldwide treatment of the genus *Lactifluus*, with a thorough geographical and taxonomical sampling. We combine a multi-gene molecular phylogeny with a morphological approach to clarify relationships within *Lactifluus*. The current classification is compared with our results, nomenclatural changes are listed and we give an overview of the revised infrageneric classification.

MATERIAL AND METHODS

Sampling

We included *Lactifluus* collections from every continent, every subgenus and every section, as well as collections with divergent morphological features. To improve species identification, we included as many type specimens as possible in our dataset. We included one collection of each species, except when sequences of only one or two genes of the type collection were available. In those cases we added an extra collection of the same species for which all four genes were sequenced. The outgroup contains nine *Russulales* species: *Amylostereum laevigatum*, *Auriscalpium vulgare*, *Bondarzewia montana*, *Echinodontium tinctorium*, *Gloeocystidiellum porosum*, *Heterobasidion annosum*, *Peniophora nuda*, *Stereum hirsutum* and *Vararia abortiphysa* (Table 1).

Morphological analyses

For each *Lactifluus* collection, several important or striking morphological characteristics were determined. The following characteristics, traditionally used to characterise infrageneric groups, are represented in the phylogenetic trees of each subgenus:

- i. fruit body type (agaricoid/pleurotoid);
- ii. presence or absence of a secondary velum;
- iii. colour reaction of the latex and/or the context when exposed to the air;

- iv. pileipellis type (Fig. 1); and
- v. presence or absence of true cystidia, together with cystidium type (macro-, lepto- or lamprocystidia, Fig. 2).

Other morphological characteristics were discussed depending on their importance as delimiting features.

Macromorphological characteristics of fresh material were described in daylight conditions and morphology of herbarium specimens was based on the notes of the collectors or was obtained from the original species descriptions. Micromorphological characteristics were studied on dried herbarium collections or derived from the original species descriptions. We follow Vellinga (1988) for general terminology and Verbeken & Walleyn (2010) for terminology concerning pileipellis structures. Basidiospores were measured in side view, in Melzer's reagent. Measurements exclude ornamentations. Elements of the pileipellis and the hymenium were measured halfway the radius of the pileus in Congo-Red in L4, using an Olympus CX31 microscope.

DNA extraction, PCR amplification, sequencing and nucleotide alignments

DNA from fresh material was extracted using the CTAB extraction described in Nuytinck & Verbeken (2003), whereas DNA of dried material was extracted using the protocol of Nuytinck & Verbeken (2003) with modifications described in Van de Putte et al. (2010). Protocols for PCR amplification follow Le et al. (2007a). In order to get support for branches at and above species level, we chose genes proven to be informative across multiple phylogenetic levels within the *Russulaceae* (Buyck et al. 2008, Van de Putte et al. 2012):

1. the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S. Primers ITS-1F/ITS5 and ITS4 were used (White et al. 1990, Gardes & Bruns 1993), together with internal primers ITS2 and ITS3 (White et al. 1990) for old type specimens and poorly dried collections;
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). As the *RPB1* fragment is over 1300 bp long, sequencing often failed for dried material. Based on existing *RPB1* sequences of milkcap species, we constructed an internal primer, with primer sequences RPB1-F3: 5'-AGTAARAYG RTY TGT GAG GC -3' and RPB1-R4: 5' - GCC TCA CAR AYC RTY TTA CT - 3'. Then, using primer pairs RPB1-Ac/RPB1-R4 and RPB1-F3/RPB1-Cr, two fragments of *RPB1* were obtained and joined for alignment and phylogenetic analyses.

PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Life Technology) at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with the Sequencher™ v. 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v. 7 (Katoh & Toh 2008), using the E-INS-I strategy. Trailing ends of the alignment were trimmed and alignments were manually edited when necessary in Mega 6 (Tamura et al. 2013). We choose not to exclude ambiguously aligned regions from the alignment (either manually or by a computer program), as it was shown by Nagy et al. (2012)

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

Species	Voucher collection (herbarium)	Country	GenBank Accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
Genus <i>Lactifluus</i>						
<i>Lactifluus</i> subg. <i>Lactariopsis</i>						
<i>Lactifluus</i> sect. <i>Lactariopsis</i>						
<i>Lactifluus annulatoangustifolius</i>	BB 00-1518 (GENT, PC)	Madagascar	AY606981	KR364253	–	–
<i>Lactifluus</i> cf. <i>zenkeri</i>	AV 11-050 (GENT)	Tanzania	KR364055	KR364182	KR364297	KR364425
<i>Lactifluus chamaeleontinus</i>	JD 946 (BR)	Congo	KR364079	KR364208	KR364267	KR364377
<i>Lactifluus heimii</i>	EDC 11-082 (GENT)	Tanzania	KR364040	KR364167	KR364286	KR364412
<i>Lactifluus heimii</i> Type	AV 94-465 (GENT)	Burundi	KR364025	KR364152	–	–
<i>Lactifluus laevigatus</i>	JD 939 (BR)	Congo	KR364077	KR364206	KR364290	KR364417
<i>Lactifluus pelliculatus</i>	JD 956 (BR)	Congo	KR364080	KR364209	KR364321	KR364449
<i>Lactifluus pruinatus</i> Type	BB 3248 (GENT)	Zambia	KR364031	KR364158	KR364328	KR364458
<i>Lactifluus sesemotani</i>	AV 94-476 (GENT)	Burundi	KR364036	KR364163	KR364345	KR364476
<i>Lactifluus</i> sp.	EDC 12-040 (GENT)	Cameroon	KR364063	KR364192	KR364289	KR364416
<i>Lactifluus uapacae</i> Type	AV 07-048 (GENT)	Cameroon	KR364007	KR364135	KR364352	KR364483
<i>Lactifluus velutissimus</i>	JD 886 (BR)	Congo	KR364075	KR364204	KR364355	KR364485
Clade 1						
<i>Lactifluus emergens</i>	AV 99-012 (GENT)	Zimbabwe	KR364021	KR364148	KR364276	KR364388
<i>Lactifluus madagascariensis</i>	BB 99-409 (PC)	Madagascar	AY606977	DQ421975	DQ421914	–
<i>Lactifluus madagascariensis</i> Type	B-E 99-417 (GENT)	Madagascar	KR364120	KR364245	–	–
Isolated species 1						
<i>Lactifluus acrisissimus</i>	EDC 11-112 (GENT)	Tanzania	KR364041	KR364168	KR364254	KR364366
<i>Lactifluus acrisissimus</i> Type	ADK2161 (GENT)	Benin	KR364126	–	–	–
Clade 2						
<i>Lactifluus annulifer</i>	TH 9014 (BRG, DUKE)	Guyana	KC155376	KC155376	–	–
<i>Lactifluus</i> sp.	RC/Guy 09-004bis (LIP)	French Guiana	KJ786643	KP691419	KP691427	–
<i>Lactifluus subiculatus</i>	SLM 10114 (BRG, RMS)	Guyana	JQ405654	–	–	–
<i>Lactifluus venezuelanus</i>	RC/Guad 11-017 (LIP)	Guadeloupe	KP691411	KP691420	KP691429	KR364393
Clade 3						
<i>Lactifluus multiceps</i>	TH 9154A (BRG, DUKE)	Guyana	JN168731	–	–	–
<i>Lactifluus</i> sp.	G3264 (MNHN)	French Guiana	KJ786706	KJ786620	KP691435	KR364400
Clade 4						
<i>Lactifluus chrysocarpus</i> Type	LE 253907 (LE)	Vietnam	JX442761	JX442761	–	–
<i>Lactifluus ramipilosus</i> Type	EDC 14-503 (GENT, MFLU)	Thailand	KR364128	–	–	–
Clade 5						
<i>Lactifluus brachystegiae</i> Type	AV 99-002 (GENT)	Zimbabwe	KR364018	KR364145	KR364262	KR364374
<i>Lactifluus leoninus</i>	DS 07-454 (GENT)	Thailand	KF220055	JN388989	JN375592	JN389188
<i>Lactifluus leoninus</i> Type	EH 72-524 (GENT)	Papua New Guinea	KR364116	–	–	–
<i>Lactifluus</i> sp.	AV 11-183 (GENT)	Togo	KR364060	KR364189	KR364277	KR364389
Isolated species 2						
<i>Lactifluus cocosmus</i> Type	ADK 4462 (GENT)	Togo	KR364013	KR364141	KR364269	KR364380
Clade 6						
<i>Lactifluus rufomarginatus</i>	ADK 3358 (BR)	Benin	KR364033	KR364160	KR364335	KR364466
<i>Lactifluus rufomarginatus</i> Type	ADK 3011 (GENT)	Benin	KR364034	KR364161	KR364336	–
<i>Lactifluus</i> sp.	AV 07-056 (GENT)	Cameroon	KR364008	KR364136	KR364293	KR364421
<i>Lactifluus</i> sp.	EDC 12-195 (GENT)	Cameroon	KR364071	KR364200	KR364301	KR364429
Clade 7						
<i>Lactifluus densifolius</i>	AV 11-111 (GENT)	Tanzania	KR364057	KR364184	KR364273	KR364385
<i>Lactifluus</i> sp.	JD 907 (GENT)	Congo	KR364076	KR364205	KR364302	KR364430
<i>Lactifluus</i> sect. <i>Russulopsidei</i>						
<i>Lactifluus cyanovirescens</i>	JD 988 (GENT)	Congo	KR364082	KR364211	KR364270	KR364382
<i>Lactifluus longipes</i>	JD 303 (BR)	Gabon	KR364009	KR364137	KR364310	KR364438
<i>Lactifluus ruvubuensis</i>	AB 305 (GENT)	Guinea	KR364035	KR364162	KR364343	KR364473
<i>Lactifluus ruvubuensis</i> Type	AV 94-599 (GENT)	Burundi	KR364122	–	–	–
<i>Lactifluus urens</i>	EDC 14-032 (GENT)	Zambia	KR364124	KR364247	KR364353	–
<i>Lactifluus</i> sect. <i>Edules</i>						
<i>Lactifluus aureifolius</i>	AV 11-074 (GENT)	Tanzania	KR364056	KR364183	KR364259	KR364371
<i>Lactifluus edulis</i>	FN 05-628 (GENT)	Malawi	KR364020	KR364147	KR364275	KR364387
<i>Lactifluus fazaoensis</i> Type	AV 11-178 (GENT)	Togo	HG426477	KR364188	KR364349	KR364481
<i>Lactifluus indusiatus</i> Type	AV 94-122 (GENT)	Burundi	KR364026	KR364153	KR364287	–
<i>Lactifluus inversus</i>	AB 063 (GENT)	Guinea	AY606976	DQ421978	DQ421917	KR364414
<i>Lactifluus latifolius</i>	SDM 037 (BR)	Gabon	KR364028	KR364155	KR364291	KR364418
<i>Lactifluus nodosicystidiosus</i>	BEM 97-273 (GENT)	Madagascar	KR364029	KR364156	KR364316	KR364444
<i>Lactifluus nodosicystidiosus</i> Type	BEM 97-072 (GENT)	Madagascar	AY606975	DQ421976	DQ421915	–
<i>Lactifluus phlebophyllus</i>	BB 00-1388 (PC)	Madagascar	AY606974	DQ421979	DQ421918	–
<i>Lactifluus roseolus</i>	AV 99-160 (GENT)	Zimbabwe	KR364032	KR364159	KR364333	KR364463
<i>Lactifluus roseolus</i> Type	AV 94-274 (GENT)	Burundi	KR364121	KR364242	–	–
<i>Lactifluus</i> sp. nov.	EDC 12-068 (GENT)	Cameroon	KR364068	KR364197	KR364299	KR364427
<i>Lactifluus</i> sect. <i>Albati</i>						
<i>Lactifluus bertillonii</i>	JN 2012-016 (GENT)	Germany	KR364087	KR364217	KR364261	KR364373
<i>Lactifluus deceptivus</i>	TENN 065854 (TENN)	North America	KR364101	–	KR364271	KR364383
<i>Lactifluus pilosus</i> Type	LTH 205 (GENT)	Thailand	KR364006	KR364134	KR364323	KR364452
<i>Lactifluus</i> sp. nov.	JN 2011-071 (GENT)	Vietnam	KR364043	KR364169	KR364255	KR364367
<i>Lactifluus</i> sp. nov.	JN 2011-077 (GENT)	Vietnam	KR364044	KR364170	KR364256	KR364368
<i>Lactifluus subvellereus</i>	AV 05-210 (GENT)	North America	KR364010	KR364138	KR364347	KR364479
<i>Lactifluus vellereus</i>	ATHU-M 8077 (ATHU-M)	Greece	KR364106	KR364237	KR364354	KR364484

Table 1 (cont.)

Species	Voucher collection (herbarium)	Country	GenBank Accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
<i>Lactifluus</i> subg. <i>Pseudogymnocarpi</i>						
<i>Lactifluus</i> sect. <i>Pseudogymnocarpi</i>						
<i>Lactifluus</i> cf. <i>longisporus</i>	AV 11-025 (GENT)	Tanzania	KR364054	KR364181	KR364311	KR364439
<i>Lactifluus</i> cf. <i>pseudogymnocarpus</i>	AV 05-085 (GENT)	Malawi	KR364012	KR364139	KR364329	KR364459
<i>Lactifluus</i> cf. <i>pumilus</i>	EDC 12-066 (GENT)	Cameroon	KR364067	KR364196	KR364332	KR364462
<i>Lactifluus gymnocarpoides</i>	JD 885 (BR)	Congo	KR364074	KR364203	KR364283	KR364409
<i>Lactifluus gymnocarpoides</i>	AV 05-184 (GENT)	Malawi	KR364024	KR364151	KR364284	KR364410
<i>Lactifluus hygrophoroides</i>	AV 05-251 (GENT)	North America	HQ318285	HQ318208	HQ328936	KR364413
<i>Lactifluus longisporus</i> Type	AV 94-557 (GENT)	Burundi	KR364118	KR364244	–	–
<i>Lactifluus luteopus</i>	EDC 11-087 (GENT)	Tanzania	KR364049	KR364176	KR364312	KR364441
<i>Lactifluus luteopus</i> Type	AV 94-463 (GENT)	Burundi	KR364119	–	KR364313	–
<i>Lactifluus medusae</i>	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198	KR364314	KR364442
<i>Lactifluus pseudoluteopus</i>	FH 12-026 (GENT)	Thailand	KR364084	KR364214	KR364331	KR364460
<i>Lactifluus rugatus</i>	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235	KR364337	KR364467
<i>Lactifluus sudanicus</i> Type	AV 11-174 (GENT)	Togo	HG426469	KR364186	KR364348	KR364480
<i>Lactifluus</i> sect. <i>Xerampelini</i>						
<i>Lactifluus</i> cf. <i>pseudovolemus</i>	ADK 2927 (GENT)	Benin	KR364113	KR364243	KR364330	KR364461
<i>Lactifluus goossensiae</i>	AB 320 (GENT)	Guinea	KR364132	KR364252	KR364281	–
<i>Lactifluus kivuensis</i> Type	JR Z 310 (GENT)	Congo	KR364027	KR364154	–	–
<i>Lactifluus rubiginosus</i>	JD 959 (BR)	Congo	KR364081	KR364210	KR364304	KR364432
<i>Lactifluus rubiginosus</i> Type	BB 3466 (GENT)	Zambia	KR364014	KR364250	–	–
<i>Lactifluus</i> sp. nov.	EDC 12-001 (GENT)	Cameroon	KR364061	KR364190	KR364298	KR364426
<i>Lactifluus</i> sp. nov.	EDC 12-176 (GENT)	Cameroon	KR364070	KR364199	KR364300	KR364428
<i>Lactifluus xerampelinus</i>	MH 201176 (GENT)	Mozambique	KR364099	KR364231	KR364364	KR364496
<i>Lactifluus xerampelinus</i> Type	TS 1116 (GENT)	Tanzania	KR364039	KR364166	–	–
Clade 8						
<i>Lactifluus armeniacus</i> Type	EDC 14-501 (GENT, MFLU)	Thailand	KR364127	–	–	–
<i>Lactifluus</i> sp. nov.	JN 2011-012 (GENT)	Vietnam	KR364045	KR364171	KR364294	KR364422
<i>Lactifluus</i> sp. nov.	TENN 065929 (TENN)	North America	KR364102	KR364233	KR364308	KR364436
<i>Lactifluus volemoides</i>	MH 201187 (GENT)	Mozambique	KR364098	KR364230	KR364363	KR364493
<i>Lactifluus volemoides</i> Type	TS 0705 (GENT)	Tanzania	KR364038	KR364165	–	–
<i>Lactifluus</i> sect. <i>Aurantifolii</i>						
<i>Lactifluus aurantiifolius</i> Type	AV 94-063 (GENT)	Burundi	KR364017	KR364144	–	–
<i>Lactifluus</i> sect. <i>Rubroviolascetini</i>						
<i>Lactifluus</i> aff. <i>rubroviolascens</i>	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195	KR364334	KR364465
<i>Lactifluus carmineus</i> Type	AV 99-099 (GENT)	Zimbabwe	KR364131	KR364251	KR364265	–
<i>Lactifluus denigricans</i>	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178	KR364272	KR364384
<i>Lactifluus</i> sp. nov.	AV 11-006 (GENT)	Tanzania	KR364052	KR364179	KR364288	KR364415
<i>Lactifluus kigomaensis</i>	EDC 11-159 (GENT)	Tanzania	KR364050	KR364177	KR364295	KR364423
<i>Lactifluus</i> sect. <i>Polysphaerophori</i>						
<i>Lactifluus pegleri</i>	PAM/Mart 12-091 (LIP)	Martinique	KP691416	KP691425	KP691433	KR364397
<i>Lactifluus</i> sp.	RC/Guy 09-036 (LIP)	French Guiana	KJ786645	KJ786550	KP752178	–
<i>Lactifluus</i> sp.	MR/Guy 13-145	French Guiana	KJ786691	KJ786595	KP752180	KR364398
<i>Lactifluus</i> sp.	MCA 3937 (GENT)	Guyana	KR364109	KR364240	KR364350	–
<i>Lactifluus veraecrucis</i> Type	M 8025 (ENCB)	Mexico	KR364112	KR364241	–	–
<i>Lactifluus</i> subg. <i>Gymnocarpi</i>						
<i>Lactifluus</i> sect. <i>Luteoli</i>						
<i>Lactifluus brunneoviolascens</i>	AV 13-038 (GENT)	Italy	KR364123	KR364246	KR364264	KR364376
<i>Lactifluus longivelutinus</i> Type	XHW 1565 (GENT)	China	KR364114	–	–	–
<i>Lactifluus luteolus</i>	AV 05-253 (GENT)	North America	KR364016	KR364142	KJ210067	KR364440
<i>Lactifluus nonpiscis</i>	AV 11-137 (GENT)	Togo	KR364058	KR364185	KR364317	KR364445
<i>Lactifluus nonpiscis</i> Type	BB 3171 (GENT)	Zambia	KR364030	KR364157	–	–
<i>Lactifluus rubrobrunnescens</i> Type	EH 7194 (GENT)	Indonesia	KR364115	–	–	–
<i>Lactifluus</i> sp. nov.	KW 392 (GENT)	Thailand	KR364091	KR364222	KR364305	KR364433
<i>Lactifluus</i> sp. nov.	REH 9398 (NY)	Australia	KR364097	KR364229	KR364307	KR364435
<i>Lactifluus</i> sect. <i>Gymnocarpi</i>						
<i>Lactifluus albocinctus</i> Type	AV 99-211 (GENT)	Zimbabwe	KR364117	KR364249	KR364258	–
<i>Lactifluus albomembranaceus</i> nom. prov.	EDC 12-046 (GENT)	Cameroon	KR364064	KR364193	KR364257	KR364369
<i>Lactifluus flammans</i>	JD 941 (BR)	Congo	KR364078	KR364207	KR364303	KR364431
<i>Lactifluus gymnocarpus</i>	EDC 12-047 (GENT)	Cameroon	KR364065	KR364194	KR364282	KR364408
<i>Lactifluus</i> cf. <i>tanzanicus</i>	AV 11-017 (GENT)	Tanzania	KR364053	KR364180	KR364296	KR364424
<i>Lactifluus tanzanicus</i> Type	TS 1277 (GENT)	Tanzania	KR364037	KR364164	KR364351	–
Isolated species 4						
<i>Lactifluus foetens</i>	ADK 3688 (BR)	Benin	KR364022	KR364149	KR364278	KR364390
<i>Lactifluus foetens</i> Type	ADK 2840 (BR)	Benin	KR364023	KR364150	KR364279	KR364391
<i>Lactifluus</i> sect. <i>Phlebonemi</i>						
<i>Lactifluus</i> aff. <i>phlebonemus</i>	EDC 12-023 (GENT)	Cameroon	KR364062	KR364191	KR364322	KR364451
<i>Lactifluus brunnescens</i>	AV 05-083 (GENT)	Malawi	KR364019	KR364146	KR364263	KR364375
Clade 9						
<i>Lactifluus</i> aff. <i>nebulosus</i>	RC/Guad 11-023 (LIP)	Guadeloupe	KP691412	KP691421	KP691430	KR364394
<i>Lactifluus caribaeus</i>	PAM/Mart 12-090 (LIP)	Martinique	KP691415	KP691424	KP691432	KR364396
<i>Lactifluus</i> cf. <i>castaneibadius</i>	CL/MART06.019 (LIP)	Martinique	KP691417	KP691426	–	–
<i>Lactifluus</i> cf. <i>murinipes</i>	F.1890 (LIP)	Martinique	KP691418	–	–	–
<i>Lactifluus</i> cf. <i>putidus</i>	PAM/Mart 11-013 (LIP)	Martinique	KP691413	KP691422	KP691431	KR364395
<i>Lactifluus chiapanensis</i>	VMB 4374A (GENT)	Mexico	GU258297	GU265580	GU258316	KR364378
Isolated species 5						
<i>Lactifluus</i> sp.	G3185	French Guiana	KJ786694	KJ786603	KP691434	KR364399

Table 1 (cont.)

Species	Voucher collection (herbarium)	Country	GenBank Accession numbers			
			ITS	LSU	RPB2	RPB1
Isolated species 6						
<i>Lactifluus brunellus</i>	TH 9130 (BRG, DUKE)	Guyana	JN168728	–	–	–
Isolated species 7						
<i>Lactifluus</i> sp.	RC/Guad 08-042 (LIP)	Guadeloupe	KP691414	KP691423	KP752179	–
Isolated species 8						
<i>Lactifluus panuoides</i>	RC/Guy 10-024 (LIP)	French Guiana	KJ786647	KJ786551	KP691428	–
<i>Lactifluus</i> sect. <i>TomENTOSI</i>						
<i>Lactifluus clarkeae</i>	MN 2004002 (L)	Australia	KR364011	HQ318205	KR364268	KR364379
<i>Lactifluus flocktonae</i>	JET1006 (MEL)	Australia	JX266621	JX266637	–	–
<i>Lactifluus</i> sp.	PGK13-130	New Caledonia	KP691436	KR605507	–	–
<i>Lactifluus subclarkeae</i>	REH 9231 (NY)	Australia	KR364095	KR364227	KR364346	KR364477
<i>Lactifluus</i> subg. <i>Lactifluus</i>						
<i>Lactifluus</i> sect. <i>Lactifluus</i>						
<i>Lactifluus acicularis</i>	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132	HQ328869	JN389131
<i>Lactifluus corrugis</i> s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143	JQ348127	–
<i>Lactifluus crocatus</i>	KVP 08-034 (GENT)	Thailand	HQ318243	HQ318151	HQ328888	JN389145
<i>Lactifluus dissitus</i>	AV-KD-KVP 09-134 (GENT)	India	JN388978	JN389026	JN375628	JN389172
<i>Lactifluus distantifolius</i>	LTH 288 (GENT)	Thailand	HQ318274	HQ318193	KR364274	JN389155
<i>Lactifluus lamprocystidiatus</i> Type	EH 72-195 (GENT)	Papua New Guinea	KR364015	–	–	–
<i>Lactifluus leptomerus</i> Type	AV-KD-KVP 09-131 (GENT)	India	JN388972	JN389023	JN375625	JN389169
<i>Lactifluus longipilus</i>	LTH 184 (GENT)	Thailand	HQ318256	HQ318169	HQ328905	JN389152
<i>Lactifluus oedematopus</i>	KVP 12-001 (GENT)	Germany	KR364100	KR364232	KR364319	KR364447
<i>Lactifluus pinguis</i> Type	AV-RW 04-023/LTH117 (GENT)	Thailand	HQ318211	HG318111	HQ328858	JN389126
<i>Lactifluus</i> sp.	SA A12 L2 (GENT)	North America	KR364088	KR364218	KR364361	KR364491
<i>Lactifluus subvolemus</i>	KVP 08-048 (GENT)	Slovenia	JQ753927	JQ348379	KR364356	KR364486
<i>Lactifluus versiformis</i> Type	AV-KD-KVP 09-045 (GENT)	India	JN388967	JN389031	JN375632	JN389177
<i>Lactifluus vitellinus</i>	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144	HQ328881	JN389138
<i>Lactifluus volemus</i>	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175	KR364360	KR364490
<i>Lactifluus volemus</i> s.l.	AV-KD-KVP 09-121 (GENT)	India	JN388979	JN389014	JN375616	JN389160
<i>Lactifluus volemus</i> s.l.	KVP 08-011 (GENT)	Thailand	HQ318232	HQ318139	HQ328876	JN389135
<i>Lactifluus volemus</i> s.l.	KVP 08-031 (GENT)	Thailand	HQ318240	HQ318148	HQ328885	JN389142
<i>Lactifluus volemus</i> s.l.	REH 9320 (NY)	Australia	KR364096	KR364228	KR364362	KR364492
<i>Lactifluus</i> sect. <i>Tenuicystidiati</i>						
<i>Lactifluus</i> aff. <i>tenuicystidiatus</i>	JN 2011-074 (GENT)	Vietnam	KR364047	KR364173	KR364358	KR364488
<i>Lactifluus</i> sp.	JN 2011-080 (GENT)	Vietnam	KR364048	KR364174	KR364359	KR364489
<i>Lactifluus subpruinosis</i>	JN 2011-061 (GENT)	Vietnam	KR364046	KR364172	KR364357	KR364487
<i>Lactifluus</i> sect. <i>Gerardii</i>						
<i>Lactifluus atrovelutinus</i>	DS 06-003 (GENT)	Malaysia	GU258231	GU265588	GU258325	JN389185
<i>Lactifluus conchatulus</i> Type	LTH 457 (GENT)	Thailand	GU258296	GU265659	GU258399	KR364381
<i>Lactifluus fuscomarginatus</i> Type	LM 4379 (XAL)	Mexico	HQ168367	HQ168367	–	–
<i>Lactifluus genevievae</i> Type	GG-DK 17-02-05 (GENT)	Australia	GU258294	GU265657	GU258397	KR364401
<i>Lactifluus</i> aff. <i>gerardii</i>	LTH 270 (GENT)	Thailand	EF560685	GU265598	GU258335	KR364402
<i>Lactifluus gerardii</i>	AV 05-375 (GENT)	North America	GU258254	GU265616	GU258353	KR364403
<i>Lactifluus</i> cf. <i>gerardii</i> var. <i>fagicola</i>	JN 2007-029 (GENT)	Canada	GU258224	GU265582	GU258318	–
<i>Lactifluus ignicululus</i> Type	LE 262983 (LE)	Vietnam	JX442759	JX442759	–	–
<i>Lactifluus leae</i>	FH 12-013 (GENT)	Thailand	KF432957	KR364213	KR364292	KR364419
<i>Lactifluus leonardii</i>	GG 07-02-04	Australia	GU258308	GU265668	GU258408	KR364495
<i>Lactifluus limbatus</i> Epitype	DS 06-247 (GENT)	Malaysia	JN388955	JN388987	JN375590	JN389186
<i>Lactifluus</i> cf. <i>ochrogalactus</i>	AV-KD-KVP 09-120 (GENT)	India	KR364130	KR364248	KR364318	KR364446
<i>Lactifluus petersenii</i>	AV 05-300 (GENT)	North America	GU258281	GU265642	GU258382	KR364450
<i>Lactifluus reticulatovenosus</i> Type	EH 6472 (GENT)	Indonesia	GU258286	GU265649	GU258389	–
<i>Lactifluus</i> sp. nov.	AV 12-050 (GENT)	Thailand	KR364086	KR364216	KR364260	KR364372
<i>Lactifluus</i> sp. nov.	AV 12-070 (GENT)	Thailand	KR364090	KR364221	KR364326	–
<i>Lactifluus</i> sp. nov.	TENN 051830 (TENN)	Nepal	KR364111	KR364140	–	–
<i>Lactifluus</i> sp. nov.	KW 304/FH 12-037 (GENT)	Thailand	KR364092	KR364223	KR364306	KR364434
<i>Lactifluus subgerardii</i>	AV 05-269 (GENT)	North America	GU258263	GU265625	GU258362	KR364478
<i>Lactifluus wirrabara</i> s.l.	PL 40509	New Zealand	GU258287	GU265650	GU258390	KR364475
<i>Lactifluus wirrabara</i> s.l.	GG 24-01-04	Australia	GU258307	GU265667	GU258407	KR364494
<i>Lactifluus</i> sect. <i>Ambicystidiati</i>						
<i>Lactifluus ambicystidiatus</i>	HKAS J7008 (HKAS)	China	KR364108	KR364239	KR364309	KR364437
Isolated species 9						
<i>Lactifluus</i> sp. nov.	PUN 7046 (PUN)	India	KM658971	–	–	–
<i>Lactifluus</i> sect. <i>Allardii</i>						
<i>Lactifluus allardii</i>	JN 2004-008 (GENT)	North America	KF220016	KF220125	KF220217	KR364370
<i>Lactifluus</i> sect. <i>Piperati</i>						
<i>Lactifluus</i> aff. <i>glaucescens</i>	AV 04-195 (GENT)	North America	KF220045	KF220146	KF220232	KR364404
<i>Lactifluus</i> aff. <i>glaucescens</i>	AV 05-374 (GENT)	North America	KF220049	KF220150	KF220236	KR364405
<i>Lactifluus</i> aff. <i>glaucescens</i>	JN 2011-014 (GENT)	Vietnam	KF220104	KF220199	KF220273	KR364406
<i>Lactifluus</i> aff. <i>glaucescens</i>	LTH 274 (GENT)	Thailand	KR364107	KR364238	KR364325	KR364457
<i>Lactifluus</i> aff. <i>piperatus</i>	JN 2011-036 (GENT)	Vietnam	KF220105	KF220200	KF220274	KR364454
<i>Lactifluus</i> aff. <i>piperatus</i>	JN 2011-072 (GENT)	Vietnam	KF220106	KF220201	KF220275	KR364455
<i>Lactifluus</i> aff. <i>piperatus</i>	TENN 064342 (TENN)	North America	KR364103	KR364234	KR364324	KR364456
<i>Lactifluus dwaliensis</i>	LTH 55 (GENT)	Thailand	KF220111	KF220204	KF220278	KR364386
<i>Lactifluus dwaliensis</i> Type	KD 612 (GENT)	India	KR364042	–	–	–
<i>Lactifluus glaucescens</i>	LGAM 2010-0132 (LGAM-AUA)	Greece	KR364105	KR364236	KR364280	KR364407
<i>Lactifluus leucophaeus</i>	LTH 182 (GENT)	Thailand	KF220059	KF220157	KF220243	KR364420
<i>Lactifluus piperatus</i>	2001 08 19 68 (GENT)	France	KF220119	KF241840	KF241842	KR364453
<i>Lactifluus roseophyllus</i>	JN 2011-076 (GENT)	Vietnam	KF220107	KF220202	KF220276	KR364464

Table 1 (cont.)

Species	Voucher collection (herbarium)	Country	GenBank Accession numbers			
			ITS	LSU	<i>RPB2</i>	<i>RPB1</i>
Genus <i>Russula</i>						
<i>Russula cyanoxantha</i>	FH 12-201 (GENT)	Germany	KR364093	KR364225	KR364341	KR364471
<i>Russula delica</i>	FH 12-272 (GENT)	Belgium	KF432955	KR364224	KR364340	KR364470
<i>Russula gracillima</i>	FH 12-264 (GENT)	Germany	KR364094	KR364226	KR364342	KR364472
<i>Russula khanchanjungae</i>	AV-KD-KVP 09-106 (GENT)	India	KR364129	JN389004	JN375607	JN389092
<i>Russula</i> sp.	EDC 12-061 (GENT)	Cameroon	KR364072	KR364201	KR364338	KR364468
<i>Russula</i> sp.	EDC 12-063 (GENT)	Cameroon	KR364073	KR364202	KR364339	KR364469
Genus <i>Lactarius</i>						
<i>Lactarius fuliginosus</i>	MTB 97-24 (GENT)	Sweden	JQ446111	JQ446180	JQ446240	KR364392
<i>Lactarius hatsudake</i>	FH 12-052 (GENT)	Thailand	KR364085	KR364215	KR364285	KR364411
<i>Lactarius miniatescens</i>	AV 11-177 (GENT)	Togo	KR364059	KR364187	KR364315	KR364443
<i>Lactarius olympianus</i>	ED 08-018 (GENT)	North America	KR364089	KR364220	KR364320	KR364448
<i>Lactarius scrobiculatus</i>	JN 2001-058 (GENT)	Slovakia	KF432968	KR364219	KR364344	KR364474
<i>Lactarius tenellus</i>	ADK 3598 (GENT)	Benin	KF133280	KF133313	KF133345	KR364482
Genus <i>Multifurca</i>						
<i>Multifurca furcata</i>	REH 7804 (NY)	Costa Rica	DQ421995	DQ421995	DQ421928	–
<i>Multifurca ochricompacta</i>	BB 02-107 (PC)	North America	DQ421984	DQ421984	DQ421940	–
<i>Multifurca</i> sp.	xp2-20120922-01 (GENT)	China	KR364125	–	–	–
<i>Multifurca stenophylla</i>	JET956 (MEL)	Australia	JX266631	JX266635	–	–
<i>Multifurca zonaria</i>	FH 12-009 (GENT)	Thailand	KR364083	KR364212	KR364365	KR364497
Outgroup						
<i>Amylostereum laevigatum</i>	CBS 623.84 (CBS)	France	AY781246	AF287843	AY218469	–
<i>Auriscalpium vulgare</i>	PBM 944 (WTU)	North America	DQ911613	DQ911614	AY218472	–
<i>Bondarzewia montana</i>	AFTOL 452 (DAOM)	No data	DQ200923	DQ234539	AY218474	DQ256049
<i>Echinodontium tinctorium</i>	AFTOL 455 (DAOM)	No data	AY854088	AF393056	AY218482	AY864882
<i>Heterobasidion annosum</i>	AFTOL 470 (DAOM)	No data	DQ206988	–	AY544206	DQ667160
<i>Stereum hirsutum</i>	AFTOL 492	No data	AY854063	AF393078	AY218520	AY864885
<i>Vararia abortiphysa</i>	CBS 630.81 (CBS)	France	KR364005	KR364133	KR364266	–

that the deletion of gapped sites universally decreases tree resolution and branch support. Four final alignments were used:

1. a combined alignment of ITS+LSU sequence data;
2. an alignment of *RPB2* sequence data;
3. an alignment of *RPB1* sequence data; and
4. a combined alignment of ITS+LSU, *RPB2* and *RPB1* sequence data.

The alignments can be acquired from the first author and TreeBASE (S17930).

Phylogenetic analyses

Sequence data were divided into the following partitions. The ITS+LSU alignment was partitioned into partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Both *RPB2*- and *RPB1*-alignments were partitioned into the intron(s) and the first, second and third codon positions of the exon. Maximum Likelihood (ML) analyses were conducted with RAxML v. 8.0.24 (Stamatakis 2014), where an ML analysis was combined with the Rapid Bootstrapping algorithm with 1 000 replicates under the GTR-CAT option (Stamatakis et al. 2008). Bayesian Inference (BI) was executed with MrBayes v. 3.2.0 (Ronquist et al. 2012). Partitionfinder v. 1.1.1 (Lanfear et al. 2012) was first used to determine the model that best fits each partition, using the Bayesian information criterion (BIC), after which we evaluated the chosen models. Models found by Partitionfinder under BIC were: 18S: JC+I, ITS1: GTR+G+I, 5.8S: K80+G+I, ITS2: GTR+G+I, 28S: GTR+G+I, *RPB1*pos1: K80+G+I, *RPB1*pos2: K80+G+I, *RPB1*pos3: GTR+G+I, *RPB1*intron1: HKY+G+I, *RPB1*intron2: GTR+G+I, *RPB1*intron3: K80+G+I, *RPB1*intron4: GTR+G+I, *RPB2*pos1: K80+G+I, *RPB2*pos2: TVM+G+I, *RPB2*pos3: GTR+G+I, *RPB2*intron: HKY+G+I. The BIC criterion mostly favoured +G+I models. However, we chose to only add the gamma model (G) and leave the estimation of invariant sites (I) out, as several studies have shown that both parameters correlate, which may not always be favourable (Jia et al. 2014, Drummond & Bouckaert 2015). Four parallel runs, each

consisting of one cold and three heated chains, were performed for 10 million generations sampling every 100th generation for the single gene trees and 20 million generations sampling every 1 000th generation for the concatenated tree. Parameter convergence for the different runs was verified in Tracer v. 1.6 (Rambaut et al. 2014) and AWTY (Nylander et al. 2008). After discarding a burn-in determined in Tracer, a majority rule consensus tree was constructed. ML and BI analyses were performed on each of the four alignments. All analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

RESULTS

Our dataset contains 213 *Russulales* collections, of which 189 are from the genus *Lactifluus*. With approximately 150 described species in *Lactifluus*, 80 % of the described taxa are represented in our dataset. Of the 20 % missing, most species are only known from collections too old for sequencing. The remainder are taxa from species complexes represented by at least 15 species in our dataset, for instance from *Lf.* subg. *Gerardii* and *Lf.* sect. *Lactifluus*. These complexes have been studied before and their absence in this analysis does not affect stability of the results (Stubbe et al. 2010, Van de Putte et al. 2010, 2012). Fifty-one of the described species we included have never been sequenced before and 46 of the described species are represented by their type specimen. Furthermore, we included 30 unidentified collections, of which at least 15 represent new species. PCR and sequencing success rate differed among the four genes, with 213, 195, 177 and 151 sequences obtained for ITS, LSU, *RPB2* and *RPB1*, respectively. A total of 493 new sequences were generated for this study, the remaining were obtained from our previous studies and GenBank. ML and BI results of the three independent datasets are similar, without any supported conflicts (support: ML > 70, BI > 0.95). We therefore used the concatenated dataset, which is 5032 bp long (including gaps).

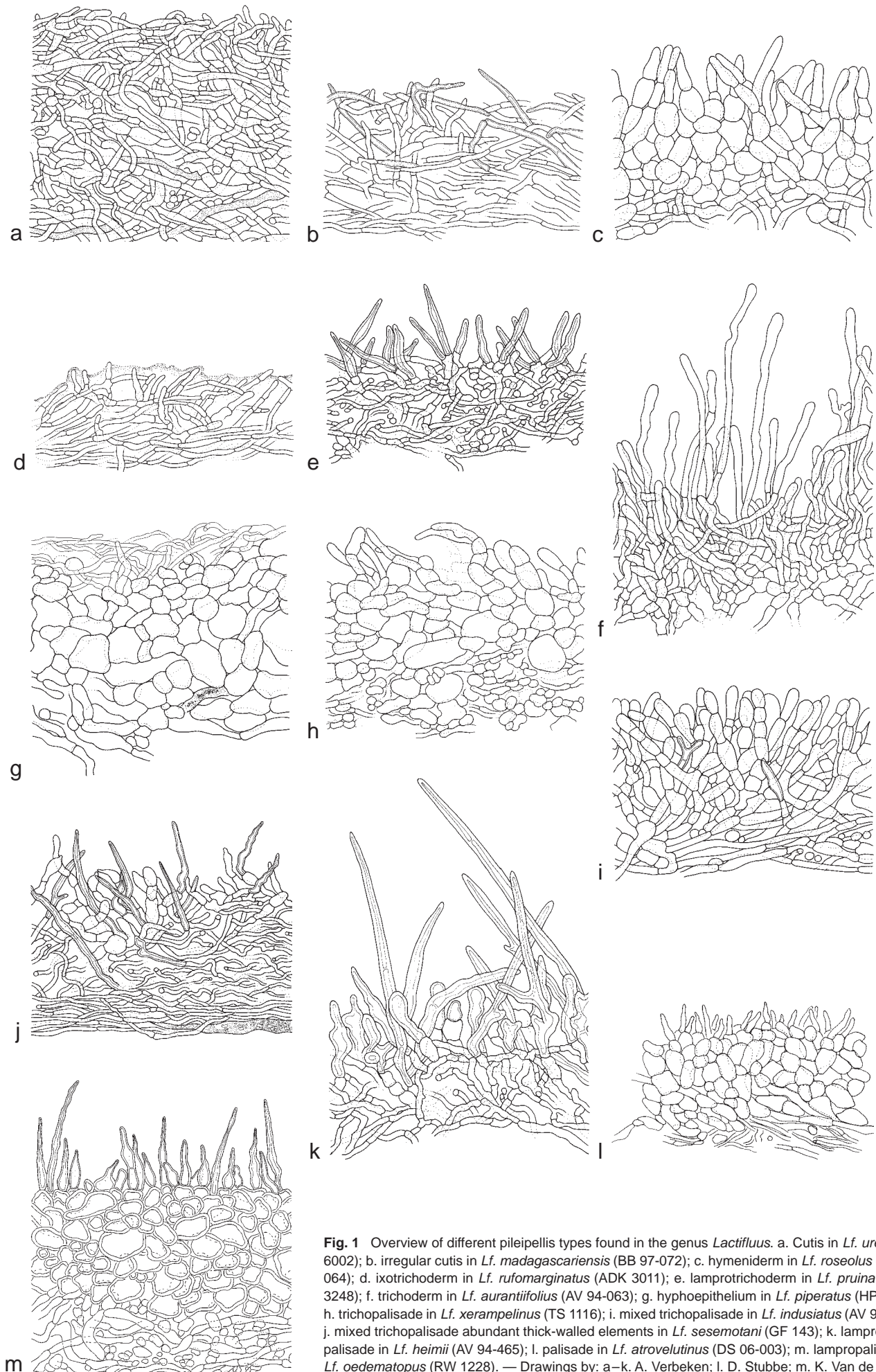


Fig. 1 Overview of different pileipellis types found in the genus *Lactifluus*. a. Cutis in *Lf. urens* (JR 6002); b. irregular cutis in *Lf. madagascariensis* (BB 97-072); c. hymeniderm in *Lf. roseolus* (AV 94-064); d. ixotrichoderm in *Lf. rufomarginatus* (ADK 3011); e. lamprotrichoderm in *Lf. pruinatus* (BB 3248); f. trichoderm in *Lf. aurantiifolius* (AV 94-063); g. hyphoepithelium in *Lf. piperatus* (HP 8475); h. trichopalisade in *Lf. xerampelinus* (TS 1116); i. mixed trichopalisade in *Lf. indusiatus* (AV 94-122); j. mixed trichopalisade abundant thick-walled elements in *Lf. sesemotani* (GF 143); k. lamprotrichopalisade in *Lf. heimii* (AV 94-465); l. palisade in *Lf. atrovelutinus* (DS 06-003); m. lampropalisade in *Lf. oedematopus* (RW 1228). — Drawings by: a–k. A. Verbeken; l. D. Stubbe; m. K. Van de Putte.



Fig. 2 Overview of different cystidium types found in the genus *Lactifluus*. — a–d. Lamprocystidia: a. in *Lf. armeniacus* (EDC 14-501); b. in *Lf. sp. nov.* (AV 11-006); c. in *Lf. cf. pumilus* (EDC 12-066); d. in *Lf. cf. volemus* (REH 9320). — e–f. Macrocystidia: e. in *Lf. sp. nov.* (JN 2011-077); f. in *Lf. roseophyllus* (JN 2011-076). — g–i. Leptocystidia: g. in *Lf. ruvubuensis* (AV 94-599); h. in *Lf. indusiatus* (AV 94-122); i. in *Lf. densifolius* (BB 3601). — Drawings by: a–f. E. De Crop; g–i. A. Verbeken.

Former, traditional classification:

- Lf. subg. Lactariopsis*
- Lf. subg. Russulopsis*
- Lf. subg. Edules*
- Lf. subg. Lactifluus*
- Lf. subg. Gerardii*
- Lf. subg. Piperati*
- Unclassified

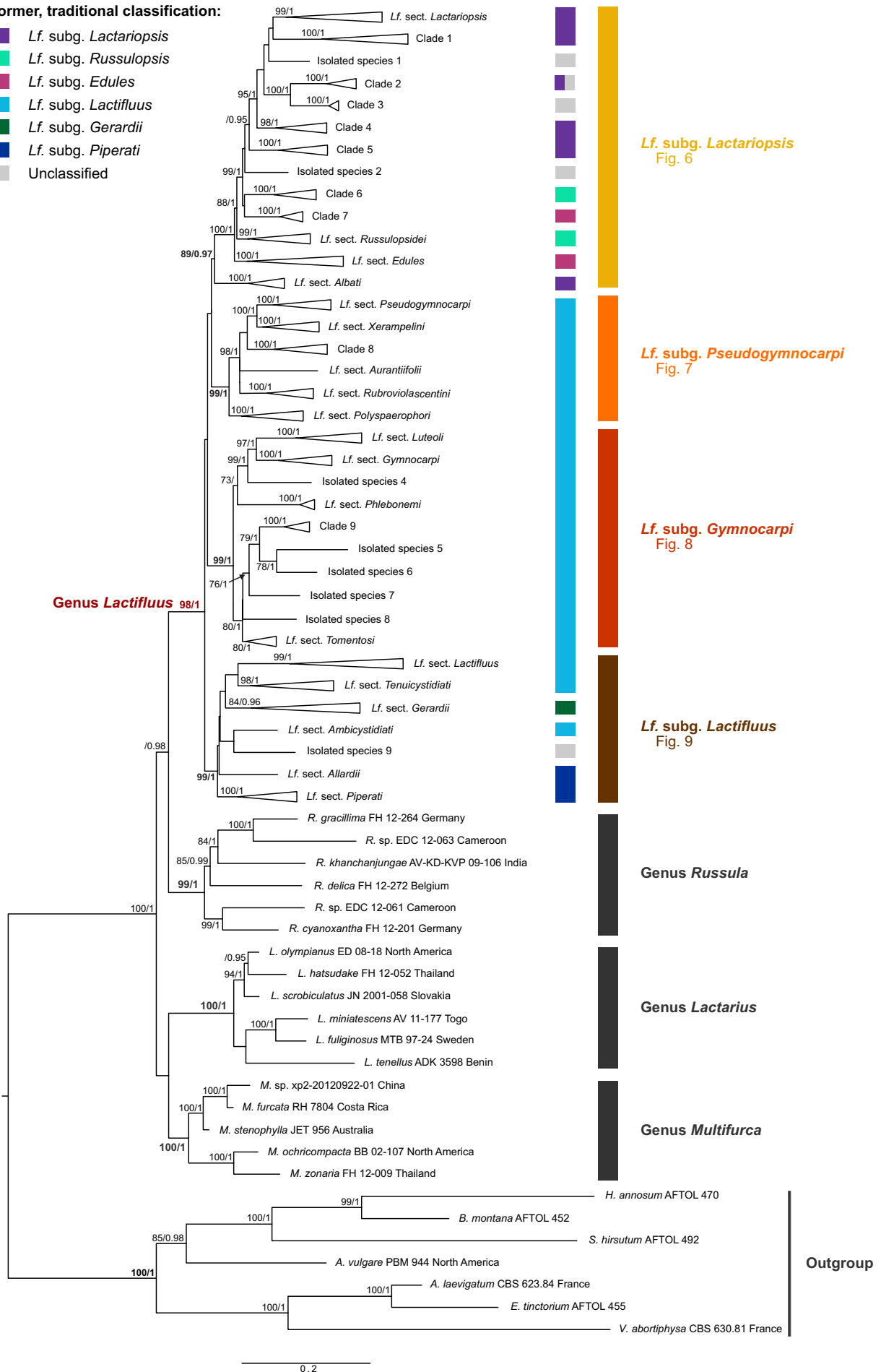


Fig. 3 Overview Maximum Likelihood tree of the genus *Lactifluus*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. The first column of colour bars represents the former, traditional classification. The second column represents the newly proposed classification. Maximum Likelihood bootstrap values > 70 and Bayesian Inference posterior probabilities > 0.95 are shown.

The phylogeny of the concatenated data is shown in Fig. 3. The outgroup is fully supported (ML: 100, BI: 1), as are the genera *Russula* (ML: 99, BI: 1), *Lactarius* (ML: 100, BI: 1) and *Multifurca* (ML: 100, BI: 1). *Lactifluus* is well-supported (ML: 98, BI: 1) and can be divided in four supported clades, corresponding to four subgenera: *Lf.* subg. *Lactariopsis* (ML: 89, BI: 0.97), *Lf.* subg. *Pseudogymnocarpi* (ML: 99, BI: 1), *Lf.* subg. *Gymnocarpi* (ML: 99, BI: 1) and *Lf.* subg. *Lactifluus* (ML: 99, BI: 1). Representatives of each subgenus are shown in Fig. 4 and 5. Each subgenus can be further divided into several sections, which are described below, together with their known morphological characteristics.

I. *Lactifluus* subg. *Lactariopsis* — Fig. 3, 4a–f, 6

Lactifluus subg. *Lactariopsis* is well-supported by molecular results. The subgenus is characterised by a variety of pileipellis types, ranging from types with abundant to scarce needle-shaped thick-walled elements. In most species true pleurocystidia are absent, but pleuromacrocytidia or pleuroleptocystidia are present in some, while pleurolamprocystidia were never observed. This is the only clade in which species with second-

ary velum occur and colour changes of the context and/or latex are only rarely observed. The subgenus consists of eleven well-supported clades and two species on isolated branches:

- In the exclusively African *Lf.* sect. *Lactariopsis*, former representatives of *Lf.* sect. *Lactariopsis* (species with velum) and *Lf.* sect. *Chamaeleontini* (species without velum) are mixed. This section can be recognised by a combination of thick-walled elements in the pileipellis and pseudocystidia that are highly emergent (up to 50 µm in *Lf. annulatoangustifolius*) and broad (up to 25 µm diam in *Lf. zenkeri*).
- Clade 1 contains two African species: *Lf. madagascariensis* and *Lf. emergens*. They can be recognised by the combination of narrow and only slightly emergent pseudocystidia, thick-walled elements in the pileipellis and the absence of secondary velum.
- *Lactifluus acrisimus*, sister to the preceding two clades, is isolated on a rather long branch. Until now, this species was considered to belong to *Lactarius* (Van Rooij et al. 2003), but our molecular study of the type sequence shows that it belongs to *Lactifluus*. It is characterised by creamy white



Fig. 4 Basidiocarps of representative species from the different subgenera and sections within the genus *Lactifluus*. — a–f. *Lf.* subg. *Lactariopsis*: a. *Lf.* sect. *Lactariopsis*: *Lf.* sp. (EDC 14-060, De Crop E); b. Clade 3: *Lf. multiceps* (TH9807, Elliot T); c. Clade 5: *Lf. leoninus* (DS 07-462, Stubbe D); d. *Lf.* sect. *Russulopsidæ*: *Lf. longipes* (EDC 12-049, De Crop E); e. *Lf.* sect. *Edules*: *Lf.* sp. nov. (EDC 12-069, De Crop E); f. *Lf.* sect. *Albati*: *Lf. vellereus* (Slos D). — g–i. *Lf.* subg. *Pseudogymnocarpi*: g. *Lf.* sect. *Pseudogymnocarpi*: *Lf. pumilus* (EDC 12-066, De Crop E); h. *Lf.* sect. *Pseudogymnocarpi*: *Lf. rugatus* (18.10.09, Pera U); i. *Lf.* sect. *Xerampelini*: *Lf.* sp. nov. (EDC 12-001, De Crop E); j. *Lf.* sect. *Xerampelini*: *Lf. kigomaensis* (EDC 11-159, De Crop E); k. Clade 8: *Lf. armeniacus* (EDC 14-501, De Crop E); l. *Lf.* sect. *Rubroviolascantini*: *Lf.* aff. *rubroviolascens* (EDC 12-051, De Crop E).

cap colours, an ixocutis to ixotrichoderm as pileipellis and a burning acrid taste.

- Clade 2 contains several agaricoid South American species. Species from this clade all have thick-walled elements in the pileipellis and comprise all known South American taxa with secondary velum on the stipe, as an annulus, and on the pileus margin.
- Clade 3 contains two pleurotoid species from South America, of which *Lf. multiceps* can be recognised by its orange cap colours, a lampropalisade and the absence of secondary velum and true cystidia.
- Clade 4 contains two Asian species: the small pleurotoid *Lf. chrysocarpus*, which was already mentioned to belong to *Lf.* subg. *Lactariopsis* in the study of Morozova et al. (2013), and the recently described *Lf. ramipilosus* (Li et al. 2016). Both are characterised by a lampropalisade and the absence of a secondary velum.
- Clade 5 is composed of African and Asian species. They all have pseudocystidia that are highly emergent (up to 40

μm in *Lf. brachystegiae*) and thick (up to 18 μm diam in *Lf. brachystegiae*), a cutis to trichopalisade as pileipellis and no secondary velum or true cystidia.

- *Lactifluus cocosmus* is another species isolated on a rather long branch. As previously mentioned by Van de Putte et al. (2009), it has a deviating morphology, with latex turning greenish and a distinct coconut odour. There are no close relatives known.
- Clade 6 contains three African agaricoid species, two of which are possible new taxa from Cameroon. *Lactifluus rufomarginatus* is characterised by an ixopalisade as pileipellis, which is rare in the genus.
- Clade 7 consists of two African representatives. Both have a cutis to a trichopalisade as pileipellis and *Lf. densifolius* is also characterised by the presence of pleuroleptocystidia.
- Species from *Lf.* sect. *Russulopsidei* are characterised by brown-red colours in cap and stipe, a cutis as pileipellis, the presence of dermatocystidia and the absence of a velum. Several species also have true pleurocystidia.



Fig. 5 Basidiocarps of representative species from the different subgenera and sections within the genus *Lactifluus*. — a–f. *Lf.* subg. *Gymnocarpi*: a. *Lf.* sect. *Luteoli*: *Lf. brunneoviolascens* (Boerio G); b. *Lf.* sect. *Gymnocarpi*: *Lf. gymnocarpus* (EDC 12-047, De Crop E); c. *Lf.* sect. *Gymnocarpi*: *Lf. albomembranaceus* nom. prov. (EDC 12-046, De Crop E); d. *Lf.* sect. *Phlebonemi*: *Lf. aff. phlebonemus* (EDC 12-067, De Crop E); e. isolated species 6: *Lf. brunellus* (TH 7684, Henkel T); f. *Lf.* sect. *Tomentosi*: *Lf. subclarkeae* (RH 9223, Halling R). — g–l. *Lf.* subg. *Lactifluus*: g. *Lf.* sect. *Lactifluus*: *Lf. volemus* (Boerio G); h. *Lf.* sect. *Tenuicystidiati*: *Lf. sp.* (JN 2011-080, Nuytinck J); i. *Lf.* sect. *Gerardii*: *Lf. bicolor* (DS 06-229, Stubbe D); j. *Lf.* sect. *Gerardii*: *Lf. sp.* (EDC 14-500, De Crop E); k. *Lf.* sect. *Allardii*: *Lf. allardii* (C.C. 3.0, Molter D); l. *Lf.* sect. *Piperati*: *Lf. aff. piperatus* (JN 2011-072, Nuytinck J).

- *Lactifluus* sect. *Edules* corresponds to the original *Lf.* subg. *Edules*. This entirely African clade is characterised by agaricoid species with firm basidiocarps, yellowish to greyish orange colours, a trichoderm to (tricho) palisade as pileipellis and the lack of conspicuous thick-walled terminal elements in the pileipellis. The smallest representative, *Lf. roseolus*, has a slightly deviating morphology with its small basidiocarps, but its microscopic characteristics perfectly fit in this section. Unexpectedly, a former representative of *Lf.* sect. *Chamaeleontini*, *Lf. indusiatus*, also belongs to this clade.
- *Lactifluus* sect. *Albati* has Northern hemisphere representatives only. They are characterised by large, white and mostly

velutinous agaricoid basidiocarps, a lamprotrichoderm as pileipellis and/or stiptipellis composed of thick-walled hairs even up to 400 µm in *Lf. vellereus* and slightly to clearly moniliform pleuromacrocytidia.

II. *Lactifluus* subg. *Pseudogymnocarpi* — Fig. 3, 4g–l, 7

Species of *Lactifluus* subg. *Pseudogymnocarpi* are all agaricoid species characterised by yellow, orange to reddish brown caps and a trichoderm to (lampro) (tricho) palisade as pileipellis. In some species, true pleurocytidia are absent, while others have pleurolamprocytidia or pleuromacrocytidia. Some species show striking colour reactions of the latex, but most species

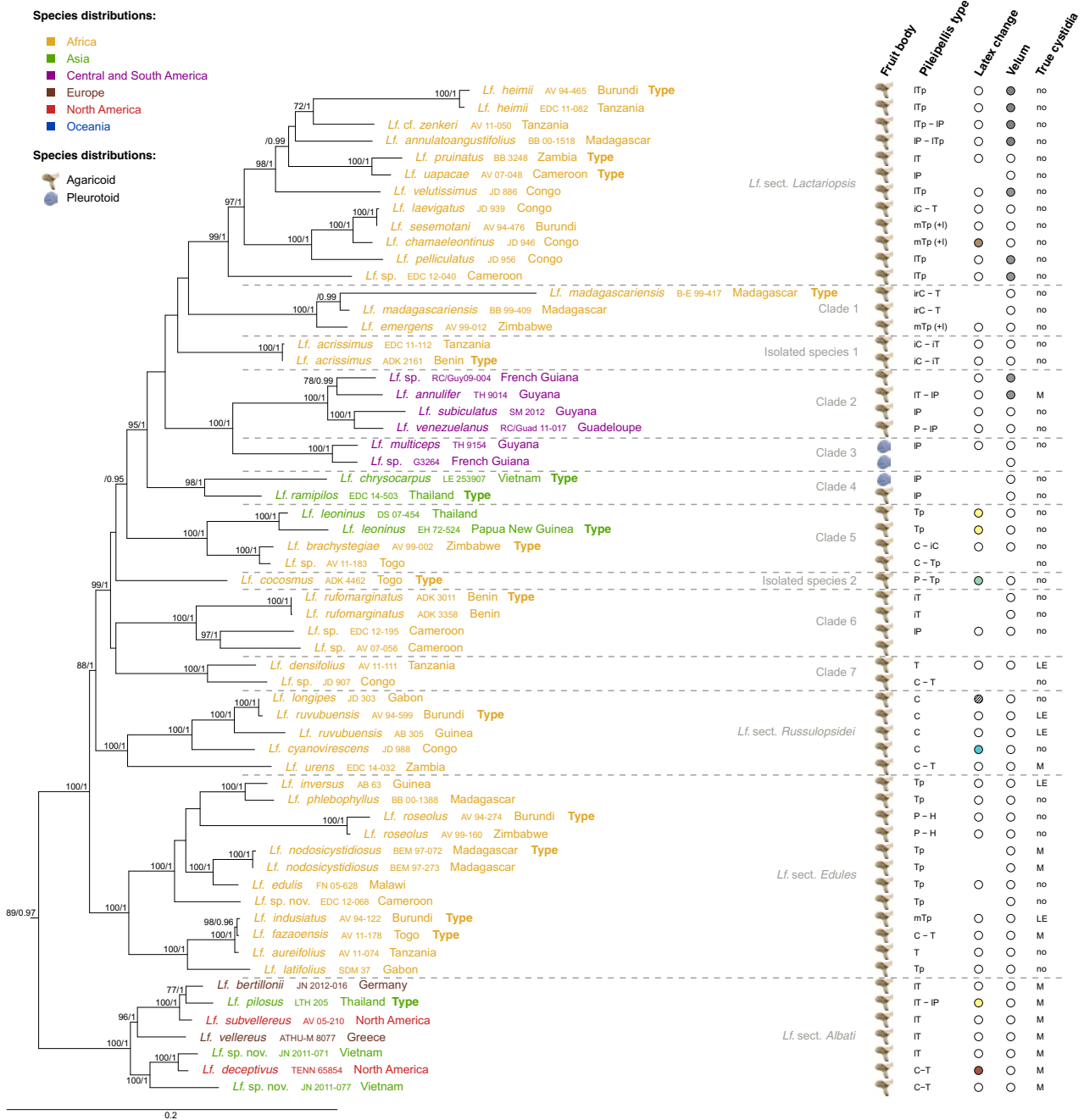


Fig. 6 Maximum Likelihood tree of *Lactifluus* subg. *Lactariopsis*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values > 70 and Bayesian Inference posterior probabilities > 0.95 are shown. Tip labels are coloured according to species' distributions, see figure for colour legend. Five morphological characteristics are plotted to the right of the tip labels. Fruit body type is represented by a symbol of an agaricoid or pleurotoid fungus. Pileipellis types are presented as a combination of the following abbreviations: C = cutis; H = hymeniderm; T = trichoderm; P = palisade; Tp = trichopalisade; i = ixo-; l = lampro-; ir = irregular; m = mixed; (+) = with abundant thick-walled elements. Latex colour change is represented by coloured circles, where white circles indicate no colour change and striped circles indicate transparent latex. Velum presence is indicated by grey, whereas velum absence is indicated by white dots. Presence of true cystidia is represented by the following abbreviations: no = no true cystidia observed; M = pleuromacrocytidia present; LE = pleurolamprocytidia present. For all characteristics, blanks indicate unknown character states.

do not. The subgenus consists of five well-supported clades and one isolated species:

- *Lactifluus* sect. *Pseudogymnocarpi* is represented by several African species and a subclade with one North American, one Asian and one European species. This section is characterised by a lampropalisade as pileipellis and some species have pleurolampro- or pleuroleptocystidia in their hymenium.
- *Lactifluus* sect. *Xerampelini* is an exclusively African clade. Species have yellowish orange to reddish brown cap colours. They have palisade-like structures as pileipellis, and only some of them have thick-walled terminal elements. They lack true pleurocystidia and spores generally have low ornamentation (usually not higher than 0.2 µm) and are verrucose or have a more or less complete reticulum.
- Clade 8 has African, Asian and North American representatives, of which several are undescribed. All representatives have palisade-like structures with thick-walled elements as

pileipellis and lack true pleurocystidia, except *Lf. armeniacus* which has pleuromacrocytostidia.

- *Lactifluus* sect. *Aurantiifolii* contains the single, isolated species *Lf. aurantiifolius*. As noted by Verbeke & Walley (2010), this species is characterised by a combination of several unique characters: bright orange lamellae, a white and fimbriate lamellar edge, a zonate and highly pruinose pileus and a chambered, tapering stipe.
- *Lactifluus* sect. *Rubroviolascensini* is an exclusively African clade. It unites species with latex that changes from cream to red and finally black, together with species that lack these colour reactions. All are characterised by pleurolamprocystidia and *Lf. carmineus* even has both pleurolampro- and pleuroleptocystidia.
- *Lactifluus* sect. *Polysphaerophori* only contains Central and South American species. Collections or their morphological descriptions were not available for most species so general characteristics are thus hard to define.

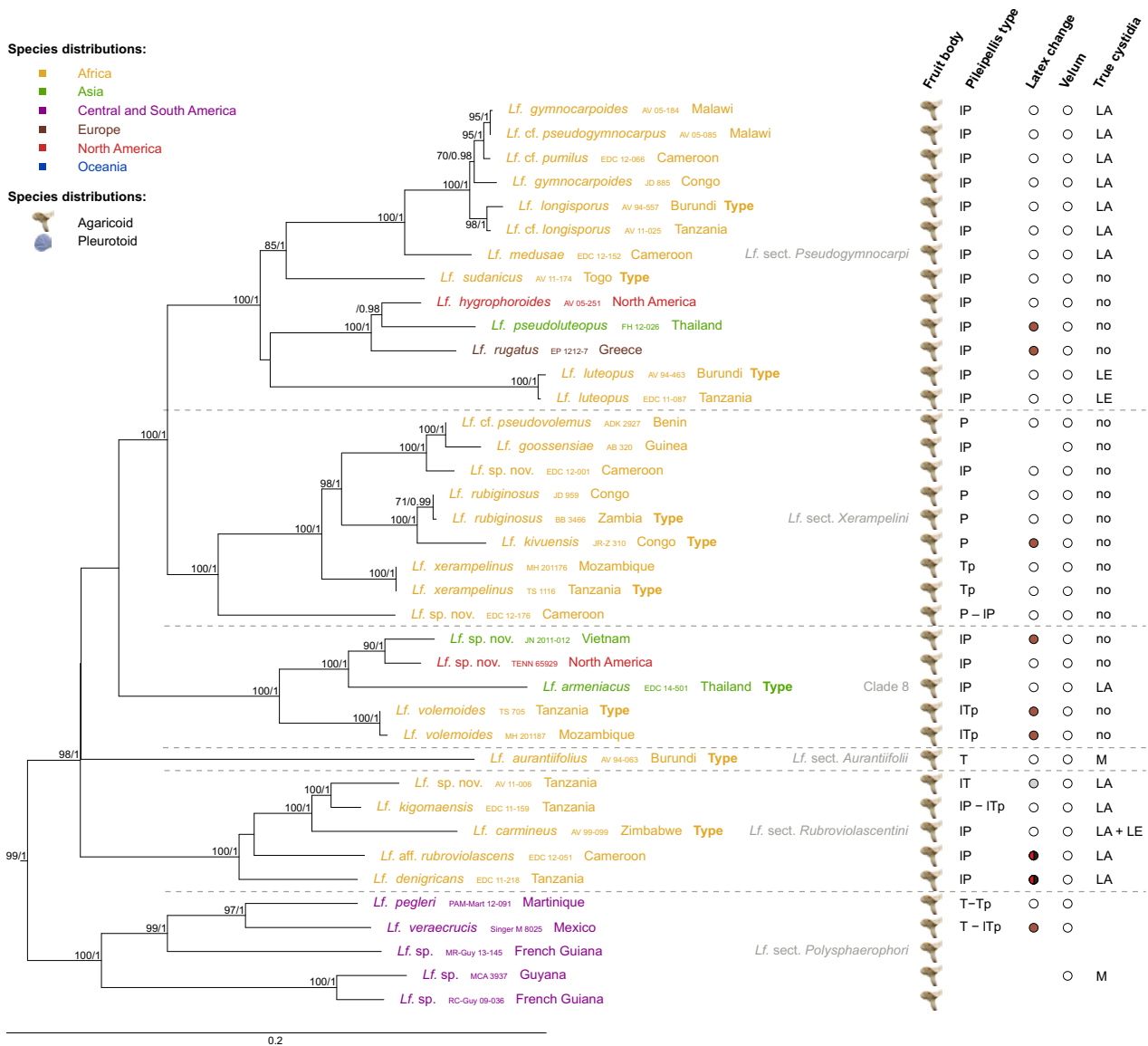


Fig. 7 Maximum Likelihood tree of *Lactifluus* subgenus *Pseudogymnocarpi*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values > 70 and Bayesian Inference posterior probabilities > 0.95 are shown. Tip labels are coloured according to species' distributions, see figure for colour legend. Five morphological characteristics are plotted to the right of the tip labels. Fruit body type is represented by a symbol of an agaricoid or pleurotoid fungus. Pileipellis types are presented as a combination of the following abbreviations: T = trichoderm; P = palisade; Tp = trichopalisade; l = lampro-. Latex colour change is represented by coloured circles, where white circles indicate no colour change and striped circles indicate transparent latex. Velum presence is indicated by grey, whereas velum absence is indicated by white dots. Presence of true cystidia is represented by the following abbreviations: no = no true cystidia observed; M = pleuromacrocytostidia present; LE = pleuroleptocystidia present; LA = pleurolamprocystidia present. For all characteristics, blanks indicate unknown character states.

III. *Lactifluus* subg. *Gymnocarpi* — Fig. 3, 5a–f, 8

Lactifluus subg. *Gymnocarpi* can be recognised by a combination of a lampropalisade as pileipellis, the absence of true pleurolamprocystidia (with discrete pleuromacrocytidia rarely present) and a brownish colour reaction of the latex and/or the context when exposed to air. The subgenus consists of five supported clades and five isolated species:

- Typical for *Lf.* sect. *Luteoli*, which consists of species from all continents except South America, are the capitate elements in the pileipellis and/or marginal cells. Verbeken & Walley (2010) already suggested that species with capitate terminal pileipellis elements might form a natural group. *Lactifluus brunneoviolascens*, the European representative, is often confused with the similar North American *Lf. luteolus*. Our study indicates that the North American species is different from the European one, which means that *Lf. luteolus* is an incorrect name for the European taxon.
- *Lactifluus* sect. *Gymnocarpi* has only African representatives. They have (slightly) thick-walled and sometimes strongly emergent marginal cells (cheilolamprocystidia) and cylindrical or irregularly shaped and often branched, thick-walled hairs in the pileipellis.
- *Lactifluus foetens* is isolated on a branch sister to the preceding two sections. Macroscopically, it resembles the recently

described species *Lf. albomembranaceus* nom. prov. (EDC 12-046) of *Lf.* sect. *Gymnocarpi*, but their microscopic characteristics do not correspond. The pileipellis of *Lf. foetens*, for example, is a lampropalisade with tufts of long, slender and regular subcylindric hairs, while the pileipellis of the undescribed species is a lampropalisade with a layer of shorter, broad and irregular subcylindric hairs.

- *Lactifluus* sect. *Phlebonemi* contains two tropical African species. They seem to have slightly different latex characteristics compared to the other species of *Lf.* subg. *Gymnocarpi*. Their latex quickly turns brownish in contact with the lamellae or the context, as well as when isolated from the flesh. Furthermore, the latex is rather whey-like and does not colour evenly.
- The remaining species form one large clade, containing several subclades with species from Oceania, Central and South America. Within this species-rich lineage, clade 9 entirely consists of Central and South American taxa. Molecularly it is well-supported, but unfortunately, thorough morphological descriptions are lacking for most of these collections. Basal to the former clade, there are four isolated species on separate branches from Central and South America: *Lf. brunellus*, *Lf. panuoides* and two undescribed species (G3185 and RC/Guad 08-042). Both *Lf. panuoides* and *Lf. brunellus* have a pleurotoid habitat, the other two

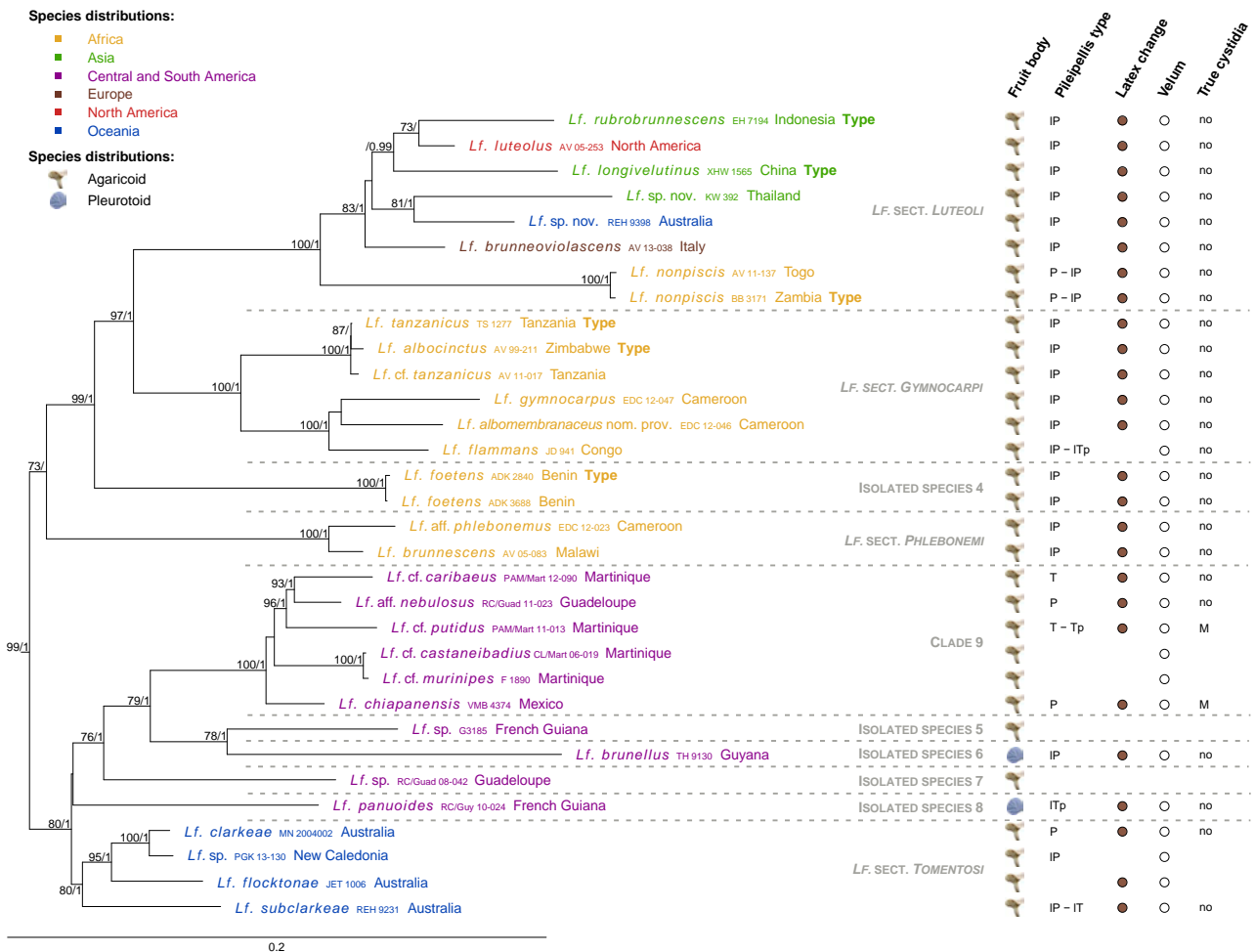


Fig. 8 Maximum Likelihood tree of *Lactifluus* subg. *Gymnocarpi*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values > 70 and Bayesian Inference posterior probabilities > 0.95 are shown. Tip labels are coloured according to species' distributions, see figure for colour legend. Five morphological characteristics are plotted to the right of the tip labels. Fruit body type is represented by a symbol of an agaricoid or pleurotoid fungus. Pileipellis types are presented as a combination of the following abbreviations: T = trichoderm; P = palisade; Tp = trichopalisade; I = lampro-. Latex colour change is represented by coloured circles, where white circles indicate no colour change and striped circles indicate transparent latex. Velum presence is indicated by grey, whereas velum absence is indicated by white dots. Presence of true cystidia is represented by the following abbreviations: no = no true cystidia observed; M = pleuromacrocytidia present. For all characteristics, blanks indicate unknown character states.

specimens are agaricoid. The Oceanian species group in *Lf.* sect. *Tomentosi*. This section is supported in both concatenated analyses, but does not get high support in the individual gene phylogenies. It includes *R. flocktonae*, originally placed in *Russula* (Cleland & Cheel 1919). Singer (1942) noted that it could be *Lactarius clarkeae* and Lebel et al. (2013) also indicated that it belongs to *Lactifluus*. In our analyses it is sister to *Lf. clarkeae* and we will recombine this in *Lactifluus*.

IV. *Lactifluus* subg. *Lactifluus* — Fig. 3, 5g–l, 9

Lactifluus subg. *Lactifluus* is characterised by a range of pileipellis types, from a hyphoepithelium over a palisade to a lampro-palisade. In some sections, true pleurocystidia are absent, while in others pleuromacrocystidia and/or pleurolamprocystidia are found. Most species are agaricoid, only *Lf.* sect. *Gerardii* has several pleurotoid representatives. For some sections, the colour reaction of the context and/or the latex upon contact with air is an important characteristic. The subgenus contains species from Asia, Europe, North America and Oceania and

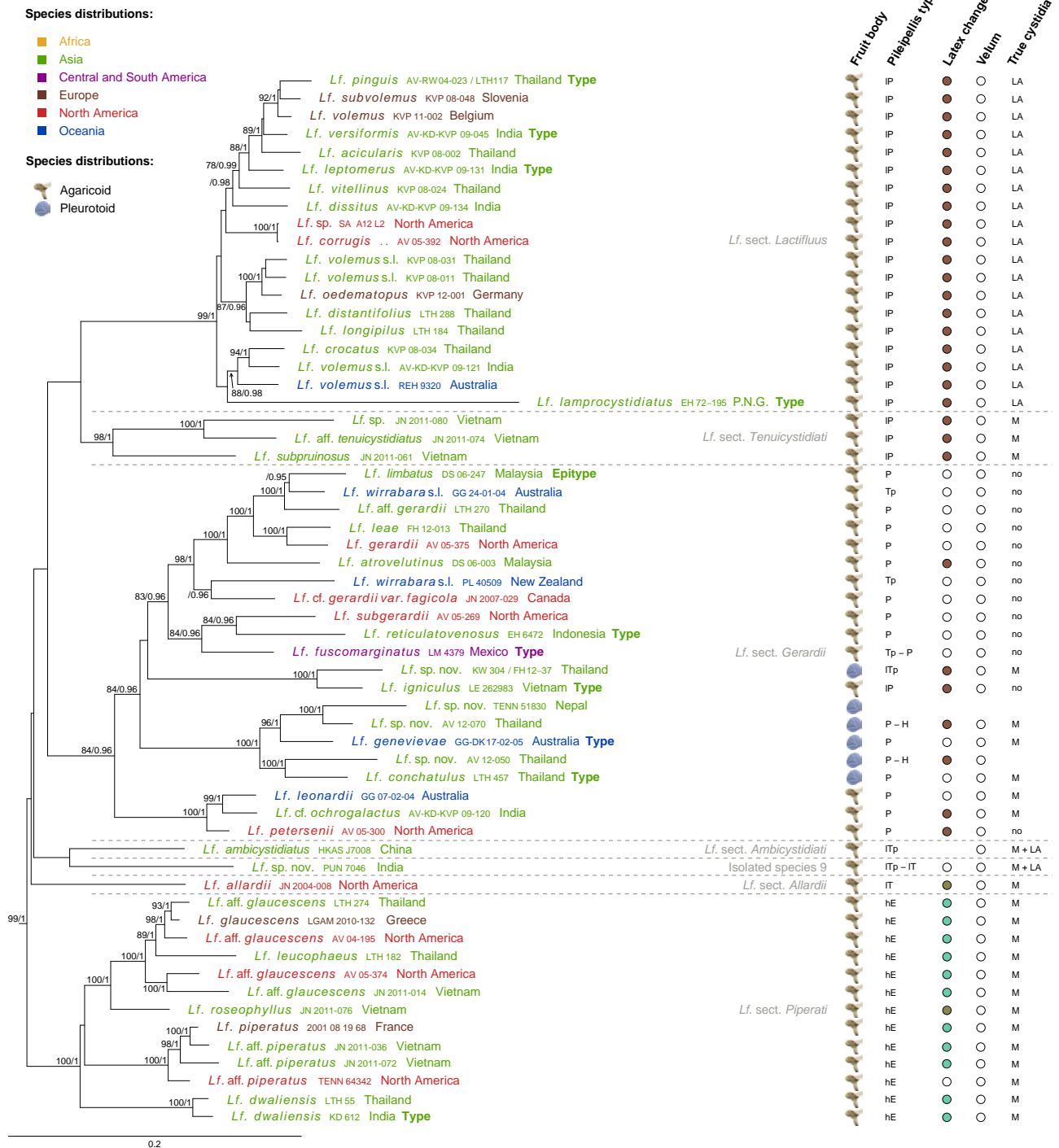


Fig. 9 Maximum Likelihood tree of *Lactifluus* subg. *Lactifluus*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values > 70 and Bayesian Inference posterior probabilities > 0.95 are shown. Tip labels are coloured according to species' distributions, see figure for colour legend. Five morphological characteristics are plotted to the right of the tip labels. Fruit body type is represented by a symbol of an agaricoid or pleurotoid fungus. Pileipellis types are presented as a combination of the following abbreviations: H = hymeniderm; T = trichoderm; hE = hyphoepithelium; P = palisade; Tp = trichopalisade; l = lampro-. Latex colour change is represented by coloured circles, where white circles indicate no colour change and striped circles indicate transparent latex. Velum presence is indicated by grey, whereas velum absence is indicated by white dots. Presence of true cystidia is represented by the following abbreviations: no = no true cystidia observed; M = pleuromacrocystidia present; LA = pleurolamprocystidia present. For all characteristics, blanks indicate unknown character states. In the tip labels, P.N.G. stands for Papua New Guinea.

consists of six separate clades, all molecularly and morphologically well-supported. These clades correspond well to current classifications and we recognize them here at section level: *Lf.* sect. *Allardii*, *Lf.* sect. *Ambicystidiati*, *Lf.* sect. *Gerardii*, *Lf.* sect. *Lactifluus*, *Lf.* sect. *Piperati* and *Lf.* sect. *Tenuicystidiati*. *Lactifluus* sect. *Gerardii* is equivalent to *Lf.* subg. *Gerardii* described in the introduction, but to limit the number of subgenera in *Lactifluus*, we decided to treat it as section. The other five sections correspond to those described in the introduction.

TAXONOMIC PART

Genus

Genus ***Lactifluus*** (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806

Basionym. *Agaricus* sect. *Lactifluus* Pers., Syn. Meth. Fung.: 429. 1801.
= *Pleurogala* Redhead & Norvell, Mycotaxon 48: 377. 1993.
≡ *Lactarius* sect. *Panuoidei* Singer, Kew Bull. 7: 301. 1952.
Type (automatic). *Agaricus lactifluus* L., Sp. Pl.: 1172. 1753 (= *Lactifluus volemus* (Fr.: Fr.) Kuntze).

Subgenera

Lactifluus* subg. *Gymnocarpi (R. Heim ex Verbeke) De Crop, *comb. nov.* — MycoBank MB814217

Basionym. *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeke, Mycotaxon 66: 374. 1998.

Type. *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946 (≡ *Lactifluus gymnocarpus* (R. Heim ex Singer) Verbeke).

Lactifluus* subg. *Lactariopsis (Henn.) Verbeke, Mycotaxon 118: 449. 2011

Basionym. *Lactariopsis* Henn., Bot. Jahrb. Syst. 30: 51. 1901.
≡ *Lactarius* subg. *Lactariopsis* (Henn.) R. Heim, Prodr. Fl. Mycologique Madagascar 1: 36. 1938.
= *Lactarius* sect. *Edules* Verbeke, Belg. J. Bot. 132: 176. 2000 (1999).
≡ *Lactifluus* subg. *Edules* (Verbeke) Verbeke, Mycotaxon 118: 448. 2011.
= *Lactarius* subg. *Russulopsis* Verbeke, Mycotaxon 77: 439. 2001.
≡ *Lactifluus* subg. *Russulopsis* (Verbeke) Verbeke, Mycotaxon 118: 452. 2011.
Type. *Lactariopsis zenkeri* Henn., Bot. Jahrb. Syst. 30: 51. 1902 (1901) (≡ *Lactifluus zenkeri* (Henn.) Verbeke).

Lactifluus subg. *Lactifluus*

≡ *Lactarius* subg. *Lactiflui* (Burl.) Hesler & A.H. Sm., N. Amer. Sp. Lactarius: 158. 1979.
= *Lactifluus* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, Mycotaxon 119: 484. 2012.
≡ *Lactarius* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, Fungal Biol. 114: 280. 2010.
≡ *Lactarius* ser. *Gerardii* A.H. Sm. & Hesler, Brittonia 14: 378. 1962.
= *Lactifluus* subg. *Piperati* Verbeke, Mycotaxon 120: 449. 2012.
Type (automatic). *Agaricus lactifluus* L., Sp. Pl.: 1172. 1753 (= *Lactifluus volemus* (Fr.: Fr.) Kuntze).

Lactifluus* subg. *Pseudogymnocarpi (Verbeke) De Crop, *comb. nov.* — MycoBank MB814193

Basionym. *Lactarius* sect. *Pseudogymnocarpi* Verbeke, Mycotaxon 66: 376. 1998.
≡ *Lactifluus* sect. *Pseudogymnocarpi* (Verbeke) Verbeke, Mycotaxon 120: 447. 2012.
≡ *Lactarius* sect. *Rugati* Verbeke, Mycotaxon 66: 372. 1998, nom illegit. (Art. 52.1).
≡ *Lactarius* subsect. *Rugati* Pacioni & Lalli, Mycotaxon 44: 190. 1998, nom illegit. (Art. 52.1).
Type. *Lactarius gymnocarpoides* Verbeke, Mycotaxon 55: 530. 1995 (≡ *Lactifluus gymnocarpoides* (Verbeke) Verbeke).

Sections

Within Lactifluus subg. Gymnocarpi:

Lactifluus* sect. *Luteoli (Pacioni & Lalli) Verbeke, *comb. nov.* — MycoBank MB814194

Basionym. *Lactarius* subsect. *Luteoli* Pacioni & Lalli, Mycotaxon 44: 190. 1992.

≡ *Lactarius* sect. *Luteoli* (Pacioni & Lalli) Pierotti, Boll. Gruppo Micol. Bres. 48: 54. 2007.

Type. *Lactarius luteolus* Peck, Bull. Torrey Bot. Club 23: 412. 1896 (≡ *Lactifluus luteolus* (Peck) Verbeke).

Lactifluus* sect. *Gymnocarpi (R. Heim ex Verbeke) De Crop, *comb. nov.* — MycoBank MB814195

Basionym. *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeke, Mycotaxon 66: 374. 1998.

Type. *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946 (≡ *Lactifluus gymnocarpus* (R. Heim ex Singer) Verbeke).

Lactifluus* sect. *Phlebonemi (R. Heim ex Verbeke) Verbeke, Mycotaxon 120: 446. 2012

Basionym. *Lactarius* sect. *Phlebonemi* R. Heim ex Verbeke, Mycotaxon 66: 378. 1998.

Type. *Lactarius phlebonemus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 38. 1955 (≡ *Lactifluus phlebonemus* (R. Heim & Gooss.-Font.) Verbeke).

Lactifluus* sect. *Tomentosi (McNabb) Verbeke, Mycotaxon 120: 448. 2012

Basionym. *Lactarius* sect. *Tomentosi* McNabb, New Zealand J. Bot. 9: 59. 1971.

Type. *Lactarius clarkeae* Cleland, Trans. & Proc. Roy. Soc. South Australia 51: 302. 1927 (as *clarkei*) (≡ *Lactifluus clarkeae* (Cleland) Verbeke).

Within Lactifluus subg. Lactariopsis:

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

Basionym. *Lactarius* (unranked) *Albati* Bataille, Fl. Monogr. Astéro.: 35. 1908.

≡ *Lactarius* sect. *Albati* (Bataille) Singer, Ann. Mycol. 40: 109. 1942.
Type. *Agaricus vellereus* Fr., Syst. Mycol. 1: 76. 1821: Fr., loc. cit. (≡ *Lactifluus vellereus* (Fr.: Fr.) Kuntze).

Lactifluus* sect. *Edules (Verbeke) Verbeke, *comb. nov.* — MycoBank MB814197

Basionym. *Lactarius* sect. *Edules* Verbeke, Belg. J. Bot. 132: 176. 2000 (1999).

Type. *Lactarius edulis* Verbeke & Buyck, Champ. Comest. Ouest Burundi: 103. 1994. (≡ *Lactifluus edulis* (Verbeke & Buyck) Buyck).

Lactifluus* sect. *Lactariopsis Verbeke, Mycotaxon 118: 450. 2011

≡ *Lactarius* sect. *Lactariopsis* (Henn.) Singer, Ann. Mycol. 40: 111. 1942.
≡ *Lactarius* sect. *Lactariopsidaei* Singer, Sydowia 15: 83. 1962.
≡ *Lactarius* sect. *Chamaeleontini* Verbeke, Mycotaxon 66: 393. 1998.
Type. *Lactariopsis zenkeri* Henn., Bot. Jahrb. Syst. 30: 51. 1902 (1901) (≡ *Lactifluus zenkeri* (Henn.) Verbeke).

Lactifluus* sect. *Russulopsidaei (Verbeke) Verbeke, Mycotaxon 118: 452. 2011

Basionym. *Lactarius* sect. *Russulopsidaei* Verbeke, Mycotaxon 77: 440. 2001.

Type. *Lactarius ruvuensis* Verbeke, Bull. Jard. Bot. Natl. Belg. 65: 208. 1996 (≡ *Lactifluus ruvuensis* (Verbeke) Verbeke).

Within *Lactifluus* subg. *Lactifluus*:***Lactifluus* sect. *Lactifluus***

Type (automatic). *Agaricus lactifluus* L., Sp. Pl.: 1172. 1753 (= *Lactifluus volemus* (Fr.: Fr.) Kuntze).

***Lactifluus* sect. *Gerardii* (A.H. Sm. & Hesler) Stubbe, *comb. nov.* — MycoBank MB814198**

Basionym. *Lactarius* ser. *Gerardii* A.H. Sm. & Hesler, Brittonia 14: 378. 1962.

Type. *Lactarius gerardii* Peck, Bull. Buffalo Soc. Nat. Sci. 1: 57. 1873 (as *L. 'gerardii'*). (≡ *Lactifluus gerardii* (Peck) Kuntze).

***Lactifluus* sect. *Piperati* (Fr.) Verbeken, Mycotaxon 120: 449. 2012**

Basionym. *Agaricus* sect. *Piperati* Fr., Syst. Mycol. 1: 73. 1821.

≡ *Lactarius* sect. *Piperati* (Fr.: Fr.) Fr., Epicr. Syst. Mycol.: 338. 1838.

Type. *Agaricus piperatus* L., Sp. Pl.: 1173. 1753: Fr., Syst. Mycol. 1: 76. 1821 (≡ *Lactifluus piperatus* (L.: Fr.) Verbeken).

***Lactifluus* sect. *Allardii* (Hesler & A.H. Sm.) De Crop, Mycotaxon 120: 450. 2012**

Basionym. *Lactarius* sect. *Allardii* Hesler & A.H. Sm., N. Amer. Sp. Lactarius: 207. 1979.

Type. *Lactarius allardii* Coker, J. Elisha Mitchell Sci. Soc. 34: 12. 1918 (≡ *Lactifluus allardii* (Coker) De Crop).

***Lactifluus* sect. *Tenuicystidiati* X.H. Wang & Verbeken, Mycologia 107, 5: 954. 2015**

Type. *Lactarius tenuicystidiatus* X.H. Wang & Verbeken, Nova Hedwigia 83, 1–2: 173. 2006 (≡ *Lactifluus tenuicystidiatus* (X.H. Wang & Verbeken) X.H. Wang).

***Lactifluus* sect. *Ambicystidiati* X.H. Wang, Mycologia 107, 5: 954. 2015**

Type. *Lactifluus ambicystidiatus* X.H. Wang, Mycologia 107, 5: 948. 2015.

Within *Lactifluus* subg. *Pseudogymnocarpi*:***Lactifluus* sect. *Aurantiifolii* (Verbeken) Verbeken, Mycotaxon 120: 450. 2012**

Basionym. *Lactarius* sect. *Aurantiifolii* Verbeken, Mycotaxon 77: 441. 2001.

Type. *Lactarius aurantiifolius* Verbeken, Bull. Jard. Bot. Natl. Belg. 65: 197. 1996 (≡ *Lactifluus aurantiifolius* (Verbeken) Verbeken).

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

Basionym. *Lactarius* sect. *Polysphaerophori* Singer, Beih. Sydowia 7: 106. 1973.

Type. *Lactarius veraecrucis* Singer, Beih. Sydowia 7: 104. 1973 (≡ *Lactifluus veraecrucis* (Singer) Verbeken).

***Lactifluus* sect. *Pseudogymnocarpi* (Verbeken) Verbeken, Mycotaxon 120: 447. 2012**

Basionym. *Lactarius* sect. *Pseudogymnocarpi* Verbeken, Mycotaxon 66: 376. 1998.

= *Lactarius* sect. *Rugati* Verbeken, Mycotaxon 66: 372. 1998, nom. illegit. (Art. 52.1).

Type. *Lactarius gymnocarpoides* Verbeken, Mycotaxon 55: 530. 1995 (≡ *Lactifluus gymnocarpoides* (Verbeken) Verbeken).

***Lactifluus* sect. *Rubroviolascens* (Singer) Verbeken, Mycotaxon 120: 447. 2012**

Basionym. *Lactarius* subsect. *Rubroviolascens* Singer, Ann. Mycol. 40: 114. 1942.

≡ *Lactarius* sect. *Rubroviolascens* (Singer) Verbeken, Mycotaxon 66: 380. 1998, as '*Rubroviolascens*'.

Type. *Lactarius rubroviolascens* R. Heim, Candollea 7: 377. 1938 (≡ *Lactifluus rubroviolascens* (R. Heim) Verbeken).

***Lactifluus* sect. *Xerampelini* De Crop, *sect. nov.* — MycoBank MB814199**

Pileus medium to large sized, firm; pellis mat, dry, with yellowish orange, red and reddish brown colours. Lamellae moderately spaced to very distant, thick, whitish, yellowish to orange; edge concolorous. Stipe central, cylindrical, firm, dry, more or less concolorous with pileus. Context white, unchanging, firm; taste mild. Latex abundant, white to watery, unchanging, sometimes drying brownish grey. Spores ellipsoid, sometimes elongate to strongly elongate, verrucose or with a more or less complete reticulum, generally low ornamented, usually not higher than 0.2 µm; plage sometimes with central amyloid spot. True pleurocystidia absent. Pileipellis a lamprospiralade to palisade or trichopalasade.

Type. *Lactarius xerampelinus* Karhula & Verbeken, Karstenia 38, 2: 59. 1998 (≡ *Lactifluus xerampelinus* (Karhula & Verbeken) Verbeken).

New combinations at species level***Lactifluus acrisimus* (Verbeken & Van Rooij) Nuytinck, *comb. nov.* — MycoBank MB814200**

Basionym. *Lactarius acrisimus* Verbeken & Van Rooij, Nova Hedwigia 77: 225. 2003.

***Lactifluus brunellus* (S.L. Mill., Aime & T.W. Henkel) De Crop, *comb. nov.* — MycoBank MB814201**

Basionym. *Lactarius brunellus* S.L. Mill., Aime & T.W. Henkel, Mycologia 94, 3: 546. 2002.

***Lactifluus castaneibadius* (Pegler) De Crop, *comb. nov.* — MycoBank MB814202**

Basionym. *Lactarius castaneibadius* Pegler, Kew Bull. 33, 4: 622. 1979.

***Lactifluus chiapanensis* (Montoya, Bandala & Guzmán) De Crop, *comb. nov.* — Mycobank MB814203**

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

***Lactifluus flocktonae* (Cleland & Cheel) T. Lebel, *comb. nov.* — MycoBank MB814204**

Basionym. *Russula flocktonae* Cleland & Cheel, Trans. & Proc. Roy. Soc. South Australia 43: 274. 1919.

***Lactifluus multiceps* (S.L. Mill., Aime & T.W. Henkel) De Crop, *comb. nov.* — Mycobank MB814205**

Basionym. *Lactarius multiceps* S.L. Mill., Aime & T.W. Henkel, Mycologia 94, 3: 549. 2002.

***Lactifluus murinipes* (Pegler) De Crop, *comb. nov.* — MycoBank MB814206**

Basionym. *Lactarius murinipes* Pegler, Kew Bull. 33, 4: 623. 1979.

***Lactifluus nebulosus* (Pegler) De Crop, *comb. nov.* — MycoBank MB814207**

Basionym. *Lactarius nebulosus* Pegler, Kew Bull. 33: 610. 1979.

***Lactifluus panuoides* (Singer) De Crop, *comb. nov.* — MycoBank MB814208**

Basionym. *Lactarius panuoides* Singer, Kew Bull. 7: 300. 1952.

***Lactifluus rufomarginatus* (Verbeken & Van Rooij) De Crop, *comb. nov.* — MycoBank MB814209**

Basionym. *Lactarius rufomarginatus* Verbeken & Van Rooij, Nova Hedwigia 77, 1: 235. 2003.

Lactifluus uapacae (Verbeken & Stubbe) De Crop, *comb. nov.* — MycoBank MB814210

Basionym. *Lactarius uapacae* Verbeken & Stubbe, *Cryptog. Mycol.* 29, 2: 140. 2008.

Lactifluus venezuelanus (Dennis) De Crop, *comb. nov.* — MycoBank MB814211

Basionym. *Lactarius venezuelanus* Dennis, *Kew Bull.*, *Addit. Ser.* 3: 467. 1970.

DISCUSSION

Translation of the phylogeny in a new infrageneric classification

In this study, we attempted to resolve the infrageneric classification of the genus *Lactifluus*. Molecular results support four major clades, which we classify as subgenera, and within these subgenera, several sections can be delimited. Not all our results are congruent with the former infrageneric classification of *Lactifluus* (Fig. 3), so we provide an overview of the nomenclatural changes evoked by these new results (Taxonomic Part). Most of the traditional subgenera are rejected; only *Lf. subg. Lactariopsis* and *Lf. subg. Lactifluus* are retained but amended. Two new subgenera are proposed here: *Lf. subg. Gymnocarpi* and *Lf. subg. Pseudogymnocarpi*. All four subgenera are supported in the concatenated and the individual gene phylogenies, with one exception: the *RPB1* phylogeny does not support the inclusion of *Lf. sect. Albatii* in *Lf. subg. Lactariopsis*. For now, we decided to include the section in *Lf. subg. Lactariopsis*, as the inclusion is supported in the other individual gene phylogenies and in the concatenated phylogeny. We also preferred to define the largest supported subgenera with an evenly balanced species diversity. The relationships between the subgenera are not yet fully resolved based on our phylogenetic results. To fully understand the relationships between the subgenera, more genes need to be sequenced. Several traditional sections are confirmed in their traditional delimitation (*Lf. sect. Albatii*, *Lf. sect. Allardii*, *Lf. sect. Ambicystidiati*, *Lf. sect. Aurantiifolii*, *Lf. sect. Edules*, *Lf. sect. Gerardii*, *Lf. sect. Lactifluus*, *Lf. sect. Piperati*, *Lf. sect. Russulopsidei* and *Lf. sect. Tenuicystidiati*), others are polyphyletic and either synonymised (*Lf. sect. Chamaeleontini* and *Lf. sect. Rugati*) or amended (*Lf. sect. Lactariopsis*, *Lf. sect. Luteoli*, *Lf. sect. Phlebonemi*, *Lf. sect. Polysphaerophori*, *Lf. sect. Pseudogymnocarpi*, *Lf. sect. Rubroviolascensini*, *Lf. sect. Tomentosii*). Our analyses show ten additional clades which we suspect may represent new sections. In the present work, we only aim to assign new sections to clades that are fully supported and characterised by several synapomorphic features. The African *Lf. sect. Xerampelini* is newly described, as it is clearly demarcated by its yellowish orange to reddish brown cap colours, a (lampro-)palisade as pileipellis, the absence of true pleurocystidia and spores with low ornamentation, usually not higher than 0.2 µm, that are verrucose or forming a more or less complete reticulum. For the remaining clades we do not yet propose infrasubgeneric ranks because a more thorough sampling and a thorough search for potential synapomorphies is necessary for this to be possible. We demonstrate the existence of at least 17 undescribed species spread across the four subgenera. This supports the hypothesis that *Lactifluus* is a species-rich genus where the diversity has not yet been adequately characterised. The new species that are phylogenetically characterised here will be described in future publications.

Conclusions at generic level

Our molecular results support the monophyly of *Lactifluus*, together with monophyly of *Lactarius*, *Russula* and *Multifurca*.

Previous analyses have shown however that this support at genus level strongly depends on outgroup choice (De Crop et al. unpubl. res.). Our phylogenies are rooted with the outgroup used in Buyck et al. (2008), with the addition of *Heterobasidion annosum* and the exclusion of *Peniophora nuda*, *Albatrellus skamianus* and *Gloeocystidiellum porosum*. Depending on the composition of the outgroup taxa, one or more of the *Russulaceae* genera receives less support. Further research within the order *Russulales* may point to better candidates as outgroup taxa for the *Russulaceae*. Additionally, to draw conclusions concerning the relationships between the *Russulaceae*-genera, the non-agaricoid genera also need to be taken into account. These are currently poorly sampled, but will be crucial to make conclusions at the generic level.

Evaluation of morphological characters

Lactifluus exhibits considerable morphological variation, with cap diameters varying from a few millimetres to more than 20 cm, agaricoid or pleurotoid fruit body types, more than ten different types of pileipellis, striking colour changes of the latex and/or context, different types of true cystidia and/or pseudocystidia, different habitats and ectomycorrhizal hosts.

In the morphological part of our study, we focus on five characteristics, which are putatively informative at the infrageneric level:

General habitus

The first characteristic is the general habitus of the basidiocarp. The majority of the studied *Lactifluus* species is agaricoid, only a minority is pleurotoid. So far, no sequestrate species are known, although more extensive explorations, targeting sequestrate fungi, might reveal sequestrate *Lactifluus* species. We confirm the results of previous studies (Miller et al. 2012, Morozova et al. 2013) which state that the pleurotoid habitus has multiple origins, since pleurotoid species occur in seven different clades in three different subgenera. Consequently, this characteristic is not informative at infrageneric level within *Lactifluus*, although it had previously been used to separate the obsolete genus *Pleurogala* (Redhead & Norvell 1993).

Presence or absence of a secondary velum

The second characteristic is the presence or absence of a secondary velum. This feature was used by Hennings (1902) as the basis for the genus *Lactariopsis* (including one species, *Lf. zenkeri*). Its importance was diminished by the definition of *L. subg. Lactariopsis* (including *Lf. annulatoangustifolius*) by Heim (1938) and later, *L. sect. Lactariopsidei* (including neotropical species *Lf. neotropicus* and *Lf. annulifer*) by Singer (1942, 1961) and Singer et al. (1983). As suggested by several other authors (Verbeken 1998b, Buyck et al. 2007, 2008, Verbeken & Walley 2010), this striking characteristic occurs in at least two clades and therefore cannot be used to delimit clades. Nevertheless, this character is phylogenetically informative, since all species with a distinct secondary velum are found within *Lf. subg. Lactariopsis*. Species with a distinct ring and velum at the pileus margin are only known from Africa and South America. Apart from species with a distinct velum, there are some African species, such as *Lf. laevigatus* and *Lf. indusiatus* that give the impression of a velum at the pileus margin. However, the feature is not as distinct as in *Lf. heimii* or *Lf. velutissimus* and these species never develop an annulus on the stipe. Further research is needed to determine whether these really are velar remnants. Anyhow, this feature is not informative at section level since it occurs in several clades within *Lf. subg. Lactariopsis*.

Colour reaction of the latex and/or the context

The third characteristic is the colour reaction of the latex and/or the context when exposed to the air. *Lactifluus* species show a wide variety of colour changes. These changes are informative and can be used together with other characteristics to distinguish

some groups. For example, in both *Lf.* subg. *Gymnocarpi* and *Lf.* sect. *Lactifluus* there are brownish colour changes of the latex and/or the context when they are exposed to air. In other groups, these changes only occur in some species, which makes the feature uninformative. For example, the beige latex of *Lf. rubroviolascens* and *Lf. denigricans* first turns bright red and later turns blackish when exposed to air, but the other species in *Lf.* sect. *Rubroviolascens* lack these striking colour changes.

Pileipellis type

The fourth characteristic is the pileipellis type. Several studies (Bon 1983, Heilmann-Clausen et al. 1998, Verbeken 1998a, Verbeken & Walley 2010) have mentioned this as one of the most important characteristics to delineate sections and subgenera within *Lactifluus*, as well as in *Lactarius*. Our study confirms this, with the restriction that the pileipellis type can only be used within some subgenera. In *Lf.* subg. *Pseudogymnocarpi* for instance, the majority of species has a lamprolalisade, which makes it difficult to use the feature within the subgenus.

Presence or absence of true pleurocystidia

The fifth characteristic is the presence or absence of true pleurocystidia, together with cystidium type (macro-, lepto- or lamprocystidia). Again, this characteristic can be used to delimit some sections in combination with other characteristics. In e.g. *Lf.* sect. *Lactifluus*, the presence of pleurolamprocystidia, together with the absence of pleuromacrocystidia, isolates it from the other sections within the subgenus.

Out of the five characteristics we focused on, three can be used, in combination with each other or other characteristics, to delimit subgenera or sections within the genus. Other morphological characteristics will need to be studied in more detail to morphologically support all subgenera and sections found in our phylogeny. Our study, together with previous ones (Verbeken 1996a, Verbeken & Walley 2010), indicates that microscopic characteristics such as the shape of pseudocystidia, the shape and ornamentation of the basidiospores (although difficult to quantify) or the shape of marginal cells might be important characteristics in certain groups. Other important characteristics that might be important in the evolution of *Lactifluus* species relate to their ecology, such as their ectomycorrhizal host trees. Within *Lf.* subg. *Lactariopsis*, the pileus development may also be an important morphological character: several species are characterised by involute pileus margins in young basidiomes, so that lamellae are protected when growing. On the contrary, in most other species pileus margins are not involute and lamellae are exposed from the beginning (De Crop et al. unpubl. res.). To know more about the evolutionary importance of this feature, a more detailed study on the ontogeny of basidiomes in the field is necessary.

Conclusions at species level

This study mainly focuses on the infrageneric relationships within *Lactifluus* and is not aimed at delimiting species within the genus. Our phylogeny cannot be used to make decisions at species level, although it can be used to draw attention to several species that need to be studied in more detail, using more collections and species delimitation techniques. The first clades within *Lf.* subg. *Lactariopsis* that draw our attention are those of *Lf. madagascariensis* and *Lf. leoninus*. For both species, the type specimen is on a longer branch than the other collection morphologically determined as the same species. This might be due to the poor quality of the type sequences. Further study is needed to verify if the latter is conspecific with the type specimens. In *Lf.* sect. *Russulopsidei*, *Lf. ruvubuensis* and *Lf. longipes* also need to be studied in more detail. The type of *Lf. ruvubuensis* is phylogenetically closest to a collection

identified as *Lf. longipes* and not closest to the other collection identified as *Lf. ruvubuensis*. Even when adding more collections to the analysis, the *Lf. ruvubuensis* type clusters together with specimens determined as *Lf. longipes*. (unpubl. res.). This could indicate misdeterminations of the non-type collections, but a more thorough study is necessary to resolve this issue. Finally, there are several clades where multiple species cluster together. For example, within *Lf.* sect. *Edules*: *Lf. aureifolius*, *Lf. indusiatus* and *Lf. fazaoensis*, in *Lf.* sect. *Pseudogymnocarpi*: *Lf. gymnocapoides*, *Lf. longisporus*, *Lf. pseudogymnocarpus* and *Lf. pumilus*, in *Lf.* sect. *Gymnocarpi*: *Lf. albocinctus* and *Lf. tanzanicus* and in *Lf.* subg. *Gymnocarpi*, clade 9: *Lf. cf. castaneibadius* and *Lf. cf. murinipes*. Some of these species might have to be synonymised, or they may represent species complexes, the occurrence of which has repeatedly been reported in *Lactifluus* (Stubbe et al. 2010, Van de Putte et al. 2010, 2012, De Crop et al. 2012).

Morphological differences between the milkcap genera *Lactifluus* and *Lactarius*

It remains difficult to find morphological synapomorphies for either *Lactarius* or *Lactifluus*. Some general trends were formulated by Verbeken & Nuytinck (2013) that can be used to distinguish both genera:

- i. thick-walled elements in the pileipellis and stipitipellis, as well as lamprocystidia, are generally present in *Lactifluus* and very rarely observed in *Lactarius*;
- ii. a hymenophoral trama composed of sphaerocytes (as in *Russula*) is common in *Lactifluus* but is rarely observed in *Lactarius*;
- iii. pleurotoid species are apparently restricted to *Lactifluus*;
- iv. sequestrate species are apparently restricted to *Lactarius*; and
- v. species with velum are apparently restricted to *Lactifluus*.

Besides these morphological trends, the genera also differ in distribution. *Lactarius* is mainly distributed in the Northern hemisphere, while *Lactifluus* has its main range in the tropics. Despite these trends, both milkcap genera remain difficult to distinguish for the time being, and can only be separated with certainty through molecular data.

Ecology

Species of the genus *Lactifluus* can be found in temperate, subtropical and tropical regions, in a wide range of vegetation types, such as tropical and subtropical rain forests, subtropical dry forests, monsoon forests, tree savannahs, Mediterranean woodlands, temperate broadleaf and coniferous forests and montane forests. Basidiocarps are commonly found on soil, but sporadically on stems or aerial roots of trees, such as *Lf. brunellus* (Fig. 5e) on stems of *Dicymbe corymbosa* (Miller et al. 2002). *Lactifluus* species are ectomycorrhizal fungi and we hypothesize that the ectomycorrhizal hosts might have played important roles in species evolution. Present data suggest that both generalists and specialists occur, but the exact mycorrhizal connection generally remains undetermined. Ecological characteristics are not commonly recorded for every collection during field work, and it is hard to find out which tree a fungal species grows with in mixed forests. Common techniques to detect the host tree in mixed forests are labour-intensive and expensive, since ectomycorrhizal roots have to be excavated and both fungus and plant have to be sequenced.

Biogeography

As previously noted (Verbeken & Nuytinck 2013), *Lactifluus* is mainly distributed in the tropics. Tropical Africa is most species-rich, followed by tropical Asia and the Neotropical region. However, the Neotropics are still largely underexplored, so

we expect the diversity of *Lactifluus* to be larger than currently known in the Neotropics. The geographical distribution of *Lactifluus* differs among the four subgenera. *Lactifluus* subg. *Lactariopsis*, *Lf.* subg. *Gymnocarpi* and *Lf.* subg. *Pseudogymnocarpi* mainly contain species from the tropics, but each contains one or two temperate lineages. *Lactifluus* subg. *Lactifluus* is mainly distributed in the northern hemisphere, with the exception of some Australian species, but with no known representatives in Africa or South America. Within *Lactifluus*, both allopatric and sympatric speciation are hypothesised to have played a role in the evolution of new species. Stubbe et al. (2010) noted that sympatric species of *Lf.* sect. *Gerardii* are often distantly related, which suggests allopatric speciation as the major mechanism responsible for the species diversity within this section. In contrast, Van de Putte et al. (2012) found that in *Lf.* subg. *Lactifluus* several closely related species occur in sympatry and therefore might have evolved reproductive barriers and/or different ways to exploit their environment. The biogeographical history of the genus will be discussed in more detail in our next publication, where we will use Bayesian techniques to date the *Lactifluus* phylogeny, to find out where the genus might have originated and how it reached its current distribution.

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