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Global phylogeny and evolutionary history of the genus Lactifluus

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DANKWOORD

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Eske

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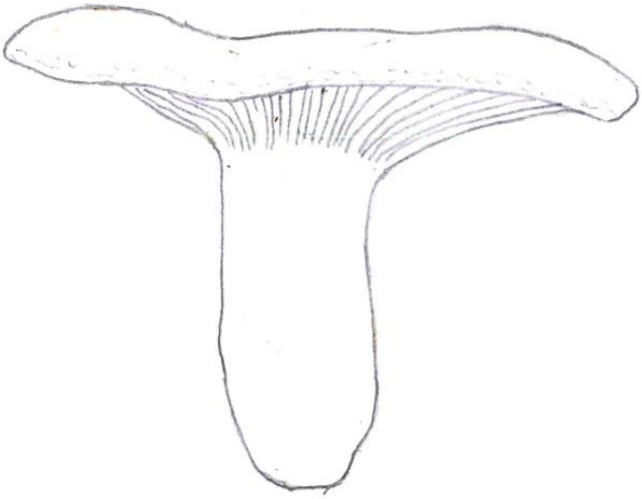
General introduction



Fungal diversity and the need for modern taxonomy

The fungi are one of the largest and most **diverse** groups of organisms on Earth. There are currently about 100.000 fungal species described (Tedersoo et al. 2014), but recent studies estimate that this is only a fraction (14–2%) of a total of 0.7–5.1 million fungal species (Hawksworth 1991, 2001; O'Brien et al. 2005; Schmit and Mueller 2007; Blackwell 2011). Compared with flowering plants or vertebrates, where respectively 10-20% and 10% of the estimated species numbers are undescribed (CBD 2006; Paton et al. 2008; Joppa et al. 2011)[1], there is still a major gap to fill for fungi. The major part of undescribed fungi are either microscopic fungi, fungi that cannot be cultured or fungi from remote and unexplored areas, but even mushroom-forming lineages contain many undescribed taxa (Blackwell 2011).

One ecological group that contains many mushroom-forming lineages is the group of ectomycorrhizal (ECM) fungi. Ectomycorrhizal fungi are a diverse group of fungi that form a mutualistic symbiotic relationship with plant roots. ECM fungi receive carbon from their host plants and in return provide enhanced nutrient uptake and resistance to stress and disease (Smith and Read 2008). Although various ECM fungi are well-studied, many species remain undiscovered or undescribed. For example, a ten-year long study of fungi in six 1-km^2 plots in the Guiana shield led to the discovery of about 120 new ECM fungi (Blackwell 2011; Smith et al. 2011). Furthermore, Verbeken and Buyck (2002) estimated the number of undescribed ECM species in tropical Africa to be double the amount of described taxa.

This large gap between the estimated number of fungal species and the actual described number of species became very obvious since the development of next generation sequencing tools, where one soil sample often reveals hundreds of potential new species. For example, Tedersoo et al. (2014) studied fungal ITS sequences from 365 global soil samples. They recovered about 45.000 non-singleton OTUs[2], of which only about one-third matched any sequences in public databases at the 97% similarity cut-off. The remaining 30.000 OTUs may thus represent possible new species, which is about 15 times the number of fungal species that were described and published during the same year (Hibbett 2016). Even if not every OTU represents new species, all these new lineages form a major challenge for taxonomists worldwide. Making detailed species descriptions is a meticulous and time-consuming task, for which at least a morphological description and a physical type specimen are needed, which are not always available in case of, for example, microscopic fungi (Taylor et al. 2006; Hibbett 2016).

Next to this large amount of undescribed fungal species, the existing knowledge on fungal diversity is unequally achieved, mainly focusing on certain regions, such as the temperate areas, or niches, such as medical mycology. For other regions or niches, fungal inventories and databases are largely non-existent, while those that exist only contain limited or basic information.

Fungi play **key roles** in almost all ecosystems on earth (Blackwell 2011; Tedersoo et al. 2014; Chambergo and Valencia 2016). They are the major decomposers in terrestrial ecosystems and thus have a critical role in the global carbon cycle. They form associations with all major groups of organisms, e.g. 92% of plant families are associated with arbuscular mycorrhizal fungi (Blackwell 2011). Some groups of fungi are important pathogens, both for humans, plants, animals or insects. For example, some mosquito-killing fungi are used as a biological control agent against mosquitoes that spread mosquito-borne diseases (Scholte et al. 2004). Many important crop pests in agriculture are also caused by fungi, such as the infection of banana plantations by the fungus *Fusarium oxysporum* f. *cubense (E.F. Sm.) W.C. Snyder & H.N. Hansen* (O'Donnell et al. 1998). Furthermore, fungi are important in biotechnology, such as the pharmaceutically important fungi that produce antibiotics as secondary metabolites (Chambergo and Valencia 2016).

This vast fungal diversity, together with the key roles they fulfil in many ecosystems, highlights that fungi are essential for everyday life. It is thus necessary to improve knowledge related to fungi in order to conserve and valorise fungal diversity and in order to recognize and respond to environmental, agricultural,

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[1] *CBD* – Convention on Biological Diversity

[2] *OTU* – operational taxonomic units, often equated with species

demographical or epidemical problems. When basic taxonomic knowledge increases, the applications of fungi in conservation, ecology, healthcare or agriculture will only increase and improve.

Taxonomy is the science of naming, describing and classifying organisms based on a combination of characteristics, such as morphological, molecular, ecological or biochemical features (CBD 2006). Taxonomy provides basic knowledge about species, which is necessary for exploring and understanding the diversity of life, but also for effective decision-making about conservation and sustainable use (CBD 2006; Costello et al. 2013). Classic taxonomy mainly focusses on morphological characteristics of species and since the last two decades, this is often combined with molecular information. The importance of combining information is emphasized by numerous studies in which morphology alone appears to be inadequate to delimit species (e.g. Dettman et al. 2006; Hibbett 2007; Buyck et al. 2008; Stubbe et al. 2010; Van de Putte et al. 2010; Van de Putte et al. 2012).

There is much information that can be gathered from a potential new species, such as morphological, molecular, physiological data or ecological data. In order to decide which data is important in describing species, one cannot go around **species concepts**. What is a species and how to define a species? The resulting answer will differ according to the biologist and his or her research field and/or interests. These differences arise due to the biological properties upon which these alternative concepts are based (de Queiroz 2007). These properties arise during different stadia of speciation and not necessarily in the same order. The biological species concept, for example, is based on interbreeding, while the ecological species concept is based on the similarities niche or adaptive zone of organisms, and the phylogenetic species concept is based on monophyly (monophyly version) or the exclusive coalescence of alleles (genealogical version).

In response to this, de Queiroz (2007) proposed a **unified species concept**, in which the only necessary property of species is that they evolve from metapopulation[3] lineages. The secondary biological properties, on which other species concepts are based, serve as evidence to assess if two lineages are indeed separating. The presence of a property does not guarantee that a population possessing that property represents a separate lineage, it only provides evidence to support the hypothesis that this population represents a separate lineage. Multiple lines of evidence, or the possession of several properties that arise during lineage divergence, result in a more highly supported hypothesis of lineage separation and thus in the existence of different species.

During the last decade of taxonomical research on fungi, mycologists started to use a variant species concept: the **consolidated species concept**. In this concept they state that conclusions based on robust multi-locus DNA data are generally unbiased and receive a high weight, while differences in morphology or ecology are given less weight in reaching a consolidated species concept conclusion (Quaedvlieg et al. 2014).

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[3] *Metapopulation* – an inclusive population made up of connected subpopulations (de Queiroz 2007)

A short history of nearly everything in Russulales

Russulales

In 1796 and 1797, Persoon describes the genera *Russula* Pers. and *Lactarius* Pers. as striking genera of agaricoid[4] fungi. Differing from other genera by their brittle context (it breaks the same way as chalk does). *Russula* species have fleshy (in Latin: *carnosus*) fruiting bodies with strikingly coloured caps and *Lactarius* species exude a milk-like solution when fruit bodies are bruised (Persoon 1796, 1797).

Due to their striking morphological characteristics, the genera *Lactarius* and *Russula* were later classified in their own order, Russulales Kreisel ex P.M. Kirk, P.F. Cannon and J.C. David, within the Agaricomycetes with pale-coloured spores (Kreisel 1969; Oberwinkler 1977). Morphologically, this classification was mainly supported by microscopical features such as sphaerocytes[5] in the trama, which are responsible for the brittle context, amyloid spore ornamentation and a gloeoplerous[6] hyphal system (Fig. 1.1). Combinations of these characters were also found in several taxa with other basidiocarp types, which were included in this order (Romagnesi 1948; Donk 1971; Oberwinkler 1977). Next to the agaricoid *Russula* and *Lactarius*, the order further comprised coral fungi (*Hericium* Pers.), poroid[7] fungi (*Heterobasidion* Bref.), hydnoid[8] fungi (*Echinodontium* Ellis & Everh.) and corticioid fungi (*Gloeocystidiellum* Donk, *Boidinia* Stalpers & Hjortstam and *Gloiothele* Bres.).

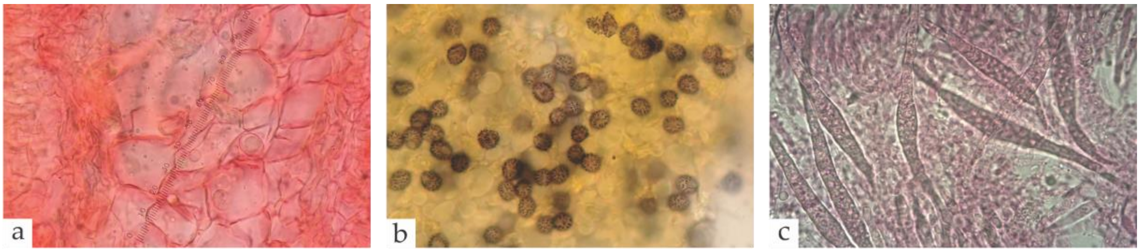

Fig. 1.1 a. Spaerocytes within the trama of *Lactifluus* sp. (EDC 14-060); **b.** amyloid spore ornamentation of *Lf.* cf. *luteolus* (REH 9398); **c.** gloeocystidia in *Gloeocystidiellum porosum* (Photographs by E. De Crop (a,b) and N. Schoutteten (c)).

Over the last two decades, molecular research strongly influenced and innovated the traditional view of the order Russulales. Molecular data showed strong support for a russuloid clade with corticioid, resupinate[9], discoid, clavarioid, pileate[10], effused-reflexed[11], and gasteroid[12] taxa with smooth, poroid, hydnoid, lamellate or labyrinthoid hymenophores (Fig. 1.2), not all of them sharing sphaerocytes and amyloid spore ornamentation (Hibbett et al. 1997; Hibbett and Binder 2002; Larsson and Larsson 2003; Larsson et al. 2004; Miller et al. 2006; Buyck et al. 2008). The Russulales clade is morphologically supported in the presence of gloeocystidia or a gloeoplerous hyphal system (Larsson & Larsson 2003, Miller et al. 2006).

Russula, *Lactarius* and some pleurotoid[13] and sequestrate[14] genera form an important group within this clade and are considered the Russulaceae Lotsy (Redhead & Norvell 1993, Miller et al. 2001, Larsson & Larsson 2003, Eberhardt & Verbeken 2004, Nuytinck et al. 2004).

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[4] *Agaricoid* – fruiting body with cap, stipe and gills.

[5] *Sphaerocytes* – globose and isodiametric cells, their presence in the trama is exceptional for fungi, as most fungi have trama composed of hyphae.

[6] *Gloeoplerous hyphae* – hyphae with long cells that contain numerous oil droplets in the cytoplasm.

[7] *Poroid* – hymenium composed of pores.

[8] *Hydnoid* – hymenium composed of spines or teeth.

[9] *Resupinate* – the hymenium grows on the top surface of the fruiting body.

[10] *Pileate* – fruiting bodies that form a pileus or cap.

[11] *Effused-reflexed* – the fruiting body is partially resupinate and partially pileate.

[12] *Gasteroid* – the hymenium grows inside the fruiting body.

[13] *Pleurotoid* – basidiocarps characterised by a lateral or absent stipe.

[14] *Sequestrate* – general term for both gasteroid and secotioid fungi (i.e. an intermediate growth form between agaricoid and gasteroid fruiting bodies).

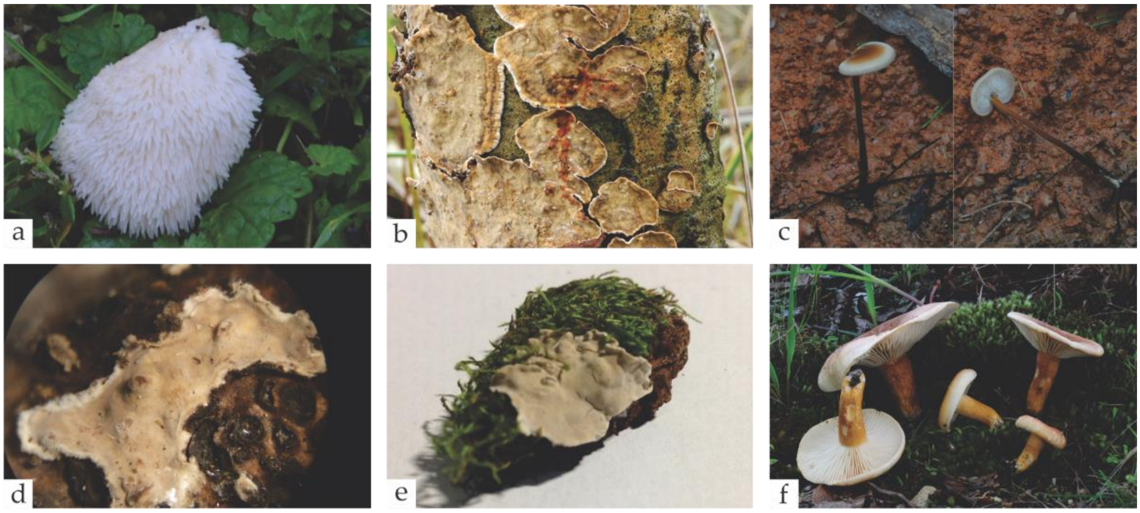

Fig. 1.2 Different types of fruiting bodies within the Russulales: **a.** coralloid fruiting body of *Hericium erinaceus* (EDC 14-463); **b.** effused-reflexed fruiting bodies of *Stereum rugosum*; **c.** pileate fruiting body with hydnoid hymenium of *Auriscalpium* sp. (EDC 14-511); **d.** corticiod fruiting body with resupinate hymenium body of *Peniophora incarnata*; **e.** discoid fruiting body of *Aleurodiscus disciforme*; **f.** pileate fruiting body with lamelloid hymenium of *Lactifluus urens* (EDC 12-032) (Photographs by J. Nuytinck (a), R. Walleyn (b), E. De Crop (c,f) and N. Schoutteten (d,e)).

Russulaceae

Before the year 2000, Russulaceae classification was mainly based on morphological characters such as fruit body type. Agaricoid species were placed within the genera *Russula* and *Lactarius*. Pleurotoid species were placed into the genus *Pleurogala* Redhead & Norvell. Sequestrate species were placed in the genera *Arcangeliella* Cavara, *Gastrolactarius* R. Heim ex J.M. Vidal, *Zelleromyces* Singer & A.H. Sm., *Cystangium* Singer & A.H. Sm., *Elasmomyces* Cavara, *Gymnomyces* Massee & Rodway, *Martellia* Mattir. and *Macowanites* Kalchbr. Veiled species were placed in the genus *Lactariopsis* Henn. Generic concepts in the mushroom-forming Russulaceae changed when it became clear that those pleurotoid, sequestrate and veiled forms originated several times, both in *Lactarius* and *Russula*. Morphological and molecular studies of pleurotoid Russulaceae species (Verbeken 1998; Buyck and Horak 1999; Henkel et al. 2000), supported their position within either *Russula* or *Lactarius*. Hence, the genus *Pleurogala*, that was erected to accommodate pleurotoid species formerly included in *Lactarius* sect. *Panuoidei* Singer (Redhead and Norvell 1993), was abandoned. Sequestrate species also occur both in *Lactarius* (formerly placed in *Arcangeliella*, *Gastrolactarius* and *Zelleromyces*) and *Russula* (formerly placed in *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Martellia* and *Macowanites*) (Calonge and Martín 2000; Miller et al. 2001; Binder and Bresinsky 2002; Desjardin 2003; Nuytinck et al. 2003; Eberhardt and Verbeken 2004; Nuytinck et al. 2004; Lebel and Tonkin 2007; Verbeken et al. 2014). Species with a secondary velum[15] occur both in *Lactarius* and *Russula*. This is in line with the standpoint of Verbeken (1998) and abandons the separate genus in which they were placed by other authors (Hennings 1902; Heim 1937; Redhead and Norvell 1993). From 2003 on, molecular analyses indicated that the Russulaceae family also contains several corticioid taxa from three genera: *Boidinia* Stalpers & Hjortstam, *Gloeopeniophorella* Rick and *Pseudoxenasma* K.H. Larss. & Hjortstam (Larsson and Larsson 2003; Miller et al. 2006).

In 2008, Buyck et al. (2008) constructed a phylogeny of the agaricoid Russulaceae genera. They focused on including more tropical taxa than previous studies, as these were lacking in most Russulaceae phylogenies

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[15] *Secondary velum* – or partial veil. This velum between the pileus margin and the stipe protects the lamellae of young fruiting bodies. The velum ruptures when fruiting bodies grow, often resulting in velar remnants at the pileus edge and an annulus around the stipe.

and as in some cases, tropical *Lactarius* and *Russula* species turned out to be hard to distinguish from each other based on morphology. Their results showed that *Lactarius* and *Russula* were not two well-defined and separate clades. *Russula* appears to be monophyletic only if a small group of species is excluded. The genus *Russula* sensu novo is the largest Russulaceae genus, with more than 750–900 species described all over the world (Kirk et al. 2008; Buyck and Atri 2011; Looney et al. 2016). The majority of *Russula* species is agaricoid, but some are pleurotoid or sequestrate and also veiled species are known (Fig. 1.3). All species lack latex production and lack pseudocystidia. They are characterised by a brittle context caused by sphaerocytes in the context and trama and by the presence of bright pigments, especially in the cap (usually contrasting with a white or whitish stipe and gills that vary from white to yellow, depending on the colour of the spores).

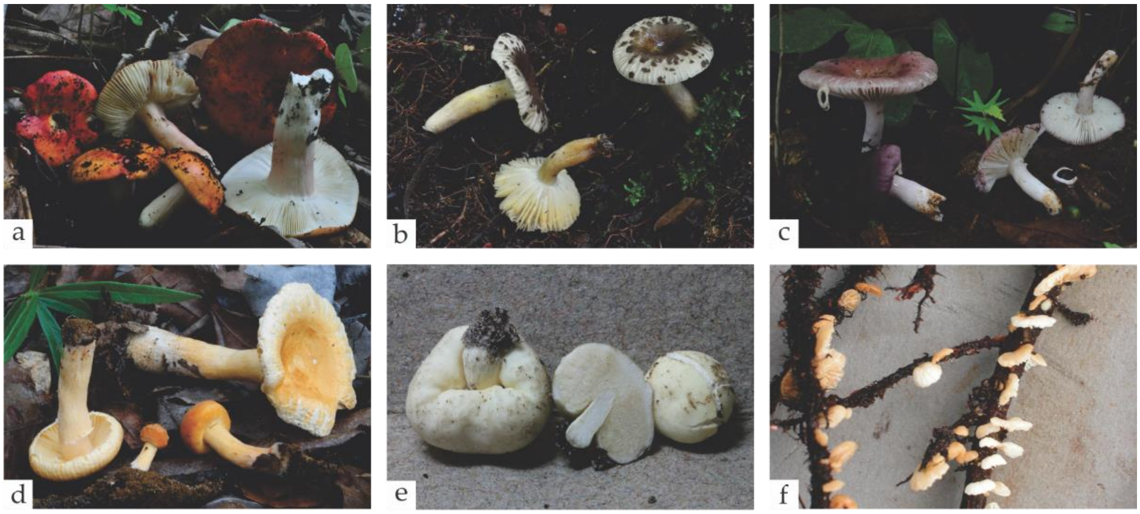

Fig. 1.3 Different Russula species: **a.** agaricoid species *Russula* sp. (EDC 12-063); **b.** agaricoid species *R.* sp. (EDC 12-058); **c.** annulate agaricoid species *R.* sp. (EDC 14-381); **d.** annulate agaricoid species *R.* sp. (EDC 14-040); **e.** secotoid species *Macowanites* sp. (REH 9496); **f.** pleurotoid species *R. campinensis* (TH 9252) (Photographs by E. De Crop (a–d), R. Halling (e) and T. Henkel (f)).

The small group of species excluded from the former *Russula* forms a clade together with some *Lactarius* species. This clade was described as the new genus *Multifurca* Buyck & V. Hofstetter (Buyck et al. 2008). The former *Russula* subsect. *Ochricompactae* Bills & O.K. Mill., the Asian *Russula zonaria* Buyck & Desjardin and the American *Lactarius furcatus* Coker 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 (Fig. 1.4). Only six *Multifurca* species are currently known (Buyck et al. 2008; Wang and Liu 2010; Lebel et al. 2013) from three continents: Asia, Oceania and North America.

Fig. 1.4 Different *Multifurca* species: **a.** *Multifurca zonaria* (FH 12-009); **b.** detail on zonate context of *M. zonaria*; **c.** *M.* sp. (xp2-20120922-01) (Photographs by F. Hampe (a), A. Verbeken (b) and G. Jiayu (c)).

The remainder of *Lactarius* falls in two different clades (Buyck et al. 2008). One large clade containing the majority of described milkcap species (about 75 % of the known milkcap species) and one smaller clade, with mainly tropical species. This smaller clade also contained the type of the former genus *Lactarius*: *Lactarius piperatus* (L.) Pers. This implicated that this smaller clade would have the name *Lactarius*, while the largest clade would receive a new name. As this would imply many name changes, a proposal was made to conserve *Lactarius*[16] with a conserved type *Lactarius torminosus* (Schaeff.: Fr.) Pers. (Buyck et al. 2010). This proposal was accepted by the 2011 International Botanical Congress (McNeill et al. 2011). The name *Lactarius* is therefore retained for the larger, mainly temperate clade (Fig. 1.5). The subgenera *L.* subg. *Lactarius* (the former *L.* subg. *Piperites* (Fr.) Kauffman), *L.* subg. *Russularia* (Fr.) Kauffman and *L.* subg. *Plinthogalus* (Burl.) Hesler & A.H. Sm. now constitute the larger milkcap genus *Lactarius* sensu novo.

Fig. 1.5 Different *Lactarius* species: **a.** *Lactarius torminosus* (JN 2011-087); **b.** *L.* sp.–*L.* sect. *Deliciosi*; **c.** *L. rubrocorrugatus* (EDC 14-505); **d.** *L. tenellus* (EDC 14-064); **e.** *L. chromospermus* (EDC 14-108); **f.** *L. stephensii* (Photographs by J. Nuytinck (a) and E. De Crop (b–f)).

The smaller milkcap genus, with approximately 150 described species, is named *Lactifluus*[17] (Pers.) Roussel and is typified by *Agaricus lactifluus* L., currently known as *Lf. volemus* (Fr.) Kuntze (Buyck et al. 2010). New combinations were made in a series of three papers for the subgenera *Lf.* subg. *Lactariopsis* (Henn.) Verbeken, *Lf.* subg. *Russulopsis* (Verbeken) Verbeken, *Lf.* subg. *Edules* (Verbeken) Verbeken, *Lf.* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, *Lf.* subg. *Lactifluus* and *Lf.* subg. *Piperati* Verbeken (Verbeken et al. 2011; Stubbe et al. 2012b; Verbeken et al. 2012).

The two milkcap genera, *Lactarius* and *Lactifluus*, are well supported molecularly, but no synapomorphic characteristics have been found to consistently separate both genera. The morphological distinction between the genera is thus far based on several trends:
Characteristics of the pileus – the genus *Lactifluus* is generally characterised by the complete absence of zonate and viscose to glutinose caps, while it contains many species with veiled and velvety caps. The genus *Lactarius* however, contains many species with zonate and viscose to glutinose caps (Verbeken and Nuytinck 2013).
Fruit body characteristics – pleurotoid milkcap species are so far only known from *Lactifluus* (Buyck et al. 2008; Verbeken and Nuytinck 2013), while sequestrate species are only known within *Lactarius*.

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[16] *Lactarius*, hereafter abbreviated as *L.*

[17] *Lactifluus*, hereafter abbreviated as *Lf.*

Hymenophoral trama – the hymenophoral trama of *Lactifluus* species is mostly composed of sphaerocytes, which is also common in *Russula* (Verbeken and Nuytinck 2013). In contrast, these sphaerocytes are only rarely observed in *Lactarius* species, in which the trama most often is composed of hyphae only.

Thick-walled elements – thick-walled elements in the pileipellis, stipitipellis and hymenophoral trama are general in the genus *Lactifluus*, while they are hardly observed in the genus *Lactarius* (Verbeken and Nuytinck 2013).

These trends might be helpful when identifying milkcap species, but they are not exclusive. There are species, especially in the tropics, in which a molecular characterisation is needed to determine which genus they belong to.

The genus *Lactifluus*

Diversity and distribution

The milkcap genus *Lactifluus* is predominantly represented in the tropics. The highest diversity of the genus is known from Africa, with 60 species described before this PhD study (Verbeken and Walleyn 2010), and Asia, with 31 species described before this PhD study (Le et al. 2007; Stubbe et al. 2010; Van de Putte et al. 2010). However, more South American habitats are being explored and new species are being described, indicating 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 and Wartchow 2013).

So far, none of the *Lactifluus* species occurs with certainty on two or more continents. Although, some species records suggest otherwise. For example, the North American *Lactifluus luteolus* has also been recorded in Europe, Asia and Australia. All collections have typical cream-beige fruit bodies, which exude white milk that quickly stains brownish. In cases like this, a molecular study is needed to examine whether it concerns one intercontinental species or if these collections represent different species. Until now, intercontinental species are assumed to occur to a lesser extent within the Russulaceae, with some exceptions. For example, Nuytinck et al. (2007) reported *Lactarius deliciosus* (L.) Gray to occur in Europe and China, Nuytinck et al. (2010) found *L. controversus* Pers. to be conspecific between Europe and North America, and Wisitrassameewong (2015) reported *L. badiosanguineus* Kühner & Romagn. to occur both in Europe and China.

Compared to its sister milkcap genus *Lactarius*, the genus *Lactifluus* is rather understudied, mainly due to its primarily tropical distribution. With an increased exploration of tropical habitats, more and more species are being recognised and described (Wang and Verbeken 2006; Van de Putte et al. 2010; Miller et al. 2012; Stubbe et al. 2012a; Van de Putte et al. 2012; Wang et al. 2012; Morozova et al. 2013; Sá et al. 2013; Sá and Wartchow 2013; Maba et al. 2014).

Ecology

Species of the genus *Lactifluus* are found in temperate (Stubbe et al. 2010; Van de Putte et al. 2016), subtropical (Stubbe et al. 2010; Van de Putte 2012) and tropical regions (Stubbe et al. 2010; Van de Putte et al. 2010; Verbeken et al. 2010; Verbeken and Walleyn 2010; Miller et al. 2012; Montoya et al. 2012; Stubbe et al. 2012a; Van de Putte et al. 2012; Wang et al. 2012; Morozova et al. 2013; Maba et al. 2014; Maba et al. 2015a; Maba et al. 2015b; Wang et al. 2015), 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* (S.L. Miller, Aime & TW Henkel) De Crop on stems of *Dicymbe corymbosa* Spruce ex Benth. (Miller et al. 2002), *Lf. multiceps* (S.L. Miller, Aime & TW Henkel) De Crop and *Lf. raspei* Verbeken & De Crop on plant seedlings (Fig. 1.6).

Fig. 1.6 *Lactifluus* species growing on trees or plant seedlings: **a.** subiculum of *Lactifluus brunellus* on the stem of a tree; **b.** *Lf. multiceps* (TH 9807); **c.** *Lf. raspei* (EDC 14-517) (Photographs by T. Henkel (a), T. Elliot (b) and E. De Crop (c)).

Lactifluus, *Lactarius*, *Multifurca* and *Russula* species are **ectomycorrhizal fungi**, the corticioid Russulaceae taxa are reported to be saprotrophic (Larsson and Larsson 2003; Miller et al. 2006; Tedersoo et al. 2010a). However, this is questioned by Miller et al. (2006), who suggest that these corticioid taxa might also be ectomycorrhizal symbionts. Typical **host plants** for *Lactifluus* are leguminous trees (Fabaceae), members of the Dipterocarpaceae and the Fagaceae, together with genera from several other families. European and North American *Lactifluus* species are mainly associated with trees of Betulaceae (e.g. *Betula*, *Carpinus*, *Corylus*), Fagaceae (e.g. *Castanea*, *Fagus*, *Quercus*), Pinaceae (e.g. *Abies*, *Picea*, *Pinus*) and Cistaceae (e.g. *Cistus*) (Hesler and Smith 1979; Heilmann-Clausen et al. 1998; Comandini et al. 2006; Van de Putte 2012). In Asia, *Lactifluus* species mainly occur with Dipterocarpaceae (e.g. *Dipterocarpus*, *Shorea*), Fagaceae (e.g. *Castanopsis*, *Lithocarpus*) (Le 2007; Van de Putte 2012). In sub-Saharan Africa, *Lactifluus* species often grow with Dipterocarpaceae (e.g. *Monotes*), Fabaceae (e.g. *Afzelia*, *Berlinia*, *Brachystegia*, *Gilbertiodendron*, *Isoberlinia*, *Julbernardia*) and Phyllanthaceae (e.g. *Uapaca*) (Verbeken and Walleyn 2010). In Central and South America, *Lactifluus* species grow with Fabaceae (e.g. *Dicymbe*), Fagaceae (e.g. *Quercus*), Nyctaginaceae (e.g. *Neea*, *Guapira*), Polygonaceae (e.g. *Coccoloba*) (Tedersoo et al. 2010c). In Australasia, *Lactifluus* species are mainly associated with Myrtaceae (e.g. *Eucalyptus* and *Leptospermum*), Nothofagaceae (e.g. *Nothofagus*) (McNabb 1971).

Present data suggest that especially generalists occur in *Lactifluus,* in contrast to *Lactarius* and *Russula* where many host specific species are known. It is hard to draw conclusions concerning hosts generalism or specialism in *Lactifluus*, as studies proving the mycorrhizal association are scarce, but for most *Lactifluus* species multiple host trees are suggested. *Lactifluus volemus*, for example, has a broad host range and is known to occur with hosts from both Fagaceae Dumort. and Pinaceae Lindley (Van de Putte et al. 2016). The few species that appear to be host specific are so far only known from a few records, such as *Lactifluus uapacae* (Verbeken & Stubbe) De Crop that is only known to occur with *Uapaca guineensis* Müller (Verbeken et al. 2008). *Lactifluus rugatus* (Kühner & Romagn.) Verbeken is known to grow with *Quercus* L. in Mediterranean areas and seems to have a restricted host association, although some authors (Brotzu 1998; Comandini et al. 2006) suggest that it also grows with *Cistus* L. For most *Lactifluus* species, 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 need to be sequenced.

Molecular diversity

The genus *Lactifluus* is characterised by a large genetic diversity (Verbeken and Nuytinck 2013). This is reflected in its phylogeny by several species complexes and species on long and isolated branches.

Two **species complexes** have been intensively studied and revealed an enormous diversity. In the complex around *Lactifluus volemus*, Van de Putte et al. (2010; 2012) applied phylogenetic species recognition and discovered about 45 different clades within this group. Some of them could be morphologically distinguished and were described as new species. Others remain cryptic[18] since no morphological differences were found. Stubbe et al. (2010; 2012a) examined the group around *Lactifluus gerardii* (Peck) Kuntze. At the start of this study, only a handful of species were known, while at the end, more than 30 clades were discovered, of which about two-third are morphologically identifiable species. Apart from these two species complexes, several other species are assumed to be part of species complexes. For example, within the African *Lf. gymnocarpoides* (Verbeken) Verbeken, *Lf. pumilus* (Verbeken) Verbeken and *Lf. longisporus* (Verbeken) Verbeken all have similar morphological characteristics and are hard to distinguish in the field. In the temperate regions, both *Lf. piperatus* and *Lf. vellereus* (Fr.) Kuntze are assumed to be part of species complexes.

Next to the species complexes, several *Lactifluus* species occur on long branches and have **isolated positions** in the phylogenetic tree, amongst them *Lf. ambicystidiatus* X. H. Wang from China (Wang et al. 2015), *Lf. aurantiifolius* (Verbeken) Verbeken from tropical Africa (Verbeken 1996; Buyck et al. 2007), *Lf. cocosmus* (Van de Putte & De Kesel) Van de Putte from Togo (Van de Putte et al. 2009) and *Lf. chrysocarpus* E. S. Popov et O. V. Morozova from Vietnam (Morozova et al. 2013).

Macromorphology

Despite the existence of species complexes, in which morphological diversity is rather limited, the genus *Lactifluus* is still characterised by a large diversity of macromorphological characters. These morphological characters are often used for species delimitation.

A first, striking character is the **fruit body type and size**. Currently, two different fruit body types are known in *Lactifluus*: the agaricoid type (with cap, gills and centrally attached stipe, e.g. Fig. 1.7a) and the pleurotoid type (with cap, gills and laterally attached stipe, e.g. Fig. 1.7l). Until now, sequestrate fruit body types within the Russulaceae are only known from the genera *Lactarius* and *Russula*. Fruit bodies of *Lactifluus* species range from miniscule fruitbodies, such as in *Lf. igniculus* O. V. Morozova et E. S. Popov (pileus 5 – 16 mm diam.), to large basidiocarps, such as in *Lf. vellereus* (pileus 50 – 300 mm diam.). Most fruit bodies grow on soil, but often the tiny agaricoid and pleurotoid species grow on subiculum (Fig. 1.6), which is an interwoven network of thick-walled hyphae from which fruiting bodies arise. This subiculum grows on saplings, roots, stems, soil or rocks, and can be intermixed with bryophyte growth and subtended by ectomycorrhizal rootlets. A subiculum can be small to very extensive, e.g. the subiculum of *Lf. multiceps* was recorded to stretch out over 15 m (Miller et al. 2002).

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[18] *Cryptic species* – genetically distinct species that are morphologically indistinguishable.

Fig. 1.7 Overview of different types of *Lactifluus* fruiting bodies: ***Lf.* subg. *Gymnocarpi***: **a.** Lf. nonpiscis (EDC 14-056); **b.** *Lf. tanzanicus* (EDC 11-224); **c.** *Lf. gymnocarpus* (EDC 12-047); **d.** *Lf. albomembranaceus* (EDC 12-046); **e.** *Lf.* cf. ▶

◄ *phlebonemus* (EDC 12-067); **f.** *Lf. panuoides*; **g.** *Lf. putidus* (PAM 05-030); **h.** *Lf. clarkeae* (REH 9871); ***Lf.* subg. *Lactifluus*: i.** *Lf. volemus*; **j.** *Lf. longipilus* (KVP 08-005); **k.** *Lf. atrovelutinus* (DS 06-003); **l.** *Lf. raspei* nom. prov. (EDC 14-517); **m.** *Lf.* cf. *piperatus* (DS 07-467); **n.** *Lf. roseophyllus* (JN 2011-076); **o.** *Lf. allardii* (C.C. 3.0); **p.** *Lf.* cf. *tenuicystidiatus* (DS 07-465); ***Lf.* subg. *Lactariopsis*: q.** *Lf.* sp. (EDC 11-068); **r.** *Lf.* sp. (EDC 14-091); **s.** *Lf. cyanovirescens* (EDC 11-021); **t.** *Lf. multiceps* (TH 9807); **u.** *Lf. longipes* (EDC 14-049); **v.** *Lf.* sp. (EDC 12-069); **w.** *Lf roseolus* (EDC 14-228); **x.** *Lf. subvellereus* (AV 13-025); ***Lf.* subg. *Pseudogymnocarpi*: y.** *Lf.* cf. *gymnocarpoides* (EDC 14-106); **z.** *Lf. medusae* (EDC 12-152); **aa.** *Lf. luteopus* (EDC 14-086); **bb.** *Lf. bicapillus* nom. prov. (EDC 12-176); **cc.** *Lf. rubiginosus* (EDC 11-067); **dd.** *Lf. armeniacus* (EDC-501); **ee.** *Lf. denigricans* (EDC 14-067); **ff.** *Lf. pegleri* (PAM/Mart 05-088) (Photographs by E. De Crop (a–e,l,q–s,u–w,y–ee), T. Henkel (f), P. A. Moreau (g,ff), R. Halling (h), G. Boerio (i), K. Van de Putte (j), D. Stubbe (k,m,p), J. Nuytinck (n), D. Molter (o), T. Elliot (t) and A. Verbeken (x)).

Within the Russulaceae, the genera *Lactifluus* and *Russula* are known to contain species with **secondary velum**. In *Lactifluus*, this velum can be present as an annulus around the stipe or as velar remnants on the pileus edge (Fig. 1.8). The annulus is fibrous, membranous, thin to almost invisible and not mobile, unlike in some *Russula* species with a mobile annulus which often sticks to the growing cap (Fig. 1.3c). Species with secondary velum, together with their closest relatives, are characterised by an involute pileus margin when young. This involute pileus margin can make contact with the stipitipellis and protects the developing lamellae (Fig. 1.8b). On the contrary, pileus margins of most other species are not involute and lamellae are exposed from the beginning.

Fig. 1.8 Overview of different types of velum in *Lactifluus*: **a.** *Lactifluus* sp. (EDC 14-060, E. De Crop); **b.** *Lf.* sp. (EDC 14-065, E. De Crop); **c.** *Lf.* sp. (EDC 11-127, E. De Crop); **d.** *Lf.* sp. (EDC 11-144, E. De Crop); **e.** *Lf.* sp. (EDC 14-172, J. Nuytinck); **f.** *Lf.* sp. (EDC 14-059, E. De Crop); **g.** *Lf.* sp. (EDC 14-146, E. De Crop); **h.** *Lf.* sp. (EDC 14-091, E. De Crop); **i.** *Lf.* sp. (EDC 14-051, E. De Crop) (Photographs by E. De Crop (a–d,f–i) and J. Nuytinck (e)).

The **pileus** shape of *Lactifluus* species varies between applanate, planoconvex, concave, infundibuliform or deeply infundibuliform. Pileus colours range from white, yellow, orange, red to brownish colours. Pileus textures range from smooth caps to chamois-leather-like to velvety or woolly (Fig. 1.9). Especially some species from *Lf.* sect. *Albati* (Bataille) Verbeken are known for their woolly pileus surface and their local names often refer to this aspect (e.g. *Lactifluus vellereus* in Dutch: schaapje, in English: fleecy milkcap, in German: Wollige Milchling, Mildmilchender Wollschwamm or Samtiger Milchling, in Spanish: lactario aterciopelado). The pileus margin is often concentrically wrinkled near the edge and can be grooved or involute. The pileus edge is either entire, crenulate or eroded. **Stipe** colours and surface mainly resemble those of the pileus, but are often slightly paler or less felted. The stipe is generally centrally attached and often tapering downwards or curved near the base.

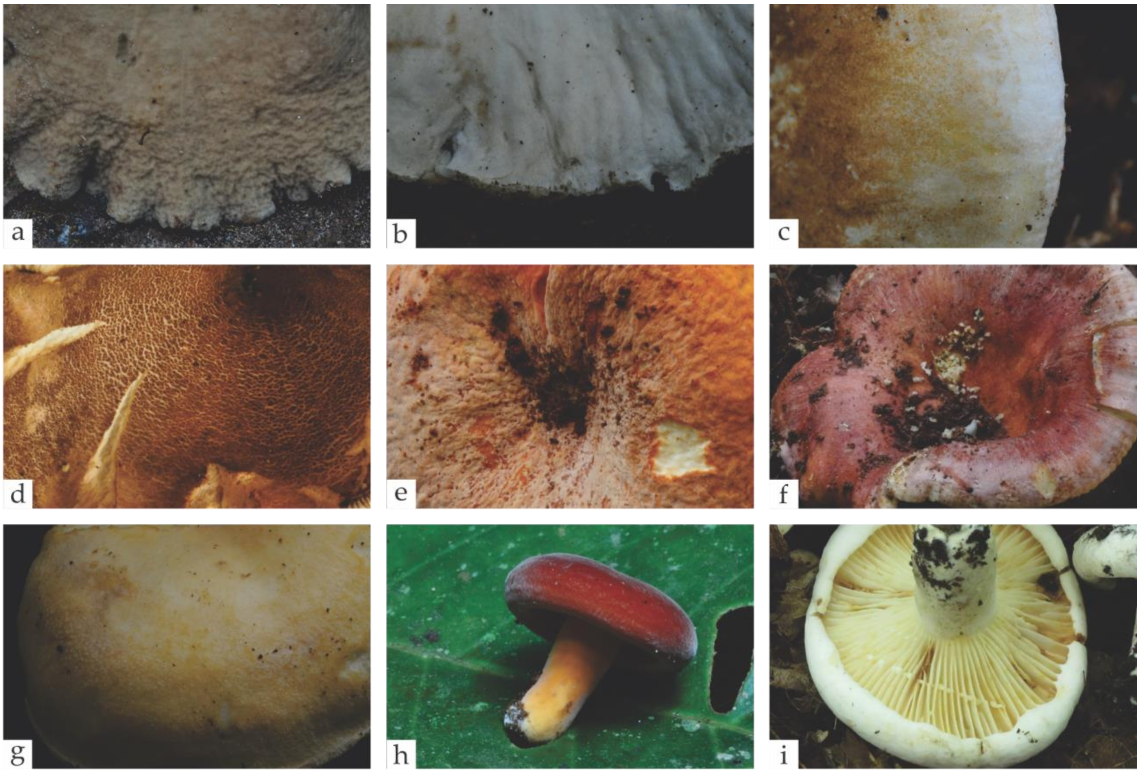


Fig. 1.9 Overview of different types of cap textures in *Lactifluus*: **a.** *Lf. brunnescens* (EDC 12-116); **b.** *Lf.* sp. (EDC 12-122); **c.** *Lf. urens* (EDC 14-032); **d.** *Lf. inversus* (EDC 12-070); **e.** *Lf.* sp. (EDC 14-153); **f.** *Lf. cyanovirescens* (EDC 11-021); **g.** *Lf. ramipilosus* (EDC 14-503); **h.** *Lf.* sp. (EDC 12-169); **i.** *Lf. subvellereus* (AV 13-025) (Photographs by E. De Crop (a–h) and A. Verbeken (i)).

Lamellae of *Lactifluus* species are mostly slightly paler than the pileus, except in some species, e.g. *Lf. aurantiifolius* with dark yellow-orange lamellae. Lamellae may be thin, almost paper-like, such as in *Lf. pelliculatus* (Beeli) Buyck; or thick and brittle, such as in *Lf. rubroviolascens* (R. Heim) Verbeken. They may be very broad, such as in *Lf. sesemotani* (Beeli) Buyck or narrow, as in *Lf. inversus* (Gooss.-Font. & R. Heim) Verbeken. Some are distant, such as in *Lf. distantifolius* (Van de Putte, Stubbe & Verbeken) Van de Putte, or very crowded, such as in *Lf. phlebophyllus* (R. Heim) Buyck (Fig. 1.10). The attachment to the stipe varies from adnate, adnate with decurrent tooth to decurrent. Generally, the lamella edge is entire and concolourous with the rest of the lamellae, however in some species, such as in *Lf. bicolor* (Massee) Verbeken, the lamella edge is concolourous with the pileus or stipe. In almost all *Lactifluus* species, lamellulae (l) are present between the lamellae (L). These lamellulae often occur in a pattern: L–l–L or L–l–l_s–l–L, with l_s the smallest lamellula. Various *Lactifluus* species have bifurcating lamellae, while others have venation patterns on their lamellae. Venation is either transvenose (when veins occur on the lamella surface) or intervenose (when veins occur between lamellae).

Fig. 1.10 Overview of different types of lamellae in *Lactifluus*: **a.** thin and paper-like lamellae of *Lactifluus urens* (EDC 14-032); **b.** thick and brittle lamellae in *Lf.* aff. *longisporus* (EDC 12-199); **c.** distant and broad lamellae in *Lf. gymnocarpus* (EDC 12-055); **d.** bifurcating narrow and crowded lamellae in *Lf. densifolius* (EDC 11-220); **e.** lamellae with venation of *Lf. persicinus* (EDC 12-002); **f.** lamellae with coloured edge in *Lf. bicolor* (DS 06-230) (Photographs by E. De Crop (a–e) and D. Stubbe (f)).

As indicated by their name, *Lactifluus* species, together with *Lactarius* species, exude **latex** when bruised. Several latex features have been important in species delimitation in both genera. In *Lactifluus*, latex is white, coloured, watery or whey-like and some species have latex changing colour (e.g. blue-green, brown or red-black) after contact with air (Fig. 1.11). In some species, the latex colours the lamellae and context after exposure to air. Species differ in latex abundance or taste. For instance, in *Lf. volemus* latex is very abundant and in *Lf. piperatus*, the latex is very acrid.

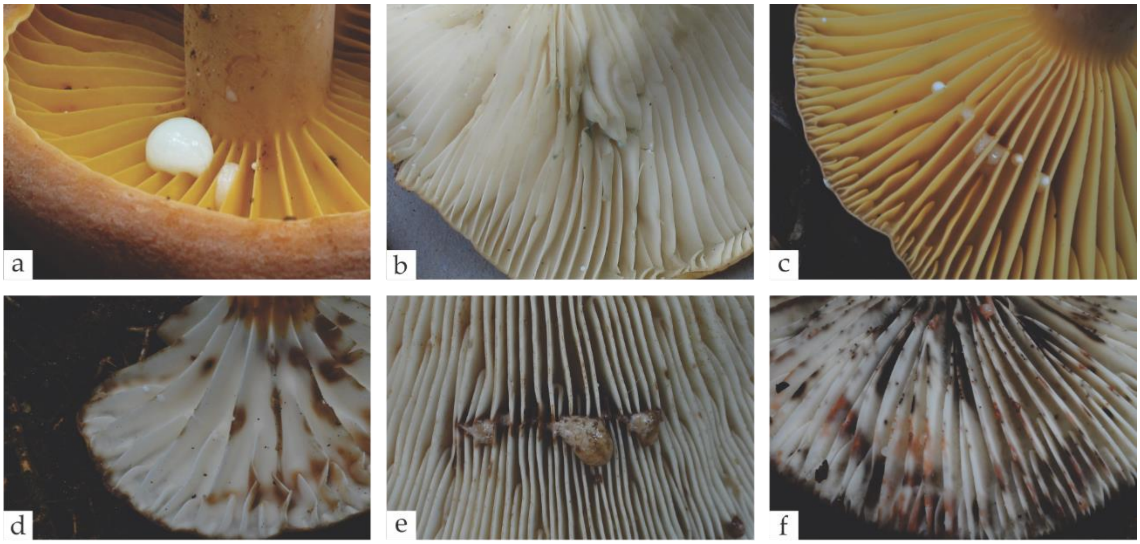


Fig. 1.11 Overview of different types of latex colourations in *Lactifluus*: **a.** unchanging white latex in *Lf.* sp. (AV 11-089); **b.** white latex changing greenish in *Lf. cyanovirescens* (EDC 11-001); **c.** unchanging watery white latex in *Lf. rubiginosus* (EDC 11-067); **d.** white latex that colours the lamellae brownish in *Lf. gymnocarpus* (EDC 12-103); **e.** brown whey-like latex in *Lf. brunnescens* (EDC 12-116); **f.** watery white latex changing red and later black in *Lf. rubroviolascens* (EDC 14-384) (Photographs by A. Verbeken (a) and E. De Crop (b–f)).

The **context** of *Lactifluus* species ranges from firm to stuffed, to partly hollow, chambered or hollow (Fig. 1.12). The context of most species is white or cream-coloured and in some species, the context changes colour after exposure to air. The context can taste mild or have a strong taste, or can have a strong odour.
The **spore print** of all *Lactifluus* species is white and therefore cannot be used to delimit *Lactifluus* species.

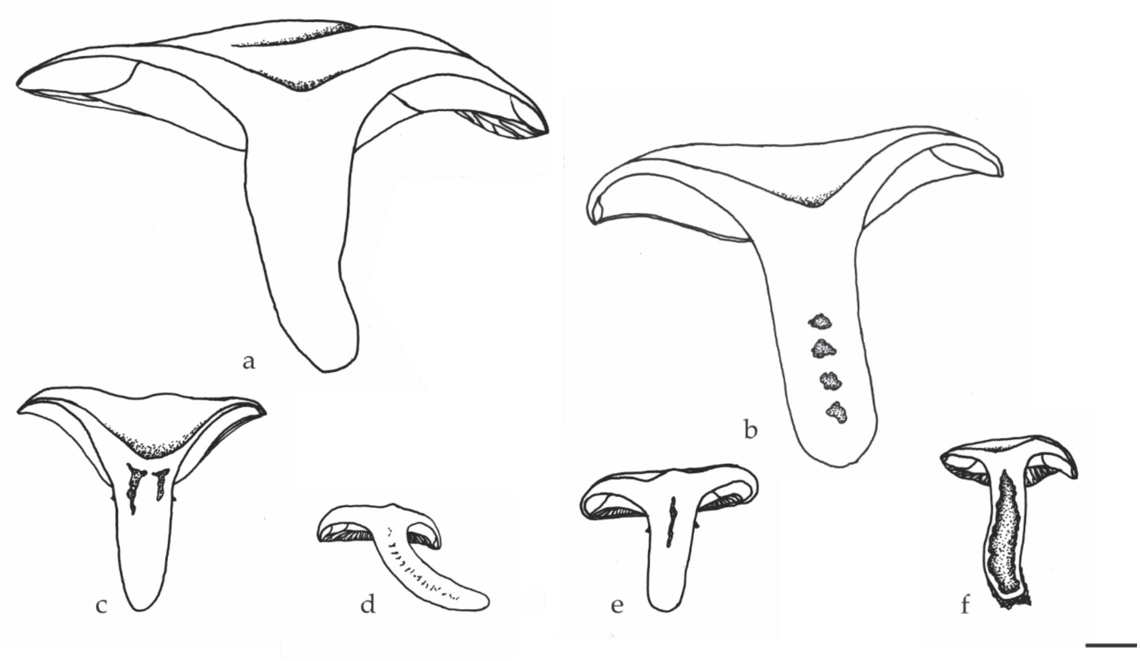


Fig. 1.12 Overview of different types of context in *Lactifluus*: **a.** firm context in *Lf. urens* (EDC 14-032); **b.** chambered context in *Lf.* sp. (EDC 14-061); **c.** chambered context in *Lf.* sp. (EDC 14-046); **d.** stuffed context in *Lf.* sp. (EDC 14-512); **e.** partly hollow context in *Lf.* sp. (EDC 14-038); **f.** hollow context in *Lf. nonpiscis* (EDC 14-056) (scale bar = 1cm; line drawings by E. De Crop).

Micromorphology

Next to the macroscopical diversity, the genus *Lactifluus* displays a variety of microscopical features. The genus is known for the occurrence of thick-walled elements in the majority of its species. For terminology concerning these characters we follow Verbeken and Walleyn (2010).

Structures of the pileipellis and stipitipellis

The structure of the pileipellis is an important character in this genus and is used to delimit species, sections or subgenera within *Lactifluus*. As **pileipellis and stipitipellis** structures slightly change during their development (Verbeken and Walleyn 2010), pellis structures in this study were observed in mature specimens. Drawings are made halfway the radius of the pileus or halfway the stipe height.
For the description of the pellis structures, we follow Heilmann-Clausen et al. (1998) and Verbeken & Walleyn (2010). In *Lactifluus*, the upper pellis layer is regularly differentiated from the underlying trama. The most important characters to look at are the presence of thick-walled elements, the presence of isodiametric cells and the orientation of the terminal elements. Thick-walled elements are present in the majority of *Lactifluus* species. In the pellis they are present as one consistent layer (this is indicated with the prefix "lampro-" in the name of that layer) or as scattered elements in a layer of thin-walled elements. Many *Lactifluus* species are characterised by the presence of isodiametric cells in the upper layer of the pellis. These are thin- or thick-walled and form one distinct layer or are mixed with hyphae. The most upper layer of the

pellis consists of terminal elements. These are either hair-like elements, hyphae or clavate elements. Their orientation is important in defining the different pellis structures.

The combination of these characters leads a differentiation between 14 pellis types (Fig. 1.13). The pellis of most species is characterised by one of these types, however, ranges of types do occur.

Pellis entirely composed of filamentous elements, without isodiametric cells

Cutis: the suprapellis consists of hyaline, thin-walled hyphae, which lay parallel, pericline or are slightly intermixed. Differentiated terminal elements are mostly lacking, although in some species of *Lf.* sect. *Russulopsidei* (Verbeken) Verbeken, there are dermatocystidia present in this layer.

Irregular cutis: the suprapellis consists of hyaline, thin-walled hyphae which are irregularly ordered.

Ixocutis: the suprapellis consists of hyaline, thin-walled hyphae which are embedded in a slime layer, which may be produced by hyphae secreting slime or by gelatinized hyphae walls.

Trichoderm: the suprapellis consists of hyaline, thin-walled hyphae, of which the terminal elements are ascending and lay anticline. These hairs often form dense turfs of hairs.

Lamprotrichoderm: the suprapellis consists of hyaline, thin-walled hyphae, of which the terminal elements are thick-walled, ascending and lay anticline.

Ixotrichoderm: the suprapellis consists of hyaline, thin-walled hyphae, of which the terminal elements are ascending, lay anticline and are embedded in a slime layer, which may be produced by hyphae secreting slime or by gelatinized hyphae walls.

Pellis with a distinct layer of isodiametric cells or sphaerocytes

Hyphoepithelium: the suprapellis consists of pericline, hyaline and thin-walled hyphae, which lay on a cellular subpellis.

Palisade: the suprapellis consists of anticline, thin-walled, elongated terminal elements, which lay on a cellular subpellis. The terminal elements are either hair-like or septate.

Lampropalisade: the suprapellis consists of anticline, thick-walled, elongated terminal elements, which lay on a cellular subpellis.

Hymeniderm: the suprapellis consists of anticline, thin-walled, short and clavate terminal elements, which lay on an often thin cellular subpellis.

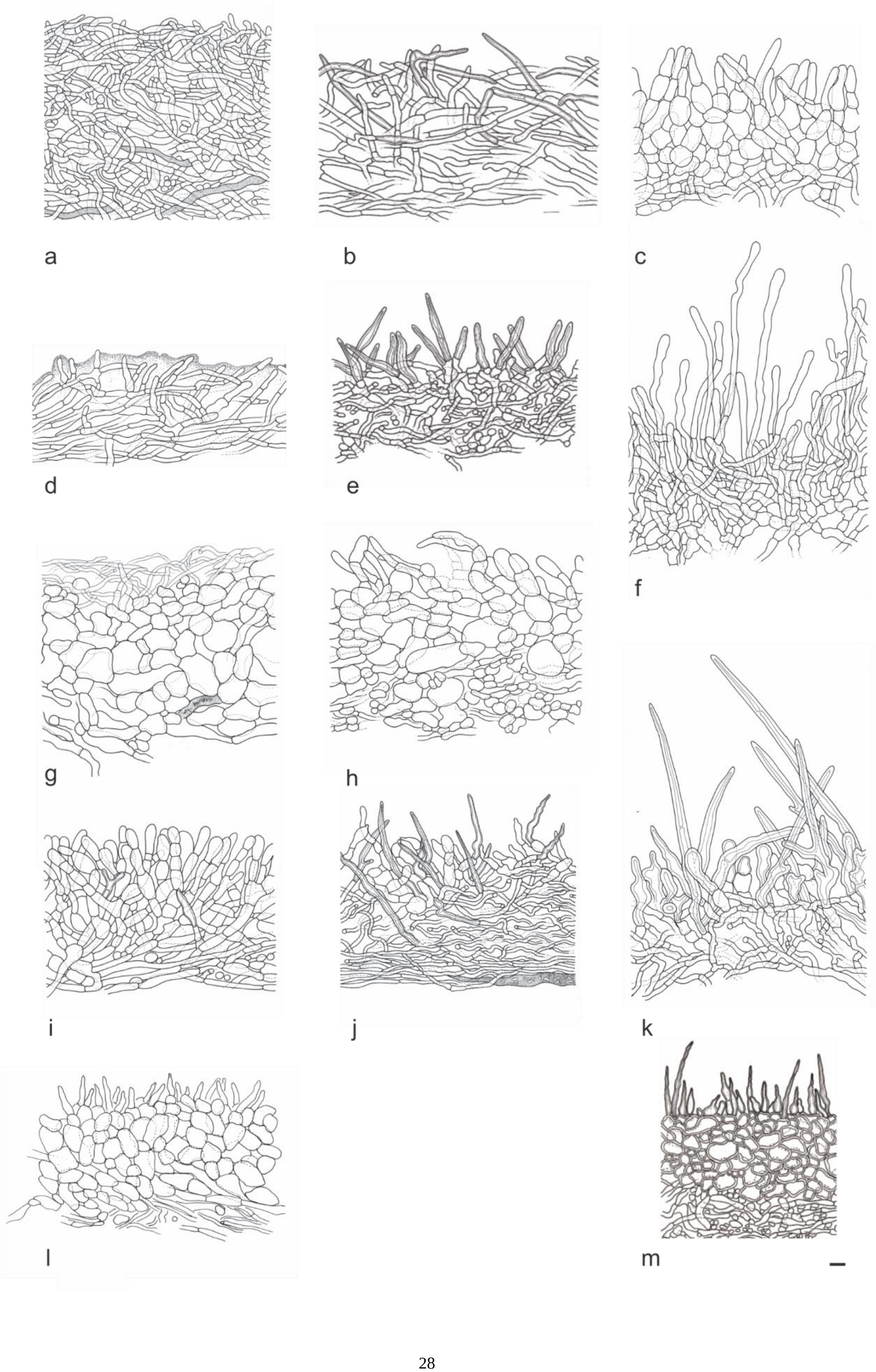
Pellis with isodiametric cells, but never forming a distinct layer

Trichopalisade: looks like a trichoderm in which some of the anticline hyphae are inflated or rounded, which gives it a palisade-like impression.

Lamprotrichopalisade: as a trichopalisade, but with thick-walled terminal elements.

Mixed trichopalisade: as a trichopalisade, in which some terminal elements are thick-walled.

Mixed trichopalisade with abundant thick-walled elements: as a trichopalisade, in which the majority of terminal elements are thick-walled.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◄ **Fig. 1.13** 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) (scale bar = 10µm; line drawings by A. Verbeken (a–k), D. Stubbe (l) and K. Van de Putte (m)). ►

Dermatocystidia rarely occur in the genus *Lactifluus*. However, they are present in *Lf.* sect. *Russulopsidei* and *Lf.* sect. *Piperati* (Fr.) Verbeken, in the upper layer of cutis-like structures or of a hyphoepithelium (Fig. 1.14).

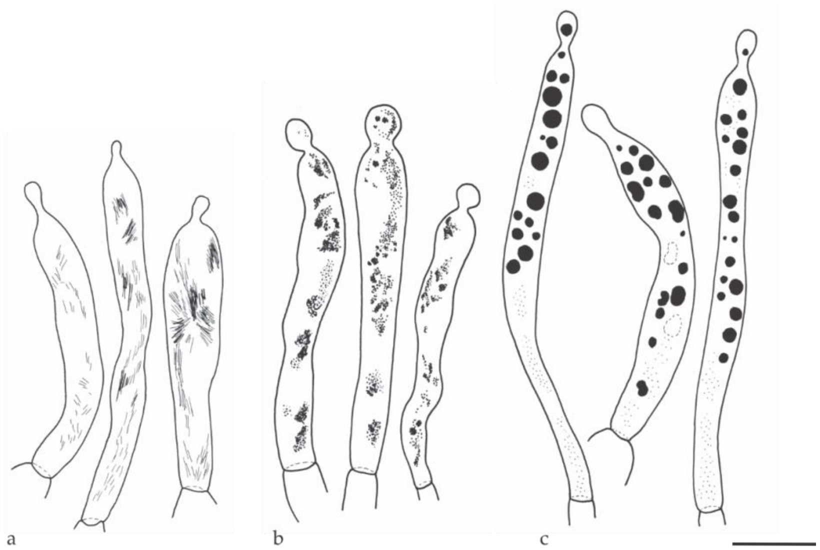

Fig. 1.14 Overview of different types of dermatocystidia found in the genus *Lactifluus*: **a.** *Lf. ruvubuensis* (AV 94-617); **b.** *Lf. longipes* (BB 1345); **c.** *Lf. claricolor* (R. Heim J18bis) (scale bar = 10µm; line drawings by A. Verbeken (a–c)).

Hymenophoral elements

Basidia and basidioles only slightly differ between species (Fig. 1.15). Some species have long and slender basidia, such as *Lf. albomembranaceus* De Wilde & Van de Putte, while others have small and almost clavate basidia, such as *Lf.* sp. nov. (EDC 14-061). Sterigmata may be short, long and slender, or very distinct, such as in *Lf.* sp. nov. (JN 2011-071). Most basidia have four sterigmata and form four spores. However, several *Lactifluus* species have two- or one-spored basidia, such as *Lf.* sp. nov. (EDC 12-071). Basidia are measured excluding sterigmata and their width is measured at the broadest place.

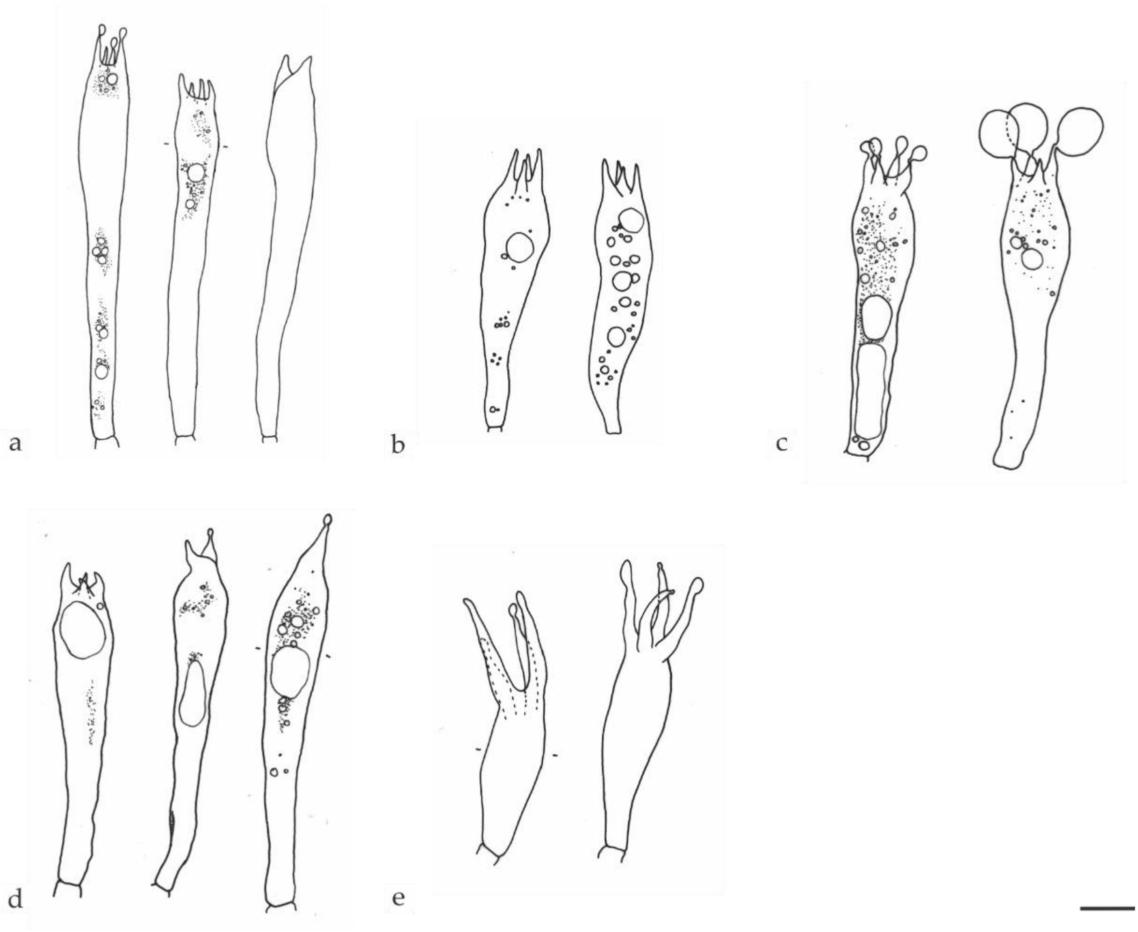

Fig. 1.15 Overview of different basidium types found in the genus *Lactifluus*: **a.** long and slender basidia in *Lf. albomembranaceus* (EDC 12-046); **b.** short and clavate basidia in *Lf.* sp. (EDC 14-061); **c.** four-spored basidia in *Lf. heimii* (EDC 11-082); **d.** one-, two- and four-spored basidia in *Lf. bicapillus* nom. prov. (EDC 12-071); **e.** short basidia with distinct sterigmata in *Lf.* sp. (JN 2011-071) (scale bar = 10µm; line drawings by E. De Crop (a–d) and S. De Wilde (e)).

The genus *Lactifluus* displays different cystidium types. A distinction between the types is made based on the presence of a septum and based on the position on the lamellae. Cystidia without septum are actually **pseudocystidia**, which are the extremities of lactiferous hyphae (Fig. 1.16). Their content therefore resembles the content of lactiferous hyphae, which is refringent, dense, oleiferic or needle-like to granular (Verbeken and Walleyn 2010). In *Lactifluus*, their abundance and form may considerably differ. In many species of *Lf.* subg. *Pseudogymnocarpi* (Verbeken) De Crop they are scarce, while in many species of *Lf.* sect. *Lactariopsis* Verbeken they are conspicuous and abundant. Pseudocystidia are slender or broad and in some species strongly emergent. Their top is rounded, tapering, moniliform or even forked. Depending on their position on the lamellae, they are called pleuropseudocystidia, when located at the lamella side, or cheilopseudocystidia, when located at the lamella edge.

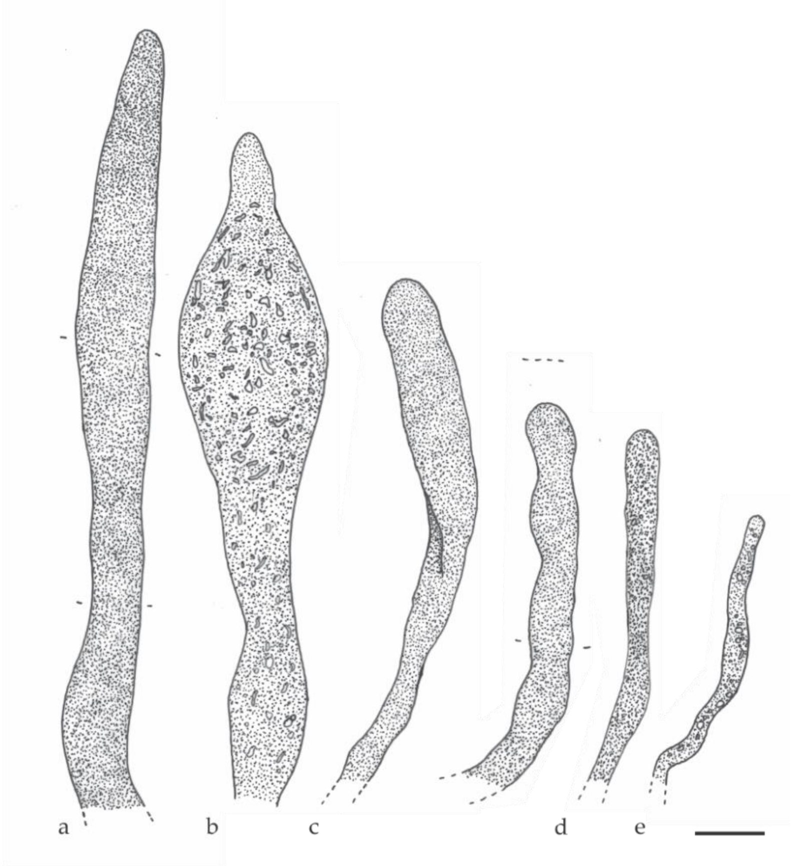

Fig. 1.16 Overview of different pseudocystidium types found in the genus *Lactifluus*: **a.** broad and emergent pseudocystidium in *Lf.* sp. (EDC 12-040); **b.** very broad pseudocystidium in *Lf.* sp (EDC 12-030); **c.** not emergent pseudocystidia in *Lf. cyanovirescens* (FN 05-631); **d.** narrow pseudocystidium in *Lf.* sp. (JN 2011-071); **e.** very narrow pseudocystidium in *Lf.* cf. *phlebonemus* (EDC 12-067) (scale bar = 10µm; line drawings by E. De Crop (a–c, e) and S. Dewilde (d)).

True pleurocystidia are located on the sides of the lamellae, true cheilocystidia on the edge of the lamellae. They always have a septum and are not connected to lactiferous hyphae. Three different types of true cystidia are known in *Lactifluus* species (Fig. 1.17).

- *Lamprocystidia:* the most common type of true cystidia in *Lactifluus*. They are thick-walled cystidia, which are often very large, frequently emergent to strongly emergent and sometimes septate. Some of the largest lamprocystidia emerge from within the hymenophoral trama, such as in species of *Lf.* sect. *Lactifluus*.
- *Macrocystidia:* thin-walled cystidia with a specific content, which is oil-like, needle-like or granular. Their top is rounded, tapering or moniliform.
- *Leptocystidia:* thin-walled cystidia, without a remarkable content, but with a deviating shape. They are rather rare in *Lactifluus*.

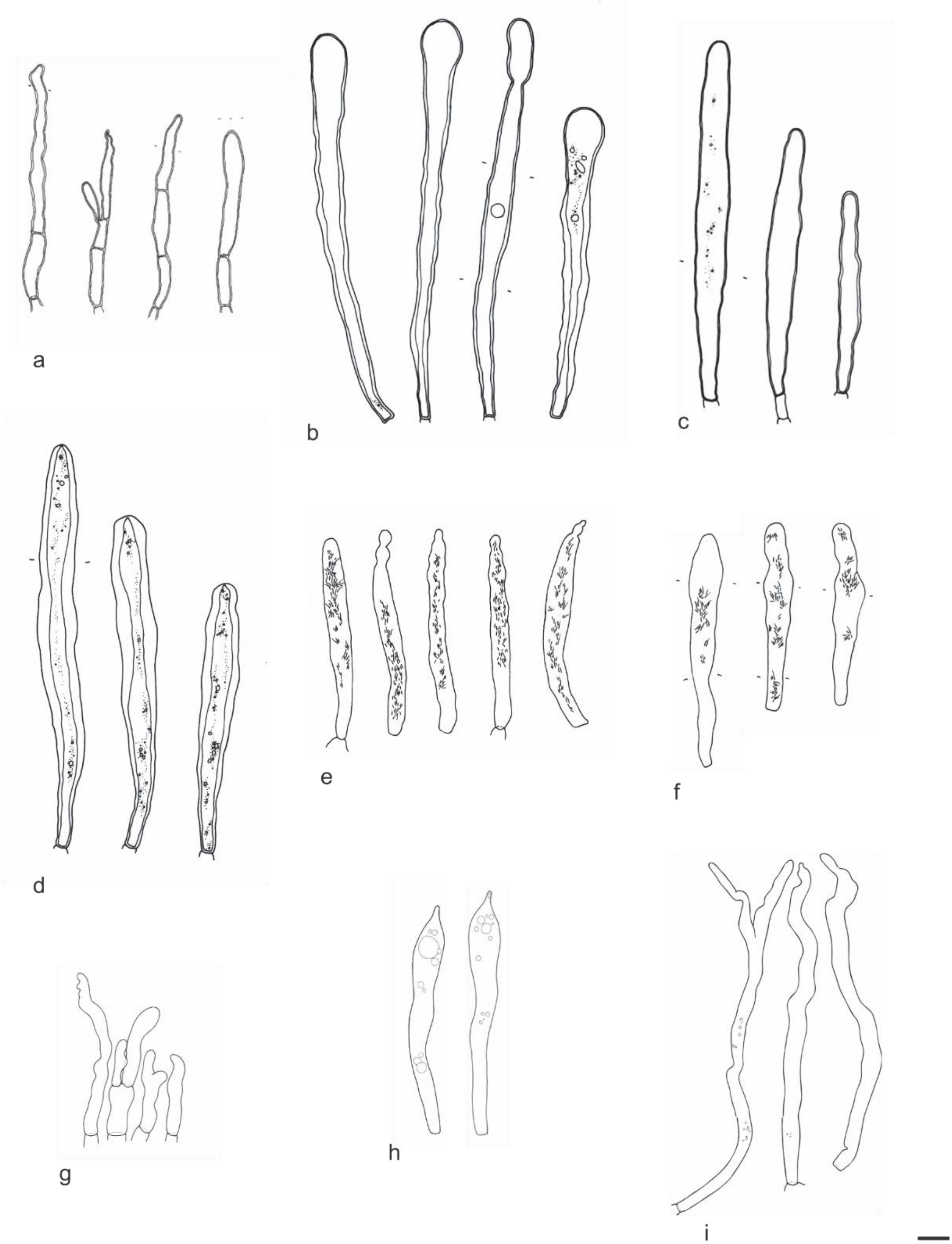

Fig. 1.17 Overview of different true cystidium types found in the genus *Lactifluus*: lamprocystidia: **a.** in *Lf. armeniacus* (EDC 14-501); **b.** in *Lf.* sp. (AV 11-006); **c.** in *Lf.* cf. *pumilus* (EDC 12-066); **d.** in *Lf.* cf. *volemus* (REH 9320); macrocystidia: **e.** in *Lf.* sp. (JN 2011-077); **f.** in *Lf. roseophyllus* (JN 2011-076); leptocystidia: **g.** in *Lf. ruvubuensis* (AV 94-599); **h.** in *Lf. indusiatus* (AV 94-122); **i.** in *Lf. densifolius* (BB 3601) (scale bar = 10µm; line drawings by E. De Crop (a–f) and A. Verbeken (g–i)).

The lamella edge can contain different elements, such as pseudocystidia, true cystidia, basidioles, basidia or sterile elements. Cheilopseudocystidia and true cheilocystidia that are present at the lamella edge are often smaller than those on the lamella sides. In several *Lactifluus* species, the lamella edge is sterile and entirely composed of sterile elements or **marginal cells** (Fig. 1.18).These marginal cells are either thin- or thick-walled, hyaline, with a clavate, fusiform to irregular shape (Verbeken and Walleyn 2010).

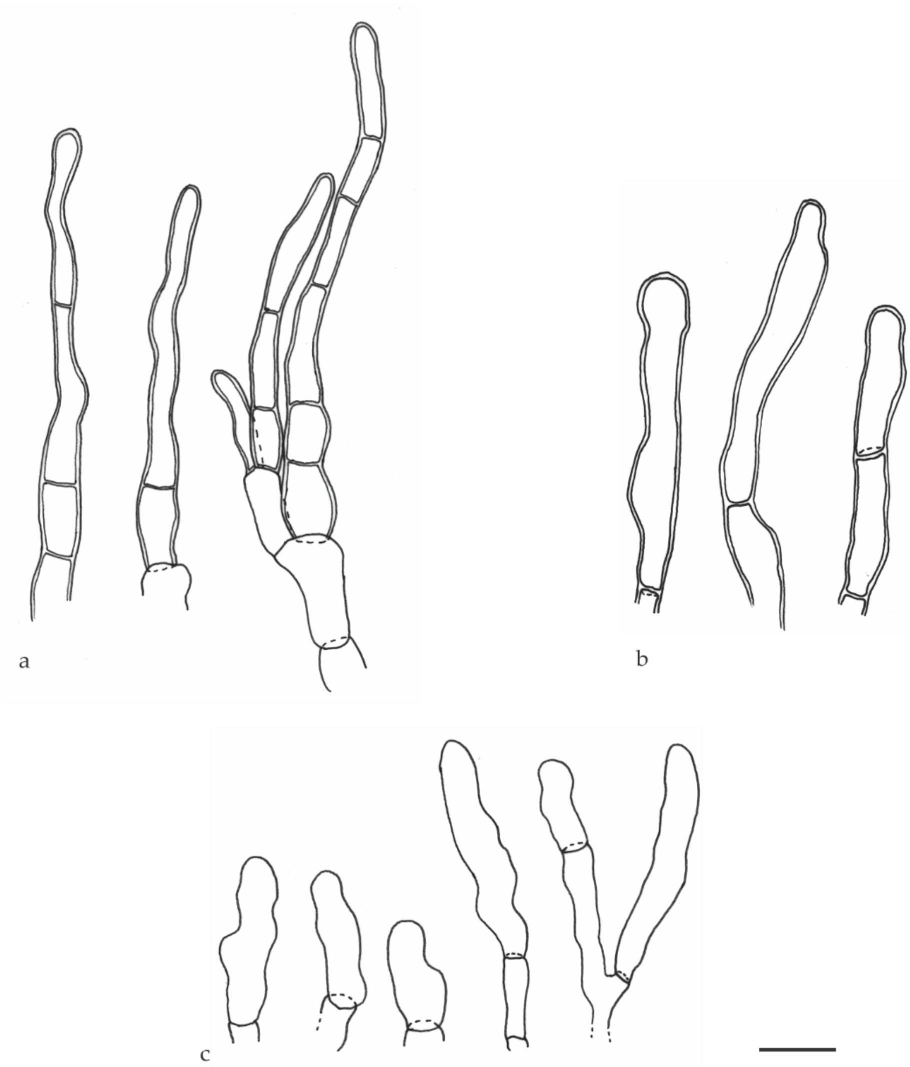

Fig. 1.18 Overview of different marginal cell types found in the genus *Lactifluus*: **a.** *Lf.* cf. *luteolus* (REH 9398); **b.** *Lf. armeniacus* (EDC 14-501); **c.** *Lf.* cf. *phlebonemus* (EDC 12-067) (scale bar = 10μm; line drawings by E. De Crop (a–c)).

Russulaceae species, together with many species of other Russulales families, are characterised by **basidiospores** with an amyloid spore ornamentation (Fig. 1.19). This ornamentation thus stains blackish-blue in Melzer's reagent[19]. In *Lactifluus*, the spore ornamentation patterns are important in delimiting species or sections. These ornamentation patterns range from isolated warts and warts connected with fine connective lines, to a complete reticulum. Spore ornamentation can be very low (<0.1 μm in *Lf. indusiatus* (Verbeken) Verbeken) to rather high (ridges up to 2.3 μm in *Lf. longipilus* (Van de Putte, H.T. Le & Verbeken)

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[19] *Melzer's reagent* – an aqueous solution containing iodine and potassium iodide, used in Russulales to stain the amyloid spore ornamentation.

Van de Putte). The plage (smooth area just above the apiculus) is either inamyloid, centrally amyloid, distantly amyloid or completely amyloid. The length and width of *Lactifluus* spores are measured in side view, excluding ornamentation. Most *Lactifluus* species have a size between 6.1–13.4 × 4.8–11.1 µm. *Lactifluus carmineus* (Verbeken & Walleyn) Verbeken has the longest spores (11.0–13.4 µm long), while *Lf. conchatulus* (Stubbe & H.T. Le) Stubbe has the shortest spores (6.1–7.8 µm long). *Lactifluus subvolemus* Van de Putte & Verbeken has the broadest spores (7.3–11.1 µm broad), while *Lf. foetens* (Verbeken) Verbeken has the narrowest spores (4.8–6.5 µm broad). The overall spore shape is determined by their length:width-ratio (quotient or Q-value): globose spores are defined by a Q-value ranging from 1.00–1.05, subglobose spores by Q between 1.06–1.12, ellipsoid spores by Q between 1.13–1.39 and elongate spores by Q >1.39 (Verbeken and Walleyn 2010). The spore shape in *Lactifluus* species ranges between subglobose to ellipsoid (average Q between 1.10–1.37), only a few species have globose spores, such as in some *Lf. oedematopus* (Scop.) Kuntze collections (Q = 1) or elongate spores, such as in some *Lf. longisporus* collections (Q = 1.6).

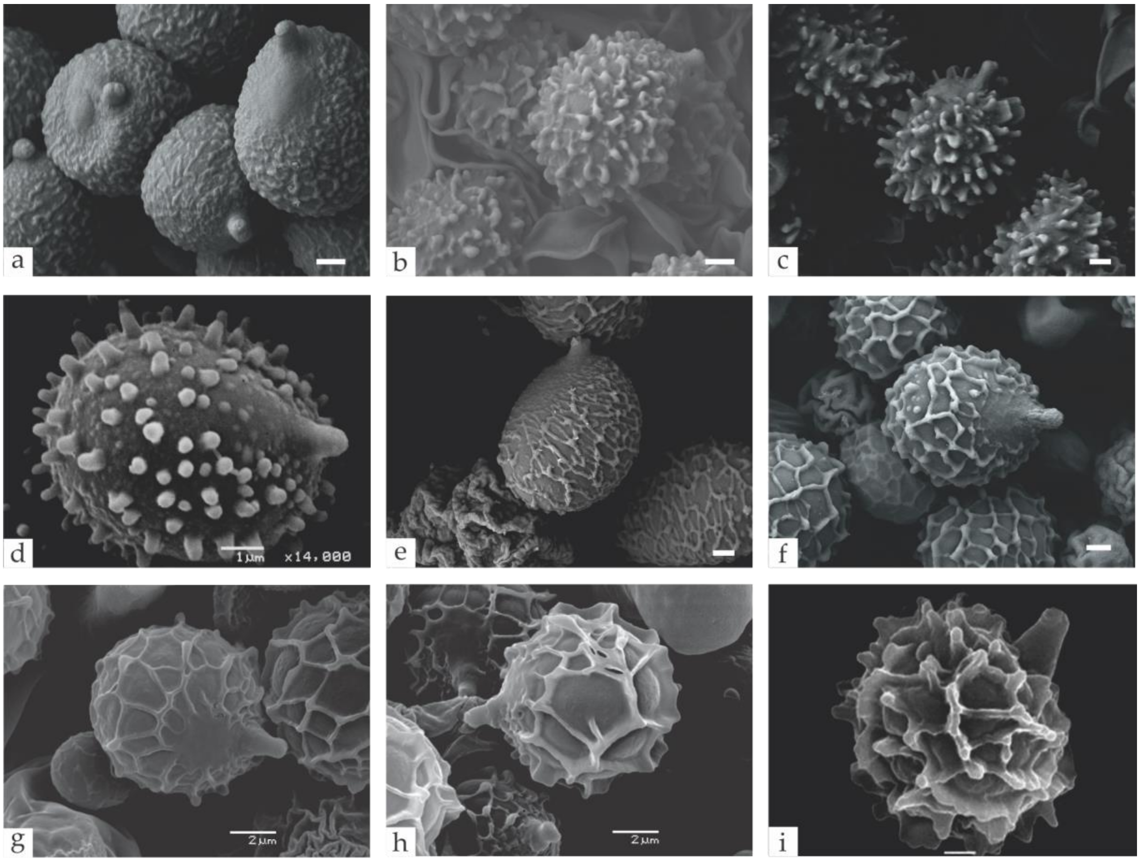

Fig. 1.19 SEM pictures of different basidiospore types found in the genus *Lactifluus*: **a.** very low ornamentation in *Lf. ramipilosus* (EDC 14-503); **b.** ornamentation of warts connected by fine connective lines in *Lf. albomembranaceus* (EDC 12-046); **c.** ornamentation of high warts connected by fine connective lines in *Lf.* cf. *luteolus* (KW 378); **d.** rounded warts in *Lf. angustus* (MGF 713); **e.** low ornamentation forming an almost complete reticulum in *Lf.* sp. (AV 11-029); **f.** ornamentation forming an almost complete reticulum in *Lf. armeniacus* (EDC 14-501); **g.** reticulated ornamentation in *Lf. volemus* (KVP 08-045); **h.** reticulated ornamentation with moderately high ridges in *Lf. oedematopus* (RW 1228); **i.** reticulated ornamentation with high ridges and warts in *Lf.* aff. *gerardii* (LTH 270) (scale bar = 1 µm).

Hymenophoral trama in *Lactifluus* typically consists of isodiametric sphaerocytes (globose cells), sometimes in combination with hyphae (Fig. 1.20). In between the trama, **lactiferous hyphae** are found. They have a refringent, dense, oleiferic or needle-like to granular content and are rather broad (4–16 µm). In some species they are abundant, while scarce in others.

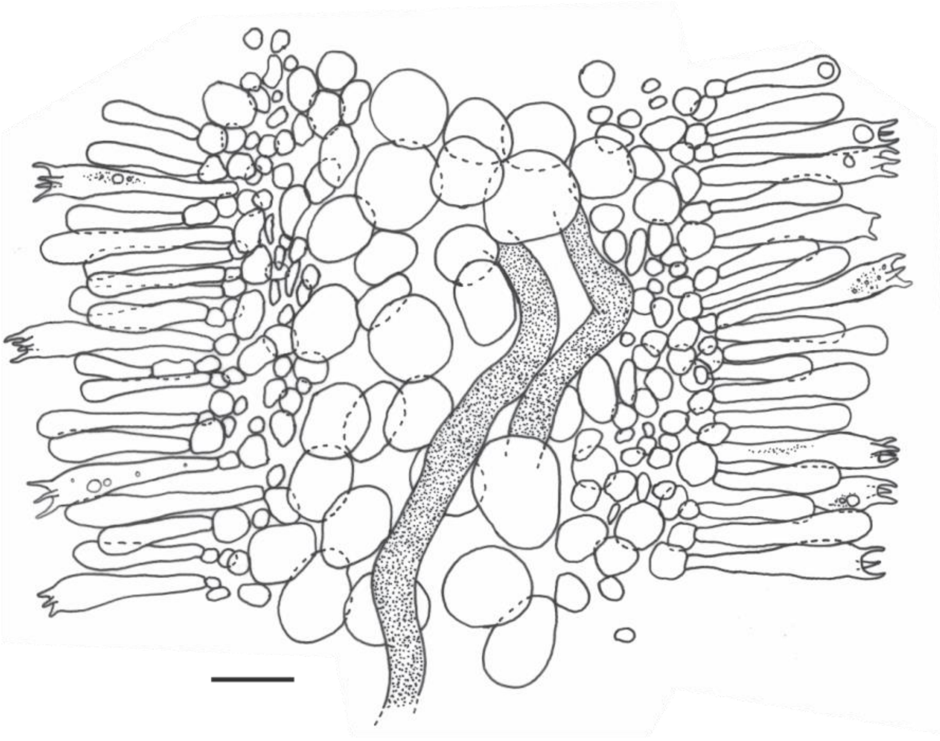

Fig. 1.20 Section through the hymenium in *Lf.* sp. (EDC 14-060) showing **a.** cellular trama and **b.** lactiferous hyphae (scale bar = 25µm; line drawing by E. De Crop).

Ethnomycological uses

In many parts of the world, fleshy mushrooms are of great importance as **seasonal food source**. Species that are consumed and the way they are prepared differ according to cultural habits. Russulaceae species are consumed in many parts of Africa, Asia, Europe, Central and North America. Milkcap species are easily recognised and often fruit in large numbers, which makes them popular at markets. Currently, no records of consumed *Lactifluus* species are known from South America and Oceania.

In **African** countries with woodlands and riparian forests, fungi, and especially ECM fungi, appear in great numbers at the beginning of the rain season. Mainly women and children go out in the forests to collect different edible species, which are then sold at the local markets and along roadsides, either fresh, dried or boiled (Fig. 1.21). The main species that are collected are from the ECM genera *Lactifluus*, *Lactarius*, *Russula*, *Amanita* Pers. and *Cantharellus* Juss., and the non-ECM genus *Termitomyces* R. Heim. Milkcap species often found at the market are: *Lf.* cf. *rubroviolascens*, *Lf. denigricans* (Verbeken & Karhula) Verbeken, *Lf. gymnocarpus* (R. Heim ex Singer) Verbeken, *Lf. albomembranaceus*, *Lf. densifolius* (Verbeken & Karhula) Verbeken, *Lf. edulis* (Verbeken & Buyck) Buyck, *Lf. gymnocarpoides*, *Lf. volemoides* (Karhula) Verbeken, *Lf. xerampelinus* (Karhula & Verbeken) Verbeken and *Lt. kabansus* Pegler & Piearce (Rammeloo and Walleyn 1993; Verbeken and Walleyn 1999; De Kesel et al. 2002; Härkönen et al. 2003; Kinge et al. 2011; Sharp 2011, 2014). Mushrooms are mostly prepared in the same way: washed, cut into pieces and cooked in a kettle with water or oil over an open fire. Onions, tomatoes and sometimes other vegetables are added, together with some salt. Milkcap species, especially the sharp-tasting species, are often parboiled, and the boiling water is thrown away (Härkönen et al. 2003).

Fig. 1.21 Edible *Lactifluus* species on African markets: **a.** cooked *Lactifluus* species for sale on Foumban market (Cameroon); **b.** our local guide with a basket full of *Lactifluus* species (Foumban, Cameroon); **c.** *Lactifluus* species for sale on Kigoma market (Tanzania); **d.** *Lf. rubroviolascens* collected for consumption (Foumban, Cameroon); **e.** cooked *Lactifluus* species (Foumban, Cameroon) (Photographs by E. De Crop (a–e)).

Several **European** countries have a long history in collecting edible fungi and milkcaps are often very popular. Although European milkcaps are mainly represented by *Lactarius*, most European *Lactifluus* species are edible and consumed in several countries. *Lactifluus volemus*, *Lf. oedematopus* and *Lf. subvolemus* for example, are popular species as they produce many large fruitbodies that are easily identified and have an excellent taste (Van de Putte 2012). The European large and white species, *Lf. vellereus*, *Lf. bertillonii* (Neuhoff ex Z. Schaef.) Verbeken, *Lf. piperatus* and *Lf. glaucescens* (Crossl.) Verbeken, have an acrid taste and are only eaten in some regions, where they are parboiled or preserved with salt before consumption to remove the acrid taste (Heilmann-Clausen et al. 1998).

Milkcap species are amongst the favourite edible mushrooms for local mushroom pickers in **North America** and among them are several *Lactifluus* species. In Pennsylvania for example, some locals go on "milkie mushroom" hunting trips, especially to collect milkcaps (Russell 2006). *Lactifluus* cf. *volemus*, *Lf. corrugis* (Peck) Kuntze and *Lf. hygrophoroides* (Berk. & M.A. Curtis) Kuntze are the most famous ones, with *Lf.* cf. *volemus* recurrently being reported as the best and most flavourful milkcap (Peck 1885; Metzler and Metzler 1992; Roody 2003; Russell 2006; Bessette 2007; Lincoff 2010; Van de Putte 2012). Some authors also mention *Lactifluus gerardii* and *Lf. luteolus* (Peck) Verbeken as edible (Roody 2003; Bessette 2007), while only a minority likes to eat the milkcaps with peppery latex, such as *Lf.* cf. *piperatus*, *Lf.* cf. *glaucescens*, *Lf. deceptivus* (Peck) Kuntze, *Lf. subvellereus* (Peck) Nuytinck and *Lf. subgerardii* (Hesler & A.H. Sm.) Stubbe. Some authors even report some of these peppery tasting species as being poisonous (Bessette 2007). For other species, such as *Lf. allardii*, the edibility is unknown (Bessette 2007). *Lactifluus* species are typically fried, baked or cooked. The species with spicy milk are cooked first and the cooking water has to be removed before consumption.

In **Central America**, both *Lf. deceptivus* and *Lf.* cf. *volemus* are known to be sold on traditional markets and used by local people in Mexico (Montoya and Bandala 1996; Van de Putte 2012).

In **Asia**, mainly members of *Lf.* sect. *Lactifluus* are known to be collected and eaten by local people of China, Japan and Thailand (Le 2007; Lincoff 2010; Van de Putte 2012).

Bioactive secondary metabolites

Lactifluus species are known to contain bioactive secondary metabolites in their fruiting bodies. Several *Lactifluus* species are reported to have anti-mutagen properties, such as *Lactifluus volemus* (Wasser 2002; Dai et al. 2009; Van de Putte 2012) or *Lf. vellereus* (Mlinaric et al. 2004). In China, *Lf.* cf. *vellereus* contains a highly functionalized lactarane sesquiterpene, velleratretraol, that shows weak anti-HIV activity (Luo et al. 2009). Some *Lactifluus* species appear effective as antioxidant agent due to their bioactive compounds, such as the Asian representatives of *Lf.* cf. *volemus* and *Lf.* cf. *piperatus* (Ferreira et al. 2009; Ozen et al. 2011; Abdullah et al. 2012; Van de Putte 2012; Joshi et al. 2013) and the European *Lf. vellereus* and *Lf. bertillonii* (Heleno et al. 2012). *Lactifluus piperatus* is reported to have possibilities as a biosorbent and can be used to remove cadmium (Cd II) and zinc (Zn II) ions from wastewater (Nagy et al. 2014a; Nagy et al. 2014b). In Turkey, *Lf. vellereus* is used as food and as traditional medicine and Dogan et al. (2013) showed that it indeed has antimicrobial properties.

Objectives and outline of this thesis

Fungi are an important part of every ecosystem on earth, however, their diversity is largely understudied compared with other organisms. Since the rise of molecular techniques, thousands of new lineages are being discovered but most of the time not accurately studied and described. Using modern taxonomy, which combines different biological properties that can serve as evidence for species divergence, species can be delimited and described. The framework of identified and described species can then be used by researchers of different disciplines to infer a variety of questions.

Amongst the mainly agaricoid Russulaceae genera, the ectomycorrhizal genus *Lactifluus* is least studied. This is due to its mainly tropical distribution and the occurrence of several species complexes. Several authors question the monophyletic status of *Lactifluus* and the current classification in subgenera and sections, in which tropical species are largely underrepresented (Buyck et al. 2008; Verbeken et al. 2014). As *Lactifluus* appears to be one of the most dominant ectomycorrhizal genera in the tropics (Tedersoo et al. 2010b; Tedersoo et al. 2011), this thesis aims to:

1. Construct a comprehensive dataset of *Lactifluus*, with an equal representation of all currently known lineages and collections from every continent.
2. Build a stable molecular phylogeny in order to test the hypothesis that *Lactifluus* is not monophyletic and to reconstruct its infrageneric relationships.
3. Compare the traditional classification, mainly based on morphology, with the newly inferred classifications and propose changes in nomenclature.
4. Reconstruct the evolutionary history of *Lactifluus*, in order to test the hypothesis that, due to its large African diversity, *Lactifluus* originated in Africa and then further diversified to the other continents.
5. Delimit species in selected clades and provide accurate descriptions of newly found species.

A multi-gene molecular phylogeny is combined with a morphological study in **Chapter 2**, in order to investigate the infrageneric relationships of the genus *Lactifluus*. An extensive dataset is constructed, comprising 80 % of all known species and 30 % of the type collections, and five important morphological characteristics for *Lactifluus* (fruit body type, presence of a secondary velum, colour reaction of the latex/context, pileipellis type and presence of true cystidia) are plotted against the phylogeny. The resulting classification is compared with the traditional one and nomenclatural changes are proposed where necessary.

Chapter 3 builds on the classification and phylogeny of *Lactifluus* obtained in chapter 2. The dataset is extended with more than 1000 *Lactifluus* ITS sequences and species delimitation is carried out on this dataset. The resulting species tree will then be dated, using a secondary calibration method based on three markers, which gives an idea of the origin date of the genus *Lactifluus*. A biogeographical study gives an overview on the history of *Lactifluus* colonising the world.

Lactifluus is known for its genetic diversity and species complexes. The group of white, large milkcaps around *Lactifluus piperatus* is assumed to be one of these species complexes and is studied in **Chapter 4**. Based on morphology alone, there was a lot of confusion about the number of European species in this group. In the first part of this study, we aimed to delimit species in Europe and compare the resulting species with the morphological results. In the second part, we construct a worldwide phylogeny, in order to verify whether there is intercontinental conspecificity in this group.

During field expeditions of recent years, several pleurotoid *Lactifluus* collections have been found. Preliminary research indicated that many of them were new to science and they are described in **Chapter 5**. A total of six pleurotoid collections was found. A phylogeny is constructed to examine if they represent new species or if they are conspecific with the known pleurotoid species.

C**hapter 6** is a compilation of recent taxonomic novelties in the genus *Lactifluus*. In the first part, an overview is given of new combinations of several sections within *Lactifluus*. Secondly, the newly discovered *Lactifluus kigomaensis* is described. In a third part, two new *Lactifluus* species from Thailand are described: *Lf. armeniacus* and *Lf. ramipilosus* and in the fourth part, two milkcap look-a-likes from tropical Africa are compared.

Chapter 7 contains the general discussion and is followed by a summary, both in English and Dutch.

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CHAPTER 2

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

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 Kreisel ex P.M. Kirk, P.F. Cannon and J.C. David (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* Pers. and *Lactarius* Pers. 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, hydnoid, 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, Eberhardt & Verbeken 2004, Nuytinck et al. 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 (Verbeken 1998b, Buyck & Horak 1999, Henkel et al. 2000), indicated that those species were placed within either *Russula* or *Lactarius*. Hence, the genus *Pleurogala* Redhead & Norvell, which was erected to accommodate pleurotoid species formerly included in *Lactarius* sect. *Panuoidei* Singer (Redhead & Norvell 1993), was abandoned. Sequestrate species also occur both in *Lactarius* (formerly placed in *Arcangeliella* Cavara, *Gastrolactarius* R. Heim ex J.M. Vidal and *Zelleromyces* Singer & A.H. Sm.) and *Russula* (formerly placed in *Cystangium* Singer & A.H. Sm., *Elasmomyces* Cavara, *Gymnomyces* Massee & Rodway, *Martellia* Mattir. and *Macowanites* Kalchbr.) (Calonge & Martín 2000, Miller et al. 2001, Binder & Bresinsky 2002, Desjardin 2003, Nuytinck et al. 2003, Eberhardt & Verbeken 2004, Nuytinck et al. 2004, Lebel & Tonkin 2007, Verbeken et al. 2014). Species with a secondary velum occur both in *Lactarius* and *Russula* and were placed in a separate genus (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* Stalpers & Hjortstam, *Gloeopeniophorella* Rick and *Pseudoxenasma* K.H. Larss. & Hjortstam (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 & V. Hofstetter (Buyck et al. 2008). The former *Russula* subsect. *Ochricompactae* Bills & O.K. Mill., the Asian *Russula zonaria* Buyck & Desjardin and the American *Lactarius furcatus* Coker 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 *Lactarius torminosus* (Schaeff.: Fr.) Pers. (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* (Fr.) Kauffman), *L.* subg. *Russularia* (Fr.) Kauffman and *L.* subg. *Plinthogalus* (Burl.) Hesler & A.H. Sm. 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* (Pers.) Roussel (hereafter abbreviated as *Lf.*) and is typified by *Agaricus lactifluus* L., currently known as *Lf. volemus* (Fr.) Kuntze (Buyck et al. 2010). New combinations were made in a series of three papers for the subgenera *Lf.* subg. *Lactariopsis* (Henn.) Verbeken, *Lf.* subg. *Russulopsis* (Verbeken) Verbeken, *Lf.* subg. *Edules* (Verbeken) Verbeken, *Lf.* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe, *Lf.* subg. *Lactifluus* and *Lf.* subg. *Piperati* Verbeken (Verbeken et al. 2011, Stubbe et al. 2012b, Verbeken et al. 2012). 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 & Walleyn 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* Baill. (Phyllanthaceae), *Eucalyptus* L'Hér and *Leptospermum* J.R. Forster & G. Forster (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, De Crop et al. 2012, Miller et al. 2012, Stubbe et al. 2012a, Van de Putte et al. 2012, 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, Van de Putte et al. 2010, Stubbe et al. 2012a, Van de Putte et al. 2012, De Crop et al. 2014, Van de Putte et al. 2016) 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 (Henn.) Verbeken

Lactifluus subg. *Lactariopsis* was traditionally divided into three sections: *Lf.* sect. *Lactariopsis* Verbeken, *Lf.* sect. *Chamaeleontini* (Verbeken) Verbeken and *Lf.* sect. *Albati* (Bataille) Verbeken (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, Buyck et al. 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 & Walleyn 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* (Verbeken) Verbeken**

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 & Walleyn 1999, 2010). When it was described, the position of *L.* sect. *Edules* Verbeken 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) Verbeken**

Verbeken et al. (2001, 2011) proposed this subgenus which includes only one section, *Lf*. section *Russulopsidei* (Verbeken) Verbeken, comprising eight species endemic to tropical Africa. Species are characterised by a dry to viscid pileus, reddish-brown 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 & Walleyn 2010).

Lactifluus* subg. *Lactifluus

Lactifluus subgenus *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, the presence of hymenial lamprocystidia and reticulate spore ornamentation. Macroscopically, they can be recognised by clay-buff to orange-brown, 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 (Singer) Verbeken is a predominantly African section, with only one South American representative, *Lf. veraecrucis* (Singer) Verbeken. Verbeken & Walleyn (2010) synonymised *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeken 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 & Walleyn 2010).

Lactifluus* sect. *Phlebonemi (R. Heim ex Verbeken) Verbeken 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 & Walleyn 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 & Walleyn (2010) state that this division might be artificial and was only conserved for practical reasons.

Lactifluus* sect. *Pseudogymnocarpi (Verbeken) Verbeken 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 & Walleyn 2010).

Lactifluus* sect. *Rubroviolascentini (Singer) Verbeken 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 & Walleyn 2010). The section was distinguished from *Lf.* sect. *Pseudogymnocarpi* based on the blackening context. However, Verbeken & Walleyn (2010) note that this distinction is artificial and was only maintained for practical reasons.

Lactifluus* sect. *Tomentosi (McNabb) Verbeken contains species from Europe, Asia and Oceania, as Verbeken et al. (2012) synonymised *L.* sect. *Rugati* Verbeken 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 & Walleyn 2010).

Lactifluus* sect. *Tenuicystidiati X.H. Wang & Verbeken is an Asian section, recently proposed by Wang et al. (2015). The type of this section was originally placed in *L.* sect. *Pseudogymnocarpi* Verbeken, 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 X.H. Wang currently contains only one species known from Asia, *Lf. ambicystidiatus* X.H. Wang. 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* (A.H. Sm. & Hesler) Stubbe**

Due to striking morphological similarities, *Lf. gerardii* (Peck) Kuntze 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* Verbeken**

This subgenus with a Northern hemispherical distribution contains two sections: ***Lf.* sect. *Piperati*** (Fr.) Verbeken and ***Lf.* sect. *Allardii*** (Hesler & A.H. Sm.) De Crop. *Lf.* 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). *Lf.* 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 (Verbeken) Verbeken has not been placed in a subgenus. The section contains only one African representative, *Lf. aurantiifolius* (Verbeken) Verbeken, 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* (R. Heim & Gooss.-Font.) Verbeken and *Lf. cocosmus* (Van de Putte & De Kesel) Van de Putte from Africa and the Australian *Lf. subclarkeae* (Grgur.) Verbeken; and the pleurotoid Neotropical *Lf. multiceps* S. L. Miller, M. C. Aime & T. W. Henkel, *Lf. brunellus* S. L. Miller, M. C. Aime & T. W. Henkel and *Lf. panuoides* Singer.

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 and type species 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* (Fr.) Boidin, *Auriscalpium vulgare* Gray, *Bondarzewia montana* (Quél.) Singer, *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh., *Gloeocystidiellum porosum* (Berk. & M.A. Curtis) Donk, *Heterobasidion annosum* (Fr.) Bref., *Peniophora nuda* (Fr.) Bres., *Stereum hirsutum* (Willd.) Pers. and *Vararia abortiphysa* Boidin & Lanq. (Table 2.1).

Morphological analyses

For each *Lactifluus* collection, several important or striking morphological characteristics were determined. The following characteristics[20], 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. 2.1) and (v) presence or absence of true cystidia, together with cystidium type (macro-, lepto- or lamprocystidia, Fig. 2.2). 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.

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[20] The five characteristics were selected based on their importance in the traditional classification of the genus *Lactifluus* and based on the possibility of dividing them into discrete categories.

Table 2.1 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. 2.3).

Species	**Voucher collection (herbarium)**	**Country**	**ITS accession no.**	**LSU accession no.**	***RPB2* accession no.**	***RPB1* accession no.**
Genus *Lactifluus*						
Lactifluus* subg. *Lactariopsis						
Lactifluus* sect. *Chamaeleontini						
Lactifluus annulatoangustifolius	BB 00-1518 (GENT,PC)	Madagascar	AY606981	KR364253	None	None
Lactifluus cf. *zenkeri*	AV 11-050 (GENT)	Tanzania	KR364055	KR364182	KR364297	KR364425
Lactifluus chamaeleontinus	JD 946 (BR)	Congo	KR364079	KR364208	KR364267	KR364377
Lactifluus heimii	EDC 11-082 (GENT)	Tanzania	KR364040	KR364167	KR364286	KR364412
Lactifluus heimii Type	AV 94-465 (GENT)	Burundi	KR364025	KR364152	None	None
Lactifluus laevigatus	JD 939 (BR)	Congo	KR364077	KR364206	KR364290	KR364417
Lactifluus pelliculatus	JD 956 (BR)	Congo	KR364080	KR364209	KR364321	KR364449
Lactifluus pruinatus Type	BB 3248 (GENT)	Zambia	KR364031	KR364158	KR364328	KR364458
Lactifluus sesemotani	AV 94-476 (GENT)	Burundi	KR364036	KR364163	KR364345	KR364476
Lactifluus sp.	EDC 12-040 (GENT)	Cameroon	KR364063	KR364192	KR364289	KR364416
Lactifluus uapacae Type	AV 07-048 (GENT)	Cameroon	KR364007	KR364135	KR364352	KR364483
Lactifluus velutissimus	JD 886 (BR)	Congo	KR364075	KR364204	KR364355	KR364485
Clade 1						
Lactifluus emergens	AV 99-012 (GENT)	Zimbabwe	KR364021	KR364148	KR364276	KR364388
Lactifluus madagascariensis	BB 99-409 (PC)	Madagascar	AY606977	DQ421975	DQ421914	None
Lactifluus madagascariensis Type	B-E 99-417 (GENT)	Madagascar	KR364120	KR364245	None	None
Isolated species 1						
Lactifluus acrissimus	EDC 11-112 (GENT)	Tanzania	KR364041	KR364168	KR364254	KR364366
Lactifluus acrissimus Type	ADK2161 (GENT)	Benin	KR364126	None	None	None
Clade 2						
Lactifluus annulifer	TH 9014 (BRG, DUKE)	Guyana	KC155376	KC155376	None	None
Lactifluus sp.	RC/Guy 09-004bis (LIP)	French Guiana	KJ786643	KP691419	KP691427	None
Lactifluus subiculatus	SLM 10114 (BRG, RMS)	Guyana	JQ405654	None	None	None
Lactifluus venezuelanus	RC/Guad 11-017 (LIP)	Guadeloupe	KP691411	KP691420	KP691429	KR364393
Clade 3						
Lactifluus multiceps	TH 9154A (BRG, DUKE)	Guyana	JN168731	None	None	None
Lactifluus sp.	G3264 (MNHN)	French Guiana	KJ786706	KJ786620	KP691435	KR364400
Clade 4						

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus chrysocarpus Type	LE 253907 (LE)	Vietnam	JX442761	JX442761	None	None
Lactifluus sp. nov.	EDC 14-503 (GENT, MFLU)	Thailand	KR364128	None	None	None
Clade 5						
Lactifluus brachystegiae Type	AV 99-002 (GENT)	Zimbabwe	KR364018	KR364145	KR364262	KR364374
Lactifluus leoninus	DS 07-454 (GENT)	Thailand	KF220055	JN388989	JN375592	JN389188
Lactifluus leoninus Type	EH 72-524 (GENT)	Papua New Guinea	KR364116	None	None	None
Lactifluus sp.	AV 11-183 (GENT)	Togo	KR364060	KR364189	KR364277	KR364389
Isolated species 2						
Lactifluus cocosmus Type	ADK 4462 (GENT)	Togo	KR364013	KR364141	KR364269	KR364380
Clade 6						
Lactifluus rufomarginatus	ADK 3358 (BR)	Benin	KR364033	KR364160	KR364335	KR364466
Lactifluus rufomarginatus Type	ADK 3011 (GENT)	Benin	KR364034	KR364161	KR364336	None
Lactifluus sp.	AV 07-056 (GENT)	Cameroon	KR364008	KR364136	KR364293	KR364421
Lactifluus sp.	EDC 12-195 (GENT)	Cameroon	KR364071	KR364200	KR364301	KR364429
Clade 7						
Lactifluus densifolius	AV 11-111 (GENT)	Tanzania	KR364057	KR364184	KR364273	KR364385
Lactifluus sp.	JD 907 (GENT)	Congo	KR364076	KR364205	KR364302	KR364430
Lactifluus* sect. *Russulopsidei						
Lactifluus urens	EDC 14-032 (GENT)	Zambia	KR364124	KR364247	KR364353	None
Lactifluus cyanovirescens	JD 988 (GENT)	Congo	KR364082	KR364211	KR364270	KR364382
Lactifluus longipes	JD 303 (BR)	Gabon	KR364009	KR364137	KR364310	KR364438
Lactifluus ruvubuensis	AB 305 (GENT)	Guinea	KR364035	KR364162	KR364343	KR364473
Lactifluus ruvubuensis Type	AV 94-599 (GENT)	Burundi	KR364122	None	None	None
Lactifluus* sect. *Edules						
Lactifluus aureifolius	AV 11-074 (GENT)	Tanzania	KR364056	KR364183	KR364259	KR364371
Lactifluus edulis	FN 05-628 (GENT)	Malawi	KR364020	KR364147	KR364275	KR364387
Lactifluus fazaoensis Type	AV 11-178 (GENT)	Togo	HG426477	KR364188	KR364349	KR364481
Lactifluus indusiatus Type	AV 94-122 (GENT)	Burundi	KR364026	KR364153	KR364287	None
Lactifluus inversus	AB 063 (GENT)	Guinea	AY606976	DQ421978	DQ421917	KR364414
Lactifluus latifolius	SDM 037 (BR)	Gabon	KR364028	KR364155	KR364291	KR364418
Lactifluus nodosicystidiosus	BEM 97-273 (GENT)	Madagascar	KR364029	KR364156	KR364316	KR364444
Lactifluus nodosicystidiosus Type	BEM 97-072 (GENT)	Madagascar	AY606975	DQ421976	DQ421915	None
Lactifluus phlebophyllus	BB 00-1388 (PC)	Madagascar	AY606974	DQ421979	DQ421918	None

Species	**Voucher collection (herbarium)**	**Country**	**ITS accession no.**	**LSU accession no.**	***RPB2* accession no.**	***RPB1* accession no.**
Lactifluus roseolus	AV 99-160 (GENT)	Zimbabwe	KR364032	KR364159	KR364333	KR364463
Lactifluus roseolus Type	AV 94-274 (GENT)	Burundi	KR364121	KR364242	None	None
Lactifluus sp. nov.	EDC 12-068 (GENT)	Cameroon	KR364068	KR364197	KR364299	KR364427
Lactifluus* sect. *Albati						
Lactifluus bertillonii	JN 2012-016 (GENT)	Germany	KR364087	KR364217	KR364261	KR364373
Lactifluus deceptivus	TENN 065854 (TENN)	North America	KR364101	None	KR364271	KR364383
Lactifluus pilosus Type	LTH 205 (GENT)	Thailand	KR364006	KR364134	KR364323	KR364452
Lactifluus sp. nov.	JN 2011-071 (GENT)	Vietnam	KR364043	KR364169	KR364255	KR364367
Lactifluus sp. nov.	JN 2011-077 (GENT)	Vietnam	KR364044	KR364170	KR364256	KR364368
Lactifluus subvellereus	AV 05-210 (GENT)	North America	KR364010	KR364138	KR364347	KR364479
Lactifluus vellereus	ATHU-M 8077 (ATHU-M)	Greece	KR364106	KR364237	KR364354	KR364484
Lactifluus* subg. *Rugati						
Lactifluus* sect. *Pseudogymnocarpi						
Lactifluus cf. *longisporus*	AV 11-025 (GENT)	Tanzania	KR364054	KR364181	KR364311	KR364439
Lactifluus cf. *pseudogymnocarpus*	AV 05-085 (GENT)	Malawi	KR364012	KR364139	KR364329	KR364459
Lactifluus cf. *pumilus*	EDC 12-066 (GENT)	Cameroon	KR364067	KR364196	KR364332	KR364462
Lactifluus gymnocarpoides	JD 885 (BR)	Congo	KR364074	KR364203	KR364283	KR364409
Lactifluus gymnocarpoides	AV 05-184 (GENT)	Malawi	KR364024	KR364151	KR364284	KR364410
Lactifluus hygrophoroides	AV 05-251 (GENT)	North America	HQ318285	HQ318208	HQ328936	KR364413
Lactifluus longisporus Type	AV 94-557 (GENT)	Burundi	KR364118	KR364244	None	None
Lactifluus luteopus	EDC 11-087 (GENT)	Tanzania	KR364049	KR364176	KR364312	KR364441
Lactifluus luteopus Type	AV 94-463 (GENT)	Burundi	KR364119	None	KR364313	None
Lactifluus medusae	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198	KR364314	KR364442
Lactifluus pseudoluteopus	FH 12-026 (GENT)	Thailand	KR364084	KR364214	KR364331	KR364460
Lactifluus rugatus	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235	KR364337	KR364467
Lactifluus sudanicus Type	AV 11-174 (GENT)	Togo	HG426469	KR364186	KR364348	KR364480
Lactifluus* sect. *Xerampelini						
Lactifluus cf. *pseudovolemus*	ADK 2927 (GENT)	Benin	KR364113	KR364243	KR364330	KR364461
Lactifluus goossensiae	AB 320 (GENT)	Guinea	KR364132	KR364252	KR364281	None
Lactifluus kivuensis Type	JR Z 310 (GENT)	Congo	KR364027	KR364154	None	None
Lactifluus rubiginosus	JD 959 (BR)	Congo	KR364081	KR364210	KR364304	KR364432
Lactifluus rubiginosus Type	BB 3466 (GENT)	Zambia	KR364014	KR364250	None	None
Lactifluus sp. nov.	EDC 12-001 (GENT)	Cameroon	KR364061	KR364190	KR364298	KR364426

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus sp. nov.	EDC 12-176 (GENT)	Cameroon	KR364070	KR364199	KR364300	KR364428
Lactifluus xerampelinus	MH 201176 (GENT)	Mozambique	KR364099	KR364231	KR364364	KR364496
Lactifluus xerampelinus Type	TS 1116 (GENT)	Tanzania	KR364039	KR364166	None	None
Clade 8						
Lactifluus sp. nov.	JN 2011-012 (GENT)	Vietnam	KR364045	KR364171	KR364294	KR364422
Lactifluus sp. nov.	TENN 065929 (TENN)	North America	KR364102	KR364233	KR364308	KR364436
Lactifluus sp. nov.	EDC 14-501 (GENT, MFLU)	Thailand	KR364127	None	None	None
Lactifluus volemoides	MH 201187 (GENT)	Mozambique	KR364098	KR364230	KR364363	KR364493
Lactifluus volemoides Type	TS 0705 (GENT)	Tanzania	KR364038	KR364165	None	None
Lactifluus* sect. *Aurantiifolii						
Lactifluus aurantiifolius Type	AV 94-063 (GENT)	Burundi	KR364017	KR364144	None	None
Lactifluus* sect. *Rubroviolascentini						
Lactifluus aff. *rubroviolascens*	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195	KR364334	KR364465
Lactifluus carmineus Type	AV 99-099 (GENT)	Zimbabwe	KR364131	KR364251	KR364265	None
Lactifluus denigricans	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178	KR364272	KR364384
Lactifluus sp. nov.	AV 11-006 (GENT)	Tanzania	KR364052	KR364179	KR364288	KR364415
Lactifluus kigomaensis	EDC 11-159 (GENT)	Tanzania	KR364050	KR364177	KR364295	KR364423
Lactifluus* sect. *Polysphaerophori						
Lactifluus pegleri	PAM/Mart 12-091 (LIP)	Martinique	KP691416	KP691425	KP691433	KR364397
Lactifluus sp.	RC/Guy 09-036 (LIP)	French Guiana	KJ786645	KJ786550	KP752178	None
Lactifluus sp.	MR/Guy 13-145	French Guiana	KJ786691	KJ786595	KP752180	KR364398
Lactifluus sp.	MCA 3937 (GENT)	Guyana	KR364109	KR364240	KR364350	None
Lactifluus veraecrucis Type	M 8025 (ENCB)	Mexico	KR364112	KR364241	None	None
Lactifluus* subg. *Gymnocarpi						
Lactifluus* sect. *Luteoli						
Lactifluus brunneoviolascens	AV 13-038 (GENT)	Italy	KR364123	KR364246	KR364264	KR364376
Lactifluus longivelutinus Type	XHW 1565 (GENT)	China	KR364114	None	None	None
Lactifluus luteolus	AV 05-253 (GENT)	North America	KR364016	KR364142	KJ210067	KR364440
Lactifluus nonpiscis	AV 11-137 (GENT)	Togo	KR364058	KR364185	KR364317	KR364445
Lactifluus nonpiscis Type	BB 3171 (GENT)	Zambia	KR364030	KR364157	None	None
Lactifluus rubrobrunnescens Type	EH 7194 (GENT)	Indonesia	KR364115	None	None	None
Lactifluus sp. nov.	KW 392 (GENT)	Thailand	KR364091	KR364222	KR364305	KR364433
Lactifluus sp. nov.	REH 9398 (NY)	Australia	KR364097	KR364229	KR364307	KR364435

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus* sect. *Gymnocarpi						
Lactifluus albocinctus Type	AV 99-211 (GENT)	Zimbabwe	KR364117	KR364249	KR364258	None
Lactifluus sp. nov.	EDC 12-046 (GENT)	Cameroon	KR364064	KR364193	KR364257	KR364369
Lactifluus cf. *tanzanicus*	AV 11-017 (GENT)	Tanzania	KR364053	KR364180	KR364296	KR364424
Lactifluus flammans	JD 941 (BR)	Congo	KR364078	KR364207	KR364303	KR364431
Lactifluus gymnocarpus	EDC 12-047 (GENT)	Cameroon	KR364065	KR364194	KR364282	KR364408
Lactifluus tanzanicus Type	TS 1277 (GENT)	Tanzania	KR364037	KR364164	KR364351	None
Isolated species 4						
Lactifluus foetens	ADK 3688 (BR)	Benin	KR364022	KR364149	KR364278	KR364390
Lactifluus foetens Type	ADK 2840 (BR)	Benin	KR364023	KR364150	KR364279	KR364391
Lactifluus* sect. *Phlebonemi						
Lactifluus aff. *phlebonemus*	EDC 12-023 (GENT)	Cameroon	KR364062	KR364191	KR364322	KR364451
Lactifluus brunnescens	AV 05-083 (GENT)	Malawi	KR364019	KR364146	KR364263	KR364375
Clade 9						
Lactifluus aff. *nebulosus*	RC/Guad 11-023 (LIP)	Guadeloupe	KP691412	KP691421	KP691430	KR364394
Lactifluus caribaeus	PAM/Mart 12-090 (LIP)	Martinique	KP691415	KP691424	KP691432	KR364396
Lactifluus cf. *castaneibadius*	CL/MART06.019 (LIP)	Martinique	KP691417	KP691426	None	None
Lactifluus cf. *murinipes*	F.1890 (LIP)	Martinique	KP691418	None	None	None
Lactifluus cf. *putidus*	PAM/Mart 11-013 (LIP)	Martinique	KP691413	KP691422	KP691431	KR364395
Lactifluus chiapanensis	VMB 4374A (GENT)	Mexico	GU258297	GU265580	GU258316	KR364378
Isolated species 5						
Lactifluus sp.	G3185	French Guiana	KJ786694	KJ786603	KP691434	KR364399
Isolated species 6						
Lactifluus brunellus	TH 9130 (BRG, DUKE)	Guyana	JN168728	None	None	None
Isolated species 7						
Lactifluus sp.	RC/Guad 08-042 (LIP)	Guadeloupe	KP691414	KP691423	KP752179	None
Isolated species 8						
Lactifluus panuoides	RC/Guy 10-024 (LIP)	French Guiana	KJ786647	KJ786551	KP691428	None
Lactifluus* sect. *Tomentosi						
Lactifluus clarkeae	MN 2004002 (L)	Australia	KR364011	HQ318205	KR364268	KR364379
Lactifluus flocktonae	JET1006 (MEL)	Australia	JX266621	JX266637	None	None
Lactifluus sp.	PGK13-130	New Caledonia	KP691436	KR605507	None	None

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus subclarkeae	REH 9231 (NY)	Australia	KR364095	KR364227	KR364346	KR364477
Lactifluus* subg. *Lactifluus						
Lactifluus* sect. *Lactifluus						
Lactifluus acicularis	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132	HQ328869	JN389131
Lactifluus corrugis s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143	JQ348127	None
Lactifluus crocatus	KVP 08-034 (GENT)	Thailand	HQ318243	HQ318151	HQ328888	JN389145
Lactifluus dissitus	AV-KD-KVP 09-134 (GENT)	India	JN388978	JN389026	JN375628	JN389172
Lactifluus distantifolius	LTH 288 (GENT)	Thailand	HQ318274	HQ318193	KR364274	JN389155
Lactifluus lamprocystidiatus Type	EH 72-195 (GENT)	Papua New Guinea	KR364015	None	None	None
Lactifluus leptomerus Type	AV-KD-KVP 09-131 (GENT)	India	JN388972	JN389023	JN375625	JN389169
Lactifluus longipilus	LTH 184 (GENT)	Thailand	HQ318256	HQ318169	HQ328905	JN389152
Lactifluus oedematopus	KVP 12-001 (GENT)	Germany	KR364100	KR364232	KR364319	KR364447
Lactifluus pinguis Type	AV-RW 04-023/LTH117 (GENT)	Thailand	HQ318211	HG318111	HQ328858	JN389126
Lactifluus sp.	SA A12 L2 (GENT)	North America	KR364088	KR364218	KR364361	KR364491
Lactifluus subvolemus nom. prov.	KVP 08-048 (GENT)	Slovenia	JQ753927	JQ348379	KR364356	KR364486
Lactifluus versiformis Type	AV-KD-KVP 09-045 (GENT)	India	JN388967	JN389031	JN375632	JN389177
Lactifluus vitellinus	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144	HQ328881	JN389138
Lactifluus volemus	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175	KR364360	KR364490
Lactifluus volemus s.l.	AV-KD-KVP 09-121 (GENT)	India	JN388979	JN389014	JN375616	JN389160
Lactifluus volemus s.l.	KVP 08-011 (GENT)	Thailand	HQ318232	HQ318139	HQ328876	JN389135
Lactifluus volemus s.l.	KVP 08-031 (GENT)	Thailand	HQ318240	HQ318148	HQ328885	JN389142
Lactifluus volemus s.l.	REH 9320 (NY)	Australia	KR364096	KR364228	KR364362	KR364492
Lactifluus* sect. *Tenuicystidiati						
Lactifluus aff. *tenuicystidiatus*	JN 2011-074 (GENT)	Vietnam	KR364047	KR364173	KR364358	KR364488
Lactifluus sp.	JN 2011-080 (GENT)	Vietnam	KR364048	KR364174	KR364359	KR364489
Lactifluus subpruinosus nom. prov.	JN 2011-061 (GENT)	Vietnam	KR364046	KR364172	KR364357	KR364487
Lactifluus* sect. *Gerardii						
Lactifluus aff. *gerardii*	LTH 270 (GENT)	Thailand	EF560685	GU265598	GU258335	KR364402
Lactifluus atrovelutinus	DS 06-003 (GENT)	Malaysia	GU258231	GU265588	GU258325	JN389185
Lactifluus limbatus Epitype	DS 06-247 (GENT)	Malaysia	JN388955	JN388987	JN375590	JN389186
Lactifluus cf. *gerardii* var. *fagicola*	JN 2007-029 (GENT)	Canada	GU258224	GU265582	GU258318	None
Lactifluus cf. *ochrogalactus*	AV-KD-KVP 09-120 (GENT)	India	KR364130	KR364248	KR364318	KR364446
Lactifluus conchatulus Type	LTH 457 (GENT)	Thailand	GU258296	GU265659	GU258399	KR364381

Species	**Voucher collection (herbarium)**	**Country**	**ITS accession no.**	**LSU accession no.**	***RPB2* accession no.**	***RPB1* accession no.**
Lactifluus fuscomarginatus Type	LM 4379 (XAL)	Mexico	HQ168367	HQ168367	None	None
Lactifluus genevievae Type	GG-DK 17-02-05 (GENT)	Australia	GU258294	GU265657	GU258397	KR364401
Lactifluus gerardii	AV 05-375 (GENT)	North America	GU258254	GU265616	GU258353	KR364403
Lactifluus igniculus Type	LE 262983 (LE)	Vietnam	JX442759	JX442759	None	None
Lactifluus leae	FH 12-013 (GENT)	Thailand	KF432957	KR364213	KR364292	KR364419
Lactifluus leonardii	GG 07-02-04	Australia	GU258308	GU265668	GU258408	KR364495
Lactifluus petersenii	AV 05-300 (GENT)	North America	GU258281	GU265642	GU258382	KR364450
Lactifluus reticulatovenosus Type	EH 6472 (GENT)	Indonesia	GU258286	GU265649	GU258389	None
Lactifluus sp. nov.	AV 12-050 (GENT)	Thailand	KR364086	KR364216	KR364260	KR364372
Lactifluus sp. nov.	AV 12-070 (GENT)	Thailand	KR364090	KR364221	KR364326	None
Lactifluus sp. nov.	TENN 051830 (TENN)	Nepal	KR364111	KR364140	None	None
Lactifluus sp. nov.	KW 304/FH 12-037 (GENT)	Thailand	KR364092	KR364223	KR364306	KR364434
Lactifluus subgerardii	AV 05-269 (GENT)	North America	GU258263	GU265625	GU258362	KR364478
Lactifluus wirrabara s.l.	PL 40509	New Zealand	GU258287	GU265650	GU258390	KR364475
Lactifluus wirrabara s.l.	GG 24-01-04	Australia	GU258307	GU265667	GU258407	KR364494
Lactifluus* sect. *Ambicystidiati						
Lactifluus ambicystidiatus nom. prov.	HKAS J7008 (HKAS)	China	KR364108	KR364239	KR364309	KR364437
Isolated species 9						
Lactifluus sp. nov.	PUN 7046 (PUN)	India	KM658971	None	None	None
Lactifluus* sect. *Allardii						
Lactifluus allardii	JN 2004-008 (GENT)	North America	KF220016	KF220125	KF220217	KR364370
Lactifluus* sect. *Piperati						
Lactifluus aff. *glaucescens*	AV 04-195 (GENT)	North America	KF220045	KF220146	KF220232	KR364404
Lactifluus aff. *glaucescens*	AV 05-374 (GENT)	North America	KF220049	KF220150	KF220236	KR364405
Lactifluus aff. *glaucescens*	JN 2011-014 (GENT)	Vietnam	KF220104	KF220199	KF220273	KR364406
Lactifluus aff. *glaucescens*	LTH 274 (GENT)	Thailand	KR364107	KR364238	KR364325	KR364457
Lactifluus aff. *piperatus*	JN 2011-036 (GENT)	Vietnam	KF220105	KF220200	KF220274	KR364454
Lactifluus aff. *piperatus*	JN 2011-072 (GENT)	Vietnam	KF220106	KF220201	KF220275	KR364455
Lactifluus aff. *piperatus*	TENN 064342 (TENN)	North America	KR364103	KR364234	KR364324	KR364456
Lactifluus dwaliensis	LTH 55 (GENT)	Thailand	KF220111	KF220204	KF220278	KR364386
Lactifluus dwaliensis Type	KD 612 (GENT)	India	KR364042	None	None	None
Lactifluus glaucescens	LGAM 2010-0132 (LGAM-AUA)	Greece	KR364105	KR364236	KR364280	KR364407
Lactifluus leucophaeus	LTH 182 (GENT)	Thailand	KF220059	KF220157	KF220243	KR364420

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.	*RPB2* accession no.	*RPB1* accession no.
Lactifluus piperatus	2001 08 19 68 (GENT)	France	KF220119	KF241840	KF241842	KR364453
Lactifluus roseophyllus	JN 2011-076 (GENT)	Vietnam	KF220107	KF220202	KF220276	KR364464
Genus *Russula*						
Russula cyanoxantha	FH 12-201 (GENT)	Germany	KR364093	KR364225	KR364341	KR364471
Russula delica	FH 12-272 (GENT)	Belgium	KF432955	KR364224	KR364340	KR364470
Russula gracillima	FH 12-264 (GENT)	Germany	KR364094	KR364226	KR364342	KR364472
Russula khanchanjungae	AV-KD-KVP 09-106 (GENT)	India	KR364129	JN389004	JN375607	JN389092
Russula sp.	EDC 12-061 (GENT)	Cameroon	KR364072	KR364201	KR364338	KR364468
Russula sp.	EDC 12-063 (GENT)	Cameroon	KR364073	KR364202	KR364339	KR364469
Genus *Lactarius*						
Lactarius hatsudake	FH 12-052 (GENT)	Thailand	KR364085	KR364215	KR364285	KR364411
Lactarius olympianus	ED 08-018 (GENT)	North America	KR364089	KR364220	KR364320	KR364448
Lactarius scrobiculatus	JN 2001-058 (GENT)	Slovakia	KF432968	KR364219	KR364344	KR364474
Lactarius fuliginosus	MTB 97-24 (GENT)	Sweden	JQ446111	JQ446180	JQ446240	KR364392
Lactarius miniatescens	AV 11-177 (GENT)	Togo	KR364059	KR364187	KR364315	KR364443
Lactarius tenellus	ADK 3598 (GENT)	Benin	KF133280	KF133313	KF133345	KR364482
Genus *Multifurca*						
Multifurca furcata	REH 7804 (NY)	Costa Rica	DQ421995	DQ421995	DQ421928	None
Multifurca ochricompacta	BB 02-107 (PC)	North America	DQ421984	DQ421984	DQ421940	None
Multifurca sp.	xp2-20120922-01 (GENT)	China	KR364125	None	None	None
Multifurca stenophylla	JET956 (MEL)	Australia	JX266631	JX266635	None	None
Multifurca zonaria	FH 12-009 (GENT)	Thailand	KR364083	KR364212	KR364365	KR364497
Outgroup						
Amylostereum laevigatum	CBS 623.84 (CBS)	France	AY781246	AF287843	AY218469	None
Auriscalpium vulgare	PBM 944 (WTU)	North America	DQ911613	DQ911614	AY218472	None
Bondarzewia montana	AFTOL 452 (DAOM)	No data	DQ200923	DQ234539	AY218474	DQ256049
Echinodontium tinctorium	AFTOL 455 (DAOM)	No data	AY854088	AF393056	AY218482	AY864882
Heterobasidion annosum	AFTOL 470 (DAOM)	No data	DQ206988	None	AY544206	DQ667160
Stereum hirsutum	AFTOL 492	No data	AY854063	AF393078	AY218520	AY864885
Vararia abortiphysa	CBS 630.81 (CBS)	France	KR364005	KR364133	KR364266	None

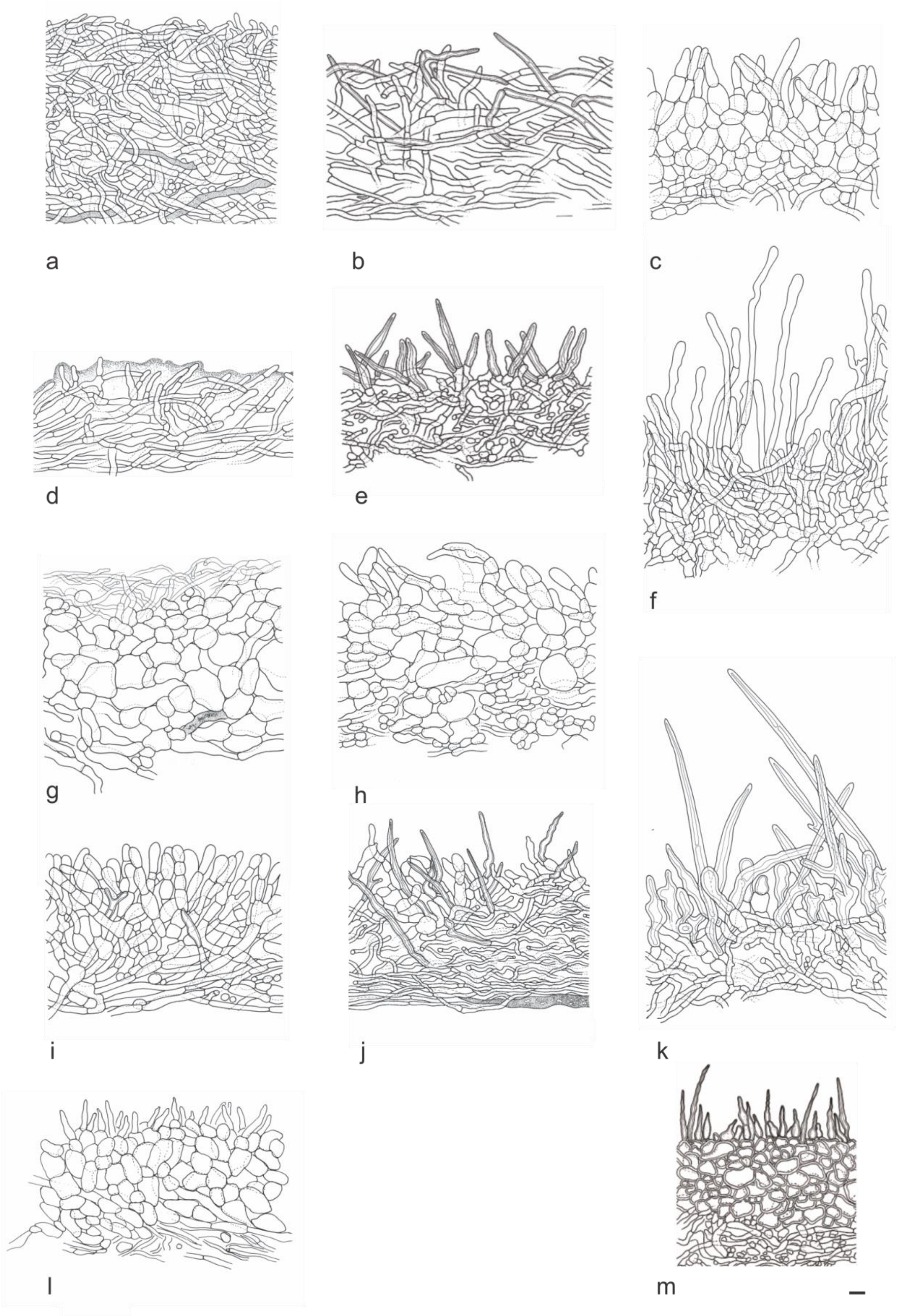


Fig. 2.1 Overview of different pileipellis types found in the genus *Lactifluus*: **a.** cutis in *Lf. urens* (JR 6002); **b.** irregular cutis in *Lf. madagascariensis* (BB 97-072); **c.** hymeniderm in *Lf. roseolus* (AV 94-064); **d.** ixotrichoderm in *Lf. rufomarginatus* (ADK 3011); **e.** lamprotrichoderm 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) (scale bar = 10µm; line drawings by A. Verbeken (a–k), D. Stubbe (l) and K. Van de Putte (m)).

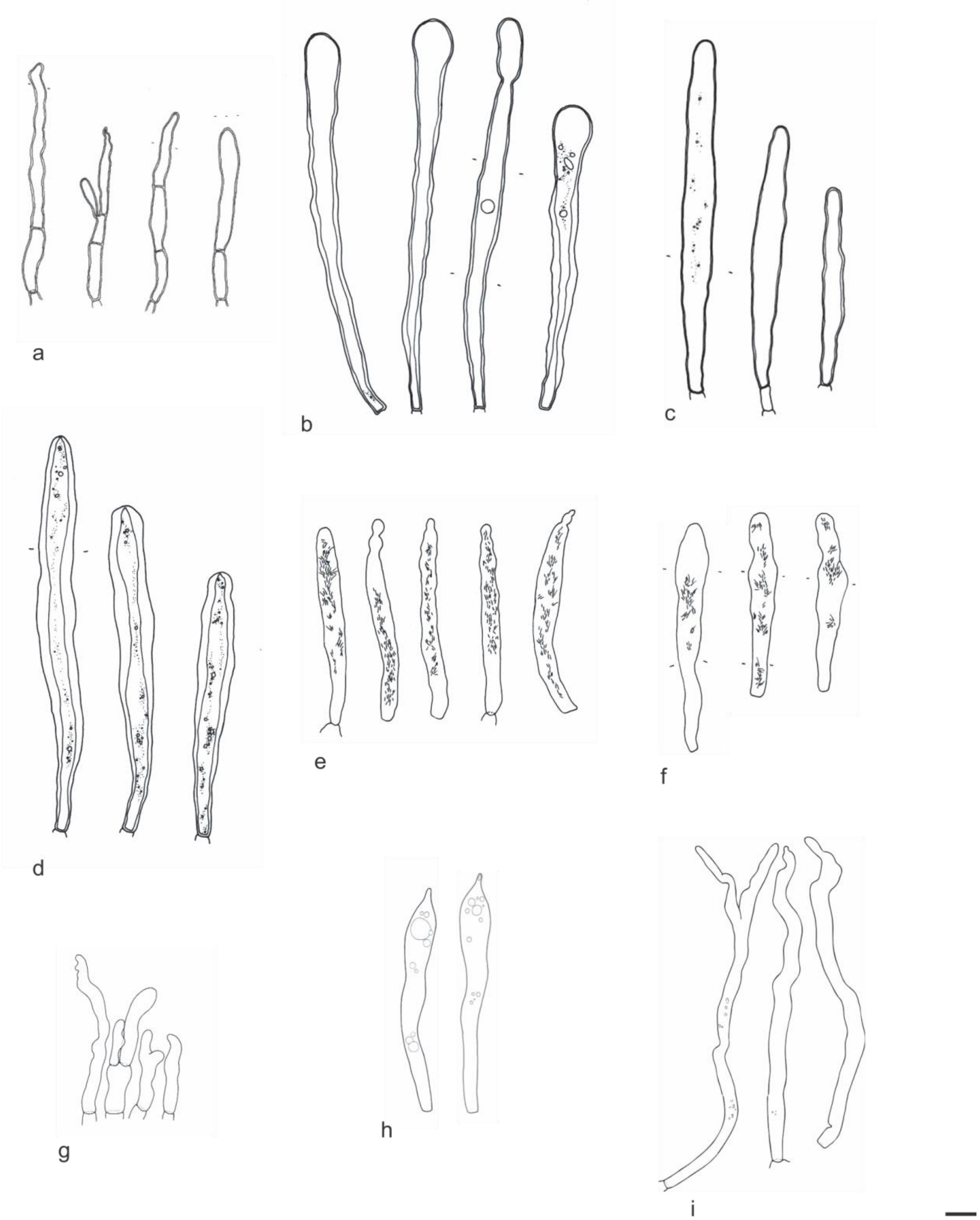


Fig. 2.2 Overview of different true cystidium types found in the genus *Lactifluus*: **lamprocystidia**: **a.** in *Lf. armeniacus* (EDC 14-501); **b.** in *Lf.* sp. nov. (AV 11-006); **c.** in *Lf.* cf. *pumilus* (EDC 12-066); **d.** in *Lf.* cf. *volemus* (REH 9320); **macrocystidia**: **e.** in *Lf.* sp. nov. (JN 2011-077); **f.** in *Lf. roseophyllus* (JN 2011-076); **leptocystidia**: **g.** in *Lf. ruvubuensis* (AV 94-599); **h.** in *Lf. indusiatus* (AV 94-122); **i.** in *Lf. densifolius* (BB 3601) (scale bar = 10µm; line drawings by E. De Crop (a–f) and A. Verbeken (g–i)).

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 1300bp 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′-AGT AAR AYG 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™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v7 (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) 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).

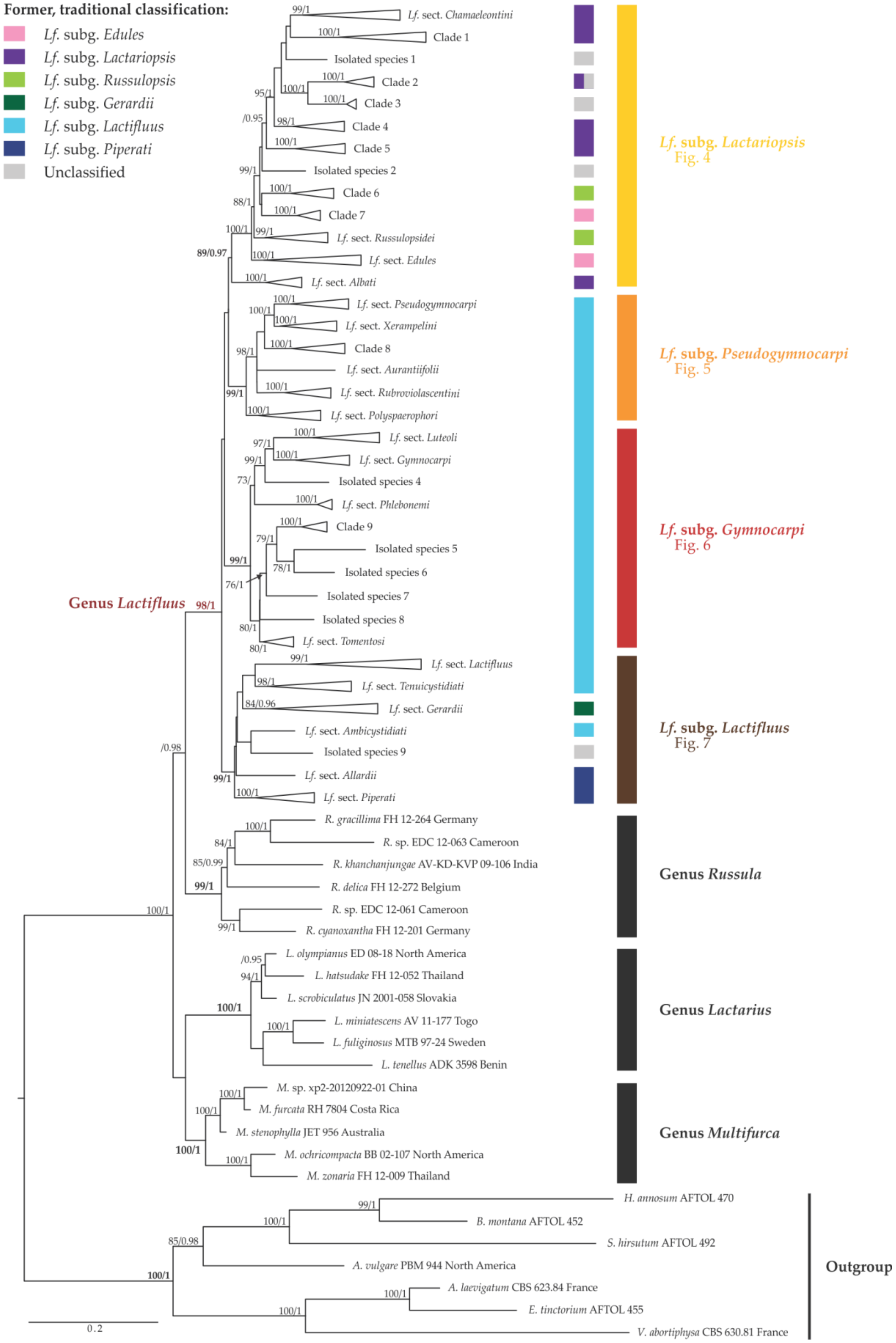
Phylogenetic analyses

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 v8.0.24 (Stamatakis 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis et al. 2008). Bayesian Inference (BI) was executed with MrBayes v3.2.0 (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 1000th generation for the concatenated tree. Parameter convergence for the different runs was verified in Tracer v1.6 (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

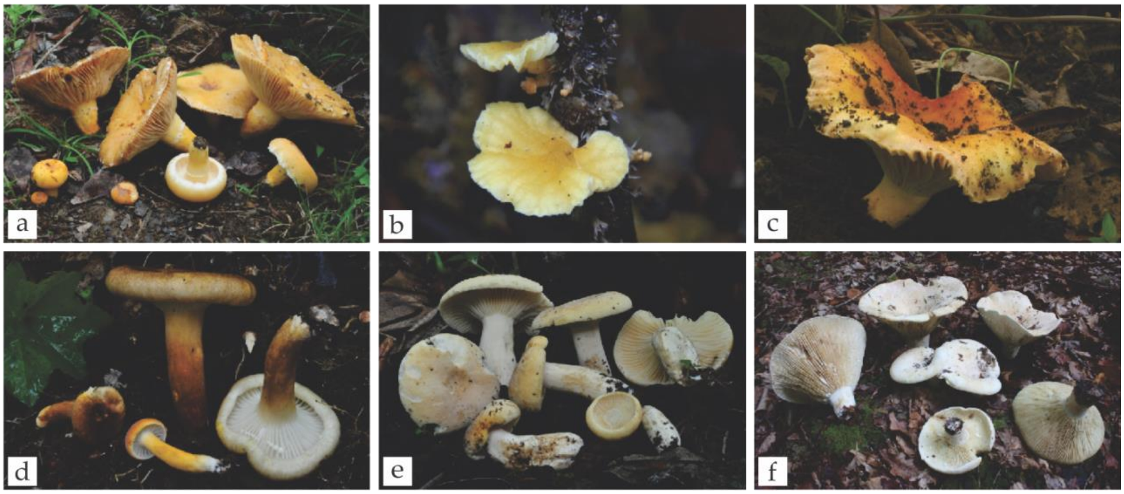
Our dataset contains 213 Russulales collections, of which 189 are from the genus *Lactifluus*. With approximately 150 described species in *Lactifluus*, 80 % of the described taxa are represented in our dataset. Of the 20 % missing, most species are only known from collections too old for sequencing. 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, Van de Putte et al. 2012). Fifty-one of the described species we included have never been sequenced before and 44 of the described species are represented by their type specimen. Furthermore, we included 32 unidentified collections, of which at least 17 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 5032bp long (including gaps).

The phylogeny of the concatenated data is shown in Fig. 2.3. 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* (Pacioni & Lalli) De Crop (ML: 99, BI: 1), *Lf.* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop (ML: 99, BI: 1) and *Lf.* subg. *Lactifluus* (ML: 99, BI: 1). Representatives of each subgenus are shown in Fig. 2.4 and 2.5. The relationships between the subgenera remain unresolved. Each subgenus can be further divided into several sections, which are described below, together with their known morphological characteristics.


Former, traditional classification:
Lf. subg. Edules
Lf. subg. Lactariopsis
Lf. subg. Russulopsis
Lf. subg. Gerardii
Lf. subg. Lactifluus
Lf. subg. Piperati
Unclassified
99/1
Lf. sect. Chamaeleontini
100/1
Clade 1
Isolated species 1
100/1
100/1
Clade 2
95/1
100/1
Clade 3
/0.95
98/1
Clade 4
100/1
Clade 5
99/1
Isolated species 2
100/1
Clade 6
88/1
100/1
Clade 7
100/1
99/1
Lf. sect. Russulopsidei
89/0.97
100/1
Lf. sect. Edules
100/1
Lf. sect. Albati
100/1
Lf. sect. Pseudogymnocarpi
100/1
100/1
Lf. sect. Xerampelini
98/1
100/1
Clade 8
Lf. sect. Aurantiifolii
99/1
100/1
Lf. sect. Rubroviolascentini
100/1
Lf. sect. Polyspaerophori
100/1
Lf. sect. Luteoli
97/1
100/1
Lf. sect. Gymnocarpi
99/1
73/
Isolated species 4
100/1
Lf. sect. Phlebonemi
100/1
Clade 9
79/1
Isolated species 5
99/1
78/1
Isolated species 6
76/1
Isolated species 7
Genus Lactifluus
98/1
Isolated species 8
80/1
80/1
Lf. sect. Tomentosi
99/1
Lf. sect. Lactifluus
98/1
Lf. sect. Tenuicystidiati
84/0.96
Lf. sect. Gerardii
Lf. sect. Ambicystidiati
Isolated species 9
/0.98
99/1
Lf. sect. Allardii
100/1
Lf. sect. Piperati
100/1
R. gracillima FH 12-264 Germany
84/1
R. sp. EDC 12-063 Cameroon
85/0.99
R. khanchanjungae AV-KD-KVP 09-106 India
99/1
R. delica FH 12-272 Belgium
100/1
R. sp. EDC 12-061 Cameroon
99/1
R. cyanoxantha FH 12-201 Germany
/0.95
L. olympianus ED 08-18 North America
94/1
L. hatsudake FH 12-052 Thailand
L. scrobiculatus JN 2001-058 Slovakia
100/1
100/1
L. miniatescens AV 11-177 Togo
L. fuliginosus MTB 97-24 Sweden
L. tenellus ADK 3598 Benin
100/1
M. sp. xp2-20120922-01 China
100/1
M. furcata RH 7804 Costa Rica
M. stenophylla JET 956 Australia
100/1
100/1
M. ochricompacta BB 02-107 North America
M. zonaria FH 12-009 Thailand
99/1
H. annosum AFTOL 470
100/1
B. montana AFTOL 452
85/0.98
S. hirsutum AFTOL 492
A. vulgare PBM 944 North America
100/1
100/1
A. laevigatum CBS 623.84 France
100/1
E. tinctorium AFTOL 455
V. abortiphysa CBS 630.81 France
0.2
Lf. subg. Lactariopsis
Fig. 4
Lf. subg. Pseudogymnocarpi
Fig. 5
Lf. subg. Gymnocarpi
Fig. 6
Lf. subg. Lactifluus
Fig. 7
Genus Russula
Genus Lactarius
Genus Multifurca
Outgroup

◄ **Fig. 2.3.** 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.

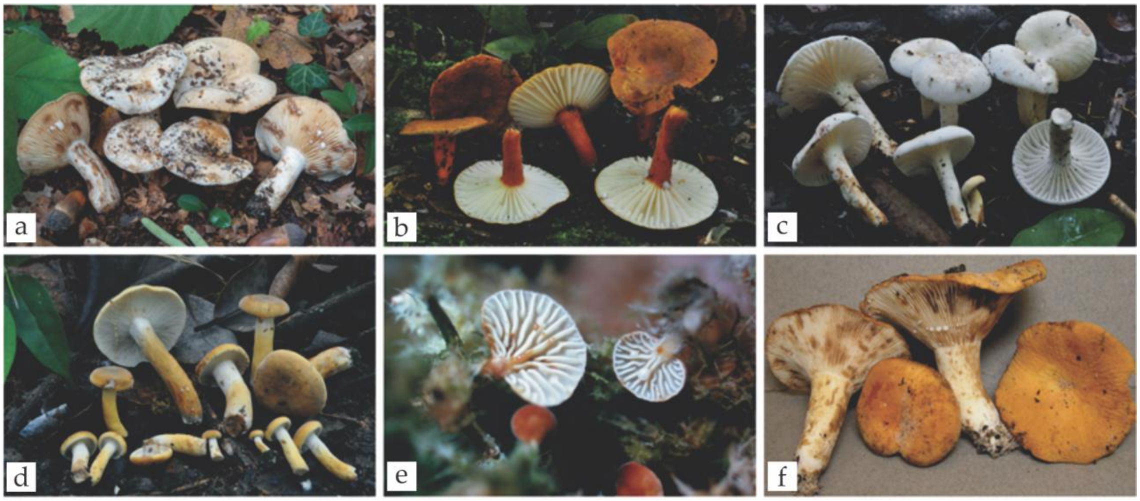
Lf.*subg. *Lactariopsis



Lf.*subg. *Pseudogymnocarpi

Fig. 2.4 Basidiocarps of representative species from the different subgenera and sections within the genus *Lactifluus*: ***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. *Russulopsidei*: *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.); ***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.* sp. (EDC 11-159, De Crop E.); **k.** Clade 8: *Lf.* sp. nov. (EDC 14-501, De Crop E.); **l.** *Lf.* sect. *Rubroviolascentini*: *Lf.* aff. *rubroviolascens* (EDC 12-051, De Crop E.).

_Lf._subg. _Gymnocarpi_



_Lf._subg. _Lactifluus_

Fig. 2.5 Basidiocarps of representative species from the different subgenera and sections within the genus *Lactifluus*: ***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.* sp. nov. (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.); ***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.).

I. *Lactifluus* subg. *Lactariopsis* (Fig. 2.3, 2.4a–f, 2.6) 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 pleuromacrocystidia or pleuroleptocystidia are present in some, while pleurolamprocystidia were never observed. This is the only clade in which species with secondary 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* (Beeli) Buyck) and broad (up to 25 µm diameter in *Lf. zenkeri* (Henn.) Verbeken).
- Clade 1 contains two African species: *Lf. madagascariensis* (Verbeken & Buyck) Buyck and *Lf. emergens* (Verbeken) Verbeken. 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.
- *Lf. acrissimus* (Verbeken & Van Rooij) Nuytinck, 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 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* E. S. Popov & O. V. Morozova, which was already mentioned to belong to *Lf.* subg. *Lactariopsis* in the study of Morozova et al. (2013), and an undescribed agaricoid specimen. 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* (Verbeken & C. Sharp) Verbeken) and thick (up to 18 µm diameter in *Lf. brachystegiae*), a cutis to trichopalisade as pileipellis and no secondary velum or true cystidia.
- *Lf. cocosmus* (Van de Putte & De Kesel) Van de Putte 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* (Verbeken & Van Rooij) De Crop 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* (Verbeken & Karhula) Verbeken 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.
- *Lf.* 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* (Verbeken) Verbeken, 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* (Verbeken) Verbeken, 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 stipitipellis composed of thick-walled hairs even up to 400 μm in *Lf. vellereus* (Fr.) Kuntze, and slightly to clearly moniliform pleuromacrocystidia.

Fig. 2.6 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 following abbreviations: C = cutis, H = hymeniderm, T = trichoderm, P = palisade, Tp = trichopalisade, i = ixo-, l = lampro-, ir = irregular, m = mixed, (+l) = 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 = pleuromacrocystidia present, LE = pleuroleptocystidia present. For all characteristics, blanks indicate unknown character states.

II. Species of ***Lactifluus* subg. *Pseudogymnocarpi*** (Fig. 2.3, 2.4g–l, 2.7) 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 pleurocystidia are absent, while others have pleurolamprocystidia or pleuromacrocystidia. Some species show striking colour reactions of the latex, but most species do not. The subgenus consists of five well-supported clades and one isolated species:

- *Lf.* 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.
- *Lf.* 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 one collection (EDC 14-501) which has pleuromacrocystidia.
- *Lf.* sect. *Aurantiifolii* contains the single, isolated species *Lf. aurantiifolius*. As noted by Verbeken & Walleyn (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.
- *Lf.* sect. *Rubroviolascentini* 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* (Verbeken & Walleyn) Verbeken even has both pleurolampro- and pleuroleptocystidia.
- *Lf.* 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.

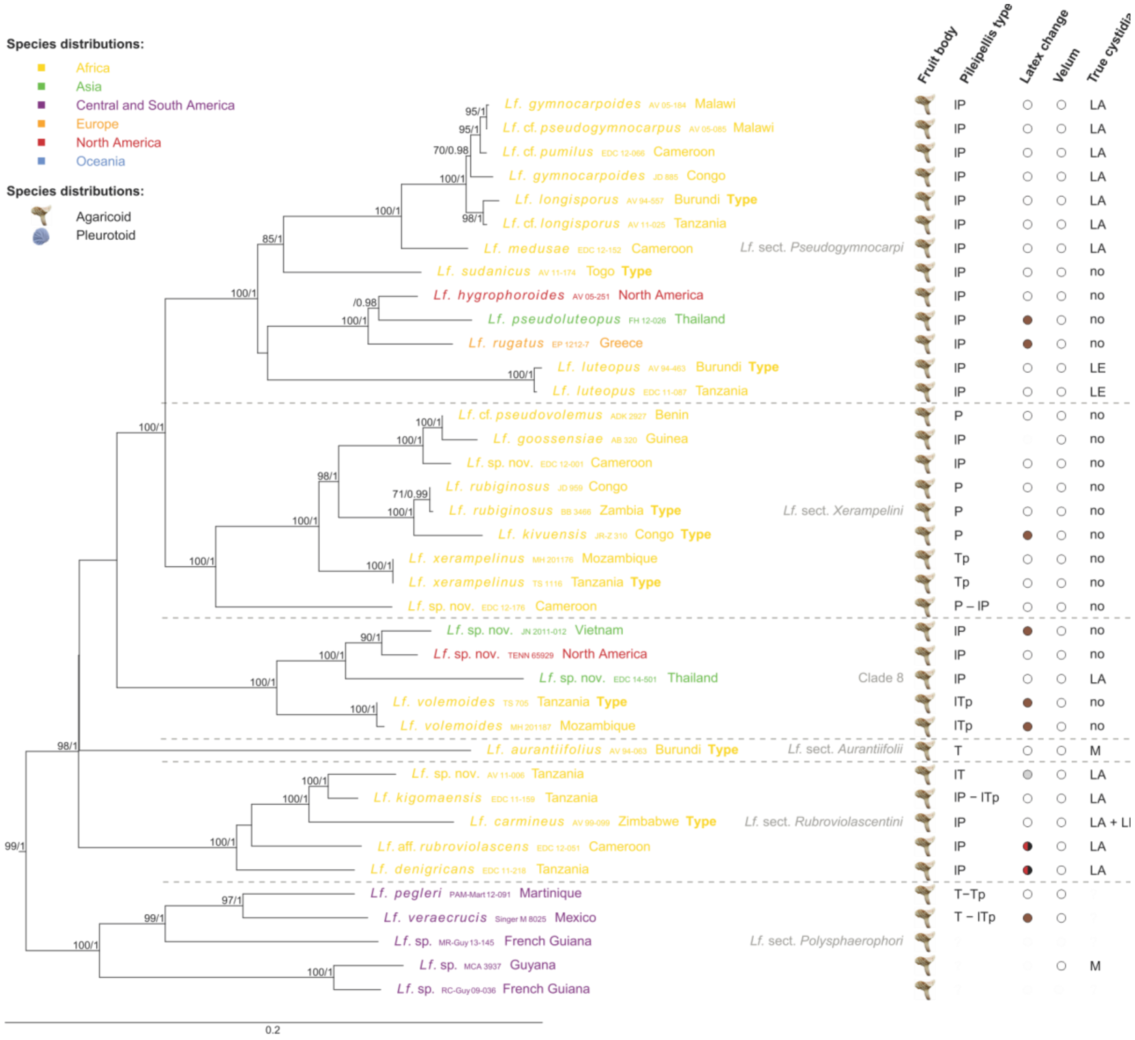


Fig. 2.7 Maximum Likelihood tree of *Lactifluus* subg. *Pseudogymnocarpi*, based on concatenated ITS, LSU, *RPB2* and *RPB1sequence* data. Maximum Likelihood bootstrap values >70 and Bayesian Inference posterior probabilities >0.95 are shown. Tip labels are coloured according to species' distributions, 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 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 = pleuromacrocystidia present, LE = pleuroleptocystidia present, LA = pleurolamprocystidia present. For all characteristics, blanks indicate unknown character states.

III. *Lactifluus* subg. *Gymnocarpi* (Fig. 2.3, 2.5a–f, 2.8) can be recognised by a combination of a lampropalisade as pileipellis, the absence of true pleurolamprocystidia (with discrete pleuromacrocystidia 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 and Walleyn (2010) already suggested that species with capitate terminal pileipellis elements might form a natural group. *Lactifluus brunneoviolascens* (Bon) Verbeken, the European representative, is often confused with the similar North American *Lf. luteolus* (Peck) Verbeken. 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.
- *Lf.* 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.
- *Lf. foetens* (Verbeken & Van Rooij) Verbeken is isolated on a branch sister to the preceding two sections. Macroscopically, it resembles the undescribed species *Lf.* sp. (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.
- *Lf.* 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* (Singer) De Crop, *Lf. panuoides* (Singer) De Crop and two undescribed species (G3185 and RC/Guad 08-042). Both *Lf. panuoides* and *Lf. brunellus* have a pleurotoid habitat, the other two specimens are agaricoid. The Oceanian species grouped 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* Cleland & Cheel, originally placed in *Russula* (Cleland & Cheel 1919). Singer (1942) noted that it could be *Lactarius clarkeae* Cleland and Lebel et al. (2013) also indicated that it belongs to *Lactifluus*. In our analyses it is sister to *Lf. clarkeae* (Cleland) Verbeken and we will recombine this species in *Lactifluus*.

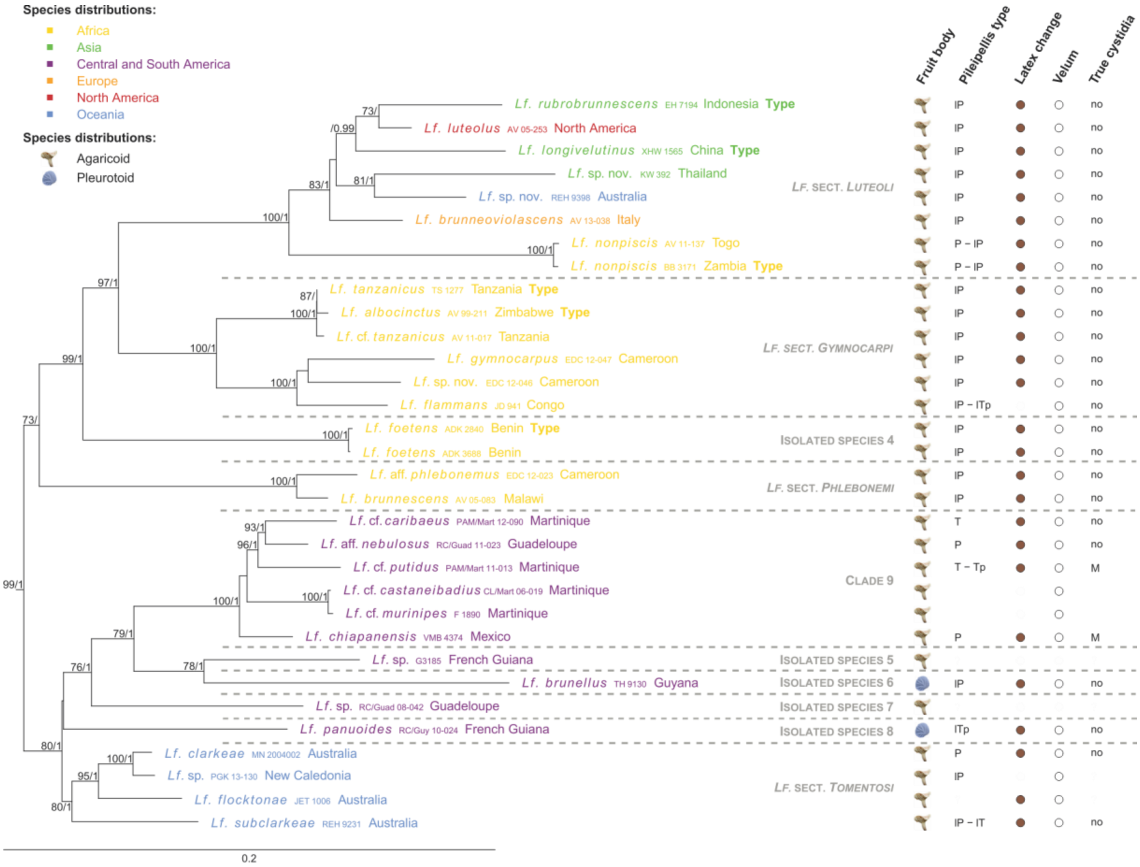


Fig. 2.8 Maximum Likelihood tree of *Lactifluus* subg. *Gymnocarpi*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values >70 and Bayesian Inference posterior probabilities >0.95 are shown. Tip labels are coloured according to species' distributions, see 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 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 = pleuromacrocystidia present. For all characteristics, blanks indicate unknown character states.

IV. *Lactifluus* subg. *Lactifluus* (Fig. 2.3, 2.5g–l, 2.9) is characterised by a range of pileipellis types, from a hyphoepithelium over a palisade to a lampropalisade. 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 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.

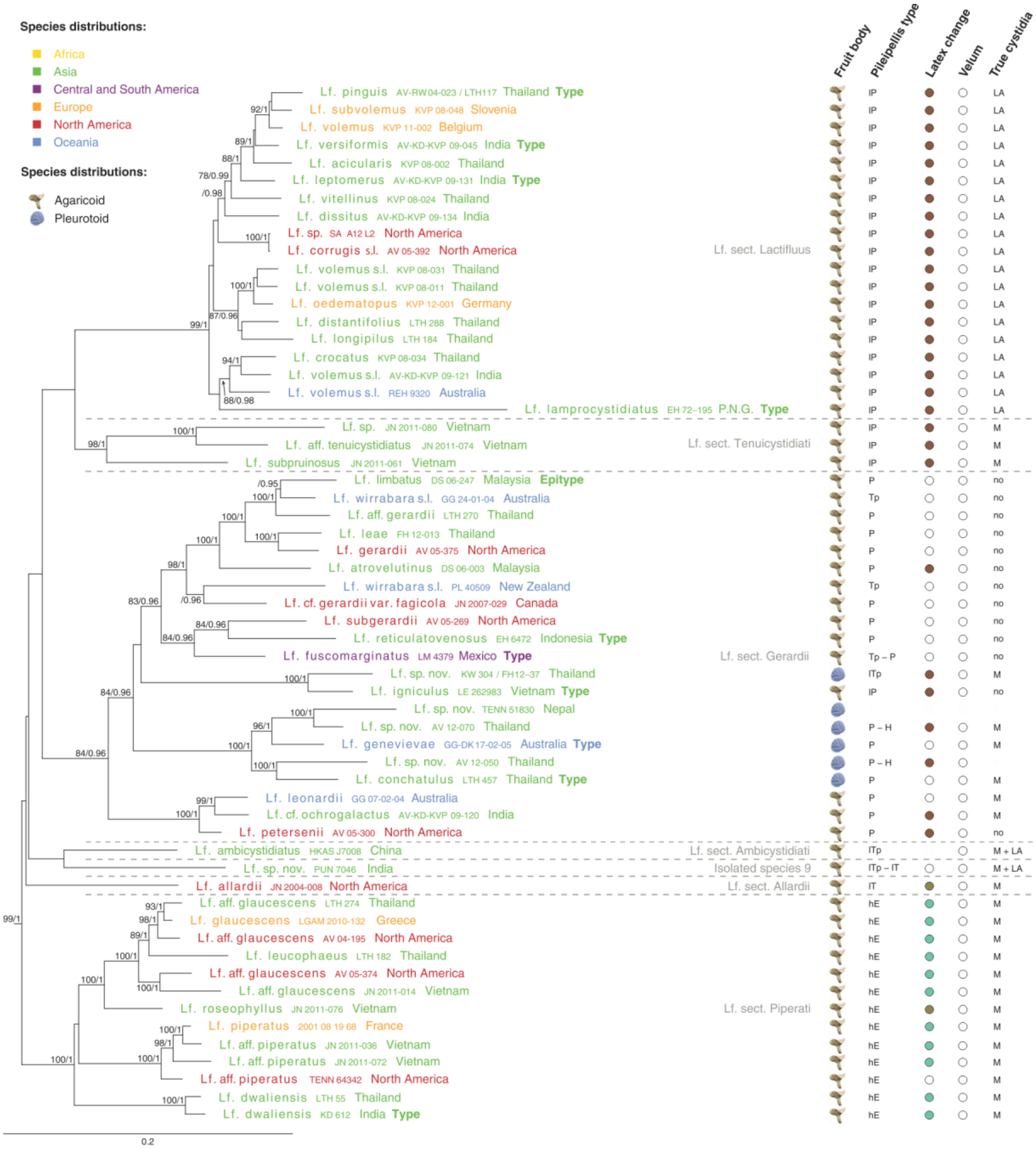


Fig. 2.9 Maximum Likelihood tree of *Lactifluus* subg. *Lactifluus*, based on concatenated ITS, LSU, *RPB2* and *RPB1* sequence data. Maximum Likelihood bootstrap values >70 and Bayesian Inference posterior probabilities >0.95 are shown. Tip labels are coloured according to species' distributions, see 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 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.

Taxonomic Part

GENUS

Genus *Lactifluus* (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806
BASIONYM: *Agaricus* sect. *Lactifluus* Pers., Syn. meth. fung. : 429. 1801.
TYPE (automatic): *Agaricus lactifluus* L., Sp. Pl.: 1172. 1753. (= *Lactifluus volemus* (Fr. : Fr.) Kuntze)
= *Pleurogala* Redhead & Norvell, Mycotaxon 48: 377. 1993
  ≡ *Lactarius* sect. *Panuoidei* Singer, Kew Bull. 7: 301. 1952

SUBGENERA

***Lactifluus* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop, comb. nov.**
MYCOBANK: MB 814217
BASIONYM: *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeken, Mycotaxon 66: 374. 1998.
TYPE: *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946. (≡ *Lactifluus gymnocarpus* (R. Heim ex Singer) Verbeken)

***Lactifluus* subg. *Lactariopsis* (Henn.) Verbeken, 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* section *Edules* Verbeken, Belg. J. Bot. 132: 176. 2000 (1999).
  ≡ *Lactifluus* subg. *Edules* (Verbeken) Verbeken, Mycotaxon 118: 448. 2011.
= *Lactarius* subg. *Russulopsis* Verbeken, Mycotaxon 77: 439. 2001.
  ≡ *Lactifluus* subg. *Russulopsis* (Verbeken) Verbeken, Mycotaxon 118: 452. 2011.
TYPE: *Lactariopsis zenkeri* Henn., Bot. Jahrb. Syst. 30: 51. 1902 (1901). (≡ *Lactifluus zenkeri* (Henn.) Verbeken)

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

***Lactifluus* subg. *Pseudogymnocarpi* (Verbeken) De Crop, comb. nov.**
MYCOBANK: MB 814193
BASIONYM: *Lactarius* sect. *Pseudogymnocarpi* Verbeken, Mycotaxon 66: 376. 1998.
≡ *Lactifluus* sect. *Pseudogymnocarpi* (Verbeken) Verbeken, Mycotaxon 120: 447. 2012.
≡ *Lactarius* sect. *Rugati* Verbeken, 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* Verbeken, Mycotaxon 55: 530. 1995 (≡ *Lactifluus gymnocarpoides* (Verbeken) Verbeken)

SECTIONS

Within *Lactifluus* subg. *Gymnocarpi:*

***Lactifluus* sect. *Luteoli* (Pacioni & Lalli) Verbeken, comb. nov.**
MYCOBANK: MB 814194
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) Verbeken)

***Lactifluus* sect. *Gymnocarpi* (R. Heim ex Verbeken) De Crop, comb. nov.**
MYCOBANK: MB 814195
BASIONYM: *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeken, Mycotaxon 66: 374. 1998.
TYPE: *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946. (≡ *Lactifluus gymnocarpus* (R. Heim ex Singer) Verbeken)

***Lactifluus* sect. *Phlebonemi* (R. Heim ex Verbeken) Verbeken, Mycotaxon 120: 446. 2012.**
BASIONYM: *Lactarius* sect. *Phlebonemi* R. Heim ex Verbeken, Mycotaxon 66: 378. 1998.
TYPE: *Lactarius phlebonemus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 38. 1955. (≡ *Lactifluus phlebonemus* (R. Heim & Gooss.-Font.) Verbeken)

***Lactifluus* sect. *Tomentosi* (McNabb) Verbeken, Mycotaxon 120: 448. 2012.**
BASIONYM: *Lactarius* sect. *Tomentosi* McNabb, New Zealand J. Bot. 9: 59. 1971.
TYPE: *Lactarius clarkeae* Cleland, Trans. & Proc. Roy. Soc. S. Australia 51: 302. 1927 (as *clarkei*). (≡ *Lactifluus clarkeae* (Cleland) Verbeken)

Within *Lactifluus* subg. *Lactariopsis:*

***Lactifluus* sect. *Albati* (Bataille) Verbeken, 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* (Verbeken) Verbeken, comb. nov.**
MYCOBANK: MB 814197
BASIONYM: *Lactarius* sect. *Edules* Verbeken, Belg. J. Bot. 132: 176. 2000 (1999).
TYPE: *Lactarius edulis* Verbeken & Buyck, Champ. Comest. Ouest Burundi: 103. 1994. (≡ *Lactifluus edulis* (Verbeken & Buyck) Buyck)

***Lactifluus* sect. *Lactariopsis* Verbeken, Mycotaxon 118: 450. 2011.**
≡ *Lactarius* sect. *Lactariopsis* (Henn.) Singer. 1942
≡ *Lactarius* sect. *Lactariopsidei* Singer. 1962
≡ *Lactarius* sect. *Chamaeleontini* Verbeken, Mycotaxon 66: 393. 1998.
TYPE: *Lactariopsis zenkeri* Henn., Bot. Jahrb. Syst. 30: 51. 1902 (1901). (≡ *Lactifluus zenkeri* (Henn.) Verbeken)

***Lactifluus* sect. *Russulopsidei* (Verbeken) Verbeken, Mycotaxon 118: 452. 2011.**
BASIONYM: *Lactarius* sect. *Russulopsidei* Verbeken, Mycotaxon 77: 440. 2001.
TYPE: *Lactarius ruvubuensis* Verbeken, Bull. Jard. Bot. Belg. 65: 208. 1996. (≡ *Lactifluus ruvubuensis* (Verbeken) Verbeken)

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: MB 814198
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. 'geradii'*). (≡ *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. Species *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, Wang et al. (2015), 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. 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. *Rubroviolascentini* (Singer) Verbeken, Mycotaxon 120: 447. 2012.**
BASIONYM: *Lactarius* subsect. *Rubroviolascentini* Singer, Ann. Mycol. 40: 114. 1942.
≡ *Lactarius* sect. *Rubroviolascentini* (Singer) Verbeken, Mycotaxon 66: 380. 1998, as *Rubroviolascentes*.
TYPE: *Lactarius rubroviolascens* R. Heim, Candollea 7: 377. 1938. (≡ *Lactifluus rubroviolascens* (R. Heim) Verbeken)

***Lactifluus* sect. *Xerampelini* De Crop, sect. nov.**
MYCOBANK: MB 814199
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 lampropalisade to palisade or trichopalisade.
TYPE: *Lactarius xerampelinus* Karhula & Verbeken, Karstenia 38 (2): 59. 1998. (≡ *Lactifluus xerampelinus* (Karhula & Verbeken) Verbeken)

NEW COMBINATIONS AT SPECIES LEVEL

Lactifluus acrissimus (Verbeken & Van Rooij) Nuytinck, comb. nov.
MYCOBANK: MB 814200
BASIONYM: *Lactarius acrissimus* Verbeken & Van Rooij, Nova Hedwigia 77: 225. 2003.

Lactifluus brunellus (S.L. Miller, Aime & TW Henkel) De Crop, comb. nov.
MYCOBANK: MB 814201
BASIONYM: *Lactarius brunellus* S.L. Miller, Aime & TW Henkel, Mycologia 94(3): 546. 2002.

Lactifluus castaneibadius (Pegler) De Crop, comb. nov.
MYCOBANK: MB 814202
BASIONYM: *Lactarius castaneibadius* Pegler, Kew Bulletin 33 (4): 622. 1979.

Lactifluus chiapanensis (Montoya, Bandala-Muñoz & Guzmán) De Crop, comb. nov.
MYCOBANK: MB 814203
BASIONYM: *Lactarius chiapanensis* Montoya, Band.-Muñoz & Guzmán, Mycotaxon 57: 412. 1996.

Lactifluus flocktonae (Cleland & Cheel) Lebel, comb. nov.
MYCOBANK: MB 814204
BASIONYM: *Russula flocktonae* Cleland & Cheel, Trans. Proc. Roy. Soc. South Australia 43: 274–275. 1919.

Lactifluus multiceps (S.L. Miller, Aime & TW Henkel) De Crop, comb. nov.
MYCOBANK: MB 814205
BASIONYM: *Lactarius multiceps* S.L. Miller, Aime & TW Henkel, Mycologia 94(3): 549. 2002.

Lactifluus murinipes (Pegler) De Crop, comb. nov.
MYCOBANK: MB 814206
BASIONYM: *Lactarius murinipes* Pegler, Kew Bulletin 33 (4): 623. 1979.

Lactifluus nebulosus (Pegler) De Crop, comb. nov.
MYCOBANK: MB 814207
BASIONYM: *Lactarius nebulosus* Pegler, Kew Bull. 33: 610. 1979.

Lactifluus panuoides (Singer) De Crop, comb. nov.
MYCOBANK: MB 814208
BASIONYM: *Lactarius panuoides* Singer, Kew Bull. 7: 300. 1952.

Lactifluus rufomarginatus (Verbeken & Van Rooij) De Crop, comb. nov.
MYCOBANK: MB 814209
BASIONYM: *Lactarius rufomarginatus* Verbeken & Van Rooij, Nova Hedwigia 77 (1): 235. 2003.

Lactifluus uapacae (Verbeken & Stubbe) De Crop, comb. nov.
MYCOBANK: MB 814210
BASIONYM: *Lactarius uapacae* Verbeken and Stubbe, Cryptogamie, Mycologie 29 (2): 140. 2008.

Lactifluus venezuelanus (Dennis) De Crop, comb. nov.
MYCOBANK: MB 814211
BASIONYM: *Lactarius venezuelanus* Dennis, Kew Bulletin Additional Series 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*, 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. *Albati* 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. *Albati*, *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. *Rubroviolascentini, Lf.* sect. *Tomentosi*). 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 demarked 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* (Fr.) Bres., *Albatrellus skamanius* (Murrill) Pouzar and *Gloeocystidiellum porosum* (Berk. & M.A. Curtis) Donk. 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 pileipellis types, 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. 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).

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, Buyck et al. 2008, Verbeken & Walleyn 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*.

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. *Rubroviolascentini* lack these striking colour changes.

The fourth characteristic is the **pileipellis type**. Several studies (Bon 1983, Heilmann-Clausen et al. 1998, Verbeken 1998a, Verbeken & Walleyn 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 lampropalisade, which makes it difficult to use the feature within the subgenus.

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 & Walleyn 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, De Crop et al. 2012, Van de Putte 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. 2.5e) on stems of *Dicymbe corymbosa* Spruce ex Benth. (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 mainly generalists and less 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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CHAPTER 3

Out of Africa: evolutionary history and global biogeography of the diverse ectomycorrhizal milkcap genus *Lactifluus* (Russulaceae)

Abstract

Compared with other groups of macro-organisms, the evolutionary histories of most groups of fungi are still largely unknown. Many ectomycorrhizal fungi display disjunct distribution patterns that might be explained by vicariance or long-distance dispersal events. The ectomycorrhizal milkcap genus *Lactifluus* (Russulaceae) displays such disjunct distributions and is characterised by many evolutionary divergent lineages in sub-Saharan Africa. In this study, we aim to reconstruct the evolutionary history of the genus *Lactifluus* and test whether it has originated in the Afrotropics. We carried out an extensive global sampling and assembled a dataset of 1306 *Lactifluus* collections. Species delimitation was performed using the GMYC method in R. Divergence times were estimated in BEAST, using a secondary calibration procedure on a dataset containing species from several Basidiomycota orders. Biogeographical ranges were inferred using BioGeoBEARS in R. Species delimitation resulted in 369–461 possible *Lactifluus* species, of which the majority are Asian and African species. Our dating analysis estimated the origin of the Russulaceae in the early Cretaceous and its major genera, *Lactifluus*, *Lactarius* and *Russula*, originated near the mid-Cenozoic. Biogeographical analyses indicated an Afrotropical origin for *Lactifluus* to be most likely, with multiple on-land migrations and long-distance dispersal events to other continents.

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Introduction

Genus-wide studies on the evolutionary history of fungi are still rather scarce due to several factors. Generally, only a small fraction of the actual fungal diversity is known, what seriously narrows our perspective and makes it difficult to reconstruct evolutionary histories. In many groups, tropical regions are under-sampled and tropical species are thus less represented in global phylogenies (Matheny et al. 2009; Tedersoo et al. 2010; Tedersoo et al. 2011; Sanchez-Ramirez et al. 2015). Furthermore, fungal taxonomy is often challenging, with the occurrence of cryptic species and species complexes with low morphological divergence, which implicates difficulties in delimiting species and assessing species richness (Taylor et al. 2000; Taylor et al. 2006). Finally, fossil records of fungi are scarce, due to the ephemeral nature of fruiting bodies, and the existing fossil records are often hard to interpret (Matheny et al. 2009; Berbee and Taylor 2010; Skrede et al. 2011).

Among the mushroom-forming fungi, studies focusing on diversification or dispersal strategies of ectomycorrhizal (ECM) fungi are increasing, mainly due to the ecological importance of ECM fungi as root-associated symbionts of many plant species (Geml et al. 2006; Geml et al. 2008; Halling et al. 2008; Matheny et al. 2009; Tedersoo et al. 2011; Geml et al. 2012; Tedersoo et al. 2012; Wilson et al. 2012; Cai et al. 2014; Tedersoo et al. 2014; Harrower et al. 2015; Sanchez-Ramirez et al. 2015; Garnica et al. 2016; Looney et al. 2016). Due to this close association with their hosts, various studies suggest that diversification of ECM fungi might depend on these host associations (den Bakker et al. 2004; Rochet et al. 2011; Wilson et al. 2012; Harrower et al. 2015). Many ECM lineages display disjunct distributions, which are explained by either vicariance or long-distance dispersal events. The boreotropical hypothesis, originally proposed for explaining plant distributions (Wolfe 1975; Lavin and Luckow 1993), states that certain disjunct ECM distributions may have originated in Palaeotropical, mixed mesophytic forests that were dominant in the northern hemisphere during the Palaeocene and Eocene (Wilson et al. 2012). During the Oligocene, continents moved further away from each other, disrupting dispersal routes via intercontinental land bridges. The boreotropical hypothesis can thus explain disjunct distributions of ECM lineages that originated before this vicariance event. This was suggested to be the case for some Inocybaceae lineages (Matheny et al. 2009), some Sclerodermatineae clades (Wilson et al. 2012) and for *Amanita* sect. *Caesareae* Singer (Sanchez-Ramirez et al. 2015). Disjunct distributions of more recently originated lineages can be explained by long-distance dispersal, which is accompanied by a change of ectomycorrhizal host for ECM fungi. Long-distance dispersal has been suggested to be important for certain lineages within the Serpulaceae (Skrede et al. 2011), *Calostoma* Desv. (Wilson et al. 2012) and *Cortinarius* sect. *Cortinarius* (Pers.) Gray (Harrower et al. 2015).

The ectomycorrhizal milkcap genus *Lactifluus* (Pers.) Roussel is one of the four mainly agaricoid genera within the Russulaceae (Russulales, Basidiomycota). The genus contains approximately 150 described species and was divided into four subgenera by De Crop et al. (acpt., chapter 2). The genus is known from all continents except Antarctica and is most species-rich in the Afrotropics, tropical Asia and the Neotropics (Verbeken and Nuytinck 2013; De Crop et al. acpt.). This is in contrast with the other milkcap genus *Lactarius* Pers., which has a more temperate distribution. The four subgenera of *Lactifluus* differ in geographical patterns and often display disjunct distributions. *Lactifluus* subg. *Lactariopsis* (Henn.) Verbeken, *Lf.* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop and *Lf.* subg. *Pseudogymnocarpi* (Verbeken) De Crop mainly contain tropical species and one or two temperate lineages; while *Lactifluus* subg. *Lactifluus* has its main distribution in the northern hemisphere, with only some Australasian species as representatives of the Southern hemisphere (chapter 2, De Crop et al. acpt.). *Lactifluus* species are found in a wide variety of vegetations in temperate, subtropical and tropical regions, such as rain forests, dry forests, monsoon forests, tree savannahs, Mediterranean vegetations, broadleaf forests, coniferous forests and montane forests. *Lactifluus* host trees include leguminous trees (Fabaceae), members of the Dipterocarpaceae and the Fagaceae, and of the genera *Uapaca* Baill. (Phyllanthaceae), *Eucalyptus* L'Hér and *Leptospermum* J.R. Forster & G. Forster (Myrtaceae).

Phylogenetic relationships within the genus *Lactifluus* are largely resolved (De Crop et al. acpt.), however, the evolutionary history of the genus is unknown. Disjunct species distributions put forward the question whether these distributions are caused by vicariance, on-land migration or long-distance dispersal events and whereas the high species diversity in sub-Saharan Africa might be in favour of the hypothesis of an Afrotropical origin for *Lactifluus*. With this study we aim to (1) estimate the date of origin of the genus *Lactifluus*, (2) reconstruct the biogeographical history of *Lactifluus* and test the possibility of an Afrotropical origin, and (3) test whether current distributions of *Lactifluus* species can be explained by vicariance, on-land migrations or long-distance dispersal events.

Material and methods

Sampling

Our aim was to include as many *Lactifluus* lineages as possible into this study. We started from the dataset of De Crop et al. (acpt., chapter 2) and included all *Lactifluus* collections with ITS sequences available in the database of Ghent University, together with all non-environmental GenBank sequences of *Lactifluus* available at the time (21/01/2016). Due to the recent nomenclatural changes within the milkcaps, *Lactifluus* species on GenBank rarely have the genus name "*Lactifluus*". Therefore, we also considered *Lactarius*, *Russula* and *Multifurca* sequences, aligned the sequences using the online version of the multiple sequence alignment program Mafft v.7 (Katoh and Toh 2008), conducted Maximum Likelihood (ML) analyses using RAxML v.8.0.24 (Stamatakis 2014) and only retained those sequences that clustered within the genus *Lactifluus* (Table S1). Analyses were carried out on the CIPRES Science Gateway (Miller et al. 2010).

DNA extraction, PCR amplification, sequencing and nucleotide alignments

Collections that were not yet deposited in GenBank consisted out of three types: dried collections, fresh collections stored on CTAB buffer or culture collections of the corticoid Russulaceae specimens on a sterilized 2% malt-agar medium (2% malt extract, 1.58% g agar, 0.0005% chloramphenicol) medium. Dry collections were extracted using the protocol described by Nuytinck & Verbeken (2003), with modifications described by Van de Putte et al. (2010). Fresh collections stored on CTAB buffer and culture collections were extracted using the CTAB extraction protocol described by Nuytinck & Verbeken (2003).

PCR amplification was done using the protocol by Le et al. (2007). Four genes were sequenced: (1) the internal transcribed spacer region of ribosomal DNA (ITS), using primers ITS-1F/ITS5 and ITS4 (White et al. 1990; Gardes and Bruns 1993) and 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 and Hall 1997; Matheny et al. 2002), together with internal primers RPB1-F3 and RPB1-R4 (De Crop et al. acpt.). 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 SequencherTM v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). Sequences were aligned using MAFFT v7.187 (Katoh and Toh 2010; Katoh and Standley 2013) on the CIPRES Science Gateway (Miller et al. 2010), using the accurate E-INS-I strategy. Trailing ends of the alignment were trimmed and sequences were manually edited when necessary in Mega 6 (Tamura et al. 2013). Partitionfinder v. 1.1.1 (Lanfear et al. 2012) was used to determine partition schemes for each gene region and nucleotide substitution models that best fits each partition, using the Bayesian information criterion.

Three different datasets were constructed for the analyses. Dataset 1 contains non-environmental ITS sequences, with multiple sequences per species. This dataset is used for species delimitation within *Lactifluus*. Dataset 2 contains three loci (LSU, *rpb2* and *rpb1*) of 95 species of the Basidiomycota that are used for fossil calibration. Dataset 3 is based on dataset 1, as it contains one sequence per species recovered after species delimitation, together with representatives of the genera *Lactarius*, *Multifurca* and *Russula* and an outgroup of seven other Russulales species. Dataset 3 contains four loci (ITS, LSU, *rpb2* and *rpb1*) and is used for the biogeographical and diversification analyses. Alignments can be acquired from the first author and TreeBASE (to be submitted).

Species delimitation

Dataset 1 was used to delimit species within *Lactifluus*. This dataset contains sequence data of a single marker (ITS) for all *Lactifluus* sequences generated by our research groups and for all *Lactifluus* species available at GenBank (excluding environmental samples). As morphological data are lacking and only ITS is available for the majority of specimens, we will delimit species exclusively on the ITS sequence data. We applied the Generalized Mixed Yule Coalescent (GMYC) method, a likelihood method for delimiting species by fitting within- and between-species branching models to reconstructed gene trees (Pons et al. 2006; Fujisawa and Barraclough 2013). Fujisawa and Barraclough (2013) proved that this method is robust as a tool for delimiting species when only single-locus data is available and it is tolerant to a moderate amount of identical sequences and singletons.

An ultrametric tree was constructed using BEAST v.2.3.0 (Bouckaert et al. 2014). The GTR+G model was used as substitution model, we chose a strict clock as clock model, since this dataset contains a lot of intra-species data (Drummond and Bouckaert 2015), and the Yule prior was chosen as tree prior. All other priors were set to default. Five independent Markov chain Monte Carlo (MCMC) chains were run for 50.000.000 generations each, sampling every 5.000th state. Convergence was verified in Tracer v1.6 (Rambaut et al. 2014). A burn-in of 10% of the trees was discarded per run. Runs were combined and resampled every 20.000th state using LogCombiner v1.8.2 (Drummond et al. 2012). A maximum clade credibility tree (MCC) was produced using TreeAnnotator v1.8.2 (Drummond et al. 2012). The GMYC analysis was performed in R (R Core Team 2016) using the 'Splits' package, under a single threshold.

Calibration and estimation of divergence times

In order to estimate the divergence times in the Russulaceae, and more specifically in *Lactifluus*, we used the secondary calibration procedure (Renner 2005; Matheny et al. 2009; Ryberg and Matheny 2011; Skrede et al. 2011; Wilson et al. 2012; Sanchez-Ramirez et al. 2015). For the **first step** of this procedure we worked with dataset 2. This dataset contains species from several Basidiomycota orders: Russulales (with a focus on *Lactifluus*), Hymenochaetales, Boletales, Agaricales, Atheliales, Polyporales, Gloeophallales, Telephorales, Corticiales and as outgroup *Gautieria otthii* Trog from the Gomphales order (Table 3.1). We calibrated this dataset based on three agaricomycete fossils: *Archaeomarasmius leggetti* Hibbett, D. Grimaldi & Donoghue (Hibbett et al. 1997), *Quatsinoporites cranhamii* S.Y. Sm., Currah & Stockey (Smith et al. 2004) and a permineralized suilloid ectomycorrhizal fossil (LePage et al. 1997). The first fossil consists of an agaricoid fruiting body, embedded in New Jersey amber from the mid-Cretaceous (90–94 My ago). The fossil resembles the extant genera *Marasmius*, *Marasmiellus*, *Mycena*, *Collybia* and other Tricholomataceae, and was used to calibrate the Agaricales in our analysis. The second fossil consists of a fragment of a poroid fruiting body from the lower Cretaceous (129.4–125 My ago), found in British Colombia. The fossil resembles extant genera of the Hymenochaetales and was therefore used to calibrate this order. The third fossil consists of a permineralized suilloid ectomycorrhiza fossil from the middle Eocene (50 My ago) found in the Princeton chert of British Columbia associated with Pinaceae roots and was used to calibrate the Suillineae.

For the **second step**, we used dataset 3 (Table S2), which contains one sequence per *Lactifluus* species, and calibrated this dataset based on the nodes of Russulaceae and *Lactifluus*, as estimated in the first step of this secondary calibration procedure.

Divergence time estimates for dataset 2 were estimated using BEAST v2.3.0 (Bouckaert et al. 2014). XML files were generated using BEAUti v2.3.0, by importing the gene partition NEXUS files separately. Partitions were unlinked for substitution models and linked for molecular-clock models and gene trees. A lognormal relaxed clock was used, with an estimated clock rate. We chose the Yule model as tree prior, as this dataset contains one specimen for each species. We chose gamma distributions for the fossil calibrations priors. Five independent MCMC chains were run for a total of 50 million generations, with a sampling frequency of 5000. Convergence was verified in Tracer v1.6 (Rambaut et al. 2014). A burn-in of 10% of the trees was discarded per run and runs were combined using LogCombiner v1.8.2 (Drummond et al. 2012). A maximum clade credibility tree (MCC) was produced using TreeAnnotator v1.8.2 (Drummond et al. 2012).

Divergence time estimates for dataset 3 were estimated using BEAST v1.8.2 (Drummond et al. 2012). XML files were generated using BEAUti v1.8.2, by importing the gene partition NEXUS files separately, together with a starting tree constructed using RAxML v.8.0.24 (Stamatakis 2014). Partitions were linked for substitution models, molecular-clock models and gene trees. We used a lognormal relaxed clock, with an estimated clock rate, and the Yule model as tree prior, as this dataset also contains one specimen for each species. The nodes for *Lactifluus, Lactarius, Multifurca* and *Russula* were calibrated using a normal distribution, in correspondence with the distributions estimated in the first step of this procedure. The mean and standard deviation of these distributions were set to approximate the age and 95% highest posterior densities of these nodes, as estimated in the first step of this procedure. One MCMC chain was run for a total of 50 million generations, with a sampling frequency of 1000. Convergence was verified in Tracer v1.6 (Rambaut et al. 2014) and a burn-in of 10% of the trees was discarded. A maximum clade credibility tree (MCC) was produced using TreeAnnotator v1.8.2 (Drummond et al. 2012). All analyses were carried out on the CIPRES Science Gateway (Miller et al. 2010).

Table 3.1 Specimens and GenBank accession numbers of LSU, *RPB1* and *RPB2* sequences used for estimating divergence times of the Russulaceae and more specifically the genus *Lactifluus*.

Order/Family	Genus	Species	Herbarium no.	LSU	*RPB2*	*RPB1*
Agaricales						
Agaricaceae	*Coprinus*	*comatus*	AFTOL 626	AY635772	AY780934	AY857983
Cortinariaceae	*Cortinarius*	*iodes*	AFTOL 285	AY702013	AY536285	AY857984
Inocybaceae	*Inocybe*	*myriadophylla*	AFTOL 482	AY700196	AY803751	DQ447916
Marasmiaceae	*Marasmius*	*rotula*	AFTOL 1505	DQ457686	DQ474118	DQ447922
Marasmiaceae	*Megacollybia*	*platyphylla*	AFTOL 560	AY635778	DQ385887	DQ447923
Marasmiaceae	*Mycetinis*	*alliaceus*	AFTOL 556	AY635776	AY786060	AY860525
Mycenaceae	*Mycena*	*aurantiidisca*	AFTOL 1685	DQ470811	DQ474122	DQ447927
Mycenaceae	*Mycena*	*galericulata*	AFTOL 727	AY647216	DQ385888	GU187491
Physalacriaceae	*Xerula*	*radicata*	AFTOL 561	AY645051	AY786067	DQ447946
Atheliales						
Atheliaceae	*Fibulorhizoctonia*	sp.	AFTOL 576	AY635779	AY885161	AY857985
Boletales						
Boletaceae	*Aureoboletus*	*projectellus*	AFTOL 713	AY684158	AY787218	AY788850
Boletaceae	*Boletus*	*edulis*	Be3	KF030282	GU187774	GU187444
Boletaceae	*Strobilomyces*	*floccopus*	AFTOL 716	AY684155	AY786065	AY858963
Gomphidiaceae	*Gomphidius*	*roseus*	AFTOL 1780	DQ534669	GU187818	GU187459
Hygrophoropsidaceae	*Leucogyrophana*	*lichenicola*	DAOM 194172	GU187583	GU187789	GU187467
Sclerodermataceae	*Calostoma*	*cinnabarinum*	AFTOL 439	AY645054	AY780939	AY857979
Serpulaceae	*Serpula*	*himantioides*	AFTOL 1387	AF518648	DQ366283	None
Serpulaceae	*Serpula*	*lacrymans*	REG 383	GU187596	GU187809	GU187485
Suillaceae	*Suillus*	*bresadolae*	REG_394	GU187598	GU187810	GU187482
Suillaceae	*Suillus*	*pictus*	AFTOL 717	AY684154	AY786066	AY858965
Tapinellaceae	*Pseudomerulius*	*curtisii*	REH8912	GU187589	GU187796	GU187472
Tapinellaceae	*Tapinella*	*atrotomentosa*	78/97	GU187603	GU187813	GU187488
Corticiales						
Punctulariaceae	*Punctularia*	*strigosozonata*	AFTOL 1248	AF518642	DQ381843	DQ831031
Gloeophyllales						
Gloeophyllaceae	*Gloeophyllum*	*striatum*	AN027866	HM536063	HM640259	None
Gomphales – outgroup						
Gomphaceae	*Gautieria*	*otthii*	AFTOL 466	AF336249	AY218486	AY864864
Hymenochaetales						
Incertae sedis	*Peniophorella*	*praetermissa*	AFTOL 518	AY707094	AY787221	AY864871

Order/Family	**Genus**	**Species**	**Herbarium no.**	**LSU**	***RPB2***	***RPB1***
Hymenochaetaceae	*Coltricia*	*perennis*	AFTOL 447	None	AY218526	AY864867
Hymenochaetaceae	*Fomitiporia*	*mediterranea*	AFTOL 688	AY684157	AY803748	AY864869
Hymenochaetaceae	*Phellinus*	*hartigii*	MUCL 53551	JX093833	JX093877	None
Repetobasidiaceae	*Cotylidia*	sp.	AFTOL 700	AY629317	AY883422	AY864868
Polyporales						
Fomitopsidaceae	*Fomitopsis*	*pinicola*	AFTOL 770	AY684164	AY786056	AY864874
Meripilaceae	*Grifola*	*sordulenta*	AFTOL 562	AY645050	AY786058	AY864877
Meruliaceae	*Climacodon*	*septentrionalis*	AFTOL 767	AY684165	AY780941	AY864872
Phanerochaetaceae	*Antrodiella*	*americana*	HHB-4100-Sp	EU232270	None	KP134885
Polyporaceae	*Coriolopsis*	*trogii*	RLG4286sp	JN164808	JN164867	JN164820
Polyporaceae	*Polyporus*	*squamosus*	AFTOL 704	AY629320	DQ408120	DQ831023
Polyporaceae	*Spongipellis*	*pachyodon*	FD-314	KP135288	KP134971	KP134875
Polyporaceae	*Trametes*	*versicolor*	FP135156sp	JN164809	JN164850	JN164825
Russulales						
Amylostereaceae	*Amylostereum*	*laevigatum*	CBS 623.84	AF287843	AY218469	None
Bondarzewiaceae	*Bondarzewa*	sp.	Cui 10724	None	KJ651720	KJ651627
Bondarzewiaceae	*Bondarzewia*	*mesenterica*	MUCL 38908	ok	ok	None
Bondarzewiaceae	*Bondarzewia*	*montana*	AFTOL 452	DQ234539	AY218474	DQ256049
Bondarzewiaceae	*Heterobasidion*	*annosum*	AFTOL 470	None	AY544206	DQ667160
Bondarzewiaceae	*Heterobasidion*	*araucariae*	65008	KJ651520	KJ651729	KJ651636
Echinodontiaceae	*Echinodontium*	*tinctorium*	AFTOL 455	AF393056	AY218482	AY864882
Echinodontiaceae	*Laurilia*	*sulcata*	MUCL 40113	ok	ok	ok
Hericiaceae	*Hericium*	*americanum*	AFTOL 469	DQ411538	DQ408127	None
Hericiaceae	*Laxitextum*	*incrustatum*	MUCL 32548	ok	ok	ok
Lachnocladiaceae	*Vararia*	*calami*	MUCL 32404	ok	ok	None
Peniophoraceae	*Peniophora*	*molesta*	MUCL 32297	ok	None	None
Russulaceae	*Boidinia*	*furfuracea*	JS16717	AF506376	None	None
Russulaceae	*Boidinia*	*propinqua*	KHL10931	AF506379	None	None
Russulaceae	*Boidinia*	sp.	KHL10303	AF506378	None	None
Russulaceae	*Gloeocystidiellum*	*aculeatum*	Wu890714-52	AF506433	None	None
Russulaceae	*Gloeopeniophorella*	*aff. convolvens*	KHL10390	AF506436	None	None
Russulaceae	*Gloeopeniophorella*	*convolvens*	KHL10103	AF506435	None	None
Russulaceae	*Gloeopeniophorella*	*laxa*	Wu911010-8	AF506440	None	None
Russulaceae	*Lactarius*	*fuliginosus*	MTB 97-24	JQ446180	JQ446240	KR364392
Russulaceae	*Lactarius*	*hatsudake*	FH 12-052	KR364215	KR364285	KR364411
Russulaceae	*Lactarius*	*lignyotus*	AFTOL 681	AY631898	DQ408128	None
Russulaceae	*Lactarius*	*miniatescens*	AV 11-177	KR364187	KR364315	KR364443
Russulaceae	*Lactarius*	*olympianus*	ED 08-018	KR364220	KR364320	KR364448
Russulaceae	*Lactarius*	*scrobiculatus*	JN 2001-058	KR364219	KR364344	KR364474
Russulaceae	*Lactarius*	*tenellus*	ADK 3598	KF133313	KF133345	KR364482
Russulaceae	*Lactifluus*	*allardii*	JN 2004-008	KF220125	KF220217	KR364370
Russulaceae	*Lactifluus*	*bicolor*	DS 06-247	JN388987	JN375590	JN389186
Russulaceae	*Lactifluus*	*caribaeus*	PAM/Mart 12-090	KP691424	KP691432	KR364396
Russulaceae	*Lactifluus*	*clarkeae*	MN 2004002	HQ318205	KR364268	KR364379
Russulaceae	*Lactifluus*	*deceptivus*	AFTOL 682	AY631899	AY803749	AY864883
Russulaceae	*Lactifluus*	*denigricans*	EDC 11-218	KR364178	KR364272	KR364384
Russulaceae	*Lactifluus*	*edulis*	FN 05-628	KR364147	KR364275	KR364387

Order/Family	Genus	Species	Herbarium no.	LSU	*RPB2*	*RPB1*
Russulaceae	*Lactifluus*	*gymnocarpus*	EDC 12-047	KR364194	KR364282	KR364408
Russulaceae	*Lactifluus*	*heimii*	EDC 11-082	KR364167	KR364286	KR364412
Russulaceae	*Lactifluus*	*luteolus*	AV 05-253	KR364142	KJ210067	KR364440
Russulaceae	*Lactifluus*	*medusae*	EDC 12-152	KR364198	KR364314	KR364442
Russulaceae	*Lactifluus*	*pegleri*	PAM/Mart 12-091	KP691425	KP691433	KR364397
Russulaceae	*Lactifluus*	*piperatus*	2001 08 19 68	KF241840	KF241842	KR364453
Russulaceae	*Lactifluus*	*rubiginosus*	JD 959	KR364210	KR364304	KR364432
Russulaceae	*Lactifluus*	*venezuelanus*	RC/Guad 11-017	KP691420	KP691429	KR364393
Russulaceae	*Lactifluus*	*volemus*	KVP 11-002	KR364175	KR364360	KR364490
Russulaceae	*Multifurca*	*furcata*	REH 7804	DQ421995	DQ421928	None
Russulaceae	*Multifurca*	*ochricompacta*	BB 02-107	DQ421984	DQ421940	None
Russulaceae	*Multifurca*	*zonaria*	FH 12-009	KR364212	KR364365	KR364497
Russulaceae	*Pseudoxenasma*	*verrucisporum*	EL34-95	AF506426	None	None
Russulaceae	*Russula*	*cyanoxantha*	FH 12-201	KR364225	KR364341	KR364471
Russulaceae	*Russula*	*delica*	FH 12-272	KR364224	KR364340	KR364470
Russulaceae	*Russula*	*gracillima*	FH 12-264	KR364226	KR364342	KR364472
Russulaceae	*Russula*	*khanchanjungae*	AV-KD-KVP 09-106	JN389004	JN375607	JN389092
Russulaceae	*Russula*	sp.	EDC 12-061	KR364201	KR364338	KR364468
Russulaceae	*Russula*	sp.	EDC 12-063	KR364202	KR364339	KR364469
Stereaceae	*Conferticium*	*insidiosum*	MUCL 32982	ok	ok	ok
Stereaceae	*Stereum*	*australe*	MUCL 32129	ok	None	None
Stereaceae	*Stereum*	*hirsutum*	AFTOL 492	AF393078	AY218520	AY864885
Thelephorales						
Bankeraceae	*Boletopsis*	*leucomelaena*	AFTOL 1527	DQ154112	GU187820	GU187494
Bankeraceae	*Hydnellum*	*geogenium*	AFTOL 680	AY631900	DQ408133	None

Biogeographical analysis

Ancestral geographical ranges were reconstructed using the BioGeoBEARS package (Matzke 2013) in R 3.3.1 (R Core Team 2016). This package implements different biogeographical history reconstruction models in a likelihood framework and makes it possible to use standard statistical model selection procedures to let the data choose the best model. Available models include the dispersal-extinction-cladogenesis model (DEC; Ree et al. 2005; Ree and Smith 2008), a likelihood version of the dispersal-vicariance analysis (DIVA; Ronquist 1997), a likelihood version of BayArea (Landis et al. 2013), as well as versions of these models in which founder-event speciation is included ("+J"; Matzke 2013).

Geographical areas were defined based on biogeographical regions combined with present-day distributions of *Lactifluus* taxa. Following areas were used: Afrotropics, Nearctic, Neotropics, Australasia, Western Palearctic (including Europe and Western Russia) and Asia (including all Southeast Asian countries together with the Eastern part of Russia, Japan, China, Iran and South Korea). The maximum number of areas any species may occupy was set to two areas, since only a handful of *Lactifluus* species are known to occur in two different areas. Model selection was based on AIC.

Results

Species diversity

Dataset 1 comprises 1306 *Lactifluus* sequences, of which 170 are GenBank sequences. Due to the large variability in quality of the GenBank sequences, we manually checked the sequence alignment for sequences showing slightly eccentric positions in the preliminary trees. Sequences with poor quality, were pruned from the alignment. GMYC species delimitation of the ITS phylogeny delimits the genus *Lactifluus* into 461 GMYC entities or probable species (confidence interval = 428-481). Of those GMYC entities, 236 represent clusters of two or more collections, 225 entities are singletons. Eighty type sequences are included, which represent 53% of the described species, but only 17% of the species delimited using GMYC.

Estimation of divergence times

Divergence time estimates are given in Table 3.2 and Fig. 3.1. The Russulales order probably originated during the late Jurassic (160.3 ± 32.5 My) and the Russulaceae family probably originated in the mid-Cretaceous (110.6 ± 23.9 My). Of the four mainly agaricoid Russulaceae genera, *Russula* and *Lactifluus* appear to be the oldest ones (34.8 ± 7.9 and 33.4 ± 7.0 My respectively) and are estimated to have originated between the Eocene and Oligocene. *Multifurca* probably originated between the Oligocene and Miocene (27.5 ± 7.1 My), while *Lactarius* appears to have originated during the Miocene (22.1 ± 5.2 My).

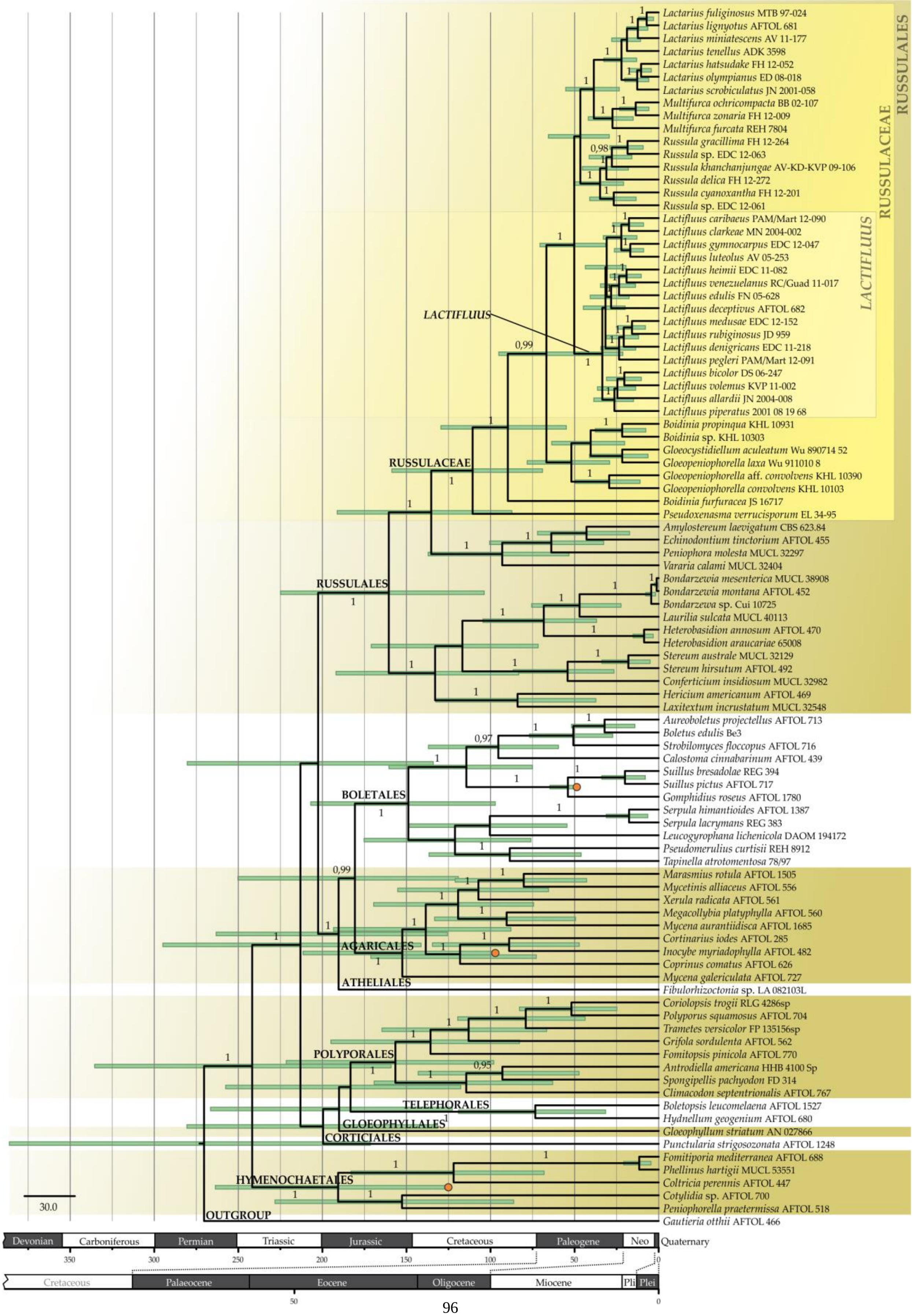
Table 3.2 Most recent common ancestor estimated divergence times for the Russulales order, the Russulaceae family and the four major Russulaceae genera acquired from a BEAST analysis. HPD = higher posterior density interval.

	Mean (My) *including crust-like Russulaceae genera*	**95% HPD (My)** *including crust-like Russulaceae genera*	**Mean (My)** *without crust-like Russulaceae genera*	**95% HPD (My)** *without crust-like Russulaceae genera*
Russulales	160.30	103.80–224.62	164.04	104.84–231.35
Russulaceae	110.56	69.00–158.37	54.62	34.86–77.74
Lactifluus	33.43	21.14–47.23	35.50	22.19–50.38
Lactarius	22.14	12.94–32.51	23.26	13.85–34.40
Multifurca	27.46	15.14–41.88	29.11	15.53–44.77
Russula	34.81	20.85–50.41	36.75	22.16–53.44

Biogeographical results

Biogeographical analyses require a species tree and therefore we performed the GMYC species delimitation on dataset 1. When comparing the GMYC species delimitations with previous delimitations performed on sections or subgenera within *Lactifluus* (either molecular of morphological), we discovered considerable discrepancies in several clades. The GMYC species delimitation resulted in the splitting of several well-studied species or in clades from which one or two single sequences were left out and again represented species themselves. This results in a species tree in which multiple species exhibit little or practically no molecular variation. The BEAST analysis to construct a dated phylogeny for the biogeographical analyses requires a species tree with enough variation between species. Using the GMYC-tree, parameters were not converging as there were too much nearly identical species (unpubl. res.). We therefore adapted the GMYC species tree according to those previous delimitations performed on sections or subgenera within *Lactifluus*. These previous delimitations are based on morphological and molecular data and support the lumping of many clades that were split up in the GMYC analysis. This second species tree contains 369 delimited species, of which 145 are singletons, and is further used in the biogeographical analyses.

When comparing the different methods for biogeographical history reconstruction (Table 3.3), the BioGeoBEARS analysis better supported models that included founder-event speciation (+J), with the highest likelihood for the dispersal-extinction-cladogenesis model with founder-event speciation included (DEC + J) and dispersal-vicariance model with founder-event speciation included (DIVA + J). Both models suggest an Afrotropical origin for the genus *Lactifluus*. Both *Lf.* subg. *Lactariopsis* and *Lf.* subg. *Gymnocarpi* most likely have an Afrotropical origin as well, while *Lf.* subg. *Pseudogymnocarpi* most likely had an Afrotropical origin, but some ancestors migrated to the Neotropics short after the origin of the subgenus, and *Lf.* subg. *Lactifluus* most likely has an Asian origin (Fig. 3.2, Fig. S3).


RUSSULALES
RUSSULACEAE
LACTIFLUUS
Lactarius fuliginosus MTB 97-024
Lactarius lignyotus AFTOL 681
Lactarius miniatescens AV 11-177
Lactarius tenellus ADK 3598
Lactarius hatsudake FH 12-052
Lactarius olympianus ED 08-018
Lactarius scrobiculatus JN 2001-058
Multifurca ochricompacta BB 02-107
Multifurca zonaria FH 12-009
Multifurca furcata REH 7804
Russula gracillima FH 12-264
Russula sp. EDC 12-063
Russula khanchanjungae AV-KD-KVP 09-106
Russula delica FH 12-272
Russula cyanoxantha FH 12-201
Russula sp. EDC 12-061
Lactifluus caribaeus PAM/Mart 12-090
Lactifluus clarkeae MN 2004-002
Lactifluus gymnocarpus EDC 12-047
Lactifluus luteolus AV 05-253
Lactifluus heimii EDC 11-082
Lactifluus venezuelanus RC/Guad 11-017
Lactifluus edulis FN 05-628
Lactifluus deceptivus AFTOL 682
Lactifluus medusae EDC 12-152
Lactifluus rubiginosus JD 959
Lactifluus denigricans EDC 11-218
Lactifluus pegleri PAM/Mart 12-091
Lactifluus bicolor DS 06-247
Lactifluus volemus KVP 11-002
Lactifluus allardii JN 2004-008
Lactifluus piperatus 2001 08 19 68
Boidinia propinqua KHL 10931
Boidinia sp. KHL 10303
Gloeocystidiellum aculeatum Wu 890714 52
Gloeopeniophorella laxa Wu 911010 8
Gloeopeniophorella aff. convolvens KHL 10390
Gloeopeniophorella convolvens KHL 10103
Boidinia furfuracea JS 16717
Pseudoxenasma verrucisporum EL 34-95
Amylostereum laevigatum CBS 623.84
Echinodontium tinctorium AFTOL 455
Peniophora molesta MUCL 32297
Vararia calami MUCL 32404
Bondarzewia mesenterica MUCL 38908
Bondarzewia montana AFTOL 452
Bondarzewa sp. Cui 10725
Laurilia sulcata MUCL 40113
Heterobasidion annosum AFTOL 470
Heterobasidion araucariae 65008
Stereum australe MUCL 32129
Stereum hirsutum AFTOL 492
Conferticium insidiosum MUCL 32982
Hericium americanum AFTOL 469
Laxitextum incrustatum MUCL 32548
Aureoboletus projectellus AFTOL 713
Boletus edulis Be3
Strobilomyces floccopus AFTOL 716
Calostoma cinnabarinum AFTOL 439
Suillus bresadolae REG 394
Suillus pictus AFTOL 717
Gomphidius roseus AFTOL 1780
Serpula himantioides AFTOL 1387
Serpula lacrymans REG 383
Leucogyrophana lichenicola DAOM 194172
Pseudomerulius curtisii REH 8912
Tapinella atrotomentosa 78/97
Marasmius rotula AFTOL 1505
Mycetinis alliaceus AFTOL 556
Xerula radicata AFTOL 561
Megacollybia platyphylla AFTOL 560
Mycena aurantiidisca AFTOL 1685
Cortinarius iodes AFTOL 285
Inocybe myriadophylla AFTOL 482
Coprinus comatus AFTOL 626
Mycena galericulata AFTOL 727
Fibulorhizoctonia sp. LA 082103L
Coriolopsis trogii RLG 4286sp
Polyporus squamosus AFTOL 704
Trametes versicolor FP 135156sp
Grifola sordulenta AFTOL 562
Fomitopsis pinicola AFTOL 770
Antrodiella americana HHB 4100 Sp
Spongipellis pachyodon FD 314
Climacodon septentrionalis AFTOL 767
Boletopsis leucomelaena AFTOL 1527
Hydnellum geogenium AFTOL 680
Gloeophyllum striatum AN 027866
Punctularia strigosozonata AFTOL 1248
Fomitiporia mediterranea AFTOL 688
Phellinus hartigii MUCL 53551
Coltricia perennis AFTOL 447
Cotylidia sp. AFTOL 700
Peniophorella praetermissa AFTOL 518
Gautieria otthii AFTOL 466
BOLETALES
AGARICALES
ATHELIALES
POLYPORALES
TELEPHORALES
GLOEOPHYLLALES
CORTICIALES
HYMENOCHAETALES
OUTGROUP
30.0
Devonian
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Paleogene
Neo
Quaternary
350
300
250
200
150
100
50
0
Cretaceous
Palaeocene
Eocene
Oligocene
Miocene
Pli
Plei
50
0

◄ **Fig. 3.1** Fossil-calibrated maximum clade credibility tree of the BEAST analysis of dataset 2. Posterior probabilities >0.95 are shown and horizontal bars represent the highest posterior density intervals. Circles indicate the place of the fossil calibrations. Time scale in million years.

Table 3.3 Resulting statistics from the BioGeoBEARS analysis in which the dispersal-extinction-cladogenesis model (DEC), the likelihood version of the dispersal-vicariance analysis (DIVA) and the likelihood version of BayArea (BAYAREALIKE), together with their version in which founder-event speciation is included (+J) , are compared.

	Log-likelihood	Dispersal	Extinction	Founder-event	AIC
DEC	-511,310	0,0044	0,0006	0,0000	1027,0
DEC+J	**-387,955**	**0,0002**	**0,0000**	**0,0256**	**781,9**
DIVALIKE	-486,090	0,0051	0,0000	0,0000	976,2
DIVALIKE+J	**-387,955**	**0,0002**	**0,0000**	**0,0256**	**781,9**
BAYAREALIKE	-647,644	0,0057	0,0318	0,0000	1299,0
BAYAREALIKE+J	-391,157	0,0002	0,0000	0,0261	788,3

Discussion

Species delimitation

We discovered a huge diversity within the genus *Lactifluus*. However, the GMYC species delimitation results only partly correspond with the species delimitation results carried out on two sections within *Lactifluus*. Van de Putte (2012) used Bayesian species delimitation to delimit species within *Lf.* sect. *Lactifluus*. The resulting species tree contained 30 supported species and 17 singletons were left out the analysis. The GMYC method in this study supported 22 of these species, six were split in two or more lineages and two clustered together as one lineage. Furthermore, we found 16 extra lineages, which were collected after and were thus not included in the study of Van de Putte (2012). European species of *Lf.* sect. *Piperati* were also delimited using Bayesian species delimitation by De Crop et al. (2014), who found two European species: *Lf. piperatus* and *Lf. glaucescens*. However, the GMYC method applied here, splits *Lf. glaucescens* into three lineages and *Lf. piperatus* into two lineages. When we compare the results of the GMYC species delimitation with morphological studies within *Lactifluus*, several well-studied species split in two or three lineages. This is the case for *Lf. volemus*, *Lf. subvolemus*, *Lf. glaucescens*, *Lf. piperatus*, *Lf. allardii* and *Lf. panuoides*. However, in more than 50 lineages, only one sequence splits off as a singleton closely related with the original species.

When comparing the GMYC results with previously studied lineages of *Lactifluus*, the GMYC method resulted in many more clades than expected. This can be due to the fact that only one locus was used for this analysis, a locus that is rather variable and often hard to align on genus level. We were aware of this shortcoming, however, no other data were available for the majority of collections and species delimitation using GMYC on one locus has proven to be robust and accurate (Fujisawa and Barraclough 2013; Payo et al. 2013). Furthermore, as many sequences of certain *Lactifluus* lineages were gathered during previous studies on infrageneric sections or in the temperate regions, where many mycologists and amateur mycologists are active, there might be a sampling bias that resulted in certain clades containing a large number of nearly identical sequences (e. g. in the clades of *Lf. volemus*, *Lf. subvolemus*, *Lf. glaucescens* and *Lf. piperatus*) in contrast with clades of which almost no sequences are available. The GMYC method is recorded to be tolerant to a moderate amount of identical sequences (Fujisawa and Barraclough 2013), but in this case it might be better to remove some sequences that are nearly identical. The GMYC method further resulted in many singletons (226 entities). Although Fujisawa and Barraclough (2013) wrote that the method is tolerant to moderate amount of singletons, our dataset might contain too many singletons in order for the species delimitation to be correct. In order to accurately estimate the number of species within the genus *Lactifluus*, we suggest carrying out an improved sampling, in order to reduce the number of singletons, and performing species delimitation analyses using multiple loci and excluding identical sequences.

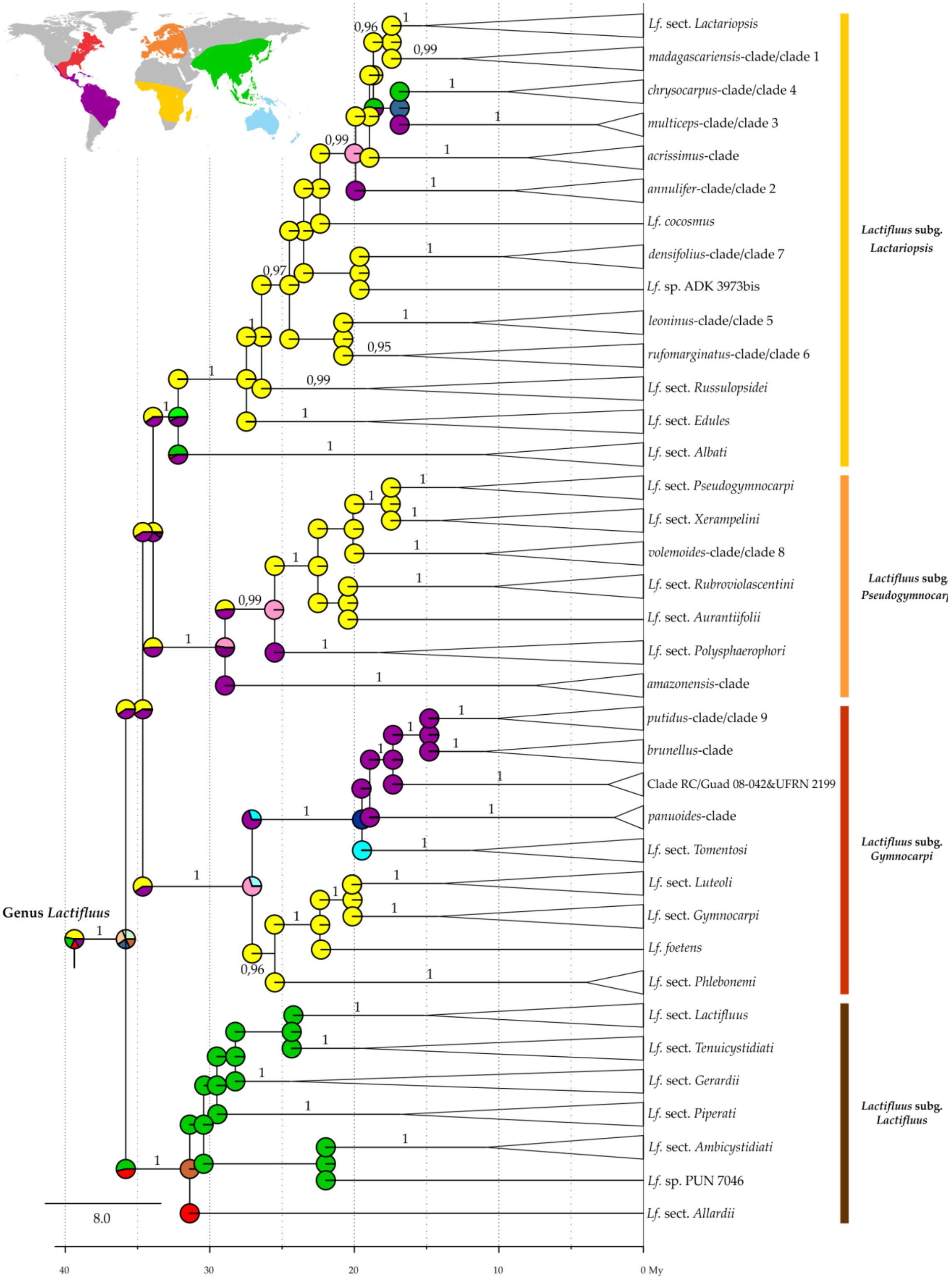


Fig. 3.2 Ancestral area reconstruction for the genus *Lactifluus* as a result of the BioGeoBEARS analysis. The results are plotted on the fossil-calibrated maximum clade credibility tree of the BEAST analysis of dataset 3. Posterior probabilities >0.95 are shown. Pie-charts represent the relative probabilities of ancestral areas at nodes as inferred under the likelihood version of the dispersal-vicariance analysis with founder-event speciation included (DIVA+J). See Fig. S3 for the full version of this tree.

Species richness

Our dataset contains 1306 *Lactifluus* collections, representing 369 species of which 160 species are already described. No sequences were available for the remaining 25 described *Lactifluus* species due to age or bad condition of the collections. Species richness analyses carried out on this dataset (Chapter 7, Fig. 7.1) estimate that *Lactifluus* contains approximately 530 species (95 % CI[21] = 461–601 species). This suggests that we have found 62–80 % of the current diversity and that 95 % of the species will have been found with a sampling of twice the number of collections we assembled for this study. However, we need to emphasize that this might be an overestimation of the actual number of *Lactifluus* species, as it is only based on collections for which DNA sequences were available. The fungal herbarium of Ghent University contains much more samples for which it was impossible to extract DNA from. Many of these collections have been morphologically studied and were appointed to *Lactifluus* species. Including these collections will increase the number of collections and will probably not substantially increase the number of known species within *Lactifluus*.

When the observed species richness is compared between the biogeographical regions (Fig. 7.2), it becomes clear that in all but one biogeographical region, a considerable amount of data is still missing. Only the Palearctic region is sufficiently sampled. When we look at the observed species richness per subgenus (Fig. 7.4), *Lf.* subg. *Lactifluus* represents the largest diversity with 148 species, followed by *Lf.* subg. *Lactariopsis* with 114 species. *Lf.* subg. *Pseudogymnocarpi* and *Lf.* subg. *Gymnocarpi* are relatively small subgenera, with 61 and 46 species respectively.

Challenges of fungal calibrations

The dating analysis based on secondary calibration using three agaricomycete fossil calibrations, estimates that the Russulales originated in the late Jurassic (160 ± 32.5 My ago) and the Russulaceae during the mid-Cretaceous (110.6 ± 23.9 My ago). This is much older than estimated by Floudas et al. (2012), where the Russulales were estimated to have originated during the mid-Cretaceous (100.9 My ago, 95%CI: 65.1–138.8 My ago), but corresponds slightly better with the findings of Chen et al. (2015), who estimated the Russulales divergence during the late Jurassic (145.4 My ago, 95%HPD: 99.6–163.5 My ago). Estimating divergence times of fungal lineages is challenging, mainly because fungal fossils are scarce and the existing fossil records are often hard to interpret (Hibbett and Matheny 2009; Matheny et al. 2009; Berbee and Taylor 2010; Skrede et al. 2011). These fossil calibrations, together with other parameters, strongly influence the inferred divergence times. In some calibration studies of Basidiomycota, only two fossils are used for secondary calibration (Wilson et al. 2012; Sanchez-Ramirez et al. 2015), while others include three (Skrede et al. 2011; Floudas et al. 2012; Chen et al. 2015). To test the influence of the number and the choice of fossil calibrations, we repeated the calibration analyses only using two fossil calibrations instead of three (excluding the ectomycorrhizal Suilloid fossil, unpubl. res.). This shifted the Russulaceae divergence time to a later date (±12 My difference), but the relative ages between the groups remained similar.

Furthermore, we tested the influence of the composition of the dataset used for calibration, by estimating the divergence times twice, with two different variations on dataset 2. The first variation comprised *Lactifluus-*, *Lactarius-*, *Multifurca-* and *Russula* species, together with members of the crust-like genera *Boidinia*, *Gloeocystidiellum*, *Gloeopeniophorella* and *Pseudoxenasma* as representatives of the Russulaceae family, while the second variation excluded these crust-like Russulaceae genera. When excluding crust-like Russulaceae taxa to dataset 2, the Russulaceae divergence time almost halved in age (54.6 ± 11.6 My; Table 3.2) compared with the analysis including the crust-like genera (110.6 ± 23.9 My). The divergence dates for the Russulales and the agaricoid Russulaceae genera only showed minor differences. These results emphasize the importance of an accurate sampling in order to infer divergence times. Due to all these shortcomings in estimating divergence times in fungi, Hibbett et al. (2009) suggest to only compare relative age estimates taken from different studies. When looking at the relative time estimates, our analyses indicate that the major Russulaceae genera originated rather recently in the history of the Russulaceae: ±75-88 My after the divergence time of the Russulaceae family.

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[21] *CI* = Confidence interval

Evolutionary history of Lactifluus

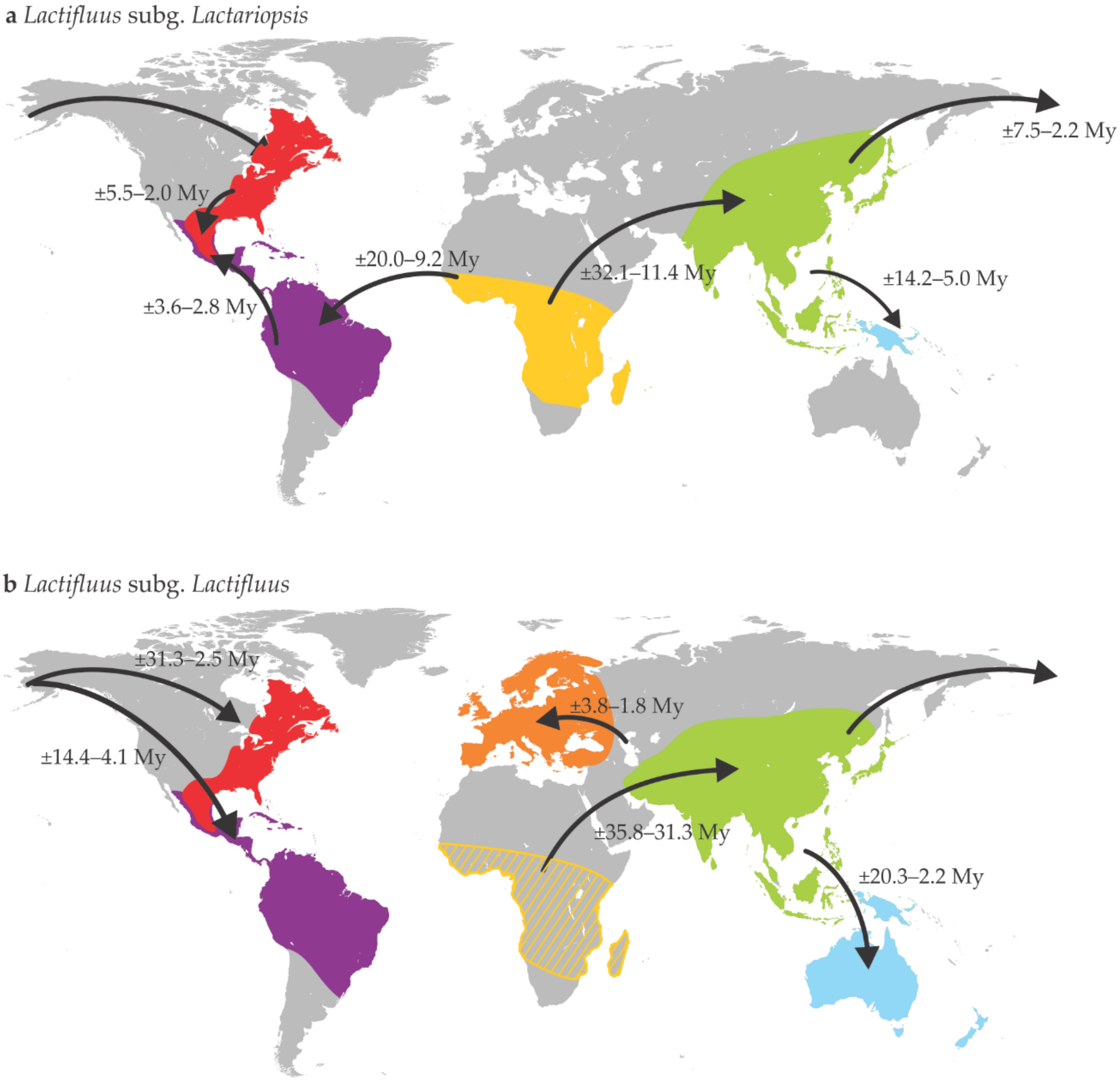
Our analyses indicate an Afrotropical origin for the genus *Lactifluus*, which most likely originated in Africa. Several ECM fungi are reported to have a Palaeotropical origin, amongst them the Inocybaceae (Matheny et al. 2009) and *Amanita* sect. *Caesareae* (Sanchez-Ramirez et al. 2015). The tropical origin of *Lactifluus* contrasts with the temperate origin of its sister genus *Russula* (Looney et al. 2016). *Lactifluus* probably originated between the Eocene and Oligocene (21–47 My). Two other Russulaceae genera have been estimated to have originated during the Eocene and Oligocene: *Russula* (33–55My) and *Lactarius* (17−42 My) (Looney et al. 2016; Wisitrassameewong et al. subm.). The main ECM hosts of African *Lactifluus* species are mainly tree species from the Phyllanthaceae (*Uapaca*) and Fabaceae (genera from within the *Berlinia* clade: *Berlinia*, *Brachystegia*, *Gilbertiodendron*, *Isoberlinia* and *Julbernardia*). The Phyllanthaceae family originated during the mid-Cretaceous, 96–114 My ago (Davis et al. 2005), which coincides with our estimate of the Russulaceae divergence (69–158 My). Within the Fabaceae, the African *Berlinia* clade originated around 48.4 ± 0.7 My ago (Bruneau et al. 2008), after which the agaricoid Russulaceae genera started to diverge.

The four subgenera of *Lactifluus* each show a different evolutionary history. Extant species of ***Lf.* subg. *Lactariopsis*** mainly occur in sub-Saharan Africa, suggesting an Afrotropical origin. Our results confirm the Afrotropics as the most likely ancestral range of this subgenus. After its origin around 32.1 My ago, there were five major dispersal events to other continents (Fig. 3.3a). Our analyses suggest that ***Lf.* subg. *Lactifluus*** originated in Asia, around 31.3 My ago. From Asia, species from this subgenus migrated multiple times to the Neotropics, Western Palearctic and Australasia (Fig. 3.3b). Our analyses imply an Afrotropical origin for ***Lf.* subg. *Pseudogymnocarpi*** around 28.9 My ago, with an early migration event to the Neotropics. This was followed by migration events from the ancestral Afrotropical region towards Asia, Western Palearctic and the Nearctic (Fig. 3.4a). The origin of ***Lf.* subg. *Gymnocarpi*** is suggested to be Afrotropical as well, around 27.1 My ago. From this ancestral range, three major dispersal events followed to Asia, Australasia and the Neotropics, from which the subgenus further diversified (Fig. 3.4b).

Our biogeographical analyses emphasized the importance of founder-event speciation in our dataset. In the case of *Lactifluus*, small numbers of individuals probably migrated away from a larger ancestral population towards other continents, which was often followed by an increased diversification after the founder-event (Templeton 2008). Founder-event speciation has also been found to be important in *Cortinarius* sect. *Cortinarius* (Harrower et al. 2015).

There were at least seven different migration events from the ancestral **Afrotropical** range **towards Asia**, divided over all subgenera. These events all occurred between 32.1–6.6 My and are all explained by migration and subsequent vicariance. During the Oligocene and Miocene, several land-bridges between Africa and Eurasia made migration possible (Allen and Armstrong 2008), which was also observed in *Amanita* sect. *Caesareae* (Sanchez-Ramirez et al. 2015). Our data suggest three major dispersal events **from the Afrotropics towards the Neotropics** and all three of them occurred between 34.0–7.9 My. The last connection between South America and Africa was around 105 My ago, after which the South American and African plate started to move away from each other (McLoughlin 2001). This means that the divergence of *Lactifluus* species into the Neotropics cannot be explained by vicariance and suggests long-distance dispersal as the most likely explanation of current distributions. This migration pattern from the Afrotropics towards the Neotropics was also found both in fungi and plants (Matheny et al. 2009; Crowl et al. 2016). There is evidence for one possible migration **from the Afrotropics towards Australasia**, between 27.1–12.4 My ago. Migration between these two regions may have occurred until 70 My via the Kerguelen platform (Raven 1979; Ali and Aitchison 2008). However, migration between those regions occurred much later in *Lactifluus*, suggesting long-distance dispersal as the most likely explanation for current distributions of extant taxa.

More than 20 different lineages migrated **from Asia towards the eastern Nearctic region** in the past 10 My. From the Miocene on, several periods of interchange between both continents were possible via land bridges in Beringia (Tiffney 1985; Wen 1999; Xiang et al. 2000). Disjunct patterns between North America and Asia are often reported and explained by on-land migration via Beringia and subsequent diversification,



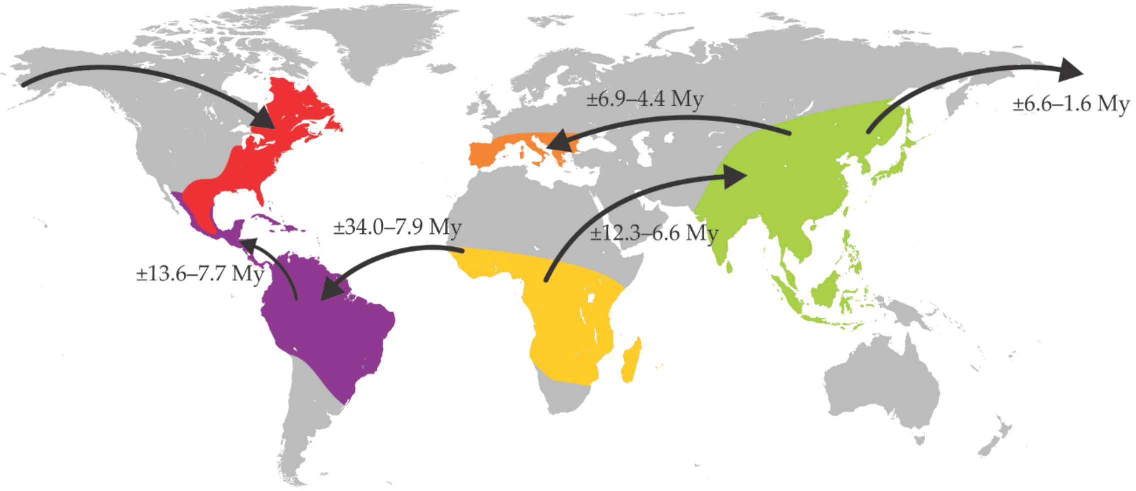
a *Lactifluus* subg. *Lactariopsis*

b *Lactifluus* subg. *Lactifluus*

Fig. 3.3 Distribution maps and major migratory events for **a.** *Lf.* subg. *Lactariopsis* and **b.** *Lf.* subg. *Lactifluus*.

boreotropical hypothesis. *Lactifluus allardii*, however, is a single extant Nearctic species at the base of *Lf.* subg. *Lactifluus* that might have reached the eastern Nearctic from 31 My ago on. The ancestor of *Lf. allardii* might have dispersed from Africa towards eastern North America via Asia and Beringia, or through Europe via the North Atlantic land bridge, which connected Europe and North America from the early Palaeocene until the late Miocene (Denk et al. 2010). Migration via the North Atlantic land bridge has also been suggested in fungi, e.g. in another Russulales genus, *Heterobasidion* (Chen et al. 2015), and in plants (Kadereit et al. 2006; Crowl et al. 2016). There is evidence for at least four migratory events between **Asia and the Neotropics** and one between **the Nearctic and the Neotropics** during the past 15 My. These might also be explained by on-land migration via Beringia and consequent migration from North to Central America, which was possible during the late Pliocene. This southwards expansion from North America into Central America resembles the patterns uncovered in *Amanita* sect. *Caesareae* (Sanchez-Ramirez et al. 2015). During

a. *Lactifluus* subg. *Pseudogymnocarpi*



b. *Lactifluus* subg. *Gymnocarpi*

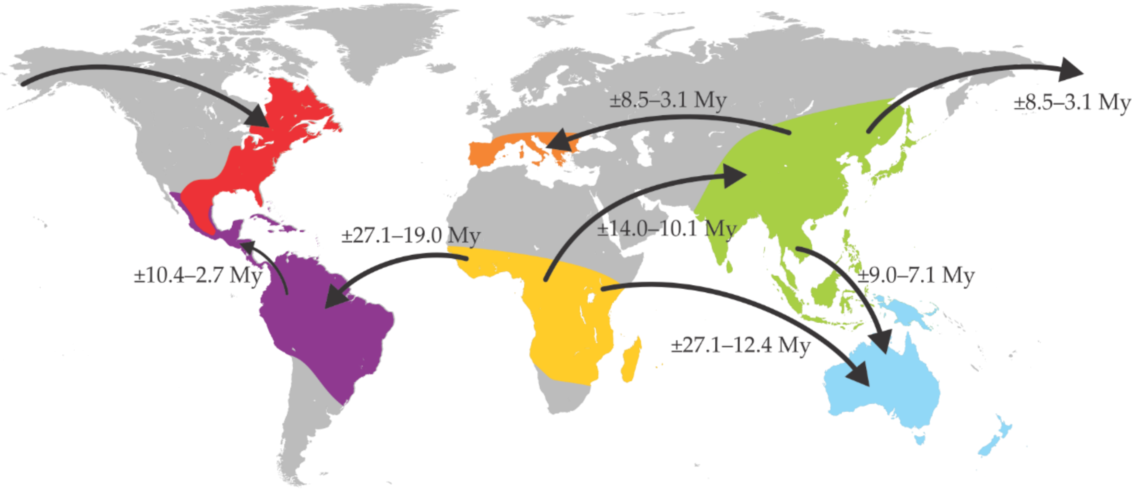


Fig. 3.4 Distribution maps and major migratory events for **a.** *Lf.* subg. *Pseudogymnocarpi* and **b.** *Lf.* subg. *Gymnocarpi*.

the past 15 My, several migratory events had taken place **within the Neotropics, from South America towards Central America**. This was possible due to the gradual closing of the Isthmus of Panama from that started in the middle Miocene (Collins et al. 1996; Huang et al. 2016).

From the late Miocene on, at least eight *Lactifluus* lineages have migrated from **Asia towards the Western Palearctic**. This dispersal was probably facilitated by the temperate vegetation in between both regions (Tiffney and Manchester 2001). We found ten lineages that have migrated from **Asia towards Australasia** in the past 10–20 My. From about 15 My ago, migration became possible between South-East Asia and Oceania, via migration over land-bridges (Raven 1979). Migration between both continents was also shown in the Inocybaceae (Matheny et al. 2009) and in *Amanita* sect. *Caesareae* (Sanchez-Ramirez et al. 2015).

We can conclude that both on-land migration and long-distance dispersal played an important role in the evolutionary history of *Lactifluus*.

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CHAPTER 4

Lactifluus piperatus (Russulales, Basidiomycota) and allied species in Western Europe and a preliminary overview of the group worldwide

Abstract

The large, white milkcaps of *Lactifluus* section *Piperati* are well known in the Northern hemisphere. Historically, there was a lot of debate about the number of European representatives and the diagnostic characteristics to delimit the species. Combining a morphological approach with a phylogenetic study, we aimed to resolve the problems in this section in Europe. Secondly, a molecular analysis of worldwide representatives of *Lactifluus* section *Piperati* was carried out, to verify whether there is intercontinental conspecificity. We compared nuclear ITS and LSU rDNA, nuclear protein-coding *RPB2* and mitochondrial protein-coding *ATP6* genealogies to delimit species, using a concatenation of genes, along with Bayesian species delimitation for the European dataset. The phylogenetic analyses show the existence of two species in Europe: *Lactifluus piperatus* and *Lactifluus glaucescens*. Morphologically, the frequently used characteristics of the colouration of the latex and the macrochemical reactions of latex and context appear not to be useful as diagnostic characteristics to discriminate the species, but the microscopical characters of the pileipellis are informative. The preliminary overview of the section worldwide shows that it comprises at least 10 possible species divided over three clades and that there is no intercontinental conspecificity.

Note: Since this chapter was published during the first year of this PhD study, this chapter still follows the traditional classification of *Lactifluus*.

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Introduction

Lactifluus piperatus and allies in Europe

Milkcaps show a striking variability in basidiocarp aspect, ranging from very small to very large, with dry to viscid, smooth to scaly or tomentose caps and different kinds of pigments in the surface structures as well as in the latex. One of the best recognizable and distinct groups commonly occurring throughout Europe is the one with large, white basidiocarps that are not sticky, viscid or bearded, and that have very acrid latex or context. Striking representatives are *Lactifluus piperatus* (L.: Fr.) Kuntze and *Lactifluus glaucescens* (Crossl.) Verbeken. After the recent splitting of the genus *Lactarius* Pers. into three genera, *Lactarius* (subsequently abbreviated as *L.*), *Lactifluus* (Pers.) Roussel (subsequently abbreviated as *Lf.*) and *Multifurca* Buyck & V. Hofst., these species are now situated in *Lactifluus* subg. *Piperati* sect. *Piperati* Verbeken (Buyck et al. 2008; Verbeken et al. 2012). *Lactarius piperatus* (L.: Fr.) Pers. had been chosen as a lectotype for both the genus *Lactarius* and the genus *Lactifluus* (Earle 1909). However, it has recently been accepted to conserve *Lactarius torminosus* (Schaeff.: Fr.) Pers. as type species for the genus *Lactarius* and *Agaricus* sect. *Lactifluus* Pers., the basionym of the genus *Lactifluus*, was automatically typified by *Agaricus lactifluus* L., which applies to the current species *Lactifluus volemus* (Fr.: Fr.) Kuntze (Buyck et al. 2010; Barrie 2011; McNeill et al. 2011; Norvell 2011; Verbeken et al. 2012). Traditionally, *Lf. piperatus* and its relatives were thought to be related to the group around *Lf. vellereus* (Fr.: Fr.) Kuntze and placed together in *L.* sect. *Albati* (Bat.) Singer (Singer 1962). However, research on a worldwide scale has shown that the group of white and big milkcaps is artificial and *L.* section *Albati* falls apart in two groups (Hesler and Smith 1979; Eberhardt 2000): *Lf. piperatus* and its relatives in *Lf.* sect. *Piperati* Verbeken, and the group around *Lf. vellereus* in *Lf.* sect. *Albati* (Bataille) Verbeken. The position as sections in two different subgenera is highly supported by morphological characteristics (Heilmann-Clausen et al. 1998; Verbeken 1998a, b), such as pileipellis structures, which are completely different in the two groups.

Lactifluus section *Piperati* has a long history of confusion because of nomenclatural and taxonomical problems and the use of different species concepts.

Nomenclature and taxonomy

Fries (1821) recognised *Lactarius piperatus* and *L. pergamenus* (Sw.: Fr.) Fr. and based the difference between the two mainly on the length and the shape of the stipe, the thickness and the aspect of the cap, and the attachment of the lamellae. Many authors after him did not believe these characters to be relevant, except for the smooth cap in *L. piperatus* versus the more rugulose and irregular cap in *L. pergamenus* (Neuhoff 1956; Romagnesi 1956; Bon 1980; Heilmann-Clausen et al. 1998; Basso 1999). Curiously, none of the original descriptions mentioned the colour change of the latex, even though the greenish discolouration of the latex when drying on the lamellae and the context is often a very striking feature. This brought Crossland (1900) to the publication of *L. glaucescens* Crossl., similar to *L. piperatus* but with distinctly greening latex. In his monograph of Central European milkcaps, Neuhoff (1956) accepts two species: *L. piperatus* without KOH (10%) reaction of the latex, and *L. glaucescens* with latex that turns yellow in KOH. He further mentions that the latex of both *L. piperatus* and *L. glaucescens* can have a greenish discolouration. According to Blum (1976), there are three species: *L. piperatus* with crowded, pinkish and really decurrent lamellae, unchanging latex and a pileipellis consisting of sphaerocytes covered by a very thin layer of hyphae; and two species with greening latex and a pileipellis consisting of sphaerocytes covered by a thick layer of hyphae: *L. pergamenus* and *L. glaucescens*. He indicates the major differences being the aspect of the pileipellis (rugulose in *L. pergamenus* versus smooth in *L. glaucescens*), the attachment of the lamellae (often almost free in *L. pergamenus* versus decurrent in *L. glaucescens*) and the shape of the stipe (bulbous and swollen at the base in *L. pergamenus* versus tapering downwards in *L. glaucescens*). *Lactarius eburneus* Z. Schaef. was proposed by Schaefer (1979) as a species with a rather long stipe and white, unchanging latex that turns yellow-orange with KOH. However, as the name was already used for an American species (Thiers 1957), Schaefer's name is illegitimate. Romagnesi (1980) proposed *L. spurius* Romagn., a species with a context that turns green, shows no reaction with KOH and has a pileipellis consisting of globose cells covered by a thin layer of narrow hyphae. This name is invalid since no Latin diagnosis was given. Based on morphological characteristics, most modern revisions (Verbeken et al. 1997; Heilmann-Clausen et al. 1998; Basso 1999) accept two species in Europe: *L. piperatus* and *L. glaucescens*. They all describe

L. piperatus as a species with white latex that is more or less unchanging on the context (at most somewhat yellowing) and unchanging with KOH. The pileipellis consists of a distinct layer of globose cells, covered with a thin layer of hyaline hyphae (10-30 µm). *Lactarius glaucescens* is characterised by white latex that dries more or less greenish on the context (sometimes very slowly) and turns yellow-orange with KOH. The pileipellis consists of a layer of globose cells, covered with a thick layer of hyaline hyphae (80-120 µm). Verbeken et al. (1997) pointed out that the name *L. pergamenus* has been used for at least two different species and should be better considered as nomen dubium. Romagnesi (1956, 1980), Damblon et al. (1956), Heinemann (1960) and several German authors used the name for a species without greening latex and a pileipellis consisting of globose cells covered by a thin layer of hyphae; whereas Blum (1966), Marchand (1980), Bon (1980) and others used it for a species with greening latex and a pileipellis consisting of globose cells covered by a thick layer of hyphae.

Macromorphology

An important feature that contributed to the confusion is the variation in macrochemical and macromorphological characteristics to distinguish between species of *Lf.* sect. *Piperati*. Verbeken et al. (1997) suggested that too much weight was traditionally given to macroscopical characters, such as the length and the shape of the stipe and the attachment of the lamellae, considering these characters as rather variable in this group. Recently we collected many specimens of this section in Western Europe and we noticed a large morphological variation. Especially the variation in macrochemical reactions was striking and the fact that specimens with different macromorphological characteristics occurred on the same location in the field, as if they were from the same mycelium.

To distinguish between *Lf. piperatus* and *Lf. glaucescens*, most commonly used characteristics are the greening of the latex, and macrochemical tests, such as the reaction of the latex with 10% KOH (Bataille 1948; Damblon et al. 1956; Neuhoff 1956; Heinemann 1960; Romagnesi 1961; Blum 1976; Schaefer 1979; Bon 1980; Marchand 1980; Romagnesi 1980; Basso 1999; Lecomte 2010) and the reaction of the context with formaldehyde and with a solution further referred to as sulphoformaldehyde (a solution of 50% formol (at 35%) and 50% sulphuric acid (at 70-80%)) (Bataille 1948; Neuhoff 1956; Bon 1980; Marchand 1980; Romagnesi 1980; Lecomte 2010). Based on carefully executed and standardized macrochemical reactions, together with the colour change of the latex, we found that we could divide the European collections in four groups, here indicated with provisional names: (i) "*Lf. piperatus*", with no colour change of the latex when drying, no reaction of the latex with KOH and no reaction of the context with (sulpho)formaldehyde; (ii) "*Lf. spurius*", with latex that turns green when drying but does not react with KOH and a context that does not react with (sulpho)formaldehyde; (iii) "*Lf. glaucescens*", with greening latex that turns pale yellow-orange with KOH and a context that turns blue with (sulpho)formaldehyde; and (iv) "*Lf. pergamenus*", with greening latex that turns bright orange with KOH and a context that turns blue after some hours with (sulpho)formaldehyde.

Microscopical features

Several microscopical characteristics can be used to distinguish between the species of *Lf.* sect. *Piperati*. The main characteristics that are used are the shape and ornamentation of the spores, the composition of the lamellar edge, the form of the cheilomacrocystidia and the structure of the pileipellis. The pileipellis structure of this section is rather unique within the genus *Lactifluus*. The pileipellis type is described as a hyphoepithelium (Heilmann-Clausen et al. 1998), with a suprapellis consisting of hyaline hyphae and abundant dermatocystidia, and a subcellular subpellis. Species of this section lack the presence of thick-walled elements in the pellis, which are typical microscopical features for the majority of the genus *Lactifluus* (Verbeken and Walleyn 2010).

As most modern revisions (Verbeken et al. 1997; Heilmann-Clausen et al. 1998; Basso 1999) accept two species in Europe, we summarize here the main microscopic features of *Lf. piperatus* and *Lf. glaucescens*. The spores of *Lf. piperatus* are subglobose to oblong and slightly larger than those of *Lf. glaucescens*, which are subglobose to ellipsoid. The ornamentation of spores of *Lf. glaucescens* consists of irregular warts that never form a reticulum, while the ornamentation of *Lf. piperatus* spores consists of irregular warts forming an incomplete reticulum. In both species, basidia are cylindric to subclavate and 2-4-spored, and pleuromacrocystidia are abundant. The lamellar edge is heterogeneous in *Lf. piperatus*, while it is almost exclusively formed by cheilomacrocystidia in *Lf. glaucescens*. Cheilomacrocystidia are more emergent in *Lf. glaucescens* than in *Lf. piperatus* (Heilmann-Clausen et al. 1998; Triantafyllou et al. 2011). The pileipellis of both species is a hyphoepithelium (Heilmann-Clausen et al. 1998). The main distinctive characteristic between both species, however, is the structure of this hyphoepithelium, as observed in surface view on mature specimens (in a scalp preparation). This way, the globose cells of the subpellis are clearly observed in between a very thin layer of hyaline hyphae in *Lf. piperatus*, but not in *Lf. glaucescens*, where the covering layer of thin, hyaline hyphae of the suprapellis is much thicker (Heilmann-Clausen et al. 1998).

Lactifluus sect. Piperati in a worldwide frame

Outside Europe, species from *Lactifluus* sect. *Piperati*, characterised by their general aspects of white to pale brownish-grey, stout basidiocarps with acrid milk and context, and by their hyphoepithelium pileipellis structure (Heilmann-Clausen et al. 1998), are known to occur in Asia and North America. In Asia, *Lf. dwaliensis* (K. Das, J.R. Sharma & Verbeken) K. Das, *Lf. leucophaeus* (Verbeken & E. Horak) Verbeken, *Lf. novoguineensis* (Henn.) Verbeken, *Lf. olivescens* (Verbeken & E. Horak) Verbeken, *Lf. paleus* (Verbeken & E. Horak) Verbeken, *Lf. roseophyllus* (R. Heim) De Crop and *Lf. subpiperatus* (Hongo) Verbeken are described as morphologically recognisable species within *Lf.* sect. *Piperati*. In addition to these morphologically distinct species, a lot of look-a-likes of the European representatives are found throughout Asia. Up to now they received the same names as their European relatives, without testing whether they are truly conspecific. In North America, Hesler and Smith (1979) recognised four species with several varieties: *Lactarius neuhoffii* Hesler & A.H. Sm., *L. neuhoffii* var. *fragrans* (Burl.) Hesler & A.H. Sm., *L. waltersii* Hesler & A.H. Sm., *L. piperatus* (Fr.) S.F. Gray, *L. piperatus* var. *glaucescens* (Crossl.) Hesler & A.H. Sm. and *L. angustifolius* Hesler & A.H. Sm. The new combinations in the genus *Lactifluus* that were not yet made are proposed here (see Nomenclature of the North American species). Again, some look-a-likes of the European species were given the same names as their European relatives, but it has never been proved for this group that they are conspecific with the European species. Species of *Lf.* sect. *Piperati* are not known to occur in Africa, South America and Australia. The only record of *Lf. piperatus* in Australia concerns probably an introduced species, as it is found under both introduced and native tree species (Fuhrer 2005). Species delimitation worldwide thus remains doubtful and confusing.

Despite the large historical confusion in this group, a targeted phylogenetic study has never been executed. Until now, species delimitation was based on morphological and macrochemical characteristics, without testing if these characteristics are supported by a molecular phylogeny and therefore reliable in delimiting the Western European species within this section. We meet this deficit by using molecular data to delimit species and by comparing the phylogenetic results with information on morphology.

As multiple gene sequence data become increasingly available for Agaricomycotina, more and more studies apply phylogenetic methods on a concatenation of alignments of different genes to reconstruct a species tree. However, research shows that topologies often differ among different genes (Knowles and Carstens 2007). Incongruence can be caused by several evolutionary processes, such as incomplete lineage sorting, hybridisation, gene duplication and horizontal gene transfer (Maddison 1997). In case of incomplete lineage sorting, the use of concatenated alignments can lead to a poor estimation of the species tree and bootstrap values can provide strong support for this incorrect phylogeny (Kubatko and Degnan 2007). To account for these inconsistencies, new methods have been constructed, such as the hierarchical Bayesian model for species tree inference implemented in *BEAST (Heled and Drummond 2010). *BEAST estimates the species tree directly from the sequence data, and it incorporates uncertainty associated with gene trees, nucleotide substitution model parameters and the coalescent process (Heled and Drummond 2010). Species can be further delimited using Bayesian species delimitation, which accommodates the species phylogeny as well as lineage sorting due to ancestral polymorphism (Yang and Rannala 2010). In this study, we will use traditional phylogenetic techniques (maximum likelihood and Bayesian inference) to check for gene-incongruence, and a species tree will be constructed using *BEAST. Additionally, we will use Bayesian species delimitation to delimit species within *Lactifluus* section *Piperati* of Western Europe.

We first studied extensively documented fresh material and herbarium collections from Western European representatives of *Lactifluus* section *Piperati*, using morphological, macrochemical and molecular analyses to test if the above groups of European collections, delimited by macroscopical and macrochemical features, represent phylogenetically distinct species. We then studied fresh material and herbarium collections from European, Asian and North American representatives of *Lactifluus* section *Piperati* using molecular analyses, to verify if there is intercontinental conspecificity in this group.

Material and methods

Sampling

This study is based on Western European, Asian and North American collections of *Lactifluus* section *Piperati* (Table 4.1). The European collections we used were mainly sampled in Belgium and France, as the herbarium specimens of these regions were provided with comprehensive macroscopic descriptions. The Asian samples were collected in Thailand, India and Vietnam, and the collections from North America mostly are from the state of Tennessee (USA). Two datasets were assembled for further analyses: a European dataset and a worldwide dataset. Two collections of *Lf.* section *Lactifluus* and two collections of *Lf.* subg. *Gerardii* were included as outgroup for the European dataset. For the worldwide phylogeny, the outgroup contains two species from *Lf.* sect. *Albati*.

Morphological analyses

Macromorphological characteristics of material collected by the authors were described in daylight conditions and those of herbarium specimens were based on the notes of the collectors. In order to allow comparison of the macrochemical reactions, the macrochemical tests were standardized. The reagents were recently prepared and were preferably from the same stock. The reagents used are KOH (10%), formaldehyde (38%) and sulphoformaldehyde (solution of 50% formol (at 35%) and 50% sulphuric acid (at 70-80%)). The tests were carried out on adult specimens that were not too old, fresh and not saturated with water. The reaction of the latex with KOH was tested by isolating a droplet of latex on a glass slide and adding a droplet of the reagent to the latex. The reaction was considered positive when there was a yellow-orange colour change within ten seconds after mixing the latex with KOH. The reaction of the context with (sulpho)formaldehyde was tested on the context of the stipe during a period of 24 hours. The reaction was noted as positive when the context colors blue; the time in which the reaction takes place was also recorded. Micromorphological characters were studied on dried herbarium collections. For general terminology we follow Vellinga (1988) and for terminology concerning pileipellis structures we follow Heilmann-Clausen et al. (1998) and Verbeken (1998a). Line-drawings were made by A. Verbeken. Basidiospores were measured and drawn in side view, in Melzer's reagent, using a Zeiss Axioscop 2 microscope and a drawing tube at a magnification of 6000×. Measurements were done excluding the ornamentation. Elements of the pileipellis and hymenial elements were measured and drawn halfway the radius of the pileus in Congo-Red in L4, using an Olympus CX31 microscope and drawing tube at a magnification of 1600×. Basidia length excludes sterigmata length.

DNA extraction, PCR amplification, sequencing and nucleotide alignments

DNA from dry collections was extracted using the protocol described by Nuytinck & Verbeken (2003), with the modifications described in Van de Putte et al. (2010). DNA from fresh material was extracted using the CTAB extraction method described in Nuytinck & Verbeken (2003). Protocols for PCR amplification follow Le et al. (2007). Three nuclear loci and one mitochondrial locus were amplified: (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, using the ITS-1F and ITS4 primers (White et al. 1990; Gardes and Bruns 1993); (2) a part of the ribosomal large subunit 28S region (LSU), using the primers LR0R and LR5 (R. Vilgalys lab 'http://www.biology.duke.edu/fungi/mycola b/primers.htm'); (3) the regions between the conserved

Table 4.1 Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses. All exsiccates are deposited in GENT, except the collections of M. Lecomte, these exsiccates are deposited in his personal herbarium.

Species	Original identification	Voucher Collector	Country	Date	ITS accession no.	LSU accession no.	*RPB2* accession no.	*ATP6* accession no.
Lactifluus section Piperati								
Group 1 - *Lf. glaucescens*-group								
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le* 66	Thailand	30-8-2003	GU258298	GU265639	KF220219	None
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le* 379	Thailand	16-10-2005	KF220019	None	None	None
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le* 383	Thailand	18-10-2005	KF220020	None	None	None
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le 237*	Thailand	5-9-2004	KF220052	KF220153	KF220238	KF219951
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le* 241	Thailand	5-9-2004	KF220053	KF220154	KF220239	KF219952
Lf. aff. glaucescens Asia 1	*L. glaucescens*	*H.T. Le* 244	Thailand	5-9-2004	KF220054	KF220155	KF220240	KF219953
Lf. aff. glaucescens Asia 1	*L. leucophaeus*	*H.T. Le* 236	Thailand	5-9-2004	KF220060	KF220158	KF220244	KF219957
Lf. aff. glaucescens Asia 1	*L.* aff. *piperatus*	*A. Verbeken/K. Das/K. Van de Putte* 09-062	India	15-8-2009	KF220096	KF220191	KF220265	KF219990
Lf. aff. glaucescens Asia 1	*L.* aff. *piperatus*	*A. Verbeken/K. Das/K. Van de Putte* 09-115	India	3-9-2009	KF220097	KF220192	KF220266	KF219991
Lf. aff. glaucescens Asia 2	*L. glaucescens*	*H.T. Le* 20	Thailand	19-7-2003	KF220018	None	None	None
Lf. aff. glaucescens Asia 2	*L. glaucescens*	*H.T. Le* 65	Thailand	30-8-2003	KF220051	KF220152	None	KF219950
Lf. aff. glaucescens Asia 2	*L. glaucescens*	*J. Nuytinck* 2011-009	Vietnam	12-6-2011	KF220103	KF220198	KF220272	KF219997
Lf. aff. glaucescens Asia 2	*L. glaucescens*	*J. Nuytinck* 2011-014	Vietnam	12-6-2011	KF220104	KF220199	KF220273	KF219998
Lf. aff. glaucescens USA 1	*L. glaucescens*	*A. Verbeken* 04-174	North America	12-7-2004	KF220044	KF220145	KF220231	KF219943
Lf. aff. glaucescens USA 2	*L. glaucescens*	*A. Verbeken* 04-195	North America	13-7-2004	KF220045	KF220146	KF220232	KF219944
Lf. aff. glaucescens USA 2	*L. glaucescens*	*A. Verbeken* 05-211	North America	10-8-2005	KF220046	KF220147	KF220233	KF219945
Lf. aff. glaucescens USA 2	*L. glaucescens*	*A. Verbeken* 05-261	North America	12-8-2005	KF220047	KF220148	KF220234	KF219946
Lf. aff. glaucescens USA 3	*L. glaucescens*	*A. Verbeken* 05-374	North America	18-8-2005	KF220049	KF220150	KF220236	KF219948
Lf. aff. leucophaeus Asia 2	*L. leucophaeus*	*H.T. Le* 360	Thailand	24-7-2004	KF220061	KF220159	KF220245	KF219958
Lf. glaucescens Europe	*L. glaucescens*	*J. Nuytinck* 2001-02	France	17-8-2001	KF220022	None	None	None
Lf. glaucescens Europe	*L. glaucescens*	*R. Walleyn* 1874	Belgium	15-8-2000	KF220023	None	None	None
Lf. glaucescens Europe	*L. glaucescens*	*A. Verbeken* 97-524	Belgium	4-8-1997	KF220024	KF220128	KF220221	KF219925
Lf. glaucescens Europe	*L. glaucescens*	*R. Walleyn* 25-08-92a	Germany	25-8-1992	KF220025	None	None	KF219926
Lf. glaucescens Europe	*L. glaucescens*	*A. Verbeken* 97-518	France	25-7-1997	KF220026	KF220129	None	KF219927

Species	Original identification	Voucher Collector	Country	Date	ITS accession no.	LSU accession no.	*RPB2* accession no.	*ATP6* accession no.
Lf. glaucescens Europe	*L. glaucescens*	*R. Walleyn* 27-08-92	Germany	27-8-1992	KF220027	KF220130	None	KF219928
Lf. glaucescens Europe	*L. glaucescens*	*N. Dam* 01024	The Netherlands	6-8-2001	KF220028	KF220131	KF220222	KF219929
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2000 09 17 01	Belgium	17-9-2000	KF220029	KF220132	None	KF219930
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 28 21	Belgium	28-8-2001	KF220030	KF220133	KF220223	KF219931
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2002 20 09 03	France	9-2-2002	KF220031	KF220134	KF220224	KF219932
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2008 08 21 01	Belgium	21-8-2008	KF220032	JN388988	JN375591	JN389041
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 28 14	Belgium	28-8-2001	KF220034	KF220136	None	KF219934
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte 2001 08 19 35*	*France*	*19-8-2001*	*KF220035*	*KF220137*	*KF220226*	*KF219935*
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 28 17	Belgium	28-8-2001	KF220036	KF220138	None	KF219936
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 28 08	Belgium	28-8-2001	KF220038	KF220140	KF220228	KF219938
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 14 02	Belgium	14-8-2001	KF220039	KF220141	KF220229	KF219939
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2001 08 28 18	Belgium	28-8-2001	KF220040	KF220142	KF220230	KF219940
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2000 09 23 29	Belgium	23-9-2000	KF220041	KF220143	None	KF219941
Lf. glaucescens Europe	*L. glaucescens*	*M. Lecomte* 2002 08 25 01	Belgium	25-8-2002	KF220043	None	None	None
Lf. glaucescens Europe	*L. pergamenus*	*A. Verbeken* 93-025 P2	France	25-7-1993	KF220062	KF220160	KF220246	KF219959
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 09 23 09	Belgium	23-9-2000	KF220063	KF220161	None	KF219960
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2002 08 25 21	Belgium	25-8-2002	KF220064	KF220162	KF220247	KF219961
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2004 08 19 02	Belgium	19-8-2004	KF220065	KF220163	KF220248	KF219962
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 05 01	France	5-10-2000	KF220066	KF220164	KF220249	KF219963
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 79	France	18-10-2000	KF220067	KF220165	None	KF219964
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 02 14	Belgium	2-10-2000	KF220068	KF220166	KF220250	KF219965
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 87	France	18-10-2000	KF220069	KF220167	KF220251	KF219966
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 09 24 01	Belgium	24-9-2000	KF220070	KF220168	None	KF219967
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 1997 07 13 01	France	13-7-1997	None	KF220169	None	KF219968
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 02	France	18-10-2000	KF220071	KF220170	None	KF219969
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 108	France	18-10-2000	KF220072	KF220171	None	KF219970
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 09 23 01	Belgium	23-9-2000	KF220073	KF220172	KF220252	KF219971
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 71	France	18-10-2000	KF220074	KF220173	None	KF219972

Species	Original identification	Voucher Collector	Country	Date	ITS accession no.	LSU accession no.	*RPB2* accession no.	*ATP6* accession no.
Lf. glaucescens Europe	*L. pergamenus*	*M. Lecomte* 2000 10 18 01	France	18-10-2000	KF220075	KF220174	None	KF219973
Lf. glaucescens Europe	*L. piperatus*	*M. Lecomte* 2000 10 18 52	France	18-10-2000	KF220086	KF220181	KF220258	KF219980
Lf. glaucescens Europe	*L. piperatus*	*M. Lecomte* 2004 08 19 01	Belgium	19-8-2004	KF220094	KF220189	KF220263	KF219988
Lf. glaucescens Europe	*L. spurius*	*M. Lecomte* 2002 07 14 01	Belgium	14-7-2002	KF220114	KF220207	KF220280	KF220005
Lf. glaucescens Europe	*L. spurius*	*M. Lecomte* 2003 06 14 01	Italy	14-6-2003	KF220117	KF220210	KF220283	KF220008
Lf. glaucescens Europe	*L. spurius*	*M. Lecomte* 2004 08 15 01	France	15-8-2004	KF220118	KF220211	KF220284	KF220009
Lf. leucophaeus Asia 1	*L. leucophaeus*	*H.T. Le/A. Verbeken & R. Walleyn* 126 / 04-075	Thailand	23-6-2004	KF220056	None	None	None
Lf. leucophaeus Asia 1	*L. leucophaeus*	*A. Verbeken* 97-382 (type)	Papua New Guinea	21-2-1997	GU258299	GU265640	KF220241	None
Lf. leucophaeus Asia 1	*L. leucophaeus*	*E. Horak* 7330	Indonesia	11-1-1999	KF220058	KF220156	KF220242	KF219955
Lf. leucophaeus Asia 1	*L. leucophaeus*	*H.T. Le* 182	Thailand	5-7-2004	KF220059	KF220157	KF220243	KF219956
Lf. roseophyllus Asia 1	*L. roseophyllus*	*J. Nuytinck* 2011-076	Vietnam	16-6-2011	KF220107	KF220202	KF220276	KF220001
Group 2 - *Lf. piperatus*-group								
Lf. aff. piperatus Asia 1	*L.* aff. *piperatus*	*A. Verbeken/K. Das/K. Van de Putte* 09-008	India	13-8-2009	KF220095	KF220190	KF220264	KF219989
Lf. aff. piperatus Asia 2	*L. piperatus*	*H.T. Le* 51	Thailand	29-8-2003	KF220076	KF220175	KF220253	None
Lf. aff. piperatus Asia 2	*L. piperatus*	*H.T. Le* 240	Thailand	8-5-2000	KF220077	None	None	None
Lf. aff. piperatus Asia 2	*L. piperatus*	*H.T. Le 88*	*Thailand*	*15-10-2003*	*KF220098*	*KF220193*	*KF220267*	*KF219992*
Lf. aff. piperatus Asia 2	*L. piperatus*	*H.T. Le* 198	Thailand	27-7-2004	KF220099	KF220194	KF220268	KF219993
Lf. aff. piperatus Asia 2	*L. piperatus*	*H.T. Le* 242	Thailand	5-9-2003	KF220100	KF220195	KF220269	KF219994
Lf. aff. piperatus Asia 2	*L.* cf. *piperatus*	*J. Nuytinck* 2011-036	Vietnam	13-6-2011	KF220105	KF220200	KF220274	KF219999
Lf. aff. piperatus Asia 3	*L. leucophaeus*	*H.T. Le* 377	Thailand	9-8-2001	KF220057	None	None	None
Lf. aff. piperatus Asia 3	*L. piperatus*	*H.T. Le* 322	Thailand	27-6-2005	KF220078	None	None	None
Lf. aff. piperatus Asia 3	*L. piperatus*	*H.T. Le* 293	Thailand	23-6-2005	KF220101	KF220196	KF220270	KF219995
Lf. aff. piperatus Asia 3	*L.* cf. *piperatus*	*J. Nuytinck* 2011-072	Vietnam	16-6-2011	KF220106	KF220201	KF220275	KF220000
Lf. aff. piperatus Asia 4	*L. piperatus*	*H.T. Le* 378	Thailand	8-10-2005	KF220102	KF220197	KF220271	KF219996
Lf. aff. piperatus Asia 4	*L. subpiperatus*	*H.T. Le* 69	Thailand	30-8-2003	KF220112	KF220205	None	KF220003
Lf. aff. piperatus Asia 5	*L. subpiperatus*	*H.T. Le/A. Verbeken & R. Walleyn* 125 / 04-072	Thailand	23-6-2004	KF220109	None	None	None
Lf. aff. piperatus USA 1	*L. glaucescens*	*A. Verbeken* 04-202	North America	14-7-2004	KF220021	KF220127	KF220220	None

Species	Original identification	Voucher Collector	Country	Date	ITS accession no.	LSU accession no.	*RPB2* accession no.	*ATP6* accession no.
Lf. aff. piperatus USA 2	*L. glaucescens*	*A. Verbeken* 05-393	North America	19-8-2005	KF220050	KF220151	KF220237	KF219949
Lf. aff. piperatus USA 3	*L. glaucescens*	*A. Verbeken* 05-295	North America	14-8-2005	KF220048	KF220149	KF220235	KF219947
Lf. piperatus Europe	*L. glaucescens*	*M. Lecomte* 2000 10 07 01	France	7-10-2000	KF220033	KF220135	KF220225	KF219933
Lf. piperatus Europe	*L. glaucescens*	*M. Lecomte* 2001 08 19 59	France	19-8-2001	KF220037	KF220139	KF220227	KF219937
Lf. piperatus Europe	*L. glaucescens*	*M. Lecomte* 2001 10 02 15	Belgium	2-10-2001	KF220042	KF220144	None	KF219942
Lf. piperatus Europe	*L. piperatus*	*R. Walleyn* 3064	Belgium	8-7-2003	KF220079	None	None	None
Lf. piperatus Europe	*L. piperatus*	*A. Fraiture* 2584	Belgium	27-7-1997	KF220080	KF220176	KF220254	KF219974
Lf. piperatus Europe	*L. piperatus*	*J. Vesterholt* 96-144	Denmark	2-9-1996	KF220081	KF220177	KF220255	KF219975
Lf. piperatus Europe	*L. piperatus*	*R. Walleyn* 25-08-92b	Germany	25-8-1992	KF220082	KF220178	KF220256	KF219976
Lf. piperatus Europe	*L. piperatus*	*J. Vesterholt* 96-074	Denmark	20-8-1996	KF220083	KF220179	None	KF219977
Lf. piperatus Europe	*L. piperatus*	*A. Verbeken* 93-023 P1	France	25-7-1993	KF220084	KF220180	None	KF219978
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2007 06 28 01	France	28-6-2007	KF220085	None	KF220257	KF219979
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2002 07 14 02	Belgium	14-7-2002	KF220087	KF220182	None	KF219981
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2001 07 20 01	France	20-7-2001	KF220088	KF220183	None	KF219982
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2003 06 29 01	France	29-6-2003	KF220089	KF220184	KF220259	KF219983
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2001 08 19 39	France	19-8-2001	KF220090	KF220185	KF220260	KF219984
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2000 09 10 14	Belgium	10-9-2000	None	KF220186	KF220261	KF219985
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2000 08 28 41	Belgium	28-8-2000	KF220091	KF220187	KF220262	KF219986
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2000 10 02 09	Belgium	2-10-2000	KF220092	JN388991	JN375594	JN389043
Lf. piperatus Europe	*L. piperatus*	*M. Lecomte* 2000 08 27 03	Belgium	27-8-2000	KF220093	KF220188	None	KF219987
Lf. piperatus Europe	*L. spurius*	*M. Lecomte* 2001 08 19 65	France	19-8-2001	KF220115	KF220208	KF220281	KF220006
Lf. piperatus Europe	*L. spurius*	*M. Lecomte* 2001 08 19 13	France	19-8-2001	KF220116	JN388993	JN375596	JN389044
Lf. piperatus Europe	*L. spurius*	*M. Lecomte* 2001 08 19 68	France	19-8-2001	KF220119	KF241840	KF241842	KF241841
Lf. piperatus Europe	*L. spurius*	*M. Lecomte* 2001 08 19 23	France	19-8-2001	KF220120	KF220212	KF220285	KF220010
Lf. piperatus Europe	*L. spurius*	*M. Lecomte* 2001 08 19 22	France	19-8-2001	None	KF220213	KF220286	KF220011
Lf. piperatus Europe	*L. spurius*	*M. Lecomte 2001 08 19 55*	*France*	*19-8-2001*	*KF220121*	*KF220214*	*KF220287*	*KF220012*
Lf. piperatus Europe	*L. spurius*	78111 (type)	France	27-8-1978	KF220122	KF220215	None	KF220013

Species	Original identification	Voucher Collector	Country	Date	ITS accession no.	LSU accession no.	*RPB2* accession no.	*ATP6* accession no.
Group 3 - *Lf. dwaliensis*-group								
Lf. aff. subpiperatus Asia	*L. subpiperatus*	*H.T. Le* 376	Thailand	8-8-2001	KF220110	None	None	None
Lf. dwaliensis Asia	*L. dwaliensis*	*K. Das* 612 (type)	India	3-10-1999	None	None	None	KF219924
Lf. dwaliensis Asia	*L.* sp.	*H.T. Le* 67	Thailand	30-8-2003	KF220108	KF220203	KF220277	None
Lf. dwaliensis Asia	*L. subpiperatus*	*H.T. Le* 55	Thailand	30-8-2003	KF220111	KF220204	KF220278	KF220002
Lf. dwaliensis Asia	*L. subpiperatus*	*H.T. Le* 346	Thailand	2-6-2005	KF220113	KF220206	KF220279	KF220004
Lactifluus* section *Allardii								
Lf. allardii	*L. allardii*	*A. Verbeken* 05-286	North America	14-8-2005	KF220015	KF220124	None	None
Lf. allardii	*L. allardii*	*J. Nuytinck* 2004-008	North America	13-7-2004	KF220016	KF220125	KF220217	None
Lf. allardii	*L. allardii*	*A. Verbeken* 05-246	North America	12-8-2005	KF220017	KF220126	KF220218	KF219923
Lactifluus* subgenus *Gerardii								
Lf. bicolor	*L. bicolor*	*D. Stubbe* 06-247	Malaysia	19-9-2006	JN388955	JN388987	JN375590	JN389040
Lf. cf. ochrogalactus	*L.* cf. *ochrogalactus*	*A. Verbeken/K. Das/K. Van de Putte* 09-120	India	4-9-2009	JN388956	JN388990	JN375593	JN389042
Lactifluus* section *Lactifluus								
Lf. crocatus	*L. crocatus*	*K. Van de Putte* 08-034	Thailand	16-7-2008	HQ318243	HQ318151	HQ328888	JN389073
Lf. volemus	*L. volemus*	90804-5	Sweden	Unknown	JN388959	JN389010	JN375612	None
Lactifluus* section *Albati								
Lf. leoninus	*L. leoninus*	*D. Stubbe* 07-454	Thailand	4-7-2007	KF220055	JN388989	JN375592	KF219954
Lf. vellereus var. hometii	*L. vellereus* var. *hometii*	Felix Hampe 5231/4	Germany	26-9-2010	KF220123	KF220216	KF220288	KF220014

domains 6 and 7 of the second largest subunit of the RNA polymerase II (*RPB2*), using the primers bRPB2-6F and fRPB2-7cR (Liu et al. 1999; Matheny 2005) and (4) the mitochondrial ATPase subunit 6 (*ATP6*), using primers ATP6-3 and ATP6-2 (Kretzer and Bruns 1999). Although all four loci were previously shown to be useful for species delimitation within the genus *Lactifluus* (Stubbe et al. 2010; Van de Putte et al. 2010; Van de Putte et al. 2012), we estimated the evolutionary divergence between the sequences by computing the number of base substitutions per site from averaging over all sequence pairs for each marker in MEGA 5 (Tamura et al. 2011). Analyses were conducted using the Maximum Composite Likelihood model. The rate variation among sites was modelled with a gamma distribution (shape parameter = 4). Standard error (S.E.) estimates were obtained by a bootstrap procedure (500 replicates). PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Macrogen). Forward and reverse sequences were assembled into contigs and edited where needed with the Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). Sequences of both the European and the worldwide datasets were aligned using the online version of MAFFT v6 (Katoh and Toh 2008), with an E-INS-I strategy, and were manually edited in MEGA 5 (Tamura et al. 2011). The alignments can be acquired from the first author and from TreeBASE (S14367).

Phylogenetic analyses

The program Gblocks 0.91b (Castresana 2000) was used to exclude ambiguously aligned positions in the alignments of both datasets, with settings allowing gaps within selected blocks, smaller blocks (minimum 5 bp) and bigger segments with contiguous non-conserved positions (maximum 10bp). ITS, *RPB2* and *ATP6* sequence data were further divided into partitions, while LSU was analysed as a whole. The ITS sequences were partitioned in the partial ribosomal gene 18S, the first spacer region ITS, the ribosomal gene 5.8S and the second spacer region ITS2. The *RPB2* sequences were partitioned into four partitions: the fourth intron of the *RPB2*-gene and the first, second and third codon positions of the exon. The *ATP6* sequences were partitioned according to the first, second and third codon positions.

A maximum likelihood (ML) analysis was executed with the program RAxML v7.0.3 (Stamatakis 2006), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 500 replicates (Stamatakis et al. 2008). Bayesian Inference analyses (BI) were executed with MrBayes v3.2.0 (Ronquist et al. 2012) on the high performance computer of Ghent University. MrModeltest v2.3 (Nylander 2004) was first used to determine the model that best fits the data of each partition, using the second order Akaike Information Criterion (AICc). Five parallel runs, each consisting of one cold and three heated chains, were run for 20 million generations, sampling every 100th generation. Convergence of the different runs was verified by checking the log-likelihoods and the effective sample sizes in Tracer v1.5 (Rambaut and Drummond 2007). A burn-in was determined in Tracer and a majority rule consensus tree was constructed, using at least three runs that converged to the same likelihood. The ML and BI analyses were performed on each marker separately and on the combination of markers of both the European and the worldwide datasets.

Bayesian species delimitation

Species tree inference

The species tree for the European dataset was estimated using the hierarchical Bayesian model implemented in *BEAST v1.6.2 (Heled and Drummond 2010). *BEAST conducts multispecies coalescent analyses to estimate the most probable species tree directly from the unlinked multi-locus sequence data. *BEAST incorporates the coalescent process, uncertainty associated with gene trees and nucleotide substitution model parameters (Heled and Drummond 2010). To examine the coalescent events for a species, the analysis requires at least two specimens per species. This was not achieved for the worldwide dataset, so Bayesian species delimitation was only used to delimit species within the European representatives of the section. Specimens were assigned to taxon subsets based on the results of the concatenated ML and BI trees. As in the BI analyses, the ITS, *RPB2* and *ATP6* sequence data were further divided into partitions, while LSU was analysed as a whole. For each partition, we manually edited the XML file to be able to use the same substitution model as determined for the BI analyses, under an uncorrelated relaxed lognormal clock model

(Drummond et al. 2006). We selected the Yule process as a tree prior, with a piecewise linear and constant root population size model. Three independent MCMC analyses were run for a total of 50 million generations, sampling every 100 steps and excluding the first 5 million generations of each run as a burn-in. Convergence was verified by checking the log-likelihoods and the effective sample sizes in Tracer v1.5 (Rambaut and Drummond 2007).

Speciation probabilities

Bayesian species delimitation was conducted using Bayesian Phylogenetics and Phylogeography (BPP v2.1a**;** (Rannala and Yang 2003; Yang and Rannala 2010). This method accommodates the species phylogeny as well as lineage sorting due to ancestral polymorphism. The rjMCMC analyses were run for 100.000 generations, sampling each fifth generation, excluding the first 50.000 generations as a burn-in. Each analysis was run twice to confirm consistency between runs. We used algorithm 0, with different fine-tune parameters to confirm stability between runs (ε = 5, 10 and 20). As prior distributions on the ancestral population size (θ) and root age (τ_0) can affect the posterior probabilities for models (Yang and Rannala 2010), we tested three different combinations of priors (Leache and Fujita 2010). The first combination of priors assumes relatively large ancestral population sizes and deep divergences: $\theta \sim G(1, 10)$ and $\tau_0 \sim G(1, 10)$, with both prior means = 0.1 and prior variances = 0.01. The second prior combination assumes relatively small ancestral population sizes and shallow divergences among species: $\theta \sim G(2, 2000)$ and $\tau_0 \sim G(2, 2000)$, with both prior means = 0.001 and variances = 5×10^{-7}. The third combination assumes large ancestral populations sizes $\theta \sim G(1, 10)$ and relatively shallow divergences among species $\tau_0 \sim G(2, 2000)$, with prior mean $\theta = 0.1$, variance = 0.01 and prior mean $\tau_0 = 0.001$, variance = 5×10^{-7}. This is a conservative combination of priors that should favour models containing fewer species. The other divergence time parameters were assigned the Dirichlet prior (Yang and Rannala 2010).

Results

Sequence alignments

In the European dataset, we included 64 European collections of *Lf.* sect. *Piperati* and 4 outgroup specimens. The worldwide dataset contains 110 collections of *Lf.* sect. *Piperati*, 7 collections from sections and subgenera closely related to *Lf.* sect. *Piperati* and 2 outgroup specimens. After aligning with MAFFT and excluding ambiguously aligned positions with Gblocks, the European dataset contained an ITS alignment with 65 sequences of 726 bases and an overall distance of 0.067 base substitutions per site (standard error (S.E.): 0.010), an LSU alignment with 62 sequences of 910 bases and an overall distance of 0.017 base substitutions per site (S.E.: 0.003), an *RPB2*-alignment with 42 sequences of 695 bases and an overall distance of 0.075 base substitutions per site (S.E.: 0.008), and an *ATP6*-alignment with 63 sequences of 622 bases and an overall distance of 0.036 base substitutions per site (S.E.: 0.006) (Table 4.1). The worldwide dataset included an ITS alignment with 115 sequences of 771 bases and an overall distance of 0.086 base substitutions per site (S.E.: 0.010), an LSU alignment with 103 sequences of 918 bases and an overall distance of 0.022 base substitutions per site (S.E.: 0.003), an *RPB2*-alignment with 80 sequences of 750 bases and an overall distance of 0.079 base substitutions per site (S.E.: 0.006), and an *ATP6*-alignment with 98 sequences of 665 bases and an overall distance of 0.036 base substitutions per site (S.E.: 0.005) (Table 4.1).

Phylogenetic analyses

In the European dataset, the single-locus ML and BI analyses show almost identical topologies, although not every clade is fully supported for each locus. In both multi-locus analyses, each clade is fully supported (Fig. 4.1). *Lactifluus* sect. *Piperati* consists of two well supported clades, *Lf. piperatus* and *Lf. glaucescens*, which cannot be further divided into supported subclades. These results thus disagree with the hypothesis of four European species.

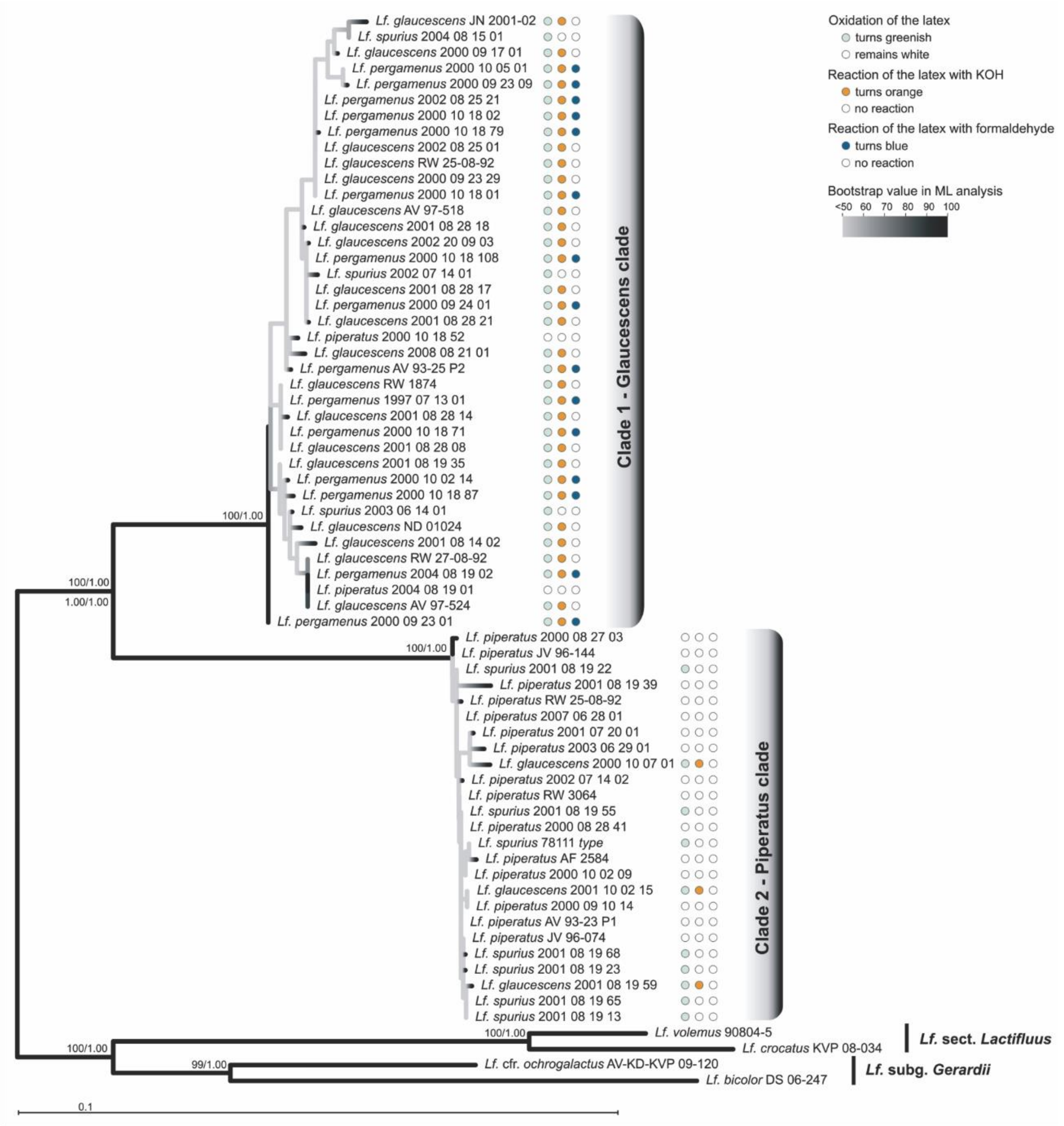

Fig. 4.1 Maximum likelihood tree of the European dataset, based on the concatenated data of ITS, LSU, *RPB2* and *ATP6* sequences. Voucher names given in the tree are the provisional names as explained in the Introduction. Branch colours indicate statistical support of the clades: black branches are strongly supported, light grey branches are poorly resolved. Intermediate shades of grey represent intermediate support (see gradient legend). Bootstrap values >50 and posterior probabilities >0.95 are shown above branches. Posterior probabilities from the *BEAST analysis and the species probabilities from the BPP analysis are plotted below the branch of the split between *Lf. glaucescens* and *Lf. piperatus*.

The multi-locus ML and BI analyses of the worldwide dataset show almost identical topologies (Fig. 4.2), with only some minor conflicts (e.g. in clade 1, the relative position of the clades *Lf. leucophaeus* Asia 1 and *Lf. glaucescens* North America 2 differs between both analyses, and the position of some singletons within clade 2 differs, but in neither analyses these positions are supported). These analyses show that worldwide, this section is divided in three clades, which we gave the working names "clade 1 – Glaucescens clade",

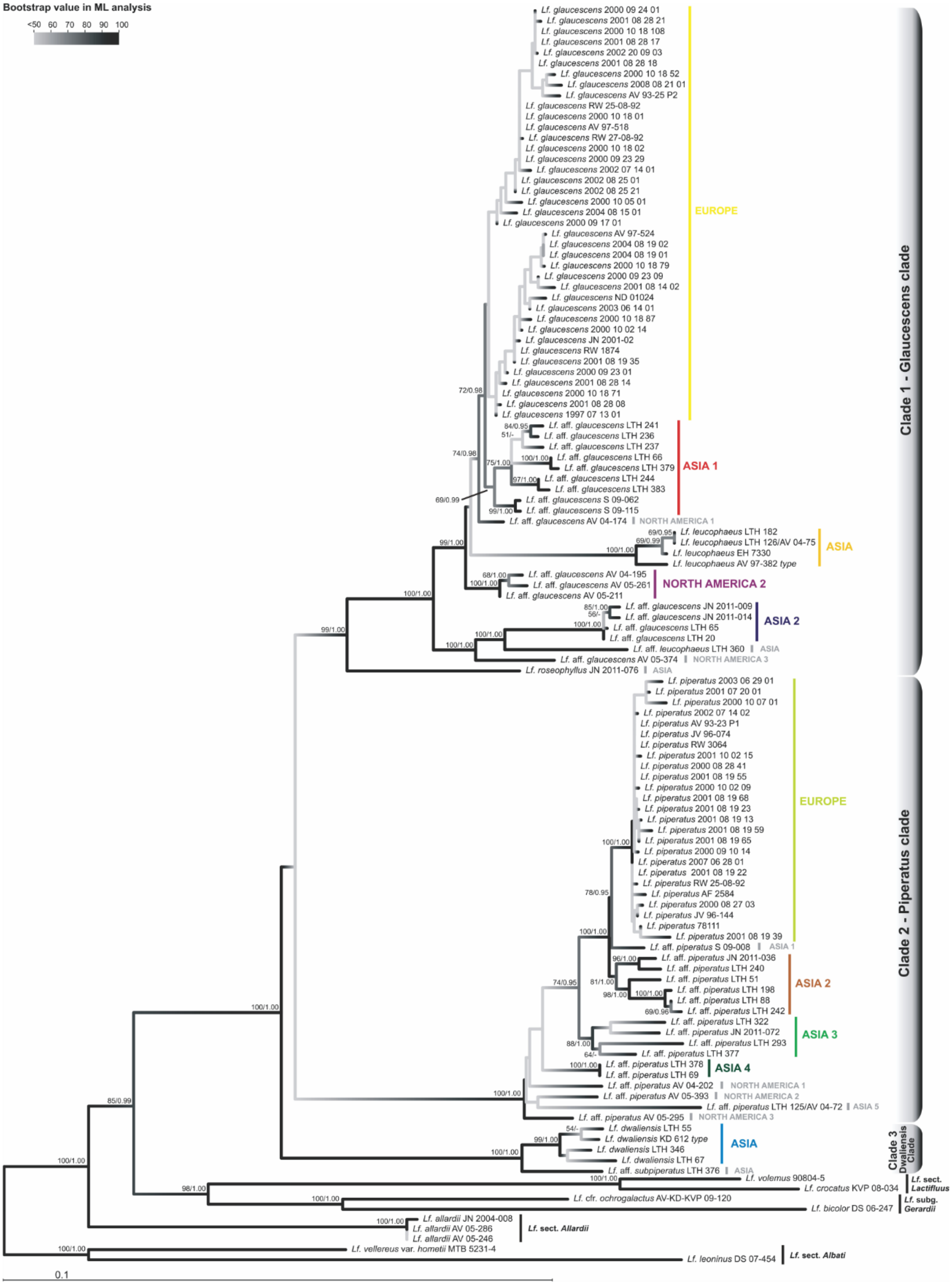

Fig. 4.2 Maximum likelihood tree of the worldwide dataset, based on the concatenated data of ITS, LSU, *RPB2* and *ATP6* sequences. Voucher names given in the tree are the revised identifications as explained in the results section. Branch colours indicate statistical support of the clades: black branches are strongly supported, branches in light grey are poorly supported. Intermediate shades of grey represent intermediate support (see gradient legend). Bootstrap values >50 and posterior probabilities >0.95 are shown.

“clade 2 – Piperatus clade” and “clade 3 – Dwaliensis clade”. All three clades are highly supported, but the position of the third clade relative to the two other clades is not resolved. In clade 1 we see some clearly delimited and highly supported subclades, such as at least three Asian subclades (*Lf.* aff. *glaucescens* Asia 1 (bootstrap value only 69), *Lf.* aff. *glaucescens* Asia 2 and *Lf. leucophaeus* Asia), one North American subclade (*Lf.* aff. *glaucescens* North America 2) and one European subclade (*Lf. glaucescens* Europe), although this latter subclade is not supported in the worldwide phylogeny. Likewise, some highly supported subclades could be delimited in clade 2, such as at least three Asian subclades (*Lf.* aff. *piperatus* Asia 2, 3 and 4) and one European subclade (*Lf. piperatus* Europe). The third clade consists of one fully supported subclade (*Lf. dwaliensis* Asia). Additionally, all three clades contain one or more single specimens which do not fall within the subclades discussed above. Further research and additional sampling may point out that they form separate subclades as well. The single-locus ML analyses show different topologies, with a considerable amount of conflict (Fig. 4.3). Likewise, the BI results show different topologies for each locus, with many conflicting clades. In each gene tree, clade 2 and 3 are monophyletic and well supported. Clade 1, however, is often paraphyletic and not supported. Within each of the three clades, the subclades often switch positions and split up.

Bayesian species delimitation

In the European analysis, the ML and BI analyses clearly showed two monophyletic clades, so we assume two species in Europe. The *BEAST analysis resulted in a species tree that highly supports the same clades and rejects the hypothesis of four European species (Fig. 4.1). BPP supports the guide tree of two species with a speciation probability of 1.0, and different prior distributions for θ and τ_0 did not affect this outcome (Fig. 4.1).

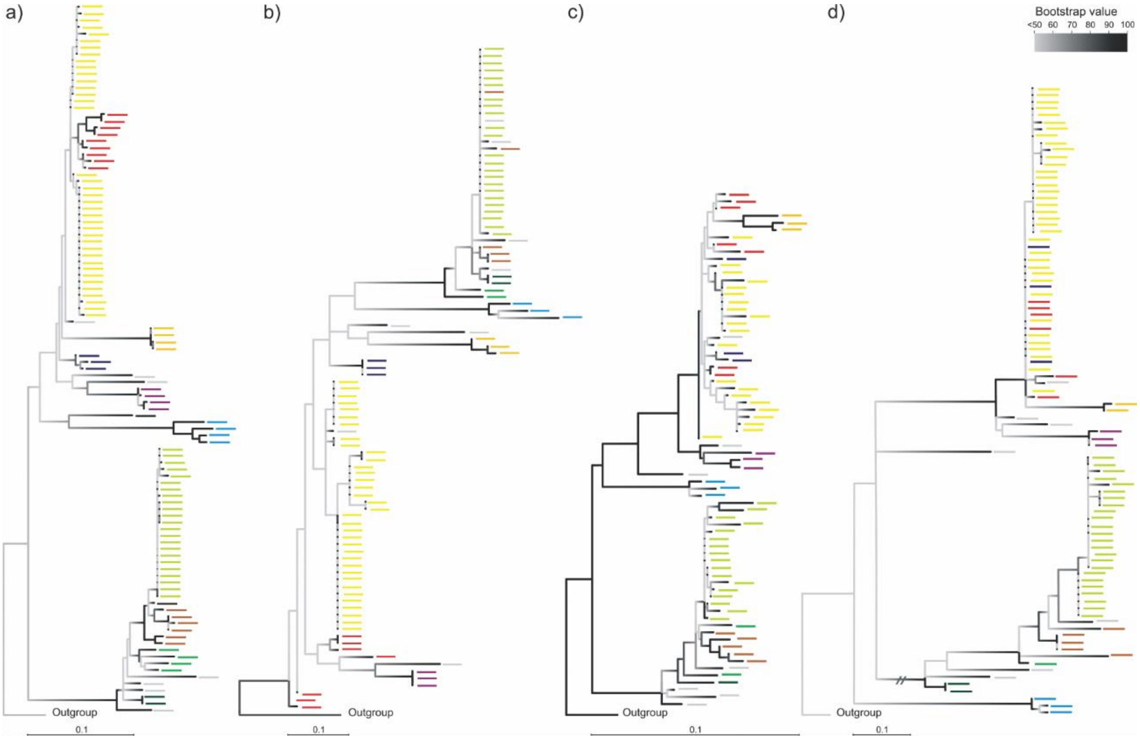

Fig. 4.3 Maximum likelihood gene trees for **a.** ITS, **b.** LSU, **c.** *RPB2* and **d.** *ATP6*, with the colour code of the provisional species as in Fig. 4.2, showing lack of monophyly for certain clades. Bootstrap values are shown by the grey scale (see gradient legend).

Taxonomy of the European species

The molecular results indicate that the current descriptions of *Lactifluus piperatus* and *Lf. glaucescens* require some adjustments. In the following paragraph, we give the new descriptions of both species, based on literature and own observations on herbarium and freshly collected specimens listed in table 4.1.

Lactifluus piperatus (L.: Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891. (Fig. 4.4)

Basionym: *Agaricus piperatus* L., Sp. pl.: 1173. 1753.
≡ *Lactarius piperatus* (L.: Fr.) Pers., Tent. disp. meth. Fung.: 64. 1797.
≡ *Galorrheus piperatus* (L.: Fr.) Fr., Stirp. agri femsion. (III): 57. 1825.
Synonym: *Lactifluus pergamenus* (Sw.: Fr.) Kuntze, Revis. gen. pl. II: 857. 1891. sensu Romagnesi (1956, 1980), Damblon et al. (1956), Heineman (1960), et al.

Neotypus (designated here): Sweden, Uppsala, Nåsten, close to Håga, N 59.84° E 17.57°. *Habitat*: shrubbery at the forest edge, in some places open, but also with some larger trees, many bushes (*Corylus avellana*) cut back earlier the same year; mixed including conifers and birch; with *Quercus* sp., *Corylus avellana* and *Populus* sp. nearby. 9 August 2004, Eberhardt U. 09.08.2004-6 (neotype UPPSALA, isoneotype GENT), GenBank accession numbers: ITS + LSU = DQ422035, *RPB2* = DQ421937. This collection was not included in the Bayesian species delimitation study, but both morphological and molecular studies (based on ITS, LSU and *RPB2*) show that this collection belongs to *Lf. piperatus*.

Pileus 40–120(–160) mm, at first convex with slightly depressed centre and decurved margin, with age expanding and becoming more depressed in the centre; surface smooth, dry, finely cracked, matt or slightly shiny, concentrically wrinkled towards margin, whitish to whitish chrome or cream, typically darkest in the centre, sometimes with buff coloured spots. *Lamellae* at first broadly adnate, then slightly decurrent to decurrent, very crowded, very narrow (1.5 mm), with some evenly distributed forkings, pale cream to cream with a pale orange tinge. *Stipe* 40–95 × 12–30 mm, cylindric or tapering downwards, smooth or uneven, dry, white, tinged whitish chrome or pale cream, becoming buff or brownish from base. *Context* firm to very firm, solid, white, tinged whitish chrome, becoming more yellow when drying, lemon-yellow in the stem base, not reacting with (sulpho)formaldehyde; taste very acrid after a short while; smell slightly acidic, distinctly honey- or apple-like when drying. *Latex* not very abundant, white, drying whitish or greyish green, usually unchanging, but sometimes yellow to orange with KOH, taste becoming very acrid after a while. *Spore deposit* white.

Basidiospores 7.0–10.4 × 5.2–7.5 µm, av. 8.0–8.5 × 5.9–6.3 µm, subglobose to oblong, Q= 1.10–1.65, av. Q= 1.28–1.40; ornamentation up to 0.2 µm high, consisting of irregularly rounded to elongate warts which are aligned or connected by lower lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 40–45 × 7–9(–10) µm, cylindric to subclavate, (2- or) 4-spored. *Pleuromacrocystidia* abundant, 50–70(–90) × 8–11 µm. *Lamellae-edge* heterogeneous. *Cheilomacrocystidia* 35–55 × 5–10 µm. *Hymenophoral trama* predominantly consisting of hyphae, with many lactiferous hyphae and sometimes sphaerocytes. *Pileipellis* a hyphoepithelium; suprapellis distinct in young specimens, of 2–4 µm broad, hyaline hyphae, becoming very thin when mature (10–30 µm) and clearly showing the underlying cellular layer; subpellis subcellular; dermatocystidia abundant in suprapellis, up to 7 µm broad, cylindric to clavate.

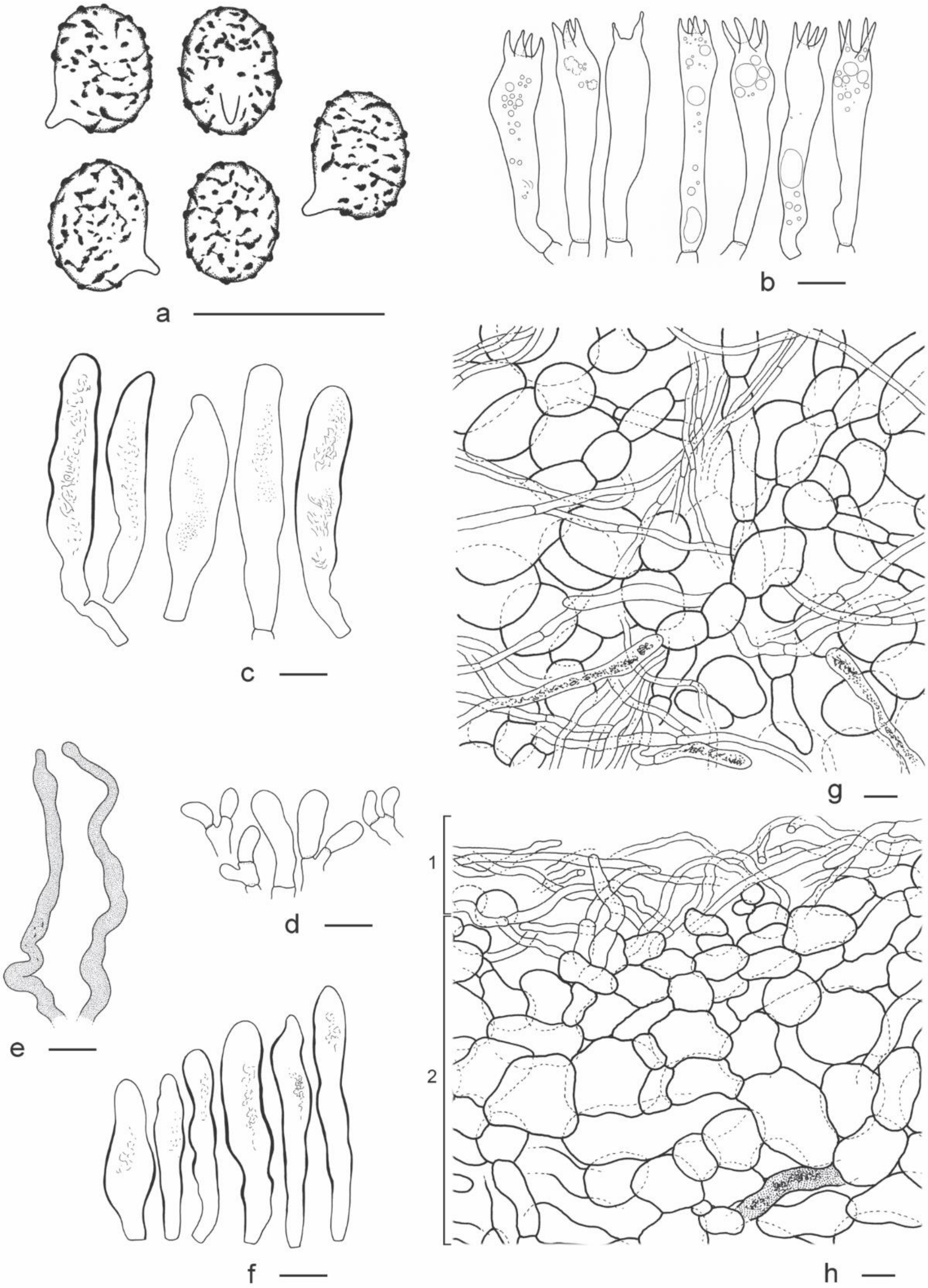

Fig. 4.4 *Lf. piperatus*, **a.** basidiospores (UE 09.08.2004-6, type), **b.** basidia (1-3: AV-RW 93-023, 4-7: JV 96-144), **c.** pleuromacrocystidia (JV 96-144), **d.** marginal cells (JV 96-144), **e.** pleuropseudocystidia (JV 96-144), **f.** cheilomacrocystidia (JV 96-144), **g.** scalp of the pileipellis, with dermatocystidia (AV-RW 93-023), **h.** cross-section of the pileipellis, with suprapellis (1) and subpellis (2) (HP 8475).

Lactifluus glaucescens (Crossl.) Verbeken (Fig. 4.5)

Basionym: *Lactarius glaucescens* Crossl., Naturalist, J. Nat. Hist. N. England 1900(516): 5. 1900.
≡ *Lactarius piperatus* var. *glaucescens* (Crossl.) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 186. 1979

Synonym: *Lactifluus pergamenus* (Sw.: Fr.) Kuntze, Revis. gen. pl. II: 857. 1891. sensu Blum (1966, 1976), Marchand (1980), Bon (1980), et al.

Holotypus: England, West Yorkshire (K), Crossland 1900

Pileus 50–150 mm, convex to plane with a depressed centre; surface smooth, dry, indistinctly velutinous, rather shiny, with irregular dots and darker spots, sometimes slightly wrinkled, white to pale cream. *Lamellae* decurrent, very narrow (2 mm broad), very crowded, whitish, turning greenish by the milk and becoming dirty brownish many hours after bruising. *Stipe* 30–90 × 10–40 mm, usually shorter than the cap diameter; surface smooth, dry, white to pale cream. *Context* very firm and thick, white, becoming bluish green after hours, sometimes becoming blue with (sulpho)formaldehyde; smell faintly honey-like when drying; taste acrid. *Latex* not very abundant, white, often becoming bluish to greyish green when drying, most often but not always yellow to orange with KOH; taste immediately very acrid. *Spore deposit* white.
Basidiospores 6.5–9.3 × 5.3–6.9 µm, av. 7.4–8.5 × 5.8–6.4 µm, subglobose to ellipsoid, Q = 1.05–1.45, av. Q = 1.26–1.33; ornamentation up to 0.2 µm high, of irregular warts, which are isolated, aligned or connected by lower lines, but never forming a reticulum; plage predominantly inamyloid, occasionally with a slightly amyloid spot. *Basidia* 45–50 × 7–9 µm, cylindric to subclavate, (2- or) 4-spored. *Pleuromacrocystidia* abundant, originating deep in the trama, mostly strongly emergent, 60–90 × 7–10 µm. *Lamella edge* almost exclusively with strongly emergent cheilomacrocystidia of 55–70 × 7–9 µm. *Hymenophoral trama* predominantly consisting of hyphae, with abundant lactiferous hyphae and sometimes sphaerocytes. *Pileipellis* a hyphoepithelium; suprapellis 80–120 µm thick, hiding the underlying cellular layer, consisting of thin, hyaline hyphae, (1–)2–4 µm broad in upper part, 3–5(–6) µm broad in lower part; subpellis almost completely cellular; dermatocystidia abundant in suprapellis, up to 4 µm broad, cylindric to subclavate.

Nomenclature of the North American species

Lactifluus angustifolius (Hesler & A.H. Sm.) De Crop, **comb. nov.**
MYCOBANK 116067

Basionym: *Lactarius angustifolius* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 190. 1979.
≡ *Lactarius albus* Thiers, Mycologia 49 (5): 712. 1957. (nom. illeg., art. 53.1 ICBN)

Lactifluus neuhoffii (Hesler & A.H. Sm.) De Crop, **comb. nov.**
MYCOBANK 116190

Basionym: *Lactarius neuhoffii* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 179. 1979.

Lactifluus neuhoffii* var. *fragrans (Burl.) De Crop, **comb. nov.**
MYCOBANK 117770

Basionym: *Lactarius piperatus* f. *fragrans* Burl., Mem. Torrey Bot. Club 14: 20. 1908.
≡ *Lactarius neuhoffii* var. *fragrans* (Burl.) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 182. 1979.

Lactifluus waltersii (Hesler & A.H. Sm.) De Crop, **comb. nov.**
MYCOBANK 116132

Basionym: *Lactarius waltersii* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 183. 1979.

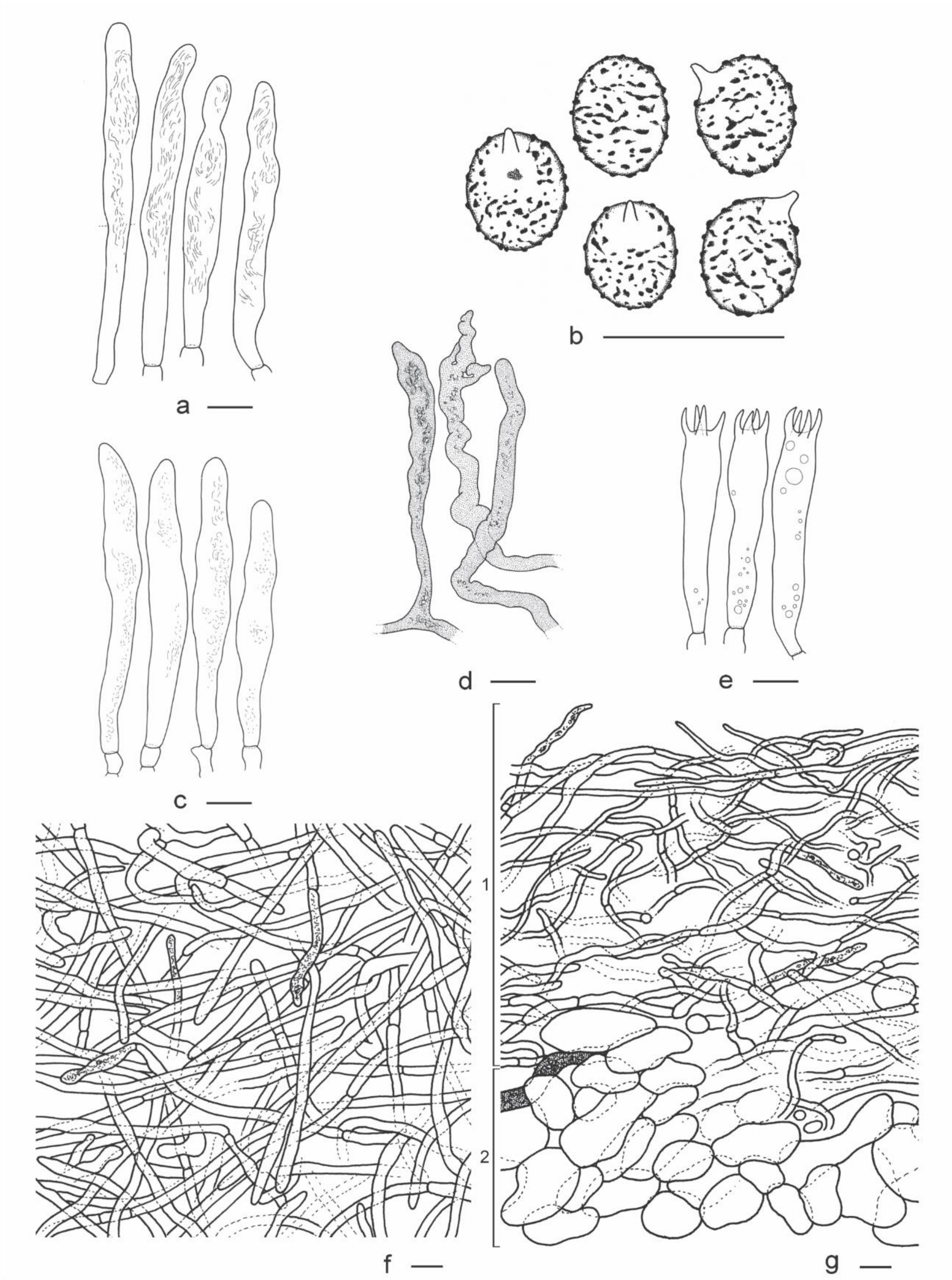

Fig. 4.5 *Lf. glaucescens*, **a.** pleuromacrocystidia (AV 93-021), **b.** basidiospores (AV-RW 93-025), **c.** cheilomacrocystidia (AF 2147), **d.** pleuropseudocystidia (AV 93-021), **e.** basidia (AV-RW 93-025), **f.** scalp of the pileipellis, with dermatocystidia (AV 93-021), **g.** cross-section of the pileipellis, with suprapellis (1) and subpellis (2) (AF 1898).

Discussion

Lactifluus section Piperati in Europe

Our study of *Lf.* sect. *Piperati* shows that the section contains two highly supported species in Europe: *Lf. glaucescens* and *Lf. piperatus* (Fig. 4.1). This result, obtained using molecular data, contradicts our starting hypothesis that this section was possibly represented by four species in Europe, a distinction based on morphological and macrochemical reactions of the latex and the context. Our findings demonstrate that a colour change of drying latex (greenish versus unchanging) is not a diagnostic characteristic. Both *Lf. piperatus* and *Lf. glaucescens* clades contain collections with the latex turning greenish when drying. Our findings reject the diagnostic value of the macrochemical characteristics of the latex and the context to delineate species within this section, since both clades of *Lf. piperatus* and *Lf. glaucescens* contain collections that display a colour reaction of the latex with KOH and the colour reaction of the context with (sulpho)formaldehyde is not a unique characteristic for either one of the species.

Our phylogenetic results support the species recognised by modern revisions (Verbeken et al. 1997; Heilmann-Clausen et al. 1998; Basso 1999), who based their conclusions mainly on the microscopical characteristics of the pileipellis (Figs. 4.4g–h, 4.5f–g). Likewise, the differences in composition of the lamella edge and the length of the cheilomacrocystidia remain good diagnostic characteristics. Contrary to the descriptions of Heilmann-Clausen et al. (1998), Basso (1999) and Verbeken et al. (1997), we show that the macrochemical reactions are not useful as a diagnostic characteristic. Our experience in determining milkcap species from both *Lactarius* and *Lactifluus* taught us that the colour change of the latex in contact with KOH is largely depending on the time interval between isolating the latex and bringing it in contact with the solution. To accommodate to this effect, we used a strict protocol for applying the chemicals, as described in the materials and methods section. Additionally, the reaction with the chemicals often varies with the age and the condition of the specimens. This is in accordance with the observations within the genus *Lactarius*, subsection *Triviales*. One of the characteristics often used to distinguish between *L. trivialis* and *L. utilis* is the reaction of the latex with KOH, which turns orange-yellow in *L. trivialis* and is unchanging in *L. utilis* (Heilmann-Clausen et al. 1998). However, this reaction appears to be strongly dependent on the time between isolating the latex and bringing it in contact with the KOH-solution. For both species, the reaction turns out more positive when the KOH is added on dry latex (unpubl. data). Romagnesi (1980) further indicated that the reaction of the context of species from *Lf.* sect. *Piperati* with sulphoformaldehyde is strongly dependent on the stage of development of the specimen.

During our European study, we predominantly focused on collections from Belgium and France, as the herbarium specimens of these regions are provided with comprehensive macroscopic descriptions and macrochemical tests according to our protocol. We realize that this is a rather limited distribution, but until now, all the samples from other European countries that we included in the study fall within one of the two European clades, so we assume that there are two species within *Lf.* section *Piperati* in Europe. However, we cannot completely rule out the possibility of another species from East or South Europe, therefore additional sampling in those regions is needed.

Lactifluus section Piperati worldwide

The worldwide phylogeny presented here suggests at least ten potential species within *Lf.* section *Piperati*, divided over three clades (Fig. 4.2). The actual number of species is likely to be higher, since by analysing the Asian and North American collections, the variation amongst those collections appeared to be much larger than previously thought by field determinations. This led to an undersampling of certain potential species, since they were only represented by one or two collections. Consequently, it was not possible to carry out a *BEAST analysis to construct a species tree, as that method requires more than one specimen per species to calculate the coalescent event for that extant species. The information on the coalescent event is needed to estimate the population size, which is in turn needed to infer speciation times and species topology (Heled and Drummond 2010). Deleting these singletons would lead to a reduction of the Asian and North American datasets and consequently to an underestimation of the actual number of species within this section. We can conclude that additional sampling is needed to get a better view on the actual species composition of *Lf.* sect. *Piperati* and to be able to construct a species tree using *BEAST, to correct for the potential amount of incomplete lineage sorting present in these data (Fig. 4.3).

Our preliminary analyses of the concatenated dataset denote that the European species are not found in North America or Asia and vice versa, so there is no intercontinental conspecificity. The first clade within the section worldwide contains three strongly supported subclades: *Lf. leucophaeus* Asia, *Lf.* aff. *glaucescens* North America 2 and *Lf.* aff. *glaucescens* Asia 2 (Fig. 4.2). The *Lf. leucophaeus*-clade is positioned on a long branch, which may indicate that this species underwent many changes since its split from the most recent common ancestor which it shares with *Lf.* aff. *glaucescens* North America 1, *Lf.* aff. *glaucescens* Asia 1 *and Lf. glaucescens* Europe. Morphologically, this species, with latex that changes from white to bluish green, differs from all European representatives of *Lf. glaucescens* by darker pileus colours (greyish brown) and a thinner layer of hyphae in the pileipellis (Verbeken and Horak 1999). So far, the other well-supported clades have not been morphologically investigated. The clade *Lf.* aff. *glaucescens* Asia1 is weakly supported (BS: 69, PP: 0.99) and the *Lf. glaucescens* clade from Europe is not supported at all (BS: 44, PP: 0.48). The latter can indicate that some other processes are going on here, such as hybridisation or a recent divergence between the European clade, the Asia 1 clade and the North America 1 clade. To elucidate this, a more thorough sampling is needed, especially from the Asian and North American representatives. There are also four singletons within clade 1 (AV 04-174 and AV 05-374 from North America, LTH 360 and JN 11-076 from Asia). Only one of these singletons is morphologically identified as a separate species, namely *Lf. roseophyllus* (JN 11-076), which differs from the remainder of clade 1 by its pink salmon and creamy coloured latex, and by its salmon orange to pale orange-brownish coloured lamellae (Heim 1966 and field observations). These morphological differences and its distant position support the delimitation of *Lf. roseophyllus*.

The second clade shows four highly supported subclades: one European and three Asian subclades (*Lf.* aff. *piperatus* Asia 2, 3 & 4; Fig. 4.2). Until now, none of those Asian subclades have been morphologically investigated. One of these subclades, *Lf. piperatus* Asia 3, contains four specimens from Thailand and Vietnam on rather long branches. Further morphological examination and Bayesian species delimitation might clarify if the specimens from this subclade really belong to the same species, or if this subclade needs to be split into separate smaller subclades. This clade also includes five singletons (AV 04-202, AV 05-393 and AV 05-295 from North America, S 09-008 and LTH 125/AV 04-072 from Asia) and none of them were previously described as a separate species.

Finally, the third clade consists of one well defined subclade, together with one singleton (LTH 376), and all collections are from Asia. The clade differentiates morphologically from the rest of the section by its distant cream-coloured lamellae. Most of the specimens in this clade were originally identified in the field as *Lf. subpiperatus*, but after microscopical examination, Le (2007) found that these specimens have bigger and more globose spores than *Lf. subpiperatus*. Additionally, she found that these specimens have distinct pleuromacrocystidia, while these are absent in *Lf. subpiperatus*. We also succeeded to sequence the *ATP6*-region for the type specimen of *Lf. dwaliensis* and it falls within this clade. This might lead to the conclusion that all those specimens are representatives of *Lf. dwaliensis*. Although the third clade itself is well supported, its position relative to the other two clades is still uncertain. In the different gene trees, this clade jumps from being a sister clade to clade 1, to being a sister clade to both clade 1 and clade 2. Additional sampling and the sequencing of more markers may elucidate the position of this third clade within *Lf.* sect. *Piperati*.

The genus *Lactifluus* is known to contain species complexes with cryptic and semi-cryptic diversity. Explicit examples are *Lf.* subg. *Gerardii* and *Lf.* sect. *Lactifluus.* Stubbe et al. (2010) uncovered at least 30 strongly supported clades in *Lf.* subg. *Gerardii*, of which only 18 are morphologically identifiable species. In their study of *Lf.* sect. *Lactifluus* from Thailand, Van de Putte et al. (2010) elucidated 18 phylogenetic species, where of six species are also morphologically distinguished; and in their study of *Lf.* sect. *Lactifluus* in India, Van de Putte et al. (2012) showed the existence of six species, of which three were newly described based on phylogeny and morphology. Preliminary studies on African *Lactifluus* sections also suggest the presence of cryptic and/or semi-cryptic diversity in *Lf.* sect. *Pseudogymnocarpi* (unpubl. data). In accordance with those results, our preliminary worldwide study suggests that *Lf.* section *Piperati* may contain cryptic and/or semi-cryptic species. To clarify this assumption, a more thorough sampling is needed, especially in Asia and North America, where a lot of countries and states are underexplored.

Because of the rather cryptic morphology and the low support in the worldwide gene trees, we will not describe the non-European clades as new species yet. First the sampling should be increased and the problem of contradicting and poorly supported gene trees should be treated in detail. In contrast to results from other studies within the genus *Lactifluus* (Stubbe et al. 2010; Van de Putte et al. 2010; Van de Putte et al. 2012), the phylogenetic markers used here appear not to be as effective to strongly support species within *Lf.* sect. *Piperati*. As indicated by their low evolutionary divergence, LSU and *ATP6* are too conservative and therefore contain not enough phylogenetic signal to delimit species within this section. ITS and *RPB2* are informative, but the amplification of *RPB2* failed for many collections. This can be explained by the fact that the majority of the herbarium specimens used for this study are between 10-20 years old. Both more recent material (preferably stored on CTAB buffer) and more informative markers could be helpful in improving these results.

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CHAPTER 5

Southeast Asia reveals new diversity in *Lactifluus* section *Gerardii*: six new species with pleurotoid or small agaricoid basidiocarps

Abstract

During several consecutive sampling expeditions in Thailand, multiple collections of milkcap species with small pleurotoid or agaricoid basidiocarps were found. Collections were morphologically compared with herbarium material. Molecular research indicated that four collections belonged to undescribed species within *Lactifluus* sect. *Gerardii*, as was also the case for herbarium collections of a pleurotoid species collected in Nepal by H. R. Bhandary. One other collection from Thailand appeared to be closely related to *Lactifluus uyedae*, known from Japan. All species are described and we propose five new species for the genus *Lactifluus*: *Lf. auriculiformis*, *Lf. gerardiellus*, *Lf. bhandaryi*, *Lf. pulchrellus* and *Lf. raspei*.

Unpublished manuscript: Eske De Crop, Felix Hampe, Komsit Wisitrassameewong, Dirk Stubbe, Jorinde Nuytinck & Annemieke Verbeken. Southeast Asia reveals new diversity in *Lactifluus* section *Gerardii*: six new species with pleurotoid or small agaricoid basidiocarps.

Introduction

Lactifluus sect. *Gerardii* (A.H. Sm. & Hesler) Stubbe was recently described as a morphologically and molecularly well supported section within *Lf*. subg. *Lactifluus*, with a disjunct distribution in America and subtropical to tropical Asia and Australasia (Stubbe et al. 2010; Stubbe et al. 2012a; De Crop et al. acpt.). Although often superficially resembling representatives of *Lactarius* subg. *Plinthogalus* (Burl.) Hesler & A.H. Sm., species of *Lf.* sect. *Gerardii* can be recognized by a combination of macro- and microscopical characters. In the original description of the section, following characteristics were mentioned as most typical: habitus combining a brown stipe and pileus contrasting with the white, mostly distant lamellae, a white spore print (important difference with *Lactarius* subg. *Plinthogalus*, where the spore print is cream to ochraceous), reticulate spore ornamentation not higher than 2 µm (with ridges or interconnected warts), a palisade structure in the pileipellis and generally the lack of macrocystidia. Besides these dark pigmented and agaricoid representatives, the group also includes small, white pleurotoid species. As in other clades, it is now accepted in the Russulales that the fruit body shape has long been overestimated as a phylogenetic feature, and that agaricoid species are very closely related to angiocarpous and pleurotoid species (De Crop et al. acpt.). Since the recent splitting of the milkcaps into three genera: *Multifurca* Buyck & V. Hofstetter, *Lactarius* Pers. (hereafter abbreviated as *L.*) and *Lactifluus* (Pers.) Roussel (hereafter abbreviated as *Lf.*), it remains a challenge to find good synapomorphic characters for the two large milkcap genera *Lactifluus* and *Lactarius*. The differences are currently based on several trends (Verbeken and Nuytinck 2013). One of these trends concerns the fruiting bodies. Milkcaps are mainly agaricoid, but angiocarpous and pleurotoid milkcaps do occur. Angiocarpous species are so far only found in *Lactarius*, while pleurotoid species are so far only found in *Lactifluus* (Verbeken and Nuytinck 2013).

The pleurotoid habitus developed more than once in *Lactifluus* (Stubbe et al. 2010; De Crop et al. acpt.) and all pleurotoid milkcaps are characterised by a white spore print and thick-walled terminal elements. Stubbe et al. (2012b) include three pleurotoid species in *Lf.* sect. *Gerardii*: *Lactifluus conchatulus* (Stubbe & H.T.Le) Stubbe from Thailand, *Lactifluus uyedae* (Singer) Verbeken from Japan and *Lactifluus genevievae* (Stubbe & Verbeken) Stubbe from Tasmania. Latha et al. (2016) recently described a new Indian pleurotoid species within the section: *Lf. indicus* K. N. A. Raj & Manim. Other pleurotoid species are placed within two other subgenera of *Lactifluus* (De Crop et al. acpt.). Two species are known from *Lf.* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop: *Lactifluus panuoides* (Singer) De Crop from both Central and South America, and *Lactifluus brunellus* (S.L. Miller, Aime & TW Henkel) De Crop from Guyana (Miller et al. 2002). Two species are known from *Lf.* subg. *Lactariopsis* (Henn.) Verbeken: *Lactifluus multiceps* (S.L. Miller, Aime & TW Henkel) De Crop from Guyana (Miller et al. 2002) and *Lactifluus chrysocarpus* E.S. Popov & O.V. Morozova from Vietnam (Morozova et al. 2013). The pleurotoid species *Lactarius campinensis* Singer from Brazil was recombined in *Russula*: *Russula campinensis* (Singer) Henkel, Aime & S.L. Mill. (Henkel et al. 2000).

Species with small agaricoid basidiocarps are also recorded several times within *Lactifluus*. Within *Lf.* sect. *Gerardii*, the Chinese *Lf. parvigerardii* X.H. Wang & Stubbe (Wang et al. 2012), the Vietnamese *Lactifluus igniculus* O. V. Morozova & E. S. Popov (Morozova et al. 2013) and the Indian *Lf. umbonatus* K. P. D. Latha & Manim. (Latha et al. 2016) are known. Within *Lf.* sect. *Lactariopsis*, one African species is known: *Lf. uapacae* (Verbeken & Stubbe) De Crop (Verbeken et al. 2008), and within *Lf.* sect. *Edules* (Verbeken) Verbeken, also one species is known: *Lf. roseolus* (Verbeken) Verbeken (Verbeken 1996).

In chapter 3, we performed GMYC species delimitation on a dataset comprising 1306 *Lactifluus* sequences, resulting in at least 461 species (CI: 428–481). Among these species, five new lineages were found, that might represent new species. Based on the molecular results of chapter 3 (De Crop et al. acpt.) and additional morphological and molecular data, this paper proposes three new Asian pleurotoid *Lactifluus* species and two species with very small agaricoid basidiocarps. Furthermore, it describes a possible new record for *Lf. uyedae*.

Material and methods

Sampling

In our dataset we included species of *Lf.* subg. *Lactifluus*, with a focus on *Lf.* sect. *Gerardii*. The majority of new specimens was collected by the authors in Thailand, Chiang Mai, and three specimens were collected in Nepal, by Hemanta Ram Bhandary. Furthermore, we added several representatives of the other *Lactifluus* subgenera and as outgroup we included five species of the genus *Lactarius* (Table 5.1).

Morphological analyses

Macroscopic characters are all based on fresh material. Colour codes refer to Kornerup and Wanscher (1978). Microscopic features were studied from dried material mainly in Congo-red in L4. Spore ornamentation is described and illustrated as observed in Melzer's reagent. For details on terminology we refer to Verbeken (1998) and Verbeken & Walleyn (2010). Line-drawings were made by A. Verbeken, with the aid of a drawing tube at original magnifications: 6000 × for spores, 1000 × for individual elements and sections. Basidia length excludes sterigmata length. Spores were measured in side view in Melzer's reagent, excluding the ornamentation, and measurements are given as described in Nuytinck and Verbeken (2005): (MIN) [Ava-2×SDa] – Ava – Avb – [Avb+2×SDb] (MAX) in which Ava = lowest mean value for the measured collections, Avb = greatest mean value and SDa/b = standard deviation of the lowest and greatest mean value respectively. MIN is the lowest value measured, MAX the highest value; MIN and MAX are only given when they exceed [Ava-2×SDa] or [Avb+2×SDb] respectively. Q stands for 'quotient length/width' and is given as MINQ – Qa – Qb – MAXQ in which Qa and Qb stand for the lowest and the highest mean quotient for the measured specimens respectively. MINQ/MAXQ stands for the minimum/maximum value over the quotients of all available measured spores.

Molecular analyses

DNA was extracted using the CTAB extraction described in Nuytinck & Verbeken (2003). PCR amplification protocols for follow Le et al. (2007). We used two nuclear markers that were previously shown informative within this genus (Stubbe et al. 2010; De Crop et al. acpt.): (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, and using primers ITS-1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) and (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000).

PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Life Technology) at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with the Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v7 (Katoh and Toh 2008), using the E-INS-I strategy. We trimmed trailing ends of the alignment and manually edited sequences when necessary in Mega 6 (Tamura et al. 2013). The alignment can be acquired from the first author and TreeBASE (to be submitted).

Following partitions were assigned to the sequence data: partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Maximum likelihood (ML) analyses were conducted with RAxML v8.0.24 (Stamatakis 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis et al. 2008). All analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

Table 5.1 Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses.

Species	**Voucher collection (herbarium)**	**Country**	**ITS accession no.**	**LSU accession no.**
Lf.* subg. *Lactifluus				
Lf. sect. Allardii				
Lf. allardii	JN 2004-008 (GENT)	USA	KF220016	KF220125
Lf. sect. Ambicystidiati				
Lf. ambicystidiatus	HKAS J7008 (HKAS)	China	KR364108	KR364239
Lf. sect. Gerardii				
Lf. atrovelutinus	DS 06-003 (GENT)	Malaysia	GU258231	GU265588
Lf. auriculiformis	AV 12-050 (GENT)	Thailand	KR364086	KR364216
Lf. bicolor Epitype	DS 06-229 (GENT)	Malaysia	GU258221	GU265577
Lf. conchatulus Type	LTH 457 (GENT)	Thailand	GU258296	GU265659
Lf. cf. conchatulus	EDC 14-502 (GENT)	Thailand	To submit	None
Lf. coniculus Type	DS 07-496 (GENT)	Sri Lanka	GU258236	GU265594
Lf. fuscomarginatus Type	LM 4379 (XAL)	Mexico	HQ168367	HQ168367
Lf. genevievae Type	GG-DK 17-02-05 (GENT)	Australia	GU258294	GU265657
Lf. gerardiellus	KW386 (GENT)	Thailand	To submit	To submit
Lf. aff. gerardii	LTH 394 (GENT)	Thailand	GU258249	GU265610
Lf. aff. gerardii	FRIM 1098 (FRIM)	Malaysia	GU258232	GU265589
Lf. gerardii	AV 05-375 (GENT)	USA	GU258254	GU265616
Lf. aff. gerardii	DS 07-390 (GENT)	Thailand	GU258252	GU265613
Lf. gerardii	TMI 15534 (TMI)	Japan	GU258229	GU265586
Lf. gerardii	AV 05-283 (GENT)	USA	GU258259	GU265621
Lf. gerardii	MC 04-259 (GENT)	Nepal	GU258234	GU265592
Lf. aff. *gerardii*	DS 07-373 (GENT)	Thailand	GU258242	GU265603
Lf. aff. *gerardii*	KIINA 126 (GENT)	China	GU258227	GU265584
Lf. cf. *gerardii* var. *fagicola*	JN 2007-029 (GENT)	Canada	GU258224	GU265582
Lf. hora Type	DS 07-502 (GENT)	Sri Lanka	GU258238	GU265596
Lf. aff. *igniculus*	LE 253908 (LE)	Vietnam	JX442760	JX442760
Lf. igniculus Type	LE 262983 (LE)	Vietnam	JX442759	JX442759
Lf. leae	FH 12-013 (GENT)	Thailand	KF432957	KR364213
Lf. leonardii	GG 07-02-04	Australia	GU258308	GU265668
Lf. limbatus	DS 06-247 (GENT)	Malaysia	JN388955	JN388987
Lf. cf. *ochrogalactus*	AV-KD-KVP 09-120 (GENT)	India	KR364130	KR364248
Lf. parvigerardii	KUN F61367 (KUN)	China	JF975641	JF975642
Lf. petersenii	AV 05-300 (GENT)	USA	GU258281	GU265642
Lf. bhandaryi Type	TENN 051830 (TENN)	Nepal	KR364111	KR364140
Lf. bhandaryi	TENN 051831 (TENN)	Nepal	To submit	To submit
Lf. bhandaryi	TENN 051832 (TENN)	Nepal	To submit	To submit
Lf. pulchrellus	KW 304/FH 12-037 (GENT)	Thailand	KR364092	KR364223
Lf. reticulatovenosus Type	EH 6472 (GENT)	Indonesia	GU258286	GU265649
Lf. sp.	EDC 14-517 (GENT)	Thailand	To submit	To submit
Lf. subgerardii	AV 05-269 (GENT)	USA	GU258263	GU265625

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
Lf. cf. *uyedae*	AV 12-070 (GENT)	Thailand	KR364090	KR364221
Lf. uyedae	MCA 584 (VPI)	Japan	None	AF218562
Lf. wirrabara s.l.	GG 24-01-04	Australia	GU258307	GU265667
Lf. wirrabara s.l.	PL 40509	New Zealand	GU258287	GU265650
Lf.* sect. *Lactifluus				
Lf. acicularis	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132
Lf. volemus	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175
Lf.* sect. *Piperati				
Lf. piperatus	2001 08 19 68 (GENT)	France	KF220119	KF241840
Lf. roseophyllus	JN 2011-076 (GENT)	Vietnam	KF220107	KF220202
Lf.* sect. *Tenuicystidiati				
Lf. subpruinosus	JN 2011-061 (GENT)	Vietnam	KR364046	KR364172
Lf.* subg. *Lactariopsis				
Lf. vellereus	ATHU-M 8077 (ATHU-M)	Greece	KR364106	KR364237
Lf. heimii	EDC 11-082 (GENT)	Tanzania	KR364040	KR364167
Lf. cyanovirescens	JD 988 (GENT)	Congo	KR364082	KR364211
Lf. multiceps	TH 9154A (BRG, DUKE)	Guyana	JN168731	None
Lf. chrysocarpus Type	LE 253907 (LE)	Vietnam	JX442761	JX442761
Lf.* subg. *Pseudogymnocarpi				
Lf. aff. *rubroviolascens*	EDC 12-051 (GENT)	Cameroon	KR364066	KR364195
Lf. luteopus	EDC 11-087 (GENT)	Tanzania	KR364049	KR364176
Lf. rugatus	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235
Lf. xerampelinus	MH 201176 (GENT)	Mozambique	KR364099	KR364231
Lf. armeniacus	EDC 14-501 (GENT)	Thailand	KR364127	None
Lf.* subg. *Gymnocarpi				
Lf. gymnocarpus	EDC 12-047 (GENT)	Cameroon	KR364065	KR364194
Lf. panuoides	RC/Guy 10-024 (LIP)	French Guiana	KJ786647	KJ786551
Lf. luteolus	AV 05-253 (GENT)	USA	KR364016	KR364142
Lf. clarkeae	MN 2004002 (L)	Australia	KR364011	HQ318205
Lf. brunellus	TH 9130 (BRG, DUKE)	Guyana	JN168728	None
***Lactarius* - Outgroup**				
Lactarius hatsudake	FH 12-052 (GENT)	Thailand	KR364085	KR364215
Lactarius olympianus	ED 08-018 (GENT)	USA	KR364089	KR364220
Lactarius scrobiculatus	JN 2001-058 (GENT)	Slovakia	KF432968	KR364219
Lactarius fuliginosus	MTB 97-24 (GENT)	Sweden	JQ446111	JQ446180
Lactarius tenellus	ADK 3598 (GENT)	Benin	KF133280	KF133313

Results

Our molecular results clearly show that all of the newly collected species, together with the species from Nepal, belong to *Lactifluus* sect. *Gerardii* (Fig. 5.1). Based on both molecular and morphological results, we describe five new species: *Lf. auriculiformis* sp. nov., *Lf. pulchrellus* sp. nov., *Lf. gerardiellus* sp. nov., *Lf. bhandaryi* sp. nov. and *Lf. raspei* sp. nov. Furthermore, we found a possible new finding of *Lf. uyedae*.

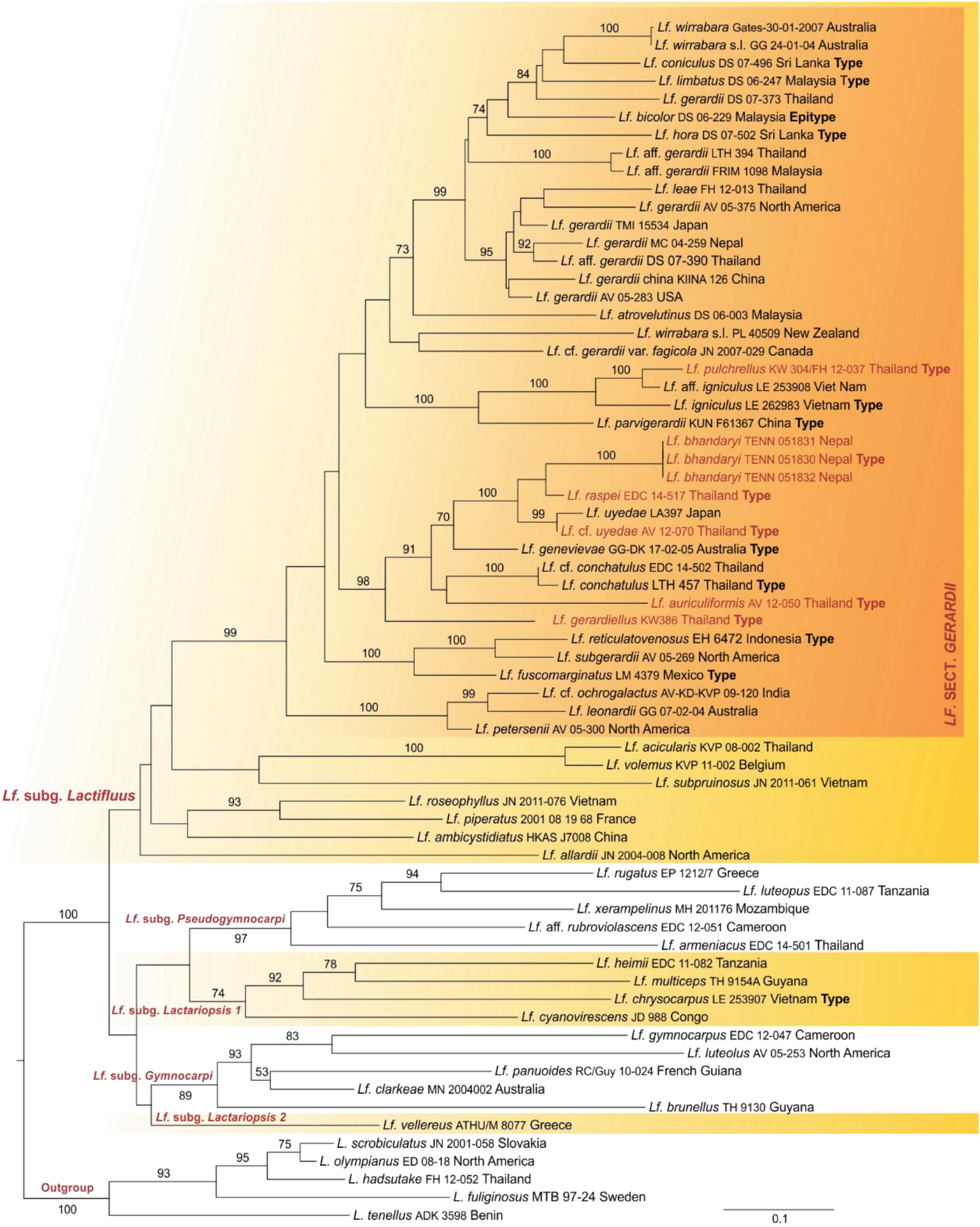

Fig. 5.1 Overview Maximum Likelihood tree of the genus *Lactifluus*, with a focus on *Lf.* sect. *Gerardii*, based on concatenated ITS and LSU sequence data. Maximum Likelihood bootstrap values >70 are shown.

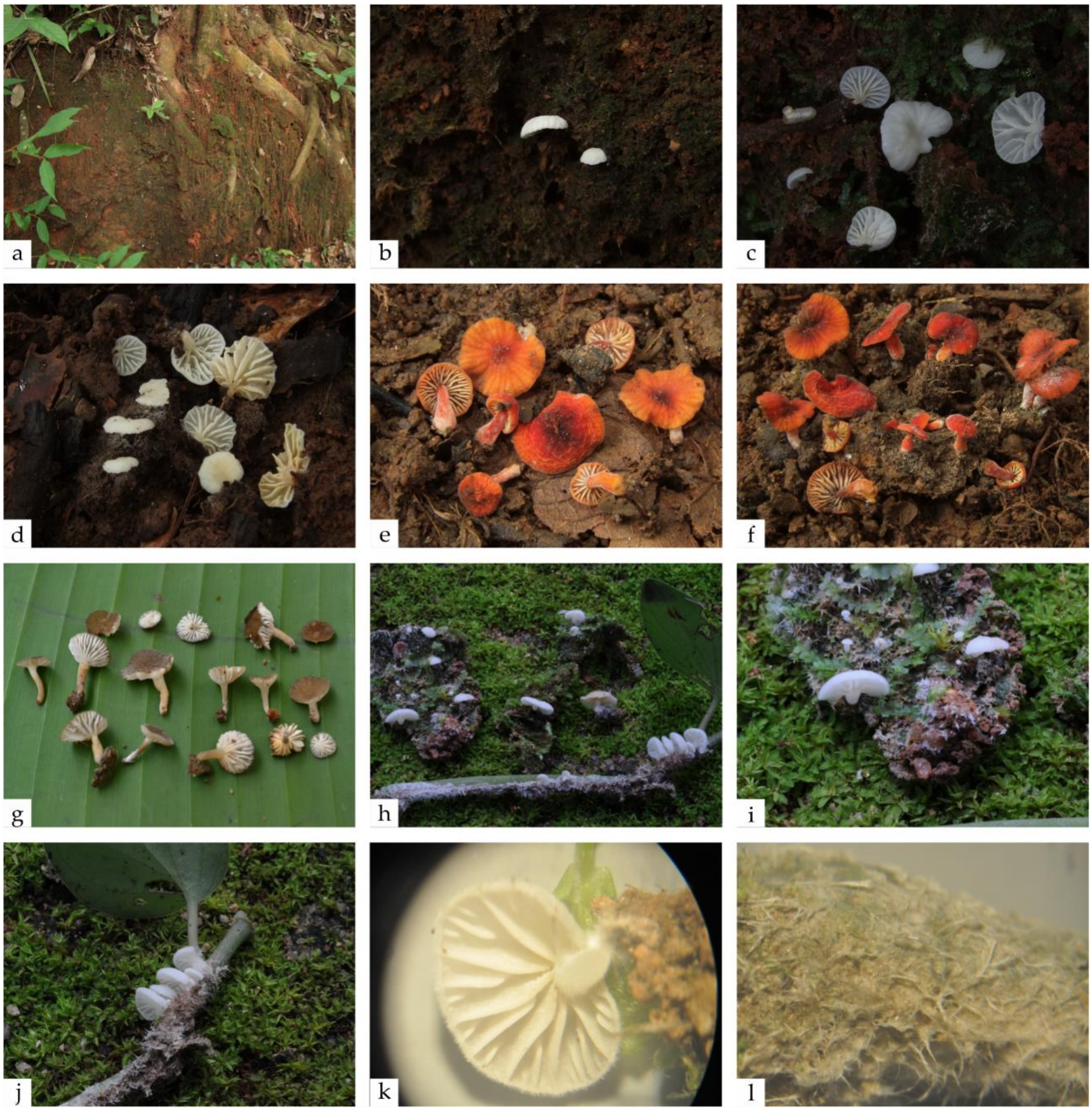

Fig. 5.2 *Lactifluus auriculiformis* (AV 12-050, photos by F. Hampe): **a.** vertically exposed collection spot showing basidiocarps in their natural environment, **b-c.** basidiocarps; *Lf.* cf. *uyedae* (AV 12-070, photo by F. Hampe): **d.** basidiocarps; *Lf. pulchrellus* (KW 304/FH 12-037, photos by F. Hampe): **e-f.** basidiocarps; *Lf. gerardiellus* (KW 386, photo by K. Wisitrassameewong): **g.** basidiocarps; *Lf. raspei* (EDC 14-517, photo by E. De Crop): **h.** overview of basidiocarps, **i.** close-up of young and fully grown basidiocarps growing on soil, **j.** close up of basidiocarps and subiculum growing on a seedling, **k.** basidiocarp under stereomicroscope, **l.** close-up of subiculum under stereomicroscope.

Lactifluus auriculiformis Verbeken & Hampe **nom. prov**., Fig. 5.2 a–c, Fig. 5.3

MycoBank: To be submitted.

Etymology: With the shape of a small ear.

Diagnosis: Lactifluus species with small, pure white, pleurotoid basidiocarps, with long and thick-walled pileipellis hairs that are even visible with a hand lens. The latex is white, but staining the lamellae brown. The pileipellis is a lampropalisade to hymeniderm, with scattered thick-walled terminal elements.

Macrocystidia are very abundant and have a needle-like content. Basidiospores are subglobose to broadly ellipsoid, with ornamentation that forms a subcomplete reticulum with rather small meshes.
Holotypus: Thailand, Chiang Mai Prov., Doi Suthep-Pui National Park, alt. 1142 m, growing on naked soil, on a vertically exposed site underneath *Lithocarpus* sp., in rainforest dominated by *Castanopsis* sp. and *Lithocarpus polystachyus*, 16 July 2012, A. Verbeken 12-050 (MFLU, GENT).

Basidiocarps pleurotoid. **Pileus** ear- or shell-shaped, max. 7–10 mm diam. but often smaller, pure white, smooth, transparently striate. **Stipe** completely eccentric and very short (1–3 mm), slightly more cream-coloured than the pileus. **Lamellae** white, staining brownish to greyish brown by the latex, in some specimens remarkably few, about 5–7 lamellae per pileus, generally a bit more, with lamellulae. **Context** white; taste mild. **Latex** scarce but visible, watery white, staining the lamellae cream to distinctly brownish-greyish after more than 30 min.
Basidiospores subglobose to broadly ellipsoid, (7.4)7.5–*8.3*–8.9 × 6.2–*6.8*–7.3 μm (Q = 1.11–*1. 22*–1.31, n = 30); ornamentation amyloid, composed of rather thick and irregular, rounded ridges, up to 0.6(0.8) μm high, ridges forming a dense and subcomplete reticulum with rather small meshes; plage slightly distally amyloid. **Basidia** 60–80 × 13–18 μm, cylindrical to narrowly clavate, 4-spored; sterigmata 5–11 × 2–5 μm. **Pleuromacrocystidia** very abundant, very emergent, 70–120 × 12–14(20) μm, cylindrical and sometimes with rounded apex, or with very small papilla, or fusiform with tapering apex, with slightly refringent walls and distinct needle-like content. **Pleuropseudocystidia** abundant, slightly emergent, 3–6 μm diam., slightly tortuous, sometimes branched. **Lamellae-edge** substerile, composed of marginal cells, abundant cheilopseudocystidia, some cheilomacrocystidia, and sometimes with small basidia; marginal cells shortly cylindrical to subclavate, 15–28(35) × 7–10 μm; cheilomacrocystidia scarce, clavate, 50–70 × 12–15 μm, with distinct needle-like content. **Hymenophoral trama** composed of hyphae, mixed with lactiferous hyphae. **Pileipellis** a lampropalisade to hymeniderm, consisting of a layer of rounded cells, up to 50 μm thick with some of them bearing rounded to subclavate terminal cells, but also with long, hair-shaped thick-walled elements that sometimes arise very basal in the subpellis; hair-like terminal elements 65–190 × 6–12 μm, broader at the base, becoming narrower at the top, with very thick walls (up to 3 μm thick).

Ecology. Found on naked soil, on a vertically exposed site underneath *Lithocarpus* sp., in rainforest dominated by *Castanopsis*, *Lithocarpus polystachyus* etc.

Distribution. Known from Thailand.

Studied material:
Thailand. Chiang Mai Prov., Doi Suthep-Pui National Park, growing on naked soil, on a vertically exposed site underneath *Lithocarpus* sp., in rainforest dominated by *Castanopsis* sp. and *Lithocarpus polystachyus*, alt. 1142 m, 16 July 2012, A. Verbeken 12-050 (Holotypus GENT; isotypus MFLU).

Notes
The pileus of *Lf. auriculiformis* is smooth, but with a good hand lens some hairs (see thick-walled terminal elements in the pileipellis) are visible. The sister species *Lf. conchatulus*, described from similar habitats in Thailand, differs by smaller spores (6.1–7.8 × 5.1–6.6 μm) and somewhat smaller macrocystidia. However, macrocystidia are mentioned to be only 40–65 μm long in the description of the type, but we did observe macrocystidia up to 80–90 μm long in *Lf. conchatulus*. Besides the larger macrocystidia, *Lf. auriculiformis* has

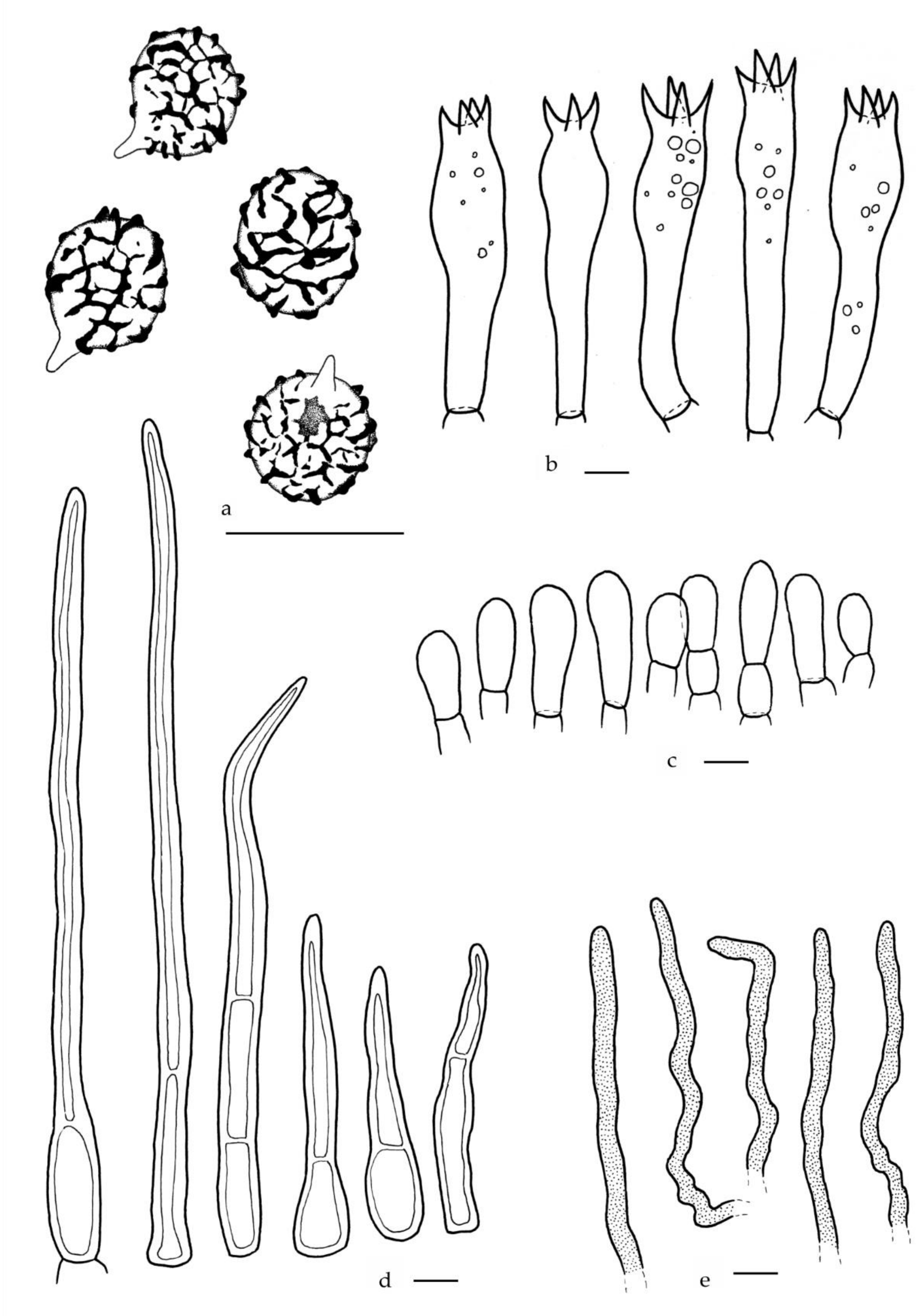

Fig. 5.3 *Lactifluus auriculiformis*: **a.** basidiospores; **b.** basidia; **c.** marginal cells; **d.** terminal elements of the pileipellis; **e.** pleuropseudocystidia, **f.** pleuromacrocystidia, **g.** section through the pileipellis (all from holotype AV 12-050, scale bar = 10 μm).

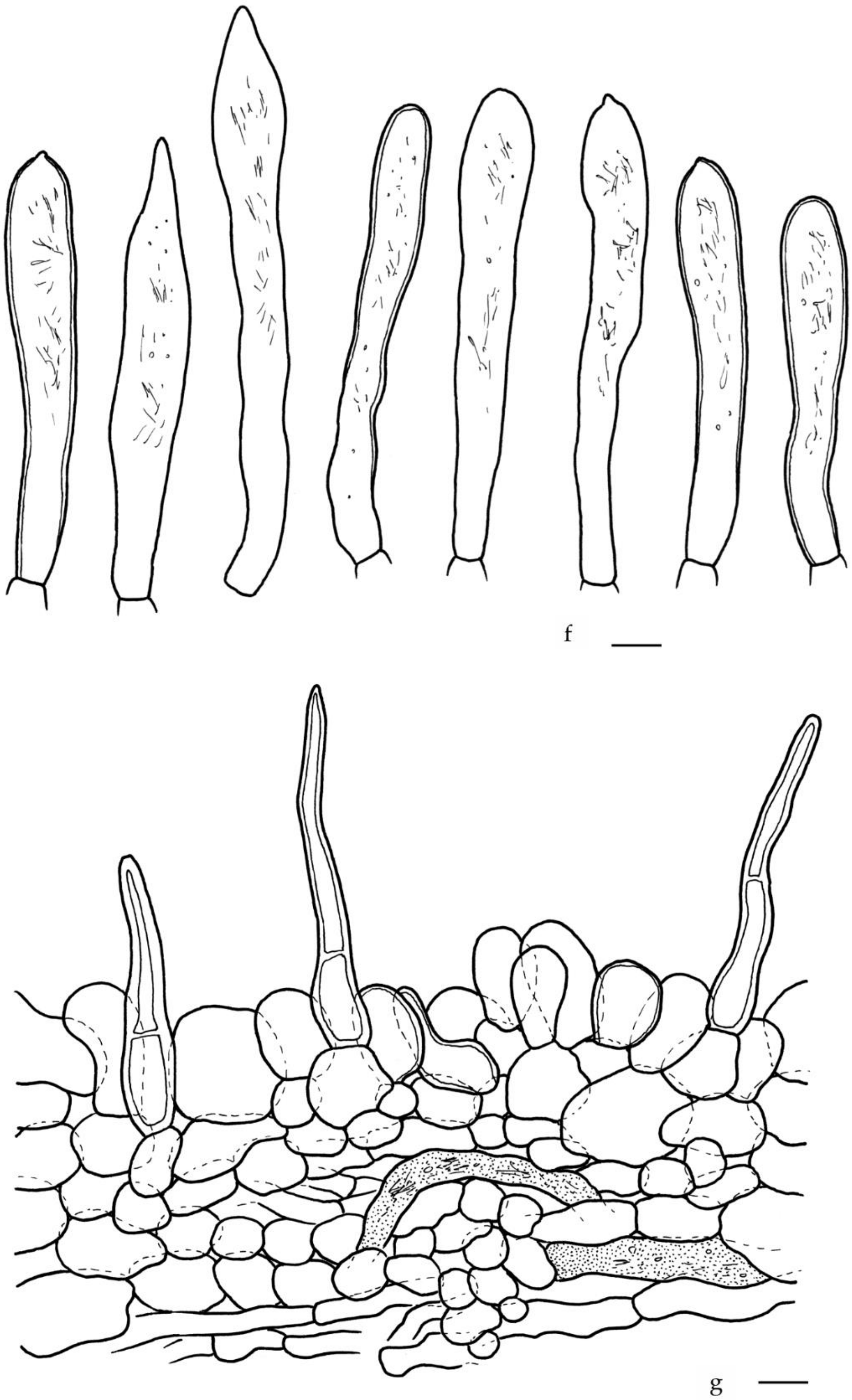

Fig. 5.3 *Lactifluus auriculiformis* – continued.

larger, more scattered, pileipellis hairs. They measure 65–190 × 6–12 µm compared with 20–70 × 3–6 µm in *Lf. conchatulus*. The ornamentation of the spores in *Lf. conchatulus* is a more regular reticulum with wider meshes and regular ridges that have a more equal height. In *Lf. auriculiformis* the reticulum is denser with distinctly smaller meshes that are more irregular, as the height of the ridges is unequal; the reticulum is subcomplete with numerous open ends. Pleuropseudocystidia are abundant and emergent in *Lf. auriculiformis*, while scarce and not emergent in *Lf. conchatulus*. The margin of the lamellae is substerile in both species, with some scarce basidia present, but mainly composed of marginal cells. In *Lf. auriculiformis,* cheilopseudocystidia are abundant, cheilomacrocystidia are scarce, while in *Lf. conchatulus* cheilomacrocystidia are abundant and cheilopseudocystidia hardly observed.

The spore ornamentation of *Lf. auriculiformis* is most similar to the ornamentation in *Lf. uyedae*, but this species differs by its shorter pileipellis hairs (25–70 × 2–6 µm). When we compare *Lf. auriculiformis* with *Lf. genevievae*, the latter has spores that are comparable in size (7.1–9.6 × 6.5–8.2), but has a complete and regular reticulum with larger meshes, similar to *Lf. conchatulus*. Furthermore, macrocystidia in *Lf. genevievae* are from a completely different type: with a more granular and dense content instead of distinct needle-like content and with a fusiform shape very gradually tapering towards the apex.

Lactifluus bhandaryi Verbeken & De Crop **nom. prov.**, Fig. 5.4
Mycobank: To be submitted
Etymology: Referring to H.R. Bhandary, who collected and described this species for his master's thesis (Bhandary 1993, unpubl.).
Diagnosis: Lactifluus species with small pleurotoid basidiocarps, with cream to orange-coloured fruiting bodies covered with small hairs, especially when young, and with an acrid taste. Growing on a white, silky subiculum with erect hairs. The pileipellis is a lampropalisade and the species has very emergent and long pleuromacrocystidia. Basidiospores subglobose to broadly ellipsoid, with ornamentation that forms a complete reticulum.
Holotypus: Bagmati, Kathmandu, Shivarpuri Watershed Management Area, Malpokhari, 27°47'30''N 085°22'40''E, 7 July 1990, H.R. Bhandary & Sunar (Holotypus TENN 051830, TENN).

Basidiocarps pleurotoid, growing on a subiculum which is thinly or densely effused around the substratum, extending up to 60 mm from the basidiocarps. **Subiculum** white, silky, with erect hairs; hairs scattered, erect, spiny, fascicles, sometimes bifurcate with pointed and curved tips. **Pileus** flabelliform or spathulate, with convex, later slightly depressed centre, up to 12 × 9 mm, broadly striate, sulcate, pure white when young, then white only between the furrow of striations, cream (4A3), pale yellow (4A4), putty (4B2), ivory (4B3), champagne (4B4), pale blonde to blonde (4C3-4) to pale orange (5AB3) or greyish orange (6D4) or greyish brown (6E4-5) all over or mostly at the ridges and in the centre; surface densely hairy when young, remaining so towards margin and pruinose or woolly in the centre; margin striate, sulcate, incurved at first, then straight, slightly lobed (sometimes rimulose and strongly lobed), with erect and silky hairs all over. **Stipe** sometimes absent, when present cylindrical, strongly eccentric or lateral, up to 1.5 mm long and 0.9 mm diam.; surface white, sometimes with hairs up to 1 mm long and base white and covered with a strigose, hairy tomentum. **Lamellae** decurrent, white to yellowish white (4A2), up to 1.5 mm broad, distant, forked up to one or two levels, with lamellulae. **Context** very thin, up to 0.3 mm in the centre of the pileus, white or greyish white, turning pink or pinkish brown with age, brittle. **Latex** abundant, white, unchanging. **Taste** immediately acrid. **Smell** inconspicuous.

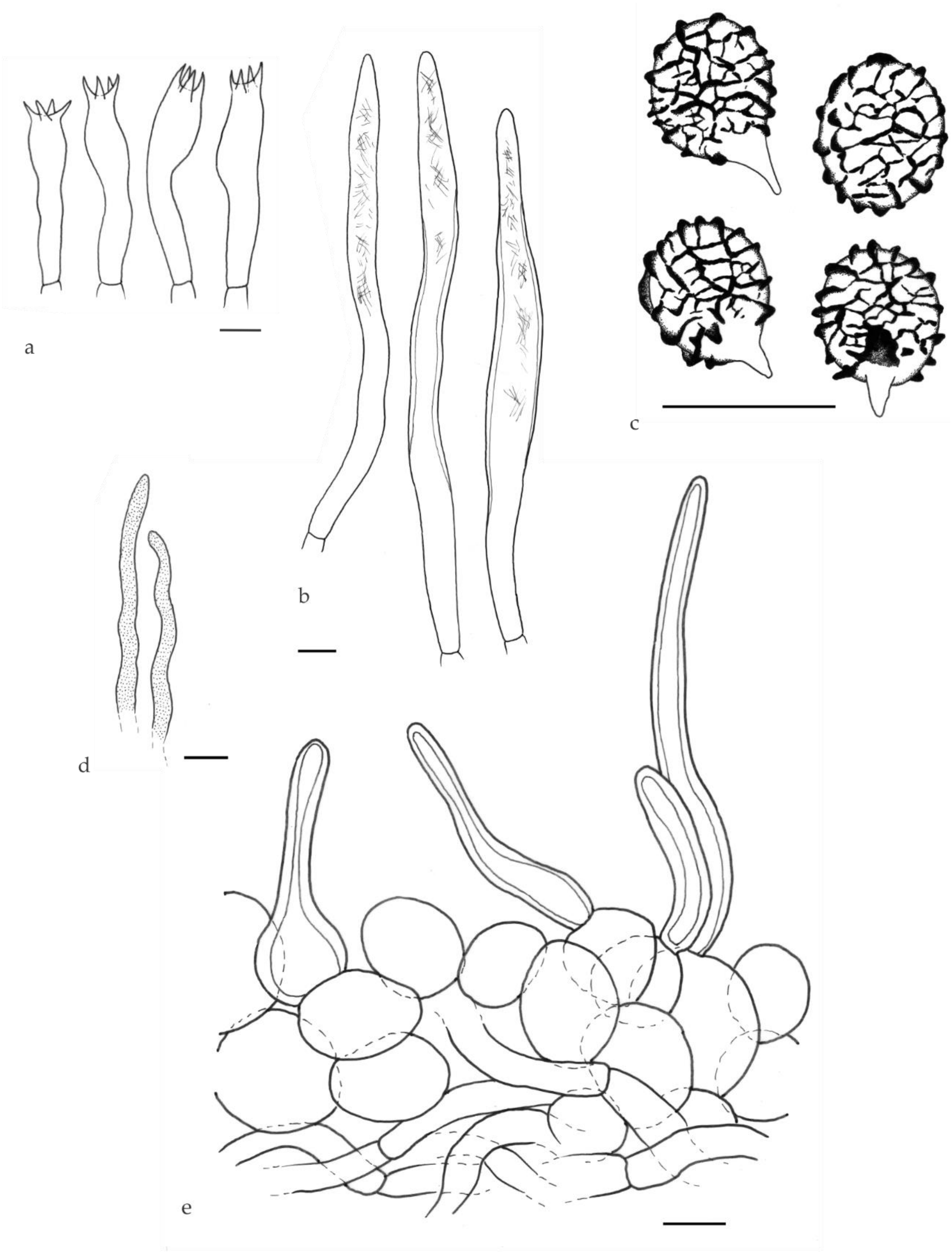

Fig. 5.4 *Lactifluus bhandaryi*: **a.** basidia; **b.** pleuromacrocystidia; **c.** basidiospores; **d.** pleuropseudocystidia; **e.** section through the pileipellis (all from holotype TENN 051830, scale bar = 10 μm).

Basidiospores subglobose to broadly ellipsoid, 6,8–*7.7*–8,5 × 6,0–*6.6*–7.3 µm (Q = 1.11–*1.1*–1.21, n = 20); ornamentation amyloid, dense, composed of ridges and warts up to 0.7(–1) µm high, usually connected by rather thick and high ridges, forming a complete reticulum; plage sometimes totally amyloid. **Basidia** 40–50 × 9–11 µm, subclavate, 4-spored. **Pleuromacrocystidia** abundant, 90–165 × 12–15 µm, very emergent, fusiform, tapering near apex, usually thin-walled, sometimes locally clearly thick-walled, with dense needle-like content. **Pleuropseudocystidia** not abundant, 2–4 µm diam., cylindrical and narrow, not emergent. **Lamellae-edge** sterile, with marginal cells and cheilomacrocystidia; marginal cells shortly cylindrical to subclavate, 17–27 × 4–7 µm; cheilomacrocystidia similar to pleuromacrocystidia. **Pileipellis** a lampropalisade; subpellis up to 30 µm thick, consisting of globose to subglobose cells, 15–25 µm diam.; suprapellis with long, hair-shaped, thick-walled, often septate elements, 40–100 × 7–9 µm, sometimes distinctly broader at the base (up to 15 µm), becoming narrower at the top but apex rounded, never acute.

Ecology. Gregarious, on roots of *Castanopsis tribuloides*, *Myrsine semiserrata* and *M. capitellata* but overgrowing on other closely associated leaf litter, soil, decaying sticks, stem base and roots of ferns and Angiosperms such as *Daphne bholua*, *Myrica esculenta*, *Quercus leucotrophora*, *Q. lamellose* etc.

Distribution. Known from Nepal.

Studied material:
Nepal. Bagmati, Kathmandu, Nagajun (Raniban) forest, 274500N 0851525E, 10 July 1991, H.R. Bhandary, TENN 051829, HRB 58. Bagmati, Kathmandu, Shivarpuri Watershed Management Area, Malpokhari, 27°47'30"N 085°22'40"E, 7 July 1990, H.R. Bhandary & Sunar, TENN 051830 (Holotypus). Bagmati, Kathmandu, Nagarjun forest, 27°47'30"N 085°22'40"E, 9 July 1990, H.R. Bhandary, TENN 51831/HBR 135. Bagmati, Kathmandu, Nagajun forest, 27°45'00"N 085°15'25"E, 15 July 1991, H.R. Bhandary, TENN 051832/HRB 83.

Lactifluus gerardiellus Wisitrassameewong & Verbeken **nom. prov.**, Fig. 5.2g & 5.5
Mycobank: To be submitted
Etymology: Resembles a small version of *Lf. gerardii*.
Diagnosis: Lactifluus species with small agaricoid basidiocarps that resembles a small version of *Lf. gerardii*. The species has a brown pileus and stipe, together with white lamellae. The pileipellis is a lampropalisade with thick-walled hair-like elements. Basidiospores are subglobose to ellipsoid, with the ornamentation forming a subcomplete reticulum.
Holotypus: Thailand, Chiang Rai province, Thasai sub-district, Muang district, Doi Pui, television repeater station, growing gregarious among leaf litter in deciduous forest dominated by *Castanopsis armata* and *Quercus* sp., N19°49'00" E99°52'03", alt. 740 m, 31 July 2012, leg. K. Wisitrassameewong, J. Chen, B. Thongbai, (Holotypus KW386, GENT, Isotypus MFLU, Mae Fah Luang University).

Basidiocarps small, fragile. **Pileus** 3–11 mm in diam., small, applanate to infundibuliform, more or less mucronate in the centre, striated at the margin; edge even; surface dry, velvety, rather smooth to slightly wrinkled, sometimes with uneven colour, ranging from dark brown to reddish brown (7E5, 7F6–7, 8E7–8). **Stipe** 5–8 mm in length, 1–2 mm in diam, cylindrical to slightly eccentric, colour often paler at apex, 5A3, 5B3–B4, white pruinose at base, with hollow pith. **Lamellae** close, white, with two series of lamellulae. **Context** <0.5 mm thick in the pileus, cream to pale yellow, not discolouring when bruised; taste mild, odour

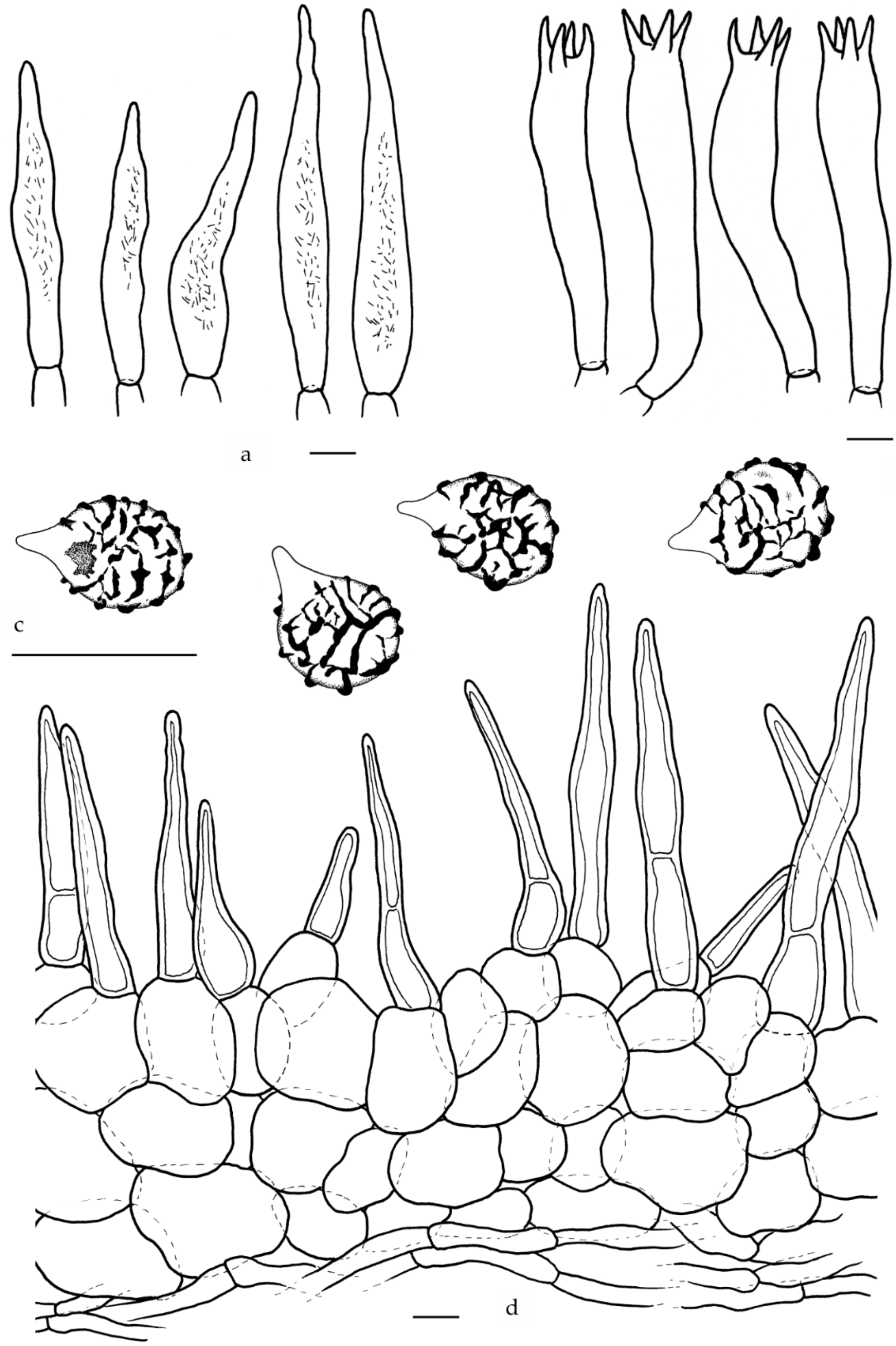

Fig. 5.5 *Lactifluus gerardiellus*: **a.** pleuromacrocystidia; **b.** basidia; **c.** basidiospores, **d.** section through the pileipellis (all from holotype KW 386, scale bar = 10 μm).

sweetish. **Latex** rather sparse, white or watery white, unchanging when isolated on glass slide; taste mild. **Spore print** white. **Macrochemical reactions** KOH 10% on latex yellow (4C6), FeSO4 unchanging.

Basidiospores subglobose to ellipsoid, mostly broadly ellipsoid, 6.7–*7.6*–8.4 × 5.4–*6.1*–6.8(6.9) µm (Q = 1.11–*1.25*–1.39, n = 30); ornamentation amyloid, composed of ridges and interconnected warts up to 0.5 µm high, forming a subcomplete reticulum; plage slightly distally amyloid. **Basidia** 65–75 × 11–14 µm, cylindrical to subcylindric, 4-spored. **Pleuromacrocystidia** absent. **Pleuropseudocystidia** 3–5 µm diam., slightly tortuous. **Lamellae-edge** sterile, composed of marginal cell and abundant cheilomacrocystidia; marginal cells shortly cylindrical to subclavate, often narrow and tortuose, 10–20 × 4–8 µm; cheilomacrocystidia 55–84 × 8–12 µm, fusiform. **Hymenophoral trama** composed of hyphae, mixed with lactiferous hyphae. **Pileipellis** a lampropalisade; subpellis consisting of globose to subglobose cells, 15–30 µm diam., forming a dense layer up to 60 µm thick; suprapellis with long, hair-shaped thick-walled elements, 25–90 × 6–10 µm, broader at the base, becoming narrower at the top, with dark intracellular pigmentation.

Ecology. Found growing gregarious among leaf litter in deciduous forest dominated by *Castanopsis armata* and *Quercus* sp.

Distribution. Known from Thailand.

Studied material:
Thailand. Chiang Rai province, Thasai sub-district, Muang district, Doi Pui, television repeater station, growing gregarious among leaf litter in deciduous forest dominated by *Castanopsis armata* and *Quercus* sp., N19°49'00" E99°52'03", alt. 740 m, 31 July 2012, leg. K. Wisitrassameewong, J. Chen, B. Thongbai, (Holotypus KW386, GENT, Isotypus MFLU, Mae Fah Luang University).

Notes
Lactifluus gerardiellus is macroscopically similar to *Lf. parvigerardii* with paler, more yellowish brown cap colours. Microscopically, *Lf. parvigerardii* has spores with a higher ornamentation and a centrally amyloid plage, and the terminal elements of the pileipellis are thin-walled and smaller than in *Lf. gerardiellus*. *Lactifluus parvigerardii* has pleuromacrocystidia with a more or less rounded apex and no cheilomacrocystidia, while *Lf. gerardiellus* has only cheilomacrocystidia with a pointed apex. Moreover, in the type collection of *Lf. gerardiellus*, considerably larger basidia have been observed.
The recently described *Lf. indicus* appears to be sister species of *Lf. gerardiellus* (unpubl. res.). *Lf. indicus* is a pleurotoid species that grows on subiculum, while *Lf. gerardiellus* is a small agaricoid species.

Lactifluus pulchrellus Hampe & Wisitrassameewong **nom. prov.**, Fig. 5.2e,f & 5.6
Mycobank: To be submitted
Etymology: Refers to the fact that the species is small and strikingly beautiful.
Diagnosis: Lactifluus species with small agaricoid basidiocarps. Both pileus and stipe are velutinous and have bright orange-red colours. The lamellae are rather thick, cream to yellow coloured and staining brownish black by the latex. The latex is watery greenish brown, staining lamellae and context brownish black when bruised. The pileipellis is a lamprotrichopalisade; basidiospores are subglobose to broadly ellipsoid, with an ornamentation of irregular warts that are sometimes connected by fine lines.
Holotypus: Thailand, Chiang Mai Prov., Mae Teang distr., Buatong waterfall and Rainbow spring, growing on naked stony soil under *Dipterocarpus tuberculatus*, N 19°04'11.78" E 99°04'48.41", alt. 507 m, 23 June 2012,

leg. F. Hampe and K. Wisitrassameewong, KW304/FH 12-037 (*Holotypus* FH 12-037, GENT, *Isotypus* MFLU12-0548).

Basidiocarps small, fragile. **Pileus** 2–11.5 mm in diam., convex at first, then applanate, occasionally lobate; in centre first sometimes papillate and with a small, pointed umbo, later depressed; margin first regular and bent downwards, later wavy; surface yellowish orange, bright orange to orange reddish (5B7, 6C7, 6C8, 6B8, 7D8), more intensive orange-red (8C8, 8D8, 9C8, 9D8) when young, often locally paler at maturity (6D6), velvety, dry, rimose and rivulose, locally with some concentrical cracks, sometimes striate almost up to the centre. **Stipe** very small, 3–5 × 0.5–1 mm, equal, cylindrical or thickening towards base, velutinous, concolourous with the cap or somewhat more pinkish; base paler, sometimes with fine white tomentum. **Lamellae** adnate, about 10 to 20 per pileus, moderately spaced with 1–3 lamellulae between two lamellae, relatively thick (1 mm), cream to yellow, staining brownish to brownish black (2F1) by the latex. **Context** 0.3–0.5 mm thick in the pileus, cream to pale yellow, not discolouring when bruised; taste mild. **Latex** abundant, watery greenish brown (2F5 to 2F3), staining the lamellae and the flesh first brownish cream, then blackish (2F1); taste mild. **Spore print** white. **Macrochemical reactions** KOH 30% on latex yellow (4C7), Guajak on stipe immediately greenish blue.

Basidiospores subglobose to broadly ellipsoid, 6,9–*7,5*–8,1 × 5,9–*6,5*–7,1 µm (Q = 1,07–*1,17*–1,28, n = 30); ornamentation amyloid, with irregular warts up to 0.3 µm high which are sometimes connected by fine lines, sometimes isolated; plage distally or almost completely and distinctly amyloid. **Basidia** 55–70 × 11–15 µm, 4-spored, sometimes 2-spored and then often with very long and irregular sterigmata. **Pleuromacrocystidia** 70–95 × 10–15 µm, cylindrical to subfusiform, often with rounded to slightly tapering apex, sometimes with long tapering apex, thin-walled. **Pleuropseudocystidia** 2–4 µm diam., cylindrical. **Lamellae-edge** sterile; cheilocystidia absent; marginal cells cylindrical to subclavate, thin-walled and hyaline, 15–50 × 7–15 µm. **Hymenophoral trama** mixed, composed of hyphae and some small sphaerocytes. **Pileipellis** a lamprotrichopalisade, with short chains of short, irregular to subglobose elements, with the terminal element of the chain clavate to globose and sometimes slightly thick-walled, mixed with long and rather slender distinctly thick-walled hairs; hair-like terminal elements up to 140 µm long, 12–14 µm broad, very thick-walled, often septate, usually tapering upwards.

Ecology. Found on naked stony soil under *Dipterocarpus tuberculatus.*

Distribution. Known from Thailand.

Studied material:
Thailand. Chiang Mai Prov., Mae Teang distr., Buatong waterfall and Rainbow spring, growing on naked stony soil under *Dipterocarpus tuberculatus,* alt. 507 m, N19°04'11.78" E99°04'48.41", 23 June 2012, leg. Felix Hampe and Komsit Wisitrassameewong, KW304/FH 12-037 (*Holotypus* FH 12-037, GENT, *Isotypus* MFLU12-0548).

Notes
This is a particular and strikingly colourful species due to the small dimensions of the basidiocarps and the warm red to orange or even somewhat pinkish colours. The species is outstanding in *Lactifluus* sect. *Gerardii,* which has mainly dark brown to blackish brown or very pale to whitish representatives. Recently, Morozova

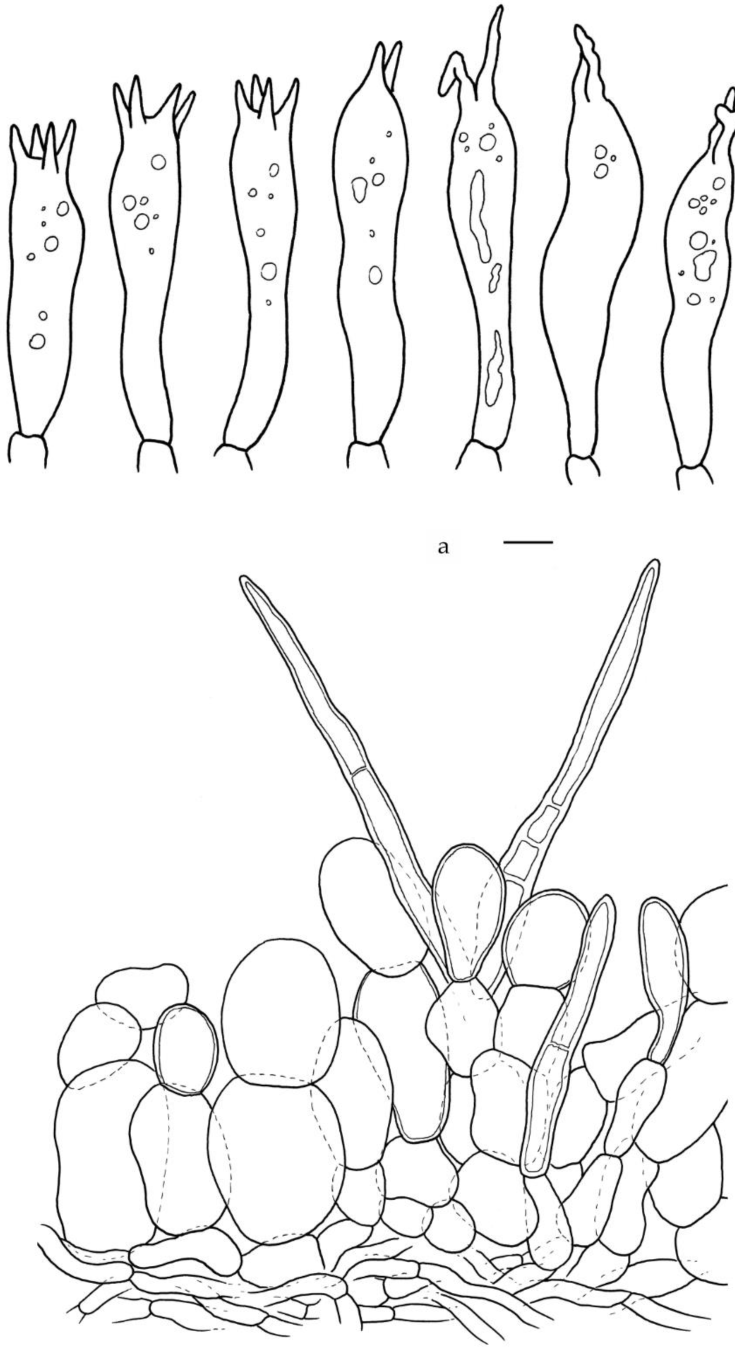

Fig. 5.6 *Lactifluus pulchrellus*: **a.** basidia; **b.** section through the pileipellis; **c.** marginal cells; **d.** basidiospores; **e.** pleuromacrocystidia; **f.** terminal elements of the pileipellis (all from holotype KW 304/FH 12-037, scale bar = 10 μm).

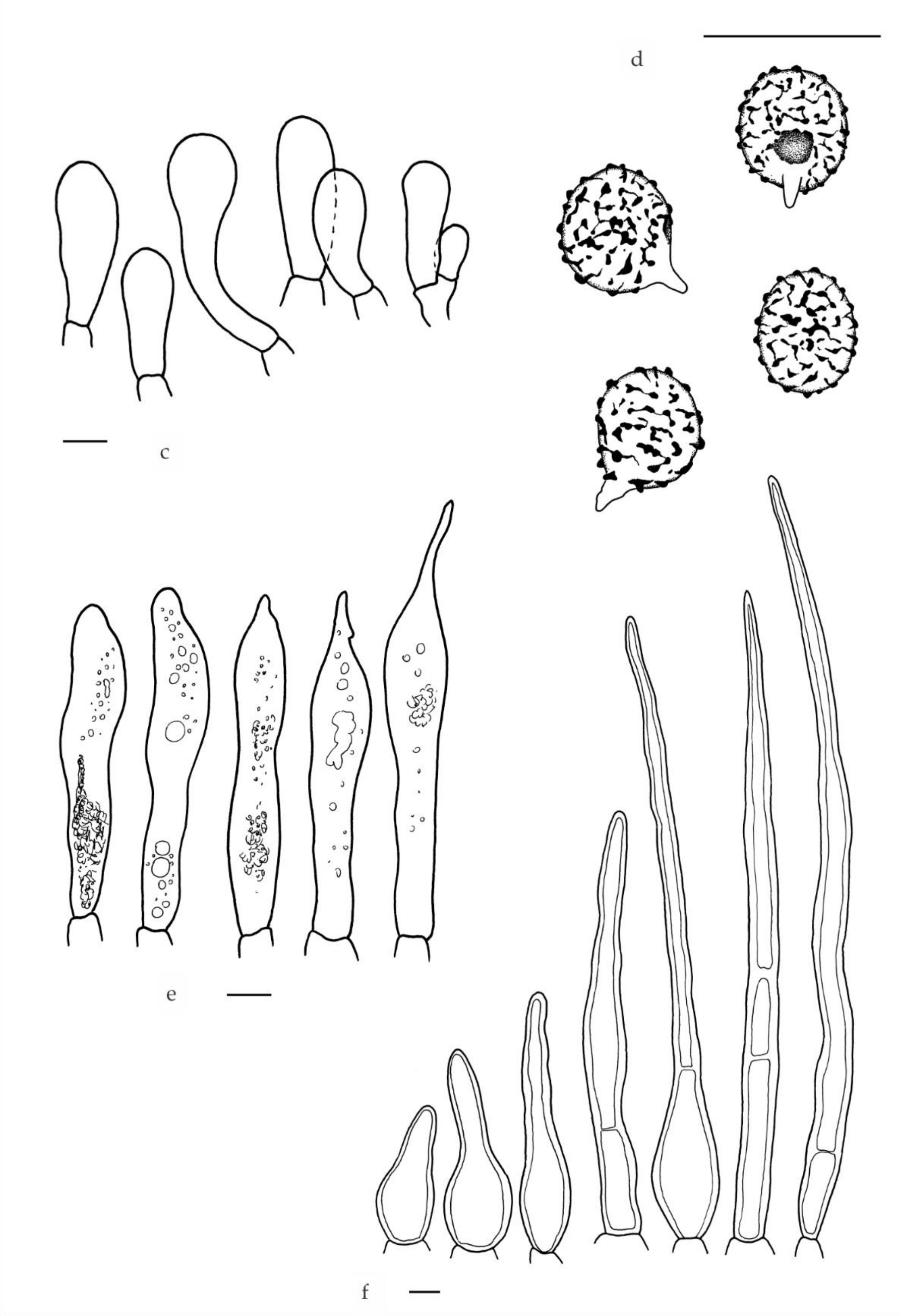

Fig. 5.6 *Lactifluus pulchrellus* – continued.

et al. (2013) described a species in this section with deep orange tinges: *Lactifluus igniculus*. They describe the species as pleurotoid, but the pictures in their description show agaricoid basidiocarps with a central to slightly eccentric stipe, which strongly reminds of our newly proposed species. However, the spore ornamentation considerably differs between the two species (Fig. 5.6): *Lf. pulchrellus* has low and irregular, rounded warts, while *Lf. igniculus* has almost echinulate spores with acute warts up to 1.6 µm high (own measurements), isolated or connected by fine lines. In their paper, Morozova et al. (2013) also describe a collection, *Lf.* aff. *igniculus*, which is close to, but not conspecific with *Lf. igniculus.* Its basidiocarps look strikingly similar and in the molecular analysis they only differ by a few base pairs from both *Lf. igniculus* and *Lf. pulchrellus*. Nonetheless, *Lf.* aff. *igniculus* represent a different species as it morphologically differs from *Lf. pulchrellus*, with echinulate spores with warts up to 1 µm.

Lactifluus raspei Verbeken & De Crop **nom. prov.**, Fig. 5.2h–l & 5.7
Mycobank: To be submitted
Etymology: Named after Dr. Olivier Raspé, who found and collected the species.
Diagnosis: Lactifluus species with small, white pleurotoid basidiocarps, forming white subiculum on soil or plant seedlings. The basidiocarps are covered with white hairs visible to the naked eye. The latex is white and unchanging. The pileipellis is a lampropalisade; basidiospores are subglobose to broadly ellipsoid, ornamentation forming a subcomplete reticulum.
Holotypus: Thailand, Chiang Mai Province, Mae Taeng district, Baan Mae Sae, on soil and seedlings in mixed forest: *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., N 19°14'26" E 98°37'60", alt. 1077 m, 1 August 2014, coll. O. Raspé (Holotypus EDC 14-517, GENT).

Basidiocarps small, pleurotoid, growing on a subiculum. **Subiculum** white to greyish-white, thinly to moderately densely effused when growing on soil, densely effused when growing on plant seedlings. **Pileus** 4–7 mm in diam., planoconvex when young, applanate when older; young basidiocarps sometimes papillate and with a small, pointed umbo in the centre, depressed when older; margin slightly inflexed; margin edge entire; surface white, with yellow tinge when old, velvety, covered with hairs. **Stipe** very small, 2–4 × 0.5–1 mm, laterally attached, cylindrical, tapering downwards near the base, velutinous, with a white and hairy tomentum at the base, concolourous with the pileus. **Lamellae** narrowly adnate, about 9–12 lamellae per pileus with 1–5 lamellulae between two lamellae, rather distant (in proportion to its size), relatively thick, white; edge concolourous and entire. **Context** 0.3–0.5 mm thick in the pileus, white, no colour change when bruised. **Latex** not abundant, white, no colour change. **Spore print** white.

Basidiospores subglobose to broadly ellipsoid, (6,8)6.9–*7.8*–8.8(9.0) × 5.7–*6.7*–7.6(8.0) µm (Q = 1.09–*1.18*–1.27, n = 20); ornamentation amyloid, dense, composed of interconnected warts up to 1 µm high, usually connected by lower ridges, forming a subcomplete reticulum; plage often totally amyloid. **Basidia** 40–45 × 10–11 µm, subcylindrical to subclavate, 4-spored. **Pleuromacrocystidia** abundant, 50–60 × 7–12 µm, emergent, irregularly subclavate, sometimes narrower near apex, usually thin-walled, sometimes slightly and locally thick-walled, with a dense needle-like content. **Pleuropseudocystidia** very abundant, 2–3 µm diam., cylindrical and very narrow, slightly tortuous, not emergent to emergent. **Lamellae-edge** mixed, with basidia, pseudocystidia and marginal cells; marginal cells shortly cylindrical to subclavate, 10–25 × 4–8 µm; basidia distinctly smaller than the basidia at the face of the lamellae, 25–30 × 7–8 µm. **Pileipellis** a lampropalisade; subpellis up to 40 µm thick, consisting of globose to subglobose cells, 10–15 µm diam.;

Fig. 5.7 *Lactifluus raspei*: **a.** section through the pileipellis; **b.** terminal elements of the pileipellis; **c.** marginal cells; **d.** elements of the lamella edge; **e.** basidia; **f.** pleuropseudocystidia; **g.** pleuromacrocystidia; **h.** basidiospores (all from holotype EDC 14-517, scale bar = 10 μm).

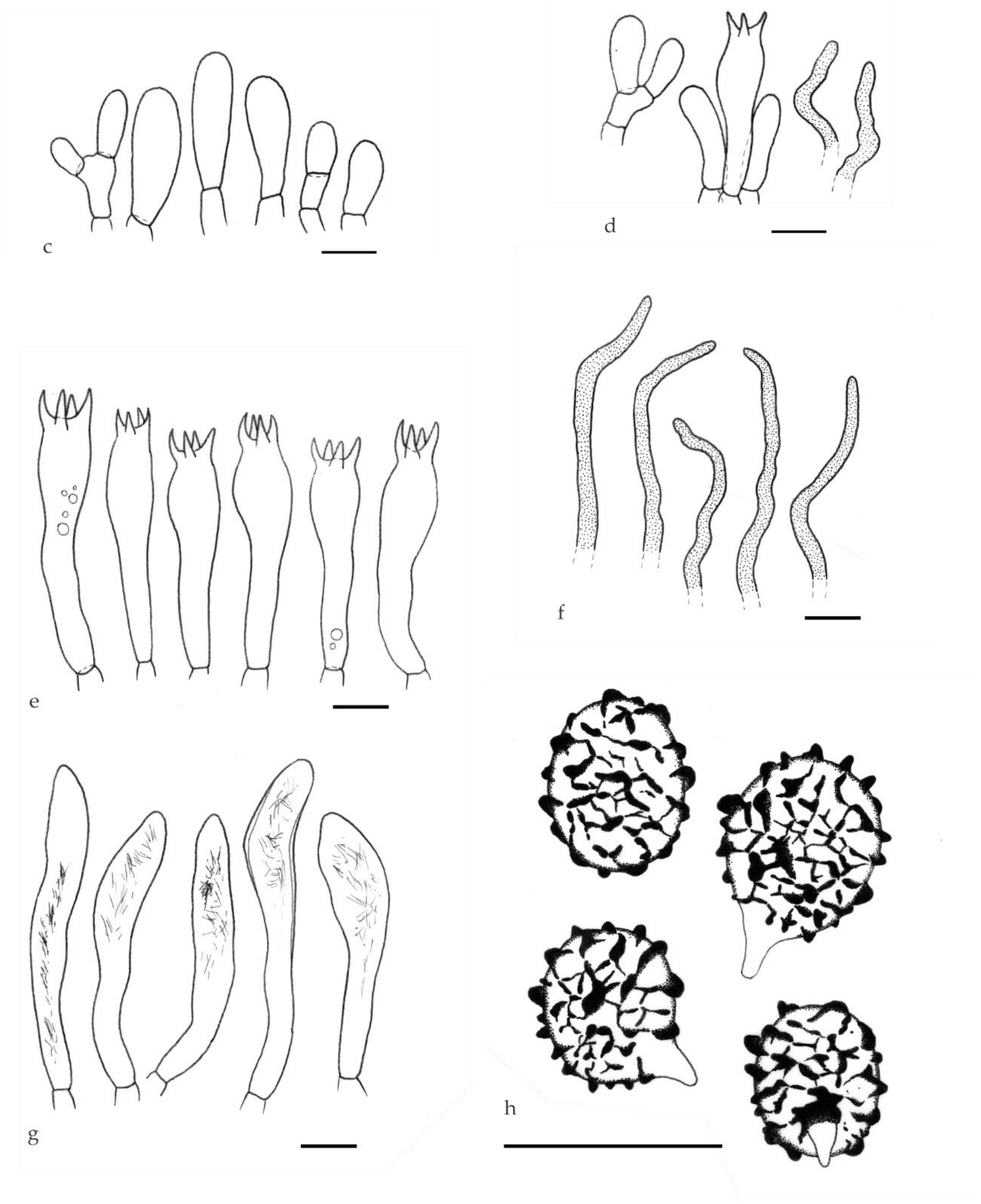

Fig. 5.7 *Lactifluus raspei* – continued.

suprapellis with long, hair-shaped thick-walled, often septate elements, 40–120 × 7–10 µm, broader at the base, becoming narrower and sometimes very acute at the top. **Subiculum** composed of linear hyaline hyphae 2–7 µm wide, septate, thick-walled ±1 µm wide.

Ecology. Found on soil and seedlings in mixed forest with *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp.

Distribution. Only known from the type locality in Thailand.**Studied material:**
Thailand. Chiang Mai Province, Mae Taeng district, Baan Mae Sae, on soil and seedlings in mixed forest: *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., N 19°14'26" E 98°37'60", alt. 1077 m, 1 August 2014, coll. O. Raspé, EDC 14-517 (Holotypus, GENT).

Lactifluus* cf. *uyedae (Singer) Verbeken, Fig. 5.2d & 5.8

Basidiocarps small, pleurotoid. **Pileus** ear-shaped, shell-shaped, max. 7–10 mm diam., often smaller, pure white, smooth, transparently striate. **Stipe** completely eccentric and very short (1–3 mm), slightly more cream-coloured than the pileus. **Lamellae** white, then staining brownish to greyish brown by the latex, in some specimens remarkably few lamellae (about 5–7 per pileus), usually a bit more and with lamellulae also present. **Context** white; taste mild. **Latex** scarce but visible, watery white, staining the lamellae first cream, but after more than 30 min. distinctly brownish-greyish.

Basidiospores subglobose to broadly ellipsoid, (7.5)7.6–*8.3*–9.1 × 6.3–*7.1*–7.8 µm (Q = 1.09–*1.18*–1.26); ornamentation amyloid, composed of rather thick and irregular, rounded ridges, up to 0.8 µm high, forming a dense, subcomplete reticulum with rather small meshes; plage slightly distally amyloid. **Basidia** 55–65(75) × 9–15 µm, mostly cylindrical, sometimes narrowly clavate, 4-spored, rarely 2-spored; sterigmata 5–8 × 1–3 µm. **Basidioles** distinctly and strikingly multiseptate. **Pleuromacrocystidia** very abundant, usually not very emergent but arising very deep in the subhymenium, 75–95(120) × 8–11 µm, clavate to fusiform with rounded or rather abruptly tapering apex, with distinct needle-like content. **Pleuropseudocystidia** abundant, not to slightly emergent, usually very narrow, 2–4(5) µm diam., slightly tortuous. **Lamellae-edge** substerile, composed of marginal cells and abundant cheilomacrocystidia, only a few basidia present; marginal cells shortly cylindrical to subclavate, multiseptate, 28–50 × 7–12 µm; cheilomacrocystidia fusiform to irregularly fusiform, 55–75 × 10–15 µm. **Hymenophoral trama** composed of hyphae, mixed with lactiferous hyphae. **Pileipellis** a palisade to hymeniderm, consisting of a layer of rounded cells, up to 60 µm thick with some of them bearing either rounded to subclavate terminal cells, or long hair-shaped thick-walled elements, hair-like terminal elements 90–180 × 6–12 µm, septate, sometimes swollen at the base, becoming narrower at the top, very thick-walled.

Studied material:
Japan. Shiga, Otsu, Kokubu, 08-1973 to 09-1974, Uyeda s.n. (holotypus, F).
Thailand. Chiang Mai Prov., Mae Teng distr., Ban Pa deng, Panthummikaram Temple, growing on naked soil under Lithocarpus sp. Shorea sp. and Castanopsis sp., alt. 1030 m, N19°06.77 E98°44.32, 18.07.2012, leg. A. Verbeken, AV 12-070 (GENT, MFLU 12-0506).

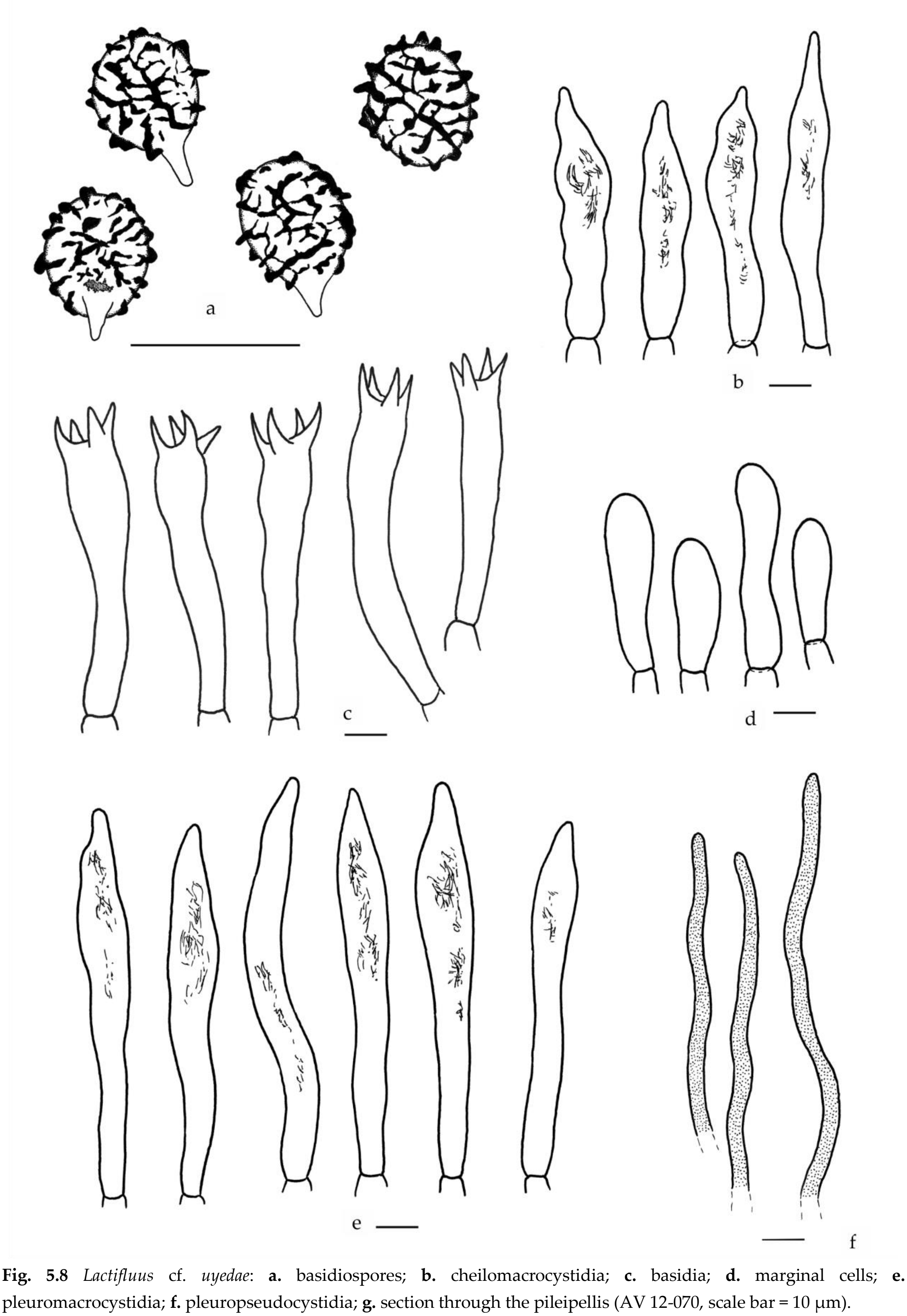

Fig. 5.8 *Lactifluus* cf. *uyedae*: **a.** basidiospores; **b.** cheilomacrocystidia; **c.** basidia; **d.** marginal cells; **e.** pleuromacrocystidia; **f.** pleuropseudocystidia; **g.** section through the pileipellis (AV 12-070, scale bar = 10 μm).

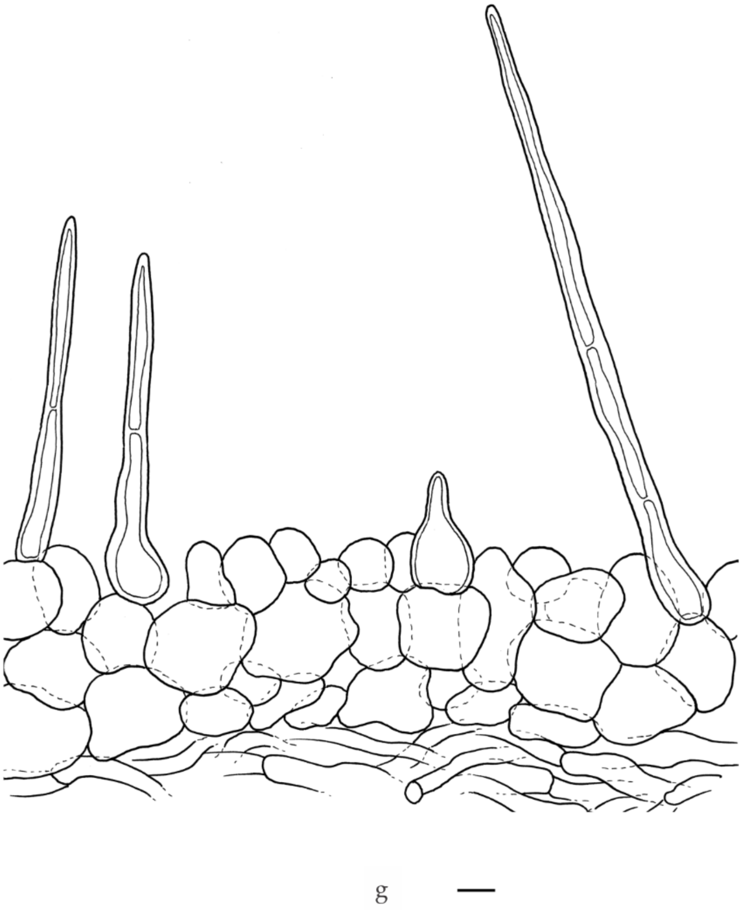

Fig. 5.8 *Lactifluus* cf. *uyedae* – continued.

Notes

The Thai collection (AV 12-070) is closely related to *Lf. uyedae* and probably conspecific with it. Molecular analyses show a very close relationship with the sequence from type-material *Lf. uyedae* (Japan). However, this result is only based on LSU sequences, as only LSU is available of the type specimen. Within the genus *Lactifluus*, LSU is known to contain less information than e.g. ITS or *RPB2*, so a more detailed molecular study is needed to confirm conspecificity. The spore size and spore ornamentation of the Thai collection are comparable with the type collection (Verbeken 1998). The macrocystidia that we measured in the Thai specimen are comparable with those of the type specimen (AV 12-070: 75–95 × 8–11 µm, type: 90–100(120) × 7–9 µm). More significant seems the difference we observe in the terminal hairs in the pileipellis. In the Thai collection, these hairs are rather long (90–180 × 6.3–7.8 µm), while those of the type collection are shorter (20–80 × 6–8 µm). More specimens are needed to find out whether this is indeed an informative feature and whether both collections are conspecific.

A striking feature of the Thai collection is the presence of abundant multiseptate basidioles in the hymenium. Often the upper cell is very short and it is not clear whether they will actually develop into true and mature basidia.

Discussion

With the description of five new species from *Lf.* sect. *Gerardii*, we confirm once more that this section contains a large and partially unknown diversity. Stubbe et al. (2010) already demonstrated the large molecular and morphological diversity of this section that mainly contains species with brownish-coloured or white basidiocarps. The findings of brightly orange-red coloured species, such as *Lf. pulchrellus* and the species found by Morozova et al. (2013), emend the characters of the group, which was thought to be characterized by either white, pleurotoid species or agaricoid species with dark brown to blackish pigments. With the results of this study, *Lf.* sect. *Gerardii* contains 20 described Asian species.

When Stubbe & al. (2010) published the first comprehensive account on *Lactifluus* sect. *Gerardii* based on world-wide sampling and a combined molecular and morphological approach, they were able to distinguish three major lineages within the section: the */gerardii* clade with the typical representatives of the section which are characterized by the lack macrocystidia, the */uyedae* clade containing species with very small, pale coloured, pleurotoid basidiocarps, and the */ochrogalactus-petersenii* clade characterized by a discolouring latex. This concept was challenged by the discovery of *Lf. parvigerardii*, a taxon with very small, dark-coloured, agaricoid basidiocarps, discolouring latex and macrocystidia. Molecular results showed that this species is closely related with the group of pleurotoid species (Wang et al. 2012), which was not expected based on morphology alone.

The new taxa presented by Morozova & al. (2013), together with those from the present paper, are decisive for a new understanding of the subdivisions within *Lf.* sect. *Gerardii*. On the basis of the presently available material, a fourth lineage can be recognized within the section, which provides a more natural position of *Lf. parvigerardii*. The */parvigerardii* clade contains taxa with tiny, agaricoid basidiocarps and is microscopically characterized by the lack of cheilomacrocystidia. In this lineage, the strikingly vivid basidiocarp colours presented by the recently described Vietnamese *Lf. igniculus* (Morozova et al. 2013) and the newly described Thai *Lf. pulchrellus* are a hitherto unique feature within the whole section.

With the newly described *Lf. gerardiellus, Lf. bhandaryi* and the recently described *Lf. indicus* (Latha et al. 2016), the *uyedae*-clade, which in Stubbe & al. (2010) exclusively contained whitish, pleurotoid taxa, is joined by agaricoid representatives with the more typical colours of the section (*Lf. gerardiellus* and *Lf. indicus*), and a pleurotoid species with cream to yellowish-orange coloured basidiocarps (*Lf. bhandaryi*). These findings extend the morphological circumscription of this lineage. On the basis of the currently available material, the */uyedae* clade contains taxa with very small basidiocarps, characterized by the presence of macrocheilocystidia and macropleurocystidia. All pleurotoid taxa still form one subclade.

Combining our results with the results of previous studies (Stubbe et al. 2010; Stubbe et al. 2012a; Wang et al. 2012), four lineages are recognised within *Lf.* sect. *Gerardii*: the */gerardii* clade contains species with normally sized basidiocarps, with dark coloured pileus and stipe, reticulate spores and no macrocystidia. In the molecular results of this study, the */gerardii* clade splits in two clades: *Lf. fuscomarginatus, Lf. reticulatovenosus* and *Lf. subgerardii* form a clade apart from the remaining species of the */gerardii* clade. However, this split is not supported and may be due to the lack of information contained in ITS and LSU to resolve relationships on this level. These relationships are resolved when including more markers (e. g. RPB2 or RPB1; De Crop et al. acpt.), which was beyond the scope of this article. Species from the */ochrogalactus-petersenii* clade have normally sized basidiocarps with latex that changes colour after contact with air, macrocystidia can be present or absent and spores have relatively high warts connected by fine lines. The */parvigerardii* clade contains species characterised by small agaricoid basidiocarps that display vivid orange-red to brown colours, macrocheilocystidia are absent while macropleurocystidia can be present or absent.

Species from the */uyedae* clade have small pleurotoid or agaricoid basidiocarps with brownish to white colours, both macrocheilocystidia and macropleurocystidia are present.

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CHAPTER 6

Exploring the diversity of the genus *Lactifluus*

A compilation of following papers:

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

De Crop E., Tibuhwa D., Baribwegure D., Verbeken A. (2012). *Lactifluus kigomaensis* sp. nov. from Kigoma province,Tanzania. Cryptogamie Mycologie 33 (4):421–426.

De Crop E., Van de Putte K., De Wilde S., Njouonkou A.L., De Kesel A., Verbeken A. Milkcap look-a-likes from gallery forests in tropical Africa: *Lactifluus foetens* and *Lf. albomembranaceus* sp. nov. (Russulaceae). Phytotaxa – Submitted.

Li G.J., Hyde K.D., Zhao R.L., Sinang H., Abdel-Aziz F.A., Abdel-Wahab M.A., Alves-Silva G., Ammirati J., Ariyawansa H.A., Baghela A., Bahkali A.H., Beug M., Bojantchev D., Boonpratuang T., Bulgakov T., Camporesi E., Castilho B.M., Ceska O., Chakraborty D., Chen J.J., Chethana K.W.T., Consiglio G., Cui B.K., Dai Y.C., Daranagama D.A., Das K., Dayarathna M.C., De Crop E., De Oliveira R.J.V., de Souza C.A.F., Dentinger B.T.M., Dissanayake A.J., Doilom M., Drechsler-Santos E.R., Ghobad-Nejhad M., Gilmore S.P., Góes-Neto A., Gorczak M., Haitjema C.H., Hapuarachchi K.K., Hashimoto A., He M.Q., Henrique J.G., Henske J.K., Hirayama K., de Souza J.I., Jayasiri S.C., Jayawardena R.S., Jeon S.J., Jesus A.L., Jones E.B.G., Josefina I.M., Karunarathna S.C., Kirk P.M., Konta S., Kuhnert E., Langer E., Lee H.S., Lee H.B., Li W.J., Li X.H., Liimatainen K., Lima D.X., Lin C.G., Luangsa-ard J.J., Lücking R., Lumbsch H.T., Lumyong S., Maharachchikumbura S.S.N., Malibiran L.E., Marano A.V., Matsumura M., McKenzie E.H.C., Nguyen T.T.T., Niskanen T., Norphanphoun C., O'Malley M.A., Pablo A., Parnmen S., Pawłowska J., Perera R.H., Phookamsak R., Phukhamsakda C., Pires-Zottarelli C.L.A., Raspé O., Reck M.A., Monteiro de Azevedo Santiago A.L.C., Setti L., Shang Q.J., Singh S.K., Sir E.B., Solomon K.V., Song J., Srikitikulchai P., Stadler M., Suetrong S., Takahashi H., Takahashi T., Tanaka K., Tang L.P., Thambugala K.M., Theodorou M.K., Thongbai B., Thummarukcharoen T., Tian Q., Tibpromma S., Verbeken A., Vizzini A., Vlasák J., Voigt K., Wanasinghe D.N., Wang Y., Weerakoon G., Wen H.A., Wen T.C., Wijayawardene N.N., Wongkanoun S., Wrzosek M., Xiao Y.P., Yan J.Y., Yang J., Yang S.D., Young J.T., Yu H., Zhang J.F., Zhao J. & Zhou L.W. (2016). Fungal diversity notes 253-366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity – DOI 10.1007/s13225-016-0366-9.

Introduction

In 2008, Buyck et al. (2008) found out that the milkcaps were paraphyletic and split into two milkcap genera: *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel. *Lactarius* contained the majority of the described species, mainly from temperate regions, while *Lactifluus* mainly contains tropical species. The names of these tropical species had to be recombined and this was done in a series of three papers. Species of the subgenera *Lf.* subg. *Lactariopsis* (Henn.) Verbeken, *Lf.* subg. *Russulopsis* (Verbeken) Verbeken and *Lf.* subg. *Edules* (Verbeken) Verbeken were combined in a first paper (Verbeken et al. 2011), species of *Lf.* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe were combined in a second paper (Stubbe et al. 2012), and species of *Lf.* subg. *Lactifluus* and *Lf.* subg. *Piperati* Verbeken were combined in a third paper (Verbeken et al. 2012). This third paper is presented here, as part A of this chapter. Since these new combinations were made at the beginning of this PhD study, this paper still follows the traditional classification of *Lactifluus*. For the most recent classification, see Chapter 2.

During the course of this study, several new species were discovered and being described. Part B, C and D each cover the description of one or two new species. In Part B, a new Tanzanian species is described from the miombo woodlands in Kigoma: *Lf. kigomaensis* De Crop & Verbeken. This species was also described at the beginning of this PhD study and the paper still follows the traditional classification. In Part C, two look-a-likes from the gallery forests in tropical Africa are studied and the new species *Lf. albomembranaceus* De Wilde & Van de Putte is described. And finally, Part D covers the description of two new Thai *Lactifluus* species: *Lf. armeniacus* De Crop & Verbeken and *Lf. ramipilosus* Verbeken & De Crop. These two species were included in a paper describing more than 100 new fungal species for Thailand.

Part A – New combinations in *Lactifluus*. 3. *Lf*. subgenera *Lactifluus* and *Piperati*

Abstract

In this last of a series of three papers, new combinations in the genus *Lactifluus* are proposed. This paper treats *Lactifluus* subg. *Lactifluus* (an autonymous subgenus) and *Lactifluus* subg. *Piperati* (proposed as a new subgenus). In *Lactifluus* subg. *Lactifluus*, six sections are recognized (five of them as new combinations) and 46 new combinations are proposed at species level. In *Lactifluus* subg. *Piperati*, two sections are recognized (as new combinations) and nine new species combinations are proposed. In addition, new combinations are proposed for an unassigned section and its single species as well as for three unassigned species.

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

Introduction

This is the third treatment of species formerly treated in *Lactarius* Pers. and now classified in *Lactifluus* (Pers.) Roussel (Buyck et al. 2008). *Lactifluus* subgenera *Edules*, *Lactariopsis*, and *Russulopsis* were discussed in the first article (Verbeken et al. 2011) and *Lactifluus* subg. *Gerardii* in the second (Stubbe et al. 2012).

Taxonomy

Lactifluus subg. *Lactifluus*

This large and diverse subgenus comprises 6 sections. *Lactifluus* sect. *Rubroviolascentini*, with 2 species, is endemic to tropical Africa. Two sections, *L.* sect. *Polysphaerophori* and *L.* sect. *Pseudogymnocarpi* (each with 8 species) are almost completely African, except for one South American species in *L.* sect. *Polysphaerophori* and one Chinese species in *L.* sect. *Pseudogymnocarpi*. *L.* sect. *Phlebonemi* (11 species) and *L.* sect. *Tomentosi* (9 species) also have their major distribution in Africa, but contain some species from North and Central America, Australia, Europe and Asia. *Lactifluus* sect. *Lactifluus* with Asian, American, and European species is the only section not represented in tropical Africa.
Five species in *Lactifluus* subg. *Lactifluus* have already been recombined: *L. corrugis*, *L. oedematopus*, *L. princeps* and *L. volemus* in *L.* sect. *Lactifluus*; and *L. hygrophoroides* in *L.* sect. *Tomentosi*.

Lactifluus (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806, **subg. *Lactifluus***
TYPE: *Agaricus lactifluus* L. [= *L. volemus* (Fr. : Fr.) Kuntze] (see Buyck et al. 2010).
= *Lactarius* subg. *Lactifluus* (Burl.) Hesler & A.H. Sm., N. Am. Species *Lactarius*: 158. 1979.
TYPE: *Agaricus volemus* Fr. : Fr.

Lactifluus (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806, **sect. *Lactifluus***
TYPE: *Agaricus lactifluus* L.
= *Lactarius* subsect. *Lactifluini* (Burl.) Singer, Ann. Mycol. 40: 114. 1942.
≡ *Lactarius* subsect. *Volemi* Pacioni & Lalli, Mycotaxon 44: 190. 1992, nom. superfl.
TYPE: *Agaricus volemus* Fr. : Fr.

Lactifluus acicularis (Van de Putte & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564580
≡ *Lactarius acicularis* Van de Putte & Verbeken, Fungal Diversity 45: 108. 2010.

Lactifluus austrovolemus (Hongo) Verbeken, **comb. nov.**
MYCOBANK MB 564581
≡ *Lactarius austrovolemus* Hongo, Rep. Tottori Mycol. Inst. 10: 362. 1973.

Lactifluus corrugis (Peck) Kuntze, Revis. Gen. Pl. 2: 856. 1891.
≡ *Lactarius corrugis* Peck, Annual Rep. New York State Mus. 32: 31. 1880 (“1878”).

Lactifluus crocatus (Van de Putte & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564582
≡ *Lactarius crocatus* Van de Putte & Verbeken, Fungal Diversity 45: 112. 2010..

Lactifluus distantifolius (Van de Putte, Stubbe & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564583
≡ *Lactarius distantifolius* Van de Putte, Stubbe & Verbeken, Fungal Diversity 45: 115. 2010.

Lactifluus lamprocystidiatus (Verbeken & E. Horak) Verbeken, **comb. nov.**
MYCOBANK MB 564584
≡ *Lactarius lamprocystidiatus* Verbeken & E. Horak, Austr. Syst. Bot. 13: 674. 2000.

Lactifluus longipilus (Van de Putte, H.T. Le & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564585
≡ *Lactarius longipilus* Van de Putte, H.T. Le & Verbeken, Fungal Diversity 45: 117. 2010.

Lactifluus oedematopus (Scop.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Agaricus oedematopus* Scop., Fl. Carniol., Ed. 2, 2: 453. 1772.

Lactifluus pallidilamellatus (Montoya & Bandala) Van de Putte, **comb. nov.**
MYCOBANK MB 564586
≡ *Lactarius pallidilamellatus* Montoya & Bandala, Cryptog. Mycol. 25: 16. 2004.

Lactifluus pinguis (Van de Putte & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564587
≡ *Lactarius pinguis* Van de Putte & Verbeken, Fungal Diversity 45: 119. 2010.

Lactifluus princeps (Berk.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Lactarius princeps* Berk., Hooker's J. Bot. Kew Gard. Misc. 4: 135. 1852.

Lactifluus vitellinus (Van de Putte & Verbeken) Van de Putte, **comb. nov.**
MYCOBANK MB 564588
≡ *Lactarius vitellinus* Van de Putte & Verbeken, Fungal Diversity 45: 121. 2010.

Lactifluus volemus (Fr.: Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Agaricus volemus* Fr.: Fr., Syst. Mycol. 1: 69. 1821.

Lactifluus* sect. *Polysphaerophori (Singer) Verbeken, **comb. nov.**
MYCOBANK MB 564589
≡ *Lactarius* sect. *Polysphaerophori* Singer, Beih. Sydowia 7: 106. 1973.
TYPE: *Lactarius veraecrucis* Singer.
= *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeken, Mycotaxon 66: 374. 1998.
TYPE: *Lactarius gymnocarpus* R. Heim ex Singer

Lactifluus albocinctus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564590
≡ *Lactarius albocinctus* Verbeken, Syst. Geogr. Pl. 70: 182. 2000.

Lactifluus brunnescens (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564591
≡ *Lactarius brunnescens* Verbeken, Bull. Jard. Bot. Belg. 65: 199. 1996.

Lactifluus flammans (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564592
≡ *Lactarius flammans* Verbeken, Mycotaxon 55: 539. 1995.

Lactifluus foetens (Verbeken & Van Rooij) Verbeken, **comb. nov.**
MYCOBANK MB 564593
≡ *Lactarius foetens* Verbeken & Van Rooij, Nova Hedwigia 77: 230. 2003.

Lactifluus goossensiae (Beeli) Verbeken, **comb. nov.**
MYCOBANK MB 564594
≡ *Lactarius goossensiae* Beeli, Bull. Soc. Roy. Bot. Belgique 60: 165. 1928.

Lactifluus gymnocarpus (R. Heim ex Singer) Verbeken, **comb. nov.**
MYCOBANK MB 564595
≡ *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946.

Lactifluus tanzanicus (Karhula & Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564596
≡ *Lactarius tanzanicus* Karhula & Verbeken, Karstenia 38: 50. 1998.

Lactifluus veraecrucis (Singer) Verbeken, **comb. nov.**
MYCOBANK MB 564597
≡ *Lactarius veraecrucis* Singer, Beih. Sydowia 7: 104. 1973.

Lactifluus* sect. *Phlebonemi (R. Heim ex Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564598
≡ *Lactarius* sect. *Phlebonemi* R. Heim ex Verbeken, Mycotaxon 66: 378. 1998.
TYPE: *Lactarius phlebonemus* R. Heim & Gooss.-Font.
= *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

Lactifluus angustus (R. Heim & Gooss.-Font.) Verbeken, **comb. nov.**
MYCOBANK MB 564599
≡ *Lactarius angustus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 67. 1955.

Lactifluus arsenei (R. Heim) Verbeken, **comb. nov.**
MYCOBANK MB 564600
≡ *Lactarius arsenei* R. Heim, Candollea 7: 380. 1938, as "*arsenii*".

Lactifluus brunneoviolascens (Bon) Verbeken, **comb. nov.**
MYCOBANK MB 564601
≡ *Lactarius brunneoviolascens* Bon, Doc. Mycol. 1 (2): 45. 1971.

Lactifluus caribaeus (Pegler) Verbeken, **comb. nov.**
MYCOBANK MB 564602
≡ *Lactarius caribaeus* Pegler, Kew Bull. 33: 617. 1979.

Lactifluus longivelutinus (X.H. Wang & Verbeken) X.H. Wang, **comb. nov.**
MYCOBANK MB 564603
≡ *Lactarius longivelutinus* X.H. Wang & Verbeken, Nova Hedwigia 83 (1-2): 168, 2006.

Lactifluus luteolus (Peck) Verbeken, **comb. nov.**
MYCOBANK MB 564604
≡ *Lactarius luteolus* Peck, Bull. Torrey Bot. Club 23: 412. 1896.

Lactifluus nonpiscis (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564605
≡ *Lactarius nonpiscis* Verbeken, Bull. Jard. Bot. Belg. 65: 204. 1996.

Lactifluus phlebonemus (R. Heim & Gooss.-Font.) Verbeken, **comb. nov.**
MYCOBANK MB 564606
≡ *Lactarius phlebonemus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 38. 1955.

Lactifluus pisciodorus (R. Heim) Verbeken, **comb. nov.**
MYCOBANK MB 564607
≡ *Lactarius pisciodorus* R. Heim, Candollea 7: 380. 1938.

Lactifluus putidus (Pegler) Verbeken, **comb. nov.**
MYCOBANK MB 564608
≡ *Lactarius putidus* Pegler, Kew Bull. 33: 620. 1979.

Lactifluus rubrobrunnescens (Verbeken, E. Horak & Desjardin) Verbeken, **comb. nov.**
MYCOBANK MB 564609
≡ *Lactarius rubrobrunnescens* Verbeken, E. Horak & Desjardin, Sydowia 53: 274. 2001.

Lactifluus* sect. *Pseudogymnocarpi (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564610
≡ *Lactarius* sect. *Pseudogymnocarpi* Verbeken, Mycotaxon 66: 376. 1998.
TYPE: *Lactarius gymnocarpoides* Verbeken

Lactifluus carmineus (Verbeken & Walleyn) Verbeken, **comb. nov.**
MYCOBANK MB 564611
≡ *Lactarius carmineus* Verbeken & Walleyn, Syst. Geogr. Pl. 70: 190. 2000.

Lactifluus gymnocarpoides (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564612
≡ *Lactarius gymnocarpoides* Verbeken, Mycotaxon 55: 530. 1995.

Lactifluus longisporus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564613
≡ *Lactarius longisporus* Verbeken, Mycotaxon 55: 527. 1995.

Lactifluus luteopus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564614
≡ *Lactarius luteopus* Verbeken, Mycotaxon 55: 536. 1995.

Lactifluus medusae (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564615
≡ *Lactarius medusae* Verbeken, Mycotaxon 55: 532. 1995.

Lactifluus pseudogymnocarpus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564616
≡ *Lactarius pseudogymnocarpus* Verbeken, Mycotaxon 55: 523. 1995.

Lactifluus pumilus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564617
≡ *Lactarius pumilus* Verbeken, Bull. Jard. Bot. Belg. 65: 205. 1996.

Lactifluus tenuicystidiatus (X.H. Wang & Verbeken) X.H. Wang, **comb. nov.**
MYCOBANK MB 564618
≡ *Lactarius tenuicystidiatus* X.H. Wang & Verbeken, Nova Hedwigia 83: 173, 2006.

Lactifluus* sect. *Rubroviolascentini (Singer) Verbeken, **comb. nov.**
MYCOBANK MB 564619
≡ *Lactarius* subsect. *Rubroviolascentini* Singer, Ann. Mycol. 40: 114. 1942.
≡ *Lactarius* sect. *Rubroviolascentini* (Singer) Verbeken, Mycotaxon 66: 380. 1998, as "*Rubroviolascentes*".
TYPE: *Lactarius rubroviolascens* R. Heim

Lactifluus denigricans (Verbeken & Karhula) Verbeken, **comb. nov.**
MYCOBANK MB 564620
≡ *Lactarius denigricans* Verbeken & Karhula, Persoonia 16: 219. 1996.

Lactifluus rubroviolascens (R. Heim) Verbeken, **comb. nov.**
MYCOBANK MB 564621
≡ *Lactarius rubroviolascens* R. Heim, Candollea 7: 377. 1938.

Lactifluus* sect. *Tomentosi (McNabb) Verbeken, **comb. nov.**
MYCOBANK MB 564622
≡ *Lactarius* sect. *Tomentosi* McNabb, New Zealand J. Bot. 9: 59. 1971.
≡ *Lactarius* subsect. *Clarkeina* McNabb, New Zealand J. Bot. 9: 59. 1971.
TYPE: *Lactarius clarkeae* Cleland
= *Lactarius* subsect. *Rugati* Pacioni & Lalli, Mycotaxon 44: 190. 1992, nom. superfl.
≡ *Lactarius* sect. *Rugati* Verbeken, Mycotaxon 66: 372. 998, 1998, nom. superfl.
TYPE: *Lactarius rugatus* Kühner & Romagn.

Lactifluus clarkeae (Cleland) Verbeken, **comb. nov.**
MYCOBANK MB 564623
≡ *Lactarius clarkeae* Cleland, Trans. & Proc. Roy. Soc. S. Australia 51: 302. 1927, as "*clarkei*".

Lactifluus hygrophoroides (Berk. & M.A. Curtis) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Lactarius hygrophoroides* Berk. & M.A. Curtis, Ann. Mag. Nat. Hist., Ser. 3, 4: 293. 1859.

Lactifluus kivuensis (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564624
≡ *Lactarius kivuensis* Verbeken, Bull. Jard. Bot. Belg. 65: 202. 1996.

Lactifluus pseudoluteopus (X.H. Wang & Verbeken) X.H. Wang, **comb. nov.**
MYCOBANK MB 564625
≡ *Lactarius pseudoluteopus* X.H. Wang & Verbeken, Nova Hedwigia 83: 171. 2006.

Lactifluus pseudovolemus (R. Heim) Verbeken, **comb. nov.**
MYCOBANK MB 564626
≡ *Lactarius pseudovolemus* R. Heim, Candollea 7: 378. 1938.

Lactifluus rubiginosus (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564627
≡ *Lactarius rubiginosus* Verbeken, Bull. Jard. Bot. Belg. 65: 207. 1996.

Lactifluus rugatus (Kühner & Romagn.) Verbeken, **comb. nov.**
MYCOBANK MB 564628
≡ *Lactarius rugatus* Kühner & Romagn., Bull. Soc. Mycol. France 69: 362. 1954 ("1953").

Lactifluus xerampelinus (Karhula & Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564629
≡ *Lactarius xerampelinus* Karhula & Verbeken, Karstenia 38: 59. 1998.

Lactifluus volemoides (Karhula) Verbeken, **comb. nov.**
MYCOBANK MB 564630
≡ *Lactarius volemoides* Karhula, Karstenia 38: 53. 1998.

Lactifluus* subg. *Piperati

This group consists of two sections, one with 9 species described from Europe and Asia (but also with records from North America), and the other with one American species. The combination *Lactifluus piperatus* has already been proposed.

Lactifluus* subg. *Piperati Verbeken, **subg. nov.**
MYCOBANK MB 564631

Pileus pallidus, saepe albus vel albidus, siccus. Pileipellis hyphoepithelium, tenue stratum hyphis hyalinis super cellullis globosis. Dermatocystidia interdum presentia.

TYPE: *Agaricus piperatus* L.: Fr.

Lactifluus* sect. *Piperati (Fr.) Verbeken, **comb. nov.**
MYCOBANK MB 564632
≡ *Agaricus* sect. *Piperati* Fr., Syst. Mycol. 1: 73. 1821.
≡ *Lactarius* sect. *Piperati* (Fr.: Fr.) Fr., Epicr. Syst. Mycol.: 338. 1838.
TYPE: *Agaricus piperatus* L.: Fr.

Lactifluus dwaliensis (K. Das, J.R. Sharma & Verbeken) K. Das, **comb. nov.** MYCOBANK MB 564633
≡ *Lactarius dwaliensis* K. Das, J.R. Sharma & Verbeken, Mycotaxon 88: 334. 2003.

Lactifluus glaucescens (Crossl.) Verbeken, **comb. nov.**
MYCOBANK MB 564634
≡ *Lactarius glaucescens* Crossl., Naturalist, J. Nat. Hist. N. England 1900(516): 5. 1900.
≡ *Lactarius piperatus* var. *glaucescens* (Crossl.) Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 186. 1979

Lactifluus leucophaeus (Verbeken & E. Horak) Verbeken, **comb. nov.**
MYCOBANK MB 564635
≡ *Lactarius leucophaeus* Verbeken & E. Horak, Austr. Syst. Bot. 12: 768. 1999.

Lactifluus novoguineensis (Henn.) Verbeken, **comb. nov.**
MYCOBANK MB 564636
≡ *Lactarius novoguineensis* Henn., Bot. Jahrb. Syst. 25: 503. 1898.

Lactifluus olivescens (Verbeken & E. Horak) Verbeken, **comb. nov.**
MYCOBANK MB 564637
≡ *Lactarius olivescens* Verbeken & E. Horak, Austr. Syst. Bot. 13: 678. 2000.

Lactifluus paleus (Verbeken & E. Horak) Verbeken, **comb. nov.**
MYCOBANK MB 564638
≡ *Lactarius paleus* Verbeken & E. Horak, Austr. Syst. Bot. 12: 771. 1999.

Lactifluus piperatus (L. : Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Agaricus piperatus* L.: Fr., Sp. Pl.: 1173. 1753.
≡ *Lactarius piperatus* (L. : Fr.) Pers., Tent. Disp. Meth. Fung.: 64. 1797.
≡ *Galorrheus piperatus* (L.: Fr.) Fr., Stirp. Agri Femsion. 3: 57. 1825.

Lactifluus roseophyllus (R. Heim) De Crop, **comb. nov.**
MYCOBANK MB 564639
≡ *Lactarius roseophyllus* R. Heim, Rev. Mycol. (Paris) 30: 237. 1966 ("1965").

Lactifluus subpiperatus (Hongo) Verbeken, **comb. nov.**
MYCOBANK MB 564647
≡ *Lactarius subpiperatus* Hongo, Mem. Fac. Liberal Arts Shiga Univ., Nat. Sci. 15: 46. 1964.

Lactifluus* sect. *Allardii (Hesler & A.H. Sm.) De Crop, **comb. nov.**
MYCOBANK MB 564640
≡ *Lactarius* sect. *Allardii* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 207. 1979.
TYPE: *Lactarius allardii* Coker

Lactifluus allardii (Coker) De Crop, **comb. nov.**
MYCOBANK MB 564641
≡ *Lactarius allardii* Coker, J. Elisha Mitchell Sci. Soc. 34: 12. 1918.

Unassigned taxa

The following section has not yet been assigned to a subgenus. Only one species, described from tropical Africa, is known.

Lactifluus* sect. *Aurantiifolii (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564642
≡ *Lactarius* sect. *Aurantiifolii* Verbeken, Mycotaxon 77: 441. 2001.
TYPE: *Lactarius aurantiifolius* Verbeken

Lactifluus aurantiifolius (Verbeken) Verbeken, **comb. nov.**
MYCOBANK MB 564643
≡ *Lactarius aurantiifolius* Verbeken, Bull. Jard. Bot. Belg. 65: 197. 1996.

The following species have an uncertain systematic position, but morphological and/or molecular data support their placement in *Lactifluus*.

Lactifluus caperatus (R. Heim & Gooss.**-**Font.) Verbeken, **comb. nov.**
MYCOBANK MB 564644
≡ *Lactarius caperatus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 36. 1955.

Lactifluus cocosmus (Van de Putte & De Kesel) Van de Putte, **comb. nov.**
MYCOBANK MB 564645
≡ *Lactarius cocosmus* Van de Putte & De Kesel, Cryptog. Mycol. 30: 40. 2009.

Lactifluus subclarkeae (Grgur.) Verbeken, **comb. nov.**
MYCOBANK MB 564646
≡ *Lactarius subclarkeae* Grgur., Larger Fungi S. Australia: 63, 1997.

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Part B – A new *Lactifluus* species from Tanzania, Kigoma province

Abstract

Lactifluus kigomaensis De Crop & Verbeken sp. nov. is described from primary miombo woodlands in the seriously underexplored Kigoma Province in North Western Tanzania. The species is consumed and offered for sale on local markets.

Published as: De Crop E., Tibuhwa D., Baribwegure D., Verbeken A. (2012). *Lactifluus kigomaensis* sp. nov. from Kigoma province,Tanzania. Cryptogamie Mycologie 33 (4):421–426.

Introduction

The diversity of the ectomycorrhizal genera *Lactarius* Pers. and *Lactifluus* (Pers.) Roussel in tropical Africa is high, with 39 and 59 species respectively (Douanla-Meli and Langer 2009; Van de Putte et al. 2009; Verbeken and Walleyn 2010). After the splitting of the genus *Lactarius*, with besides some representatives in *Multifurca*, the remaining species divided over *Lactarius* sensu novo and *Lactifluus*, we know that the genus *Lactarius* has its main distribution in the Northern hemisphere, while the genus *Lactifluus* mainly occurs in the tropics with a major distribution in tropical Africa. *Lactarius* seems a large genus with a relatively low genetic diversity while *Lactifluus* is a smaller group with very high genetic diversity and subgroups in very different and distant clades. This is also illustrated by the recent discovery of *Lactifluus cocosmus (Van de Putte et al. 2009)*, which turns out to have a phylogenetically very isolated position and to represent an unknown subgroup of the genus.

One of the most important and rich ectomycorrhizal vegetations, where *Lactifluus* is one of the major genera, is the miombo woodland. The miombo woodland covers an estimated area of 2.7 million km^2 on nutrient-poor soils in sub-Saharan Africa that receives less than 700 mm of precipitation per year (Campbell et al. 1996). It is also characterized by the local codominance of ectomycorrhizal trees of different genera of the Caesalpinaceae, especially *Julbernardia, Brachystegia* and *Isoberlinia*, as well as trees of the genus *Uapaca* (Phyllantaceae).

In Tanzania, studies focusing on edible mushrooms in miombo woodland have been rather well-explored compared to other countries in the region (Härkönen et al. 1993; Härkönen et al. 1994; Saarimäki et al. 1994; Härkönen et al. 1995; Calonge et al. 1997; Karhula et al. 1998; Tibuhwa et al. 2008; Tibuhwa et al. 2012). However, the Kigoma province in North-West Tanzania, situated at the border of Burundi and Lake Tanganyika, is rich in miombo forest, but has been poorly explored concerning the presence and the use of edible fungi. This region contains the largest untouched miombo zones in the country and was the focus region of our study. This work describes a new *Lactifluus* species from the Kigoma province in Tanzania.

Material and methods

Macroscopic characters are all based on fresh material. Microscopic features were studied from dried material mainly in Congo-red in L4. Spore ornamentation is described and illustrated as observed in Melzer's reagent. For details on terminology we refer to Verbeken (1998) and Verbeken & Walleyn (2010). Line-drawings were made by A. Verbeken, with the aid of a drawing tube at original magnifications: 6000 × for spores, 1000 × for individual elements and sections. Basidia length excludes sterigmata length. Spores were measured in side view in Melzer's reagent, excluding the ornamentation, and measurements are given as [AVa-2*SD] - *AVa* - [AVa + 2*SD], in which AVa = mean value for the measured collection and SD = standard deviation. Q stands for "quotient length/width" and is given as MINQ - *AvQ* – MAXQ, in which AvQ stands for the mean quotient for the measured spores. Colour codes refer to Kornerup & Wanscher (1978).

Results

Lactarius kigomaensis **De Crop & Verbeken sp. nov.** Fig. 6.1
Etymology: from the Kigoma region

Pileus 65 mm diam., firm, moderately thick, planoconvex, irregularly shaped, somewhat knotty; surface dry, somewhat felty or chamois-leather-like, strongly and irregularly cracking, with concentrical wrinkles at the extreme margin, almost unicolorous, only paler in the cracks, pale brown, brownish orange or brown (7CD6-7, 7DE7), slightly paler towards margin. **Stipe** 45 x 17 mm, irregularly cylindric, with some folds and ridges, curved; surface smooth, dry, pale reddish orange, 6AB4-5. **Lamellae** decurrent with teeth,

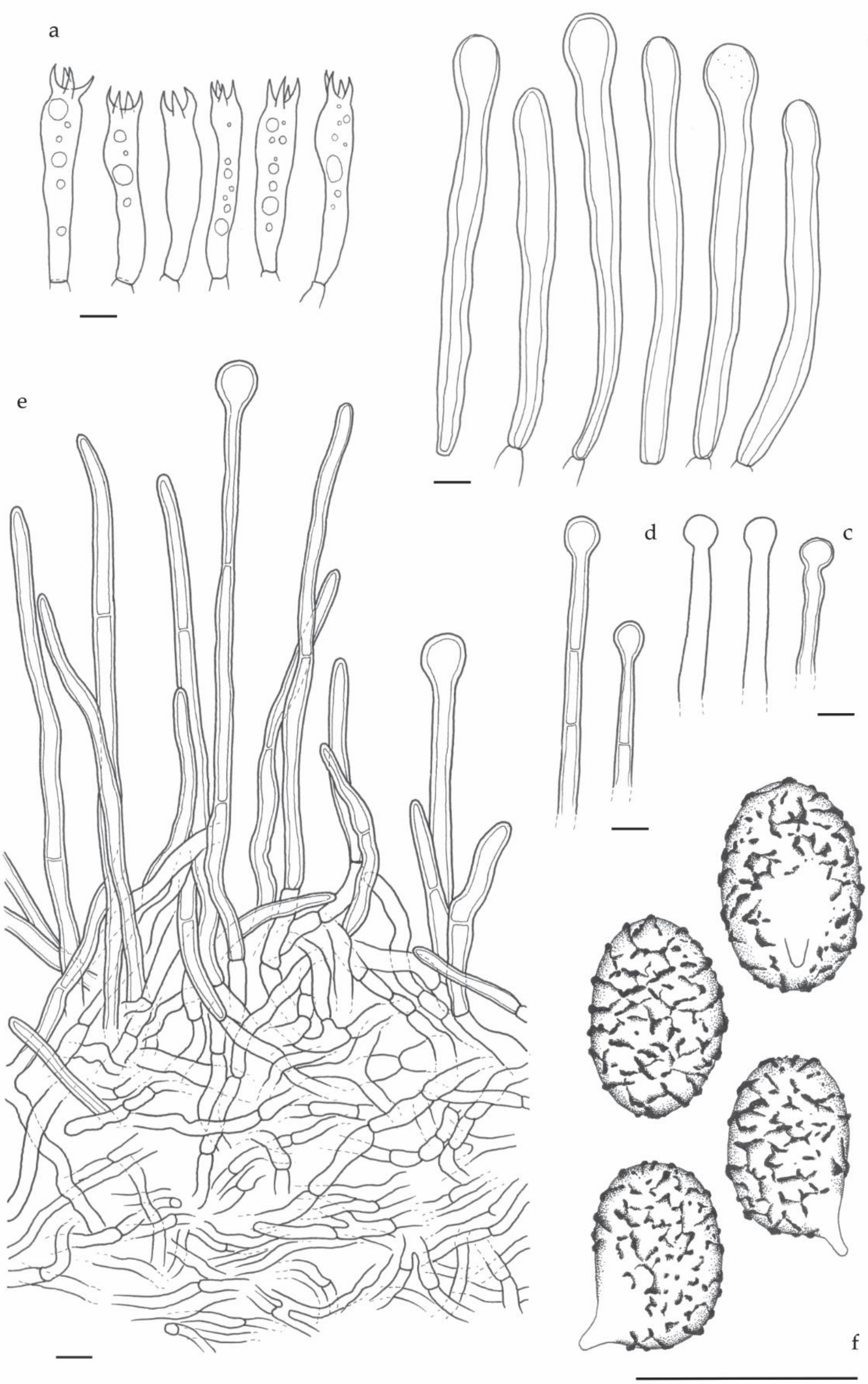

Fig. 6.1 *Lactifluus kigomaensis*: **a.** basidia, **b.** pleuromacrocystidia, **c.** capitate elements of the stipitipellis, **d.** capitate elements of the pileipellis, **e.** section through the pileipellis, **f**. basidiospores (all from holotype AV 11-066, scale bar = 10 μm).

moderately distant, 9 L+l/cm, with abundant lamellulae of different lengths, pale yellow (4A4-5A4), staining purplish-brown by the latex (pale, not dark). **Context** white, very solid and firm in stipe and in pileus, slightly changing flesh-coloured to pale orange when cut, dirty salmon to greyish with Fe_2SO_4, unchanging with gaiac; smell very much like *Lactifluus volemus*, agreeable, lobster-like; taste agreeable, nut-like. **Latex** rather abundant, semitransparent, between watery and white, staining the lamellae pale purplish brown to greyish; taste mild.

Basidiospores broadly ellipsoid to ellipsoid, 7.5-*8.4*-9.3 x 5.2-*6.2*-7.0 µm (Q = 1.13-*1.37*-1.53); ornamentation amyloid, composed of low, up to 0.3 µm high, ridges forming an incomplete reticulum; many isolated warts and short ridges present; plage inamyloid. **Basidia** 45-50 x 8-11 µm, cylindric to narrowly clavate, 4-spored. **Pleurolamprocystidia** very abundant, very emergent and arising deep in the hymenium, 90-120 x 7-11 µm, cylindrical and typically capitate, distinctly swollen at the top; very thick-walled. **Pleuropseudocystidia** rare, usually not emergent, 3-5 µm diam., slightly tortuose. **Lamellae-edge** fertile, composed of basidia and occasionally a cheilocystidium. **Hymenophoral trama** cellular, with lactifers and sphaerocytes. **Pileipellis** a lamprotrichoderm, up to 220 µm thick; terminal elements cylindric to distinctly capitate, 50-170 x 4-6 µm, thick-walled; subpellis composed of intricate, hyaline hyphae. **Stipitipellis** a lamprotrichoderm, also with distinctly capitate terminal elements present.

Studied material:
Tanzania, Kigoma Province, Mboyogo Kigoma, Kitwe, alt. 780 m, S04°54.96′ E29°36.51′, purchased from Katonga market, sold in a mixture with *Cantharellus* spp., *Amanita loosii* Beeli, *Russula* spp., 15 March 2011, Verbeken, AV 11-006[22] (Holotypus, GENT) – Tanzania, Kigoma Province, near Kigoma, Msitwa Katara, alt. 816 m, S04°54.52' E29°36.06', young and managed miombo forest with *Brachystegia* sp., 16 March 2011, De Crop, EDC 11-012 (GENT) – Tanzania, Kigoma Province, near Kigoma, Zungu beach, alt. 781 m, S04°54.51' E29°33.08', young and managed miombo forest with *Brachystegia* sp., 16 March 2011, De Crop, EDC 11-013 (GENT).

Discussion

Lf. kigomaensis can be recognized in the field by strongly cracking pileus, the lamellae that are staining purplish brown by the latex and the smell of *Lactifluus volemus* (agreeable fishy, lobster-like). Microscopically, the capitate elements are very striking, both in the hymenium as pleurocystidia, as in the pilei- and stipitipellis as terminal elements in a trichoderm. In African species, such capitate elements are only observed in *Lactifluus nonpiscis* (Verbeken) Verbeken and *Lactifluus rubroviolascens* (R. Heim) Verbeken. In *Lf. nonpiscis*, they are very abundant as terminal elements in a lampropalisade (pileipellis) or lamprotrichoderm (stipitipellis), but lamprocystidia are absent. In *Lf. rubroviolascens* they only occur in the stipitipellis near the base of the stipe, while the lamprocystidia and the terminal elements in the pileipellis are never capitate.

Morphologically, the species seems to belong to *Lf.* sect. *Pseudogymnocarpi* because of the thick-walled hairs in the pileipellis and the lamprocystidia. Exceptional for this species is the trichodermic structure of the pileipellis, while all other representatives have a palisadic structure of the pileipellis. Preliminary phylogenetic results show that *Lf. kigomaensis* has an isolated position within the phylogeny of *Lactifluus*.

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[22] *Erratum:* the actual holotype of *Lf. kigomaensis* is AV 11-066, we will correct this mistake in a publication concerning *Lf. kigomaensis* and its sister species (Delange, in prep.).

Part C – Milkcap look-a-likes from gallery forests in tropical Africa: *Lactifluus foetens* and *Lf. albomembranaceus* sp. nov. (Russulaceae)

Abstract

The ectomycorrhizal milkcap genus *Lactifluus* is commonly found within Central and West African gallery forests. During recent field expeditions in Cameroon and Togo, several collections of white *Lactifluus* species were found, resembling *Lactifluus foetens*. Molecular and morphological research indicates that these collections belong to unrelated species, *i.e. Lactifluus foetens* and an undescribed taxon. The latter is here described as *Lactifluus albomembranaceus sp. nov.* from the gallery forests in Central and Western Africa. At least in Cameroon, *Lactifluus albomembranaceus* is a popular edible fungus that is harvested for own consumption and offered for sale on local markets.

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Introduction

Central and West African vegetation is characterised by a forest-savanna mosaic between the Guineo-Congolian rainforest in the south and the Sudanian woodland to the north of the rainforest (White 1983). This forest-savanna mosaic consists of (drier) forests islands and gallery forests along rivers and streams, interspersed with open woodlands and savannas. Open woodlands and savannas generally have dry soils, abundant light availability and a grassy understory. In gallery forests, rivers and streams provide water to the soil and due to closed canopies there is competition for light, a grassy understory is limited and the relative humidity is increased (Natta et al. 2003; Hoffmann et al. 2009; Azihou et al. 2013). Common tree species within West African gallery forests are broad-leaved Caesalpinioideae (e.g. *Berlinia* sp.) and Phyllanthaceae (e.g. *Uapaca* sp.), which are typical hosts for ectomycorrhizal fungi. These humid gallery forests thus provide an ideal habitat for ectomycorrhizal fungi.

Amongst the ectomycorrhizal fungi, members of the Russulaceae family are commonly found within gallery forests in Central and West Africa (Van Rooij et al. 2003; Verbeken and Walleyn 2010; Ba et al. 2012; Maba et al. 2014). The agaricoid Russulaceae genera *Russula* Pers. and *Lactifluus* (Pers.) Roussel are found in large amounts during the rainy season. Many of these species are edible and thus harvested and sold at local markets.

The milkcap genus *Lactifluus* (hereafter abbreviated as *Lf.*) is mainly distributed in the tropics. This ectomycorrhizal genus is species-rich (about 160 species worldwide) and the majority of species is found in tropical Africa (Van de Putte et al. 2009; Verbeken and Walleyn 2010; De Crop et al. 2012; Maba et al. 2014; Maba et al. 2015a; Maba et al. 2015b), tropical Asia (Le et al. 2007b; Stubbe et al. 2010; Van de Putte et al. 2010) and in the Neotropics (Henkel et al. 2000; Miller et al. 2002; Smith et al. 2011; Sá et al. 2013; Sá and Wartchow 2013). Due to its mainly tropical diversity, the genus is relatively understudied and many species remain undescribed. In their study, De Crop *et al.* (acpt.) show that *Lactifluus* consists of four subgenera, in which at least 17 new species were discovered and are waiting to be described. About 40 *Lactifluus* species are known from West Africa (Van de Putte et al. 2009; Verbeken and Walleyn 2010; Maba et al. 2014; Maba et al. 2015a; Maba et al. 2015b), however, based on the large area covered by ectomycorrhizal vegetation in tropical Africa, together with the lack of mycological studies in most countries of the region, this number is expected to be higher.

During field work in Togo (2007) and Cameroon (2012), a white *Lactifluus* species was found, with latex staining brownish when in contact with air, typical for *Lf.* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop. The species is macromorphologically similar to *Lf. foetens* (Verbeken & Van Rooij 2003:230) Verbeken (2012: 445), which was recorded before from Benin (Van Rooij et al. 2003) and Togo (Verbeken and Walleyn 2010), but had not yet been reported from Cameroon. Field notes and a preliminary microscopical study, however, indicated some differences with *Lf. foetens*, which initiated a more detailed study of all available material. In this study, we make a molecular and morphological comparison between this newly found white *Lactifluus* species and *Lf. foetens*.

Material and methods

Sampling

Our dataset consists of species of *Lf.* subg. *Gymnocarpi* extracted from the dataset of De Crop *et al.* (acpt.). We added five more collections of the possible new species and five more collections of *Lf. foetens*, including the type collection. The outgroup consists of five species of *Lf.* subg. *Lactifluus* (Table 6.1). The studied collections are deposited in herbarium Universitatis Gandavensis (GENT) and Herbarium Botanic Garden Meise (BR).

Table 6.1 Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses.

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
Lf.* subg. *Gymnocarpi				
Lactifluus albocinctus **Type**	AV 99-211 (GENT)	Zimbabwe	KR364117	KR364249
Lactifluus albomembranaceus **Type**	EDC 12-046 (GENT)	Cameroon	KR364064	KR364193
Lactifluus albomembranaceus	EDC 12-052 (GENT)	Cameroon	*To be submitted*	*To be submitted*
Lactifluus albomembranaceus	EDC 12-045 (GENT)	Cameroon	*To be submitted*	*To be submitted*
Lactifluus albomembranaceus	EDC 12-054 (GENT)	Cameroon	*To be submitted*	*To be submitted*
Lactifluus albomembranaceus	ADK 4284 (BR)	Togo	*To be submitted*	*To be submitted*
Lactifluus albomembranaceus	DM 355B	Burkina Faso	LN651269	None
Lactifluus brunellus	TH 9130 (BRG, DUKE)	Guyana	JN168728	None
Lactifluus brunneoviolascens	AV 13-038 (GENT)	Italy	KR364123	KR364246
Lactifluus brunnescens	AV 05-083 (GENT)	Malawi	KR364019	KR364146
Lactifluus caribaeus	PAM/Mart 12-090 (LIP)	Martinique	KP691415	KP691424
Lactifluus cf. *castaneibadius*	CL/MART06.019 (LIP)	Martinique	KP691417	KP691426
Lactifluus chiapanensis	VMB 4374A (GENT)	Mexico	GU258297	GU265580
Lactifluus clarkeae	MN 2004002 (L)	Australia	KR364011	HQ318205
Lactifluus flammans	JD 941 (BR)	Congo	KR364078	KR364207
Lactifluus flocktonae	JET1006 (MEL)	Australia	JX266621	JX266637
Lactifluus foetens	ADK 3486 (GENT)	Togo	*To be submitted*	*To be submitted*
Lactifluus foetens	ADK 3688 (BR)	Benin	KR364022	KR364149
Lactifluus foetens **Type**	ADK 2840 (BR)	Benin	KR364023	KR364150
Lactifluus foetens	AV 11-176 (GENT)	Togo	*To be submitted*	*To be submitted*
Lactifluus foetens	ADK 3526 (BR)	Benin	*To be submitted*	*To be submitted*
Lactifluus foetens	ADK 4283 (BR)	Togo	*To be submitted*	*To be submitted*
Lactifluus foetens	ADK 4411 (BR)	Togo	*To be submitted*	*To be submitted*
Lactifluus gymnocarpus	EDC 12-047 (GENT)	Cameroon	KR364065	KR364194
Lactifluus longivelutinus **Type**	XHW 1565 (GENT)	China	KR364114	None
Lactifluus luteolus	AV 05-253 (GENT)	North America	KR364016	KR364142
Lactifluus cf. *murinipes*	F.1890 (LIP)	Martinique	KP691418	None
Lactifluus aff. *nebulosus*	RC/Guad 11-023 (LIP)	Guadeloupe	KP691412	KP691421
Lactifluus nonpiscis **Type**	BB 3171 (GENT)	Zambia	KR364030	KR364157
Lactifluus nonpiscis	AV 11-137 (GENT)	Togo	KR364058	KR364185
Lactifluus panuoides	RC/Guy 10-024 (LIP)	French Guiana	KJ786647	KJ786551
Lactifluus aff. *phlebonemus*	EDC 12-023 (GENT)	Cameroon	KR364062	KR364191
Lactifluus cf. *putidus*	PAM/Mart 11-013 (LIP)	Martinique	KP691413	KP691422
Lactifluus rubrobrunnescens **Type**	EH 7194 (GENT)	Indonesia	KR364115	None
Lactifluus sp.	RC/Guad 08-042 (LIP)	Guadeloupe	KP691414	KP691423
Lactifluus sp.	G3185	French Guiana	KJ786694	KJ786603
Lactifluus sp.	KW 392 (GENT)	Thailand	KR364091	KR364222
Lactifluus sp.	RH 9398 (NY)	Australia	KR364097	KR364229
Lactifluus sp.	PGK13-130	New Caledonia	KP691436	Toulouse
Lactifluus subclarkeae	RH 9231 (NY)	Australia	KR364095	KR364227
Lactifluus cf. *tanzanicus*	AV 11-017 (GENT)	Tanzania	KR364053	KR364180
Lactifluus tanzanicus **Type**	TS 1277 (GENT)	Tanzania	KR364037	KR364164

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
***Lf.* subg. *Lactifluus* - Outgroup**				
Lactifluus acicularis	KVP 08-002 (GENT)	Thailand	HQ318226	HQ318132
Lactifluus corrugis s.l.	AV 05-392 (GENT)	North America	JQ753822	KR364143
Lactifluus crocatus	KVP 08-034 (GENT)	Thailand	HQ318243	HQ318151
Lactifluus vitellinus	KVP 08-024 (GENT)	Thailand	HQ318236	HQ318144
Lactifluus volemus	KVP 11-002 (GENT)	Belgium	JQ753948	KR364175

Morphological analyses

Macroscopic characters are all based on fresh material. Colour codes refer to Kornerup & Wanscher (1978). Microscopic features were studied from dried material. See Verbeken & Walleyn (2010) for details on the terminology used. Elements of the pileipellis and hymenial elements were either mounted in 10% KOH (enhances cell expansion), after which Congo-Red in L4 was added, or directly mounted in Congo-Red in L4. Hairs of the pileipellis were measured from scalps and line drawings of the pileipellis were made from sections. Basidia length excludes sterigmata. Spores were studied in Melzer's reagent and measured in side view, excluding ornamentation (minimum 20 spores per collection). Spore measurements are given as described in Nuytinck and Verbeken (2005). Line drawings were made with the aid of a drawing tube at following magnifications: 6000× for spores (Zeiss axioscop 2 microscope), 1600× for other hymenial elements and sections (Olympus cx31 microscope).

Molecular analysis

DNA from dry collections was extracted using the protocol described by Nuytinck & Verbeken (2003), with modifications described in Van de Putte *et al.* (2010). DNA from fresh material was extracted using the CTAB extraction described in Nuytinck & Verbeken (2003). Protocols for PCR amplification follow Le *et al.* (2007a). Two nuclear markers that were previously shown informative within this subgenus (De Crop et al. acpt.) were used: (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, and using primers ITS-1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) and (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000).

PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Life Technology) at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with the Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v7 (Katoh and Toh 2008), using the E-INS-I strategy. Trailing ends of the alignment were trimmed and sequences were manually edited when necessary in Mega 6 (Tamura et al. 2013). The alignment can be acquired from the first author and TreeBASE (S19376).

Sequence data were divided into the following partitions: partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Maximum likelihood (ML) analyses were conducted with RAxML v8.0.24 (Stamatakis 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis et al. 2008). All analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

Results

Our molecular results clearly show that the newly collected species differs from *Lactifluus foetens* (Fig. 6.2). The new species falls within *Lf.* sect. *Gymnocarpi* and is a sister species of *Lf. gymnocarpus* (R. Heim ex Singer Singer 1948: 107) Verbeken (2012: 445), whilst *Lf. foetens* is still a species on an isolated branch (Fig. 6.2). This is also supported by morphological differences (see Discussion). Based on these morphological and molecular differences, the new species is here described as *Lactifluus albomembranaceus sp. nov*. A revised description of the microscopical characteristics of *Lf. foetens* is given as well.

Fig. 6.2 Overview Maximum Likelihood tree of the *Lactifluus* subg. *Gymnocarpi*, based on concatenated ITS and LSU sequence data. Maximum Likelihood bootstrap values >70 are shown.

Fig. 6.3 Basidiocarps of *Lactifluus albomembranaceus*: **a.** holotypus EDC 12-046 (photo by E. De Crop) and **b.** ADK 4284 (photo by A. De Kesel) and *Lf. foetens*: **c.** ADK 4283 (photo by A. De Kesel) and **d.** AV 11-176 (photo by A. Verbeken).

Lactifluus albomembranaceus De Wilde & Van de Putte *sp. nov*. Fig. 6.3a–b, 6.4, 6.5, 6.6a–b

Mycobank: MB 815846

Diagnosis: A medium-sized white species, which resembles *Lactifluus foetens* at first sight but differs on several levels. Macroscopically it is characterised by a white and often translucent pileus when fresh, together with white lamellae and a white to cream-yellow coloured stipe. Microscopically, this species has a lampropalisade as pileipellis with terminal hair-like elements shorter than those of *Lf. foetens*, broadly ellipsoid spores, a spore ornamentation of isolated warts that are often connected by fine lines and never forming a reticulum, slender basidia and rather long marginal cells.

Etymology: Contraction of 'albo' and 'membranaceus', referring to the white colour and translucent aspect of the pileus.

Holotypus: CAMEROON. Western region: Noun division, Koutaba subdivision, Mamevouo village, N5°38.97′ E10°51.08′, elev. 1111m, gallery forest in savannah region near a river and surrounded by farmland, *Uapaca guineensis* Müller Argoviensis (1864 : 517), 10 May 2012, E. De Crop 12-046 (GENT!)

Pileus 40–55 mm diam., firm, planoconvex with central depression, translucent at maturity and when fresh; margin slightly involute when young, deflexed when older, concentrically wrinkled, often striate to sulcate up to around 1 cm from the margin; pellis chamois leather-like, wrinkled and granulose, yellowish when juvenile, then becoming pure white, after collecting becoming yellowish-cream (2–3A2), becoming brownish after bruising. *Lamellae* adnate with distinct decurrent tooth, with lamellulae of different lengths,

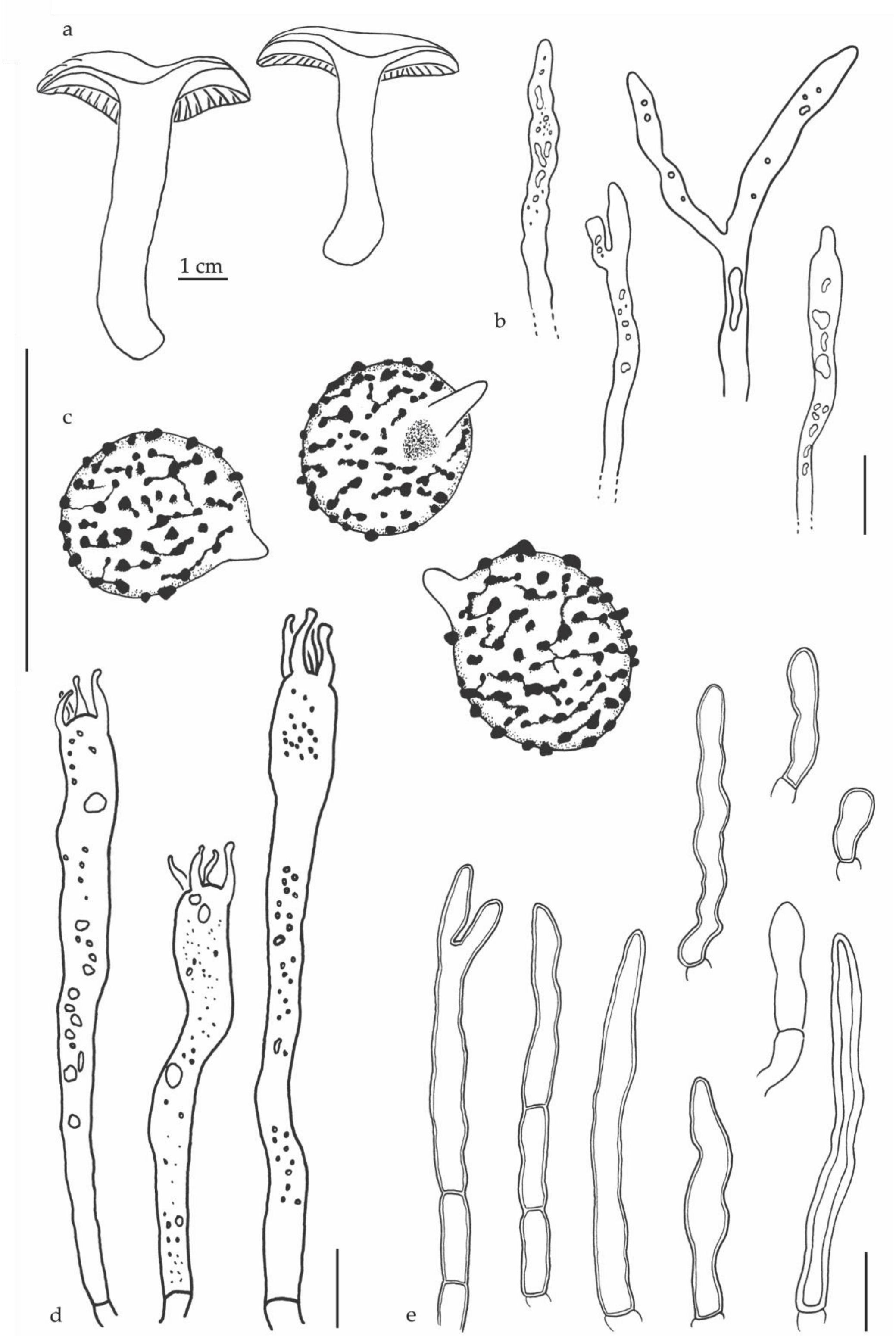

Fig. 6.4 *Lactifluus albomembranaceus*: **a.** basidiocarps, **b.** pleuropseudocystidia, **c.** basidiospores, **d.** basidia, **e.** marginal cells (all from holotype EDC 12-046, scale bar = 10 μm, illustrations by K. Van de Putte and S. De Wilde)

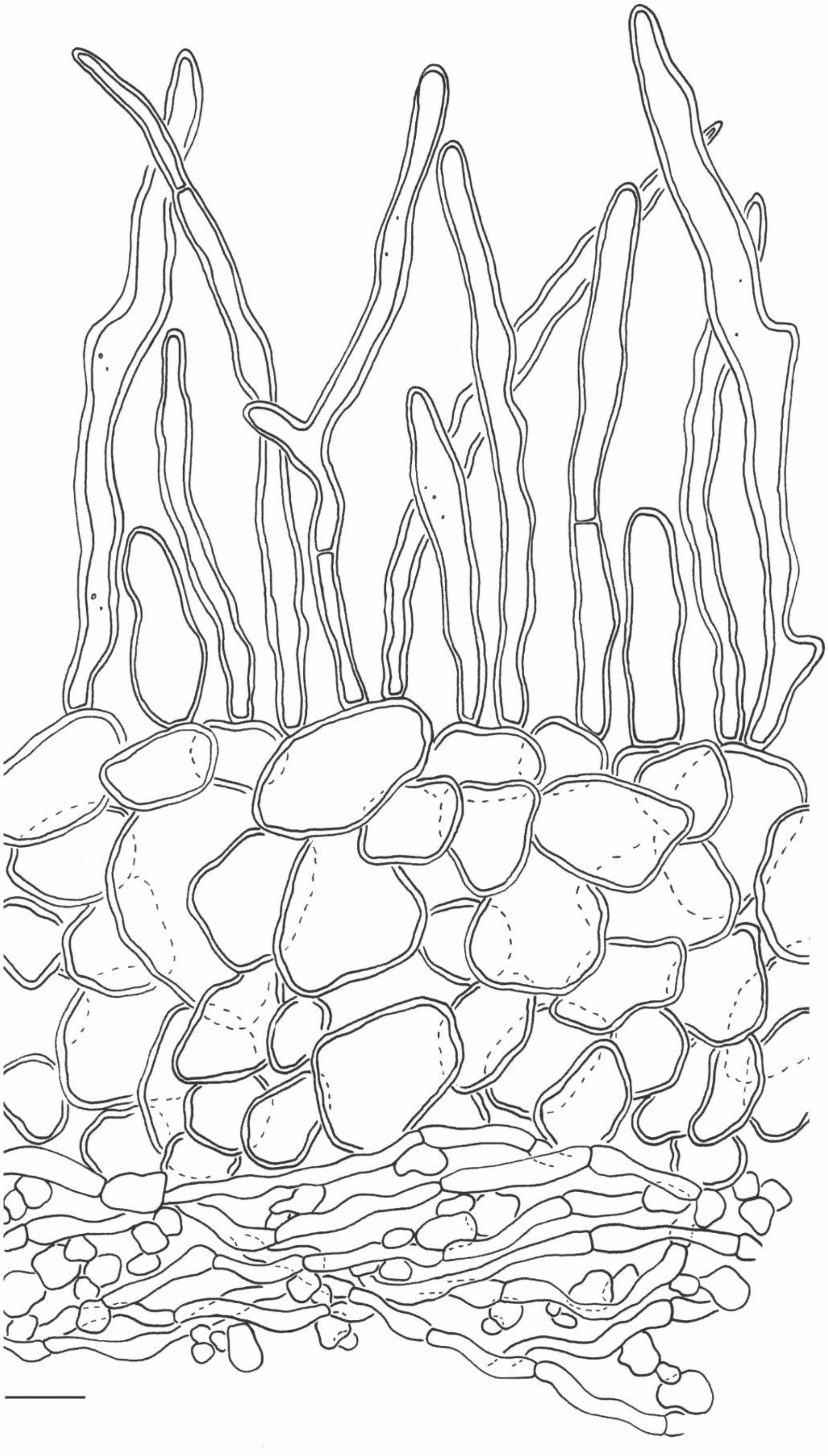

Fig. 6.5 *Lactifluus albomembranaceus*: section through the pileipellis (all from holotype EDC 12-046, scale bar = 10 μm, illustration by K. Van de Putte).

rather distant (6 L+11 l/cm), whitish, concolorous with pileus, becoming brownish after bruising, thin but not brittle; edge entire, concolourous. *Stipe* 40–50 × 7–10 mm, irregularly cylindrical to tapering downwards, sometimes slightly swollen at the base, sometimes curved, centrally attached to pileus; pellischamois leather-like, slightly longitudinally wrinkled, concolourous with pileus or with a cream to yellowish colour, becoming brownish after bruising. *Context* firm, solid when young, becoming stuffed when older; white, not changing with age, becoming brownish when cut (especially pileus); sometimes with a strong, slightly unpleasant smell, slightly sweetish to nutty taste, quickly turning pink with FeSO4, no reaction with gaiac. *Latex* abundant, white, slowly turning brownish (6E7–8) when staying in contact with the basidiocarp, taste mild.

Basidiospores globose to broadly ellipsoid 5.9–*6.9–7.3*–8.1(–8.4) × 5.0–*5.7–6.1*–7.0(–7.1) µm (Q = 1.09–*1.17–1.22*–1.36(–1.41), n = 138); ornamentation amyloid, composed of isolated warts (up to 1 µm high), often connected by low ridges, but not forming a reticulum; plage centrally to almost totally amyloid. *Basidia* 53–80(–90) × 7–9(–10) µm, very slender, subcylindrical to subclavate, thin-walled, 4-spored; content oil-like to granular or needle-like. *True pleurocystidia* absent. *Pleuropseudocystidia* very abundant, mostly emerging, 3–7 µm diam., cylindrical to irregularly cylindrical, occasionally branched; apex obtuse to subcapitate; content oil-like to granular or needle-like. *Lamellae-edge* sterile; marginal cells 7–55(–65) × 4–6(–9) µm, often septate to multiseptate with terminal cells up to 50 µm, subclavate to cylindric or tortuous, occasionally tapering upwards, obtuse, thin-walled to refringent or slightly thick-walled (up to 0.5 µm, rarely 1 µm). *Hymenophoral trama* mixed, with abundant lactifers. *Pileipellis* a lampropalisade, up to 255 µm thick; terminal elements 20–120(–150) × 5–12 µm, subcylindrical to subclavate, often irregularly shaped, obtuse, occasionally septate, thick walled (up to 1 µm); subpellis composed of mostly rounded to elongated, thick walled cells, 10–35 × 8–20 µm. *Stipitipellis* a lampropalisade. *Clamp connections* absent.

Ecology: Found in the Guineo-Sudanian transition zone in gallery forests with *Berlinia grandiflora* (Vahl) Hutchinson & Dalziel (1928: 343) and *Uapaca guineensis*.

Distribution: Known from Burkina Faso, Cameroon and Togo.

Studied material

CAMEROON**.** Western region: Noun division, Koutaba subdivision, Mamevouo village, N5°38.88′ E10°51.05′, elev. 1118m, gallery forest in savannah region near a river and surrounded by farmland, *Uapaca guineensis*, 10 May 2012, E. De Crop 12-045 (GENT!); Ibidem, N5°38.97′ E10°51.08′, elev. 1111m, gallery forest in savannah region near a river and surrounded by farmland, *Uapaca guineensis*, E. De Crop 12-046 (Holotypus, GENT!); Ibidem, N5°39.1′ E10°50.88′, elev. 1129m, gallery forest in savannah region near a river and surrounded by farmland, *Uapaca guineensis*, E. De Crop 12-052 (GENT!); Ibidem, N5°38.97′ E10°51.03′, elev. 1113m, gallery forest in savannah region near a river and surrounded by farmland, *Uapaca guineensis*, E. De Crop 12-054 (GENT!).
TOGO. Central province: Alédjo Wildlife Reserve, N09°16.460' E01°12.416', gallery forest, *Berlinia grandiflora*, 11 July 2007, De Kesel A., ADK 4284 (BR MYCO 158446–45!).

Lf. foetens (Verbeken & Van Rooij 2003:230) Verbeken (2012: 445). Fig. 6.3c–d, 6.6c–d

Mycobank: MB 564593
Holotypus: BENIN. Atacora Province: Bassila, 21 June 2000, A. De Kesel 2840 (Holotypus BR 126393–02; isotypus GENT).

Basidiospores ellipsoid, sometimes subglobose or elongate (6.0–)6.1–*7.1–7.4*–8.8 × (4.0–)4.4–*5.6–5.8*–6.5 µm (Q = 1.07–*1.24–1.33*–1.54(–1.56), n = 177); ornamentation amyloid, composed of irregular, subspherical to subconical warts, up to 1 µm high, aligned and connected by fine connective lines, forming a distinct and incomplete to complete reticulum, warts seldom isolated; plage often centrally to almost totally amyloid. *Basidia* 55–77 × 9–12 µm, subclavate, thin-walled, 4-spored (seldom 2-spored); content oil-like or granular, sometimes needle-like. *True pleurocystidia* absent. *Pleuropseudocystidia* 4–8 µm diameter, regularly cylindric with rounded, sometimes subcapitate apex; content needle-like and granular, sometimes with oil-like droplets. *Lamellae-edge* sterile; marginal cells 15–30(–38) × 2–5 μm, subclavate to irregularly cylindric, sometimes branched, thin-walled to refringent, hyaline, sometimes with oil-like droplets. *Hymenophoral trama* mixed, with rosettes and abundant lactifers. *Pileipellis* a lampropalisade, composed of a distinct pseudoparenchymatous layer (subpellis) covered with distinctly developed tufts of hair-shaped thick-walled elements (discontinuous suprapellis); elements of the suprapellis (terminal elements) 25–225× 3–5 μm, cylindric, hair-shaped, sometimes tapering towards the apex, septate, with thickened walls (0.5–1 μm); subpellis pseudoparenchymatous, with spherical cells (10–)15–25(–30) μm, sometimes with thickened walls. *Stipitipellis* idem, without developed pseudoparenchymatous layer, terminal elements usually longer and thin-walled (< 0.5 μm). *Clamp-connections* absent.

Ecology: Found both in gallery forests within the Guineo-Sudanian transition zone, with *Berlinia grandiflora*, *Uapaca guineensis* and *U. somon* Aubréville & Leandri (1935: 50) and in Sudanian woodland, with *Isoberlinia doka* Craib & Stapf (1911: 267), *Monotes* sp. and *Uapaca* sp.

Distribution: Known from Benin, Burkina Faso and Togo.

Studied material

BENIN. Donga province: Bassila, 21 June 2000, A. De Kesel 2840 (Holotypus BR MYCO 126393–02; isotypus GENT); Atacora province: Kota falls, gallery forest dominated by *Berlinia grandiflora* and *Uapaca somon*, 18 June 2004, A. De Kesel 3688 (BR MYCO 157117–74).

Togo. Central province: West of Alédjo Wildlife Reserve, 28 June 2002, A. De Kesel 3486 (BR MYCO 152042–43!); ibidem, N09°16.460' E01°12.416', gallery forest, *Berlinia grandiflora*, 11 July 2007, De Kesel A., ADK 4283 (BR MYCO 163675-36!); Fazao, Fazao Malfakassa National Park, primary Sudanian woodland with *Isoberlinia*, *Monotes* and *Uapaca*, 19 June 2011, A. Verbeken 11-176 (GENT!).

Discussion

Lactifluus albomembranaceus can be confused with *Lf. foetens* in the field, as they both grow in exactly the same environment and both have white basidiocarps and latex that stains the lamellae and context brownish when exposed to air. However, a more detailed study reveals several differences: basidiocarps of *Lf. foetens* (cap: 60–70 mm diam., stipe 11–16 mm diam.) are generally larger and more robust than those of *Lf. albomembranaceus* (cap: 40–50 mm diam., stipe: 7–10 mm diam.) although we mainly found young fruiting bodies of the latter, the pileus of *Lf. albomembranaceus* often has a translucent aspect that is not present in *Lf. foetens*, the undisturbed pileus of mature *Lf. foetens* basidiocarps is never entirely white such as in *Lf. albomembranaceus*, and the latex is more abundantly present in *Lf. albomembranaceus*. In addition, there are several distinctive microscopical features that discriminate between both species. The spores of *Lf. foetens* are ellipsoid to broadly ellipsoid (Q = 1.07–*1.24–1.33*–1.54(–1.56)), while the spores of *Lf. albomembranaceus* are broadly ellipsoid (Q = 1.09–*1.17–1.22*–1.36(–1.41)), due to their difference in length. *Lactifluus foetens* has a reticulate spore ornamentation with almost no isolated warts, while the spore ornamentation of *Lf. albomembranaceus* is characterized by isolated warts that are often connected by fine lines, but never forming a reticulum. Basidia of *Lf. albomembranaceus* are more slender (most basidia are 9 μm broad, very rarely up to 10 μm) than those of *Lf. foetens* (mostly 10 μm, up to 11 μm, rarely less than 10 μm). Marginal cells of *Lf. albomembranaceus* (up to 55(–65) μm long) are distinctly longer than those of *Lf. foetens* (up to 23–30(–38) μm long). *Lactifluus foetens* is characterized by a discontinuous suprapellis, consisting of tufts of hair-shaped thick-walled elements, while the terminal elements are evenly distributed in the suprapellis of *Lf. albomembranaceus*. Finally, the terminal hair-like elements of the suprapellis are distinctly longer in *Lf. foetens* (up to 225 μm) than in *Lf. albomembranaceus* (up to 120 μm, rarely up to 150 μm). Despite their morphological and molecular differences, the two species have a rather similar distribution and ecology. Both species were recorded from the gallery forests in the Guineo-Sudanian transition zone (sometimes even the same day on exact the same locality), while only *Lf. foetens* has been recorded from Sudanian woodland.

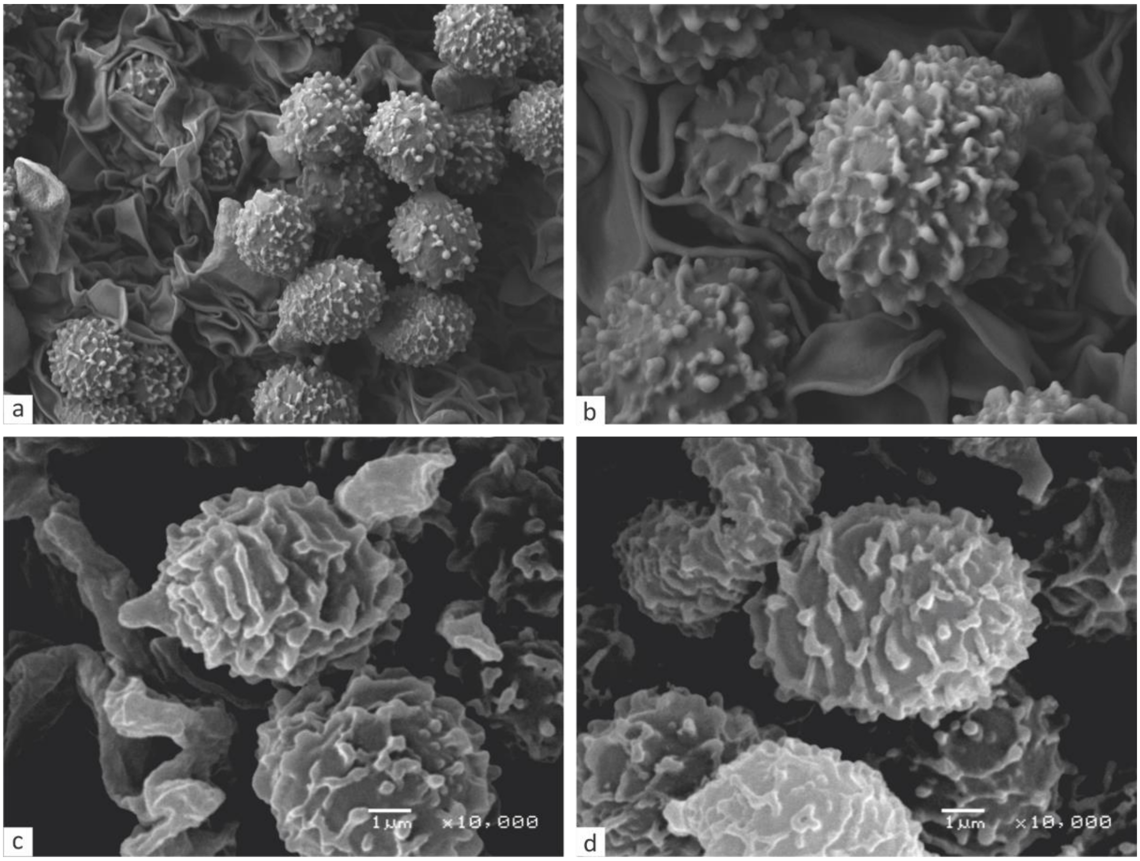

Fig. 6.6 Scanning electron microscope images of basidiospores of *Lactifluus albomembranaceus* (all from holotype EDC 12-046): a) overview, b) detail on basidiospores and *Lf. foetens* (all from holotype ADK 2840): c–d) detail on basidiospores (scale bars = 1 µm).

The chamois-leather-like aspect of the cap, the presence of hymenophoral sphaerocytes and a lampropalisade as pileipellis are in accordance with the general morphological trends for the genus *Lactifluus*. A lampropalisade as pileipellis, together with the absence of true pleurolamprocystidia and a brownish colour reaction of the latex and/or the context when exposed to air are consistent with the morphological trends of *Lf.* subg. *Gymnocarpi*. Our molecular results place *Lf. albomembranaceus* in *Lf.* sect. *Gymnocarpi* (Fig. 6.2), which is also suggested by the emergent marginal cells and the thick-walled hairs.

Several collections of this species were found in the gallery forests in West Cameroon, at a site where local women were collecting basidiocarps of various ectomycorrhizal fungi. This species was collected in large amounts for consumption or trade at a local market. Many ectomycorrhizal fungi are known to be edible and *Lactifluus* is one of the most edible genera in Africa (Rammeloo and Walleyn 1993). In West Cameroon, at least 9 *Lactifluus* species are reported to be edible (Njouonkou et al. acpt.). Njouonkou *et al.* (acpt.) also reported *Lf. albomembranaceus* (as *Lactifluus* sp. 1) as edible and in the Noun region it is known by its local name “Puo' nga' lare fü” (phonetic: pwɔ̋ ngaʹ lărə́ fɨ), which means white exocarp of passion fruit (*Passiflora edulis* Sims) mushroom.

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Part D – Two new *Lactifluus* species from Thailand

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Introduction

The ectomycorrhizal genus *Lactifluus* is the smaller of the two milkcap genera (Russulaceae). The genus is mainly distributed in the tropics and is well represented in Thailand (Le et al. 2007b; Stubbe et al. 2010; Van de Putte et al. 2010; De Crop et al. 2014). In a recent study (De Crop et al. acpt.), the genus is revised and four subgenera are proposed: *Lf.* subg. *Lactariopsis*, *Lf.* subg. *Pseudogymnocarpi*, *Lf.* subg. *Gymnocarpi* and *Lf.* subg. *Lactifluus*. The two species from Thailand that are presented here belong to *Lf.* subg. *Lactariopsis* and *Lf.* subg. *Pseudogymnocarpi*. The phylogenetic tree is presented in Fig. 6.7.

Material and methods

Morphological study

For macromorphological characters, specimens were described and photographed in fresh conditions during daylight hours. Colours are according to Kornerup and Wanscher (1978).

Microscopic features were studied from dried material. Most microscopic characters were observed in congo red SDS solution, except the basidiospore ornamentation which was observed in melzer reagens. Basidiospore measurements are based on 20 spores, excluding the ornamentation and are represented as {(MIN) [AVa-2×SD]–*AVa*– [AVa+2×SD] (MAX)} × {(MIN) [AVb-2×SD]–*AVb*– [AVb+2×SD] (MAX)}, in which MIN = the minimum value, MAX = the maximum value, AVa = average value for the length, AVb = average value for the width and SD = standard deviation. Q corresponds to spore "length/width ratio" and is given as (MINQ) Qa (MAXQ), where Qa is the average length/wide ratio of the 20 measured spores, MINQ is the lowest value measured and MAXQ the highest. Basidiospores were studied using a Zeiss Axioscop 2 microscope, other hymenial elements were studied using an Olympus CX31 microscope.

Molecular study

Our dataset was composed based on the recent revision of the genus *Lactifluus* (De Crop et al. acpt.). The two species we describe here belong to two different subgenera within the genus *Lactifluus*: *Lf.* subg. *Lactariopsis* and *Lf.* subg. *Pseudogymnocarpi*. We included representatives of both subgenera, focusing on the closest relatives of each new species. Our ingroup thus consists of 29 species and we added an outgroup of six *Lactifluus* species belonging to *Lf.* subg. *Gymnocarpi* (Table 6.2).

Genomic DNA was extracted from fresh material stored in 2×CTAB buffer using the protocol described by Nuytinck & Verbeken (2003), with modifications described in Van de Putte et al. (2010). Two regions were amplified: the internal transcribed spacer of the nuclear ribosomal DNA (ITS) region, using primers ITS-1F and ITS4 (White et al. 1990; Gardes and Bruns 1993), and a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al. 2000). PCR products were sequenced using an automated ABI 3730 XL capillary sequencer (Life Technology) at Macrogen.

Obtained sequences were manually edited and assembled using the software Sequencer™ v5.0 (Gene Code Corporation, Ann Arbor, Michigan, U.S.A.). Nucleotide sequence alignment was made using MAFFT v7 (Katoh and Standley 2013) and later manually edited in MEGA6 (Tamura et al. 2013). The alignment was partitioned into partial 18S, ITS1, 5.8S, ITS2 and partial 28S. Maximum likelihood (ML) algorithm was executed using RAxML v8.2.4, where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates, using the GTRCAT option (Stamatakis 2014). All analyses were performed on the CIPRES Science Gateway (Miller et al. 2010).

Table 6.2 Specimens and GenBank accession numbers of DNA sequences used in the molecular analyses.

Species	Voucher collection (herbarium)	Country	ITS accession no.	LSU accession no.
INGROUP				
Lactifluus acrissimus	EDC 11-112 (GENT)	Tanzania	KR364041	KR364168
Lactifluus annulifer	TH 9014 (BRG, DUKE)	Guyana	KC155376	KC155376
Lactifluus armeniacus sp. nov.	EDC 14-501 (MFLU, GENT)	Thailand	KR364127	None
Lactifluus aurantiifolius Type	AV 94-063 (GENT)	Burundi	KR364017	KR364144
Lactifluus aureifolius	AV 11-074 (GENT)	Tanzania	KR364056	KR364183
Lactifluus brachystegiae Type	AV 99-002 (GENT)	Zimbabwe	KR364018	KR364145
Lactifluus chrysocarpus Type	LE 253907 (LE)	Vietnam	JX442761	JX442761
Lactifluus cyanovirescens	JD 988 (GENT)	Congo	KR364082	KR364211
Lactifluus denigricans	EDC 11-218 (GENT)	Tanzania	KR364051	KR364178
Lactifluus densifolius	AV 11-111 (GENT)	Tanzania	KR364057	KR364184
Lactifluus goossensiae	AB 320 (GENT)	Guinea	KR364132	KR364252
Lactifluus gymnocarpoides	AV 05-184 (GENT)	Malawi	KR364024	KR364151
Lactifluus heimii	EDC 11-082 (GENT)	Tanzania	KR364040	KR364167
Lactifluus leoninus	DS 07-454 (GENT)	Thailand	KF220055	JN388989
Lactifluus madagascariensis	BB 99-409 (PC)	Madagascar	AY606977	DQ421975
Lactifluus medusae	EDC 12-152 (GENT)	Cameroon	KR364069	KR364198
Lactifluus multiceps	TH 9154A (BRG, DUKE)	Guyana	JN168731	None
Lactifluus nodosicystidiosus	BEM 97-273 (GENT)	Madagascar	KR364029	KR364156
Lactifluus pilosus Type	LTH 205 (GENT)	Thailand	KR364006	KR364134
Lactifluus ramipilosus sp. nov.	EDC 14-503 (MFLU, GENT)	Thailand	KR364128	None
Lactifluus rufomarginatus	ADK 3358 (MEISE)	Benin	KR364033	KR364160
Lactifluus rugatus	EP 1212/7 (LGAM-AUA)	Greece	KR364104	KR364235
Lactifluus sesemotani	AV 94-476 (GENT)	Burundi	KR364036	KR364163
Lactifluus sp. nov.	JN 2011-012 (GENT)	Vietnam	KR364045	KR364171
Lactifluus sp. nov.	TENN 065929 (TENN)	USA	KR364102	KR364233
Lactifluus vellereus	ATHU-M 8077 (ATHU-M)	Greece	KR364106	KR364237
Lactifluus veraecrucis Type	M 8025 (ENCB)	Mexico	KR364112	KR364241
Lactifluus volemoides	MH 201187 (GENT)	Mozambique	KR364098	KR364230
Lactifluus xerampelinus	MH 201176 (GENT)	Mozambique	KR364099	KR364231
OUTGROUP				
Lactifluus brunnescens	AV 05-083 (GENT)	Malawi	KR364019	KR364146
Lactifluus chiapanensis	VMB 4374A (GENT)	Mexico	GU258297	GU265580
Lactifluus clarkeae	MN 2004002 (L)	Australia	KR364011	HQ318205
Lactifluus foetens	ADK 3688 (MEISE)	Benin	KR364022	KR364149
Lactifluus gymnocarpus	EDC 12-047 (GENT)	Cameroon	KR364065	KR364194
Lactifluus luteolus	AV 05-253 (GENT)	USA	KR364016	KR364142

Results

Our dataset contains of 35 *Lactifluus* collections, for which we obtained 35 ITS and 32 LSU sequences. Figure 6.7 shows the maximum likelihood (ML) topology based on the ITS-LSU sequence data. The result shows that both new species are well-delimited and show considerable genetic differences with their sister species. *Lactifluus armeniacus* is sister species of two undescribed species, *Lf.* sp. (JN 2011-012) from Vietnam and *Lf.* sp. (TENN 065929) from North America. The species belongs to *Lf.* subg. *Pseudogymnocarpi*. *Lactifluus ramipilosus* is sister to the pleurotoid *Lf. chrysocarpus* from Vietnam and belongs to *Lf.* subg. *Lactariopsis.* The molecular evidence is in accordance with the morphology (see taxonomic part).

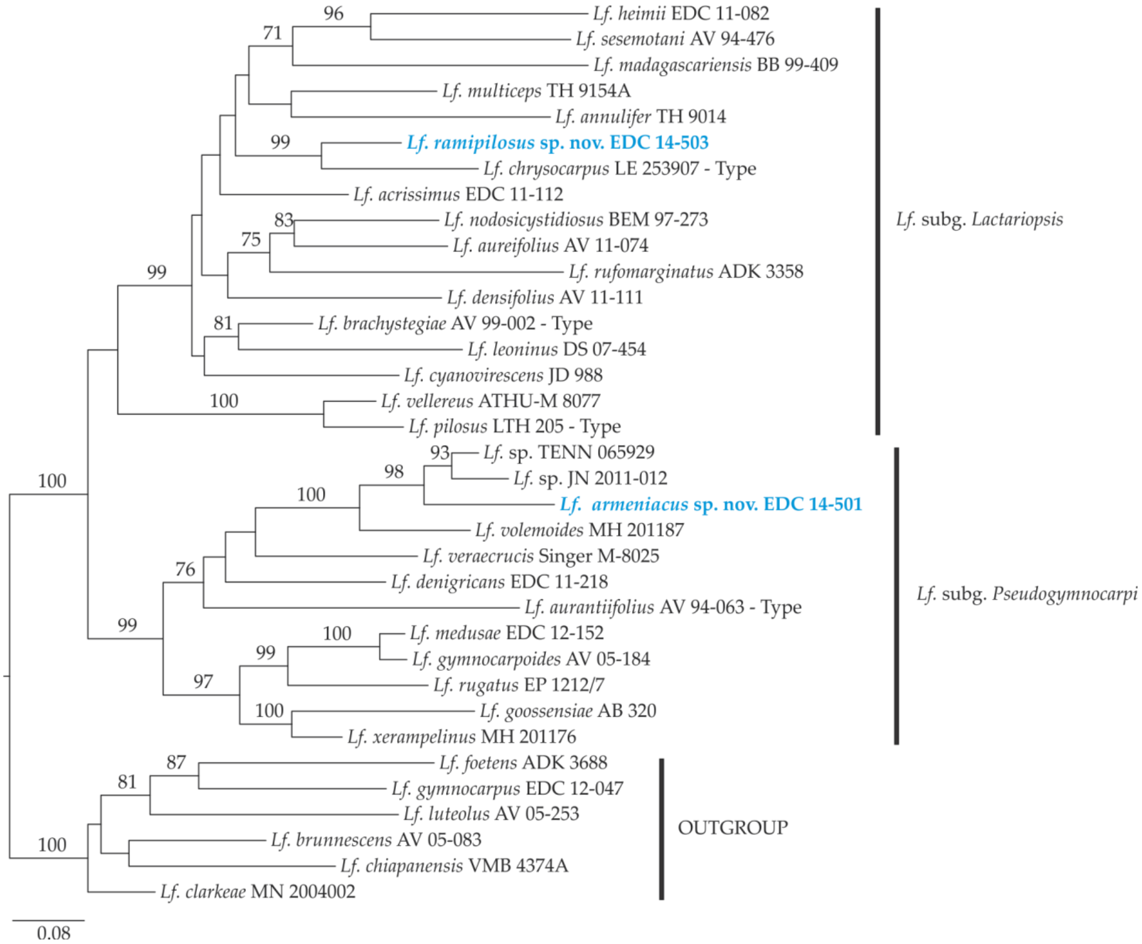

Fig. 6.7 Maximum Likelihood tree of *Lactifluus* subg. *Lactariopsis* and *Lf.* subg. *Pseudogymnocarpi*, based on ITS-LSU sequence data. ML bootstrap values >70 are shown. Green tip labels represent the new species. GenBank accession numbers are given between brackets, respectively ITS and LSU accession numbers.

Taxonomy

Lactifluus armeniacus **De Crop & Verbeken nov. sp.** Fig. 6.8a, 6.9
Diagnosis: A medium-sized, warm apricot-coloured species which is microscopically characterized by septated lamprocystidia, low ornamented spores and a lampropalisade as pileipellis structure, with small to medium-sized, thick-walled hairs in the suprapellis and a thick layer of spherical cells in the subpellis.
Etymology: Referring to the apricot-coloured basidiocarps.

Holotypus: Thailand, Chiang Mai Province, Mae Taeng district, Baan Tapa (22km marker along road 1095), N19°7'45" E98°46'1", alt. 766.8 m, on soil in mixed forest, with *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., 31 July 2014, E. De Crop 14-501 (MFLU, Isotypus in GENT)
Mycobank: MB 815137

Pileus 69–72 mm diam., planoconvex with central depression to slightly infundibuliform; margin sometimes slightly striate, sometimes concentrically wrinkled; edge rather irregular, sometimes crenulate or locally undulate; surface chamois leather-like, locally wrinkled but smooth in the centre, pruinose, bright orange (as 5B5/6, but more yellow), unicolourous. **Lamellae** adnate with decurrent tooth to subdecurrent, distant (2L + 1l / cm – 4L + 3l / cm), bright orange to yellow (4A3 to 4/5A4), very broad, rather thick and brittle, slightly intervenose; edge entire and concolourous. **Stipe** 27–28 x 11–18 mm, cylindrical to slightly tapering downwards, sometimes curved, centrally attached to pileus; surface very soft, pruinose and finely striate, concolourous with pileus (bright orange 5B5/6 with a more yellowish tinge). **Context** solid and quite firm, white, unchanging; taste sweet, mild; smell not distinctive. **Latex** abundant, white, unchanging; taste sweet.

Basidiospores broadly ellipsoid, sometimes subglobose, sometimes ellipsoid, 6.4–7.7–9.0 x 5.1–6.2–6.7 µm (n = 20, Q = 1.11–1.24–1.41); ornamentation amyloid, forming an almost complete reticulum, composed of very low warts connected by fine ridges, up to 0.2 µm high; plage inamyloid. **Basidia** 4-spored, sometimes 2-spored, 59–71 x 8–9 µm, cylindric to subclavate, with refringent to slightly thickened walls; content guttate to granular. **Pleurolamprocystidia** abundant, slightly emergent up to 17 µm, cylindrical, septate, 50–80 x 4–8 µm, with slightly thickened walls (<1 µm). **Pleuropseudocystidia** very scarce, 7–9 µm, cylindrical, mostly collapsed at apex; content granular. **Lamellae-edge** sterile; completely composed of cheilolamprocystidia which are 41–45 x 4–7 µm, cylindrical, septate, thick-walled. **Hymenophoral trama** cellular, with abundant lactifers and sphaerocytes. **Pileipellis** a lampropalisade; elements of the suprapellis 28–64 x 3–5 µm, cylindrical, obtuse, thick-walled; subpellis 132–174 µm thick, spherical cells 9–22 µm diam., with thickened wall. **Stipitipellis** hymeniderm; elements of the suprapellis 15–26 x 5–11 µm, cylindrical to clavate, sometimes with strong congophilous content, thick-walled.

Studied material – Thailand, Chiang Mai Province, Mae Taeng district, Baan Tapa (22km marker along road 1095), N19°7'45" E98°46'1", alt. 766.8 m, on soil in mixed forest, with *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., 31 July 2014, E. De Crop 14-501 (Holotypus in MFLU, Isotypus in GENT).

Fig. 6.8 Basidiocarps of **a.** *Lactifluus armeniacus* sp. nov. (EDC 14-501) and **b.** *Lactifluus ramipilosus* sp. nov. (EDC 14-503)

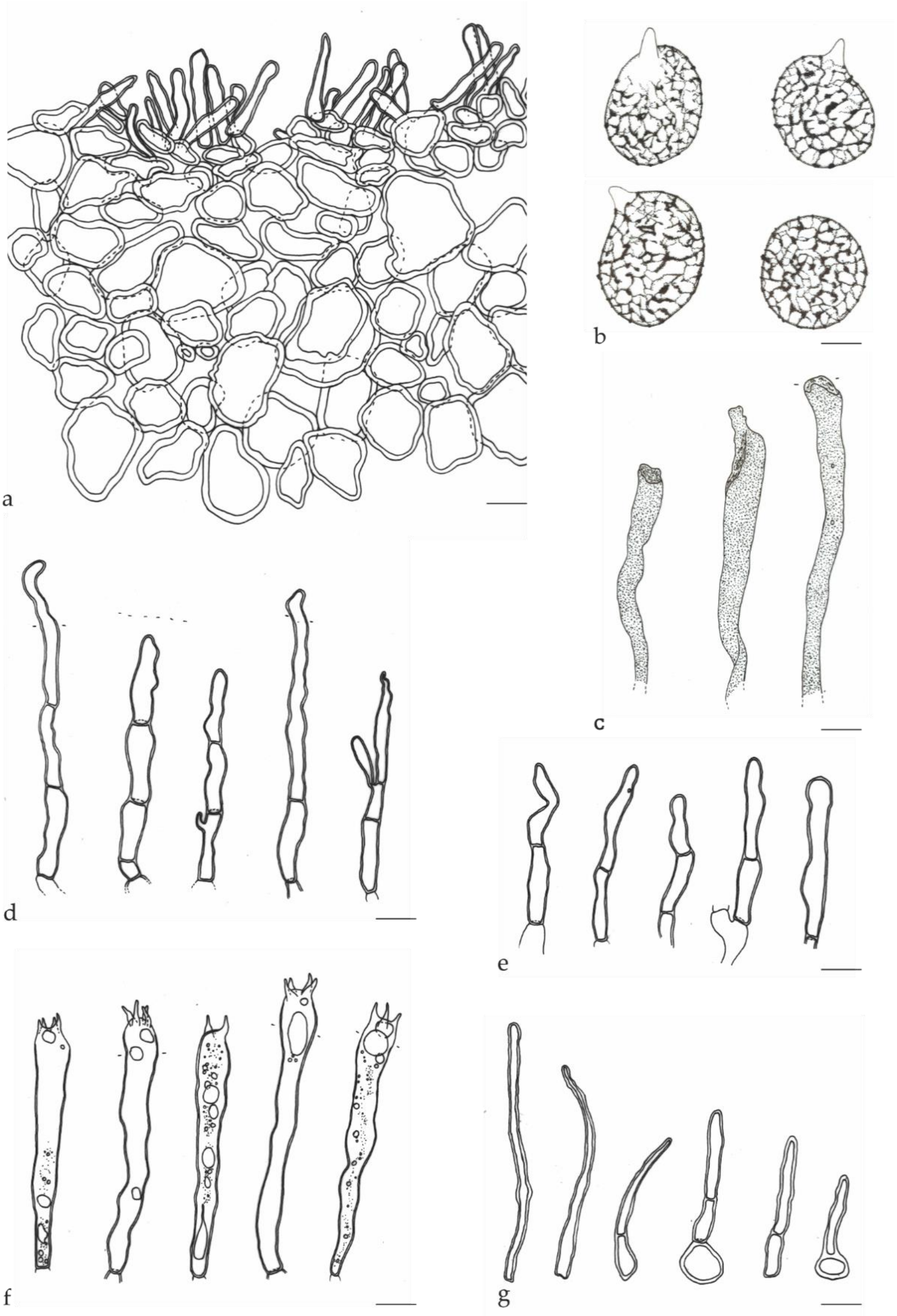

Fig. 6.9 *Lactifluus armeniacus* sp. nov.: **a.** section through pileipellis, **b.** basidiospores, **c.** pleuropseudocystidia, **d.** pleurolamprocystidia, **e.** marginal cells, **f.** basidia, **g.** terminal elements of the pileipellis (all from holotype EDC 14-501, scale bar = 10 μm).

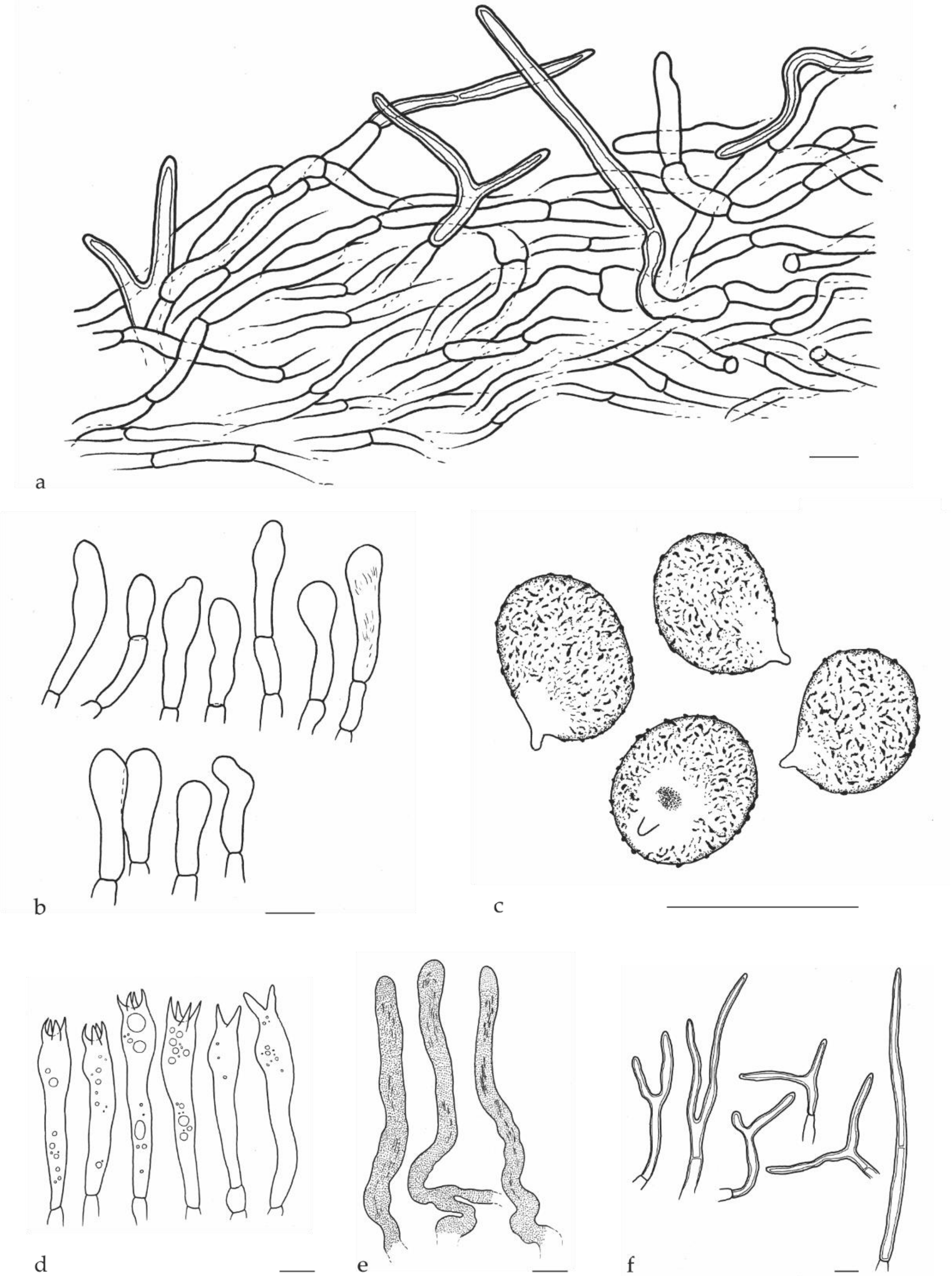

Fig. 6.10 *Lactifluus ramipilosus* sp. nov.: **a.** section through pileipellis, **b.** marginal cells, **c.** basidiospores, **d.** basidia, **e.** pleuropseudocystidia, **f.** terminal elements of the pileipellis (all from holotype EDC 14-503, scale bar = 10 μm).

Lactifluus ramipilosus **Verbeken & De Crop nov. sp.** Fig. 6.8b, 6.10
Diagnosis: A medium-sized, warm yellowish orange species which is microscopically characterized by the very lowly and indistinctly ornamented spores, the absence of true cystidia and ramified thick-walled hairs in the pileipellis structure.
Etymology: with branched (rami-) hairs (-pilosus), referring to the striking hairs in the pileipellisstructure.
Holotypus: Thailand, Chiang Mai Province, Mae Taeng district, Baan Tapa (22km marker along road 1095), N19°8'0" E98°46'15", alt. 829.6 m, on soil in mixed forest, with *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., 31 July 2014, E. De Crop 14-503 (MFLU, Isotypus in GENT).
Mycobank nr.: 815138

Pileus 55 mm diam., convex to planoconvex with undeep depression in the center; surface soft, chamois-leather like and pruinose, almost smooth but slightly irregular, yellowish orange (5A3-4A4); margin entire, straight to slightly deflexed. **Stipe** 25 x 17 mm, strongly tapering downwards; surface pale yellow (4A2), slightly paler towards the lamellae, very finely fibrillose. **Lamellae** broadly adnate to decurrent, up to 4 mm broad, medium thick, brittle, yellow (4A3). **Context** whitish yellow. **Latex** not observed.

Basidiospores 5,6–*7,2*–8,9(9,1) x 5,5–6,2–7,2(7,3) µm, Q = 1,03-1,16-1,32, broadly ellipsoid, sometimes subglobose; ornamentation amyloid but very low and weakly developed, composed of low and irregular warts that are often connected by very fine ridges forming a partial reticulum; plage mostly not amyloid, but sometimes with a very weak central amyloid spot. **Basidia** 4-spored, with some rare 2-spored basidia present, 45-55 x 8-10 µm, subcylindrical to subclavate, with guttate contents. **True cystidia** absent. **Pleuropseudocystidia** abundant, not emergent to slightly but distinctly abundant, 6-8 µm diam., cylindric but often swollen at the apex, with rounded apex, with needle-like to granular content. **Hymenophoral trama** mixed with some hyphae present but especially abundant sphaerocytes of up to 25 µm diam., with abundant lactifers. **Subhymenium** cellular. **Lamellar edge** sterile; marginal cells 15-28 x 6-8 µm, subclavate to irregular, mostly hyaline, sometimes with refringent walls, sometimes with slightly needle-like content. **Pileipellis** lamprotrichoderm-like, composed of a layer of hyphae with 3-5 µm diam., which are mainly horizontally arranged and often terminating in remarkable thick-walled hairs which are pericline to oblique; hairs thick-walled, 35-125 x 3-5 µm, often branched, sometimes septate, sometimes tapering near paex, sometimes with rounded apex.

Studied material – Thailand, Chiang Mai Province, Mae Taeng district, Baan Tapa (22km marker along road 1095), N19°8'0" E98°46'15", alt. 829.6 m, on soil in mixed forest, with *Dipterocarpus* sp., *Castanopsis* sp., *Lithocarpus* sp. and *Quercus* sp., 31 July 2014, E. De Crop 14-503 (Holotypus in MFLU, Isotypus in GENT)

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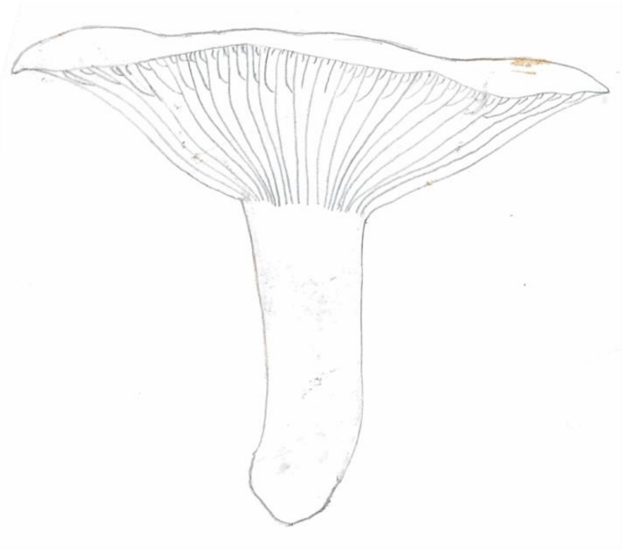
General discussion



The merit and challenge of exploring a fungal genus

When researchers, such as ecologists or biochemists, want to study the ecological or biochemical functions of an organism or a group of organisms, they rely on a **framework** of species descriptions or classifications available for these organisms, which is provided by taxonomists. In fungi, this framework is lacking or incomplete for the majority of lineages. With this thesis, we aimed to provide such a framework for a diverse genus of ectomycorrhizal fungi, the milkcap genus *Lactifluus*. Contrary to several studies that focus on a geographical region or on a subgroup of a genus (e.g. subgenus, section or lineage), we aimed at studying the genus in its totality, including as many geographical regions and lineages as possible. This demands a well-considered approach, both in terms of sampling as techniques, from statistics to data management.

Our **global sampling** was accomplished by collaborating with mycologists all over the world. These collaborations helped us to assess the current diversity of the genus and made it possible to request loans and organise field expeditions in a targeted way, with a focus on *Lactifluus*. The Herbarium Universitatis Gandavensis partim Mycology already comprises a large amount of especially European, African and South-East Asian *Lactifluus* specimens, including several type collections. However, certain geographical regions were lacking and many of the present collections were rather old (>20 years), which hampered the successful extraction of DNA or the subsequent amplification of DNA. Therefore collaborations were essential in order to get access to recent material. By combining all data, we assembled a vast dataset, with recent collections from all continents, covering many lineages within *Lactifluus*.

In order to explore the diversity of *Lactifluus*, we needed to consider the **species concept** we wanted to use. We decided to work with the consolidated species concept (Quaedvlieg et al. 2014), a variant of the unified species concept (de Queiroz 2007), in which conclusions based on robust multi-locus DNA data receive a high weight, while differences in morphology or ecology are given less weight in reaching a consolidated species concept conclusion (see chapter 1). Several lines of evidence, e. g. morphological, ecological or biochemical data, are needed to delimit species, but we start from the molecular data by constructing phylogenies and delimit lineages or species based on the information available in the DNA.

Once sampling more or less covered the large lineages within the genus, we constructed robust and accurate **phylogenies**, using the newest or most adequate techniques. These resolved phylogenies served as a basis for building a new or revised classification. This **new classification** implied changing or choosing new names for subgenera, sections or species. This was done by checking relevant literature and consulting nomenclatural experts, in order to make decisive changes conform the International Code of Nomenclature for algae, fungi, and plants.

The resulting classification forms the basis of a solid framework for the genus *Lactifluus*, which can further be used by researchers of different disciplines to infer a variety of questions. In order to make this framework available to other researchers, we aim to add our data to several **publicly available databases**. All sequences are or will be submitted on GenBank and UNITE. Moreover, we will appoint reference or representative sequences for the described *Lactifluus* species in UNITE, which will improve future species determinations. Finally, we will update the current information concerning the genus *Lactifluus* on "Russulales News", a web portal that is dedicated to the study of Russulales.

Current status of the diversity of the milkcap genus *Lactifluus*

Total species diversity

Our study confirms the results of preliminary studies, as *Lactifluus* turns out to have a large and mainly undescribed diversity. When this study started, 129 species were known within the genus *Lactifluus*. These species were divided over 6 subgenera, 13 sections, and three species were unassigned: *Lf. caperatus* (R. Heim & Gooss.-Font.) Verbeken, *Lf. cocosmus* (Van de Putte & De Kesel) Van de Putte and *Lf. subclarkeae* (Grgur.) Verbeken (Stubbe et al. 2010; Van de Putte et al. 2010; Verbeken and Walleyn 2010; Verbeken et al. 2011; Stubbe et al. 2012b; Verbeken et al. 2012).

From the results of chapter 3, we acquired two different *Lactifluus* species trees. They are both made from dataset 3 of chapter 3. For the first species tree, species were delimited based on Generalized Mixed Yule Coalescent (GMYC) species delimitation; for the second, species delimitations were also based on the GMYC results, but adapted according to previous delimitations performed on sections or subgenera within *Lactifluus* (molecular and/or morphological). In the first species tree, we uncovered 461 putative *Lactifluus* species, of which 226 are singletons. In the second species tree, 369 species were delimited, of which 145 are singletons. In order to avoid an overestimation of the total number of *Lactifluus* species, we decided to work with the results of the second species delimitation method.

In total, we assembled a dataset of 1306 *Lactifluus* collections, for which we have DNA sequences for one or more loci. These collections represent 369 species, of which 160 species are already described. There are currently 183 described *Lactifluus* species (see S4), but no sequences were available for the remaining 23 described species. This is mainly due to the age or bad condition of the collections. We did not include these species in the following calculations, as their species-status cannot be verified by the phylogeny and we did not examine them morphologically.

In Fig. 7.1, we calculated the species accumulation curve, where the number of delimited species is plotted against the number of collections, and estimated the total number of *Lactifluus* species by extrapolating the rarefaction curve beyond sampling size in EstimateS v9.1 (Colwell 2013). The results suggest that *Lactifluus* contains approximately 530 species (95 % CI[23] = 461–601 species). This means that we have found 62–80 % of the current diversity and suggests that 95 % of the species will have been found with a sampling of twice the number of collections we assembled for this study. There is still a relatively large number of species to be found, which is in line with the number of new species found during field expeditions. Expeditions to remote or underexplored areas frequently result in the discovery of multiple new species. New collections are also found when collecting multiple times in the same locality, but during a different moment of the

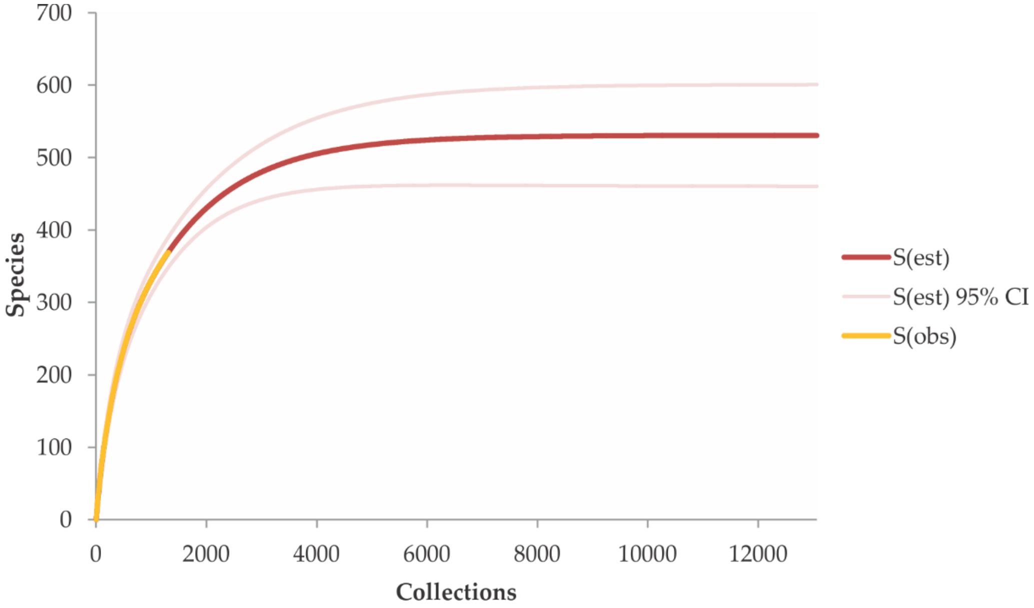


Fig. 7.1 Species accumulation curve of observed and estimated species richness of the genus *Lactifluus*. Species richness (S) was estimated by extrapolation of the rarefaction curve, with 95% confidence interval (pink lines).

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[23] *CI* = Confidence interval

fruiting season. For example, several members of our lab explored the forests along the Mushroom Research Centre in Chiang Mai, Thailand, in the middle of the mushroom season, during various years. In 2014, a collecting trip of one week at the end of the season yielded in twelve *Lactifluus* collections, of which four represent new species.

However, we need to emphasize that these results are purely made on a dataset of collections for which one or more DNA loci are available. The fungal herbarium of Ghent University contains much more samples for which it was impossible to extract DNA from. Many of these collections have been morphologically studied and were appointed to *Lactifluus* species. Including these collections will increase the number of collections and will probably not substantially increase the number of known species within *Lactifluus*. As a result of this, we should consider the estimated number of *Lactifluus* species as calculated above, as an indicative number, which will probably be an overestimation of the actual number of extant *Lactifluus* species.

Species diversity per biogeographic region

The majority of *Lactifluus* species only occur in a single biogeographic region, only three Lactifluus specie are known from two regions. *Lactifluus leoninus*, *Lf. leucophaeus* and *Lf. austrovolemus* are all known from both Asia (China, India, Indonesia and Thailand) and Australasia (Papua New Guinea).

Of the 369 *Lactifluus* lineages, **Asia** comprises the largest number of species and *Lf.* subg. *Lactifluus* is by far the most dominant lineage in Asia. During her PhD study of the hidden diversity of *Lf.* sect. *Lactifluus*, Van de Putte (2012) discovered at least 21 putative Asian species within the section and confirmed and described nine species (Van de Putte et al. 2010; Van de Putte 2012; Van de Putte et al. 2012; Van de Putte et al. 2016). In chapter 3, we found 16 lineages within this section that were not included in previous analyses but might represent putative new species. Likewise, a huge diversity of 20 Asian lineages was discovered in *Lf.* sect. *Gerardii* during the PhD study of Stubbe (2012). Today, 15 Asian species are described in *Lf.* sect. *Gerardii* (Stubbe et al. 2010; Stubbe et al. 2012a; Wang et al. 2012; Latha et al. 2016; Zhang et al. 2016, Chapter 5). In chapter 3, we found 14 *Lf.* cf. *gerardii*-lineages that were not included in previous analyses but might represent possible new species. Five of them are described in chapter 5. In chapter 4, we found 21 Asian lineages of *Lf.* sect. *Piperati* and in chapter 3, we found an additional 13 lineages (De Crop et al. 2014). Recently, Wang et al. (2015) described two new sections within *Lf.* subg. *Lactifluus*: *Lf.* sect. *Ambicystidiati* and *Lf.* sect. *Tenuicystidiati*, containing one and three species respectively. In chapter 3, we found out that both sections contain one and six extra lineages respectively.

The **Afrotropics** were long believed to contain the majority of *Lactifluus* species (Verbeken and Nuytinck 2013; Maba et al. 2015a; De Crop et al. acpt.). This study indicates that next to Asia, sub-Saharan Africa contains the second highest number of known *Lactifluus* species, all from *Lf.* subg. *Lactariopsis*, *Lf.* subg. *Gymnocarpi* and *Lf.* subg. *Pseudogymnocarpi*. Until today, no African representatives of *Lf.* subg. *Lactifluus* are known. Many African *Lactifluus* species were studied and described by Verbeken and colleagues (Verbeken 1995, 1996, 1998b, a; Verbeken and Walleyn 1999; Van Rooij et al. 2003; Buyck et al. 2007; Verbeken et al. 2008; Van de Putte et al. 2009; Verbeken and Walleyn 2010). The West African Russulaceae species were the topic of the PhD study of Maba (2015). During this study, he found and described 11 new *Lactifluus* species (Maba et al. 2014; Maba et al. 2015a; Maba et al. 2015b). In chapter 6, we described two more African *Lactifluus* species: *Lf. kigomaensis* and *Lf. albomembranaceus* (De Crop et al. 2012) and three more new African species are described and in preparation for publication (unpubl. res.). The analyses of chapter 3 indicate the existence of at least 45 extra *Lactifluus* lineages in tropical Africa.

This study confirmed what several studies suggested: the **Neotropics** contain a large diversity of Russulaceae species (Miller et al. 2002; Smith et al. 2011; Miller et al. 2012, unpubl. res.). In South America, several *Lactifluus* species have been found. A study of the macrofungi of the Guyana shield revealed three new *Lactifluus* species (Miller et al. 2002, 2012). In the course of her on-going PhD study of Brazilian ectomycorrhizal fungi, M. Sá and her professor F. Wartchow discovered three new *Lactifluus* species in Brazil (Sá et al. 2013; Sá and Wartchow 2013; Wartchow et al. 2013). But the Neotropical diversity appears much larger than this. Together with our colleague M. Roy and her team from the University of Toulouse, who explored several vegetation types in the Neotropics, we found approximately 32 new Neotropical *Lactifluus* lineages in chapter 3. Many of these new lineages will be studied and described during the PhD studies of M. Sá (Brazil) and L. Delgat (Ghent University). Just like the African diversity, no species from *Lf.* subg. *Lactifluus* are known from the Neotropics. The Central American diversity partly overlays with the South American diversity and contains species from all subgenera (Chapter 3), of which only a few are described (Singer 1973, 1975; Montoya et al. 1996; Montoya and Bandala 2004; Montoya et al. 2011).

Our results indicated that *Lactifluus* is less represented in the extratropical regions. Nonetheless, we found several lineages within **the Nearctic region**. Only a few North American *Lactifluus* species have been described (Berkeley and Curtis 1859; Peck 1896; Coker 1918; Hesler and Smith 1979), most other species are known by the names of their European look-a-likes (Methven 2010). Van de Putte (2012) found several North American lineages within *Lf.* sect. *Lactifluus* and Stubbe et al. (2010) found many new lineages within the *Lf.* sect. *Gerardii*. In chapter 4, we reported six lineages of North American members within *Lf*. sect. *Piperati*; and in chapters 2 and 3, we found ten more clades spread over all subgenera of *Lactifluus*. The **Australasian** diversity was long believed to be rather small. Only representatives of *Lf*. sect. *Gerardii*, *Lf.* sect. *Piperati*, *Lf.* sect. *Tomentosi* and *Lf. leoninus* (Clade 5, Fig. 2.6) were described before (Verbeken and Horak 1999; Verbeken et al. 2010; Stubbe et al. 2012a). Our results from chapters 2 and 3 show the existence of several more lineages, spread over five sections in two subgenera: *Lf*. sect. *Gerardii*, *Lf*. sect. *Lactifluus*, *Lf*. sect. *Piperati*, *Lf*. sect. *Luteoli* and *Lf*. sect. *Tomentosi*. *Lactifluus* species from the **Western Palearctic region** are well studied and nine species have been described. Three in *Lf*. sect. *Lactifluus* (Van de Putte et al. 2016), two in *Lf*. sect. *Piperati* (Linnaeus 1753; Fries 1821; Crossland 1900, chapter 4), one in *Lf*. subg. *Gymnocarpi* (Bon 1971, chapter 2), one in *Lf*. subg. *Pseudogymnocarpi* (Kühner and Romagnesi 1953) and two in *Lf*. sect. *Albati* (Fries 1838; Bon 1979; Schaefer 1979).

The **observed species richness per biogeographical region** was plotted in Fig. 7.2. The results indicate that the number of species does not reach convergence for all continents, except for Europe. This means that the sampling effort in Europe has been large and in spite of this large sampling effort, almost no new species are found. We therefore might conclude that nearly all European *Lactifluus* species are found. In contrast, no convergence is reached in the other continents and many more samples are likely to be needed in order to reach convergence.

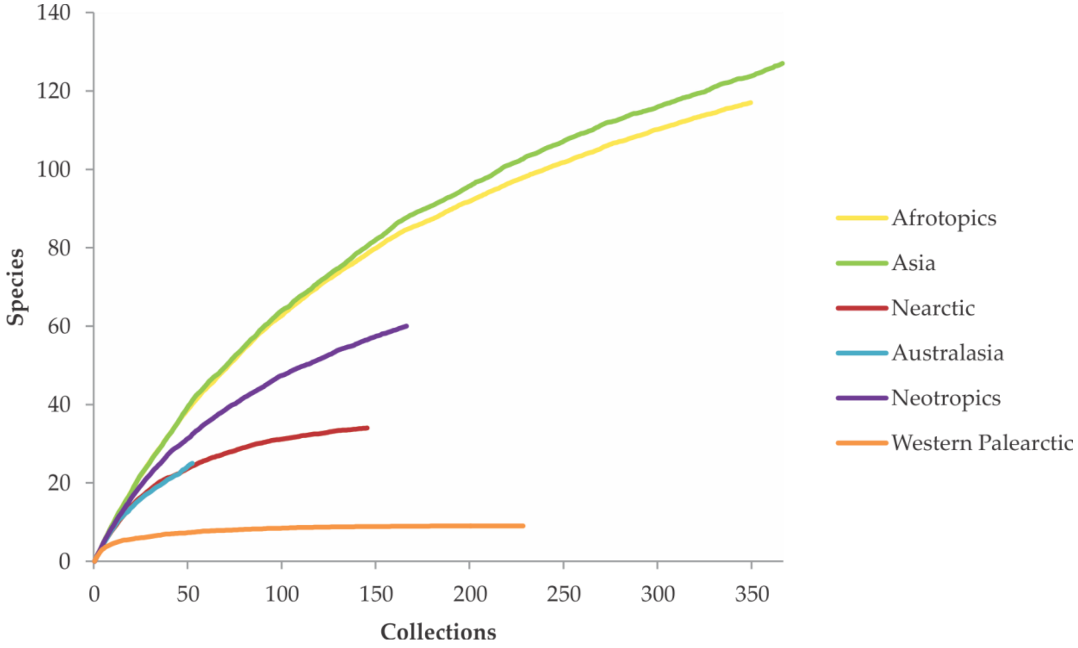

Fig. 7.2 Observed species richness per biogeographic region of the genus *Lactifluus*.

Species diversity per subgenus

In chapter 2, we found support for four subgenera within the genus *Lactifluus*: *Lf.* subg. *Gymnocarpi*, *Lf.* subg. *Lactariopsis*, *Lf.* subg. *Lactifluus* and *Lf.* subg. *Pseudogymnocarpi*. Each subgenus contains several supported sections and all subgenera, except *Lf.* subg. *Lactifluus*, also contain several unknown clades that probably represent new sections (Fig. 7.3).

When we look at the observed species richness per subgenus (Fig. 7.4), *Lf.* subg. *Lactifluus* represents the largest diversity with 148 species, followed by *Lf.* subg. *Lactariopsis* with 114 species. *Lf.* subg. *Pseudogymnocarpi* and *Lf.* subg. *Gymnocarpi* are relatively small subgenera, with 61 and 46 species respectively. This might be partially explained because of the rather recent divergence of several lineages within the largest subgenera. In *Lf.* subg. *Gymnocarpi*, for example, the 46 extant species descend from 30 lineages that already existed 5 My ago; while in *Lf.* subg. *Lactifluus*, the 150 extant species descend from 75 lineages that already existed 5 My ago. This shows that several lineages within *Lf.* subg. *Lactifluus* have only recently diverged. This divergence may be linked to the diversification of ectomycorrhizal hosts or to host shifts. For example, several lineages within *Lf.* sect. *Lactifluus* also form associations with members of Pinaceae. It can be hypothesized that this expansion of the host range may have driven diversification, such as reported for the genus *Russula* (Looney et al. 2016). However, to test whether this is the case for *Lf.* subg. *Lactifluus*, diversification analyses need to be performed.

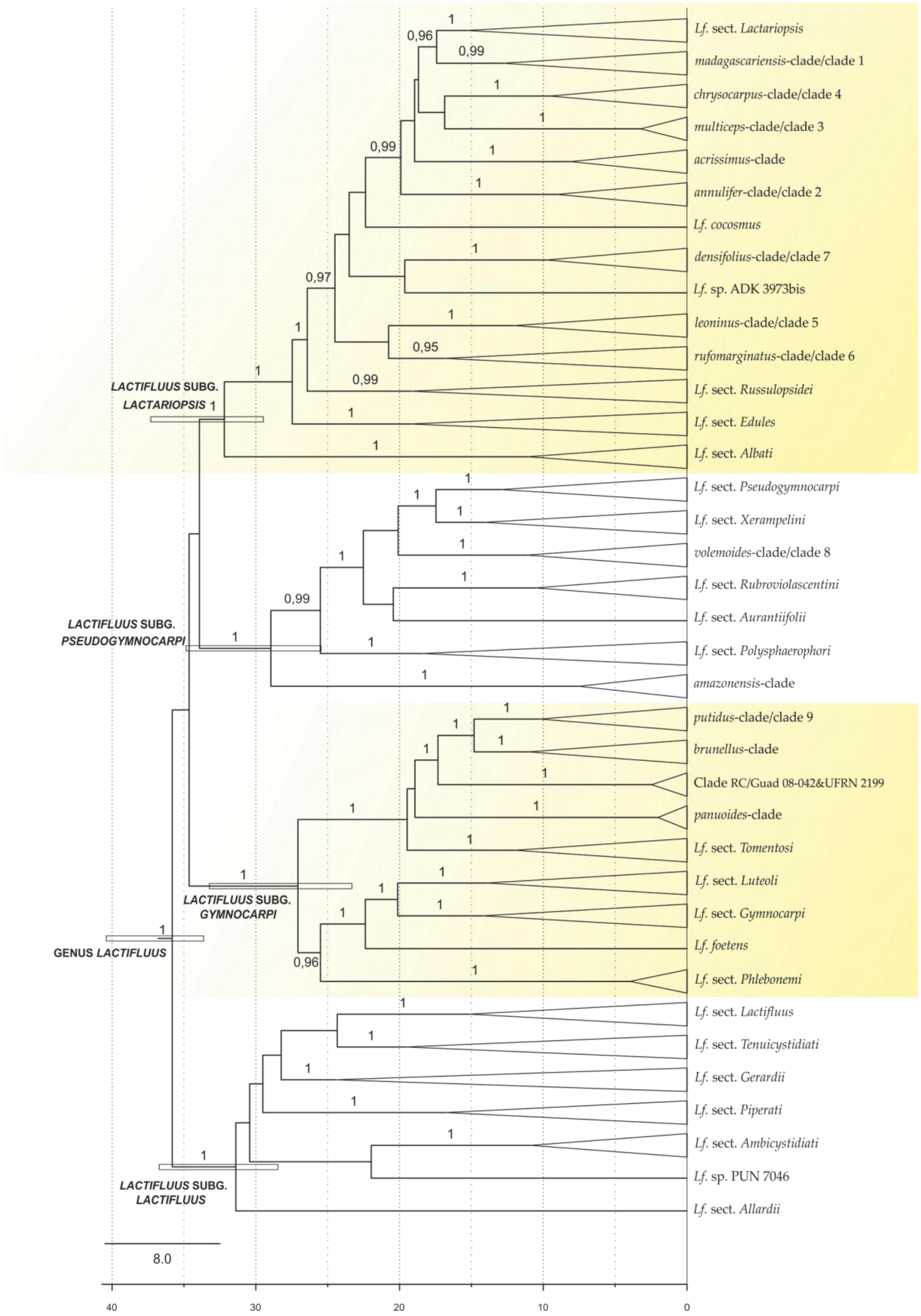

Fig. 7.3 Overview of the genus *Lactifluus*, inferred from the dated BEAST phylogeny of chapter 3 (time scale = million years). Undescribed clades are named after one representative inside that clade and clades that correspond with the phylogeny in chapter 3 are represented by the corresponding clade numbers.

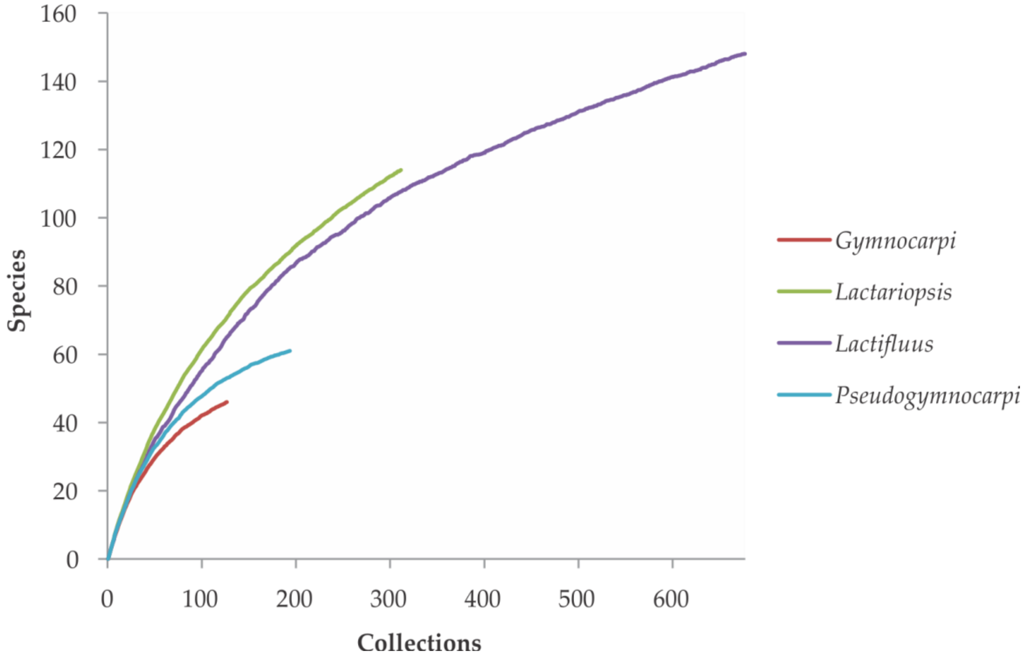

Fig. 7.4 Observed species richness per subgenus of the genus *Lactifluus*.

Molecular diversity

Despite our huge sampling effort, there are still some species that are represented in the phylogeny by one or a few collections on long and isolated branches: *Lf. allardii*, *Lf. ambicystidiatus*, *Lf. aurantiifolius*, *Lf. cocosmus*, *Lf. concentricus*, *Lf. foetens*, *Lf. lamprocystidiatus*, together with some unidentified taxa. These might be the only extant lineages of a once larger group, or other extant samples are yet to be found.

Furthermore, there are several species complexes which require a more detailed study. ***Lactifluus* subg. *Lactifluus*** is well-known for its species complexes. Despite the enormous work of Van de Putte (2012) and Stubbe (2012) to resolve *Lf.* sect. *Lactifluus* and *Lf.* sect. *Gerardii* respectively, still many undescribed lineages occur within both sections. Some of these lineages are characterised by a clear genetic diversity, but consist of species that are hard to distinguish morphologically. This suggests that they represent species complexes. In chapter 4, we explored the diversity of *Lf.* sect. *Piperati* and revealed many new lineages that are yet to be described. Wang (2015) described and studied *Lf.* sect. *Tenuicystidiati*, containing three taxa. Our results show at least six more lineages within this section (chapter 3). All four sections are genetically diverse, but are believed to contain a considerable amount of cryptic species.

Also within ***Lf.* subg. *Gymnocarpi***, we uncovered several species complexes with possible cryptic species. Within the Australian *Lf.* sect. *Tomentosi*, three species have been described, but our results suggest at least six more taxa. *Lf.* sect. *Luteoli* also contains several described (five) and undescribed (six) lineages that all are morphologically very similar. Interestingly, this section has a wide distribution with species occurring on five different continents. Also the African *Lf.* sect. *Phlebonemi* was believed to represent a species complex, as species are rather difficult to identify in the field, however, we only found three lineages within this section.

Within ***Lf.* subg. *Lactariopsis***, we found eight new clades that might represent new sections (chapter 2 and 3) and some of them are probably species complexes, such as the Neotropical /*annulifer*-clade (Clade 2, Fig. 2.6, 3.2 & 7.3) that contains species with secondary velum. This clade contains 22 lineages, of which only three have been described (Dennis 1970; Singer et al. 1983; Miller et al. 2012). In *Lf.* sect. *Albati*, which is predominantly distributed in the Northern hemisphere, only six species were described before this study. Our results suggest that this section is genetically very diverse, with 14 additional lineages found (chapter 3). This section might represent a species complex, which is confirmed by preliminary research that indicates it contains several cryptic species (unpubl. res.). Species of *Lf.* sect. *Lactariopsis*, the African section that contains species with secondary velum, are hard to distinguish in the field and this often leads to misidentifications. We found twenty lineages within this section, of which only eleven have been described. Within ***Lf.* subg. *Pseudogymnocarpi***, we also found one possible species complex. The African *Lf.* sect. *Pseudogymnocarpi* is characterised by lineages that are morphologically very similar, such as *Lf. longisporus*, *Lf. pumilus* and *Lf. gymnocarpoides*. Our study found 16 lineages within this group, of which the majority still needs to be described.

When we look at the results of the biogeographical study, we see that most clades that are indicated as putative species complexes are characterised by a recent divergence (<10My), indicated by very short branch lengths in the calibrated tree (chapter 3).

Ecological diversity

Lactifluus species are known from a diverse 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. *Lactifluus* is an ectomycorrhizal (ECM) genus recorded to form associations with many different plant families (chapter 1). Field observations of both *Lactifluus* fruit bodies and associated host trees suggest that most *Lactifluus* species are generalists and even associate with different hosts tree families. For example, at least 45% of the African *Lactifluus* species in our dataset are recorded to occur both with *Uapaca* species (Phyllanthaceae) as with members of the Fabaceae (Fig. 7.5). This is in accordance with the findings of previous studies in tropical African ecosystems (Diedhiou et al. 2010; Tedersoo et al. 2011), in which ECM fungi are found to associate with multiple hosts and ECM hosts associate with multiple mycobionts. The lack of specificity in plant-fungal interactions are hypothesised to provide resistance to the effects of habitat fragmentation by increasing the chance on re-establishment in disturbed areas (Tedersoo et al. 2010b; Tedersoo et al. 2011). It is hypothesised that associations between generalist ECM fungi and early-successional ECM hosts facilitate the establishments of secondary colonizing hosts by providing secondary colonizers with compatible ECM fungal symbionts (Nara 2006). In tropical Africa, *Uapaca* species are an example of these early successional ECM hosts. Tedersoo et al. (2011) hypothesise that *Uapaca* species, together with their generalist ECM communities, facilitate the establishment of late colonisers of the Fabaceae (e.g. *Afzelia*, *Berlinia*, *Brachystegia*, *Gilbertiodendron*, *Isoberlinia*, *Julbernardia*) in disturbed areas.

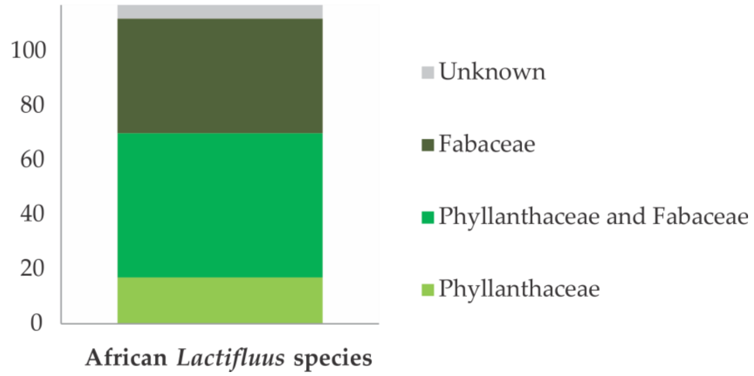

Fig. 7.5 Ectomycorrhizal (ECM) host tree family for the African *Lactifluus* species. ECM host tree family records are based on field observations and are not confirmed by molecular tools.

Future perspectives

This study revealed that the milkcap genus *Lactifluus*, once believed to be relatively small, contains a large diversity, especially in tropical regions. Based on a global molecular phylogeny, the monophyletic status of the genus is supported and infrageneric relationships were resolved. This led to new views on the traditional classification of the genus, and a new, revised classification was proposed. Furthermore, more than 200 undescribed lineages or putative species were discovered. Nine of those lineages are published or will be submitted to be published, while five others are in preparation for publication. Calibration analyses and biogeographical analyses indicated that *Lactifluus* originated between the Eocene and Oligocene in sub-Saharan Africa. However, in order to fully map the diversity of this large genus, additional research is required.

Contributions to an improved knowledge on the history of Lactifluus

In this study, a large effort was done to **improve sampling** of *Lactifluus* collections, both by the organisation of field expeditions to remote areas, as by requesting loans from fungal herbaria worldwide. Strikingly, a large portion of these collected and received specimens consisted of new lineages within the *Lactifluus* phylogeny. This, together with the results of the estimated number of species within the genus, indicates the need for further sampling in different countries, vegetation types or seasons.

Our biogeographical study revealed several areas for which the information on the occurrence of *Lactifluus* species is scarce or non-existing. The Western Palearctic appears to be well-sampled, but the border between the Western Palearctic and Asia, together with the North-Eastern part of the Palearctic is undersampled and may accommodate interesting *Lactifluus* species. Our biogeographical analyses further indicated potential dispersal patterns of *Lactifluus* species from Asia to the Nearctic and Neotropics, through Beringia. However, no *Lactifluus* species are known from the North-Western part of the Nearctic. Collecting is this region might elucidate this part of the evolutionary history of *Lactifluus*. Almost no collections are known from the Middle East, except one collection from Iran, *Lf.* cf. *glaucescens*, which is conspecific with collections from India, Japan and Thailand. It would be interesting to examine whether collections from that region are mainly related to Asian collections, or if completely new lineages would be discovered. Our study further showed a large Neotropical diversity. However, the Neotropics are only recently being explored and might contain a much larger *Lactifluus* diversity than previously thought. Next to these regions, also the Afrotropics, Australasia and Southeast Asia might benefit from additional sampling and, especially in a biogeographical point of view, islands are worth a more detailed look. If they contain a *Lactifluus* diversity differing from the mainland, conclusions may be drawn on the age of these lineages, independent from ages deduced from fossil calibrations.

A first step to achieve an improved sampling, is building networks with mycologists worldwide in order to exchange collections as loans or information on where and when to collect in their countries. Sampling in some regions, such as the Neotropics, is challenging, as fruiting is less dependent on the seasons compared to other regions, meaning that there is no real mushroom season, and vegetation with ECM hosts are rather restricted to certain areas in the forests. This makes it more time-consuming and expensive to collect in these regions. In order to get a quick idea of the *Lactifluus* diversity, it might be interesting to take soil samples and use next generation sequencing (NGS) techniques to reveal the *Lactifluus* diversity in these regions.

In order to properly delimit species within *Lactifluus*, more **molecular data** is needed from the present collections. For most collections only ITS is available, while this marker has proven to be rather variable in *Lactifluus* and species delimitation based on this locus alone resulted doubtful delimitations (see Chapter 3). More markers need to be sequenced in order to thoroughly delimit species within the genus (e.g. LSU, *RPB1*, *RPB2*, *TEF 1-α*). Another shortcoming is the large amount of type species for which no sequence data is available. In this study, we assembled 80 type sequences, but we were not able to retrieve sequence data for the remainder of type collections. Most of these type collections are old or improperly dried. New techniques (e.g. using more DNA cleaning protocols or use NGS techniques to construct a type ITS sequence) may help to sequence old material or the designation of an epitype can be considered, when it is impossible to extract DNA and the type material is in very bad condition (Botanical code art. 9.7.; e.g. in Buyck and Hofstetter 2011).

Next to molecular information, other characters are needed to properly describe species, e.g. morphological, ecological, biochemical, geographical or functional data. Especially ecological data is missing for most *Lactifluus* species. The majority of *Lactifluus* species are believed to associate with many tree hosts and very few specialists are believed to occur within the genus. In order to be sure of the correct host association(s), research on **ECM associations** in *Lactifluus* should be carried out on a global scale. These techniques encompass the time-consuming digging out of root samples from the vicinity of basidiocarps, from which both the fungus as the hosts can be determined, or the use of next generation techniques (NGS), in which large samples of roots can be studied. The ECM host association is believed to be one of the major drivers of diversification within ectomycorrhizal fungi (Rochet et al. 2011; Wilson et al. 2012; Harrower et al. 2015), unfortunately we lack this information for nearly all *Lactifluus* species.

When ecological data or other data are available, **diversification analyses** can be carried out to investigate the major drivers of diversification within *Lactifluus*. For example, Looney et al. (2016) found out that host switching and host expansion are driving diversification within the genus *Russula*. It would be interesting to examine whether it sister genus *Lactifluus* displays the same patterns.

Despite the efforts of several mycologists the past few years, our results suggest that several *Lactifluus* lineages remain unstudied and are in need for a **careful molecular and morphological investigation**. Within subgenera and sections, species should be delimited using robust techniques based on multiple gene markers and several lines of evidence (e.g. morphological, ecological or functional) should be investigated in order to support the delimitations.

Filling the gap

Notwithstanding our efforts to improve sampling for the genus *Lactifluus*, the results of the estimated number of *Lactifluus* species suggest many species are still to be found. It can even be the case that entire clades are lacking in our phylogenies. It might be interesting to examine whether we mainly lack species from regions that were not yet covered by field expeditions or whether we also lack species in the places we examined during the past years. This can be done by comparing our basidiocarp-based phylogeny with soil-sample-based phylogenies in order to see if the aboveground diversity is a good estimate of the **underground diversity**. Many ecological studies pointed out that the */russula-lactarius-lactifluus* lineage is one of the dominant ectomycorrhizal lineages in many vegetation types worldwide (Peay et al. 2010; Tedersoo et al. 2010a; Jairus et al. 2011; Tedersoo et al. 2011), so it would be interesting to connect those phylogenies with our basidiocarp-based phylogeny.

Furthermore, the results of our integrated approach to study the genus *Lactifluus* might be able to **fill the current gap** between taxonomical and ecological knowledge. A large drawback of ecological metagenomic studies is that the amount of unnamed species detected by metabarcoding is unclear since there is no unified way of naming these sequences. Taxonomists on the other hand, tend to focus on small groups of taxa. Our genus-wide approach, combining molecular, morphological and biogeographical data, might build bridges between both research fields.

Beyond Lactifluus

Next to the exploration of the genus *Lactifluus*, it would be interesting to focus on the bigger picture and compare the phylogenies and evolutionary histories of the **different Russulaceae genera**. For example, the two milkcap genera, *Lactarius* and *Lactifluus*, resemble each other on many levels, nonetheless they do display differences in distribution, morphological diversity, genetic diversity or host preference. It would be worthwhile to investigate what the basis of these differences is. Furthermore, relationships between all

Russulaceae genera are basically unknown, mainly due to the understudied crust-like genera. Species from these genera lack in current phylogenies, but they might shed a different light on the Russulaceae history.
Ectomycorrhizal fungi, such as *Lactifluus* species, play a major role in tropical and subtropical African forest ecosystems, where many trees, often growing on N- and P-poor soils, completely depend on these associations. Both above-ground and soil sample records confirm the */russula-lactarius-lactifluus* lineage is one of the dominant ectomycorrhizal lineages in many African vegetation types (Tedersoo et al. 2010a; Tedersoo et al. 2011). The next important groups, based on basidiocarp diversity, are the Boletales (> 200 species), *Amanita* (> 70 species) and *Cantharellus* (> 60 species). Soil diversity, however, showed that the */tomentella-thelephora* lineage follows the */russula-lactarius-lactifluus* lineage in species-richness. This indicates a discrepancy between basidiocarp diversity and soil diversity, and it is hypothesised that part of the Russulaceae and other ectomycorrhizal diversity remains hidden underground and the environment and the microclimate select what fructifies. Recent metagenomic research in Europe (Geml et al. 2014) reveals that some species that have long been considered to be restricted to arctic-alpine habitats, do occur in soil samples in the temperate zone, where they may only rarely, if at all, fructify. In order to reveal the **hidden diversity of Russulaceae species** and other ectomycorrhizal fungi, ectomycorrhizal root tip samples were be sampled and will be studied using NGS techniques. For this on-going project, we sampled ectomycorrhizal root tip samples from three African vegetation types in which the *Lactifluus* basidiocarp diversity is high (Zambezian miombo woodlands in southern to central and eastern Africa, Sudanian woodlands in West Africa and Guineo-Congolian rainforest in Central Africa). Using NGS techniques, DNA of the root samples will be sequenced and will be compared with the current Russulaceae phylogenies based on basidiocarps to fully characterize and document Russulaceae biodiversity for three African vegetation types.

General conclusions

This study aimed to explore the diversity of the mainly tropical milkcap genus *Lactifluus*. We largely improved coverage of *Lactifluus* specimens from the tropics, mainly by conducting field expeditions in remote tropical areas and by requesting loans from fungal herbaria worldwide. Because of this improved sampling, we were able to explore the global diversity of the genus and conclude that only part of this diversity has been found. The largest diversity is found in tropical Asia, where many cryptic species occur, and tropical Africa.
After conducting meticulous molecular analyses, we can conclude that the genus *Lactifluus* is monophyletic and can be divided into four well-supported subgenera: *Lf*. subg. *Gymnocarpi*, *Lf*. subg. *Lactariopsis*, *Lf*. subg. *Lactifluus* and *Lf*. subg. *Pseudogymnocarpi*. These subgenera only partly correspond with the traditional, mainly morphology-based classification and therefore, a new classification was proposed. Five morphological characters that were traditionally believed to be very informative for delimiting groups within *Lactifluus* (fruit body type, presence of a secondary velum, colour reaction of the latex/context, pileipellis type and presence of true cystidia) were studied more closely and we found out that they are important at different evolutionary levels within the genus, but other characteristics need to be studied to find morphological support for each clade.
Dating analysis indicated a mid-Cretaceous origin of the Russulaceae, and a Eocene-Oligocene origin of the genus *Lactifluus*. Although absolute divergence time estimates from fossil-calibrated phylogenies of fungi can be doubtful, relative dates can be informative. Our analyses indicate that the major Russulaceae genera originated rather recently in the history of the Russulaceae: ±75-88 My after the divergence time of the Russulaceae family. The biogeographical analyses estimated an African origin for the genus *Lactifluus* to be most likely. From this ancestral range, *Lactifluus* migrated towards other continents via both vicariance and long-distance dispersal.

When focusing on particular lineages within the genus *Lactifluus*, we discovered a large diversity within *Lf.* sect. *Piperati*. Only two species occur in Europe, but both Asia and North America contain many lineages, which are morphologically hard to distinguish and might represent cryptic species. Within *Lf.* sect. *Gerardii*, we discovered a large diversity of pleurotoid and agaricoid species with small basidiocarps, with five new species being described.

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Summary

Within the Russulales order, the Russulaceae family contains seven genera of which four are mainly agaricoid and dominant ectomycorrhiza formers in major vegetation types around the world. Due to their ecological importance and due to the fact that they are macrofungi with several striking characteristics, both the genus *Russula* and *Lactarius* (milkcaps) were often studied by mycologists worldwide. In 2008, molecular research pointed out that the milkcaps were paraphyletic and consisted out of the genera *Lactarius* and *Lactifluus*, and a few representatives belonged to *Multifurca*, a small genus also containing some former *Russula* species. *Lactarius* sensu novo is the largest milkcap genus, has a mainly temperate distribution and is characterised by a large morphological diversity. In contrast, *Lactifluus* is smaller, has a predominantly tropical distribution and displays a large genetic diversity, with many species complexes and cryptic species. In the meantime, it has also been proven that the agaricoid genera contain angiocarp (*Russula* and *Lactarius*) and pleurotoid (*Russula* and *Lactifluus*) representatives. Out of the two milkcap genera, *Lactifluus* is less-studied, mainly due to its distribution.

This thesis aims to (i) explore the diversity of the genus *Lactifluus*, (ii) test whether the genus is monophyletic, (iii) resolve infrageneric relationships and compare these with the traditional, morphology-based classification, (iv) reconstruct the evolutionary history of the genus and (v) further explore lineages within the genus by delimiting species and carefully describing newly found taxa.

Chapter 2 explores the diversity of the genus *Lactifluus* and proposes a new classification for the genus. First, an extensive global dataset was assembled, covering all major regions where *Lactifluus* was known to occur, together with all known lineages within the genus. This dataset contains 80 % of all known *Lactifluus* species and 30 % of the type collections. A four-gene molecular phylogeny was constructed in which *Lactifluus* was proven to be monophyletic and infrageneric relationships were almost fully resolved. These results were combined with a morphological study, focusing on five key-characteristics for *Lactifluus* (fruit body type, presence of a secondary velum, colour reaction of the latex/context, pileipellis type and presence of true cystidia). The resulting classification was compared with the traditional classification and nomenclatural changes were proposed where necessary.

Chapter 3 reconstructs the evolutionary history of the genus *Lactifluus*. The dataset of chapter 1 was complemented with all *Lactifluus* collections with ITS sequences available from the Ghent University fungal herbarium, together with all non-environmental GenBank sequences of *Lactifluus* available at the time. This resulted in a dataset of 1306 *Lactifluus* collections, including 80 type collections. Species delimitation was carried out on this dataset, in order to objectively assign one specimen per species. The resulting species tree was dated, using the secondary calibration procedure, and used for biogeographical analyses. The calibration analysis suggested the Russulaceae to have diverged during the mid-Cretaceous (±110.6 My) and *Lactifluus* between the Eocene and Oligocene (±33.4 My). The biogeographical analysis suggest an African origin for *Lactifluus*, which then later diversified to other continents, probably by vicariance and long-distance dispersal.

Chapter 4 explores the diversity of *Lactifluus* sect. *Piperati*. Due to the confusing morphology of the European members of this group, a morphological and molecular study were combined in order to delimit the European species. Two species were confirmed: *Lf. piperatus* and *Lf. glaucescens*. The frequently used morphological characteristics of the colouration of the latex and the macrochemical reactions of latex and context appear not to be useful as diagnostic characteristics to discriminate both species, but the microscopical characters of the pileipellis are informative. A preliminary worldwide phylogeny shows that this section comprises at least ten possible species divided over three clades and that there is no intercontinental conspecificity.

Chapter 5 investigates some Asian *Lactifluus* species from *Lf.* sect. *Gerardii*. Several *Lactifluus* collections have been found during field expeditions, both in Thailand and Nepal, of which some are pleurotoid and some

have tiny basidiocarps. Morphological research indicated that many of them were new to science, which was confirmed by our molecular phylogeny. Five new species are described: *Lf. auriculiformis*, *Lf. gerardiellus*, *Lf. pleurotoideus*, *Lf. pulchrellus* and *Lf. raspei*; and one new finding of *Lf.* cf. *uyedae* is reported.

Chapter 6 compiles recent taxonomic novelties in the genus *Lactifluus*. First, an overview is given of new combinations *Lactifluus* subg. *Lactifluus* and *Piperati*. Secondly, a new species that was found in the miombo woodlands of Kigoma (Tanzania) is described: *Lactifluus kigomaensis*. In a third part, two milkcap look-a-likes from tropical Africa were compared and this resulted in the finding and description of a new *Lactifluus* species: *Lf. albomembranaceus*. In the fourth part of this chapter, two recently found Thai *Lactifluus* species are described: *Lf. armeniacus* and *Lf. ramipilosus*.

Chapter 7 is a general discussion about the diversity of the genus *Lactifluus*. We discuss the global observed *Lactifluus* diversity and estimate the total number of *Lactifluus* species. Following these estimates, the genus *Lactifluus* might contain between 461–601 species and 62–80% of these species are represented in our phylogenies. Furthermore, we compare the observed number of species per continent and subgenus. All continents, except Europe, need additional sampling in order to approach the total number of *Lactifluus* species and both *Lf.* subg. *Lactifluus* and *Lf.* subg. *Lactariopsis* are most species rich. We confirm that *Lactifluus* is molecularly diverse, with several species complexes and species on isolated positions in the phylogeny.

Samenvatting

Binnen de orde Russulales bestaat de Russulaceae-familie uit zeven genera. Vier van deze genera zijn voornamelijk agaricoid en ectomycorrhizavormers die dominant zijn in de voornaamste vegetatietypes wereldwijd. De genera *Russula* en *Lactarius* (melkzwammen) zijn frequent bestudeerd door mycologen over de hele wereld vanwege hun groot ecologisch belang en omdat het opvallende macrofungi zijn. Moleculair onderzoek uit 2008 heeft duidelijk gemaakt dat de melkzwammen een parafyletische groep vormen en bestaan uit de genera *Lactarius*, *Lactifluus* en *Multifurca*. *Multifurca* is een klein genus, dat enkele voormalige *Lactarius* en *Russula* soorten bevat. *Lactarius* sensu novois het grootste melkzwamgenus, komt voornamelijk voor in de tropen en wordt gekenmerkt door een grote morfologische diversiteit. Dit in tegenstelling tot het kleinere genus *Lactifluus*, dat hoofdzakelijk in de tropen voorkomt en een grote genetische diversiteit vertoont. Daarnaast werd ook aangetoond dat de agaricoide genera ook angiocarpe (*Russula* en *Lactarius*) en pleurotoide (*Russula* en *Lactifluus*) soorten bevatten. *Lactifluus* is het minst bestudeerde melkzwamgenus, wat grotendeels te wijten is aan zijn distributie.

Deze thesis heeft als doel om (i) de diversiteit van het genus *Lactifluus* te onderzoeken, (ii) te testen of het genus monofyletisch is, (iii) de infragenerische verwantschappen op te lossen en deze te vergelijken met de traditionele classificatie, (iv) de evolutionaire geschiedenis van het genus te reconstrueren en (v) verschillende secties binnen het genus beter te onderzoeken door middel van soortsafbakeningstechnieken en de resulterende nieuwe soorten te beschrijven.

Hoofdstuk 2 onderzoekt de diversiteit van het genus *Lactifluus* en stelt een nieuwe infragenerische classificatie voor. Allereerst werd een uitgebreide wereldwijde dataset opgesteld, met daarin collecties uit alle regio's waar *Lactifluus* voorkomt en collecties uit alle gekende secties en subgenera. Deze dataset bevat 80 % van alle gekende *Lactifluus*-soorten en 30 % van de type collecties. Een fylogenie gebaseerd op vier merkers toonde aan dat *Lactifluus* monofyletisch is en maakte de infragenerische verwantschappen duidelijk. Deze resultaten werden gecombineerd met een morfologische studie, waarin gefocust werd op vijf belangrijke kenmerken voor het genus (type vruchtlichaam, aanwezigheid van velum, kleurreactie van de melk of de context, type hoedhuid en aanwezigheid van echte cystiden). De resulterende classificatie werd vergeleken met de traditionele classificatie en nomenclaturale aanpassingen werden voorgesteld waar nodig.

Hoofdstuk 3 reconstrueert de evolutionaire geschiedenis van het genus *Lactifluus*. De dataset uit hoofdstuk 1 werd aangevuld met alle *Lactifluus*-collecties waarvoor een ITS sequentie beschikbaar was, zowel uit het mycologisch herbarium van Universiteit Gent als uit GenBank. Dit resulteerde in een dataset van 1306 collecties, waaronder 80 type sequenties. Soorten werden afgebakend door middel van GMYC om op een objectieve manier één collectie per soort te selecteren voor verdere analyses. Na selectie werd de fylogenie gedateerd door middel van secondaire calibratie en werd een biogeografische studie uitgevoerd. Uit de analyses blijkt dat de Russulaceae ontstaan zijn tijdens het midden-Krijt (±110.6 My) en *Lactifluus* tijdens het Eoceen/Oligoceen (±33.4 My). De biogeografische analyses suggereren een Afrikaanse oorsprong van *Lactifluus*, waarna het genus verder diversifieerde naar andere continenten, waarschijnlijk door middel van geografische vicariantie en lange-afstands dispersie.

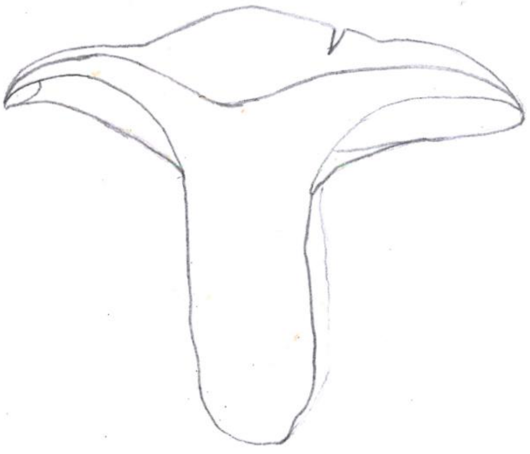
Hoofdstuk 4 onderzoekt de diversiteit van *Lactifluus* sect. *Piperati*. Aangezien de morfologie binnen de Europese soorten uit deze groep verwarrend bleek doorheen de geschiedenis, hebben we een morfologische en een moleculaire studie gecombineerd, met als doel het afbakenen van de Europese soorten. We bevestigden het bestaan van twee Europese soorten: *Lf. piperatus* en *Lf. glaucescens*. Het verkleuren van de melk en de macrochemische reacties van de melk en de context blijken geen diagnostische kenmerken voor deze soorten. De microscopische opbouw van de hoedhuid blijkt wel informatief te zijn. Daarnaast toonde de wereldwijde fylogenie van deze groep aan dat er minstens 10 mogelijke soorten tot deze groep behoren, verspreid over drie clades, en geen van deze soorten komt op meerdere continenten voor.

Hoofdstuk 5 onderzoekt enkele Aziatische *Lactifluus*-soorten uit *Lf.* sect. *Gerardii*. Verschillende collecties werden gevonden tijdens inzamelexpedities in Thailand en Nepal, waaronder een aantal pleurotoide en heel kleine agaricoide collecties. Morfologisch onderzoek wees uit dat meerdere van deze collecties nieuwe soorten waren, wat bevestigd werd door de moleculaire analyses. We beschrijven vijf nieuwe soorten: *Lf. auriculiformis*, *Lf. gerardiellus*, *Lf. pleurotoideus*, *Lf. pulchrellus* en *Lf. raspei*; en rapporteren een nieuwe vindplaats voor *Lf.* cf. *uyedae*.

Hoofdstuk 6 verzamelt nieuwe taxonomische vondsten binnen het genus *Lactifluus*. In een eerste deel wordt een overzicht gegeven van nieuwe combinaties binnen een aantal subgenera en secties van *Lactifluus*. In het tweede deel wordt een nieuwe soort beschreven die gevonden werd in de miombo boomsavannes in Kigoma (Tanzania): *Lf. kigomaensis*. In het derde deel worden twee Afrikaanse melkzwam dubbelgangers met elkaar vergeleken en blijkt één daarvan een nieuwe soort te zijn: *Lf. albomembranaceus*. Tot slot worden in het vierde deel twee nieuwe Thaise soorten beschreven: *Lf. armeniacus* en *Lf. ramipilosus*.

Hoofdstuk 7 bevat een algemene discussie over de diversiteit van het genus *Lactifluus*. We bespreken de globaal geobserveerde diversiteit van *Lactifluus* en schatten het totaal aantal soorten binnen het genus. Volgens deze schattingen bevat *Lactifluus* ongeveer 461–601 soorten, waarvan 62–80% vertegenwoordigd is in onze analyses. Daarnaast vergelijken we het waargenomen aantal soorten per continent, subgenus en vegetatietype. Voor alle continenten, behalve Europa, zijn aanvullende collecties nodig om de totale diversiteit van *Lactifluus* te kennen en de subgenera *Lf.* subg. *Lactifluus* en *Lf.* subg. *Lactariopsis* zijn het soortenrijkst. We bevestigen dat het genus *Lactifluus* een grote moleculaire diversiteit kent, met verschillende soortencomplexen en soorten op geïsoleerde posities in de fylogenie.

Addenda



S1 – Table Dataset 1 Chapter 3

Available by request from the author.

S2 – Table Dataset 3 Chapter 3

Table S2 Specimens and GenBank accession numbers of ITS, LSU, *RPB1* and *RPB2* sequences used for reconstructing the biogeographical history of the genus *Lactifluus*.

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*acicularis*	KVP 08-002	Thailand	HQ318226	HQ318132	HQ328869	JN389131
Lactifluus		*acrissimus*	EDC 11-112	Tanzania	KR364041	KR364168	KR364254	KR364366
Lactifluus		*albocinctus*	AV 11-181	Togo	To submit	To submit	To submit	To submit
Lactifluus		*albomembranaceus*	EDC 12-046	Cameroon	KR364064	KR364193	KR364257	KR364369
Lactifluus		*allardii*	AV 05-246	USA	KF220017	KF220126	KF220218	To submit
Lactifluus		*allardii*	JN 2004-008	USA	KF220016	KF220125	KF220217	KR364370
Lactifluus		*amazonensis*	F1037055	Brazil	To submit	None	None	None
Lactifluus	cf.	*amazonensis*	AMV1874	Colombia	KR364004	None	None	None
Lactifluus		*ambicystidiatus/volemus* var. *asiaticus*	HKAS J7008	China	KR364108	KR364239	KR364309	KR364437
Lactifluus		*annulatoangustifolius*	AB 360	Guinea	To submit	None	None	None
Lactifluus		*annulatoangustifolius*	BB 00-1518	Madagascar	AY606981	KR364253	None	None
Lactifluus		*annulatoangustifolius*	MD145	Togo	HG426475	None	None	None
Lactifluus		*annulatoangustifolius*	SDM 017	Gabon	To submit	None	None	None
Lactifluus		*annulatolongisporus*	MD123	Togo	HG426470	None	None	None
Lactifluus		*annulifer*	TH 9014	Guyana	KC155376	KC155376	None	None
Lactifluus		*armeniacus*	EDC 14-501	Thailand	KR364127	None	None	None
Lactifluus		*atrovelutinus*	DS 06-003	Malaysia	GU258231	GU265588	GU258325	JN389185
Lactifluus		*aurantiifolius*	AV 94-063	Burundi	KR364017	KR364144	None	None
Lactifluus		*aurantiifolius*	AV 99-186	Zimbabwe	To submit	To submit	To submit	

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*aureifolius/indusiatus/fazaoensis*	AV 11-074	Tanzania	KR364056	KR364183	KR364259	KR364371
Lactifluus		*auriculiformis*	AV 12-050	Thailand	KR364086	KR364216	KR364260	KR364372
Lactifluus		*bertillonii*	JN 2012-016	Germany	KR364087	KR364217	KR364261	KR364373
Lactifluus		*bhandaryi* nom. prov.	TENN 051832/HRB 83	Nepal	To submit	None	To submit	None
Lactifluus		*bicapillus* nom. prov.	EDC 12-176	Cameroon	KR364070	KR364199	KR364300	KR364428
Lactifluus		*bicolor*	DS 06-229	Malaysia	GU258221	GU265577	GU258313	None
Lactifluus		*brachystegiae*	AV 99-002	Zimbabwe	KR364018	KR364145	KR364262	KR364374
Lactifluus	aff.	*brasiliensis*	TH7677	Guyana	KT339245	None	None	None
Lactifluus		*brunellus*	TH 9130	Guyana	JN168728	None	None	None
Lactifluus		*brunneocarpus*	AB 185	Guinea	To submit	To submit	To submit	To submit
Lactifluus		*brunneoviolascens*	AV 13-038	Italy	KR364123	KR364246	KR364264	KR364376
Lactifluus		*brunnescens*	AV 05-083	Malawi	KR364019	KR364146	KR364263	KR364375
Lactifluus	cf.	*brunnescens*	EDC 12-116	Cameroon	To submit	To submit	To submit	To submit
Lactifluus		*burkinabei*	MD 355	Burkina Faso	LK392609	None	None	None
Lactifluus		sp.	MJ 99	Brazil	To submit	None	None	None
Lactifluus		*caribaeus*	CL/Mart 06-014	Martinique	To submit	To submit	None	None
Lactifluus		*carmineus*	AV 05-146	Malawi	To submit	To submit	To submit	To submit
Lactifluus		*chamaeleontinus*	JD 946	Democratic Republic of the Congo	KR364079	KR364208	KR364267	KR364377
Lactifluus		*chiapanensis*	VMB 4374A	Mexico	GU258297	GU265580	GU258316	KR364378
Lactifluus		*chrysocarpus*	LE 253907	Viet Nam	JX442761	JX442761	None	None
Lactifluus		*clarkeae*	MN 2004002	Australia	KR364011	HQ318205	KR364268	KR364379
Lactifluus		*clarkeae*	MN 2004122	Australia	To submit	To submit	To submit	To submit
Lactifluus		*clarkeae*	REH 8830	Australia	To submit	None	To submit	To submit
Lactifluus		*clarkeae*	REH 8853	Australia	To submit	None	To submit	None
Lactifluus		*clarkeae*	REH 9326	Australia	To submit	None	To submit	None
Lactifluus		*clarkeae*	RH 9557	Australia	To submit	None	None	None
Lactifluus		*cocosmus*	ADK 4462	Togo	KR364013	KR364141	KR364269	KR364380
Lactifluus		sp.	MJ 100	Brazil	To submit	None	None	None
Lactifluus		*conchatulus*	LTH 457	Thailand	GU258296	GU265659	GU258399	KR364381
Lactifluus		*coniculus*	DS 07-496	Sri Lanka	GU258236	GU265594	GU258331	None
Lactifluus		sp.	MJ 112	Brazil	To submit	None	None	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*corrugis*	AV 04-209	USA	To submit	To submit	To submit	To submit
Lactifluus		*corrugis*	AV 05-337	USA	To submit	To submit	To submit	None
Lactifluus	aff.	*corrugis*	OSA-My-4016	Japan	To submit	AB238668	To submit	None
Lactifluus	aff.	*corrugis*	OSA-My-4019	Japan	To submit	AB238671	To submit	None
Lactifluus	aff.	*corrugis*	OSA-My-4021	Japan	To submit	AB238674	To submit	None
Lactifluus	aff.	*corrugis*	OSA-My-4017	Japan	To submit	AB238669	To submit	None
Lactifluus		*crocatus*	KVP 08-035	Thailand	To submit	HQ318152	HQ328889	To submit
Lactifluus		*cyanovirescens*	EDC 11-021	Tanzania	To submit	To submit	To submit	To submit
Lactifluus		*cyanovirescens*	JD 930	Democratic Republic of the Congo	To submit	To submit	To submit	None
Lactifluus		*cyanovirescens*	JD 978	Democratic Republic of the Congo	To submit	To submit	To submit	To submit
Lactifluus		*deceptivus*	AV 05-249	USA	To submit	None	To submit	None
Lactifluus		*deceptivus*	JN 2007-012	Canada	To submit	To submit	None	None
Lactifluus		*deceptivus*	NVE 396	Colombia	KF937340	None	None	None
Lactifluus		*deceptivus*	PC BB2004-259	USA	EU598200	None	None	None
Lactifluus		*deceptivus*	REH 7938	Costa Rica		None	None	None
Lactifluus		*deceptivus*	TENN 065854	USA	KR364101	None	KR364271	KR364383
Lactifluus		*denigricans*	EDC 11-218	Tanzania	KR364051	KR364178	KR364272	KR364384
Lactifluus		*densifolius*	AV 11-111	Tanzania	KR364057	KR364184	KR364273	KR364385
Lactifluus		*dissitus*	AV-KD-KVP 09-134	India	JN388978	JN389026	JN375628	JN389172
Lactifluus		*distantifolius*	DS 07-461	Thailand	HQ318124	HQ318223	To submit	To submit
Lactifluus		*dunensis*	MAN 219	Brazil	To submit	None	None	None
Lactifluus		*dunensis*	UFRN-Fungos 1882	Brazil	To submit	To submit	None	None
Lactifluus		*dwaliensis*	LTH 55	Thailand	KF220111	KF220204	KF220278	KR364386
Lactifluus		*dwaliensis*	LTH 67	Thailand	KF220108	KF220203	KF220277	To submit
Lactifluus		*dwaliensis*	LTH 346	Thailand	KF220113	KF220206	KF220279	To submit
Lactifluus	cf.	*edulis*	AV 11-187	Togo	To submit	To submit	To submit	To submit
Lactifluus		*edulis*	ADK 3127	Benin	To submit	To submit	To submit	None
Lactifluus		*edulis*	FN 05-628	Malawi	KR364020	KR364147	KR364275	KR364387
Lactifluus		*emergens*	AV 99-012	Zimbabwe	KR364021	KR364148	KR364276	KR364388
Lactifluus		*emergens*	DPM04	Togo	HG426467	None	None	None

Genus		Species epithet	Herbarium no.	Country	ITS	LSU	*RPB2*	*RPB1*
Lactifluus		*fazaoensis*	MD152	Togo	HG426477	None	None	None
Lactifluus		*flammans*	JD 941	Democratic Republic of the Congo	KR364078	KR364207	KR364303	KR364431
Lactifluus		*flammans*	MD124	Togo	HG426471	None	None	None
Lactifluus		*flavellus*	MD393	Togo	LK392594	None	None	None
Lactifluus		*flavellus*	MD397	Togo	LK392595	None	None	None
Lactifluus		*flocktonae*	JET1006	Australia	JX266621	JX266637	None	None
Lactifluus		*foetens*	ADK 3688	Benin	KR364022	KR364149	KR364278	KR364390
Lactifluus		*foetens*	C1822	Togo	HG917382	None	None	None
Lactifluus		*fuscomarginatus*	LM 4379	Mexico	HQ168367	HQ168367	None	None
Lactifluus		*fuscomarginatus*	LM4640	Mexico	HQ168369	None	None	None
Lactifluus		*genevievae*	GG-DK 17-02-05	Australia	GU258294	GU265657	GU258397	KR364401
Lactifluus		*gerardiellus*	KW386	Thailand	To submit	To submit	None	None
Lactifluus	aff.	*gerardii*	Halling 6800	Australia	To submit	To submit	None	None
Lactifluus	aff.	*gerardii*	LTH 270	Thailand	EF560685	GU265598	GU258335	KR364402
Lactifluus	aff.	*gerardii*	DS 07-390	Thailand	GU258252	GU265613	GU258350	None
Lactifluus	aff.	*gerardii*	FRIM 1357	Malaysia	To submit	To submit	To submit	None
Lactifluus	aff.	*gerardii*	LTH 394	Thailand	GU258249	GU265610	GU258347	None
Lactifluus	aff.	*gerardii*	LTH 400	Thailand	To submit	To submit	To submit	None
Lactifluus	aff.	*gerardii*	TMI 15558	Japan	GU258230	GU265587	GU258324	None
Lactifluus	aff.	*gerardii*	JN 2011-062	Viet Nam	To submit	To submit	To submit	To submit
Lactifluus	aff.	*gerardii*	289-361	Japan	AB531470	None	None	None
Lactifluus		*gerardii*	AV 05-309	USA	To submit	To submit	To submit	None
Lactifluus		*gerardii*	AV 05-375	USA	GU258254	GU265616	GU258353	KR364403
Lactifluus	aff.	*gerardii*	DS 07-373	Thailand	GU258242	GU265603	GU258340	None
Lactifluus	aff.	*gerardii*	Halling 8262	Costa Rica	To submit	To submit	To submit	None
Lactifluus	aff.	*gerardii*	HKAS 42260	China	To submit	None	To submit	None
Lactifluus		*gerardii*	P.R.Leacock 5770	USA	To submit	To submit	To submit	None
Lactifluus	aff.	*gerardii*	TMI 15534	Japan	GU258229	GU265586	GU258323	None
Lactifluus	aff.	*gerardii*	Watling 24783	Malaysia	To submit	To submit	To submit	None
Lactifluus	aff.	*gerardii*	MC 04-259	Nepal	GU258234	GU265592	GU258329	
Lactifluus	aff.	*gerardii china*	KIINA 126	China	GU258227	GU265584	GU258321	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	cf.	*gerardii* var. *fagicola*	JN 2007-029	Canada	GU258224	GU265582	GU258318	None
Lactifluus		*gerardii* var. *subrubescens*	DED 5275	USA	To submit	To submit	To submit	None
Lactifluus		*gerardii* var. *subrubescens*	KD 4062	India	To submit	To submit	None	None
Lactifluus		*gerardii* var. *subrubescens*	Watling 24828	Malaysia	To submit	To submit	To submit	None
Lactifluus	aff.	*glaucescens*	AV 04-195	USA	KF220045	KF220146	KF220232	KR364404
Lactifluus	aff.	*glaucescens*	AV 05-374	USA	KF220049	KF220150	KF220236	KR364405
Lactifluus	aff.	*glaucescens*	JN 2011-014	Viet Nam	KF220104	KF220199	KF220273	KR364406
Lactifluus	aff.	*glaucescens*	LTH 274	Thailand	KR364107	KR364238	KR364325	KR364457
Lactifluus	aff.	*glaucescens*	293-58	Japan	AB531463	None	None	None
Lactifluus	aff.	*glaucescens*	293-61	Japan	AB509515	None	None	None
Lactifluus	aff.	*glaucescens*	F_PRL5812	USA	GQ166898	None	None	None
Lactifluus	aff.	*glaucescens*	LaGl	Iran	KT833866	None	None	None
Lactifluus		*glaucescens*	LGAM 2010-0132	Greece	KR364105	KR364236	KR364280	KR364407
Lactifluus	aff.	*glaucescens*	LTH 66	Thailand	To submit	To submit	To submit	To submit
Lactifluus	aff.	*glaucescens*	NEHU.MBSR.07	India	KM282287	None	None	None
Lactifluus	aff.	*glaucescens*	No117	Thailand	LC008296	None	None	None
Lactifluus	aff.	*glaucescens*	LTH 236	Thailand	KF220060	KF220158	KF220244	None
Lactifluus	aff.	*glaucescens*	LTH 237	Thailand	KF220052	KF220153	KF220238	None
Lactifluus	aff.	*glaucescens*	LTH 244	Thailand	KF220054	KF220155	KF220240	None
Lactifluus	aff.	*glaucescens*	AV 04-174	USA	KF220044	KF220145	KF220231	None
Lactifluus	aff.	*glaucescens*	S 09-115	India	KF220097	KF220192	KF220266	None
Lactifluus		*glaucescens*	2000 10 05 01	France	KF220066	KF220164	KF220249	None
Lactifluus		*glaucescens*	2008 08 21 01	Belgium	KF220032	JN388988	JN375591	To submit
Lactifluus		*goossensiae*	AB 320	Guinea	KR364132	KR364252	KR364281	None
Lactifluus		*guellii*	C2157	Togo	HG426466	None	None	None
Lactifluus	cf.	*gymnocarpoides*	JD 931	Democratic Republic of the Congo	To submit	To submit	To submit	To submit
Lactifluus		*gymnocarpoides*	AV 05-011	Malawi	To submit	To submit	None	None
Lactifluus		*gymnocarpoides*	AV 11-186	Togo	To submit	To submit	To submit	None
Lactifluus		*gymnocarpoides*	JD 885	Democratic Republic of the Congo	KR364074	KR364203	KR364283	KR364409
Lactifluus		*gymnocarpoides*	MD 301	Benin	LK392601	None	None	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*gymnocarpoides*	MD 318	Benin	LK392600	None	None	None
Lactifluus		*gymnocarpus*	EDC 12-047	Cameroon	KR364065	KR364194	KR364282	KR364408
Lactifluus	aff.	*gymnocarpus*	MD125	Togo	HG426472	None	None	None
Lactifluus		*heimii*	C2018	Togo	LK392612	None	None	None
Lactifluus		*heimii*	EDC 11-082	Tanzania	KR364040	KR364167	KR364286	KR364412
Lactifluus		*hora*	DS 07-502	Sri Lanka	GU258238	GU265596	GU258333	None
Lactifluus		sp.	MJ 26	Brazil	To submit	None	None	None
Lactifluus		sp.	MJ 51	Brazil	To submit	None	None	None
Lactifluus	aff.	*hygrophoroides*	CU_Micro_Nan-MN22	Thailand	AB451978	None	None	None
Lactifluus	aff.	*hygrophoroides*	285-352	Japan	AB509713	None	None	None
Lactifluus		*hygrophoroides*	AV 05-251	USA	HQ318285	HQ318208	HQ328936	KR364413
Lactifluus	aff.	*hygrophoroides*	MRNo224	Thailand	LC008528	None	None	None
Lactifluus	aff.	*hygrophoroides*	No115	Thailand	LC008295	None	None	None
Lactifluus	aff.	*igniculus*	LE 253908	Viet Nam	JX442760	JX442760	None	None
Lactifluus		*igniculus*	LE 262983	Viet Nam	JX442759	JX442759	None	None
Lactifluus		*ignifluus*	5213	India	xxx	None	None	None
Lactifluus	cf.	*inversus*	EDC 12-070	Cameroon	To submit	To submit	To submit	To submit
Lactifluus		*inversus*	AB 063	Guinea	AY606976	DQ421978	DQ421917	KR364414
Lactifluus		*kigomaensis*	EDC 11-159	Tanzania	KR364050	KR364177	KR364295	KR364423
Lactifluus		*kivuensis*	JR Z 310	Democratic Republic of the Congo	KR364027	KR364154	None	None
Lactifluus		*lamprocystidiatus*	EH 72-195	Papua New Guinea	KR364015	None	None	None
Lactifluus		*latifolius*	SDM 037	Gabon	KR364028	KR364155	KR364291	KR364418
Lactifluus		*leae*	FH 12-013	Thailand	KF432957	KR364213	KR364292	KR364419
Lactifluus		*leonardii*	GG 07-02-04	Australia	GU258308	GU265668	GU258408	KR364495
Lactifluus	aff.	*leoninus*	KVP 08-003	Thailand	To submit	To submit	To submit	None
Lactifluus		*leoninus*	DS 07-454	Thailand	KF220055	JN388989	JN375592	JN389188
Lactifluus		*leoninus*	EH 72-524	Papua New Guinea	KR364116	None	None	None
Lactifluus		*leptomerus*	AV-KD-KVP 09-131	India	JN388972	JN389023	JN375625	JN389169
Lactifluus		*leucophaeus*	LTH 182	Thailand	KF220059	KF220157	KF220243	KR364420
Lactifluus	aff.	*leucophaeus*	LTH 360	Thailand	KF220061	KF220159	KF220245	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*limbatus*	DS 06-247	Malaysia	JN388955	JN388987	JN375590	JN389186
Lactifluus		*longibasidius*	MD141	Togo	HG426473	None	None	None
Lactifluus		*longibasidius*	MD156	Togo	LK392596	None	None	None
Lactifluus		*longipes*	EDC 12-049	Cameroon	To submit	To submit	To submit	To submit
Lactifluus		*longipilus*	AV-RW 04-160	Thailand	HQ318235	HQ318143	HQ328880	To submit
Lactifluus		*longipilus*	LTH 206	Thailand	HQ318258	HQ318171	HQ328907	None
Lactifluus	cf.	*longisporus*	AV 11-025	Tanzania	KR364054	KR364181	KR364311	KR364439
Lactifluus		*longisporus*	EDC 11-208	Tanzania	To submit	To submit	To submit	None
Lactifluus		*longivelutinus*	XHW 1565	China	KR364114	None	None	None
Lactifluus	cf.	*luteolus*	KUN_F73536	South Korea	KC154099	KC154125	KC154151	None
Lactifluus	cf.	*luteolus*	KUN_F73547	China	KC154098	KC154124	KC154150	None
Lactifluus		*luteolus*	ASM 13476	USA	To submit	None	None	None
Lactifluus		*luteolus*	AV 05-253	USA	KR364016	KR364142	KJ210067	KR364440
Lactifluus		*luteopus*	EDC 11-087	Tanzania	KR364049	KR364176	KR364312	KR364441
Lactifluus		*luteopus*	MD102	Togo	LK392602	None	None	None
Lactifluus		*luteopus*	MD212	Guinea	LN849749	None	None	None
Lactifluus		*madagascariensis*	BB 99-409	Madagascar	AY606977	DQ421975	DQ421914	None
Lactifluus	aff.	*medusae*	MD142	Togo	HG426474	None	None	None
Lactifluus		*medusae*	EDC 12-152	Cameroon	KR364069	KR364198	KR364314	KR364442
Lactifluus		*melleus*	EDC 12-030	Cameroon	To submit	To submit	To submit	To submit
Lactifluus		*membranaceus*	C2349	Togo	HG426478	None	None	None
Lactifluus		*membranaceus*	MD234	Guinea	LK392610	None	None	None
Lactifluus		*multiceps*	TH 9154A	Guyana	JN168731	None	None	None
Lactifluus		*murinipes*	LD15-015	Martinique	To submit	To submit	To submit	To submit
Lactifluus	aff.	*nebulosus*	RC/Guad 11-023	Guadeloupe	KP691412	KP691421	KP691430	KR364394
Lactifluus		*nebulosus*	LD15-059	Martinique	To submit	To submit	To submit	To submit
Lactifluus		*nodosicystidiosus*	BEM 97-273	Madagascar	KR364029	KR364156	KR364316	KR364444
Lactifluus		*nonpiscis*	AV 11-137	Togo	KR364058	KR364185	KR364317	KR364445
Lactifluus	aff.	*nonpiscis*	MD101	Togo	HG426468	None	None	None
Lactifluus	aff.	*ochrogalactus*	AV-KD-KVP 09-120	India	KR364130	KR364248	KR364318	KR364446
Lactifluus	aff.	*ochrogalactus*	AV-KD-KVP 09-093	India	To submit	To submit	To submit	To submit
Lactifluus		*ochrogalactus*	TMI 26088	Japan	To submit	None	None	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*oedematopus*	KVP 12-001	Germany	KR364100	KR364232	KR364319	KR364447
Lactifluus		*paleus*	EH 72-385	Papua New Guinea	To submit	None	None	None
Lactifluus		*pallidilamellatus*	Montoya 4716	Mexico	JQ753824	JQ348268	To submit	To submit
Lactifluus		*panuoides*	MCA 2109	Guyana	To submit	None	None	None
Lactifluus		*panuoides*	MR-GUY-14-093	Guyane	To submit	To submit	None	None
Lactifluus		*panuoides*	RC/Guy 10-024	French Guiana	KJ786647	KJ786551	KP691428	None
Lactifluus		*paraensis*	UFRN-Fungos 2192	Brazil	To submit	To submit	None	None
Lactifluus		*parvigerardii*	KUN_F61367	China	JF975641	JF975642	JF975643	None
Lactifluus		*pectinatus*	MD140	Togo	LK392599	None	None	None
Lactifluus		*pegleri*	LD15-014	Martinique	To submit	To submit	To submit	To submit
Lactifluus		*pelliculatus*	JD 956	Democratic Republic of the Congo	KR364080	KR364209	KR364321	KR364449
Lactifluus		*persicinus*	EDC 12-001	Cameroon	KR364061	KR364190	KR364298	KR364426
Lactifluus		*petersenii*	AV 05-300	USA	GU258281	GU265642	GU258382	KR364450
Lactifluus	aff.	*phlebonemus*	EDC 12-023	Cameroon	KR364062	KR364191	KR364322	KR364451
Lactifluus		*phlebophyllus*	BB 00-1388	Madagascar	AY606974	DQ421979	DQ421918	None
Lactifluus		*pilosus*	LTH 205	Thailand	KR364006	KR364134	KR364323	KR364452
Lactifluus		*pinguis*	AV-RW 04-023/LTH117	Thailand	HQ318211	HG318111	HQ328858	JN389126
Lactifluus	aff.	*piperatus*	AV 05-295	USA	KF220048	KF220149	KF220235	None
Lactifluus	aff.	*piperatus*	AV13-018	Canada	To submit	None	None	None
Lactifluus	aff.	*piperatus*	JN 2011-036	Viet Nam	KF220105	KF220200	KF220274	KR364454
Lactifluus	aff.	*piperatus*	JN 2011-072	Viet Nam	KF220106	KF220201	KF220275	KR364455
Lactifluus	aff.	*piperatus*	TENN 064342	USA	KR364103	KR364234	KR364324	KR364456
Lactifluus	aff.	*piperatus*	S 09-063	India	To submit	To submit	To submit	None
Lactifluus	aff.	*piperatus*	HKAS 39333	China	To submit	None	None	None
Lactifluus		*piperatus*	2001 08 19 68	France	KF220119	KF241840	KF241842	KR364453
Lactifluus	aff.	*piperatus*	291-835	Japan	AB509984	None	None	None
Lactifluus	aff.	*piperatus*	KVP 08-009	India	To submit	To submit	To submit	To submit
Lactifluus	aff.	*piperatus*	LTH 51	Thailand	To submit	To submit	To submit	None
Lactifluus	aff.	*piperatus*	MSY13	China	KM069459	None	None	None
Lactifluus	aff.	*piperatus*	LTH 88	Thailand	KF220098	KF220193	KF220267	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	aff.	*piperatus*	LTH 293	Thailand	KF220101	KF220196	KF220270	None
Lactifluus	aff.	*piperatus*	LTH 322	Thailand	KF220078	None	None	None
Lactifluus	aff.	*piperatus*	LTH 377	Thailand	KF220057	None	None	None
Lactifluus	aff.	*piperatus*	LTH 378	Thailand	KF220102	KF220197	KF220271	None
Lactifluus	aff.	*piperatus*	AV-RW 04-072 = LTH 125	Thailand	KF220109	None	None	None
Lactifluus	aff.	*piperatus*	AV 05-393	USA	KF220050	KF220151	KF220237	None
Lactifluus	aff.	*piperatus*	S 09-008	India	KF220095	KF220190	KF220264	None
Lactifluus		*piperatus*	2001 08 19 39	France	KF220090	KF220185	KF220260	None
Lactifluus		sp.	UFRN-Fungos 2199	Brazil	To submit	None	None	None
Lactifluus		*pruinatus*	BB 3248	Zambia	KR364031	KR364158	KR364328	KR364458
Lactifluus		*pseudoluteopus*	FH 12-026	Thailand	KR364084	KR364214	KR364331	KR364460
Lactifluus		*pseudoluteopus*	JN 2011-008	Viet Nam	To submit	To submit	To submit	None
Lactifluus		*pseudoluteopus*	QCai29	China	KC154100	KC154126	KC154152	None
Lactifluus	cf.	*pseudovolemus*	ADK 2927	Benin	KR364113	KR364243	KR364330	KR364461
Lactifluus		*pulchrellus*	KW 304/FH 12-037	Thailand	KR364092	KR364223	KR364306	KR364434
Lactifluus	cf.	*pumilus*	EDC 12-066	Cameroon	KR364067	KR364196	KR364332	KR364462
Lactifluus	cf.	*pumilus*	AV 11-114	Tanzania	To submit	To submit	To submit	None
Lactifluus	cf.	*pumilus*	EDC 11-061	Tanzania	To submit	To submit	To submit	To submit
Lactifluus		*putidus*	LD15-004	Martinique	To submit	To submit	To submit	To submit
Lactifluus		*raspei* nom. prov.	EDC 14-517	Thailand	To submit	None	None	None
Lactifluus		*reticulatovenosus*	EH 6472	Indonesia	GU258286	GU265649	GU258389	None
Lactifluus		*robustus*	JPZhang119	China	KC154102	KC154128	KC154154	None
Lactifluus		*robustus*	XHWang3513	China	KC154104	KC154130	KC154156	None
Lactifluus		*roseolus*	AV 94-274	Burundi	KR364121	KR364242	None	None
Lactifluus		*roseolus*	AV 99-160	Zimbabwe	KR364032	KR364159	KR364333	KR364463
Lactifluus		*roseophyllus*	JN 2011-076	Viet Nam	KF220107	KF220202	KF220276	KR364464
Lactifluus		*rubiginosus*	JD 959	Democratic Republic of the Congo	KR364081	KR364210	KR364304	KR364432
Lactifluus		*rubiginosus*	MD389	Togo	HG917386	None	None	None
Lactifluus		*rubiginosus*	MD394	Togo	LN849750	None	None	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		*rubrobrunnescens*	EH 7194	Indonesia	KR364115	None	None	None
Lactifluus		*rubrobrunnescens*	KD 7004	India	To submit	None	None	None
Lactifluus	aff.	*rubroviolascens*	EDC 12-051	Cameroon	KR364066	KR364195	KR364334	KR364465
Lactifluus		*rubroviolascens*	JD 872	Democratic Republic of the Congo	To submit	To submit	To submit	To submit
Lactifluus		*rufomarginatus*	ADK 3358	Benin	KR364033	KR364160	KR364335	KR364466
Lactifluus		*rugatus*	EP 1212/7	Greece	KR364104	KR364235	KR364337	KR364467
Lactifluus		*ruvubuensis*	FN 05-562	Malawi	To submit	To submit	To submit	To submit
Lactifluus		*ruvubuensis*	JD 303	Gabon	KR364009	KR364137	KR364310	KR364438
Lactifluus		*sainii*	PUN 7046	India	KM658971	None	None	None
Lactifluus	aff.	*sepiaceus*	PL 34204	New Zealand	To submit	To submit	To submit	None
Lactifluus	aff.	*sepiaceus*	PL 10409	New Zealand	To submit	To submit	To submit	None
Lactifluus		*sepiaceus*	MEL 2218964	Australia	To submit	To submit	To submit	None
Lactifluus		*sesemotani*	AB77	Cameroon	KR819081	None	None	None
Lactifluus		*sesemotani*	AV 94-476	Burundi	KR364036	KR364163	KR364345	KR364476
Lactifluus		sp.	JLC 06031001	French Guiana	To submit	To submit	None	None
Lactifluus		sp.	4930	Malaysia	KP071178	None	None	None
Lactifluus		sp.	61916	Malaysia	KP071192	None	None	None
Lactifluus		sp.	A12 L2 - Slavomir	USA	KR364088	KR364218	KR364361	KR364491
Lactifluus		sp.	AB50	Cameroon	KR819054	None	None	None
Lactifluus		sp.	ACM 1024	Brazil	To submit	None	None	None
Lactifluus		sp.	ADK 3973bis	xxx_Africa	To submit	None	None	None
Lactifluus		sp.	AV 07-056	Cameroon	KR364008	KR364136	KR364293	KR364421
Lactifluus		sp.	AV 11-006	Tanzania	KR364052	KR364179	KR364288	KR364415
Lactifluus		sp.	AV 11-020	Tanzania	To submit	To submit	To submit	To submit
Lactifluus		sp.	AV 11-022	Tanzania	To submit	To submit	To submit	None
Lactifluus		sp.	AV 11-104	Tanzania	To submit	To submit	To submit	To submit
Lactifluus		sp.	AV 11-172	Togo	To submit	To submit	To submit	None
Lactifluus		sp.	AV 15-057	Laos PDR	To submit	None	None	None
Lactifluus		sp.	AV 15-107	Laos PDR	To submit	None	None	None
Lactifluus		sp.	AV 99-036	Zimbabwe	To submit	To submit	To submit	None
Lactifluus		sp.	AV13-015	Canada	To submit	None	None	None

Genus	**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	sp.	AVM 474	Colombia	To submit	None	None	None
Lactifluus	sp.	AVM-2003	Colombia	To submit	To submit	None	None
Lactifluus	sp.	AVM-2204	Colombia	To submit	None	None	None
Lactifluus	sp.	AVM-2209	Colombia	To submit	To submit	None	None
Lactifluus	sp.	C2163	Togo	LN849747	None	None	None
Lactifluus	sp.	CMMy30_M1	New Caledonia	To submit	None	None	None
Lactifluus	sp.	EDC 11-018	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 11-121	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 11-127	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 11-141	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 11-220	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 11-223	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 12-040	Cameroon	KR364063	KR364192	KR364289	KR364416
Lactifluus	sp.	EDC 12-068	Cameroon	KR364068	KR364197	KR364299	KR364427
Lactifluus	sp.	EDC 12-122	Cameroon	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 12-134	Cameroon	To submit	To submit	To submit	To submit
Lactifluus	sp.	EDC 12-195	Cameroon	KR364071	KR364200	KR364301	KR364429
Lactifluus	sp.	EDC 14-106	Zambia	To submit	None	None	None
Lactifluus	sp.	EDC 14-186	Zambia	To submit	None	None	None
Lactifluus	sp.	EDC 14-503	Thailand	KR364128	None	None	None
Lactifluus	sp.	EDC 14-508	Thailand	To submit	None	None	None
Lactifluus	*sp.*	G3185	French Guiana	KJ786694	KJ786603	KP691434	KR364399
Lactifluus	sp.	G3264	Guyane	To submit	To submit	None	None
Lactifluus	sp.	G4797	Guyane	To submit	None	None	None
Lactifluus	sp.	G4804	Guyane	To submit	None	None	None
Lactifluus	sp.	G5117	Guyane	To submit	To submit	None	None
Lactifluus	sp.	JD 907	Democratic Republic of the Congo	KR364076	KR364205	KR364302	KR364430
Lactifluus	sp.	JN 2011-010	Viet Nam	To submit	To submit	To submit	To submit
Lactifluus	sp.	JN 2011-012	Viet Nam	KR364045	KR364171	KR364294	KR364422
Lactifluus	sp.	JN 2011-035	Viet Nam	To submit	To submit	To submit	None
Lactifluus	sp.	JN 2011-071	Viet Nam	KR364043	KR364169	KR364255	KR364367

Genus	**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	sp.	JN 2011-077	Viet Nam	KR364044	KR364170	KR364256	KR364368
Lactifluus	sp.	JN 2011-079	Viet Nam	To submit	To submit	To submit	None
Lactifluus	sp.	JN 2011-080	Viet Nam	KR364048	KR364174	KR364359	KR364489
Lactifluus	sp.	JOH-468	xxx_SouthAmerica	To submit	To submit	To submit	None
Lactifluus	sp.	KW 392	Thailand	KR364091	KR364222	KR364305	KR364433
Lactifluus	sp.	LD15-066	Martinique	To submit	To submit	To submit	To submit
Lactifluus	sp.	LM-UNAH 0072	Honduras	HM639277	None	None	None
Lactifluus	sp.	LM-UNAH 0073	Honduras	HM639278	None	None	None
Lactifluus	sp.	LTH 240	Thailand	To submit	None	None	None
Lactifluus	sp.	MAN 696	Brazil	To submit	None	None	None
Lactifluus	sp.	MAN_BZL16	Brazil	To submit	None	None	None
Lactifluus	sp.	MAN_DLK900	Brazil	To submit	None	None	None
Lactifluus	sp.	MAN_DS769	Brazil	To submit	None	None	None
Lactifluus	sp.	MAN_MAN919	Brazil	To submit	None	None	None
Lactifluus	sp.	MD154	Togo	LK392607	None	None	None
Lactifluus	sp.	MD166	Burkina Faso	LN849748	None	None	None
Lactifluus	sp.	MD307	Benin	LN849741	None	None	None
Lactifluus	sp.	MD320	Benin	LN849742	None	None	None
Lactifluus	sp.	MD326	Benin	LM999911	None	None	None
Lactifluus	sp.	MD366	Togo	LN849746	None	None	None
Lactifluus	sp.	MEL_2383003	Australia	KP012857	None	None	None
Lactifluus	sp.	MEL_2383012	Australia	KP012864	None	None	None
Lactifluus	sp.	MR/Guy 13-145	Guyane	KJ786691	KJ786595	KP752180	KR364398
Lactifluus	sp.	MR-GUY-13-033	Guyane	To submit	To submit	None	None
Lactifluus	sp.	MR-GUY-13-038	Guyane	To submit	To submit	None	None
Lactifluus	sp.	MR-GUY-14-011	Guyane	To submit	To submit	None	None
Lactifluus	sp.	Nan MN15	Thailand	AB458892	None	None	None
Lactifluus	sp.	NC-5-6004	USA	AY456367	None	None	None
Lactifluus	sp.	NC-5-7289/1	USA	AY456368	None	None	None
Lactifluus	sp.	NC-5-8601	USA	AY456366	None	None	None
Lactifluus	sp.	PGK13-130	New Caledonia	KP691436	To submit	None	None
Lactifluus	sp.	RC/Guad 08-042	Guadeloupe	KP691414	KP691423	KP752179	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus		sp.	RC/Guy 09-004bis	French Guiana	KJ786643	KP691419	KP691427	None
Lactifluus		sp.	RC/Guy 09-036	Guyane	KJ786645	KJ786550	KP752178	None
Lactifluus		sp.	REH 9398	Australia	KR364097	KR364229	KR364307	KR364435
Lactifluus		sp.	TENN 065929	USA	KR364102	KR364233	KR364308	KR364436
Lactifluus		sp.	TH7880	Guyana	KT339212	None	None	None
Lactifluus		sp.	ZD 578	China	To submit	None	None	None
Lactifluus		sp.	ZD 815	China	To submit	None	None	None
Lactifluus		sp.	G6839	Guyane	To submit	To submit	None	None
Lactifluus		sp.	G6848	Guyane	To submit	To submit	None	None
Lactifluus		*subclarkeae*	REH 9231	Australia	KR364095	KR364227	KR364346	KR364477
Lactifluus		*subgerardii*	AV 05-269	USA	GU258263	GU265625	GU258362	KR364478
Lactifluus		*subiculatus*	MCA 4276	Guyana	To submit	None	None	None
Lactifluus		*subpiperatus*	HKAS 41909	China	To submit	None	None	None
Lactifluus		*subpiperatus*	LTH 204	Thailand	To submit	To submit	To submit	None
Lactifluus	aff.	*subpiperatus*	LTH 376	Thailand	KF220110	None	None	None
Lactifluus		*subpruinosus*	JN 2011-061	Viet Nam	KR364046	KR364172	KR364357	KR364487
Lactifluus		*subpruinosus*	QZhao282	China	KC154107	KC154133	KC154159	None
Lactifluus		*subvellereus*	ASM 12-075	USA	To submit	None	None	None
Lactifluus		*subvellereus*	AV 05-210	USA	KR364010	KR364138	KR364347	KR364479
Lactifluus		*subvellereus*	AV 05-226	USA	To submit	None	None	None
Lactifluus		*subvellereus*	TENN 065593	USA	To submit	To submit	To submit	None
Lactifluus		*subvellereus*	TENN 066157	USA	To submit	To submit	To submit	To submit
Lactifluus		*subvellereus* var. *subdistans*	ASM 10-383	USA	To submit	To submit	None	None
Lactifluus		*subvolemus*	KVP 08-048	Slovenia	JQ753927	JQ348379	KR364356	KR364486
Lactifluus		*subvolemus*	KVP R 12-007	Germany	To submit	To submit	To submit	To submit
Lactifluus		*subvolemus*	kvp08-50	Slovenia	To submit	To submit	To submit	None
Lactifluus		*sudanicus*	AV 11-174	Togo	HG426469	KR364186	KR364348	KR364480
Lactifluus		*sudanicus*	EDC 14-323	Cameroon	To submit	None	None	None
Lactifluus		*sudanicus*	MD148	Togo	HG426476	None	None	None
Lactifluus		*sulcatipes*	MCA 3937	Guyana	KR364109	KR364240	KR364350	None
Lactifluus		*tanzanicus/albocinctus*	EDC 11-011	Tanzania	To submit	To submit	To submit	To submit
Lactifluus	aff.	*tenuicystidiatus*	JN 2011-074	Viet Nam	KR364047	KR364173	KR364358	KR364488

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	aff.	*tenuicystidiatus*	JN 2011-075	Viet Nam	To submit	To submit	To submit	To submit
Lactifluus		*tenuicystidiatus*	XHWang3512	China	KC154118	KC154144	KC154170	None
Lactifluus		*tenuicystidiatus*	XTZhu477	China	KC154119	KC154145	KC154171	None
Lactifluus		*tenuicystidiatus*	YCLi1878	China	KC154120	KC154146	KC154172	None
Lactifluus		*uapacae*	AV 07-048	Cameroon	KR364007	KR364135	KR364352	KR364483
Lactifluus		*urens*	EDC 14-032	Zambia	KR364124	KR364247	KR364353	None
Lactifluus		*urens*	JD742	Democratic Republic of the Congo	To submit	None	None	None
Lactifluus		*vellereus*	ATHU-M 8077	Greece	KR364106	KR364237	KR364354	KR364484
Lactifluus	aff.	*vellereus*	Geen vouchernr	China	DQ011144	None	None	None
Lactifluus		*vellereus*	RW 1658	France	To submit	None	None	None
Lactifluus		*vellereus* var. *hometii*	FH 5231/4	Germany	KF220123	KF220216	KF220288	None
Lactifluus	cf.	*velutissimus*	AV 11-097	Tanzania	To submit	To submit	To submit	None
Lactifluus		*velutissimus*	FN 05-538	Malawi	To submit	To submit	None	None
Lactifluus		*velutissimus*	JD 886	Democratic Republic of the Congo	KR364075	KR364204	KR364355	KR364485
Lactifluus	aff.	*venezuelanus*	RC/Guy 12-007	French Guiana	To submit	To submit	None	None
Lactifluus		*venezuelanus*	RC/Guad 11-017	Guadeloupe	KP691411	KP691420	KP691429	KR364393
Lactifluus		sp.	UFRN-Fungos 2197	Brazil	To submit	None	None	None
Lactifluus		*veraecrucis*	M 8025	Mexico	KR364112	KR364241	None	None
Lactifluus		*versiformis*	AV-KD-KVP 09-006	India	JN388965	JN389033	JN375633	JN389179
Lactifluus		*vitellinus*	KVP 08-024	Thailand	HQ318236	HQ318144	HQ328881	JN389138
Lactifluus		*volemoides*	MH 201187	Mozambique	KR364098	KR364230	KR364363	KR364493
Lactifluus		*volemus*	BB 2699	Germany	HQ318219	HQ318119	JQ348134	None
Lactifluus		*volemus*	IK 83568	Sweden	JQ753900	JQ348350	JQ348212	None
Lactifluus		*volemus*	KVP 11-002	Belgium	JQ753948	KR364175	KR364360	KR364490
Lactifluus	aff.	*volemus*	287-46	Japan	AB509502	None	None	None
Lactifluus	aff.	*volemus* s.l.	AV 04-165	USA	To submit	To submit	JQ348139	None
Lactifluus	aff.	*volemus* s.l.	AV 04-166	USA	JQ753829	None	None	None
Lactifluus	aff.	*volemus*	AV 04-194	USA	To submit	To submit	To submit	None
Lactifluus	aff.	*volemus*	AV 05-227	USA	JQ753832	JQ348284	JQ348150	None
Lactifluus	aff.	*volemus*	AV 05-293	USA	JQ753834	JQ348287	JQ348153	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	aff.	*volemus*	AV 05-294	USA	To submit	JQ348286	JQ348152	None
Lactifluus	aff.	*volemus*	AV 05-298	USA	To submit	To submit	To submit	None
Lactifluus	aff.	*volemus*	AV 05-384	USA	JQ753826	HQ318127	JQ348136	None
Lactifluus	aff.	*volemus*	AV 15-055	Laos PDR	To submit	None	None	None
Lactifluus	aff.	*volemus*	CUB_Microbiology M4	Thailand	AB458687	None	None	None
Lactifluus	aff.	*volemus*	DS 07-465	Thailand	To submit	To submit	To submit	None
Lactifluus	aff.	*volemus*	HKAS 39022	China	To submit	None	To submit	None
Lactifluus	aff.	*volemus*	HKAS 44012	China	To submit	None	None	None
Lactifluus	aff.	*volemus*	KIINA158	China	To submit	To submit	None	None
Lactifluus	aff.	*volemus*	KVP 08-008	Thailand	HQ318231	HQ318138	HQ328875	None
Lactifluus	aff.	*volemus*	KVP 08-031	Thailand	HQ318240	HQ318148	HQ328885	JN389142
Lactifluus	aff.	*volemus*	LTH 247	Thailand	HQ318261	HQ318175	HQ328911	None
Lactifluus	aff.	*volemus*	OSA-My-3995	Japan	To submit	AB238647	To submit	None
Lactifluus	aff.	*volemus*	OSA-My-4001	Japan	To submit	AB238653	To submit	None
Lactifluus	aff.	*volemus*	OSA-My-4004	Japan	To submit	AB238656	To submit	None
Lactifluus	aff.	*volemus*	OSA-My-4007	Japan	To submit	AB238659	To submit	None
Lactifluus	aff.	*volemus*	OSA-My-4009	Japan	To submit	AB238661	To submit	None
Lactifluus	aff.	*volemus*	OSA-My-4010	Japan	To submit	AB238662	To submit	None
Lactifluus	aff.	*volemus*	PKSR5	India	KF293401	None	KJ411968	KJ411959
Lactifluus	aff.	*volemus*	REH 9320	Australia	KR364096	KR364228	KR364362	KR364492
Lactifluus	aff.	*volemus*	RH 9665	Australia	To submit	None	None	None
Lactifluus	aff.	*volemus*	TFB11981	USA	JQ358925	JN940232	JN985475	None
Lactifluus	aff.	*volemus*	TFB12115	USA	JQ358926	JN940230	JN985477	None
Lactifluus	aff.	*volemus*	TFB12263	USA	JQ358927	JN940229	JN985460	None
Lactifluus	aff.	*volemus*	TMI 26125	Japan	To submit	None	To submit	None
Lactifluus	aff.	*volemus*	TMI 26126	Japan	To submit	To submit	To submit	None
Lactifluus	aff.	*volemus*	TMI 26128	Japan	To submit	None	To submit	None
Lactifluus	aff.	*volemus* sp. 1	LTH 359	Thailand	HQ318255	HQ318168	HQ328904	None
Lactifluus	aff.	*volemus* sp. 12	LTH 251	Thailand	HQ318262	HQ318177	HQ328913	None
Lactifluus	aff.	*volemus* sp. 14	KVP 08-006	Thailand	HQ318136	HQ318229	HQ328873	None
Lactifluus	aff.	*volemus* sp. 16	LTH 275	Thailand	HQ318275	HQ318194	HQ328924	None

Genus		**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Lactifluus	aff.	*volemus* sp. 17	LTH 214	Thailand	HQ318249	HQ318158	HQ328894	None
Lactifluus	aff.	*volemus* sp. 21	AV-KD-KVP 09-137	India	JN388958	JN389027	JN375629	JN389173
Lactifluus	aff.	*volemus* sp. 22	AV-KD-KVP 09-129	India	JN388957	JN389021	JN375623	JN389167
Lactifluus	aff.	*volemus* sp. 24	AV-KD-KVP 09-123	India	JN388980	JN389015	JN375617	JN389161
Lactifluus	aff.	*volemus* sp. 5	LTH 313	Thailand	HQ318272	HQ318190	None	None
Lactifluus	aff.	*volemus* sp. 6	LTH 294	Thailand	HQ318273	HQ318191	HQ328923	None
Lactifluus	aff.	*volemus* sp. 4	FH 12-059	Thailand	To submit	To submit	To submit	To submit
Lactifluus		*volemus* var. *flavus*	AV13-023	Canada	To submit	None	None	None
Lactifluus	aff.	*wirrabara*	GG 24-01-04	Australia	GU258307	GU265667	GU258407	KR364494
Lactifluus	aff.	*wirrabara*	PL 40509	New Zealand	GU258287	GU265650	GU258390	KR364475
Lactifluus		*xerampelinus*	MH 201176	Mozambique	KR364099	KR364231	KR364364	KR364496
Lactifluus	cf.	*zenkeri*	AV 11-050	Tanzania	KR364055	KR364182	KR364297	KR364425
Lactarius		*fuliginosus*	MTB 97-24	Sweden	JQ446111	JQ446180	JQ446240	KR364392
Lactarius		*hatsudake*	FH 12-052	Thailand	KR364085	KR364215	KR364285	KR364411
Lactarius		*miniatescens*	AV 11-177	Togo	KR364059	KR364187	KR364315	KR364443
Lactarius		*olympianus*	ED 08-018	USA	KR364089	KR364220	KR364320	KR364448
Lactarius		*scrobiculatus*	JN 2001-058	Slovakia	KF432968	KR364219	KR364344	KR364474
Lactarius		*tenellus*	ADK 3598	Benin	KF133280	KF133313	KF133345	KR364482
Multifurca		*furcata*	RH 7804	Costa Rica	DQ421995	DQ421995	DQ421928	None
Multifurca		*ochricompacta*	BB 02-107	USA	DQ421984	DQ421984	DQ421940	None
Multifurca		sp.	xp2-20120922-01	China	KR364125	None	None	None
Multifurca		*stenophylla*	JET956	Australia	JX266631	JX266635	None	None
Multifurca		*zonaria*	FH 12-009	Thailand	KR364083	KR364212	KR364365	KR364497
Russula		*cyanoxantha*	FH 12-201	Germany	KR364093	KR364225	KR364341	KR364471
Russula		*delica*	FH 12-272	Belgium	KF432955	KR364224	KR364340	KR364470
Russula		*gracillima*	FH 12-264	Germany	KR364094	KR364226	KR364342	KR364472
Russula		*khanchanjungae*	AV-KD-KVP 09-106	India	KR364129	JN389004	JN375607	JN389092
Russula		sp.	EDC 12-061	Cameroon	KR364072	KR364201	KR364338	KR364468
Russula		sp.	EDC 12-063	Cameroon	KR364073	KR364202	KR364339	KR364469

Genus	**Species epithet**	**Herbarium no.**	**Country**	**ITS**	**LSU**	***RPB2***	***RPB1***
Amylostereum	*laevigatum*	CBS 623.84	France	AY781246	AF287843	AY218469	None
Auriscalpium	*vulgare*	PBM 944	USA	DQ911613	DQ911614	AY218472	None
Bondarzewia	*montana*	AFTOL 452	No data	DQ200923	DQ234539	AY218474	DQ256049
Echinodontium	*tinctorium*	AFTOL 455	No data	AY854088	AF393056	AY218482	AY864882
Heterobasidion	*annosum*	AFTOL 470	No data	DQ206988	None	AY544206	DQ667160
Stereum	*hirsutum*	AFTOL 492	No data	AY854063	AF393078	AY218520	AY864885
Vararia	*abortiphysa*	CBS 630.81	France	KR364005	KR364133	KR364266	None

S3 – Full version of Fig. 3.2

Available at the end of this thesis.

S4 – The genus *Lactifluus*: described species

Table S4 List of described *Lactifluus* species, together with the current authors, the original publication, year of publication and biogeographical region of origin. Western Palearctic includes for Europe and the Western part of Russia, Asia includes Southeast Asia, China, Japan, South Korea, The Eastern part of Russia and Iran.

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
1	*Lactifluus*	*acicularis*	(Van de Putte & Verbeken) Van de Putte	Van de Putte et al. (2010)	2010	Asia
2	*Lactifluus*	*acrissimus*	(Verbeken & Van Rooij) Nuytinck	Van Rooij et al. (2003)	2003	Afrotropics
3	*Lactifluus*	*albocinctus*	(Verbeken) Verbeken	Verbeken et al. (2000)	2000	Afrotropics
4	*Lactifluus*	*albomembranaceus*	De Wilde & Van de Putte	De Crop et al. (Subm.)	Subm.	Afrotropics
5	*Lactifluus*	*allardii*	(Coker) De Crop	Coker (1918)	1918	Nearctic
6	*Lactifluus*	*amazonensis*	Singer – not combined in Lactifluus yet	Singer et al. (1983)	1983	Neotropics
7	*Lactifluus*	*ambicystidiatus*	X.H. Wang	Wang et al. (2015)	2015	Asia
8	*Lactifluus*	*angustifolius*	Hesler & A.H. Sm. – not combined in *Lactifluus* yet	Hesler and Smith (1979)	1979	Nearctic
9	*Lactifluus*	*angustus*	(R. Heim & Gooss.-Font.) Verbeken	Heim (1955)	1955	Afrotropics
10	*Lactifluus*	*annulatoangustifolius*	(Beeli) Buyck	Beeli (1936)	1936	Afrotropics
11	*Lactifluus*	*annulatolongisporus*	Maba	Maba et al. (2015a)	2015	Afrotropics
12	*Lactifluus*	*annulifer*	(Singer) Nuytinck	Singer et al. (1983)	1983	Neotropics
13	*Lactifluus*	*armeniacus*	De Crop & Verbeken	Li et al. (2016)	2016	Asia
14	*Lactifluus*	*arsenei*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
15	*Lactifluus*	*atrovelutinus*	(J.Z. Ying) X.H. Wang	Ying (1991)	1991	Asia
16	*Lactifluus*	*aurantiifolius*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
17	*Lactifluus*	*aurantiorugosus*	Sá & Wartchow	Sá and Wartchow (2013)	2013	Neotropics
18	*Lactifluus*	*aureifolius*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
19	*Lactifluus*	*auriculiformis*	Verbeken & Hampe	De Crop et al. (In prep)	In prep.	Asia
20	*Lactifluus*	*austrovolemus*	(Hongo) Verbeken	Hongo (1973)	1973	Australasia
21	*Lactifluus*	*batistae*	Wartchow, J.L. Bezerra & M. Cavalc.	Wartchow et al. (2013)	2013	Neotropics
22	*Lactifluus*	*bertillonii*	(Neuhoff ex Z. Schaef.) Verbeken	Schaefer (1979)	1979	Western Palearctic
23	*Lactifluus*	*bhandaryi*	Verbeken & De Crop	De Crop et al. (In prep)	In prep.	Asia

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
24	*Lactifluus*	*bicapillus*	Lescroart & De Crop	In prep.	In prep.	Afrotropics
25	*Lactifluus*	*bicolor*	(Massee) Verbeken	Massee (1914)	1914	Asia
26	*Lactifluus*	*brachystegiae*	(Verbeken & C. Sharp) Verbeken	Verbeken et al. (2000)	2000	Afrotropics
27	*Lactifluus*	*brasiliensis*	Singer – not combined in *Lactifluus* yet	Singer et al. (1983)	1983	Neotropics
28	*Lactifluus*	*brunellus*	(S.L. Mill., Aime & T.W. Henkel) De Crop	Miller et al. (2002)	2002	Neotropics
29	*Lactifluus*	*brunneocarpus*	Maba	Maba et al. (2015a)	2015	Afrotropics
30	*Lactifluus*	*brunneoviolascens*	(Bon) Verbeken	Bon (1971)	1971	Western Palearctic
31	*Lactifluus*	*brunnescens*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
32	*Lactifluus*	*burkinabei*	Maba	Maba et al. (2015a)	2015	Afrotropics
33	*Lactifluus*	*caperatus*	(R. Heim & Gooss.-Font.) Verbeken	Heim (1955)	1955	Afrotropics
34	*Lactifluus*	*caribaeus*	(Pegler) Verbeken	Pegler and Fiard (1979)	1979	Neotropics
35	*Lactifluus*	*carmineus*	(Verbeken & Walleyn) Verbeken	Verbeken et al. (2000)	2000	Afrotropics
36	*Lactifluus*	*castaneibadius*	(Pegler) De Crop	Pegler and Fiard (1979)	1979	Neotropics
37	*Lactifluus*	*chamaeleontinus*	(R. Heim) Verbeken	Heim (1955)	1955	Afrotropics
38	*Lactifluus*	*chiapanensis*	(Montoya, Bandala-Muñoz & Guzmán) De Crop	Montoya et al. (1996)	1996	Neotropics
39	*Lactifluus*	*chrysocarpus*	E. S. Popov et O. V. Morozova	Morozova et al. (2013)	2013	Asia
40	*Lactifluus*	*claricolor*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
41	*Lactifluus*	*clarkeae*	(Cleland) Verbeken	Cleland (1927)	1927	Australasia
42	*Lactifluus*	*coccolobae*	O.K. Mill. & Lodge – not combined in *Lactifluus* yet	Miller et al. (2000)	2000	Neotropics
43	*Lactifluus*	*cocosmus*	(Van de Putte & De Kesel) Van de Putte	Van de Putte et al. (2009)	2009	Afrotropics
44	*Lactifluus*	*conchatulus*	(Stubbe & H.T. Le) Stubbe	Stubbe et al. (2012)	2012	Asia
45	*Lactifluus*	*coniculus*	Stubbe & Verbeken	Stubbe et al. (2012)	2012	Asia
46	*Lactifluus*	*corbula*	(R. Heim & Gooss.-Font.) Verbeken	Heim (1955)	1955	Afrotropics
47	*Lactifluus*	*corrugis*	(Peck) Kuntze	Peck (1879)	1879	Nearctic
48	*Lactifluus*	*crocatus*	Van de Putte & Verbeken	Van de Putte et al. (2010)	2010	Asia
49	*Lactifluus*	*cyanovirescens*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
50	*Lactifluus*	*deceptivus N.Am.*	(Peck) Kuntze	Peck (1885)	1885	Nearctic
51	*Lactifluus*	*denigricans*	(Verbeken & Karhula) Verbeken	Verbeken (1996b)	1996	Afrotropics
52	*Lactifluus*	*densifolius*	(Verbeken & Karhula) Verbeken	Verbeken (1996a)	1996	Afrotropics
53	*Lactifluus*	*dinghuensis*	Jianbin	Zhang et al. (2016)	2016	Asia
54	*Lactifluus*	*dissitus*	Van de Putte, K. Das & Verbeken	Van de Putte et al. (2012)	2012	Asia
55	*Lactifluus*	*distans*	(Peck) Kuntze	Peck (1873)	1873	Nearctic
56	*Lactifluus*	*distantifolius*	Van de Putte, Stubbe & Verbeken	Van de Putte et al. (2010)	2010	Asia
57	*Lactifluus*	*dunensis*	Sá & Wartchow	Sá et al. (2013)	2013	Neotropics
58	*Lactifluus*	*dwaliensis*	(K. Das, J.R. Sharma & Verbeken) K. Das	Das et al. (2003)	2003	Asia

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
59	*Lactifluus*	*edulis*	(Verbeken & Buyck) Buyck	Buyck (1994)	1994	Afrotropics
60	*Lactifluus*	*emergens*	(Verbeken) Verbeken	Verbeken et al. (2000)	2000	Afrotropics
61	*Lactifluus*	*fazaoensis*	Maba, Yorou & Guelly	Maba et al. (2014)	2014	Afrotropics
62	*Lactifluus*	*flammans*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
63	*Lactifluus*	*flavellus*	Maba & Guelly	Maba et al. (2015b)	2015	Afrotropics
64	*Lactifluus*	*flocktonae*	(Cleland & Cheel) Lebel	Cleland and Cheel (1919)	1919	Australasia
65	*Lactifluus*	*foetens*	(Verbeken) Verbeken	Van Rooij et al. (2003)	2003	Afrotropics
66	*Lactifluus*	*fuscomarginatus*	Montoya, Bandala & Haug – not combined in *Lactifluus* yet	Montoya et al 2012	2012	Neotropics
67	*Lactifluus*	*genevievae*	(Stubbe & Verbeken) Stubbe	Stubbe et al. (2012)	2012	Australasia
68	*Lactifluus*	*gerardiellus*	Wisitrassameewong & Verbeken	De Crop et al. (In prep)	In prep.	Asia
69	*Lactifluus*	*gerardii s.s.*	(Peck) Kuntze	Peck (1874)	1874	Nearctic
70	*Lactifluus*	*glaucescens s.s.*	(Crossl.) Verbeken	Crossland (1900)	1900	Western Palearctic
71	*Lactifluus*	*goossensiae*	(Beeli) Verbeken	Beeli (1928)	1928	Afrotropics
72	*Lactifluus*	*guellii*	Maba	Maba et al. (2015a)	2015	Afrotropics
73	*Lactifluus*	*gymnocarpoides*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
74	*Lactifluus*	*gymnocarpus*	(R. Heim ex Singer) Verbeken	Singer (1948)	1948	Afrotropics
75	*Lactifluus*	*heimii*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
76	*Lactifluus*	*hora*	Stubbe & Verbeken	Stubbe et al. (2012)	2012	Asia
77	*Lactifluus*	*hygrophoroides N.Am.*	(Berk. & M.A. Curtis) Kuntze	Berkeley and Curtis (1859)	1859	Nearctic
78	*Lactifluus*	*igniculus*	O. V. Morozova et E. S. Popov	Morozova et al. (2013)	2013	Asia
79	*Lactifluus*	*indicus*	K. N. A. Raj & Manim.	Latha et al. (2016)	2016	Asia
80	*Lactifluus*	*indusiatus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
81	*Lactifluus*	*inversus*	(Gooss.-Font. & R. Heim) Verbeken	Heim (1955)	1955	Afrotropics
82	*Lactifluus*	*kigomaensis*	De Crop & Verbeken	De Crop et al. (2012)	2012	Afrotropics
83	*Lactifluus*	*kivuensis*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
84	*Lactifluus*	*laevigatus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
85	*Lactifluus*	*lamprocystidiatus*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (2000)	2000	Australasia
86	*Lactifluus*	*latifolius*	(Gooss.-Font. & R. Heim) Verbeken	Heim (1955)	1955	Afrotropics
87	*Lactifluus*	*leae*	Stubbe & Verbeken	Stubbe et al. (2012)	2012	Asia
88	*Lactifluus*	*leonardii*	Stubbe & Verbeken	Stubbe et al. (2012)	2012	Australasia
89	*Lactifluus*	*leoninus*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (1999)	1999	Australasia
90	*Lactifluus*	*leptomerus*	Van de Putte, K. Das & Verbeken	Van de Putte et al. (2012)	2012	Asia
91	*Lactifluus*	*leucophaeus*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (1999)	1999	Australasia
92	*Lactifluus*	*limbatus*	Stubbe & Verbeken	Stubbe et al. (2012)	2012	Asia
93	*Lactifluus*	*longibasidius*	Maba & Verbeken	Maba et al. (2015b)	2015	Afrotropics

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
94	*Lactifluus*	*longipes*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
95	*Lactifluus*	*longipilus*	Van de Putte, Le & Verbeken	Van de Putte et al. (2010)	2010	Asia
96	*Lactifluus*	*longisporus*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
97	*Lactifluus*	*longivelutinus*	(X.H. Wang & Verbeken) X.H. Wang	Wang and Verbeken (2006)	2006	Asia
98	*Lactifluus*	*luteolus N. Am.*	(Peck) Verbeken	Peck (1896)	1896	Nearctic
99	*Lactifluus*	*luteopus*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
100	*Lactifluus*	*madagascariensis*	(Verbeken & Buyck) Buyck	Buyck et al. (2007)	2007	Afrotropics
101	*Lactifluus*	*medusae*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
102	*Lactifluus*	*melleus*	Maba	Maba et al. (2015b)	2015	Afrotropics
103	*Lactifluus*	*membranaceus*	Maba	Maba et al. (2015a)	2015	Afrotropics
104	*Lactifluus*	*multiceps*	(S.L. Miller, Aime & TW Henkel) De Crop	Miller et al. (2002)	2002	Neotropics
105	*Lactifluus*	*murinipes*	(Pegler) De Crop	Pegler and Fiard (1979)	1979	Neotropics
106	*Lactifluus*	*nebulosus*	(Pegler) De Crop	Pegler and Fiard (1979)	1979	Neotropics
107	*Lactifluus*	*neotropicus*	(Singer) Nuytinck	Singer (1952)	1952	Neotropics
108	*Lactifluus*	*neuhoffii*	Hesler & A.H. Sm. – not combined in *Lactifluus* yet	Hesler and Smith (1979)	1979	Nearctic
109	*Lactifluus*	*nodosicystidiosus*	(Verbeken & Buyck) Buyck	Buyck et al. (2007)	2007	Afrotropics
110	*Lactifluus*	*nonpiscis*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
111	*Lactifluus*	*novoguineensis*	(Henn.) Verbeken	Hennings (1898)	1898	Australasia
112	*Lactifluus*	*ochrogalactus*	(Hashiya) X.H. Wang	Wang et al. (2006)	2006	Asia
113	*Lactifluus*	*oedematopus*	(Scop.) Kuntze	Scopoli (1772)	1772	Western Palearctic
114	*Lactifluus*	*olivescens*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (2000)	2000	Australasia
115	*Lactifluus*	*paleus*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (1999)	1999	Australasia
116	*Lactifluus*	*pallidilamellatus*	(Montoya & Bandala) Van de Putte	Montoya and Bandala (2004)	2004	Neotropics
117	*Lactifluus*	*panuoides*	(Singer) De Crop	Singer (1952)	1952	Neotropics
118	*Lactifluus*	*parvigerardii*	X.H. Wang & D. Stubbe	Wang et al. (2012)	2012	Asia
119	*Lactifluus*	*pectinatus*	Maba & Yorou	Maba et al. (2015b)	2015	Afrotropics
120	*Lactifluus*	*pegleri*	Pacioni & Lalli – not combined in *Lactifluus* yet	Lalli and Pacioni (1992)	1992	Neotropics
121	*Lactifluus*	*pelliculatus*	(Beeli) Buyck	Buyck (1989)	1989	Afrotropics
122	*Lactifluus*	*persicinus*	Delgat & De Crop	Delgat et al. (In prep.)	In prep	Afrotropics
123	*Lactifluus*	*petersenii*	(Hesler & A.H. Sm.) Stubbe	Hesler and Smith (1979)	1979	Nearctic
124	*Lactifluus*	*phlebonemus*	(R. Heim & Gooss.-Font.) Verbeken	Heim (1955)	1955	Afrotropics
125	*Lactifluus*	*phlebophyllus*	(R. Heim) Buyck	Heim (1938)	1938	Afrotropics
126	*Lactifluus*	*pilosus*	(Verbeken, H.T. Le & Lumyong) Verbeken	Le et al. (2007)	2007	Asia
127	*Lactifluus*	*pinguis*	Van de Putte & Verbeken	Van de Putte et al. (2010)	2010	Asia
128	*Lactifluus*	*piperatus s.s.*	(L.: Fr.) Kuntze	Linnaeus (1753)	1753	Western Palearctic

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
129	*Lactifluus*	*pisciodorus*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
130	*Lactifluus*	*princeps*	(Berk.) Kuntze	Berkeley (1852)	1852	Asia
131	*Lactifluus*	*pruinatus*	(Verbeken & Buyck) Verbeken	Verbeken (1998)	1998	Afrotropics
132	*Lactifluus*	*pseudogymnocarpus*	(Verbeken) Verbeken	Verbeken (1995)	1995	Afrotropics
133	*Lactifluus*	*pseudoluteopus*	(X.H. Wang & Verbeken) X.H. Wang	Wang and Verbeken (2006)	2006	Asia
134	*Lactifluus*	*pseudotorminosus*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
135	*Lactifluus*	*pseudovolemus*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
136	*Lactifluus*	*puberulus*	(H.A. Wen & J.Z. Ying) Nuytinck	Wen and Ying (2005)	2005	Asia
137	*Lactifluus*	*pulchrellus*	Hampe & Wisitrassameewong	De Crop et al. (In prep)	In prep.	Asia
138	*Lactifluus*	*pumilus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
139	*Lactifluus*	*putidus*	(Pegler) Verbeken	Pegler and Fiard (1979)	1979	Neotropics
140	*Lactifluus*	*ramipilosus*	Verbeken & De Crop	Li et al. (2016)	2016	Asia
141	*Lactifluus*	*raspei*	Verbeken & De Crop	De Crop et al. (In prep)	In prep.	Asia
142	*Lactifluus*	*reticulatovenosus*	(Verbeken & E. Horak) Verbeken	Verbeken et al. (2001)	2001	Asia
143	*Lactifluus*	*roseolus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
144	*Lactifluus*	*roseophyllus*	(R. Heim) De Crop	Heim (1966)	1966	Asia
145	*Lactifluus*	*rubiginosus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
146	*Lactifluus*	*rubrobrunnescens*	(Verbeken, E. Horak & Desjardin) Verbeken	Verbeken et al. (2001)	2001	Asia
147	*Lactifluus*	*rubroviolascens*	(R. Heim) Verbeken	Heim (1938)	1938	Afrotropics
148	*Lactifluus*	*rufomarginatus*	(Verbeken & Van Rooij) De Crop	Van Rooij et al. (2003)	2003	Afrotropics
149	*Lactifluus*	*rugatus*	(Kühner & Romagn.) Verbeken	Kühner and Romagnesi (1953)	1953	Western Palearctic
150	*Lactifluus*	*ruvubuensis*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
151	*Lactifluus*	*sepiaceus*	(McNabb) Stubbe	McNabb (1971)	1971	Australasia
152	*Lactifluus*	*sesemotani*	(Beeli) Buyck	Buyck (1989)	1989	Afrotropics
153	*Lactifluus*	*subclarkeae*	(Grgur.) Verbeken	Grgurinovic (1997)	1997	Australasia
154	*Lactifluus*	*subgerardii*	(Hesler & A.H. Sm.) Stubbe	Hesler and Smith (1979)	1979	Nearctic
155	*Lactifluus*	*subiculatus*	S.L. Mill., Aime & T.W. Henkel	Miller et al. 2012	2012	Neotropics
156	*Lactifluus*	*subpallidipes*	Singer – not combined in *Lactifluus* yet	Singer et al. (1983)	1983	Neotropics
157	*Lactifluus*	*subpiperatus*	(Hongo) Verbeken	Hongo (1964)	1964	Asia
158	*Lactifluus*	*subpruinosus*	X.H. Wang	Wang et al. (2015)	2015	Asia
159	*Lactifluus*	*subreticulatus*	Singer – not combined in *Lactifluus* yet	Singer et al. (1983)	1983	Neotropics
160	*Lactifluus*	*subtomentosus*	(Berk. & Ravenel) Kuntze	Berkeley and Curtis (1859)	1859	Nearctic
161	*Lactifluus*	*subvellereus*	(Peck) Nuytinck	Peck (1898)	1898	Nearctic
162	*Lactifluus*	*subvolemus*	Van de Putte & Verbeken	Van de Putte et al. (2016)	2016	Western Palearctic
163	*Lactifluus*	*sudanicus*	Maba, Yorou & Guelly	Maba et al. (2014)	2014	Afrotropics

	Genus	Species	Current authors	Original publication	Year of description	Biogeographical region
164	*Lactifluus*	*tanzanicus*	(Karhula & Verbeken) Verbeken	Karhula et al. (1998)	1998	Afrotropics
165	*Lactifluus*	*tenuicystidiatus*	(X.H. Wang & Verbeken) X.H. Wang	Wang and Verbeken (2006)	2006	Asia
166	*Lactifluus*	*tropicosinosus*	X.H. Wang	Wang et al. (2015)	2015	Asia
167	*Lactifluus*	*uapacae*	(Verbeken & Stubbe) De Crop	Verbeken et al. (2008)	2008	Afrotropics
168	*Lactifluus*	*umbonatus*	K. P. D. Latha & Manim.	Latha et al. (2016)	2016	Asia
169	*Lactifluus*	*urens*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
170	*Lactifluus*	*uyedae*	(Singer) Verbeken	Singer (1984)	1984	Asia
171	*Lactifluus*	*vellereus*	(Fr.) Kuntze	Fries (1838)	1838	Western Palearctic
172	*Lactifluus*	*velutissimus*	(Verbeken) Verbeken	Verbeken (1996a)	1996	Afrotropics
173	*Lactifluus*	*venezuelanus*	(Dennis) De Crop	(Dennis 1970)	1970	Neotropics
174	*Lactifluus*	*venosus*	(Verbeken & E. Horak) Verbeken	Verbeken and Horak (2000)	2000	Australasia
175	*Lactifluus*	*veraecrucis*	(Singer) Verbeken	Singer (1973)	1973	Neotropics
176	*Lactifluus*	*versiformis*	Van de Putte, K. Das & Verbeken	Van de Putte et al. (2012)	2012	Asia
177	*Lactifluus*	*vitellinus*	Van de Putte & Verbeken	Van de Putte et al. (2010)	2010	Asia
178	*Lactifluus*	*volemoides*	(Karhula) Verbeken	Karhula et al. (1998)	1998	Afrotropics
179	*Lactifluus*	*volemus s.s.*	(Fr.: Fr.) Kuntze	Fries (1838)	1838	Western Palearctic
180	*Lactifluus*	*waltersii*	Hesler & A.H. Sm. – not combined in *Lactifluus* yet	Hesler and Smith (1979)	1979	Nearctic
181	*Lactifluus*	*wirrabara*	(Grgur.) Stubbe	Grgurinovic (1997)	1997	Australasia
182	*Lactifluus*	*xerampelinus*	(Karhula & Verbeken) Verbeken	Karhula et al. (1998)	1998	Afrotropics
183	*Lactifluus*	*zenkeri*	(Henn.) Verbeken	Singer (1942)	1942	Afrotropics

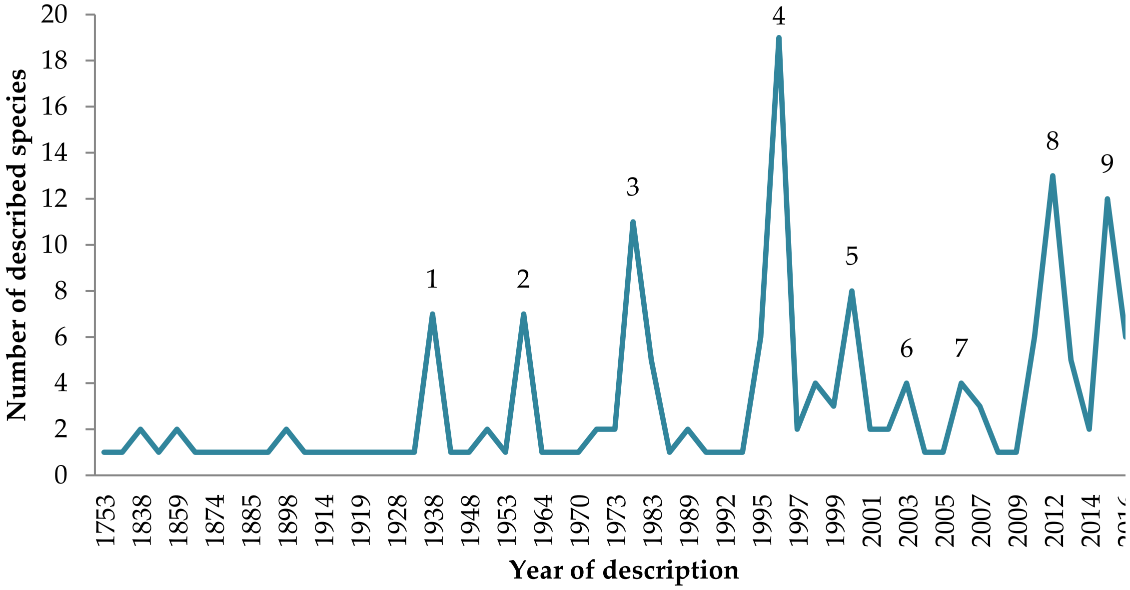


Fig. S4.1 Publication history of species within the genus *Lactifluus*. 1. Heim (1938), 2. Heim (1955), 3. Hesler & Smith (1979), 4. Verbeken et al. (1996), 5–7. various authors, 8. Stubbe et al. (2012) & Van de Putte et al. (2012), 9. Maba et al. (2015) & Wang et al. (2015).

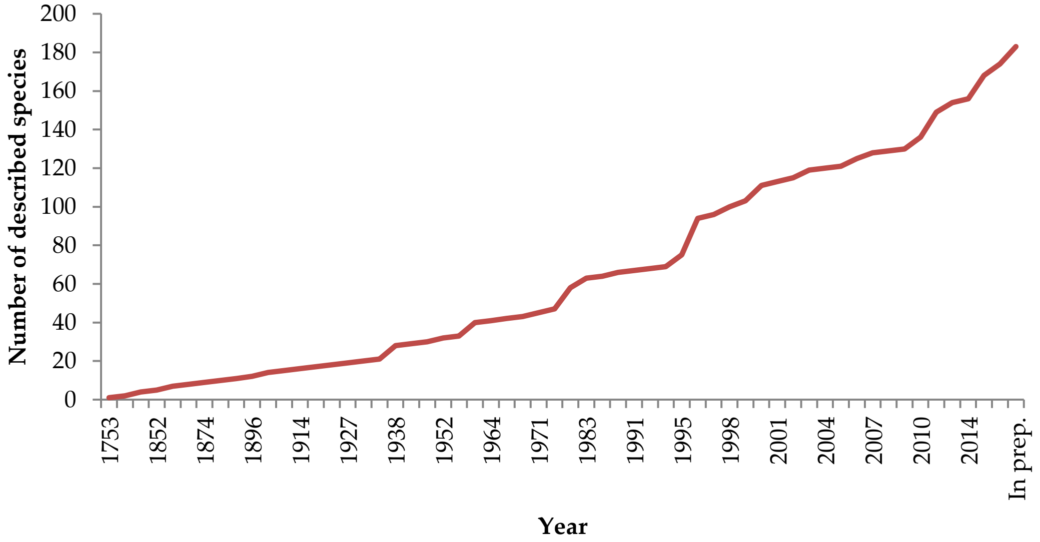


Fig. S4.2 Accumulative curve of described species within the genus *Lactifluus*.

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Wang XH, Buyck B, Verbeken A (2015) Revisiting the morphology and phylogeny of *Lactifluus* with three new lineages from southern China. Mycologia 107 (5):941–958

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Wen HA, Ying JZ (2005) Studies on the genus *Lactarius* from China II. Two new taxa from Guizhou. Mycosystema 24 (2):155–158

Ying YZ (1991) Studies on the genus *Lactarius* S.F. Gray from China I. New taxa of *Lactarius*. Acta Mycologica Sinica 10:190–199

Zhang JB, Huang HW, Qiu LH (2016) *Lactifluus dinghuensis* sp nov from southern China. Nova Hedwigia 102 (1-2):233-240. doi:10.1127/nova_hedwigia/2015/0305

Curriculum vitae

PERSONALIA

Name	Eske De Crop
Date and place of birth	4 February 1986, Gent
Nationality	Belgian
Address	Driesstraat 41, 9050 Ledeberg, Belgium
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EDUCATION

2013-2016	BOF research project, Ghent University, Research Group Mycology 'Molecular phylogenetic biodiversity assessment of tropical African ectomycorrhizal fungi, with an emphasis on Russulaceae.' *Supervisor*: Prof. Dr. Annemieke Verbeken – *Co-supervisor*: Dr. Jorinde Nuytinck *External researcher:* Dr. József Geml
2010-2012	BOF research project, Ghent University, Research Group Mycology 'Contrasting evolutionary patterns in two sister genera of macrofungi: *Lactarius* and *Lactifluus*.' *Supervisor*: Prof. Dr. Annemieke Verbeken – *Co-supervisor*: Dr. Jorinde Nuytinck
2008-2010	Master of biology, Ghent University (great distinction) Major Biodiversity & Ecology. Internship at the Research Institute for Nature and Forest: 'Research on forest reserves' Master dissertation: 'The impact of the specialized pollinator *Stenoptilia zophodactylus* on the pollination system and reproductive success of common centaury (*Centaurium erythraea*) in a fragmented landscape.' *Supervisor*: Prof. Dr. Maurice Hoffmann – *Co-supervisor*: Dr. Rein Brys
2005-2008	Bachelor of biology, Ghent University (great distinction) Bachelor dissertation: '*Lactarius fuliginosus* and *Lactarius picinus*.' *Supervisor*: Prof. Dr. Annemieke Verbeken
2004-2005	First year of Bachelor of mathematics, KULAK – Kortrijk
1998-2004	Science-Mathematics, Sint-Franciscusinstituut Melle

SKILLS

Software knowledge	MS Office, CorelDRAW, Sequencher, Mafft, Mega, RAxML, MrBayes and (*)BEAST, R, BioGeoBears.
Phylogenetic methods	Multiple sequence alignments, ML analysis, Bayesian Inference, GMYC method and Bayesian species delimitation.
Laboratory techniques	DNA extraction from dried and fresh material, PCR, gel electrophoresis, NGS library preparation.
Microscopical techniques	Light microscopy, making hand slides, making microscopic line drawings with drawing tube.
Languages	Dutch (mother tongue), English (very good) and French (good)

TRAININGS

Doctoral schools training programme, Ghent University, November 2010 – May 2016.

Leiden University, The Netherlands, 29 October – 23 November 2012: DEST - Taxonomy Training: Trends in Biodiversity in Time and Space.

Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom, 29 April – 10 May 2013: Wellcome Trust Advanced Course: Computational Molecular Evolution.
Funding: travel grant from the Research Foundation – Flanders (FWO).

Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom, 12-19 April 2015: Wellcome Trust Advanced Course: Next Generation Sequencing.

PUBLICATIONS

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14. Delgat L., De Crop E., Njouonkou A.L. & Verbeken A. A new milkcap species discovered in the gallery forests of West Cameroon: *Lactifluus persicinus* sp. nov. (Russulaceae). To be submitted in Cryptogamie, Mycologie.

13. De Crop E., Hampe F., Wissitrassameewong K., Stubbe D., Nuytinck J. & Verbeken A. (2016). Southeast Asia reveals new diversity in *Lactifluus* section *Gerardii*: six new species with pleurotoid or small agaricoid basidiocarps. In prep.

12. De Crop E., Van de Putte K., De Wilde S., Njouonkou A.L., De Kesel A. & Verbeken A. Milkcap look-a-likes from gallery forests in West Africa: *Lactifluus foetens* and *Lf. albomembranaceus* sp. nov. Submitted to Phytotaxa.

11. Wisitrassameewong K., Looney B., Le H.T., De Crop E., Das K., Van de Putte K., Eberhardt U., Jiayu G., Stubbe S., Hyde K.D., Verbeken A. & Nuytinck J. *Lactarius* subgenus *Russularia* (Basidiomycota, Russulales): biodiversity, molecular phylogeny and evolutionary relationships. Submitted in Fungal Biology.

10. De Crop E., Nuytinck J., Van de Putte K., Wisitrassameewong K., Hackel J., Stubbe D., Hyde K. D., Roy M., Halling R. E., Wang X., Moreau P., Eberhardt U. & Verbeken A. A multi-gene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. Persoonia – Accepted.

9. Njouonkou A. L., De Crop E., Mbenmoun A. M., Kinge T. R., Biyé E. H. & Verbeken A. Diversity of wild mushrooms exploited in the Noun Division of the West Region of Cameroon. Journal of Medicinal Mushrooms – Accepted.

8. Li G.J., Hyde K.D., Zhao R.L., Sinang H., Abdel-Aziz F.A., Abdel-Wahab M.A., Alves-Silva G., Ammirati J., Ariyawansa H.A., Baghela A., Bahkali A.H., Beug M., Bojantchev D., Boonpratuang T., Bulgakov T., Camporesi E., Castilho B.M., Ceska O., Chakraborty D., Chen J.J., Chethana K.W.T., Consiglio G., Cui B.K., Dai Y.C., Daranagama D.A., Das K., Dayarathna M.C., De Crop E., De Oliveira R.J.V., de Souza C.A.F., Dentinger B.T.M., Dissanayake A.J., Doilom M., Drechsler-Santos E.R., Ghobad-Nejhad M., Gilmore S.P., Góes-Neto A., Gorczak M., Haitjema C.H., Hapuarachchi K.K., Hashimoto A., He M.Q., Henrique J.G., Henske J.K., Hirayama K., de Souza J.I., Jayasiri S.C., Jayawardena R.S., Jeon S.J., Jesus A.L., Jones E.B.G., Josefina I.M., Karunarathna S.C., Kirk P.M., Konta S., Kuhnert E., Langer E., Lee H.S., Lee H.B., Li W.J., Li X.H., Liimatainen K., Lima D.X., Lin C.G., Luangsa-ard J.J., Lücking R., Lumbsch H.T., Lumyong S., Maharachchikumbura S.S.N., Malibiran L.E., Marano A.V., Matsumura M., McKenzie E.H.C., Nguyen T.T.T., Niskanen T., Norphanphoun C., O'Malley M.A., Pablo A., Parnmen S., Pawłowska J., Perera R.H., Phookamsak R., Phukhamsakda C., Pires-Zottarelli C.L.A., Raspé O., Reck M.A., Monteiro de Azevedo Santiago A.L.C., Setti L., Shang Q.J., Singh S.K., Sir E.B., Solomon K.V., Song J., Srikitikulchai P., Stadler M., Suetrong S., Takahashi H., Takahashi T., Tanaka K., Tang L.P., Thambugala K.M., Theodorou M.K., Thongbai B., Thummarukcharoen T.,

Tian Q., Tibpromma S., Verbeken A., Vizzini A., Vlasák J., Voigt K., Wanasinghe D.N., Wang Y., Weerakoon G., Wen H.A., Wen T.C., Wijayawardene N.N., Wongkanoun S., Wrzosek M., Xiao Y.P., Yan J.Y., Yang J., Yang S.D., Young J.T., Yu H., Zhang J.F., Zhao J. & Zhou L.W. (2016). Fungal diversity notes 253-366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity – DOI 10.1007/s13225-016-0366-9.

7. Van de Putte K., Nuytinck J., De Crop E. & Verbeken A. (2016). *Lactifluus volemus* in Europe: three species in one - revealed by a multilocus genealogical approach, bayesian species delimitation and morphology. Fungal biology 120 (1):1–25.

6. Wissitrassameewong K., Nuytinck J., Le H.T., De Crop E., Hampe F., Hyde K.D., Verbeken A. (2015). *Lactarius* subgenus *Russularia* (Russulaceae) in South-East Asia: 3. new diversity in Thailand and Vietnam. Phytotaxa 207 (3):215–241

5. De Crop E., Nuytinck J., Van de Putte K., Lecomte M., Eberhardt U. & Verbeken A. (2014). *Lactifluus piperatus* (Russulales, Basidiomycota) and allied species in Western Europe and a preliminary overview of the group worldwide. Mycological Progress 13(3): 493-511.

4. De Crop E., Tibuhwa D., Baribwegure D. & Verbeken A. (2012). *Lactifluus kigomaensis*, a new species from Tanzania. Cryptogamie, Mycologie 33(4): 421-426.

3. Verbeken A., Van de Putte K. & De Crop E. (2012). New combinations in *Lactifluus*. 3. *L.* subgenera *Lactifluus* and *Piperati*. Mycotaxon 120: 443–450(8).

2. De Crop E., Brys R. & Hoffmann M. (2012). The impact of habitat fragmentation on the interaction between *Centaurium erythraea* (Gentianaceae) and its specialized seed predator *Stenoptilia zophodactylus* (Pterophoridae, Lepidoptera). Ecological Research 27(5): 967-974.

1. Brys R., De Crop E., Hoffmann M. & Jacquemyn H. (2011). Importance of autonomous selfing is inversely related to population size and pollinator availability in a monocarpic plant. American Journal of Botany 98(11): 1834-1840.

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2. De Crop E. & Verbeken A. (2011). Paddenstoelen zoeken in Afrika... net even anders. Sporen 4(2): 20-22.

1. De Keersmaeker L., Vandekerkhove K., De Crop E., Demolder H., Opstaele B. & Martens L. (2011). Schipperen tussen oud bos en schraalgrasland in het Vroenenbos. De levende natuur 112(1): 32-37.

CONFERENCES & ABSTRACTS

Young Botanists' Forum, National Botanic Garden, Meise, Belgium, 19 November 2010.

XVI Congress of European Mycologists, Halkidiki, Greece, 19 – 23 September 2011.
Poster: De Crop E., Nuytinck J. & Verbeken A. – *Lactifluus piperatus* and company (Russulaceae).

21^{e} Vlaamse Mycologendag, KVMV, Leuven, Belgium, 17 March 2012.
Presentation: Van de Putte K., De Crop E. & Verbeken A. Nieuws uit Russulales-land.

Sondershausen, Germany, 26 – 31 August 2012: Russulales workshop.
Presentation: Nuytinck J., Stubbe D., Van de Putte K., De Crop E. & Verbeken A. Current updates in *Lactarius*.

1st Annual meeting on plant ecology and evolution, Royal Botanical Society of Belgium and the National Botanic Garden of Belgium, Meise, Belgium, 20 November 2012.

23^{e} Vlaamse Mycologendag, KVMV, Gent, Belgium, 15 March 2014.
Presentation: De Crop E. Op zoek naar *Lactifluus* in Afrika.

10th International Mycological Congress, Bangkok, Thailand, 3 – 8 August 2014.
Poster: De Crop E., Nuytinck J. Van de Putte K. & Verbeken A. – The milkcap genus *Lactifluus* (Russulaceae) unravelled.

Poster: Hackel J., Moreau P.A., Courtecuisse R., Buyck B., Henkel T.W., Miller S.L., De Crop E., Verbeken A., Neves M.A., Jaeger M.C.W., Duque J., Wartchow F., Sà M., Cheype J.L., Louisanna E., Schimann H., Garnica S., Mueller G.M., Hofstetter V., Manzi S., Gardes M., Roy M. – Origins and diversification of neotropical taxa in a cosmopolitan lineage of ectomycorrhizal fungi (Basidiomycota: Russulaceae).

Funding: travel grant from the Research Foundation – Flanders (FWO).

Areál Zdravia, Jedľové Kostoľany, Slovakia, 8 – 13 September 2014: Russulales workshop.

XVII Congress of European Mycologists, Funchal, Madeira, Portugal, 21 – 25 September 2015.

Presentation: De Crop E., Nuytinck J., Van de Putte K. & Verbeken A. – Unravelling the milkcap genus *Lactifluus* (Russulaceae).

Funding: travel grant from the Research Foundation – Flanders (FWO).

3rd Annual meeting on plant ecology and evolution, Royal Botanical Society of Belgium and the National Botanic Garden of Belgium, Gent, Belgium, 5 February 2016.

Presentation: De Crop E., Nuytinck J., Van de Putte K. & Verbeken A. – Exploring the diversity of the milkcap genus *Lactifluus* (Russulaceae).

FIELDWORK

Kigoma, Tanzania, 12 March – 14 April 2011: collecting ectomycorrhizal macrofungi in Tanzanian miombo woodland, with special attention for species from the genera *Lactifluus* and *Lactarius*, in collaboration with Dr. Donatha Tibuhwa (University of Dar es Salaam, Tanzania) and Dr. Deo Baribwegure (Director of Kicora, Kigoma, Tanzania).

Rhodope Mountains, Greece, 24 – 27 September 2011: collecting macrofungi with special attention for ectomycorrhizal fungi.

Cameroon, 5 May – 3 June 2012: collecting ectomycorrhizal macrofungi in diverse regions in Cameroon (Foumban region, Dja Biosphere Reserve, Kribi region), with special attention for species from the genera *Lactifluus* and *Lactarius*, in collaboration with Dr. André-Ledoux Njouonkou (University of Bamenda, Cameroon).

Zambia, 20 January – 10 February 2014: collecting ectomycorrhizal macrofungi and root tips in the Mutinondo Wilderness Area, with special attention for Russulaceae species, in collaboration with the Biology Department of the University of Zambia and Naturalis Biodiversity Center.
Funding: travel grant from the Research Foundation – Flanders (FWO).

Cameroon and Togo, 11 May – 23 June 2014: collecting ectomycorrhizal macrofungi and root tips in diverse regions in Cameroon and Togo, with special attention for Russulaceae species, in collaboration with Dr. André-Ledoux Njouonkou (University of Bamenda, Cameroon) and Prof. A.K. Guelly (University of Lome, Togo).
Funding: travel grant from the Research Foundation – Flanders (FWO) and the Leopold III-fund.

Thailand, 28 July – 3 August 2014: Pre IMC Workshop on Northern Thailand Mushroom Diversity.

MEMBERSHIPS & COMMITTEES

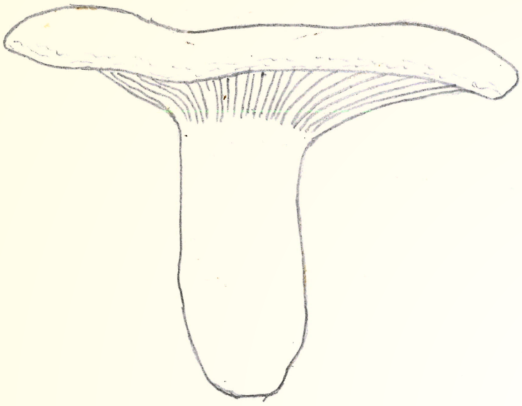
Member of the Royal Flemish Mycological Society (Koninklijke Vlaamse Mycologische Vereniging) since 2011.

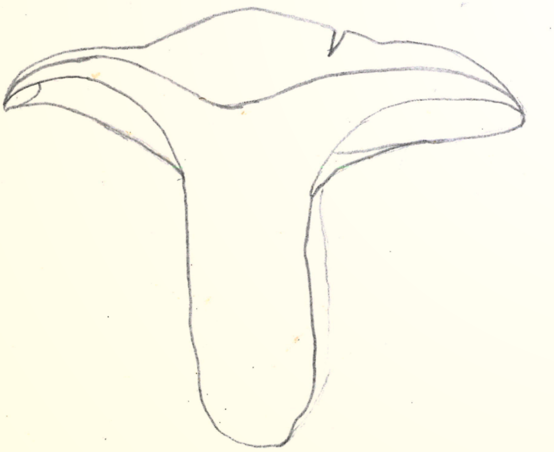
Member of the International Mycological Association since 2014.

Member of the European Mycological Society since 2015.

Treasurer of the European Mycological Society, 2015-2019.

Member of the Royal Botanical Society of Belgium since 2016.





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