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SISTEMÁTICA FILOGENÉTICA DE *LACTIFLUUS* (RUSSULACEAE) COM  
ÊNFASE EM ESPÉCIES DO NORTE E NORDESTE BRASILEIRO

Alexandre Gonçalves dos Santos e Silva Filho

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Tese de Doutorado  
Natal/RN, Setembro de 2021.

**ALEXANDRE GONÇALVES DOS SANTOS E SILVA FILHO**

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**Orientador: Prof. Dr. Felipe Wartchow**

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EM ESPÉCIES DO NORTE E NORDESTE BRASILEIRO**

Tese apresentada ao Programa de Pós-Graduação em Sistemática e Evolução como requisito parcial para obtenção de título de Doutor em Sistemática e Evolução.

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## RESUMO

*Lactifluus* (Russulaceae, Russulales) reúne fungos que produzem em sua forma reprodutiva basidioma agaricoide, pleurotoide e gasteroide; que se caracterizam principalmente pela exudação de látex. O gênero possui aproximadamente 210 espécies distribuídas principalmente nos trópicos, onde frequentemente faz associação ectomicorrízica com uma ampla variedade de plantas. A diversidade do gênero vem sendo mais bem investigada na África e na Ásia, enquanto permanece ainda pouco explorada na região Neotropical. No Brasil, apenas nove espécies de *Lactifluus* foram descritas ou reportadas para a Amazônia, Mata Atlântica e Caatinga, e outras dez espécies ainda tratadas como *Lactarius* necessitam ser revisadas e confirmadas. Com isso, o projeto proposto visou sistematizar e interpretar o gênero *Lactifluus* a partir de análises morfológicas e moleculares, com o incremento de novas coletas e a revisão de coleções de herbário provenientes dos diferentes biomas das regiões Norte e Nordeste do Brasil. As análises basearam-se em dados morfológicos e moleculares de dez espécimes depositados nos Herbários PACA, INPA JPB, UFRN-Fungos e URM; e de 31 espécimes coletados em diferentes ecossistemas da Amazônia, Caatinga e Mata Atlântica. A revisão morfológica das coleções herborizadas resultou na recombinação de *Lactifluus amazonensis*, *Lf. brasiliensis*, *Lf. braunii*, *Lf. mamorensis*, *Lf. rupestres* e *Lf. russula*; e na designação de um lectótipo para *Lf. annulifer*, *Lf. braunii* e *Lf. russula*. Novas amostras coletadas resultaram na identificação e no sequenciamento de *Lf. amazonensis*, *Lf. annulifer*, *Lf. dunensis*, *Lf. lepus*, *Lf. mamorensis* e *Lf. neotropicus*. Outras 11 espécies são propostas como novas para a ciência, e são elas: *Lf. aurantioceraceus* nom. prov., *Lf. brunneoruber* nom. prov., *Lf. candidus* nom. prov., *Lf. flavoscaesпитosus* nom. prov., *Lf. guttulatus* sp. nov., *Lf. perforatus* nom. prov., *Lf. piperogalactus* sp. nov., *Lf. reniformis* nom. prov., *Lf. restingae* nom. prov., *Lf. sphauliformis* sp. nov. e *Lf. umbilicatus* sp. nov. A análise molecular das regiões ITS, LSU e RPB2 do DNA dos espécimes coletados confirmam as espécies citadas como linhagens distintas em *Lactifluus*, estando elas distribuídas em cinco diferentes clados nos subgêneros *Gymnocarpi*, *Lactariopsis* e *Pseudogymnocarpi*. Além disso, os estudos filogenéticos dos espécimes coletados deram suporte para a delimitação de duas seções: *Lf.* seção *Piperogalacti* sec. nov. e *Lf.* seção *Multiceps* nom. prov.; e para a emenda de *Lf.* sec. *Panuoidei*.

**Palavras-chave:** Agaricomycetes, filogenia, fungos neotropicais, Russulaceae, taxonomia

## ABSTRACT

*Lactifluus* (Russulaceae, Russulales) comprises fungi that produce in their reproductive form agaricoid, pleurotoid and gasteroid basidiomata; mainly characterized by latex exudation. The genus has approximately 210 species distributed mostly in the tropics, where they frequently form ectomycorrhizal association with a wide variety of plants. The diversity of the genus has been better investigated in Africa and Asia, while remains underexplored in Neotropical region. In Brazil, only nine *Lactifluus* species were described or reported from Amazon, Atlantic Forest and Caatinga, and another ten species still treated as *Lactarius* needed to be revised and confirmed. Thus, the proposed project aimed to systematize and interpret the genus *Lactifluus* from morphological and molecular analysis, with the increase of new collections and review of collection of Herbaria from different biomes in the North and Northeast regions of Brazil. The analyzes were based on morphological and molecular data from ten specimens deposited in the PACA, INPA, JPB UFRN-Fungos and URM Herbaria; and also from additional 31 specimens collected in different ecosystems of Amazonia, Caatinga and Atlantic Forest. The morphological review of herborized collections resulted in recombination of *Lactifluus amazonensis*, *Lf. brasiliensis*, *Lf. braunii*, *Lf. mamorensis*, *Lf. russula* and *Lf. rupestris*; and in the designation of one lectotype for *Lf. annulifer*, *Lf. braunii* and *Lf. russula*. New sample resulted in the identification and sequencing of *Lf. amazonensis*, *Lf. annulifer*, *Lf. dunensis*, *Lf. lepus*, *Lf. neotropicus* and *Lf. mamorensis*. Another 11 species were proposed as new to science: *Lf. aurantioceraceus* nom. prov., *Lf. brunneoruber* nom. prov., *Lf. candidus* nom. prov., *Lf. flavuscaespitosus* nom. prov., *Lf. guttulatus* sp. nov., *Lf. perforatus* nom. prov., *Lf. piperogalactus* sp. nov., *Lf. reniformis* nom. prov., *Lf. restingae* nom. prov., *Lf. sphauliformis* sp. nov. and *Lf. umbilicatus* sp. nov. Molecular analysis of the ITS, LSU and RPB2 DNA regions of the collected specimens confirm the distinct lineages of the cited species in *Lactifluus*, in which are distributed in five different clades in the subgenera *Gymnocarpi*, *Lactariopsis* and *Pseudogymnocarpi*. Furthermore, the phylogenetic analyses of the collected specimens provided support for delimitation of two sections: *Lf.* section *Piperogalacti* sect. nov. and *Lf.* section *Multiceps* nom. prov.; and emendation of *Lf.* sect. *Panuoidei*.

**Key Words:** Agaricomycetes, filogeny, Neotropical fungi, Russulaceae, taxonomy

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## 1 INTRODUÇÃO

### 1.1 Os cogumelos quebradiços de Russulales

*Russula* Pers. e *Lactarius* Pers. *sensu lato* são os gêneros mais representativos em Russulales Kreisel ex P.M. Kirk, P.F. Cannon e J.C. David. Ambos os nomes foram descritos por Person (1796, 1797) para abrigar táxons agaricoides e com o contexto frágil. *Russula* produz basidiomas carnudos com píleo de cores fortes que contrastam com as lamelas e estipe, enquanto *Lactarius* exudam látex (PERSOON, 1796, 1797).

Apenas no século XX, *Lactarius sensu lato* e *Russula* foram classificados em Russulales (KREISEL, 1969; OBERWINKLER, 1977). Morfologicamente, a ordem é caracterizada pelos esferocistos na trama, o que torna o contexto do píleo e do estipe frágil; pela ornamentação dos esporos amiloides e pelo sistema gloeopleuro, que inclui as hifas oleíferas e laticíferas (KREISEL, 1969; OBERWINKLER, 1977). A combinação desses caracteres também foi encontrada em espécies com outros hábitos, como os corticioide, ressupinado, discoide, efuso-reflexo, clavarioide, secotioides e gasteroide, que foram incluídos posteriormente em Russulales (ROMAGNESI, 1948; OBERWINKLER, 1977; LARSSON & LARSSON, 2003). Dados moleculares reconhecem com forte suporte Russulales no clado ‘russuloide’ que, atualmente, é formado por sete famílias (MILLER et al., 2001).

### 1.2 Russulaceae

Russulaceae foi inicialmente proposta para abrigar os gêneros agaricoides *Lactarius*, *Russula* e *Russulina* J. Schröt. (= *Russula*). Posteriormente, outros grupos com diferentes morfologias foram incluídos na família: espécies com hábito pleurotoide foram inicialmente classificadas em *Lactarius* seção *Panuoidei* Singer e depois transferidas ao gênero *Pleurogala* Redhead & Norvell (SINGER, 1984; REDHEAD & NORVELL, 1993); os gêneros *Arcangeliella* Cavara, *Cystangium* Singer & A.H. Sm., *Elasmomyces* Cavara, *Gastrolactarius* R. Heim ex J.M. Vidal, *Gymnomyces* Masee & Rodway *Macowanites* Kalchbr, *Martellia* Mattir e *Zelleromyces* Singer & A.H. Sm. foram erguidos para abrigar as espécies sequestradas; e por fim, as espécies veladas foram inicialmente propostas no gênero *Lactariopsis* Henn (HENNING, 1902; HEIM, 1937; REDHEAD & NORVELL, 1993; CALODGE & MARTÍN, 2000).

Estudos moleculares alteraram o conceito genérico em Russulaceae, revelando origens múltiplas dos fungos pleurotoides, sequestrados e velados dentro de *Lactarius*, *Lactifluus* e *Russula* (MILLER et al., 2001, 2006; DE CROP et al. 2017; ELLIOTT & TRAPPE, 2018; LEBEL et al., 2021). Atualmente a família é formada pelos gêneros *Russula*, *Lactarius*, *Lactifluus* (Pers.) Roussel, e *Multifurca* Buyck & V. Hofst., adicionados de outros três gêneros corticioides: *Boidinia* Stalpers & Hjortstam, *Gloeopeniophorella* Rick e *Pseudoxenasma* K.H. Larss. & Hjortstam (LARSSON & LARSSON, 2003; MILLER et al., 2001).

### 1.3 Desconstruindo *Lactarius* e reerguendo *Lactifluus*

A inclusão de dados moleculares aos estudos de taxonomia alterou consideravelmente a sistemática de Russulaceae. Buyck et al. (2008) construíram uma filogenia com táxons agaricoides de Russulaceae, e incluíram mais espécies tropicais. Os resultados mostraram que *Lactarius* e *Russula* não eram suportados como clados monofiléticos. *Russula* passa a ser monofilético quando um pequeno grupo de espécies pertencendo a *Russula* subseção *Ochricomptae* Bills & O.K. Mill. é removido. Este pequeno grupo de espécies formou um clado juntamente com *Lactarius furcatus* Coker, e todos foram transferidos ao novo gênero *Multifurca* (BUYCK et al., 2008).

Além desta alteração nos representantes pertencentes à *Multifurca*, *Lactarius* passa a ser dividido em dois clados, sendo um maior e outro menor. No maior, é proposto conservar o nome *Lactarius sensu novo* (abreviação *L.*) com *Lactarius torminosus* (Schaeff: Fr.) Pers. como espécie tipo do gênero. O clado menor passou a ser denominado *Lactifluus* (abreviação *Lf.*), e foi tipificado por *Agaricus lactifluus* L., atualmente conhecido como *Lf. volemus* (Fr.) Kuntze (Buyck et al. 2010).

Com a nova classificação e proposta nomenclatural dos fungos lactarioides, *Lactarius* passa a ser composto por espécies dos subgêneros *Lactarius*, *Russularia* Fr. ex Burl. e *Plinthogali* (Burl.) Singer, enquanto *Lactifluus* agrega membros dos subgêneros *Lactariopsis* (Henn.) Verbeken, *Russulopsis* (Verbeken) Verbeken, *Lactifluus*, *Gerardii* (A.H. Sm. & Hesler) Stubbe, *Piperati* Verbeken e da seção *Edules* (Verbeken) Verbeken (VERBEKEN et al., 2011, 2012; STUBBE et al., 2012).

*Lactarius* e *Lactifluus* em questão são difíceis de serem definidos por meio de estados de caracteres sinapomórficos. A distinção entre os gêneros é com base em alguns poucos caracteres morfológicos: espécies de *Lactarius* possuem píleo zonado, viscoso a glutinoso,

característica ausente em espécies de *Lactifluus* (DE CROP et al., 2017). A distribuição geográfica, aliada a dados moleculares, é melhor utilizada para defini-los: *Lactarius* possui distribuição predominantemente em áreas temperadas e boreais, *Lactifluus*, nos trópicos e subtropicais (DE CROP et al., 2017).

#### 1.4 Classificação subgenérica de *Lactifluus*

Um estudo filogenético utilizando sequência das regiões ITS, LSU, *RPB1* e *RPB2*, aliado às principais características morfológicas de 80% das espécies conhecidas, levou a um rearranjo infragenérico e ao reconhecimento de quatro subgêneros e 13 seções em *Lactifluus*. (DE CROP et al., 2017). A filogenia também mostrou a formação de outros nove ramos ainda não delimitados, e nove espécies isoladas. Mesmo assim, os limites morfológicos dos quatro subgêneros ainda não foram bem estabelecidos. A partir de então, apenas dois estudos realizados com amostras da América Central e América do Sul priorizaram a sistemática com foco na definição dos clados ainda não delimitados e nas espécies isoladas (BARBOSA et al., 2020; DELGAT et al., 2020). Os subgêneros de *Lactifluus* possuem distribuição geográfica bem definida: *Lactifluus* subgênero *Lactariopsis*, *Lf.* subgênero *Gymnocarpi* (R. Heim ex Verbeken) De Crop e *Lf.* subgênero *Pseudogymnocarpi* (Verbeken) De Crop contêm principalmente espécies dos trópicos, mas com alguns poucos representantes originais da região temperada (DE CROP et al., 2017). *Lactifluus* subg. *Lactifluus* é distribuído principalmente no Hemisfério Norte, com exceção de algumas espécies australianas e sem representantes conhecidos na África ou na América do Sul.

#### 1.5 A morfologia das espécies de *Lactifluus*

*Lactifluus* é caracterizado por produzir basidioma agaricoide, pleurotoide e raro gasteroide (VERBEKEN & NUYTINCK, 2013; LEBEL et al., 2021). O píleo em *Lactifluus* pode variar entre seco às vezes viscido, com a superfície enrugada, escribiculada, velutinoso, rivuloso e algumas vezes rachada (Figura 1); as lamelas quase nunca são bifurcadas, podem ser próximas a muito distantes, apresentam lamélulas de tamanhos variados e eventualmente são veiadadas; o estipe pode ser central, lateral reduzido ou ausente e quando presente são cilíndricos e mais finos na base (VERBEKEN & WALLEN, 2010; DE CROP et al., 2017).

Espécies de *Lactifluus* produzem esporos globosos a subelipsoide, com ornamentação amiloide em reagente de Melzer (VERBEKEN & NUYTINCK, 2013). A ornamentação pode

Tabela 1. Morfologia dos subgêneros e distribuição geográfica das seções de acordo com De Crop et al. (2017), Barbosa et al. (2020), Delgat et al. (2020) e Lebel et al. (2021).

	morfologia do subgênero	seções	distribuição
subgênero	<i>Gymnocarpi</i>		
	Hábitos agaricoide e pleurotoide. Ausência de pleurolamprocistídios, pileipelis composta por uma (lampro) paliçada e reação de cor acastanhada no látex e ou no contexto quando exposto ao ar.	<i>Luteoli</i> (Pacioni & Lalli) Verbeken	Amplamente distribuído com exceção da América Central e América do Sul
		<i>Gymnocarpi</i> (R. Heim ex Verbeken) De Crop	África
		<i>Phlebonemy</i> R Heim ex Verbeken	África
		<i>Tomentosi</i> (McNabb) Verbeken	Oceania
		<i>Nebulosi</i> Delgat	América Central
		<i>Panuoidei</i> Singer (Delgat)	América Central e América do Sul
clados não delimitados		Três espécies isoladas	
subgêneros	<i>Lactariopsis</i>		
	Hábitos agaricoide e pleurotoide. Pileipelis uma (lampro) paliçada ausência de pleurolamprocistídios. Látex brancos e imutáveis.	<i>Albati</i> (Bataille) Verbeken	Hemisfério norte
		<i>Edules</i>	África
		<i>Lactariopsis</i> Verbeken,	África
		<i>Neotropici</i> J. Duque, L. Delgat, A. Verbeken, M. A. Neves & Anibal A. Carvalho Jr.	América Central e América do Sul
		<i>Russulopsidei</i> (Verbeken) Verbeken	África
clados não delimitados		Seis clados e uma espécie isolada	
subgêneros	<i>Lactifluus</i>		
seções	Hábitos agaricoide, pleurotoide e gasteroide. Desenvolvem uma grande variedade de tipos de pileipelis.	<i>Allardii</i> (Hesler & A.H. Sm.) De Crop	América do Norte
		<i>Ambicystidiati</i> X.H. Wang	Ásia

Tabela 1 (continuação). Morfologia dos subgêneros e distribuição geográfica das seções de acordo com De Crop et al. (2017), Barbosa et al. (2020), Delgat et al. (2020) e Lebel et al. (2021).

	Em algumas seções os cistídios podem estar presentes. Látex pode ser imutável ou ter reação de alteração para o marrom ou verde.	<i>Gerardii</i> (A.H. Sm. & Hesler) Stubbe	Amplamente distribuído com exceção da América Central e América do Sul
		<i>Lactifluus</i>	Ásia, Europa, Oceania e América do Norte
		<i>Piperati</i> (Fr.) Verbeken	Ásia, Europa e América do Norte
		<i>Tenuicystidiati</i> X.H. Wang & Verbeken	Ásia
clados não delimitados		Uma espécie isolada	
subgêneros	<i>Pseudogymnocarpi</i>		
	Hábito agaricoide e gasteroide. Basidioma amarelo, laranja a marrom-avermelhados. Pileipelis uma (lampro) ou (tricho) paliçada. Cistídios presentes ou ausentes. O látex na maioria das vezes é imutável, com exceção de algumas espécies que alteram do branco para o marrom.	<i>Aurantifolii</i> (Verbeken) Verbeken	África
		<i>Pseudogymnocarpi</i> (Verbeken) Verbeken	África, América do Norte, Europa e Ásia
		<i>Polysphaerophori</i> (Singer) Verbeken	América Central e América do Sul
		<i>Rubroviolascetini</i> (Singer) Verbeken	África
			África
		<i>Xerampelini</i> De Crop	
clados não delimitados		Um clado	

Fonte: Elaborado pelo autor (2021).

variar entre verrucosa, reticulada ou, como em grande parte das espécies neotropicais, uma transição entre verrucosa e reticulada. Outra característica importante dos esporos é o *plage hilar*, que pode variar entre inamiloide, parcialmente amiloide ou totalmente amiloide (VERBEKEN & WALLEN, 2010) (Figura 2).

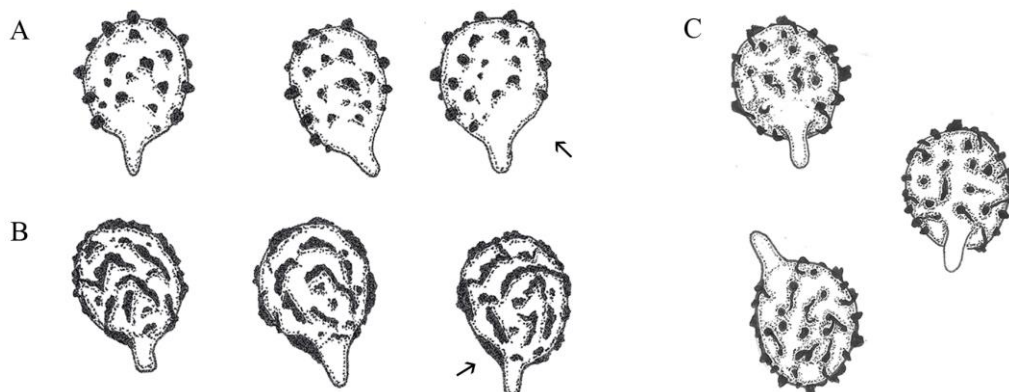
Os basídios em *Lactifluus* são quase sempre tetraesporados, mas em algumas espécies são encontrados basídios uni, bi e triesporados, podendo ser clavados, cilíndricos, cilindro-clavados ou fusiformes (VERBEKEN & WALLEN, 2010) (Figura 3). Alguns são hialinos,

Figura 1. Hábitos dos basidiomas de *Lactifluus*. A. *Lf. spathuliformis* (D.L. Komura 1891). B. *Lf. brunneoruber* nom prov. (D.L. Komura 2595). C. *Lf. neotropicus* (FW21/2017) D. *Lf. dunensis* (AGS 103/2019). E. *Lf. piperogalactus* (FW 22/2017) F. *Lf. wirrabara* (JET 943).



Fotos: A-B. D.L. Komura C. F. Wartchow D. A.G.S. Silva-Filho. E. F. Wartchow F. J.E. Tonkin (foto F: alterado de LEBEL et al. 2021).

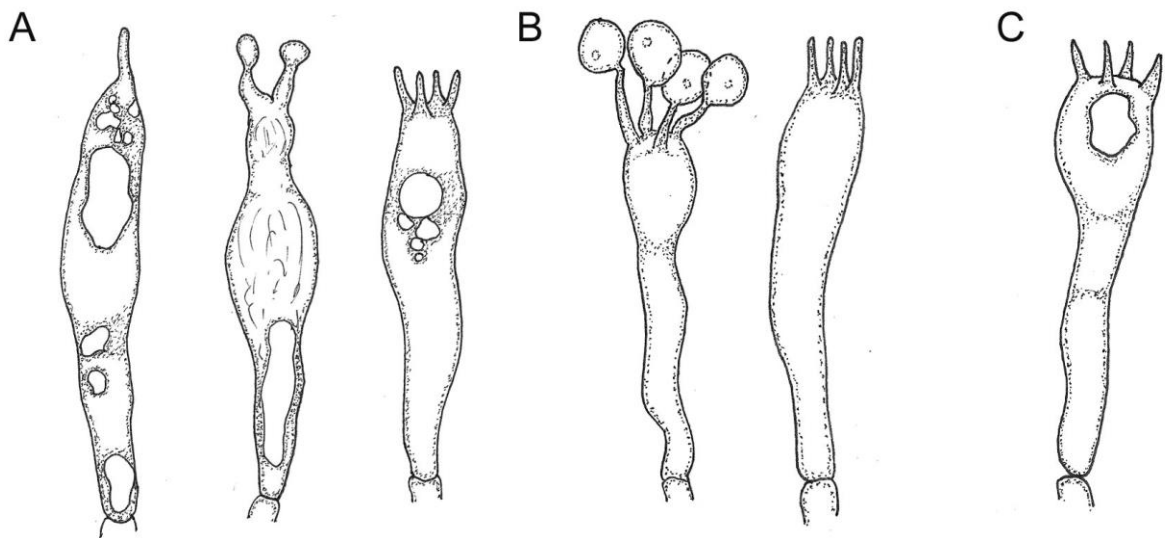
Figura 2. Esporos das espécies que ocorrem no Brasil. A. Esporos verrucosos de *Lf. lepus* (D.L. Komura 1854), seta indica o plage inamiloide. B. Esporos reticulado de *Lf. spathuliformis* (D.L. Komura 116), seta indica o plage totalmente amiloide. C. Esporos com ornamentação entre verrucosa e reticulada de *Lf. umbilicatus*. (D.L. Komura 1964).



Fonte: O elaborado pelo autor (2021).

mas a grande maioria apresenta conteúdo refringente (que se destacam pelo brilho, contendo gúttulas heterogêneas) ou oleífero que se destacam pelo brilho, contendo conteúdo homogêneo). Em algumas espécies, os basídios são mais alongados, como em *Lf. dunensis* Sá & Wartchow (SÁ & WARTCHOW 2013).

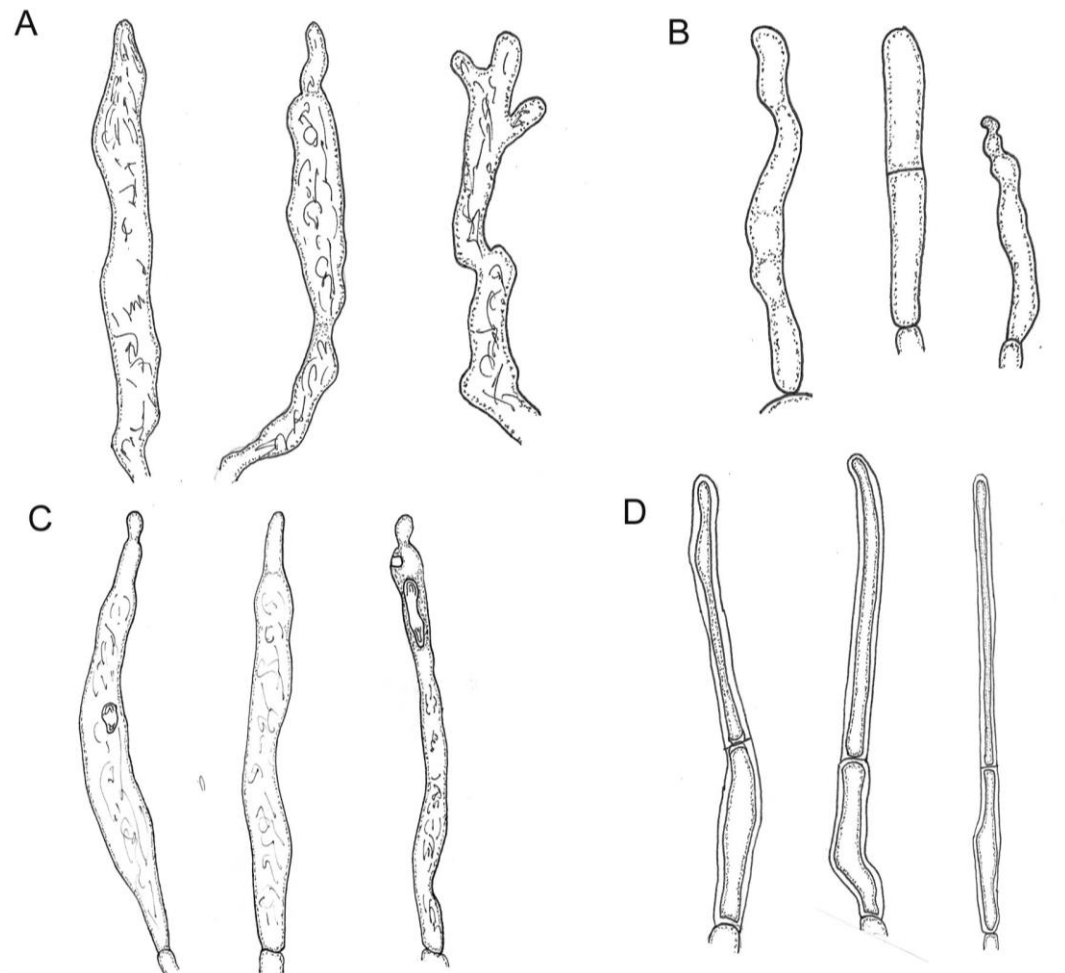
**Figura 3.** Basídios que ocorrem nas espécies do Brasil. **A.** Basídio uni, bi e tetra esporados levemente fusiformes e com conteúdo refringente de *Lf. amazonensis*. (D.L. Komura 2618). **B.** Basídio tetra esporados, cilíndricos a levemente clavados e hialinos de *Lactifluus* sp. (FW 145) **C.** Basídio clavado com conteúdo refringente de *Lf. neotropicus* (FW 23/2010).



Fonte: Elaborado pelo autor (2021)

Os cistídios em *Lactifluus* são importantes elementos com peso taxonômico a nível específico. Os pseudocistídios são aqueles que estão ligados a hifas lactíferas originadas da trama da lamela, e podem estar presentes na lateral e/ou na borda lamelar (VERBEKEN & WALLEN, 2010) (Figura 4A–5). Os pseudocistídios são medidos em largura, podendo variar consideravelmente no tamanho, formato, na abundância, projeção para o himênio e nos elementos internos. Os macrocistídios são similares aos pseudocistídios, entretanto eles têm origem em uma célula do subhímênio. Quando presentes, também estão dispostos na lateral ou na borda da lamela, podendo variar em formato, tamanho, abundância, projeção para o himênio e elementos internos (Figura 4C–5). Os macrocistídios com parede espessa são chamados de lamprocistídios, e são raros em espécies neotropicais (Figura 4D). Os leptocistídios são elementos estéreis sem conteúdo interno, diferenciando dos basidiolos no formato, geralmente têm origem em uma hifa celular da subhímênio e possuem paredes finas (Figura 4B).

Figura 4. Cistídios das espécies que ocorrem no Brasil. A. Pseudocistídios de *Lf. brunneoruber* (D.L. Komura 2585). B. Leptocistídios de *Lf. candidus* nom. prov. (JCB 145). C. Macroclistídios de *Lf. restingae* nom. prov. (AGS 104/2019) D. Lamprocistídios de *Lf. brunneoruber* nom. prov. (D.L. Komura 2585).



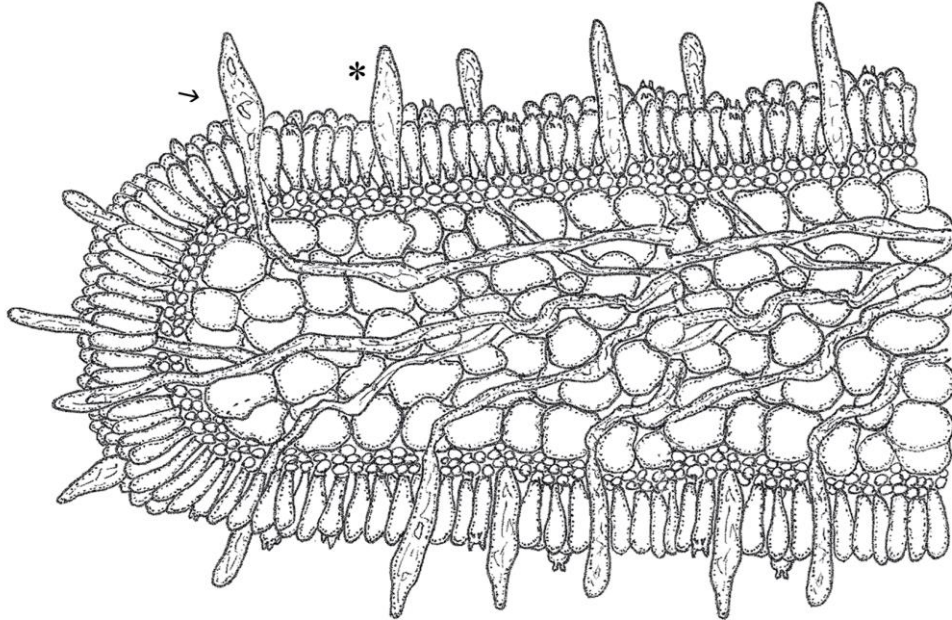
Fonte: Elaborado pelo autor (2021).

A trama lamelar é geralmente composta por esferocistos, hifas cilíndricas hialinas e hifas lactíferas (Figura 5). Já as espécies pleurotoides neotropicais de *Lactifluus* subgênero *Gymnocarpi* não produzem esferocistos na trama da lamela, ou elas são escassas, estando os esferocistos presentes apenas na trama do píleo.

A superfície do píleo em *Lactifluus* pode variar entre uma cútis, himenoderme, tricoderme, ixotricoderme, ixocutis, paliçada, tricopaliçada, lampropaliçada e tricolampropaliçada (VERBEKEN, 1989). O subgênero *Lactifluus* abriga espécies com maior diversidade de tipos de pileipellis (DE CROP, et al. 2017). Nas espécies neotropicais, encontra-se majoritariamente a configuração paliçada e lampropaliçada (Figura 6). A

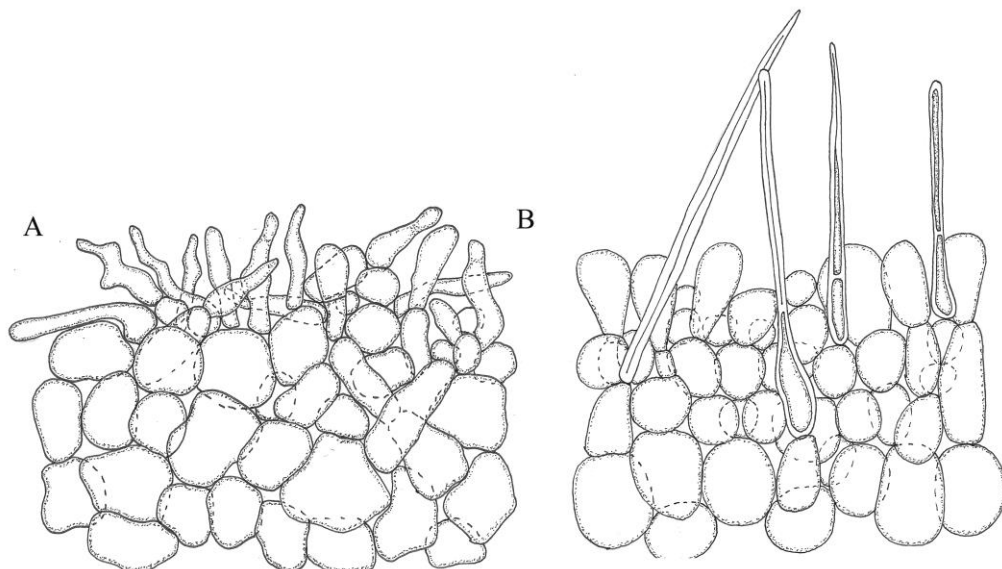
superfície do estipe possui, na maioria das vezes, a mesma configuração da superfície do píleo.

**Figura 5.** Trama Lamelar de *Lactifluus* sp. (MS 01/2015) com a seta mostrando um pleuropseudocistídio e o \* mostrando um pleuromacrocistídio.



Fonte: Elaborado pelo autor (2021).

**Figura 6.** Diferentes tipos de superfície do píleo das espécies que ocorrem no Brasil. **A.** Paliçada de *Lf. flavuscaespitosus* nom. prov. (FW 08/2017). **B.** Lampropaliçada de *Lf. reniformis* nom. prov. (D.L. Komura 1094).



Fonte: Elaborado pelo autor (2021).

## 1.6 Ecologia

Basidiomas de *Lactifluus* são geralmente encontrados no solo, fazendo associação ectomicorrízica com plantas simbiontes. *Lactifluus* vêm sendo encontrados em uma ampla variedade de tipos de vegetação, em florestas tropicais e subtropicais, florestas de monções, savanas arbóreas, florestas mediterrâneas, florestas temperadas de folha larga, florestas montanas e coníferas (DE CROP et al., 2021).

Algumas espécies com hábito pleurotoide, ou agaricoide reduzido, crescem em troncos de árvores, desconhecendo-se completamente a ecologia dessas espécies. No Brasil, muitas espécies de *Lactifluus* foram coletadas em áreas de restinga na Mata Atlântica e em áreas de Campinarana e Campina na Amazônia. Ambos os locais são caracterizados pelo solo arenoso, pobre em nutrientes e com uma grande diversidade de plantas ectomicorrízicas (por exemplo, SINGER et al., 1983; SÁ et al., 2013; SULZBACHER et al., 2013a).

No Brasil, espécies de *Lactifluus* são encontradas em ambientes onde ocorrem plantas ectomicorrízicas nativas, possivelmente crescendo junto a espécies de Fabaceae, (por exemplo, *Dicymbe* spp.), Fagaceae, Nyctaginaceae (por exemplo, *Guapira* Aubl.) e Polygonaceae (por exemplo, *Coccoloba* P. Browne) (TEDERSOO et al., 2010; SULZBACHER et al., 2013). Espécies de *Lactifluus* nunca foram encontradas no Brasil associadas a árvores introduzidas, como o *Pinus* e o *Eucaliptus*. Por outro lado, espécies de *Lactarius* vêm sendo coletados em plantações de *Pinus*, onde até o momento foram referidas cinco espécies, sendo uma nova com hábito gasteroide (SULZBACHER et al., 2013b; SILVA-FILHO et al., 2018, 2020).

Até o momento, não há registro na literatura sobre a confirmação de associação ectomicorrízica de espécies de *Lactifluus* no Brasil. Os dados ecológicos sobre esse tipo de associação ainda são escassos devido à dificuldade de identificar com qual árvore o micobionte está associado.

## 1.7 Estudos com *Lactifluus* no Brasil

Até o início desta pesquisa, em abril de 2017, eram conhecidas seis espécies de *Lactifluus* para o Brasil. O primeiro registro de um fungo lactarioide foi *Panus reticulatus* Berk. para o estado do Amazonas (BERKELEY, 1856), transferido posteriormente por Singer (1955) para *Lactarius*. Rick (1906, 1907, 1930, 1938) descreveu quatro espécies para o Rio Grande do Sul: *Lactarius adustus* Rick, *L. braunii* Rick, *L. russula* Rick e *L. steffenii* Rick.

Mais tarde, Singer et al. (1983) and Singer (1984) descreveram *L. amazonensis* Singer, *L. annulifer* Singer, *L. brasiliensis* Singer, *L. campinensis* Singer, *L. gigasporus* Singer, *L. igapoensis* Singer, *L. mamorensis* Singer, *L. pallidipes* Singer, *L. paulensis* Singer e *L. subpallidipes* Singer para os estados do Amazonas e São Paulo. Dentre essas espécies *L. campinensis* foi transferida para *Russula* (HENKEL et al., 2000; DE CROP et al. 2021).

Outros trabalhos reportam a ocorrência de espécies de *Lactifluus* para o Brasil, todos ainda nomeados como *Lactarius*: *L. reticulatus* foi revisado por Pegler & Fiard (1979); *L. paulensis* foi reportado por Pegler (1997) novamente para o Estado de São Paulo; e *L. venezuelanus* Dennis foi registrado por Buyck & De Meijer (1999) e por De Meijer (2006) para o Paraná.

A partir de 2010, novas espécies foram descritas para o Sul, Sudeste e Nordeste: *L. rupestris* Wartchow foi descrito para o Estado de Pernambuco (WARTCHOW & CAVALCANTI, 2010); *Lactifluus aurantiorugosus* Sá & Wartchow para o Rio Grande do Sul (SÁ & WARTCHOW, 2013), *Lf. batistae* Wartchow, J.L. Bezerra & M. Cavalc. para a Bahia (WARTCHOW et al., 2013), *Lf. dunensis* para o Rio Grande do Norte (SÁ et al., 2013). No ano de 2019, *Lf. caatingae* Sá & Wartchow foi descrita para o Piauí (SÁ et al., 2019). Em seguida, *Lf. catarinensis* J. Duque, M.A. Neves & M. Jaegger foi descrita para o Estado de Santa Catarina, *Lf. marielleae* J. Duque & M.A. Neves para os Estados do Rio de Janeiro e Minas Gerais e *Lf. neotropicus* (Singer) Nuytinck teve sua ocorrência confirmada para o Brasil (BARBOSA et al., 2020)

A partir da premissa de que *Lactifluus* encontra-se amplamente distribuída nos trópicos, que o Brasil é um país megadiverso e que apenas seis espécies eram reportadas para o país em 2017, havia uma grande lacuna para a revisão de exsicatas, a descoberta e a inclusão de novas espécies e seus caracteres morfológicos e moleculares para tentar resolver alguns agrupamentos de espécies isoladas ou clados ainda não delimitados.

## 2 OBJETIVOS

### 2.1 Geral

Sistematizar e interpretar, a partir de análises morfológicas e moleculares, os caracteres informativos do gênero *Lactifluus*, com o incremento de novas coletas, incluindo material de herbário proveniente de diferentes biomas das regiões Norte e Nordeste do Brasil.

## 2.2 Específicos

- Ilustrar, descrever e identificar os espécimes coletados;
- Relacionar os espécimes coletados com os possíveis hospedeiros;
- Revisar material das regiões Norte e Nordeste depositados nos herbários INPA, JPB, UFRN-Fungos e URM;
- Realizar estudos comparativos com materiais já identificados e depositados em herbários;
- Verificar padrões de distribuição geográfica das espécies analisadas;
- Gerar sequências de DNA a partir de novos materiais coletados, e se possível, os de herbário, para incrementar a filogenia e sistemática do gênero, assim como incluí-los no banco de nucleotídeos (GenBank);
- Gerar árvores filogenéticas a fim de avaliar o status subgenérico de *Lactifluus*.

## 3 MATERIAL E MÉTODOS

### 3.1 Revisão de coleções de herbário

Os espécimes das coleções de Singer et al. (1983), Singer (1984), Wartchow & Cavalcanti (2010), Sá & Wartchow (2013) e Wartchow et al. (2013), disponíveis nos herbários (Instituto Nacional de Pesquisas da Amazônia), JPB (Lauro Pires Xavier, Universidade Federal da Paraíba), UFRN-Fungos (Universidade Federal do Rio Grande do Norte) e URM (Pe. Camille Torrend, da Universidade Federal de Pernambuco) foram criteriosamente revisados. O mesmo foi realizado para coleções de Rick (1906, 1907, 1930, 1938) disponíveis no Herbário PACA (Anchieta, do Instituto Anchietano de Pesquisas/Unisinos).

### 3.2 Áreas de coleta

As regiões Norte e Nordeste do Brasil estão sobre o domínio dos Biomas Amazônia, Mata Atlântica, Caatinga e Cerrado (IBGE 2004). Todos esses biomas apresentam certas complexidades em relação a sua flora. A riqueza biológica está relacionada às oscilações de tipos de solos, relevo, hidrografia e aos níveis de umidade estabelecidos em determinadas

regiões. A Mata Atlântica e o Cerrado são considerados dois *hotspots* mundiais, abrigando um grande número de espécies endêmicas (Oslon & Dinerstein 2002). A Floresta Amazônica é a maior floresta tropical do mundo e também abriga uma grande biodiversidade devido a sua grande extensão e seu ambiente heterogêneo (HUBBELL et al., 2008). Em todos esses três biomas ocorrem plantas ectomicorrízicas representantes de Fabaceae, Fagaceae, Nyctaginaceae e Polygonaceae (FREIRE 1990; BARBOSA et al. 2004; STROPP et al. 2011; DEMARCHI et al., 2018).

Para este estudo, foram realizadas 13 expedições a campo entre os anos de 2017 a 2019, em seis áreas de Mata Atlântica e Caatinga nos Estados da Paraíba e do Rio Grande do Norte (Tabela 2). Basidiomas coletados nos anos anteriores nos Estados da Bahia, de Pernambuco, do Amazonas e do Pará foram incorporados ao estudo, de acordo com a tabela 2.

### **3.3 Método de coleta e herborização**

No momento da coleta foram anotados dados como número de coletor e substrato. Os espécimes encontrados foram fotografados utilizando câmera fotográfica digital Nikon D3100. Posteriormente foram acondicionados em caixas organizadoras e/ou sacos de papel e levados ao laboratório para realização de análises morfológicas. A desidratação foi realizada em secadora de alimentos a uma temperatura de 40°C, e acondicionamento do material coletado em saco ziplock e por fim, devidamente depositados nos Herbários INPA, JPB e UFRN-Fungos.

### **3.4 Análise morfológica**

#### **3.4.1 Análise Macroscópica**

As análises macroscópicas foram realizadas logo após a coleta, contemplando características fundamentais dos basidiomas e seguindo a terminologia de Verbeken & Walley (2010). As cores das macroestruturas foram designadas de acordo com a carta de cores de Kernerup & Wanscher (1978).

#### **3.4.2 Análise Microscópica**

Tabela 2. Locais de coletas, origem dos materiais incorporados ao estudo com referência ao bioma, ecossistemas, estado, município e coordenadas geográficas.

Local	Bioma	Ecossistema	Estado	Cidade	Coordenadas
<b>Locais de coleta</b>					
Parque Estadual das Dunas	Mata Atlântica	Restinga	Rio Grande do Norte	Natal	5°49'12"S, 35°11'16"O
Reserva Biológica Guaribas	Mata Atlântica	Restinga	Paraíba	Mamanguape	6°43'17"S, 35°10'49"O
Parque Boca da Mata	Mata Atlântica	Restinga	Rio Grande do Norte	Boca da Mata	9°38'31" S, 36°13'11"O
RPPN Mata Estrela	Mata Atlântica	Restinga	Rio Grande do Norte	Canguaretama	6°22'58" S, 35°7'30'O
Parque estadual Mata do Pau Ferro	Caatinga	Brejo de altitude	Paraíba	Areias	6°57'58.9"S, 35°44'55.15"O
RPPN Fazenda das Almas	Caatinga		Paraíba	São José dos Cordeiros	7°28'45"S, 36°54'18"O
<b>Local de origem dos espécimes incorporados</b>					
Mata do Tijuquinho	Caatinga	Campos rupestres	Bahia	Abairá	13°15'S, 41°39'50"O
'Reserva Biológica Charles Darwin'	Mata Atlântica	Floresta ombrofila densa	Pernambuco	Igarassu	07°48'37"S, 34°56'5"O
'CIAPA'	Amazônia	Terra firme	Amazonas	Manaus	2°38'20.02"S, 60°56'22.15"O
Balbina	Amazônia	Campinarana	Amazonas	Presidente Figueiredo	1°55'49.69"S, 59°29'44.94"O
Itacoatiara Mirim	Amazônia	Campinarana	Amazonas	São Gabriel da Cachoeira	0°7'48"S, 67°5'20"O
Estação Experimental de Manejo Florestal ZF-2	Amazônia	Terra firme	Amazonas	Manaus	02°37';02°38'S, 60°09';60°11'O
Floresta Nacional do Tapajós	Amazônia	Terra firme	Pará	Belterra	2°48'14"S, 54°59'50"O

As análises microscópicas foram realizadas através de secções dos basidioma secos. Para reidratação e visualização de microestruturas, foram montadas lâminas com hidróxido de potássio (KOH) 3% e corante vermelho congo 1% (Largent et al. 1977). O reagente de Melzer foi utilizado para observar a reação amiloide da ornamentação dos esporos. As terminologias utilizadas para descrever as estruturas microscópicas observadas foram de acordo com a proposta de Verbeken & Walley (2010). As estruturas microscópicas foram desenhadas através de sistema de câmara clara adaptado ao microscópio óptico. Para a identificação dos espécimes foram utilizadas literaturas especializadas que contêm descrição das espécies do Neotrópico, como as de Pegler & Fiard 1979, Singer et al. (1983) e Singer (1984), dentre outras.

### 3.4.3 Microscopia Eletrônica de Varredura

A análise por Microscopia Eletrônica de Varredura foi realizada a fim de detalhar a morfologia das estruturas microscópicas da ornamentação dos esporos. Para isso, uma porção da lamela foi destacada e fixada por fita adesiva de carbono em suportes de alumínio. Os suportes com as amostras foram submetidos a metalização em metalizador Balzers SCD030 – Balzers Union FL9496, revestindo o material com uma camada de ouro. Finalmente, o material foi observado ao MEV Jeol JSM-6360LV sob tensão de 15-20 Kv, em magnificação variável (2.000-30.000x), dependendo do tamanho do esporo a ser examinado.

## 3.5 Análises moleculares

### 3.5.1 Extração, amplificação (PCR), purificação e sequenciamento de DNA

Para extração de DNA, foram retirados pequenos fragmentos da lamela dos basidiomas secos. Os fragmentos foram triturados com o auxílio de uma lâmina estéril. Foi utilizado o kit da QIAGEN DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A). Os protocolos seguiram conforme o fabricante, com uma modificação: as amostras foram submetidas ao 'Buffer 1' em temperatura de 55–60°C durante 24 horas. Para extração do DNA de espécimes mais antigos, como os coletados por Singer em 1978 a 1980 e Rick 1890, foram utilizados kits forenses de extração (FTA Cards), seguindo a metodologia de extração baseada em Telleria et al. (2014). O produto da extração foi verificado em gel agarose a 1% a fim de verificar a presença do DNA extraído.

A amplificação foi realizada por meio de PCR, utilizando ‘PCR beads’ (Illustra™ PuReTaq Ready-To-Go PCR Beads). Nos tubos de ‘PCR beads’ foram adicionados 1 µl de cada primer, amostra de DNA e água ultrapura estéril, resultando em um volume final de 25 µl. Em algumas amostras foi utilizado o taq polimerase ‘FIREPol 5x Master Mix’, ao qual foi adicionado 0,6 µl de cada primer, 4 µl de taq polimerase, amostra e água ultrapura estéril, resultando em um volume final de 23 µl. Os produtos da PCR foram analisados por eletroforese em gel de agarose 0,5 %.

As regiões alvo do DNA foram as regiões rDNA ITS (ITS1 + 5.8S+ ITS2), rDNA LSU (= 28S) e a segunda maior subunidade da RNA polimerase II (*RPB2*). Para a região ITS foram utilizados os pares de iniciadores ITS1F/ITS4B (WHITE et al., 1990; GARDENS & BRUNS, 1993) e ITS1/ITS4 (GARDES & BRUNS, 1993). Para a região LSU foram utilizados os iniciadores LR0R/LR7 ou LR5 (VIGALYS & HESTER, 1990; REHNER & SAMUELS, 1994). E, por fim, para a região *RPB2* foram utilizados os primers RPB2-5F/RPB2-7.1R (Matheny, 2005) e os internos BRPB2-6F/BRPB2-7R2 (MATHENY, 2005). As amostras amplificadas foram purificadas utilizando o kit de enzimas ‘ExoSAP-IT1’ (USB Corporation, OH, USA), de acordo com o protocolo do fabricante. Posteriormente, as amostras foram enviadas à Coreia do Norte para sequenciamento na empresa Macrogen.

As sequências consenso foram editadas e obtidas com auxílio do programa Sequencher 5.2.4 (Gene Codes Corp, USA). Os consensos obtidos foram submetidos a uma busca no BLAST (Genbank/ncbi.nlm.nih.gov) a fim de verificar possíveis contaminações.

### 3.5.2 Análises filogenéticas

Os alinhamentos das sequências obtidas e das sequências selecionadas do GenBank foram inicialmente realizados no MAFFT, utilizando a estratégia de configurações avançadas E-INS-i (KATOH & STANDLEY, 2013). Quando necessário, as sequências da matriz alinhada foram editadas manualmente no software Seaview version versão 4.7 (GOUY et al., 2010).

As análises filogenéticas foram realizadas sobre dois critérios: Máxima Verossimilhança (MV) e Inferência Bayesiana (IB). Uma primeira análise particionada foi realizada a fim de verificar a topologia das árvores de acordo com cada marcador. As análises finais foram realizadas a partir de uma matriz concatenada. As análises de MV utilizaram RAxML-HPC v.8 com os modelos evolutivos GTRCAT (análise concatenada) ou GTRGAMMA (apenas um marcador), bootstrap (MVbs) de mil replicações. Os modelos

evolutivos para a IB foram selecionados com AIC (*Akaike Information Criterion*) usando jModelTest 2v.1.6 (DARRIBA et al., 2012). A IB foi realizada usando Mr Bayes 3.1.2 (RONQUIST & HUELSENBECK, 2003), com duas corridas independentes, cada uma começando a partir de árvores aleatórias com quatro cadeias independentes simultâneas, realizando gerações  $2 \times 10^7$  mcmc, amostrando uma árvore em cada geração  $1 \times 10^3$ . As primeiras  $5 \times 10^3$  árvores amostradas foram descartadas como *burn-in*, enquanto as restantes foram usadas para reconstruir uma árvore de consenso de regra (maioria de 50%) e para calcular as probabilidades posteriores (BPP) dos clados. O RAxML-HPC v.8, jModelTest 2v.1.6 e MrBayes 3.1.2 foram usados no CIPRES Science Gateway 3.1 (MILLER et al., 2010).

As probabilidades posteriores (PP) e os valores de *bootstrap* foram levados em conta para identificar o grau de confiabilidade dos clados (LUTZONI et al., 2004). As árvores obtidas foram visualizadas e editadas no FigTree e depois exportadas para o COREL DRAW. Todas as sequências matrizes e árvores geradas foram depositadas no GenBank e TreeBase.

Para avaliar a posição filogenética das espécies nos subgêneros e seções, foi realizada inicialmente uma análise MV a partir de uma matriz concatenada com sequências selecionadas do GenBank de novas de ITS, LSU e *RPB2* (apêndice 1, Figuras 7–10). A matriz contém espécies dos gêneros *Lactarius*, *Multifurca* e *Russula*; e as sequências de *Lactifluus* utilizadas na análise de De Crop et al. (2017) com inclusão de algumas outras de Barbosa et al. (2020), e Delgat et al. (2020). Para essa análise foram utilizadas as espécies *Auriscalpium vulgare* Gray, *Bondarzewia montana* (Qué.) Singer, *Heterobasidion annosum* (Fr.) Bref. e *Stereum hirsutum* (Willd.) Pers como *outgroup*. As análises filogenéticas incluídas nos artigos foram realizadas a fim de mostrar as relações filogenéticas dos subgêneros, e seguiram o método de acordo com cada matriz de sequências analisadas.

## 4 RESULTADOS

Inicialmente foram revisadas dez exsiccatas de sete diferentes espécies das coletas publicadas por Rick (1906, 1907 e 1938), Singer et al. (1983), Singer (1984), Wartchow & Cavalcanti (2010). A revisão morfológica dessas coleções resultou na transferência de seis espécies de *Lactarius* para *Lactifluus*: *Lactifluus amazonensis* (Singer) Silva-Filho & Wartchow, *Lf. brasiliensis* (Singer) Silva-Filho & Wartchow, *Lf. braunii* (Rick) Silva-Filho & Wartchow, *Lf. mamorensis* (Singer) Silva-Filho & Wartchow, *Lf. rupestres* (Wartchow) Silva-Filho & Wartchow e *Lf. russula* (Rick) Silva-Filho & Wartchow. Além disso foi

determinado a designação de um lectótipo para *Lf. annulifer* (Singer) Nuytinck, *Lf. braunii* e *Lf. russula*.

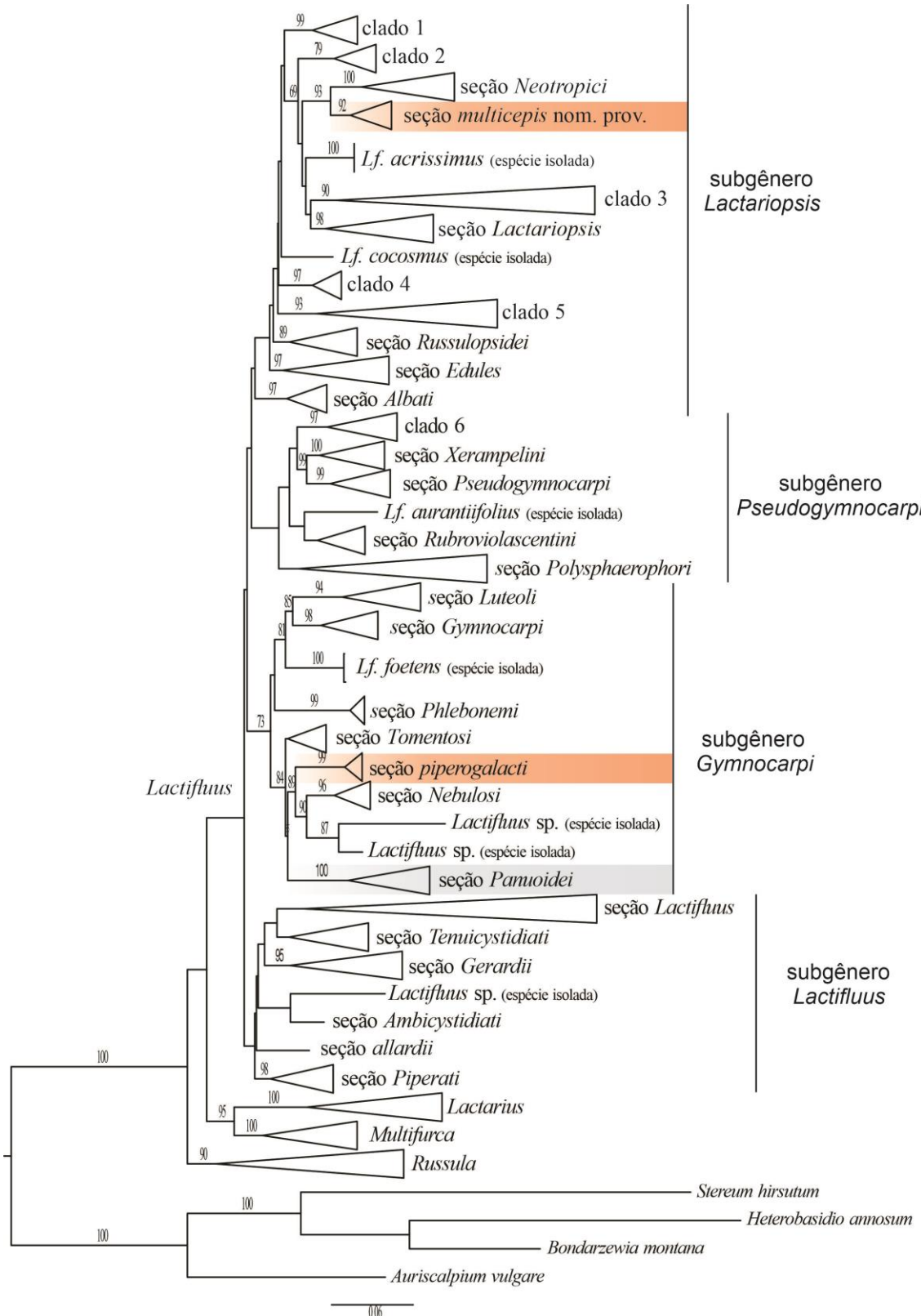
Além dessas exsiccatas, foram analisados outros 14 espécimes coletados pelo autor e seu orientador no Parque Estadual das Dunas (RN), Parque Estadual Mata do Pau Ferro (PB) e na Reserva Biológica Guaribas (PB) entre os anos de 2017 e 2019. Outros 17 espécimes previamente coletados a partir do ano de 2012 na Amazônia, Mata Atlântica e Caatinga (Tabela 2) foram incorporados ao estudo. A partir de tais amostras foram geradas 21 sequências de ITS, seis de LSU e 10 de *RPB2*. As amostras herborizadas não foram sequenciadas devido ao estado de conservação das exsiccatas, pois muitas delas continham DNA degradado ou estavam contaminadas por *Aspergillus* spp. As análises morfológicas e moleculares resultaram na identificação de 17 diferentes espécies, sendo 11 delas ainda novas para a ciência.

A análise filogenética inicial resultou em uma árvore (Figuras 7–10) com topologia similar da gerada por De Crop et al. (2017). Os espécimes coletados no Nordeste e Norte brasileiros estão ancorados em cinco diferentes clados nos subgêneros *Gymnocarpi*, *Lactariopsis* e *Pseudogymnocarpi*. Não foram encontradas espécies do subgênero *Lactifluus*, corroborando a hipótese de que as espécies do subgênero *Lactifluus* não se diversificaram na América do Sul (DE CROP et al., 2017).

No subgênero *Gymnocarpi*, três espécies foram descritas: *Lactifluus guttulatus* Silva-Filho, D.L. Komura & Wartchow, *Lf. spathuliformis* Silva-Filho, D.L. Komura & Wartchow e *Lf. piperogalactus* Silva-Filho, Sá & Wartchow. *Lactifluus lepus* Delgat & Courtec. originalmente descrito para Guadalupe (Caribe) passa a ter sua distribuição ampliada para a América do Sul. As espécies pleurotoides *Lf. guttulatus* e *Lf. spathuliformis* formam, juntamente com a espécie tipo *Lf. panuoides*, a seção *Panuoidei*. Essa seção foi emendada a partir da morfologia dessas três espécies. As espécies agaricoides *Lf. piperogalactus* e *Lf. lepus* passaram a formar um clado fortemente suportado, que foi delimitado e nomeado como *Lactifluus* seção *Piperogalacti* Silva-Filho & Wartchow (Figura 10).

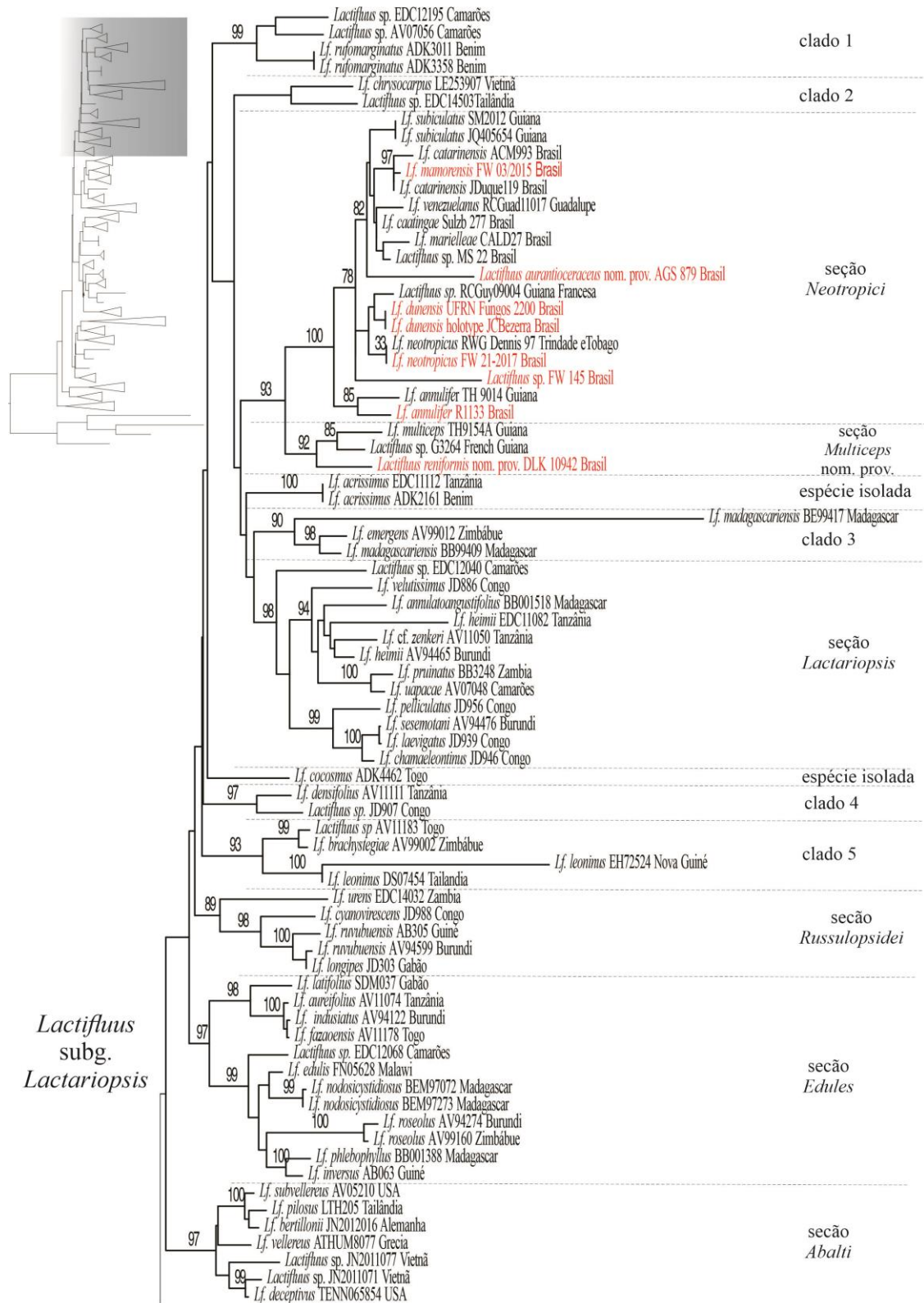
No subgênero *Lactariopsis*, *Lf. aurantioceraceus* nom. prov. será proposta como nova em *Lf.* seção *Neotropici*. Adicionalmente, foram incluídas na seção sequências de *Lf. mamorensis*, *Lf. annulifer*, *Lf. neotropicus* e a sequência da espécie tipo de *Lf. dunensis*. *Lactifluus reniformis* nom. prov. será proposto como novo em um clado irmão à seção *Neotropici*. O clado abriga também *Lf. multiceps* (S.L. Mill., Aime & T.W. Henkel) De Crop e uma espécie da Guiana Francesa ainda não descrita (G3264). Esse clado é fortemente suportado e será delimitado e nomeado: *Lf.* seção *Multicipis* nom. prov. (Figura 8).

Figura 7. Arvore filogenética gerada a partir da análise de Máxima Verossimilhança dos marcadores ITS, LSU e *RPB2* de seqüências de *Lactarius*, *Lactifluus*, *Multifurca* e *Russula*. Novas seções delimitadas foram marcadas em vermelho, a seção emendada marcada em cinza. A barra de escala representa o número esperado de mudanças nucleotídicas.



Fonte: Elaborado pelo autor (2021).

Figure 8. Árvore filogenética gerada a partir da análise de Máxima Verossimilhança dos marcadores ITS, LSU e *RPB2* de sequências de *Lactifluus* subg. *Lactariopsis*. Espécies em vermelho representam sequências de espécies geradas neste trabalho.



Fonte: Elaborado pelo autor (2021)

Figure 9. Árvore filogenética a partir da análise de Máxima Verossimilhança dos marcadores ITS, LSU e *RPB2* de seqüências de *Lactifluus* subg. *Pseudogymnocarpi*. Espécies em vermelho representam seqüências de espécies geradas neste trabalho.

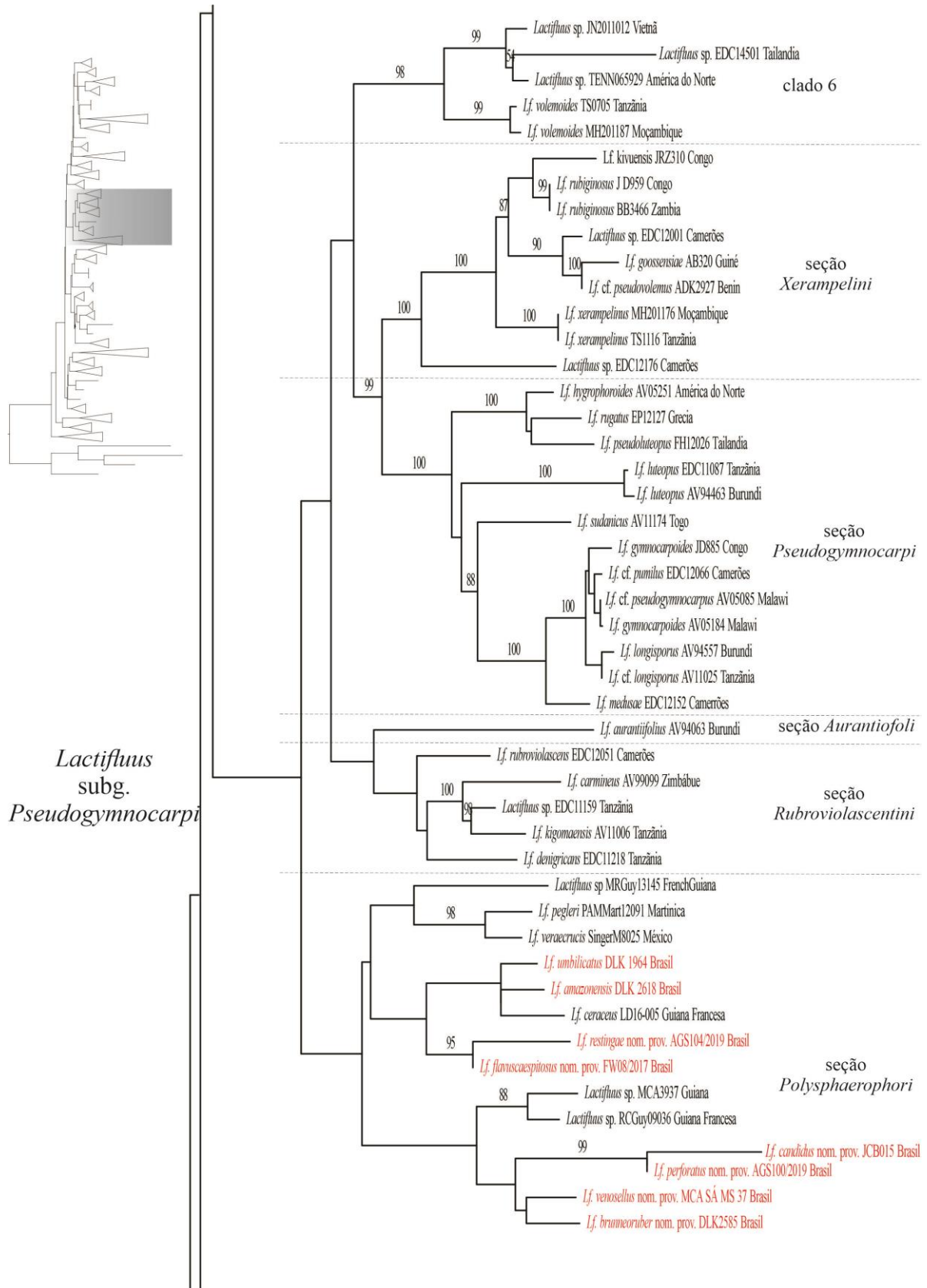
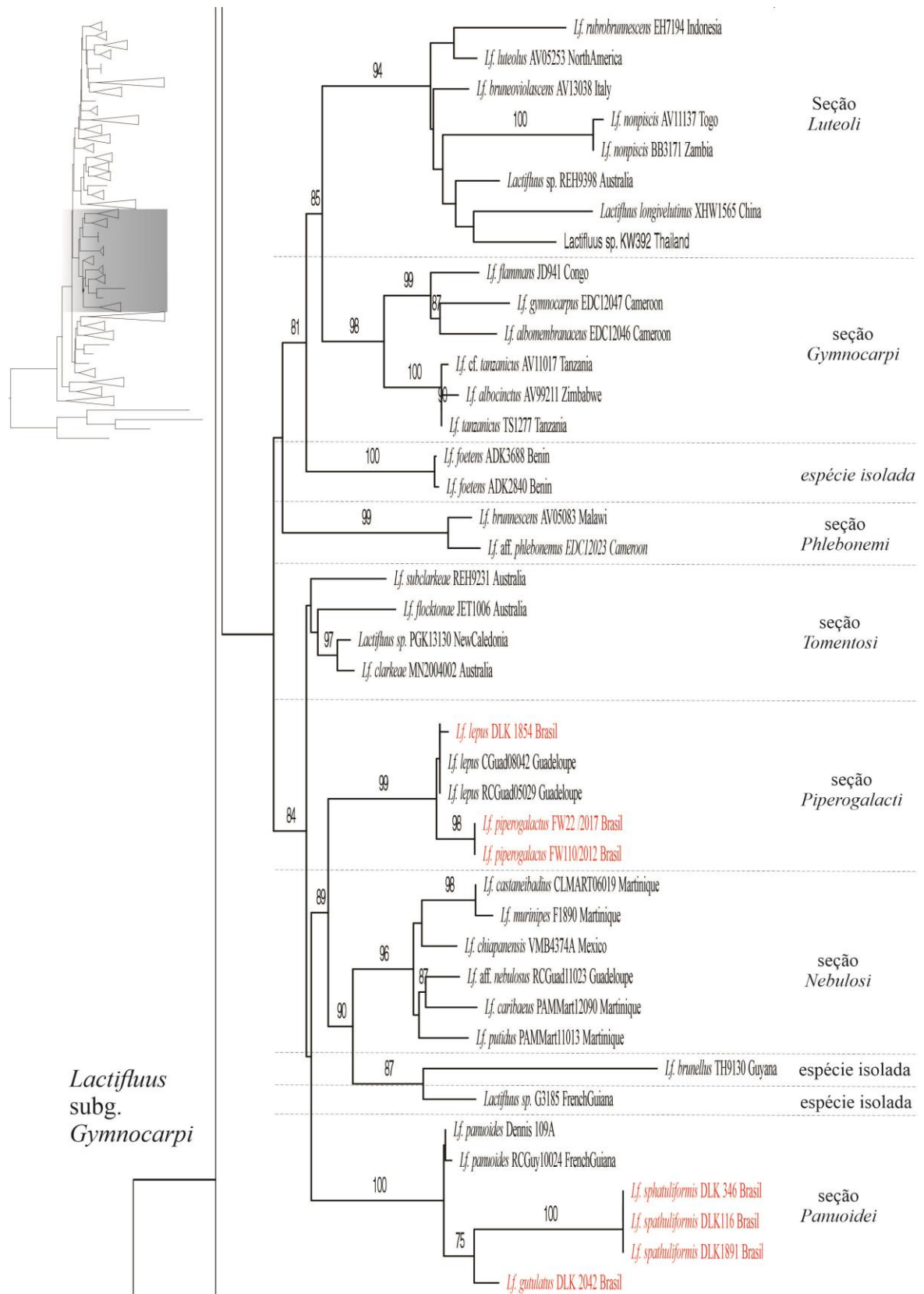


Figure 10. Árvore filogenética a partir da análise de Máxima Verossimilhança dos marcadores ITS, LSU e *RPB2* de sequências de *Lactifluus* subg. *Gymnocarpi*. Espécies em vermelho representam sequências de espécies geradas neste trabalho.



Fonte: Elaborado pelo autor (2021).

O subgênero *Pseudogymnocarpi* abriga a maior diversidade na região tropical do Brasil. Seis novas espécies estão ancoradas na seção *Polysphaerosphori*: *Lf. umbilicatus* Silva-Filho D. L. Komura & Wartchow foi proposta como nova espécie enquanto *Lf. brunneoruber* Silva-Filho D. L. Komura & Wartchow nom. prov., *Lf. candidus* Silva-Filho, Sá & Wartchow nom. prov., *Lf. flavuscaespitosus* Silva-Filho & Wartchow nom. prov., *Lf. restingae* Silva-Filho & Wartchow nom. prov. e *Lf. perforatus* Silva-Filho & Wartchow nom. prov. serão propostas como novas espécies. Além dessas, *Lf. venosellus* Silva-Filho, Sá & Wartchow, incorporada a partir dos estudos realizados por Sá (2018), também foi descrita como nova espécie. Já *Lf. amazonensis* foi identificada e confirmada na seção (Figura 9).

Os resultados obtidos serão apresentados em formas de capítulos. Os três primeiros capítulos já foram publicados com o resultado da revisão das coleções dos herbários, a descrição de novas espécies nas seções *Polysphaerophori*, *Panuoidei* e *Piperogalacti*, a delimitação das seções *Piperogalacti* e a emenda da seção *Panuoidei*. O quarto e quinto capítulos ainda serão submetidos como apresentados a seguir, e trazem novidades para o subgênero *Lactariopsis* e também para a seção *Polysphaerosphori*.

Capítulo 1: Type studies and new combinations in Brazilian milkcaps (*Lactifluus*, Russulaceae)

Artigo publicado no ano de 2019 no periódico New Zealand Journal of Botany (Qualis B1). Este manuscrito apresenta uma revisão dos tipos das espécies de *Lactifluus* publicadas por Rick, Singer e coautores, e Wartchow & Cavalcanti.

Capítulo 2: Two novel species of *Lactifluus* subg. *Pseudogymnocarpi* (Russulaceae) from Brazil

Artigo publicado no ano de 2020 na Revista Phytotaxa (Qualis B1). Duas novas espécies, *Lactifluus umbilicatus* da Amazônia e *Lactifluus venosellus* da Mata Atlântica foram descritas a partir da morfologia e inferência filogenética da região ITS. Ambas pertencem à *Lf.* sub. *Pseudogymnocarpi*.

Capítulo 3: Novelties in *Lactifluus* subg. *Gymnocarpi* (Russulales, Basidiomycota) from Brazilian tropical forests

Artigo publicado no ano de 2021 no periódico Mycological Progress (Qualis A3). Com base em dados morfológicos e moleculares (ITS e *RPB2*) a seção *Piperogalacti* foi delimitada, a seção *Panuoidei* emendada e três novas espécies foram descritas como novos táxons: *Lf. guttulatus*, *Lf. piperogalactus* e *Lf. spathuliformis*; *Lf. lepus* também registrada como nova ocorrência para a América do Sul.

Capítulo 4: Integrative taxonomy of *Lactifluus* subg. *Lactariopsis* (Russulales/Basidiomycota) from Brazilian tropical forests

Artigo a ser submetido à revista Botany (Qualis A3). Baseado em dados morfológicos e moleculares (ITS e LSU) a seção *Multiceps* nom. prov. é delimitada e nomeada, *Lf. aurantioceraceus* nom. prov. e *Lf. reniformis* nom. prov. são propostas como novas, *Lf. mamorensis* e *Lf. dunensis* são confirmados na seção *Neotropici* e *Lf. netropicus* é um novo registro para a Caatinga.

Capítulo 5: New diversity of *Lactifluus* section *Polysphaerosphori* (Russulales/Basidiomycota) from Brazilian tropical forests

Artigo será submetido ao periódico Systematic Diversity (Qualis A2). O manuscrito traz a descrição de cinco novas espécies na seção *Polysphaerosphori*.

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**6 Capítulo 1 - Type studies and new combinations in Brazilian milkcaps (*Lactifluus*, Russulaceae)**

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## Abstract

In the tropics, the diversity of ectomycorrhizal milkcaps is predominantly represented by genus *Lactifluus*. In Brazil, species traditionally described as *Lactarius* need to be revised and recombined. Thus, seven milkcaps from Amazonia and Rio Grande do Sul, Brazil, published by Rick, Singer and co-authors were reviewed. *Lactarius amazonensis*, *L. brasiliensis*, *L. braunii*, *L. mamorensis* and *L. russula*, along with the recently described *L. rupestris*, are combined in *Lactifluus*. In addition, *Lactifluus annulifer* together with *Lf. braunii* and *Lf. russula*, have lectotypes designated. A modern and detailed microscopic description, drawings and taxonomic discussion are provided for each species.

**Keywords:** Agaricomycetes; *Lactarius*; Neotropics; nomenclature; Russulales; taxonomy

## Introduction

Milkcaps are represented by three phylogenetically well-supported genera: *Lactarius* Pers., *Lactifluus* (Pers.) Roussel and *Multifurca* Buyck & V. Hofst. (Buyck et al. 2008, 2010). In tropical South America, the diversity of these mushrooms are well represented by the genus *Lactifluus* (Smith et al. 2011; Sá and Wartchow 2013; Wartchow et al. 2013; Crous et al. 2017; Sá et al. 2019), *Multifurca* does not occur in the region, and *Lactarius* s.s. refers to species associated with *Pinus* spp. (e.g., Giachini et al. 2004; Chávez et al. 2015). However, before multilocus phylogenies (Buyck et al. 2008) and nomenclatural changes in Russulaceae (Buyck et al. 2010), all milkcaps were taxonomically treated as *Lactarius*, including taxa published by Rick, Singer and co-authors from Brazil.

In Brazil, the first milkcap described was *Panus reticulatus* Berk. from the State of Amazonas (Berkeley 1856), later transferred by Singer (1955) to *Lactarius reticulatus* (Berk.) Singer. Rick (1906, 1907, 1930) described *L. braunii* Rick, *L. russula* Rick and *L. steffenii* Rick from Rio Grande do Sul, but only the first two species have preserved exsiccatae (Singer 1953). Singer et al. (1983) and Singer (1984) carried out the most relevant studies on Brazilian milkcaps, in which *L. amazonensis* Singer, *L. annulifer* Singer, *L. brasiliensis* Singer, *L. campinensis* Singer, *L. gigasporus* Singer, *L. igapoensis* Singer, *L. mamorensis* Singer, *L. pallidipes* Singer, *L. paulensis* Singer and *L. subpallidipes* were described mostly from Brazilian Amazon lowlands. Furthermore, other taxa were reported from Brazil: *L. reticulatus*

was reviewed by Pegler and Fiard (1979), *L. paulensis* was reported by Pegler (1997) from São Paulo, *Lactifluus venezuelanus* (Dennis) De Crop was recorded by Buyck and de Meijer (1999, as *Lactarius venezuelanus* Dennis) and by de Meijer (2006, as *L. venezuelanus*) from Paraná, and *Lactarius rupestris* Wartchow was described from Pernambuco (Wartchow and Cavalcanti 2010). The most recent studies described additional milkcap species from Brazil, but now with the correct taxonomic treatment: *Lf. aurantiorugosus* Sá & Wartchow from Rio Grande do Sul (Sá and Wartchow 2003), *Lf. batistae* Wartchow, J.L. Bezerra & M. Cavalc. from Bahia (Wartchow et al. 2013), *Lf. dunensis* Sá & Wartchow from Rio Grande do Norte (Sá et al. 2013) and *Lf. caatingae* Sá & Wartchow from Piauí (Sá et al. 2019). Additionally, five known species of *Lactarius* s.s. were listed and one new species, *L. taedae* Silva-Filho, Sulzbacher & Wartchow was described in *Pinus* plantations from southern Brazil (Sulzbacher et al. 2013; Silva-Filho et al. 2018).

Brazilian milkcaps described between 1906 and 2010, and formerly treated as *Lactarius*, need to be reviewed and recombined. Therefore, our aim is to review the *Lactarius* species described by Rick, Singer and co-authors, housed in Field Museum of Natural History (F), Instituto Anchieta de Pesquisas/UNISINOS (PACA), Instituto Nacional de Pesquisas da Amazônia (INPA), Royal Botanic Gardens (K) and Universidade Federal de Pernambuco (URM) herbaria (Thiers 2019, continuously updated), and to provide a modern microscopic description and elucidate their generic placement. All preserved species were analysed, described and discussed. Drawings are provided and a new nomenclatural treatment is proposed. Basidiospores of *L. mamorensis* and *L. braunii* were viewed under scanning electron microscopy (SEM).

### **Materials and methods**

Exsiccated specimens deposited at F, INPA, K, PACA and URM were requested. Studies were performed using the emended methodology of Verbeken (1998) and Verbeken and Walley (2010). In Singer's original descriptions, the code colour 'M&P' refers to Maerz &

Paul (1950) colour chart. Basidiospores were mounted in Melzer's reagent and measured in lateral view, excluding ornamentation. Dimensions of basidiospores with  $n \geq 20$  are given as (<10%, minimum length) average minimum length – average maximum length (<10%, maximum length)  $\times$  (<10%, minimum width) average minimum width – average maximum width (<10%, maximum width) and with  $n < 20$  are given as minimum length – average maximum length  $\times$  average minimum width – average maximum width. In the basidiospore description, L(W) = length (width) average from a single basidioma, Q is the quotient between length and width, Qm is the median value of Q, 'n' is the number of measured basidiospores/number of analyzed basidiomata/number of collections. Other structures were mounted in 5% KOH and Congo red, ten structures were measured and the values given as minimum – maximum. To visualize cystidia, cresyl blue and sulpho-vanillin were used. For scanning electron microscopy (SEM), the dried lamellae were directly stuck on an adhesive layer on a small clean coverslip, then coated with gold in a metalizer (Bal-Tec SCD-050, Balzers, Schaan, Liechtenstein) and observed with a scanning electron microscope (JSM-6360LV, JEOL Ltda, Akishima, Tokyo, Japan).

## Results

The following species were analysed: *Lactarius braunii* and *L. russula* from Rio Grande do Sul (Rick 1907, 1930), *L. amazonensis*, *L. brasiliensis*, *L. mamorensis*, and *Lactifluus annulifer* from Amazonia (Singer et al. 1983).

***Lactifluus amazonensis*** (Singer) Silva-Filho & Wartchow comb. nov. (Figure 1 & Figure 7A)

$\equiv$  *Lactarius amazonensis* Singer in Singer et al., Beih. Nova Hedwigia 77: 295 (1983).

Mycobank: MB831814

**Material examined:** Brazil, Amazonas, Manaus, Manaus-Caracaraí road, km 125, 20 July 1978, leg. R. Singer B11315 (INPA 82756, holotype).

**Original macroscopic description:** ‘Pileus deep yellow (deeper than “Spanish y”, dried 11G6 on margin and “Alamo” in the center, or “deep chrome” and “nugget” on margin reaching “orange pell” at centre), not viscid, glabrous, with shallowly and somewhat coarsely sulcate, or short-folded margin, convex with depressed, 33–45 mm broad. Lamellae whitish, cream-white, then cream (M&P), the edges entire and concolorous, the sides dried whitish pulverulent only when seen under a lens, unchanging when injured, distant to very distant, moderately broad, didymous, not or scarcely intervenose, some forked, irregularly adnexed, to adnate with some short decurrent, intermixed. Spore print not obtained. Stipe concolorous with the pileus when fresh, concolorous with the dried margin of the pileus, when dried, short ribbed at apex only, dry, not viscid, equal 19–48 × 6.5–9.5 mm; veil none. Context almost concolorous with the surfaces but paler, odor none, taste mild. Latex watery scanty.’

**Microscopic description** – Basidiospores 8–9 × 7–8 μm; (n = 15/1/1); L = 8.6 μm, W = 7.4 μm; Q = 1.06–1.21, Qm = 1.16; subglobose to broadly ellipsoid, predominantly subglobose, thin-walled, hyaline; ornamentation amyloid, up to 0.5 μm high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid; hilar appendix up to 1 μm long (Figure 1A). Basidia 50–64 × 10–15 μm, cylindrical to cylindrical-clavate, 2-or 4-spored, thin-walled, hyaline with refractive contents in the lower half; sterigmata up to 8 μm long (Figure 1B). Pleuromacrocystidia frequent to abundant, 8–13 μm diam., cylindrical, fusoid, thin-walled; refractive contents in lower half; projecting up to 63 μm above the hymenium (Figure 1D). Pleuropseudocystidia not found. Hymenophoral trama poorly rehydrated, composed of abundant sphaerocytes 15–35 μm diam., hyaline and scattered lactifers 6–7 μm diam. Lamellar edge not rehydrated. Pileipellis well rehydrated; a lampropalisade, the subpellis consisting of a layer of isodiametric cells, 9–14 μm diam., thin-walled, hyaline and the suprapellis composed by abundant hair-shaped, thick-walled elements, hair-like terminal elements 20–63 × 4–6 μm, cylindrical to cylindrical-clavate, sometimes fusoid, sometimes flexuous, mostly thick-walled, (up to 3 μm thick), hyaline, sometimes

arising deeply from subpellis (Figure 1C). Pileus trama not rehydrated. Stipitipellis and stipitirama not rehydrated. Clamp connections absent in all parts examined.

**Habitat:** ‘On raw humus in campinarana vegetation in Amazonia, fruiting late, in the rain season, associated with Leguminosae and Sapotaceae’ (Singer et al. 1983).

**Known distribution:** Known only from Brazilian Amazonia (Singer et al. 1983).

**Observation on exsiccata:** The holotype consists of a single deteriorated basidioma (Figure 7A).

**Notes:** In the protologue, *Lactifluus amazonensis* was described as having small basidiome, orange pileus with striate to sulcate margin, pileipellis with abundant cylindrical to fusoid thick-walled elements with obtuse apex and frequent to abundant macropleurocystidia, which are projected above the hymenium (Singer et al. 1983). In addition, also described with short ( $16\text{--}25 \times 5\text{--}6 \mu\text{m}$ ) and ‘thin- to firm-walled dermatocystidia on the pileipellis’, but the holotype analysed by us has longer ( $20\text{--}63 \mu\text{m}$  long) terminal elements with a thicker wall (up to  $3 \mu\text{m}$  thick). Although the presence of pleuromacroscystidia was confirmed, the poorly rehydrated lamellar trama makes observation of the base of this structure difficult. Singer et al. (1983) considered *L. amazonensis* in sect. *Polysphaerophori* due to its agaricoid habit, absence of velum and presence of sphaerocytes in lamellar and pileus trama. Obviously, it corresponds to an artificial group, since a heteromerous lamellar trama possessing sphaerocytes is frequent in all current *Lactifluus*. Actually, *Polysphaerophori* is a clade/section of the subg. *Pseudogymnocarpi* (Verbeken) De Crop, which corresponds to a not yet morphologically delimited group with Neotropical species (De Crop et al. 2017). Based on the new infrageneric classification proposed by De Crop et al. (2017), *Lf. amazonensis* might be a member of the subg. *Pseudogymnocarpi* due to the orange pileus, unchanging context and latex, and the lampropalisade pileipellis (De Crop et al. 2017), probably a member of the sect. *Polysphaerophori* sensu De Crop et al. (2017).

*Lactifluus ceraceus* Delgat & M. Roy from French Guiana is a similar Neotropical entity of subg. *Pseudogymnocarpi*, confirmed in the sect. *Polysphaerophori* by molecular data. *Lactifluus ceraceus* differs in the more robust basidiomata (pileus 64–98 mm, stipe 83–112 × 18–26 mm), smaller basidiospores (5.4–8.1 × 4.4–6.2 μm) and thinner (5–9 μm wide) pleuromacrocystidia (Crous et al. 2017).

*Lactifluus annulifer* (Singer) Nuytinck in Verbeken et al., Mycotaxon 118: 450 (2011). (Figure 2 & Figure 7B)

≡ *Lactarius annulifer* Singer in Singer et al., Beih. Nova Hedwigia 77: 290 (1983).

**Material examined:** Brazil, Amazonas, Manaus, Manaus-Caracará road, km 45, 20 July 1978, leg. E. Freire and R. Singer B 11353 (INPA 82326, lectotype, here designated).

**Original macroscopic description:** ‘Pileus flesh color (“toltec” M&P) then ochraceous (“desert” M&P) not hygrophanous, not viscid, subtomentose, when fresh, tomentose, when dried, 56–117 mm broad when mature and then convex with depressed center, young more convex and often with lobed or coarsely crenate margin which is sterile-projecting (about 2 mm wide) not rugose anywhere. Lamellae (“willow” M&P), narrow when young, intermixed, close, short decurrent. Stipe very pale orange, smooth, not radicate, cylindrical, or attenuated, in lower half 25–40 × 12–19 mm; veil annuliform, strictly apical, well-formed but narrow, concolorous with continuous margin of the pileus at first. Context pale orange, unchanging but in worm hole of the stipe eventually discolored (e.g., “willow” M&P). Odor agreeable, “mushroomy”.’

**Microscopic description:** Basidiospores 9–12 × 8–10 μm; L = 9.8 μm, W = 8.9 μm, (n = 15/1/1); Q = 1.00–1.33 Qm = 1.18; subglobose to broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 2 μm high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid; hilar appendix short, up to 2 μm long (Figure 2A). Basidia 50–62 × 11–16 μm, clavate, 4-spored, thin-walled, hyaline, sometimes with refractive contents; sterigmata up to 9 μm long.

Pleurocystidia absent. Pleuropseudocystidia frequent to scattered, 8.5–22.5  $\mu\text{m}$  diam., fusoid to fusiform, thin-walled; with refractive contents; projecting up to 32  $\mu\text{m}$  above the hymenium; arising deeply from hymenophoral trama (Figure 2B). Hymenophoral trama moderately rehydrated; composed of abundant sphaerocytes 13–21  $\mu\text{m}$  diam. and scattered lactifers 6–13  $\mu\text{m}$  diam. Lamella edge not rehydrated. Pileipellis rehydrated only the suprapellis; the suprapellis composed by hair-like terminal elements, 37–79  $\times$  5–12  $\mu\text{m}$ , abundant, cylindrical to fusoid slightly flexuous, sometimes forked, thick-walled (2–4  $\mu\text{m}$  thick), acute apex, hyaline to yellowish (Figure 2C). Pileus trama not rehydrated. Stipitipellis and stipitirama not rehydrated. Clamp connections absent in all parts examined.

**Habitat:** ‘On raw humus in campinarana vegetation in Amazonia, fruiting late in the rainy season, associated with Leguminosae and Sapotaceae’ (Singer et al. 1983).

**Known distribution:** Known only from Amazonia of Brazil and Guyana (Singer et al. 1983; De Crop et al. 2017).

**Observation on exsiccata:** The exsiccata ‘Singer B 11353’ is composed of two basidiomata (Figure 7B). Most tissues were poorly rehydrated.

**Notes:** *Lactifluus annulifer* is characterized by the orange pileus, presence of a secondary velum, pileipellis with abundant thick-walled elements with forked apex, and non-abundant pleuropseudocystidia (Singer et al. 1983). *Lactifluus annulifer* along with the annulate *Lactifluus neotropicus* (Singer) Nuytinck from Trinidad were previously grouped in the sect. *Lactariopsidei* Singer (Singer 1942), later combined in *Lactifluus* and transferred to the sect. *Lactariopsis* Verbeken (Verbeken et al. 2011). More recently, studies revealed that these taxa together with other Neotropical species form a natural grouping within subg. *Lactariopsis* (Henn.) Verbeken (De Crop et al. 2017). Despite both species possess a secondary velum, *Lf. neotropicus* differs in the more delicate basidiomata with pileus up to 40 mm and stipe 25–40  $\times$  12–19 mm (Pegler and Fiard 1979), somewhat smaller basidiospores (6.5–10  $\times$  6–8  $\mu\text{m}$ ,

Pegler and Fiard 1979) and not forked thick-walled elements on pileipellis (Singer 1952; Pegler and Fiard 1979; Pegler 1983).

The holotype ('R. Singer B 12087') was not found at INPA, and has possibly been lost. Thus, a lectotype needs to be designated in accordance with Art. 9.11 of the Code (Turland et al. 2018). Morphological analyses of the paratype 'R. Singer B 11353' agrees with the protologue, and since it is the only known paratype, we designate it as the lectotype.

***Lactifluus brasiliensis*** (Singer) Silva-Filho & Wartchow comb. nov. (Figure 3 & Figure 7C,D)

≡ *Lactarius brasiliensis* Singer, in Singer et al., Beih. Nova Hedwigia 77: 308 (1983)

Mycobank: MB831815

***Material examined:*** Brazil, Amazonas, Manaus, road Manaus-Caracaraí Km 45, 28 February 1978, leg. St. John, Araujo and R. Singer B10729 (INPA 77475, holotype). Brazil, Amazonas, Manaus, Manaus-Caracaraí road Km 45, 12 January 1979, leg. R. Singer B 11479 (INPA 82380, paratype).

***Original macroscopic description:*** 'Pileus reminding one of *Lactarius volemus* (Fr.) Fr. in color but often more yellow, "Maia" to "ginger" reaching "chutney" in the center, or "nugget bronze y" tending to "burnt sienna" or 5G12 (M&P), somewhat hygrophanous and fading to paler, either smooth or more frequently slightly finely rugulose at the centre, and with coarsely sulcate or shallowly and distantly furrowed margin up to one third of the radius when adult, not viciid, glabrous macroscopically, convex, soon with depressed center, eventually often concave, 43–70 mm broad. Lamellae "nugget" to "mango" (M&P), eventually reaching "Mohawk", when old, distinctly but thinly whitish powdered, unchanging when bruised, narrow to medium broad, subdistant to distant, all simple or all forked, arcuate, then horizontal to descendant, decurrent. Spore print nearly white. Stipe "nugget" to "leather br" or "arabian br", eventually with "mohawk" or "chutney" areas, not viciid, glabrous, at least macroscopically, smooth, solid, then narrowly and eventually broadly hollow, equal or

tapering up or down, or both up and downwards, 39–54 × 8–13 mm; veil none; basal mycelium usually scanty, white pruinose. Context in rind region subconcolorous with the surface, and otherwise buff pallid, or pale to dark cinnamon in parts, unchanging when bruised. Odor agreeable, “parfumé”, but often so weakly so that it is imperceptible. Taste moderately to decidedly acrid, not bitter. Latex scanty, watery to white, unchanging.’

**Microscopic description:** Basidiospores 8–10 × (5.5–)6.5–8 μm; (n = 20/2/2); L = 8.4 μm, W = 7.0 μm; Q = 1.00–1.33 Qm = 1.18; globose to broadly ellipsoid, predominantly subglobose, thin-walled, hyaline, ornamentation amyloid, up to 1 μm high, composed of irregular short ridges and warts connected by fine lines but not forming a complete reticulum; plage inamyloid; hilar appendix up to 4 μm long (Figure 3A). Basidia not seen, collapsed. Pleuromacrocytidia scattered, 4.5–8 μm diam., fusoid to fusiform, sometimes cylindrical, thin-walled, with few refractive contents; mostly not projecting, but sometimes projecting up to 5 μm above the hymenium; arising deeply from hymenophoral trama (Figure 3B). Pleuropseudocystidia not found. Hymenophoral trama poorly rehydrated; composed of abundant sphaerocytes 8–25 μm diam., abundant lactifers, 5–8 μm diam. Lamellar edge not rehydrated. Pileipellis well rehydrated; a palisade, the subpellis composed by a layer of isodiametric cells 7–17 μm diam., thin-walled, hyaline and the suprapellis composed of cylindrical to clavate terminal hyphae, 20–33 × 5–8 μm, thin-walled, hyaline to yellowish (Figure 3C). Pileus trama moderately rehydrated; composed of abundant sphaerocytes 15–31 μm diam., scattered lactifers 5–8 μm diam. Stipitipellis and stipititrampa not rehydrated. Clamp connections absent in all parts examined.

**Habitat:** ‘In campina and campinarana vegetation on humus mixed with rotten pieces of wood, gregarious, associated with Leguminosae and Sapotaceae. Fruiting early in the rainy season’ (Singer et al. 1983).

**Known distribution:** Known only from Brazilian Amazonia.

**Observation on *exsiccata*:** The holotype gathered as ‘Singer B 10729’ consists of a single basidiome; the sample ‘Singer B 11479’ apparently has more than one broken basidiome (Figure 7C,D).

**Notes:** *Lactifluus brasiliensis* was described as having orange pileus, a palisade pileipellis and frequent to abundant pleuromacrocystidia projecting above the hymenium (Singer et al. 1983). However, were seen scattered pleuromacrocystidia, possible due the poorly rehydrated hymenophoral trama.

As observed in other Amazonian species, *Lf. brasiliensis* was described belonging to sect. *Polysphaerophori*, but as previously discussed, the section is currently considered a clade of the subg. *Pseudogymnocarpi* (De Crop et al. 2017). The yellowish to orange cap, unchanging context and latex, and the palisade structure of the pileipellis suggest the placement of *Lf. brasiliensis* in this subgenus, which probably belongs to the sect. *Polysphaerophori* sensu De Crop et al. (2017), together with other Neotropical species.

*Lactifluus amazonensis* and *Lf. ceraceus* can be considered similar entities to *Lf. brasiliensis*. The former has more slender basidiomata, wider pleuromacrocystidia and a lampropalisade pileipellis while *Lf. ceraceus* differs in the smaller basidiospores ( $5.4\text{--}8.1 \times 4.4\text{--}6.2 \mu\text{m}$ ), slightly thick-walled pleuromacrocystidia with an obtuse to mucronate and slightly rostrate or moniliform apex, and a lampropalisade that forms the pileipellis structure (Crous et al. 2017).

***Lactifluus braunii*** (Rick) Silva-Filho & Wartchow comb. nov. (Figure 4; 8A; 9A)

≡ *Lactarius braunii* Rick, Brotéria Série Botânica 24: 118 (1930).

Mycobank: MB831816

**Material examined:** Brazil, Rio Grande do Sul, Porto Alegre, 1930, leg. Braun 8710 (PACA 13764, lectotype, here designated). Brazil, Rio Grande do Sul, Porto Alegre, 1929, leg. Rick 8707 (PACA 13757); São Leopoldo, 1930, leg. Rick 8706 (PACA 13756); ibid. 1931, leg. Rick 8705 (PACA 13756).

**Original description:** ‘Pileo citrino, granuloso, maculis rubri spicto, sicco fragile carnosus, 4 cm. lato; lamellis longe decurrentibus, valde distantibus polymacriis, ventricosis, acie serratis fragilibus, concoloribus; stipites albo, versus apicem tomentosus, curtos, 8 cm. altos, crassos; lactealbo; spora 8–9  $\mu\text{m}$  grosse echinulata.’

**Microscopic description:** Basidiospores  $7\text{--}9 \times (5\text{--})6\text{--}7(-7.5) \mu\text{m}$ ; ( $n = 20/1/4$ );  $L = 7.4 \mu\text{m}$ ,  $W = 6.3 \mu\text{m}$ ;  $Q = 1.00\text{--}1.33$   $Q_m = 1.18$ ; globose to ellipsoid, predominantly subglobose, thin-walled, hyaline; ornamentation amyloid, up to  $0.5 \mu\text{m}$  high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid; hilar appendix short, up to  $2 \mu\text{m}$  long (Figs. 4A; 9A). Basidia  $62\text{--}86 \times 8\text{--}11 \mu\text{m}$ , cylindrical to cylindrical-clavate, 4-spored, hyaline, sometimes with refractive contents; sterigmata up to  $9 \mu\text{m}$  (Figure 4B). Pleurocystidia absent. Pleuropseudocystidia frequent to scarce,  $5\text{--}8 \mu\text{m}$  diam., cylindrical to cylindrical-clavate, thin-walled, with refractive contents, not projecting above the hymenium, arising deeply from lamellar trama (Figure 4D). Hymenophoral trama moderately rehydrated; composed of abundant sphaerocytes  $15\text{--}28 \mu\text{m}$  diam., and scattered lactifers  $10\text{--}16 \mu\text{m}$  diam. Lamellar edge not rehydrated. Pileipellis well rehydrated; a trichopalisade, the subpellis composed of few isodiametric cells  $9\text{--}16 \mu\text{m}$  diam., thin-walled, hyaline, cylindrical hyphae  $5\text{--}8 \mu\text{m}$  diam., smooth, thin-walled, hyaline and cylindrical-inflated hyphae  $10\text{--}14 \mu\text{m}$  diam., smooth, thin-walled, hyaline, the suprapellis composed of cylindrical, clavate, fusoid, mostly flexuous, sometimes septate hyphae,  $29\text{--}40 \times 4\text{--}6 \mu\text{m}$ , thin-walled, sometimes slightly thickened (to  $0.5 \mu\text{m}$  diam.), hyaline to yellowish (Figure 4 C). Pileus trama not rehydrated. Stipitipellis and stipititrampa not rehydrated. Clamp connections absent in all parts examined.

**Distribution:** Known only from Rio Grande do Sul State, Brazil.

**Habitat:** Unknown host (Rick 1930).

**Observation on exsiccata:** The Braun exsiccata ‘Rick 8710’ has three well preserved basidiomata (Figure 8A). Other samples ‘Rick 8707’, ‘Rick 8706’ and ‘Rick 8705’ are well preserved. The SEM analysis confirms basidiospore ornamentation.

**Notes:** *Lactifluus braunii* is characterized by lemon yellow pileus with red spots, white latex, non-abundant pseudocystidia that do not project above the hymenium and a trichopalisade pileipellis. However, these features are not clear enough to define its taxonomic position. The lemon yellow pileus and the trichopalisade pileipellis might suggest the species as a member of subg. *Pseudogymnocarpi*, but new collections and molecular evidence are needed before reaching this conclusion.

*Lactifluus braunii* was named based on Braun’s collection from Porto Alegre in 1930, represented by two exsiccatae: ‘Rick 8710’ (PACA 13764) analysed here and PACA 13751 analysed by Singer (1953), which was considered a ‘typus duvidosus’ according to annotation on the herbarium sheet. Rick (1930) did not designate a holotype, and no did Singer (1953) when reviewing the species. Since no material was designated as type, the exsiccata 8710 (PACA 13764), which comprises a well preserved original material collected by Braun in 1930 in Porto Alegre is chosen here as lectotype (Turland et al. 2018, Art. 9.11, Art. 9.12).

*Lactifluus mamorensis* (Rick) Silva-Filho & Wartchow comb. nov. (Figure 5, Figure 8B,C & Figure 9B)

≡ *Lactarius mamorensis* Singer, in Singer et al., Beih. Nova Hedwigia 77: 302 (1983).

Mycobank: MB831816

**Material examined:** Brazil: Amazonas, Manaus, Igarapé do Turumãzinho, 26 March 1980, leg. R. Singer B 12085 (INPA 102015, paratype); 30-IV-1980, leg. R. Singer B 12118A (INPA 102038, paratype).

**Original macroscopic description:** ‘Pileus at first a pink cinnamon to tan (“Maya” to “Titian gold” Cinnamon” or “spice” or 12B10, M&P) neither viscid not hygrophanous, but later becoming more or less hygrophanous or simply fading to flesh ochre or cream (“auteuil”,

“toltec”, “sunset”, “leghorn” 11B8, M&P) glabrous and matt, sometimes in places somewhat rivulose, with the margin at first strongly incurved, acute, smooth, becoming striate when wet or sulcate over a distance of 2–10 mm from the rim, otherwise smooth, early depressed at the centre, otherwise remaining convex or becoming entirely concave, 18–51 mm broad. Lamellae cinnamon within with cinnamon shade, then cream to pale salmon with an “onion skin pink” shade, 10C/D6, 9B3 (M&P), unchanging when bruised, close, more rarely subclose, narrow to medium broad (mostly about 2–3 mm broad), strongly intermixed with lamellulae but not intervenose, but sometimes rugulose in the upper (near to pileus trama) half, decurrent. Spore print white (A1 Romangnesi). Stipe concolorous with the pileus or slightly paler when fresh, the apex colored like the margin, the rest like the pileus centre, or apex an orangy yellow-cream (10D5) and below orange yellow (10G7 M&P), more rarely entirely almost whitening, in age becoming pale flesh colored (9B5) at apex, otherwise light cinnamon (12C9), macroscopically glabrous, but often finely pubescent under a lens in places at least when young, characteristically longitudinally ribbed-rugose over the upper third 4/5’s of its length, but occasionally ribs present, only one side of stipe, the ribs in the lower part often anastomosing, in the upper part continuing the lamellae, in the lower fifth to 2/3’s smooth, not viscid, hollow when mature, equal, later equal or slightly ventricose or tapering downwards, 15–40 × 3–13 mm; veil none; basal mycelium well developed, strigose-tomentose, white, in dried material remaining white or becoming cinnamon-fulvous. Context white or concolorous with the surfaces although paler than these, unchanging on exposure. Odor none. Taste mild, more rarely slightly and slowly acrid-peppery, in older specimens always mild. Latex scanty, aqueous, colorless, unchanging.’

**Microscopic description:** Basidiospores 7–9.5 × (5.5–)7–8 μm; (n = 20/2/2); L = 8 μm, W = 7.3 μm; Q = 1.00–1.27, Qm = 1.18; globose to broadly ellipsoid, predominantly subglobose, thin-walled, hyaline; ornamentation amyloid, up to 2 μm high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid;

hilar appendix up to 3  $\mu\text{m}$  long (Figs. 5B; 9B). Basidia 32–56  $\times$  10–13  $\mu\text{m}$ , clavate, 4-spored, thin-walled, hyaline; sterigmata up to 6  $\mu\text{m}$  (Figure 5D). Pleurocystidia absent. Pleuropseudocystidia scattered, 12–24  $\mu\text{m}$  diam., clavate, clavate-mucronate to ventricose-rostrate, thin-walled, with oleiferic contents; projecting up to 20  $\mu\text{m}$  above the hymenium; arising deeply from hymenophoral trama; found only in the lamellar upper portion (Figure 5A). Hymenophoral trama moderately rehydrated; composed of abundant sphaerocytes 10–23  $\mu\text{m}$  diam., and scattered lactifers 3.5–7  $\mu\text{m}$  diam. Lamellar edge not rehydrated. Pileipellis well rehydrated; a lampropalisade, the subpellis composed by isodiametric cells, 8–40  $\mu\text{m}$  diam., thin-walled, hyaline and the suprapellis composed by scattered hair-shaped, thick-walled terminal elements 33–99.5  $\times$  4–5  $\mu\text{m}$ , aciculate to aculeate, thick-walled (up to 1.5  $\mu\text{m}$  thick), hyaline, anticlinal sometimes periclinal or oblique or disposed, sometimes arise deeply from subpellis (Figure 5C). Pileus trama composed of sphaerocytes 10–35  $\mu\text{m}$  diam. and rare cylindrical hyphae 3–7  $\mu\text{m}$  diam., hyaline, smooth thin-walled. Stipitipellis and stipititrma not rehydrated. Clamp connections absent in all parts examined.

**Habitat:** ‘Along the riverside, mostly on detritus or dead wood over sandy soil, but also on soil poorer in sandy matter, in dense tropical rain forest along the rivers, mostly where temporarily inundated (igapó), fruiting in March and April; Amazonia’ (Singer et al. 1983).

**Known distribution:** Known only from Brazilian and Bolivian Amazonia.

**Observation on exsiccata:** The paratype ‘Singer B 12085’ is composed of five basidiomata in fairly good condition; ‘Singer B 12118A’ consists of one damaged basidioma (Figs. 8B, 8C).

**Notes:** *Lactifluus mamorensis* was originally described in sect. *Polysphaerophori* (Singer et al. 1983). However, based on the new taxonomic rearrangement by de Crop et al. (2017), the species can be considered a good member of subg. *Lactariopsis* due to the reddish brown pileus with striate pileus margin, unchanging context and latex, and thick-walled hair-shaped elements on pileipellis. *Lactifluus mamorensis* differs from other taxa of the subgenus by its set of unique features, for example: ribbed stipe, scarce and wider pleuropseudocystidia with

oleiferic contents, lampropalisade pileipellis with scattered hair-shaped thick-walled elements.

The Neotropical milkcaps *Lf. annulifer*, *Lf. caatingae*, *Lf. subiculatus* S.L. Mill., Aime & T.W. Henkel and *Lf. venezuelanus* form a monophyletic clade/section in the subg. *Lactariopsis* (De Crop et al. 2017; Sá et al. 2019). Possibly, other species that do not have available molecular data also belong to this group, such as *Lf. dunensis*, *Lf. mamorensis*, *Lf. neotropicus* and *L. paulensis*.

Among these species, *Lf. annulifer* and *Lf. neotropicus* primarily differ by the presence of velum remnants, lacking in *Lf. mamorensis* (Singer et al. 1983, Pegler 1983). *Lactifluus caatingae* differs in the brownish orange pileus, non-ribbed stipe, smaller basidiospores ( $6.1\text{--}7.1 \times 5.6\text{--}6.6 \mu\text{m}$ ) and thinner thick-walled hairs ( $3\text{--}4 \mu\text{m}$  wide, Sá et al. 2019). *Lactifluus subiculatus* arises from a mycelial subiculum and has a much smaller pileus (9–30 mm diam.), no emergent pseudocystidia and longer terminal thick-walled elements with swollen base on the lampropalised pileipellis structure (Miller et al. 2012). *Lactifluus venezuelanus* also arises from a subiculum and differs in the more delicate basidiomata, pileus 15–23 mm in diam. and stipe  $12 \times 1.5\text{--}3$  mm and slightly or not emergent pleuropseudocystidia (Buyck and de Meijer 1999).

***Lactifluus rupestris*** (Wartchow) Silva-Filho & Wartchow comb. nov.

≡ *Lactarius rupestris* Wartchow, in Wartchow & Cavalcanti, Mycotaxon 112: 56 (2010).

Mycobank: MB831819

**Original description:** Complete description in Wartchow & Cavalcanti (2010).

**Notes:** *Lactifluus rupestris*, recently described from Pernambuco, was regarded by Wartchow and Cavalcanti (2010) as an isolated species that does not fit entirely into any tropical infrageneric taxon proposed by Verbeken (2001). However, *Lf. rupestris* can be placed in the subg. *Pseudogymnocarpi* sensu De Crop et al. (2017) due to orange to brownish orange pileus and the trichodermial pileipellis. ‘*Lactarius pegleri* Pacioni & Lalli’, a non-recombined

species from Martinique, is similar to *Lf. rupestris*. Both have orange to brownish pileus, cream lamellae, pale ochraceous salmon stipe, homologous basidiospores and a trichoderm pileipellis (Lalli and Pacioli 1992; Wartchow and Cavalcanti 2010). However, *L. pegleri* has smaller pileus, 25–57 mm in diam., with sulcate to plicate margin, smaller stipe 25–38 x 6–12 mm, distant lamellae and abundant pseudocystidia with acute apex (Lalli and Pacioli 1992); on the other hand, *Lf. rupestris* has larger (60–70 mm), non-striate pileus, crowded lamellae, larger stipe (35–45 × 18–21 mm) and very scarce pseudocystidia with obtuse apex (Wartchow and Cavalcanti 2010).

***Lactifluus russula*** (Rick) Silva-Filho & Wartchow comb. nov. (Figure 6 & Figure 8D)

≡ *Lactarius russula* Rick, Brotéria Série Botânica 5: 20 (1906).

Mycobank: MB831820

**Material examined:** Brazil, Rio Grande do Sul, São Leopoldo, no date, Leg. Rick 8717 (PACA 13762, lectotype, here designated). Brazil, no place, no date, J. Rick s/n (URM 6197).

**Original description:** ‘Pileo depresso, usque 1 dm lato, carnosus-fragilis, rugosus, glabrus, cinereus fuliginosus, margine depresso; stipite 5 cm, alto, cavo, brunneo, subtiliter velutino. Lamellis latis, adnatis, rigidis, fragilibus, late distantibus; lacte albo acerrimo. Pileo fracto nigrescente. Sporis subrotundis, guttulatis, 6 diam., eckinulatis, albis.’

**Microscopic description:** Basidiospores 7.5–9 × 5.5–7 μm; (n=10/1/1); L = 7.9 μm, W = 6.5 μm; Q = 1.14–1.36 Qm = 1.22; subglobose to broadly ellipsoid, predominantly broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 1 μm high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid; hilar appendix up to 4 μm long (Figure 6A). Basidia not seen, collapsed. Pleurocystidia absent. Pleuropseudocystidia 6.5–11.5 μm in diam., abundant, cylindrical, cylindrical-clavate, sometimes fusiform, thin-walled, with refrigent contents; mostly not projecting, rarely projecting up to 10 μm above the hymenium; arising deeply from hymenophoral trama (Figure 6B). Hymenophoral trama moderately rehydrated; composed of

abundant sphaerocytes, 7.5–14  $\mu\text{m}$  diam. and abundant lactifers 4.5–7  $\mu\text{m}$  diam. Lamellar edge not rehydrated. Pileipellis moderately rehydrated; a lampropalisade, the subpellis composed by a layer of isodiametric cells 6–19  $\mu\text{m}$  diam., hyaline, thin-walled, cylindrical inflated hyphae, 10–17  $\mu\text{m}$  diam., smooth, thin-walled, hyaline and by lactifers 6–8  $\mu\text{m}$  diam. and the suprapellis composed by abundant hair-shaped, thick-walled terminal 65–108  $\times$  4–10  $\mu\text{m}$ , mostly fusoid, fusiform, sometimes cylindrical to clavate, sometimes flexuous, thick-walled (up to 4.5  $\mu\text{m}$  thick), hyaline (Figure 6C). Pileus trama not rehydrated. Stipitipellis and stipititrana not rehydrated. Clamp connections absent in all parts examined.

**Habitat:** In forest (Rick 1906).

**Known distribution:** Known only from Rio Grande do Sul, Brazil.

**Observation on exsiccata:** The exsiccata ‘Rick 8717’ (PACA 13762) is composed of two moderately preserved basidiomata (Figure 8D) and ‘Rick s/n’ (URM 6197) of two broken basidiomata lacking basidiospores, but the pileipellis, and pseudocystidia resemble the ones observed in the lectotype.

**Notes:** *Lactifluus russula* is characterized by the dark greyish cracked pileus, white latex, abundant pseudocystidia and a lampropalisade pileipellis bearing abundant thick-walled elements with obtuse apex. The protologue by Rick (1906) was later complemented by a photo published in Rick (1907), which probably corresponds to the type material collected in 1904. Singer (1953) reviewed an exsiccata (Rick 148) corresponding to a collection from 1906 deposited at Farlow Herbarium and possibly does not correspond to the original material. The specimens analysed by us agree in some aspects with Singer’s descriptions as, for example, in the basidiospore size (8.5–10  $\times$  6.5–8.3  $\mu\text{m}$ ) and the thick-walled elements with obtuse apex forming a lampropalisade pileipellis.

Rick did not designate a type, and Singer (1953) did not report any decision regarding the status of the typification. Although the exsiccata ‘Rick 8717’ (PACA 13762) has no collection date, we strongly suspect that it might comprise the original material used in the protologue of

*Lf. russula*, and thus we chose to designate this material as the lectotype (Turland et al. 2018, Art. 9.11, 9.12).

Rick (1906, 1907) compared *Lf. russula* with *Russula nigricans* Fr., a species with darkening context after exposure (Shaffer 1962). It suggests some colour reaction in *Lf. russula* as seen in members of subgenera *Gymnocarpi* (R. Heim ex Verbeken) De Crop and *Lactifluus* (Pers.) Roussel (De Crop et al. 2017) and in some species of subg. *Pseudogymnocarpi*. Therefore, although *Lf. russula* is a member of *Lactifluus*, its taxonomic position will only be elucidated with more morphological data or phylogenetic inferences.

### **Discussion**

In this research, six Brazilian milkcaps described by Rick (1906, 1907, 1930), Singer et al. (1983) and Singer (1984) were reviewed. Three lectotypes were designated and six new combinations into *Lactifluus* were made. Unfortunately, it was not possible to review other Brazilian milkcaps: *Lactarius reticulatus* (exsiccata Singer B 10986), housed at the INPA herbarium, has been damaged and was in poor condition for analysis. The type species of *L. reticulatus* (as *Panus reticulatus*) was requested from K, but was not available. *Lactarius paulensis* and *L. subpallidipes* from F were also unavailable for our studies, and *L. steffenii* described by Rick had no available exsiccata (Singer 1953). The requested specimens of *L. pallidipes* and *L. gigasporus* were lost in the INPA herbarium, and neotypes need to be designated for these species.

In future work, we will try to include molecular data from these analysed specimens in integrative taxonomic research. If the sequencing is unsuccessful, morphological data provided by us will allow the identification and epitypification of these collections.

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Figura 11 - Figure 1. *Lactifluus amazonensis* (R. Singer B11315, holotype): A. Basidiospores. B. Basidia. C. Pileipellis. D. Macropleurocystidia. Scale bars = 10  $\mu$ m. Drawing by A.G.S. Silva-Filho

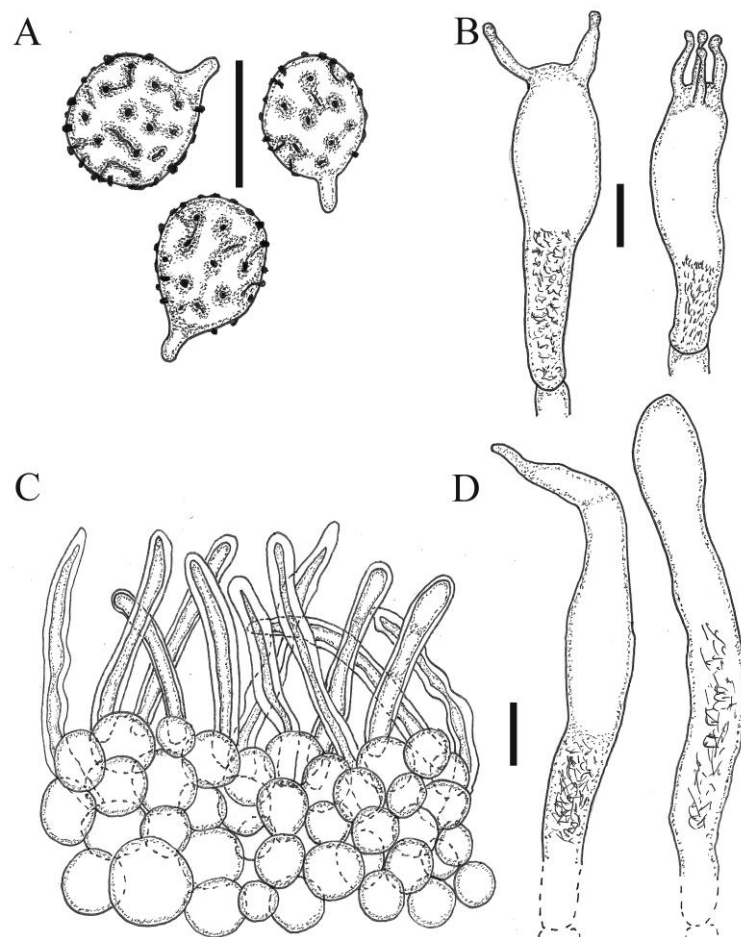


Figura 12 - Figure 2. *Lactifluus annulifer* (R. Singer B11353, lectotype): A. Basidiospores. B. Pleuropseudocystidia; C. Terminal elements of pileipellis. Scale bars = 10  $\mu$ m. Drawing by A.G.S. Silva-Filho

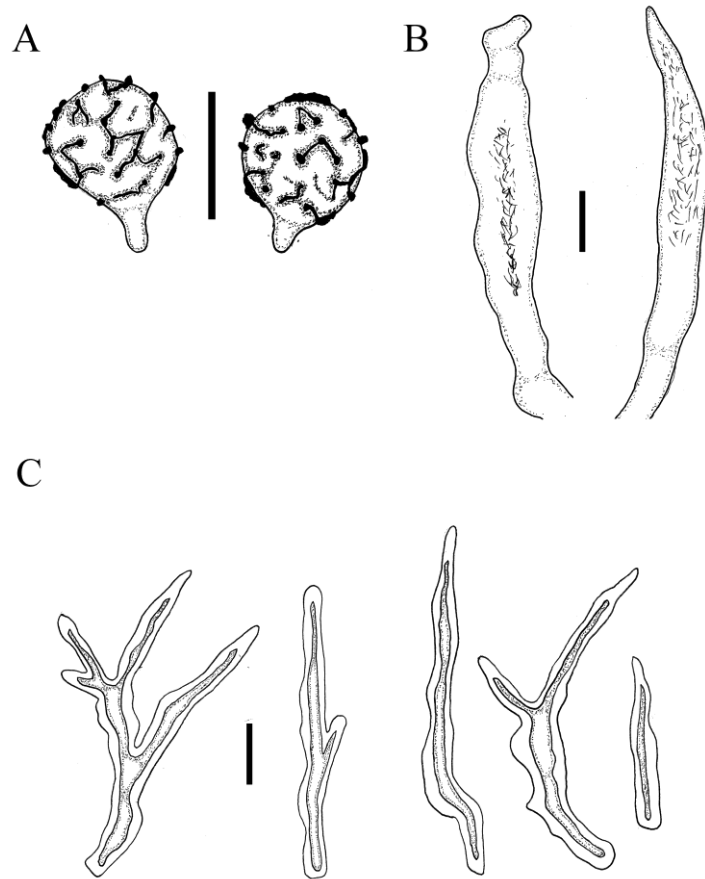


Figura 13 - Figure 3. *Lactifluus brasiliensis* (R. Singer B10729, holotype): A. Basidiospores. B. Macropleurocystidia. C. Pileipellis. Scale bars = 10  $\mu$ m. Drawing by A.G.S. Silva-Filho

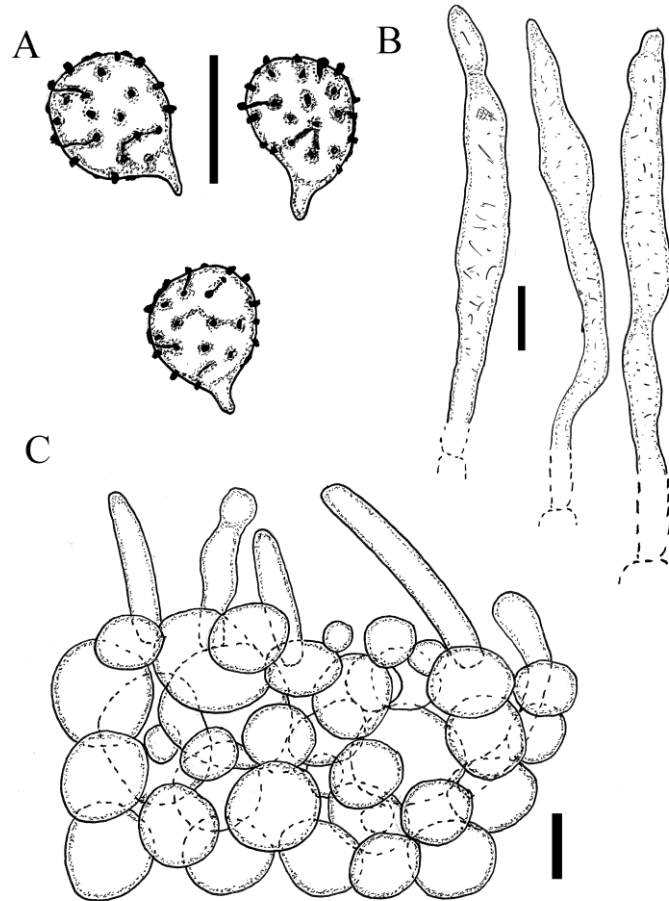


Figura 14 - Figure 4. *Lactifluus braunii* (Braun 8710, lectotype): A. Basidiospores. B. Basidium. C. Pileipellis. D. Pleuropseudocystidia. Scale bars = 10  $\mu$ m. Drawing by A.G.S. SilvaFilho

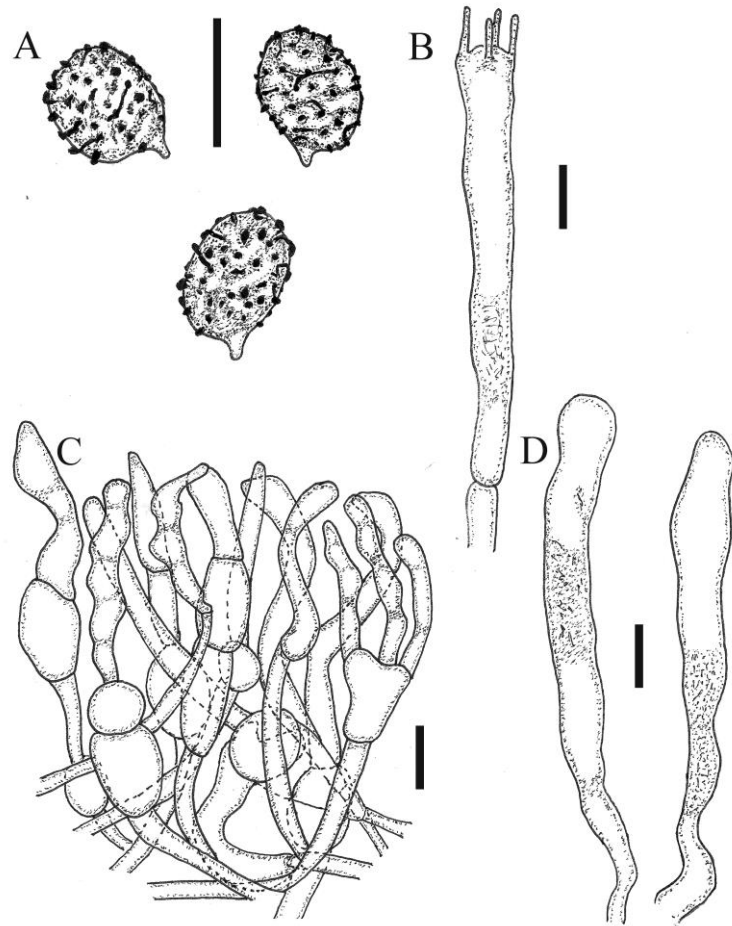


Figura 15 - Figure 5. *Lactifluus mamorensis* (R. Singer B12085, paratype). A. Pleuropseudocystidia. B. Basidiospores. C. Pileipellis. D. Basidium. Scale bars = 10  $\mu\text{m}$ . Drawing by A.G.S. Silva-Filho

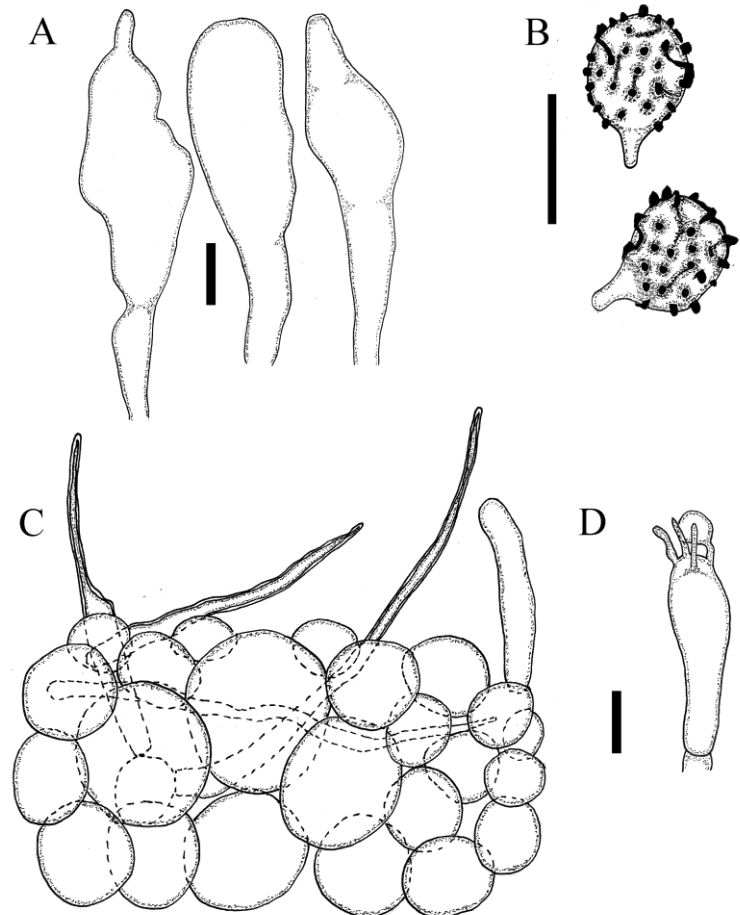


Figura 16 - Figure 6. *Lactifluus russula* (Rick 8717). A. Basidiospores. B. Pleuropseudocystidia. C. Pileipellis. Scale bars = 10  $\mu$ m. Drawing by A.G.S. Silva-Filho.

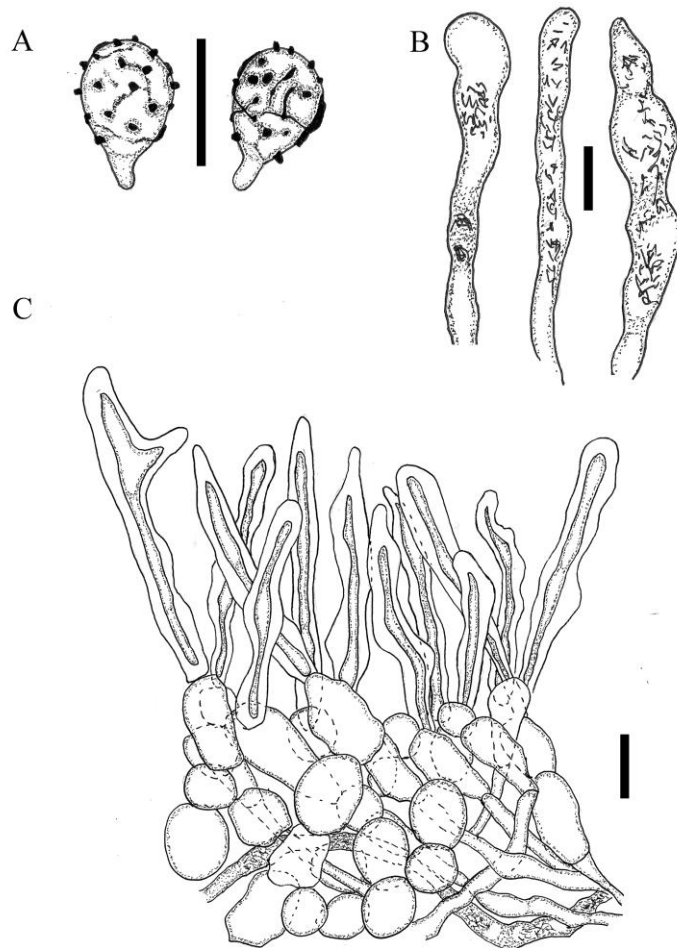


Figura 17 - Figure 7. Exsiccateae. A. *Lactifluus amazonensis* (R. Singer B11315, holotype). B. *Lactifluus annulifer* (R. Singer B11353, lectotype). C. *Lactifluus brasiliensis* (R. Singer B10729, holotype). D. *Lactifluus brasiliensis* (R. Singer B10729, paratype).

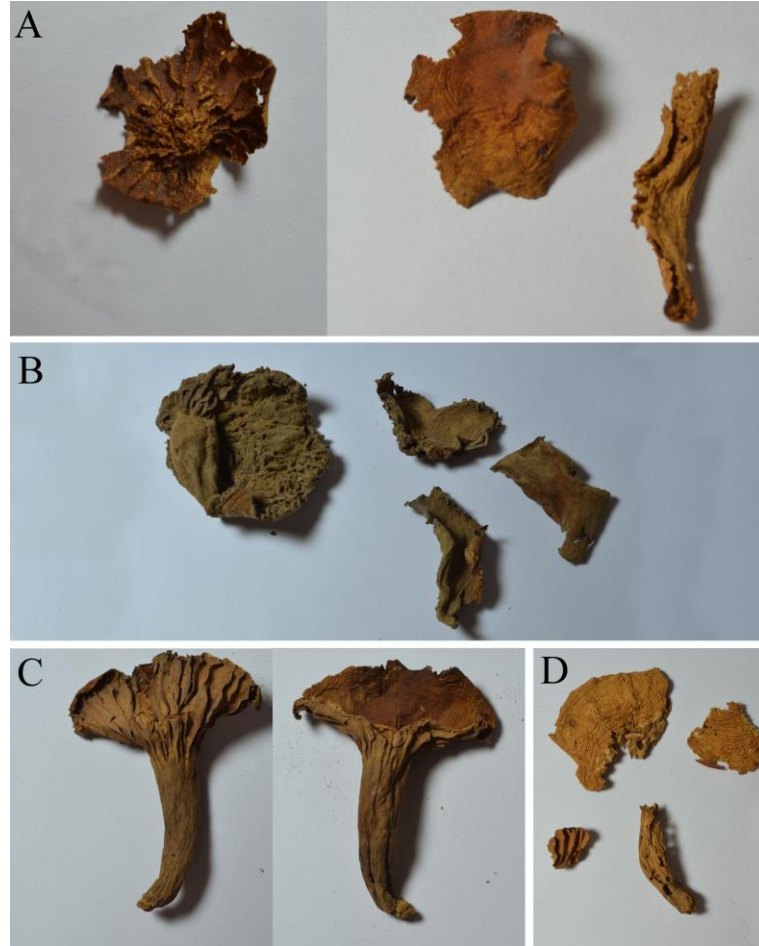
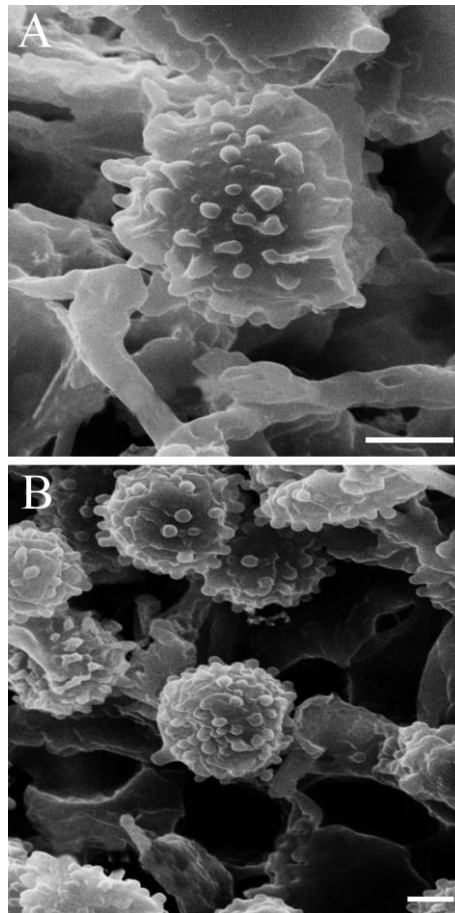


Figura 18 - Figure 8. Exsiccatae. A. *Lactifluus braunii* (Braun 8710, lectotype). B. *Lactifluus mamorensis* (R. Singer B12085, paratype). C. *Lactifluus mamorensis* (R. Singer B12118, paratype). D. *Lactifluus russula* (Rick 8717, lectotype).



Figura 19 - Figure 9. SEM micrographs of the basidiospores. A. *Lactifluus braunii* (Braum 8710, lectotype). B. *Lactifluus ma9morensis* (R. Singer B12118, paratype). Scale bars = 2  $\mu$ m.



**7 CAPÍTULO 2 - Two novel species of *Lactifluus* subg. *Pseudogymnocarpi* (Russulaceae) from Brazil**

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## Abstract

Two new species in *Lactifluus* subg. *Pseudogymnocarpi*, *Lf. umbilicatus* and *Lf. venosellus* were collected from Amazonian and Atlantic forests of Brazil, respectively. Detailed macro- and micromorphological descriptions, including scanning electron microscopy images of the basidiospores, are provided. The putative phylogenetic placement of these taxa was investigated from ITS sequence analyses. Comparisons with related taxa are discussed.

**Key words:** Agaricomycetes, milkcaps, Neotropic, new taxa, phylogeny, rDNA ITS, Russulales

## Introduction

Russulaceae is a family of mushrooms that form ectomycorrhizal associations with diverse shrubs and trees in temperate and tropical forested biomes (Looney *et al.* 2018). Recent molecular phylogenetic studies in Russulaceae have shown that the two traditional core genera of this family, *Lactarius* Persoon (1797: 63, abbreviate *L.*) and *Russula* Persoon (1796: 100) are paraphyletic, and should be divided into four distinct genera: *Lactarius*, *Lactifluus* (Persoon 1801: 429) Roussel (1806: 66, abbreviate *Lf.*), *Multifurca* Buyck & V. Hofst. in Buyck *et al.* (2008: 37) and *Russula* (Buyck *et al.* 2008). All genera, except *Multifurca*, have been recorded in South America (e.g., Miller *et al.* 2012, Wang *et al.* 2018) and several new species have been revealed recently from Brazil (e.g., Sá & Wartchow 2016, Sá *et al.* 2019). The genus *Russula*, despite being poorly studied in Brazil, is now represented by 17 species (Sá *et al.* 2013a). *Lactarius* has been related to the introduction of ectomycorrhizal hosts and five species were reported in *Pinus* plantations (Sulzbacher *et al.* 2013, Silva-Filho *et al.* 2018). *Lactifluus* is a common tropical genus (Verbeken & Walley 2010, De Crop *et al.* 2017) but yet underexplored in Brazil, and has recently received more attention (Sá *et al.* 2013a, 2019). Indeed, the diversity of this genus has been studied in the Amazon, Atlantic forest and Caatinga biomes and revealed already 20 species (Sá *et al.* 2013a). The genus is still unexplored in other Brazilian biomes, such as the Cerrado or Pampa biomes.

Most Brazilian *Lactifluus* species were described from Amazon forests, notably from Singer's studies (Sá *et al.* 2013a). The first species, described as *Lactarius reticulatus* (Berkeley 1856: 142) Singer (1955: 426), was initially named *Panus reticulatus* Berkeley (1856: 142) by Berkeley (1856). Later, Singer *et al.* (1983) and Singer (1984) described nine species from Central and Western Amazonia regions (all described as *Lactarius*): *L. amazonensis* Singer in Singer *et al.* (1983: 295), *L. annulifer* Singer in Singer *et al.* (1983: 290), *L. brasiliensis* Singer in Singer *et al.* (1983: 308), *L. campinensis* Singer (1994: 438), *L. gigasporus* Singer in Singer *et al.* (1983: 323), *L. igapoensis* Singer (1984: 441), *L. mamorensis* Singer in Singer

*et al.* (1983: 302), *L. pallidipes* Singer in Singer *et al.* (1983: 299) and *L. subpallidipes* in Singer *et al.* (1983: 298). Among these, *L. amazonensis*, *L. annulifer*, *L. brasiliensis*, and *L. mamorensis* were recombined in *Lactifluus* (Verbeken *et al.* 2011, Silva-Filho & Wartchow 2019), *L. campinensis* transferred to *Russula* (Henkel *et al.* 2000) and *L. igapoensis* was synonymized in *Lf. panuoides* (Singer, 1952: 300) De Crop in De Crop *et al.* 2017: 76). Since then, no new species have been described from the Amazon.

The Atlantic forest in Brazil also harbors some *Lactifluus* species. In the last century, Rick (1906, 1907, 1930) described *Lactarius braunii* Rick (1930: 118), *L. russula* Rick (1906: 20) and *L. steffanii* Rick (1930: 117) from Rio Grande do Sul. Only *L. braunii* and *L. russula* have preserved exsiccatæ and were currently recombined in *Lactifluus* (Silva-Filho & Wartchow 2019). Singer *et al.* (1983) described *L. paulensis* Singer in Singer *et al.* (1983: 305) from São Paulo. *Lactifluus venezuelanus* (Dennis 1970: 467) De Crop in De Crop *et al.* (2017: 77) was recorded by Buyck & de Meijer (1999, as *Lactarius venezuelanus* Dennis 1970: 467) and de Meijer (2006, as *L. venezuelanus*) from Paraná. More recently, new species have been described after new taxonomic treatment: *Lf. aurantiorugosus* Sá & Wartchow (2013: 56), *Lf. batistae* Wartchow, J.L. Bezerra & M. Cavalc. in Wartchow *et al.* (2013: 104) and *Lf. dunensis* Sá & Wartchow in Sá *et al.* (2013b: 263) from Rio Grande do Sul, Bahia and Rio Grande do Norte respectively (Sá & Wartchow 2013, Wartchow *et al.* 2013, Sá *et al.* 2013b). Recent field trips in various ecosystems in Brazil have led to the discovery of two additional new *Lactifluus* species from the Caatinga domain in the semiarid regions of Brazil: *Lf. rupestris* (Wartchow in Wartchow & Cavalcanti 2010: 56) Silva-Filho & Wartchow (2019: in press) from Pernambuco and *Lf. caatingae* Sá & Wartchow in Sá *et al.* (2019: 171) from Piauí.

This work proposes two new species of *Lactifluus*, collected in Amazon and Atlantic Forests. Both species are described based on morphological and molecular characters, and are placed in *Lf.* subg. *Pseudogymnocarpi* (Verbeken in Verbeken *et al.* 2012: 447) De Crop (in De Crop *et al.* 2017: 75).

### **Materials and Methods**

*Collecting area:*—Fresh specimens were collected in two distinct areas: (1) ‘Reserva Biológica Guaribas’, municipality of Mamanguape, (6°44'27.89" S 35°8'53.82" W) State of Paraíba, Northeast of Brazil. This is an Atlantic Forest protected reserve, which essentially consists of diverse representatives of Poaceae, Cyperaceae, Rubiaceae, Asteraceae, Malvaceae, Melastomataceae, Myrtaceae and Polygonaceae (Barbosa *et al.* 2011); (2) ‘Floresta Nacional do Tapajós’, municipality of Belterra (2°48'14" S, 54°59'50" W), State of Pará, North of

Brazil, which is a dense ombrophilous Amazon Forest predominantly composed by several species of Fabaceae, Euphorbiaceae, Annonaceae, Apocynaceae, Lecythidaceae, Myrtaceae and Polygonaceae (Gualberto *et al.* 2014).

*Morphological analysis:*—Macroscopic features were based on fresh material. Color names and codes follow Kornerup & Wanscher (1978). Studies were performed using the methodology of Verbeken (1998) and Verbeken & Walley (2010). Forty basidiospores were measured excluding ornamentation, in lateral view using Melzer's reagent, and dimensions are reported as (<10%, minimum length) average minimum length – average maximum length (<10%, maximum length) × (<10%, minimum width) average minimum width – average maximum width (<10%, maximum width). For biometric measurements of the basidiospores we follow the methodology of Wartchow & Gamboa-Trujillo (2012). Other microscopic structures were measured and visualized in 5% KOH and Congo red. For scanning electron microscopy (SEM), dried lamellae were directly stuck onto an adhesive layer on a small clean coverslip, then coated with gold and observed under Jeol JSM-6360LV and JSM-6010LA. Specimens were deposited in the Herbaria JPB, INPA (Thiers 2019) and in the Mycological collection at the 'Universidade Federal do Rio Grande do Norte' (UFRN-Fungos).

*Molecular methods:*—DNA was extracted from fresh tissue blotted on FTA® cards (Dentinger *et al.* 2010) or from dried basidioma following Nuytinck & Verbeken (2003) with modifications described in Van de Putte *et al.* (2010). The nuclear ribosomal internal transcribed spacer region (ITS) was amplified and sequenced using primers ITS1-F and ITS4-B (White *et al.* 1990). Sequencing reactions were conducted by MACROGEN (KOREA) and resulting sequences were assembled and edited in BioEdit (Hall 1999).

*Phylogenetic analyses:*— The newly generated ITS sequences from this study were used as BLAST queries to NCBI (GenBank) in order to retrieve similar sequences. Most of the retrieved sequences represent species of *Lactifluus* subg. *Pseudogymnocarpi* from De Crop *et al.* (2017). Three sequences of *Lf.* subg. *Lactariopsis* (Henn.) Verbeken were used as outgroup: *Lactifluus bertillonii* (Neuhoff 1956: 93) Verbeken in Verbeken *et al.* (2011: 451), *Lf. pilosus* (Verbeken, H.T. Le & Lumyong, in Le *et al.* 2007: 287) Verbeken in Verbeken *et al.* (2011: 451) and *Lf. subvellerus* (Peck 1898: 369) Nuytinck in Verbeken *et al.* (2011: 451). Our dataset consisted of 46 ITS sequences that were aligned using MAFFT v.7 (Kato & Standley 2013) under the Q-INS-i criteria. Seaview v.4 (Gouy *et al.* 2010) was used to visualize and adjustment the alignment. Gblocks v0.91b (Castresana 2000) was used to eliminate poorly aligned positions in the alignment, with settings allowing gaps within selected blocks, smaller blocks and bigger segments with contiguous non-conserved positions.

Maximum Likelihood analyses were performed in RAxML v8.2.X (Stamatakis 2006). The best nucleotide substitution model, TIM2f+I+G was selected with AIC (Akaike Information Criterion) using jModelTest 2v.1.6 (Guindon & Gascuel 2003, Darriba *et al.* 2012). Bayesian inferences (BI) were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), performing  $2 \times 10^7$  MCMC generations, sampling one tree every  $1 \times 10^3$  generation. The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 softwares were implemented in the CIPRES Science Gateway 3.1 (Miller *et al.* 2010). A node was considered significantly supported if it received a BPP  $\geq 0.95$  and/or BS  $\geq 80\%$ . The final alignment and the resulting topologies were deposited in TreeBASE, ID: 24560.

### Results

The dataset included sequences from 46 *Lactifluus* specimens, with 942 characters (including introduced gaps). For the analysis, the putatively unreliable alignment regions were removed by the Gblocks method, thus the final dataset resulted in 530 characters. The ITS phylogenetic inferences using either RAxML or Bayesian analyses yielded similar tree topologies. The figure 1 shows the tree generated in the ML analysis, including bootstrap and posterior probabilities values of well supported branches.

Our analyses place the two new species described in *Lf.* sect. *Polysphaerophori* (Singer 1973: 106) Verbeken in Verbeken *et al.* (2012: 445), which consists of four branches. The type species of the section, *Lf. veraecrucis* (Singer 1973: 104) Verbeken in Verbeken *et al.* (2012: 445), clusters strongly with '*Lactarius pegleri*' Pacioni & Lalli' (1992: 192) (99% BS / 1.0 BPP). Another branch with good support (97% BS / 1.0 BPP) groups *Lactifluus ceraceus* Delgat & M. Roy in Crous *et al.* (2017: 347), *Lf. umbilicatus* sp. nov. and two undescribed species (AMV1874) from Colombia and (TH7677) from Guyana. *Lactifluus venosellus* sp. nov. and six more undescribed collections (G4799) from French Guiana, (G082, G4258, MCA 3937 and TH7677), from Guyana and (AMV2209) from Colombia are clustered in another well-supported branch (99% BS / 1.0 BPP). The undescribed species (63144) from French Guiana also forms a branch.

### Taxonomy

***Lactifluus umbilicatus*** Silva-Filho, D.L.Komura & Wartchow, *sp. nov.* (Figs. 2–3–6a)

Mycobank:— MB832851

Genbank accession nrITS: MK929291

Type:—BRAZIL. Pará: Belterra, Floresta Nacional do Tapajós, Trilha da Comunidade Maguari, 2°48'14" S, 54°59'50" W, on soil close to *Tachigali myrmecophila* (Ducke) Ducke, 30 March 2014, (1922, 94), D.L. Komura, T.S. Cabral, I.R. Fonseca DLK1964 (INPA 284001 holotype, JPB isotype).

Diagnosis:—Distinguished from other species by: pileus umbilicate, orange, surface slightly wrinkly, margin slightly striated; lamellae slightly decurrent and whitish; stipe orange. Basidiospores  $7\text{--}8.5 \times 6\text{--}7 \mu\text{m}$ ; ornamentations up to  $1 \mu\text{m}$  high, composed of irregular short warts connected by fine lines; pseudocystidia absent; macrocystidia (pleuro- and cheilo-) fusoid to slightly fusiform; pileipellis a lampropalisade with frequent to abundant thick-walled terminal elements and frequent to abundant lactifers and unique ITS sequence.

Description:—Pileus 55–63 mm diam., plane convex, umbilicate, dark orange (5A8), brownish orange (5C6) to grayish orange (6B7); surface slightly wrinkly, dry, slightly opaque; margin slightly striated, straight to decurved, edge sometimes entire, sometimes eroded (Figs. 2a–d); context firm, 4–6 mm at the disc to very thin towards at the margin, light orange (5A4), unchanging. Lamellae short decurrent, distant, white (1A1) to yellowish white (1A2); edge even, concolorous with the face; lamellulae frequent, with three lengths (Figs. 2b–c). Stipe 35–41  $\times$  6–9 mm, central, cylindrical, equal to slightly tapered towards the base, dry, light orange (5A4/5) to orange (5A6); surface smooth, slightly ribbed (Figs. 2b–c); context solid. Veil absent. Latex not found. Odor and taste not determined.

Basidiospores  $7\text{--}8.5(9) \times (4\text{--})6\text{--}7 \mu\text{m}$ ;  $L = 7.5 \mu\text{m}$ ,  $W = 6.2 \mu\text{m}$ ;  $Q = 1.14\text{--}1.26$ ,  $Q_m = 1.21$ ; subglobose to broadly ellipsoid; predominantly broadly ellipsoid, thin-walled, hyaline, ornamentations amyloid, up to  $1 \mu\text{m}$  high, composed of irregular short warts connected by fine lines, not forming a complete reticulum; plage non-amyloid; hilar appendix up to  $3.5 \mu\text{m}$  long, inamyloid (Figs 3a, 6a). Basidia  $53\text{--}64 \times 7\text{--}10 \mu\text{m}$ , cylindrical, slightly clavate, sometimes slightly fusiform, 1-2- and 4-spored, predominantly 4-spored, thin-walled, hyaline, sometimes with refractive contents; sterigmata up to  $7 \mu\text{m}$  long (Fig. 3b). Pleuromacrocystidia abundant,  $55\text{--}75 \times 7\text{--}10 \mu\text{m}$ , fusoid to slightly fusiform, thin-walled, with refractive contents; projecting up to  $10 \mu\text{m}$  above the hymenium (Fig. 3c). Pleuropseudocystidia absent. Lamellar edge sterile, composed of abundant cheilomacrocystidia and marginal cells: cheilomacrocystidia  $45\text{--}52 \times 6\text{--}8 \mu\text{m}$ , fusoid to slightly fusiform, thin-walled, with refractive contents (Fig 3d); marginal cells  $10\text{--}30 \times 4\text{--}7 \mu\text{m}$ , cylindrical to clavate, thin-walled, hyaline. (Fig. 3f). Hymenophoral trama composed of abundant sphaerocytes,  $11\text{--}33 \mu\text{m}$  diam. and scattered lactifers,  $7\text{--}10 \mu\text{m}$  diam., thin-walled. Subhymenium cellular. Pileipellis a lampropalisade up to  $75 \mu\text{m}$  thick, consisting of a layer of isodiametric cells with frequent to abundant lactifers and frequent to abundant thick-walled elements (Fig. 3h): isodiametric cells,  $7\text{--}17 \mu\text{m}$  diam., thin-walled; lactifers  $7\text{--}13 \mu\text{m}$  diam, thin-walled, with refractive contents, sometimes emergent, arising deeply from pileus trama, clinal to oblique oriented (Figs. 3e,h); thick-walled terminal elements  $37\text{--}98 \times 3\text{--}6 \mu\text{m}$ , aciculate, filiform, cylindrical,

sometimes flexuous, sometimes septate, sometimes forked, thick-walled (up to 2  $\mu\text{m}$  thick), hyaline, clinal to oblique oriented (Figs. 3g,h). Pileus trama composed of abundant sphaerocytes 9–30  $\mu\text{m}$  diam., thin-walled, cylindrical hyphae, 2–4.5  $\mu\text{m}$  diam. and frequent lactifers, 7–12  $\mu\text{m}$  diam., thin-walled. Stipitipellis a lampropalisade similar to pileipellis. Clamp connections absent from all tissues examined.

**Etymology:**—From Lat. ‘umbilicatus’. In reference to the umbilicate pileus.

**Habitat:**—Scattered, on clay soil in dense ombrophilous forest in Amazon, close to *Tachigali myrmecophila* (Fabaceae).

**Distribution:**—Known only from type locality.

**Materials examined:**—BRAZIL. Pará: Belterra, Floresta Nacional do Tapajós, trilha da comunidade Maguari, 2°48'14" S, 54°59'50" W, 113 m a.s.l., on soil, close to *Tachigali myrmecophila*, 30 March 2014, D.L. Komura, T.S. Cabral, I.R. Fonseca DLK 1964 (INPA 284001 holotype; JPB isotype).

**Additional material examined:**—*Lactifluus amazonensis* Singer in Singer *et al.* (1983: 295) Silva-Filho & Wartchow (2019: in press): Brazil. Amazonas: Manaus, Manaus-Caracarái road, km 125, 20 July 1978, R. Singer B11315 (**holotype** INPA 82756).

**Comment:**—*Lactifluus umbilicatus* belongs to *Lf.* subg. *Pseudogymnocarpi* (De Crop *et al.* 2017) based on both molecular and morphological evidence, such as: its orange pileus color, unchanging context and the lampropalisade structure of the pileipellis. Phylogenetic analysis places the species in *Lf.* sect. *Polysphaerophori*, a clade/section composed by Neotropical entities, but not yet characterized morphologically. This new species clusters with *Lf. ceraceus* from French Guiana and two undescribed species (Th678) from Guyana and (AMV1874) from Colombia. Despite being phylogenetically closely related, *Lf. ceraceus* has a more robust basidiomes, (pileus 64–98 mm diam., and stipe 83–112  $\times$  18–26 mm), smaller basidiospores (5.4–8.1  $\times$  4.4–6.2  $\mu\text{m}$ ), smaller thick-walled elements on the pileipellis (8–42  $\times$  3.5–6  $\mu\text{m}$ ) that are predominantly cylindrical or subclavate and the lacks lactifers on the pileipellis (Crous *et al.* 2017).

Also related are ‘*Lactarius pegleri*’, a species from Martinique, still to be recombined into *Lactifluus*, and *Lf. veraecrucis* (Singer 1973: 104) Verbeken in Verbeken *et al.* (2012: 445) from Mexico. These two species have similar basidiospore sizes, but ‘*Lactarius pegleri*’ has more fragile basidioma, with a smaller pileus (25–57 mm in diam.) and shorter stipe (25–38 mm long), and absence of true pleurocystidia (Lalli & Pacioli 1992); *Lactifluus veraecrucis* differs in the smaller pileus (25–45 mm diam.), smaller basidiospore

ornamentation (up to 0.5  $\mu\text{m}$  high), shorter thick-walled elements on the pileipellis structure (20–44  $\times$  2.5–5.5  $\mu\text{m}$ ) and thick-wall isodiametric cells in pileipellis (Montoya *et al.* 1996). *Lactifluus amazonensis*, *Lf. brasiliensis* (Singer in Singer *et al.* 1983: 308) Silva-Filho & Wartchow (2019: in press) and *Lf. rupestris* also have yellowish to orange pileus, unchanging context and latex and a trichoderm to (lampro)palisade pileipellis, suggesting the placement of these species in *Lf.* subg. *Pseudogymnocarpi* (De Crop *et al.* 2017). These taxa have no molecular data available so far. *Lactifluus amazonensis* is the only species that has thick-walled elements on the pileipellis, similar to *Lf. umbilicatus*, differing in more fragile basidiomata with smaller pileus (33–45 mm diam.) and shorter stipe (19–48 mm length) (Singer *et al.* 1983). In addition, we analyzed the holotype (R. Singer B11315), which exhibits larger (8.5–10.5  $\times$  7–8.0  $\mu\text{m}$ ) and predominantly subglobose basidiospores, abundant cylindrical to subclavate thick-walled elements on pileipellis and absence of lactifers in the pileipellis.

***Lactifluus venosellus*** Silva-Filho, Sá & Wartchow, *sp. nov.* (Figs. 4–5–6b,c)

Mycobank:—MB832852

Genbank accession nrITS: MK929292

Type:—BRASIL. Paraíba: Mamanguape, Reserva Biológica Guaribas, 6°44'27.89" S 35°8'53.82" W, on soil, 30 June 2012, M.C.A Sá MS37 (UFRN-fungos 2197 **holotype**, JPB **isotype**).

Diagnosis:—Distinguished from other species by: pileus convex to slightly depressed, plane-depressed, infundibuliform, surface slightly velutinous, rivulose, reddish brown; lamellae slightly decurrent, distant. Basidiospores 8–9  $\times$  6–7  $\mu\text{m}$ , subglobose to broadly ellipsoid; ornamentations up to 0.3  $\mu\text{m}$  high, composed of irregular short ridges; cystidia absent; scattered to abundant cylindrical, fusoid to slightly fusiform pseudocystidia; pileipellis a palisade with scattered lactifers and unique ITS sequence.

Description:—Pileus 20–40 mm, convex slightly depressed, becoming plane-depressed to infundibuliform, reddish brown (8E4) with dark brown (8F7) spots; surface slightly velutinous, rivulose near to margin, slightly cracked, dry, dull; edge even to slightly eroded, incurved (Figs. 4a–c); context firm, 4–5 mm at the disc, very thin towards at margin, pastel red (7A4), unchanging (Fig. 4c). Lamellae short decurrent, distant, brownish (7D5/6); edge even, concolorous with the face; lamellulae frequent with two lengths, sometimes forked. (Figs. 4a–c). Stipe 15–20  $\times$  7–9 mm, central, cylindric, equal to tapered to the base, dry, reddish brown (8E4), dull red (8B3) sometimes with brown (6E6) spots; surface smooth, sometimes veined (Figs. 4a–c); context solid. Veil absent. Latex not found. Odor and taste not determined.

Basidiospores (7.5–)8–9(–10) × (5.5–)6–7(–7.5) μm; L = 8.5 μm, W = 6.5 μm; Q = 1.18–1.47, Qm = 1.37; subglobose to broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 0.3 μm high, composed of irregular short ridges and warts; plage non-amyloid; hilar appendix up to 1 μm long (Figs. 5a, 6b,c). Basidia 53.5–80 × 6–8 μm cylindrical to slightly clavate, 4-spored, thin-walled, hyaline, sometimes with refractive contents; sterigmata up to 6 μm high (Fig. 5b). Pleuromacrocystidia absent. Pleuropseudocystidia abundant, 5.5–10 μm diam., cylindrical to slightly fusiform, thin-walled, with refractive contents; projecting up to 35 μm above the hymenium; arising deeply from hymenophoral trama (Fig. 5c). Lamellae edge sterile, composed of scattered cheilopseudocystidia and common marginal cells: cheilopseudocystidia 4–8 μm diam., cylindrical and fusoid, thin-walled, with refractive contents, projecting up to 18 μm above the hymenium, arising deeply from hymenophoral trama (Fig. 5d); marginal cells 28–35 × 7.5–7 μm, clavate, cylindro-clavate, sphaeropendunculate, catenulate, sometimes flexuous, thin-walled, hyaline (Fig. 5e). Hymenophoral trama composed of abundant sphaerocytes 9–17 μm diam. and frequent lactifers 7–12 μm diam., thin-walled. Pileipellis a palisade up to 85 μm thick, composed of isodiametric cells with cylindrical, clavate and obclavate terminal cells and scattered lactifers: isodiametric cells, 9–22 μm diam., thin-walled; lactifers, 7–10 μm diam., with refractive contents, sometimes emergent, arising deeply from pileus trama (Figs. 5f,g); terminal cells 12.5–50 × 3.5–11.5 μm, cylindrical, clavate sometimes obclavate, thin-walled, hyaline (Fig. 5g). Pileus trama composed of abundant sphaerocytes 6–21 μm diam., cylindrical hyphae, 2–5 μm diam. and frequent to abundant lactifers, 7–12 μm diam., thin-walled. Stipitipellis a palisade similar to pileipellis. Clamp connections absent from all tissues examined.

Etymology:—In reference to the veined surface of the pileus.

Habitat:—Scattered, on sand soil, in a savanna area of Atlantic Forest, under unknown host, but growing surrounded by tree species of *Coccoloba alnifolia* Casaretto (1842, 71), *Coccoloba laevis* Casaretto (1842, 71), *Coccoloba mollis* Casaretto (1842, 72) *Coccoloba ramosissima* Weddel (1850, 257) and *Coccoloba scandens* Casaretto (1842, 70).

Distribution:—Known only from type locality.

Materials examined:—BRASIL. Paraíba: Mamanguape, Reserva Biológica Guaribas, 6°44'27.89" S 35°8'53.82" W, on soil, 30 June 2012, *M.C.A Sá MS37* (UFRN-fungos 2197 holotype, JPB isotype); *ibid.*, 05 July 2015, *FW 15/2017* (JPB).

Additional material examined:—*Lactifluus brasiliensis*: BRAZIL. Amazonas: Manaus, road Manaus-Caracaraí Km 45, 28 February 1978; *St. John, Araujo and R. Singer B10729* (holotype INPA 77475), *ibid.*, 12 January 1979, *R. Singer B 11479* (paratype INPA 82380).

**Comment:**— *Lactifluus venosellus* also belongs to *Lf.* subg. *Pseudogymnocarpi* (De Crop *et al.* 2017) based on molecular and morphological evidence. In our phylogeny *Lf. venosellus* clusters with other five undescribed South American species, forms a branch in *Lf.* sect. *Polysphaerophori*. Among them, the collection (TH7677) from Guyana was identified as *Lf.* aff. *brasiliensis*. *Lactifluus brasiliensis* as mentioned above, perhaps belongs to *Lf.* subg. *Pseudogymnocarpi* (Singer *et al.* 1983). Both species share a similar pileipellis structure, but *Lf. brasiliensis* differs at least in the larger pileus (20–40 mm diam.) and stipe (15–20 × 7–9 mm) (Singer *et al.* 1983). We also analyzed the holotype (St. John, Araujo and R. Singer B10729) and paratype (R. Singer B11479) of *Lf. brasiliensis*, which possess predominantly subglobose basidiospores ( $Q_m = 1.18$ ) with higher ornamentation (up to 1  $\mu\text{m}$  high) and lacks pleuropseudocystidia. *Lactifluus rupestris* described from Caatinga, is distinguished from *Lf. venosellus* by larger pileus (60–70 mm) with red tint, larger stipe (35–45 × 18–21 mm), crowded lamellae, scarce pleuropseudocystidia and a trichoderm as pileipellis (Wartchow & Cavalcanti 2010).

### Discussion

This study has revealed two new species of *Lactifluus* from the Amazon and Atlantic forests and highlights the phylogenetic diversity of *Lactifluus* in South America. As many Neotropical Russulaceae species, *both* were sampled only in a single location (Roy *et al.* 2016). This points the need for more field and taxonomic surveys to understand the distribution of *Lactifluus* diversity in Brazilian biomes and reveal new species. The ITS phylogeny revealed well-supported branches in *Lactifluus* subg. *Pseudogymnocarpi*, with possibly new sections and/or subsections to be recognized in the future. The further combination of taxonomic and phylogenetic studies will help identifying the evolutionary history of *Lactifluus* in South American biomes, and the processes shaping their diversity.

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Tabela 3. **TABLE 1.** Specimens, voucher collection, origin and GenBank accession numbers of nrITS sequences used in the molecular analyses. Accession numbers in bold were newly generated for this study.

Species	Origing	Voucher Collection	ITS accession n°
<i>'Lactarius pegleri'</i>	Martinique	PAM/Mart 12-091	KP691416
<i>Lactifluus armeniacus</i>	Thailand	EDC 14-501	KR364127
<i>Lf. bertillonii</i>	Germany	JN 2012-016	KR364087
<i>Lf. carmineus</i>	Zimbabwe	AV 99-099	KR364131
<i>Lf. ceraceus</i>	French Guiana	LD16-005	KY884995
<i>Lf. ceraceus</i>	French Guiana	M. Roy, PC0713390	KY884996
<i>Lf. denigricans</i>	Tanzania	EDC 11-218	KR364051
<i>Lf. goossensiae</i>	Guinea	AB 320	KR364132
<i>Lf. gymnocarpoides</i>	Congo	JD 885	KR364074
<i>Lf. gymnocarpoides</i>	Malawi	AV 05-184	KR364024
<i>Lf. hygrophoroides</i>	North America	AV 05-251	HQ318285
<i>Lf. kigomaensis</i>	Tanzania	AV 11-006	KR364052
<i>Lf. kigomaensis</i>	Tanzania	EDC 11-012	KY562731
<i>Lf. kivuensis</i>	Congo	JR Z 310	KR364027
<i>Lf. longisporus</i>	Burundi	AV 94-557	KR364118
<i>Lf. cf. longisporus</i>	Tanzania	AV 11-025	KR364054
<i>Lf. luteopus</i>	Tanzania	EDC 11-087	KR364049
<i>Lf. luteopus</i>	Burundi	AV 94-463	KR364119
<i>Lf. medusae</i>	Cameroon	EDC 12-152	KR364069
<i>Lf. pilosus</i>	Thailand	LTH 205	KR364006
<i>Lf. pseudoluteopus</i>	Thailand	FH 12-026	KR364084
<i>Lf. cf. pseudogymnocarpus</i>	Malawi	AV 05-085	KR364012
<i>Lf. cf. pseudovolemus</i>	Benin	ADK 2927	KR364113
<i>Lf. cf. pumilus</i>	Cameroon	EDC 12-066	KR364067
<i>Lf. rubiginosus</i>	Congo	JD 959 (BR)	KR364081
<i>Lf. rubiginosus</i>	Zambia	BB 3466	KR364014
<i>Lf. aff. rubroviolascens</i>	Cameroon	EDC 12-051	KR364066
<i>Lf. rugatus</i>	Greece	EP 1212/7	KR364104
<i>Lf. subkigomaensis</i>	Tanzania	AV 11-066	KY562728
<i>Lf. subvellereus</i>	North America	AV 05-210	KR364010
<i>Lf. umbilicatus</i> sp. nov.	Brazil	D.L.K. 1964	<b>MK929291</b>

<i>Lf. venosellus</i> sp. nov.	Brazil	M.C.A. Sá MS 37	<b>MK929292</b>
<i>Lf. veraecrucis</i>	Mexico	M 8025	KR364112
<i>Lf. volemoides</i>	Mozambique	MH 201187	KR364098
<i>Lf. volemoides</i>	Tanzania	TS 0705	KR364038
<i>Lf. xerampelinus</i>	Tanzania	TS 1116	KR364039
<i>Lf. xerampelinus</i>	Mozambique	MH 201176	KR364099
<i>Lactifluus</i> sp.	Guyana	MCA 3937	KR364109
<i>Lactifluus</i> sp.	Guyana	G4258	KJ786715
<i>Lactifluus</i> sp.	Guyana	G3144	KJ786691
<i>Lactifluus</i> sp.	Guyana	G082	KJ786645
<i>Lactifluus</i> sp.	Guyana	TH7658	KT339247
<i>Lactifluus</i> sp.	Guyana	TH7677	KT339245
<i>Lactifluus</i> sp.	French Guiana	G4799	KM073088
<i>Lactifluus</i> sp.	Colombia	AMV2209	KT354752
<i>Lactifluus</i> sp.	Colombia	AMV1874	KT354738

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Figura 20 - **FIGURE 1.** The ML phylogeny based on ITS sequences. *Lactifluus umbilicatus* sp. nov. and *Lf. venosellus* sp. nov. are in red. The thicker branches represent those ones with maximum values of bootstrap values and posterior probabilities (100 % BS / 1.0 BPM). Bootstrap values and Bayesian posterior probabilities are indicated if they exceed 80% and 0.95, respectively. The bar scale represents the expected number of nucleotide changes per site.

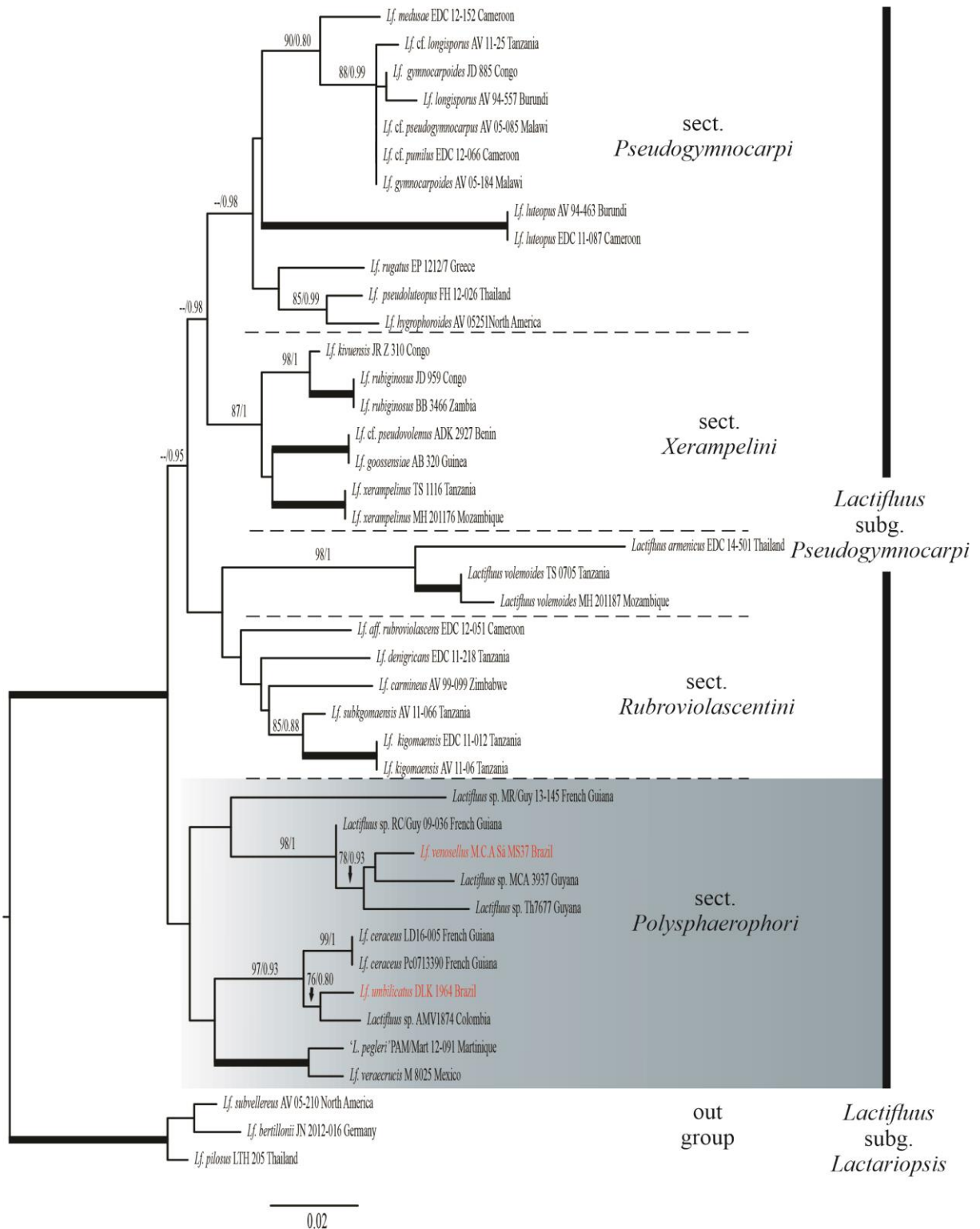


Figura 21 - **FIGURE 2.** *Lactifluus umbilicatus*. **a-d** D.L. Komura 1964 (holotype). **a**) basidiomata *in situ*, **b-d**) basidiomata *ex situ*. (bars = 10  $\mu$ m). Photos by: Dirce L. Komura.

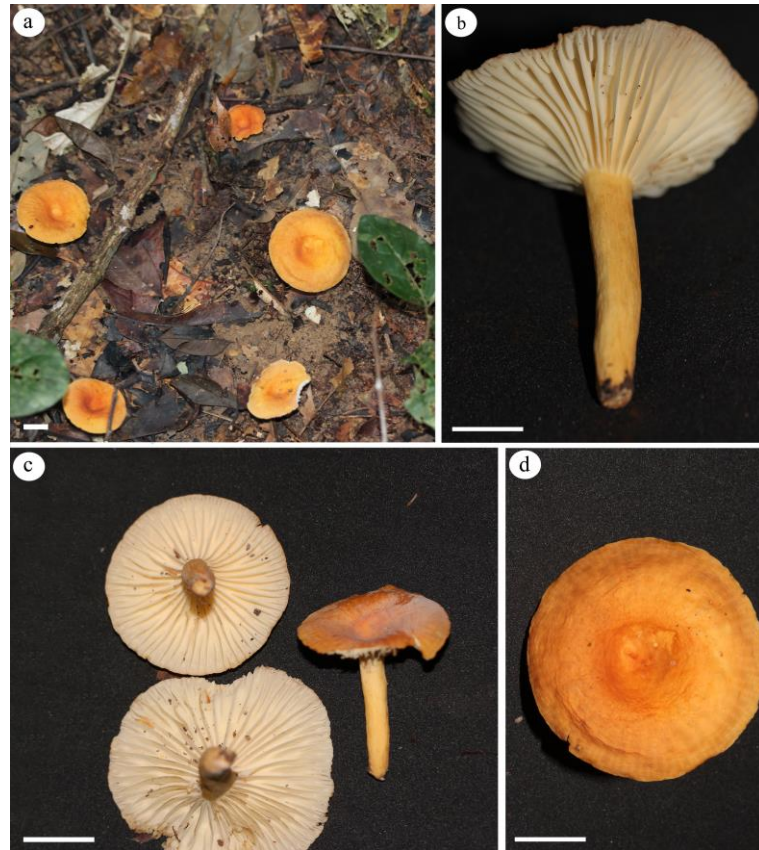


Figura 22 - **FIGURE 3.** *Lactifluus umbilicatus*. **a–h** *D.L. Komura 1964* (holotype). **a**) basidiospores **b**) basidia, **c**) pleuromacrocytidia, **d**) cheilomacrocytidia, **e**) lactifers of the pileipellis, **f**) marginal cells of the lamellar edge, **g**) thick-walled elements of the pileipellis, **h**) pileipellis. (bars = 10  $\mu$ m). Drawings by: Alexandre G. S. Silva-Filho.

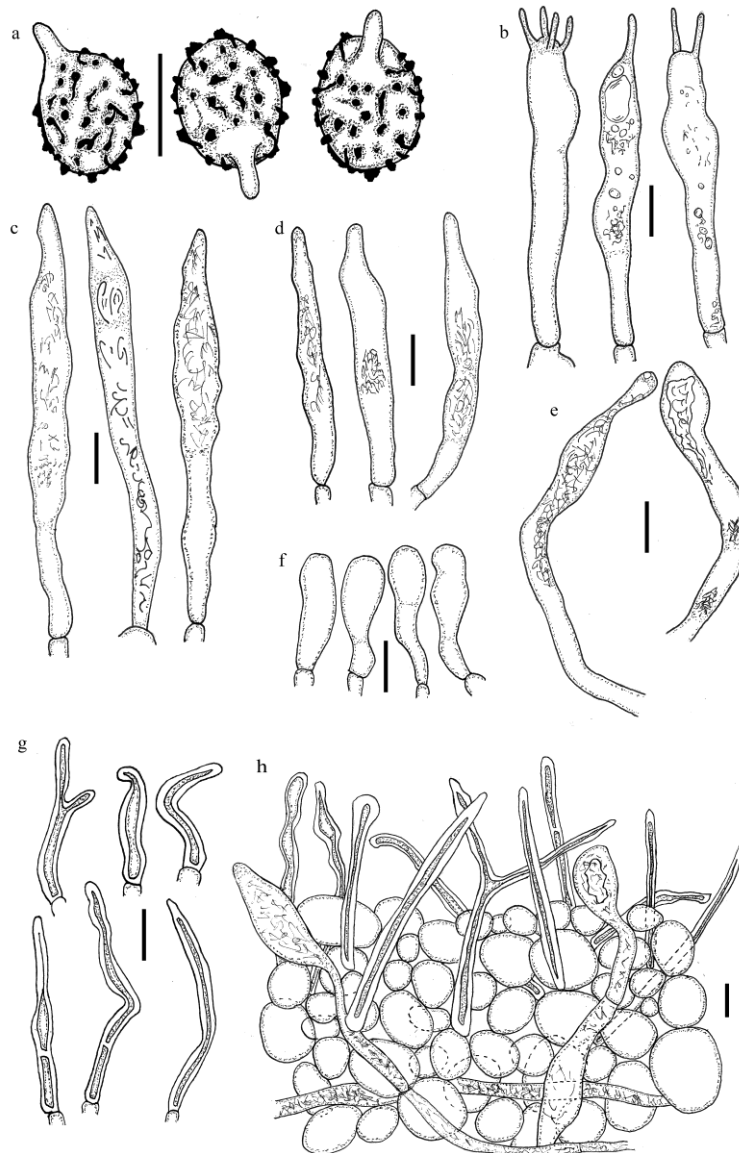


Figura 23 - **FIGURE 4.** *Lactifluus venosellus*. **a** M.C.A. Sá MS 37 (holotype), **b,c** F. Wartchow 15/2017 (Paratype). **a** basidiomata *in situ*, **b,c** basidiomata *ex situ* (bars = 10  $\mu$ m). Photos: **a**) Mariana C A. Sá. **b**) Felipe Wartchow.



Figura 24 - **FIGURE 5.** *Lactifluus venosellus*. **a–g** M.C.A. Sá MS 37 (holotype). **a)** basidiospores **b)** basidia, **c)** pleuropseudocystidia, **d)** cheilopseudocystidia, **e)** marginal cells of the lamellar edge, **f)** lactifers of the pileipellis, **g)** pileipellis. (bars = 10  $\mu$ m). Drawings by: Alexandre G. S. Silva-Filho

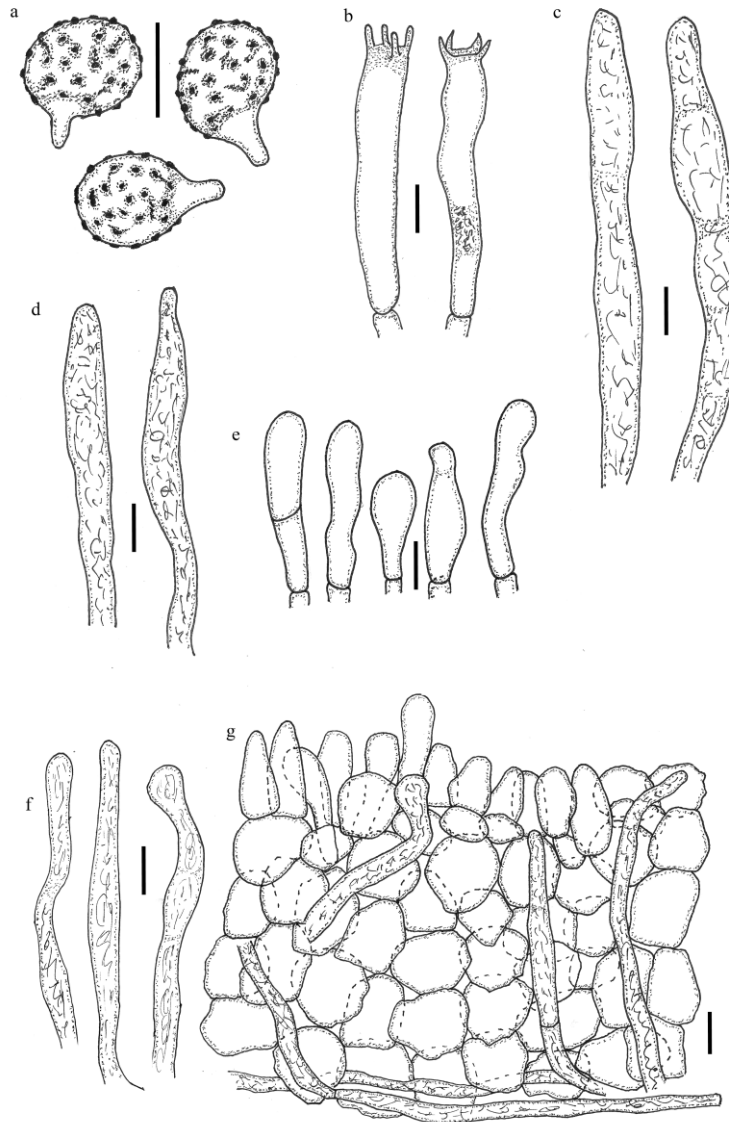
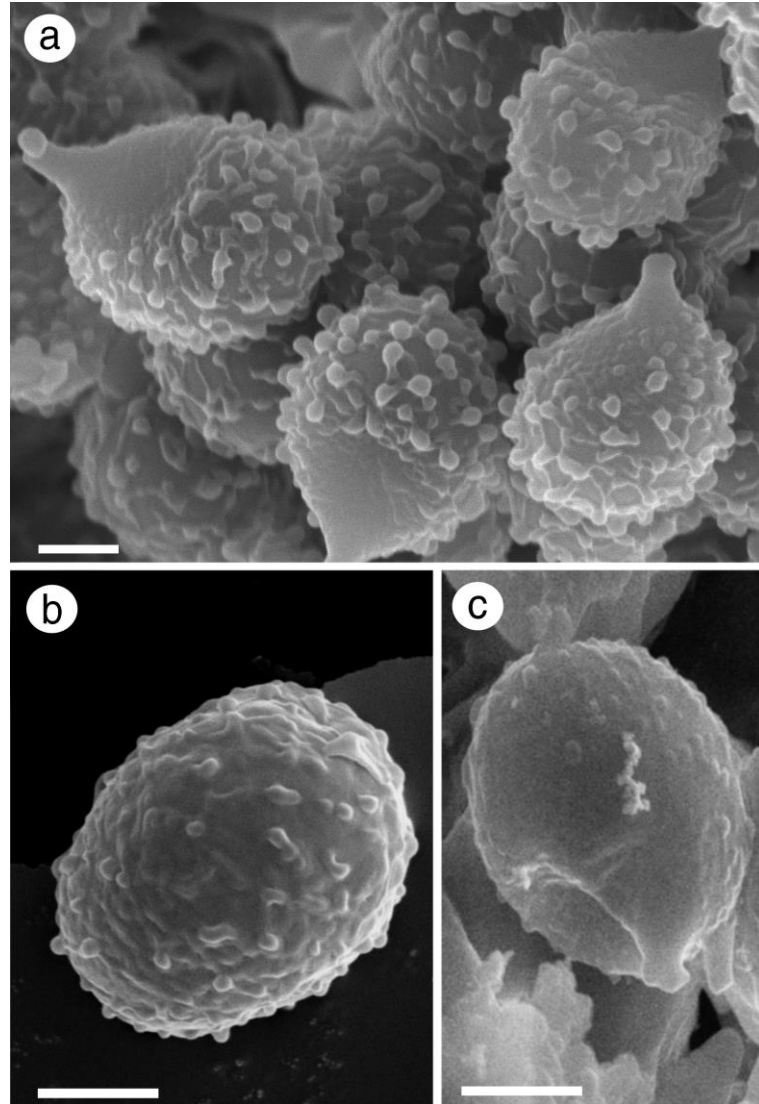


Figura 25 - **FIGURE 6.** SEM micrographs of the basidiospores. **a)** *Lactifluus umbilicatus* (D.L. Komura 1964 holotype **b,c)** *Lactifluus venosellus* (M.C.A. Sá MS 37 holotype). (bars = 5  $\mu$ m).



### **8 CAPÍTULO 3 - Novelty in *Lactifluus* subg. *Gymnocarpi* (Russulales, Basidiomycota) from Brazilian tropical forests**

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## Abstract

*Lactifluus* is an ectomycorrhizal genus that occurs predominantly in the tropical and subtropical regions. In Brazil, knowledge of species diversity in *Lactifluus* is restricted to the Amazon, Atlantic Forest and Caatinga biomes, where several species have been described in subgenera *Lactariopsis* and *Pseudogymnocarpi*. Until now, subg. *Gymnocarpi* was represented in Brazil only by *Lf. panuoides*. Based on morphological and phylogenetic analyses, three novel species of *Lf.* subg. *Gymnocarpi* are described from Brazilian tropical forest: *Lf. guttulatus*, *Lf. piperogalactus* and *Lf. spathuliformis*. *Lactifluus lepus*, originally described in the Caribbean, has been found in a dense ombrophilous forest of Amazon and is also described, illustrated and discussed. A combined dataset of nuclear rDNA internal transcribed spacer region (ITS) and RNA polymerase II second largest subunit (*RPB2*) was analyzed. Based on phylogenetic and morphological data, the agaricoid sect. *Piperogalacti* is erected and the pleurotoid sect. *Panuoides* is emended.

Key words: ITS, milkcaps, Neotropics, new taxa, phylogeny, *RPB2*

## Introduction

The ectomycorrhizal milkcap lineage is comprised of three genera: *Lactarius* Pers. (abbreviated *L.*) with approximately 450 spp., *Lactifluus* (Pers.) Roussel (abbreviated *Lf.*) composed of about 200 spp. and *Multifurca* Buyck & V. Hofst. with 10 spp. (He et al. 2019). Among them, *Lactifluus* is predominantly represented in the tropics (De Crop et al. 2017) with the highest known diversity in Africa (Verbeken and Walley 2010, Delgat et al. 2017, De Lange et al. 2018) and Asia (Le et al. 2007; Van De Putte et al. 2010; Stubbe et al. 2012). The Neotropical region remains underexplored (Roy et al. 2016), although this situation is changing with the discovery of new species (e.g. Henkel et al. 2000; Miller et al. 2002; Miller et al. 2012; Sá et al. 2013; Sá and Wartchow 2013; Wartchow et al. 2013; Crous et al. 2017; Sá et al. 2019; Barbosa et al. 2020; Silva-Filho et al. 2020).

In the recent classification of *Lactifluus* (De Crop et al. 2017), four subgenera were recognized: *Lf.* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop, *Lf.* subg. *Lactariopsis* (Henn.) Verbeken, *Lf.* subg. *Lactifluus*, and *Lf.* subg. *Pseudogymnocarpi* (Verbeken) De Crop. Subgenus *Gymnocarpi* is distributed worldwide with high diversification in the African continent (Verbeken 1998; Verbeken and Walley 2010) and comprises six described sections (De Crop et al. 2017; Dierickx et al. 2019; Delgat et al. 2020): (1) *Lf.* sect. *Luteoli* (Pacioni & Lalli) Verbeken includes species distributed in all continents except South America and Antarctica; (2) *Lf.* sect. *Gymnocarpi* (R. Heim ex Verbeken) De Crop and (3) *Lf.* sect. *Phlebonemi* (R. Heim ex Verbeken) Verbeken harbor exclusively African species; (4) *Lf.* sect. *Tomentosi* (McNabb) Verbeken is formed by representatives

from Oceania; (5) *Lf.* sect. *Nebulosi* Delgat is composed of Central American and Caribbean species; and (6) *Lf.* sect. *Panuoidei* (Singer) Delgat includes the Neotropical species *Lf. panuoides* (Singer) De Crop. In addition, subg. *Gymnocarpi* contains an unnamed clade that includes the Neotropical species *Lf. lepus* Delgat & Courtec., and three other isolated species: *Lf. foetens* (Verbeken) Verbeken from Africa, *Lf. brunellus* (S.L. Mill., Aime & T.W. Henkel) De Crop and an undescribed species, both from South America.

Members of *Lf.* subg. *Gymnocarpi* exhibit considerable morphological variation, with both agaricoid and pleurotoid basidiomata; however, they are characterized by latex that changes to brown and the absence of true pleurolamprocystidia (De Crop et al. 2017). Except for *Lf.* sect. *Nebulosi*, the pileipellis structure in most species is composed of a lampropalisade (Pegler and Fiard 1979; Montoya et al. 1996; Delgat et al. 2020).

*Lactarius* sect. *Panuoidei* Singer was originally formed to accommodate the pleurotoid milkcap *L. panuoides* Singer (Singer 1952). Later, Singer (1984) described *L. uyedae* Singer from Japan and *L. campinensis* Singer and *L. igapoensis* Singer from Brazil. Miller et al. (2002) described *L. brunellus* S.L. Mill., Aime & T. W. Henkel and *L. multiceps* S.L. Mill., Aime & T.W. Henkel from Guyana. All these species were recombined in *Lactifluus*, except for *L. campinensis*, which was transferred to *Russula* (Henkel et al. 2000; Miller et al. 2002). Further studies using a phylogenetic approach revealed the polyphyletic nature of pleurotoid milkcaps (Stubbe et al. 2012) which have evolved several times, independently, and are embedded in the phylogeny among species with an agaricoid habit, in three different subgenera (De Crop et al. 2017). Seven Asian species were subsequently described within *Lf.* subg. *Lactifluus* (De Crop et al. 2018). Furthermore, *Lactifluus chrysocarpus* E.S. Popov & O.V. Morozova from Vietnam and *Lf. multiceps* from Guyana within *Lf.* subg. *Lactariopsis*, *Lf. panuoides* (Singer) De Crop and *Lf. brunellus* (S.L. Miller, Aime & T.W. Henkel) De Crop within *Lf.* subg. *Gymnocarpi* (De Crop et al. 2017). Recently, *L.* sect. *Panuoidei* was transferred to *Lactifluus* (Delgat et al. 2020).

The five Brazilian biomes (Atlantic Forest, Amazon Forest, Caatinga, Pampa, Cerrado and Pantanal) include two of thirty-four recognized global hotspots (Forza et al. 2012) and constitute an important center of biodiversity (Myers et al. 2000). *Lactifluus* is represented best in the Amazon and Atlantic rain forest, with ten taxa (Singer et al. 1983; Singer 1984; Silva-Filho et al. 2019) and 11 taxa, respectively (Rick 1906, 1907, 1930; Buyck and de Meijer 1999; de Meijer 2006; Sá et al. 2013; Sá and Wartchow 2013; Wartchow et al. 2013; Barbosa et al. 2020; Silva-Filho et al. 2020). In the Caatinga biome, two taxa were described (Wartchow & Cavalcanti 2010; Sá et al. 2019), while in Cerrado, Pampa and Pantanal, there are no records of *Lactifluus* species.

Specimens of *Lf.* subg. *Gymnocarpi* were collected during field trips in the Amazon, Atlantic forest and Caatinga biomes and investigated using phylogenetic and morphological analyses; they represent novelties within *Lf.* subg. *Gymnocarpi*.

## Materials and Methods

### Collecting area

Fresh specimens were collected in different areas of the Amazon and Atlantic forests and Caatinga biomes (Table 1, Fig. 1).

### Morphological analysis

Macroscopic features were recorded from fresh material. Color names and codes follow Kornerup and Wanscher (1978). Micromorphological analyses were performed using the methodology of Verbeken and Walley (2010). Basidiospores were measured excluding ornamentation, in lateral view, using Melzer's reagent. Biometric measurements of the basidiospores follow the methodology of Wartchow and Gamboa-Trujillo (2012), where 'n' is the number of measured basidiospores/analyzed basidiomata/collections. Other microscopic structures were hydrated in 5% KOH and then measured. For scanning electron microscopy (SEM), dried lamellae were directly stuck to an adhesive layer on a small clean coverslip, coated with gold and observed under Jeol JSM-6360LV and JSM-6010LA. Specimens were deposited in the Herbaria JPB, INPA (Thiers, 2020, continuously updated) and in the UFRN-Fungos Herbarium.

### Molecular methods

Total fungal DNA was extracted using the Qiagen DNeasy® Plant Mini Kit (Germantown, MD, USA) according to the manufacturer's instructions. The nuclear ribosomal internal transcribed spacer (ITS) region was amplified and sequenced using primers ITS1-F and ITS4-B (White et al. 1990) and 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). Sequencing reactions were conducted by MACROGEN (KOREA) and the resulting sequences were assembled and edited in Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were deposited in Genbank public database (Table 1).

### Phylogenetic analyses

Six new ITS and four novel *RPB2* sequences were generated in this study. Most of the retrieved sequences were downloaded from NCBI (Genbank) and represent species of *Lf.* subg. *Gymnocarpi*. Four sequences of *Lf.* subg. *Pseudogymnocarpi* were used as outgroup: *Lf. carmineus* (Verbeken and Walley) Verbeken, *Lf. denigricans* (Verbeken and Karhula) Verbeken, *Lf. kigomaensis* De Crop & Verbeken and *Lf. aff. rubroviolascens*. Our dataset was constructed with combined ITS + *RPB2* with 57 sequences of 41 species, which was aligned using

MAFFT v.7 (Katoh 2013) under the E-INS-i criteria. Seaview v.4 (Gouy et al. 2010) was used to visualize and adjust the alignment. Gblocks v0.91b (Castresana 2000) was used to eliminate poorly aligned positions in the alignment, with settings allowing gaps within selected blocks, smaller blocks and bigger segments with contiguous non-conserved positions. In order to compute the best fit model of nucleotide evolution, ITS was subdivided into three data partitions, ITS1, 5.8S and ITS2, and the *RPB2* into the intron and the first, second and third codon positions of the exon. Maximum Likelihood analyses were performed in RAxML v8.2.X (Stamatakis 2006). The best nucleotide substitution model was selected with BIC (Bayesian Information Criterion) using jModelTest 2v.1.6 [46–47]. The evolutionary models selected for the dataset were: ITS1: TIM2ef+G, 5.8S: TrNef+I, ITS2: TPM3uf+G, *RPB2* Intron: K80+G, *RPB2* 1st codon: HKY+I, *RPB2* 2nd codon TPM1uf+I and *RPB2* 3rd codon: TrNef+G. Bayesian inferences (BI) were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), performing 2 x 10<sup>7</sup> MCMC generations, sampling one tree every 1 x 10<sup>3</sup> generation. The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 software were implemented in the CIPRES Science Gateway 176 3.1 (Miller et al. 2010). The final alignment and the resulting topologies were deposited in TreeBASE, ID: 26818.

## Results

### Phylogenetic results

The dataset included sequences from 57 *Lactifluus* specimens with 1734 characters (including introduced gaps). The ITS and *RPB2* phylogenetic inferences using either RAxML or Bayesian analyses yielded similar tree topologies. Fig. 2 shows the tree generated in the ML analysis, including bootstrap and posterior probabilities. *Lactifluus* subg. *Gymnocarpi* has good support (100% BS, 1.0 BPP) in accordance with worldwide molecular analysis of *Lactifluus* by De Crop et al. (2017). The Neotropical species are clustered in six branches: sections *Nebulosi* (100% BS, 1.0 BPP) and *Panuoidei* (100% BS, 1.0 BPP), the newly described section *Piperogalacti* (100% BS, 1.0 BPP), and two isolated species: *Lactifluus* sp. (G3185) from French Guiana and *Lf. brunellus* from Guyana. The two new pleurotoid species are included in *Lf.* sect. *Panuoidei*, representing distinct lineages and the new agaricoid species belongs in sect. *Piperogalacti* with *Lf. lepus*.

### Taxonomy

#### ***Lactifluus* sect. *Panuoidei* (Singer) Delgat, *Persoonia* 44: 292 (2020) emend.**

Original diagnosis: ‘Epicute pilei ex pilis crasse tunicatis flavis composita; hypodermio ex hyphis inflatis saepe catenulatis composito; sporis heterotropis, cristatis vel reticulatis; superficie pilei adulti scrobiculato-venosa; stipite laterali: lattice albescente-aquoso, gloeo-cystidiis absentibus; sapore miti: lignicolae’.

Remarks: The section is emended here to include species with exclusively Neotropical distribution, white latex that changes to brown and ectomycorrhizal associations.

***Lactifluus guttulatus* Silva-Filho, D.L. Komura & Wartchow, sp. nov. (Figs 3, 4 and 5a)**

Mycobank: MB 836712

Type: Brazil. Amazonas, Novo Airão, CIAPA, trail returning to point 1, 16 Apr 2014, leg. Komura D.L., Cabral, T.S., DLK 2042 (INPA 284002 holotype!, JPB 64508 Isotype!)

Diagnosis: Characterized among *Lf.* sect. *Panuoidei* by a combination of the following: Pileus 10–35 mm diam., subreniform to petaloid. Basidiospores (6–)7–9(–10.5) x 6–8.5(–10)  $\mu\text{m}$ , ornamented with irregular large warts mixed with irregular and short warts and ridges connected by fine lines, not forming a complete reticulum. True cystidia absent. Pseudocystidia scattered to frequent, thin-walled. Pileipellis a lampropalisade with abundant thick-walled terminal elements and partially covered by yellow-green droplets/incrustations in KOH mounts.

Etymology: From Latin ‘guttula’= drop. In reference to yellow-green droplets on pileipellis.

Basidiomata pleurotoid, densely gregarious (Fig. 3). Pileus 10–35 mm diam., subreniform, petaloid, white (1A1), dark orange (5A8), brownish orange (5C6) to grayish orange (6B7); surface smooth, moist to slightly glutinous; margin smooth, decurved to slightly incurved, regular, entire, neither sulcate or striate (Fig. 3); context firm, up to 1 mm thick, white (1A1), changing to brown (6E8) when exposed or touched. Lamellae short decurrent, subdistant, white (1A1), yellowish white (1A2) dark orange (5A8), brownish orange (5C6) to grayish orange (6B7); edges even, concolorous with faces; lamellulae frequent, of one length (Fig. 3). Stipe 8–12 x 8–12 mm, lateral, reduced, compressed, equal, concolorous with the pileus; surface smooth, moist to slightly glutinous (Fig. 3); context solid. Subiculum white (1A1), interwoven, texture sponge-like (Fig. 3). Latex exuding from lamellae white (1A1), changing to brown (6E8). Odor and taste not determined.

Basidiospores (6–)7–9(–10.5) x 6–8.5(–10)  $\mu\text{m}$ ,  $L = 7.3 \mu\text{m}$ ,  $W = 6.7 \mu\text{m}$ ,  $Q = 1–1.16$   $Q_m = 1.07$ ;  $n = 80/4/1$ ; globose to broadly ellipsoid, predominantly subglobose; thin-walled, hyaline; ornamentation amyloid, up to 0.8  $\mu\text{m}$  high, composed of irregular large warts mixed with short warts and ridges; plage completely amyloid; hilar appendix to 2  $\mu\text{m}$  long (Figs 4a, 5a). Basidia 36–47 x 7–11  $\mu\text{m}$ , cylindrical to slightly clavate, 4-spored, thin-walled, hyaline, sometimes with refringent contents in the lower portion (Fig. 4b); sterigmata up to 11  $\mu\text{m}$  long. Pleurocystidia absent. Pleuropseudocystidia scattered to frequent, 4–9  $\mu\text{m}$  diam., cylindrical to clavate, sometimes with forked apex, thin-walled, with refractive contents, projecting up to 15  $\mu\text{m}$  above the hymenium, arising deeply from the hymenophoral trama (Fig. 4c). Lamellar edge sterile, composed of scattered cheilopseudocystidia and marginal cells: cheilopseudocystidia 4–9  $\mu\text{m}$  diam., cylindrical to clavate, occasionally with forked apex, thin-walled, with refractive contents, not projecting above the hymenium, arising deeply from

the hymenophoral trama (Fig. 4f); marginal cells 15–23 x 6–9  $\mu\text{m}$ , cylindrical to clavate, thin-walled, hyaline (Fig. 4e). Hymenophoral trama subregular, composed of scattered to frequent cylindrical, 4–7  $\mu\text{m}$  diam., thin-walled hyphae, and abundant lactiferous hyphae, 5–10  $\mu\text{m}$  diam. Subhymenium composed of thin-walled cylindrical hyphae. Pileipellis a lampropalisade up to 80  $\mu\text{m}$ , consisting of a layer of isodiametric cells with abundant thick-walled terminal elements (Fig. 4d): isodiametric cells 12–59  $\mu\text{m}$  diam., thin-walled, hyaline, the upper layers partially covered by yellow green droplets/incrustations in KOH; terminal elements 50–139 x 5–11  $\mu\text{m}$ , aciculate, filiform, cylindrical, sometimes with broad base, thick-walled, up to 3  $\mu\text{m}$  thick, sometimes septate, hyaline, sometimes with yellow-green droplets incrustated on apex, vertically oriented, sometimes diagonally oriented (Fig. 4g). Pileus trama composed of cylindrical 5–8  $\mu\text{m}$  diam., thin-walled hyphae and abundant lactiferous hyphae, 4–11  $\mu\text{m}$  diam. Stipitipellis a lampropalisade similar to the pileipellis. Clamp connections absent in all tissues examined.

Ecology and distribution: Gregarious on trunks, between bryophyte and algae; collected in May, in ‘terra-firme’ forest in the Amazon. Known only from type locality.

Remarks: *Lactifluus guttulatus* is macromorphologically similar to *Lf. panuoides*, because of its small (up to 35 mm diam.), pleurotoid and gregarious habit, and subreniform to petaloid basidiomata (Singer 1952). Micromorphologically it can be identified by its larger basidiospores measuring 7–9 x 6–8.5  $\mu\text{m}$ , ornamented with connected irregular and large warts with short warts and ridges, lampropalisade structure with abundant thick-walled terminal elements covered by yellow-green drops and thin-walled pseudocystidia. In contrast, *Lf. panuoides* produces basidiospores with more reticulated ornamentation, thick-walled pseudocystidia and pileipellis terminal elements lacking yellow-green drops (Singer 1952; Verbeken 1998).

***Lactifluus spatuliformis* Silva-Filho, D.L. Komura & Wartchow, sp. nov. (Figs. 5b, 6 and 7).**

Mycobank: MB 836713

Type: Brazil. Pará, Belterra, Floresta Nacional do Tapajós, Trilha da Comunidade Jamaraguá, 25 Mar 2014, leg. Komura D.L., Cabral, T.S., Fonseca, I.R. DLK 1891 (INPA 284000 holotype!, JPB Isotype!).

Diagnosis: Distinguished from other species of *Lf.* sect. *Panuoides* by a combination of the following: Basidiomata sometimes caespitose. Pileus 80–180 mm diam., petaloid to spatulate. Basidiospores (5–)6–7(–8) x 5–6(–7)  $\mu\text{m}$ , ornamentation composed of connected irregular short ridges and warts forming a complete reticulum. Pseudocystidia abundant, occasionally with thick-walled apex.

Etymology: In reference to spatulate shape of the basidiomata.

Basidiomata pleurotoid, gregarious, sometimes caespitose (Fig. 6). Pileus (70)80–180 mm diam., petaloid, subspatulate to spatulate, white (1A1) becoming yellowish grey (3B2), grayish orange (5B4) light brown (6D8)

or brown (6E8); surface glabrous, initially slightly glutinous, becoming dry, mat, waxy; margin decurved to incurved, becoming straight, edge smooth, entire, sometimes lobed, (Fig. 6); context firm, up to 2 mm thick, white (1A1), changing to brown (6E8). Lamellae short decurrent to decurrent, subdistant, white (1A1), becoming yellowish grey (3B2), grayish orange (5B4) light brown (6D8) or brown (6E8); edges entire, concolorous; lamellulae frequent, of three lengths (Fig. 6a). Stipe 2.5–3.5 x 2–3 mm, sessile, lateral, reduced or absent, concolorous with the pileus; surface glutinous becoming dry, mat, waxy (Fig. 6). Subiculum white (1A1), densely interwoven, texture sponge-like. Latex white (1A1), changing to brown (6E8). Smell and taste not determined.

Basidiospores (5–)6–7(–8) x 5–6(–7)  $\mu\text{m}$ ,  $L = 6.4 \mu\text{m}$ ,  $W = 5.7 \mu\text{m}$ ,  $Q = 1.04–1.20(–1.27)$ ,  $Q_m = 1.13$ ;  $n = 80/4/2$ ; subglobose to broadly ellipsoid, predominantly subglobose; thin-walled, hyaline; ornamentation amyloid, up to 0.5  $\mu\text{m}$  high, composed of irregular short ridges and warts connected by lines forming a complete reticulum; plage completely amyloid; hilar appendix to 5  $\mu\text{m}$  long (Figs 7a, 5B). Basidia 29.5–43 x 4–7  $\mu\text{m}$ , cylindrical to slightly clavate, 4-spored, hyaline, sometimes with refringent contents; sterigmata up to 5  $\mu\text{m}$  long (Fig. 7a). Pleurocystidia absent. Pleuropseudocystidia abundant, 6–8.5  $\mu\text{m}$  diam., cylindrical to clavate, sometimes forked, slightly flexuous, thin-walled, occasionally with thick-walled apex, with refractive contents, projecting up to 20  $\mu\text{m}$  above the hymenium, arising deeply from the hymenophoral trama (Fig. 7c). Lamellar edge fertile, composed of scattered basidia, scattered cheilopseudocystidia, and marginal cells: cheilopseudocystidia, 5–7  $\mu\text{m}$  diam., cylindrical to clavate, sometimes forked, slightly flexuous, thin-walled, occasionally with thick-walled apex, with refractive contents, not projecting above the hymenium, arising deeply from the hymenophoral trama (Fig. 7f); marginal cells 22–29 x 5–8.5  $\mu\text{m}$ , cylindrical to clavate, irregular, thin-walled, hyaline (Fig. 7e). Hymenophoral trama subregular composed of scattered cylindrical, 4–7  $\mu\text{m}$  diam., thin-walled hyphae and abundant lactiferous hyphae 6–10  $\mu\text{m}$  diam., sinuous then oriented toward hymenium. Subhymenium composed of thin-walled cylindrical hyphae. Pileipellis a lampropalisade up to 115  $\mu\text{m}$ , consisting of a layer of isodiametric cells with scattered thick-walled terminal elements (Fig. 7d): isodiametric cells, 9–31  $\mu\text{m}$  diam., thin-walled; terminal elements 47–95 x 5–11  $\mu\text{m}$ , aciculate, filiform, cylindrical, thick-walled (walls up to 1.3  $\mu\text{m}$  thick) hyaline, vertically oriented (Fig. 7g). Pileus trama composed of scattered sphaerocytes 5–13  $\mu\text{m}$  diam., and abundant lactiferous hyphae 10–20  $\mu\text{m}$  diam. Stipitipellis a lampropalisade similar to the pileipellis. Clamp connections absent in all tissues examined.

Ecology and distribution: Solitary to gregarious, on trunks between bryophytes and algae, arising from a short subiculum; collected in March, in 'terra-firme', a mixture of clay and sandy soil forest, in the Pará state in Amazonas. Known only in the North of Brazil (Amazonas and Pará states).

Additional material examined: Brazil, Amazonas, Manaus, ‘Estação Experimental de Manejo Florestal ZF-2’, área BIONTE, bloco IV- SB1, 14 Mar 2012, leg. Komura D.L., Araújo, F.R., DLK 116 (INPA 284000 paratype, JPB 64080 paratype); ibid. 19 Apr 2012, Komura D.L, Moncalvo, J.M., Zartman, C.E., DLK 346 (INPA 283995 paratype).

Remarks: *Lactifluus spathuliformis* can be easily recognized in the field by its pleurotoid, gregarious, caespitose habit, and large (80–180 mm diam.), petaloid to spathulate basidiomata. *Lactifluus panuoides* has smaller (up to 35 mm diam.), subreniform to spathulate basidiomata (Singer 1952). The microscopy of *Lf. spathuliformis* resembles *Lf. panuoides* in basidiospore size (6–7 x 5–6 µm), ornamentation of connected warts and ridges forming a complete reticulum and pileipellis structure with scattered, thick-walled, terminal elements (Singer 1952). However, *Lf. spathuliformis* produces thin-walled pleuropseudocystidia with an occasional thick-walled apex, while *Lf. panuoides* produces thick-walled pleuropseudocystidia (Singer 1952).

*Lactifluus guttulatus* differs from *Lf. spathuliformis* in its smaller basidiomata (up to 35 mm diam), larger basidiospores (7–9 x 6–8.5 µm), scattered to frequent, thin-walled forked pseudocystidia, and the presence of droplets/incrustations on the terminal elements of the pileipellis.

***Lactifluus* sect. *Piperogalacti* Silva-Filho & Wartchow, sect. nov.**

Mycobank: MB 839026

Diagnosis: Characterized in *Lf.* subg. *Gymnocarpi* by a combination of the following: Neotropical distribution. Basidiomata agaricoid, whitish, yellow to light brown, lamellae distant. Basidiospore ornamentation composed of isolated rounded warts, plage non-amyloid. True cystidia occasionally present. Pseudocystidia occasionally branched. Pileipellis with thick-walled terminal elements.

Type: *Lactifluus piperogalactus* Silva-Filho, Sá & Wartchow

Register identifier type: MB 839026

Etymology: Due to the epithet of type species *Lactifluus piperogalactus*.

Remarks: This new section is morphologically delimited by its Neotropical distribution, whitish, yellowish or light brown, agaricoid basidiomata, distant lamellae, occasionally branched pseudocystidia, true cystidia and a pileipellis with thick-walled terminal elements. *Lactifluus* sect. *Nebulosi* (Fig. 2), another section from this region, is composed of agaricoid species with subdistant to distant lamellae, basidiospores ornamented by rounded warts sometimes connected by fine lines, presence of macrocystidia, absence of branched pseudocystidia and a pileipellis lacking thick-walled terminal elements (Delgat et al. 2020). *Lactifluus* sect. *Piperogalacti* is composed by *Lf. lepus*, *Lf. piperogalactus* and an undescribed species (JLC06031001) from French Guiana.

***Lactifluus lepus* Delgat & Courtec. in Delgat et al. *Persoonia* 44: 292 (2020) (Figs. 8 and 9)**

Basidiomata agaricoid. Pileus 39–55 mm diam., plano-convex, depressed, with a small papilla in the depression, yellowish white (1A2), grayish yellow (2B4), (4B5) to brownish orange (5C5, 5C4); surface slightly rugulose to slightly scrobiculate, moist, viscid; margin, smooth to slightly striate, decurved to incurved, edge crenate (Fig. 8); context firm, 3–4 mm thick, white (1A1), changing to brownish orange (5C4). Lamellae decurrent, distant, white (1A1) to yellowish white (1A2); edges entire, concolorous; lamellulae frequent, of three lengths. Stipe 33–44 x 8–10 mm (at apex) and 6–7.5 mm (at base), central, tapering toward the base, pale yellow (4A3), pale orange (5A3) to brownish orange (5C5, 5C6) with brown (6E7) spots; surface slightly scrobiculate, moist, slightly viscid (Fig. 8); context solid, white (1A1) changing to brownish orange (5C4). Veil absent. Latex white (1A1) changing to brown (6E8) on exposure. Smell and taste not determined.

Basidiospores (5.5–)6–7 × (4.5–)5–6(–6.5) μm; L = 6.4 μm, W = 5.4 μm; Q = 1.08–1.3, Qm = 1.18; n = 80/2/1; subglobose to broadly ellipsoid; thin-walled, hyaline; ornamentation amyloid, up to 1 μm high, composed of regular short ridges; plage inamyloid; hilar appendage up to 2 μm long, inamyloid (Fig. 9a). Basidia 47–62 × 5.5–11 μm, clavate to long-clavate, 4-spored, thin-walled, hyaline, sometimes with refringent contents; sterigmata up to 7.5 μm long (Fig. 9b). Pleurocystidia not observed. Pleuropseudocystidia abundant, 3.5–7 μm diam., cylindrical, frequently forked at apex, immersed or projected parts in the hymenium, thin-walled, with refringent contents, mostly not projecting above the hymenium, rarely projecting up to 73 μm above, arising deeply from hymenophoral trama (Fig. 9c). Lamellar edge sterile, composed of scattered cheilopseudocystidia and abundant marginal cells: cheilopseudocystidia 3–4 μm diam., fusoid to cylindrical clavate, thin-walled, not projecting above the hymenium, arising deeply from hymenophoral trama (Fig. 9f); marginal cells 20.5–35.5 x 6–10 μm, cylindrical, sometime flexuous, catenulate, thin-walled, hyaline (Fig. 9g). Hymenophoral trama composed of scattered sphaerocytes up to 19 μm diam., abundant, cylindrical, 3–7.5 μm diam., thin-walled, hyaline, hyphae and lactiferous hyphae. Subhymenium cellular. Pileipellis a lampropalisade up to 116 μm thick, consisting of a layer of isodiametric cells with abundant thick-walled terminal elements (Fig. 9d): isodiametric cells, 12–30.5 μm diam., thin-walled; terminal elements 28.5–96 x 4–7 μm, cylindrical, fusoid, clavate, sometimes slightly flexuous, thick-walled (up to 2 μm thick), hyaline, vertically oriented (Fig. 9e). Pileus trama composed of abundant sphaerocytes, 15–24 μm diam., and abundant lactifers up 5–8 μm diam. Stipitipellis a lampropalisade similar to pileipellis. Clamp connections absent in all tissues examined.

Ecology and distribution: Scattered on clay soil in dense ombrophilous forest, collected in March, in Amazon, under unknown host, growing in forest composed of potential ectomycorrhizal hosts including the Fabaceae,

Polygonaceae and Sapotaceae (Espirito-Santo et al. 2005). Known from Guadeloupe Island in hygrophytic forests and now in Brazil.

Material examined: Brazil, Pará, Belterra, Floresta Nacional do Tapajós, Trilha da Comunidade Jamaraquá, 24 Mar 2014, leg. Komura D.L., Cabral, T.S., Fonseca, I.R. Komura DLK 1854 (INPA 283999, JPB 6418).

Remarks: *Lactifluus lepus* is diagnosed by its striate margin, widely spaced lamellae, small basidiospores, thick-walled terminal elements of the pileipellis and the presence of many 1- and 2-spored basidia (Delgat et al. 2020). The Brazilian specimens showed two slight differences in comparison with the original description: the absence of 1- and 2 spored basidia and true cystidia. Regardless of these differences, the ITS sequence supports the identification of the Brazilian collections.

*Lactifluus lepus* is a unique milkcap of the Brazilian Amazon with whitish basidiomata which exude white milk that changes to brown. Except for *L. pallidipes* Singer and *L. subpallidipes* Singer, all agaricoid milkcaps described from the Amazon by Singer et al. (1983) and Silva-Filho et al. (2020) have basidiomata with orange tints. *Lactarius pallidipes* and *L. subpallidipes* have unchanged color context and scant, practically absent milk, suggesting their placement in *Lf.* subg. *Lactariopsis*.

***Lactifluus piperogalactus* Silva-Filho, Sá & Wartchow, sp. nov. (Figs. 5c-d, 10 and 11)**

Mycobank: MB 836714

Type: Brasil, Paraíba, Areia, Parque Estadual Mata do Pau-Ferro, Trilha do Cumbre, 10 Jul 2017, leg. F. Wartchow FW 22/2017 (JPB 64502 holotype!, UFRN-Fungos 3287 isotype!).

Diagnosis: Distinguished from *Lf. lepus* by a combination of the following: Pileus 51–100 mm diam., convex to plane convex, slightly depressed. Stipe 10–15 mm diam., surface glabrous, slightly velutinous, waxy, mat. Basidiospores with ornamentation up to 0.5  $\mu\text{m}$  high, irregularly disposed. Pileipellis a lamprolalisade with shorter (15–37  $\mu\text{m}$  high), wider (8–13  $\mu\text{m}$  diam.) and septate thick-walled terminal elements.

Etymology: From Latin: ‘Pipero’= peppery and ‘galactus’= milk. In reference to peppery tasting latex.

Pileus 51–100 mm diam., convex to plane convex, slightly depressed at center, yellow (4C8) grayish yellow (4C6) to golden (4C7) then cream yellow (4B1,4A4, 4A3); surface subglabrous, moist, waxy and mat in young specimens, becoming dry, brittle and slightly radially wrinkled; margin broadly sulcate to plicate, incurved to decurved, edge crenate becoming eroded (Fig. 10); context firm, up to 9 mm thick at the disc, pale cream (4A2), changing to brownish orange (6C5) to light brown (7D7). Lamellae short-decurrent to decurrent, very distant, cream (4A5) to pale buff (4C3), sometimes with light brown (7D7) to brown (5C7) spots; edges entire and concolorous; lamellulae frequent, of two lengths (Fig. 10). Stipe 30–55 x 10–15 mm, central, cylindrical, subequal, to slightly tapered towards the base, white (1A1) to yellowish white (1A3) sometimes with brown

(5C7) spots (Fig. 10); surface glabrous, slightly velutinous, waxy, matt; context solid, yellowish white (1A2), changing to brown (5C7) (Fig. 10). Veil absent. Odor not determined. Taste peppery. Latex scanty, white (1A1) changing to brown (5C7) on exposure with peppery taste.

Basidiospores  $(5.5-6-7.5 \times 4.5-6.5(-7) \mu\text{m}$ ;  $L = 6.4 \mu\text{m}$ ,  $W = 5.5 \mu\text{m}$ ;  $Q = 1.01-1.23$ ,  $Q_m = 1.16$ ;  $n = 60/3/3$ ); subglobose to broadly ellipsoid, predominantly subglobose; thin-walled, hyaline; ornamentation amyloid, up to  $0.5 \mu\text{m}$  high, composed of isolated short warts; plage non-amyloid; hilar appendix up to  $2.5 \mu\text{m}$  long, inamyloid (Figs. 11a, 5c-d). Basidia  $63-76 \times 7.5-9 \mu\text{m}$ , slightly clavate, 4-spored, thin-walled, with refractive contents; sterigmata up to  $9 \mu\text{m}$  long (Fig. 11b). Pleurocystidia absent. Pleuropseudocystidia abundant,  $5-7.5 \mu\text{m}$  diam., cylindrical, sometimes with apex slightly acute, sometimes forked, flexuous, thin-walled, with refractive contents, mostly not projecting above the hymenium, rarely projecting up to  $20 \mu\text{m}$  above, arising deeply from hymenophoral trama (Fig. 11c). Lamellae edge sterile, composed of scattered cheilopseudocystidia and abundant marginal cells: cheilopseudocystidia,  $4-7 \mu\text{m}$  diam., cylindrical, fusoid, sometimes forked, slightly flexuous, thin-walled with refractive contents (Fig. 11g); marginal cells  $29-56.5 \times 4-6.5 \mu\text{m}$ , fusoid, sometimes flexuous, thin-walled, hyaline (Fig. 11d). Hymenophoral trama composed of abundant sphaerocytes  $5.5-25 \mu\text{m}$  diam., and abundant lactiferous hyphae  $3-7 \mu\text{m}$  diam. Subhymenium cellular. Pileipellis a lampropalisade up to  $169 \mu\text{m}$  thick, consisting of a layer of isodiametric cells with abundant thick-walled terminal elements (Fig. 11e): isodiametric cells,  $10.5-18.5 \mu\text{m}$ , thin-walled; thick-walled terminal elements  $15-37 \times 8-13 \mu\text{m}$ , cylindrical, fusoid to slightly fusiform, slightly flexuous, sometimes septate, thick-walled (up to  $3.5 \mu\text{m}$  thick) hyaline, vertically oriented (Fig. 11f). Pileus trama composed of abundant sphaerocytes  $12.5-38.5 \mu\text{m}$ , diam., and scattered lactiferous hyphae,  $2.1-6.5 \mu\text{m}$  diam. Stipitipellis a lampropalisade similar to pileipellis. Clamp connections absent in all tissues examined.

Ecology and distribution: Solitary or scattered in Atlantic forest and ‘brejo de altitude’, a tropical wet mountain forest in the Caatinga Domain, collected in July, under unknown host. In the Atlantic forest this species grows surrounded by ectomycorrhizal tree species of Caesalpinioideae, Euphorbiaceae, Nyctaginaceae and Polygonaceae (Alves-Araújo et al. 2008), and in the Caatinga by Fabaceae, Nyctaginaceae (species of *Guapira* Aubl.) and Polygonaceae (Barbosa et al. 2004). Known from Pernambuco and Paraíba States, Northeastern Brazil.

Additional material examined: Brazil, Pernambuco, Igarassu, Reserva Biológica Charles Darwin, 04 July 2012, *ibid*, N.A. Silva & F. Wartchow FW110/2012 (UFRN-Fungos 2199 paratype!); PARAIBA, Areia, Parque Estadual Mata do Pau-Ferro, Trilha do Cumbre, 17 July 2017, *ibid*, F. Wartchow FW 36/2017 (JPB 64495 paratype!).

Remarks: *Lactifluus piperogalactus* is characterized by its robust basidiomata, convex to plano-convex, slightly depressed pileus, glabrous, slightly velutinous, waxy, matt stipe, shorter basidiospore ornamentation and absence of macrocystidia and septate thick-walled terminal elements on the pileipellis. *Lactifluus lepus*, the only other described member of *Lf.* sect. *Piperogalacti*, has a smaller pileus (39–55 mm diam.), thinner (8–10 mm diam.) and slightly scrobiculate, viscid stipe, higher basidiospore ornamentation (up to 1.0  $\mu\text{m}$  high), and longer (28.5–96  $\mu\text{m}$  high), thinner (4–7  $\mu\text{m}$  diam.) and non-septate thick-walled terminal elements on pileipellis. In addition, *Lf. lepus* was originally described with abundant macrocystidia and uni- and bi-spored basidia (Delgat et al. 2020), elements not seen in *Lf. piperogalactus*. *Lactifluus piperogalactus* is a unique species from the Atlantic forest and Caatinga biomes with whitish and brownish stained basidiomata and peppery taste. All reported species from the Atlantic forest have more vivid colors ranging between orange and brown.

Among the species described from Atlantic Forest, *Lf. russula* (Rick) Silva-Filho & Wartchow most closely resembles *Lf. piperogalactus* due to its non-vivid color (Rick 1906, 1907, 1930) and a possible color change in its context. However, *Lf. russula* produces larger basidiospores (7.5–9 x 5.5–7  $\mu\text{m}$ ) and non-branched pseudocystidia (Silva-Filho and Wartchow 2019).

## Discussion

The two pleurotoid species described form well-supported separate lineages with good support in the combined ITS and *RPB2* analysis and morphologically in their basidiomata shape and size, basidiospore ornamentation, pseudocystidia shape and walls and elements of the pileipellis. The agaricoid milkcaps of *Lactifluus* subg. *Gymnocarpi* are more diverse in Central America and the Caribbean, with nine species described by Pegler and Fiard (1979), Montoya et al. (1996) and Delgat et al. (2020) as compared to the two species that occur in South America. Despite the limited knowledge of *Lactifluus* in South America, this hypothesis can be corroborated by considering the high diversification within the subgenera *Lactariopsis* and *Pseudogymnocarpi* (Sá et al. 2019; Barbosa et al. 2020; Silva Filho et al. 2020). *Lactifluus piperogalactus* was sampled in the Atlantic Forest and Caatinga, these two areas belong to different biomes, but both are relatively close and have similar environments with the presence of ectomycorrhizal members of the Nyctaginaceae and Polygonaceae (Barbosa et al. 2004; Alves-Araújo et al. 2008). The other species were collected in the Amazon forests, including two new species and one new record from ‘terra firme’ forest. These represent a few species of the ectomycorrhizal fungi that occur in ‘terra-firme’ forest. Many ectomycorrhizal fungi were found in white-sand (Campina and Campinarana) and ‘Igapó forests’ in Brazil (Singer et al. 1983). These three biomes are very distinct forest types which harbor endemic species of plants (Andrade-Lima 1961, Espirito-Santo et al. 2005; Alves-Araújo et al. 2008). The question remains about how geographic distance and host association play a role in the speciation of *Lactifluus*

in South America. Increased sampling in different habitats and locations in Brazil may reveal new species and could also allow us to better understand the process shaping *Lactifluus* diversification in the Neotropics.

## Declarations

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**Consent to participate:** Not applicable

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**Code availability:** Not applicable

**Authors' contributions:** Alexandre G. S. Silva-Filho described the morphological characteristics of the specimens, ran the molecular analyzes, produced the Figures and map and wrote the manuscript. Mariana C. A. Sá, Dirce L. Komura and Felipe Wartchow collected the samples and took the photos. Iuri G. Baseia, Paulo Marinho, Melanie Roy and Jean-Marc Moncalvo made DNA sequences available. All reviewed the manuscript.

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Tabela 4. **Table 1.** Site, state, municipality, biome, ecosystem and geographic coordinates of *Lactifluus* collections.

Site	State	Municipality	Biome	Ecosystem	Geographic coordinates
'CIAPA'	Amazonas	Manaus	Amazon forest	terra firme forest	2°38'20.02" S 60°56'22.15" W
'Estação Experimental de Manejo Florestal do INPA' (ZF-2)	Amazonas	Manaus	Amazon forest	terra firme forest	02°37' and 02°38' S; 60°09' and 60°11' W
'Floresta Nacional do Tapajós'	Pará	Belterra	Amazon forest	terra firme forest	2°48'14" S, 54°59'50" W
'Reserva Biológica Charles Darwin'	Pernambuco	Igarassu	Atlantic forest	dense ombrophilous forest	07°48'37" S 34°56'5" W
'Mata do Pau-Ferro'	Paraíba	Areias	Caatinga	tropical wet mountain	6°57'58.9" S and 35°44'55.15" W

Tabela 5. **Table 2** Species, voucher collection, country and GenBank accession numbers of ITS and *RPB2* sequences used in molecular analyses. Accession numbers in bold were newly generated for this study. Lack of sequences are marked with ‘-’.

Species	Voucher collection	Country	GenBank Acession n°	
			ITS	<i>RPB2</i>
<i>Lactifluus albocinctus</i>	AV 99-211	Zimbabwe	KR364117	KR364258
<i>Lf. brunellus</i>	TH 9130	Guyana	JN168728	-
<i>Lf. brunneoviolascens</i>	AV 13-038	Italy	KR364123	KR364264
<i>Lf. brunnescens</i>	AV 05-083	Malawi	KR364019	KR364263
<i>Lf. carmineus</i>	AV 99-099	Zimbabwe	KR364131	KR364265
<i>Lf. caribaeus</i>	JP Fiard 827B	Martinique	MK046853	-
<i>Lf. caliendrifer</i>	KW 392	Thailand	KR364091	KR364305
<i>Lf. chiapanensis</i>	VMB 4374A	Mexico	GU258297	GU258316
<i>Lf. coccolobae</i>	OKM-27240	Puerto Rico	MK774805	-
<i>Lf. clarkeae</i>	MN 2004002	Australia	KR364011	KR364268
<i>Lf. denigricans</i>	EDC 11-218	Tanzania	KR364051	KR364384
<i>Lf. flammans</i>	JD 941	Congo	KR364078	KR364303
<i>Lf. flocktonae</i>	JET1006	Australia	JX266621	-
<i>Lf. foetens</i>	ADK 3688	Benin	KR364022	KR364278
<i>Lf. foetens</i>	ADK 2840	Benin	KR364023	KR364279
<i>Lf. guadeloupensis</i>	RC/Guad11-023	Guadeloupe	KP691412	KP691430
<b><i>Lf. guttulatus</i></b>	<b>DLK 2042</b>	<b>Brazil</b>	<b>MT611966</b>	<b>MT903963</b>
<i>Lf. gymnocarpus</i>	EDC 12-047	Cameroon	KR364065	KR364282
<i>Lf. kigomaensis</i>	AV 11-006	Tanzania	KR364052	KR364288
<i>Lf. lepus</i>	RC/Guad 08-042	Guadeloupe	KP691414	KP752179
<i>Lf. lepus</i>	RC/Guad 05-029	Guadeloupe	MK046811	-
<b><i>Lf. lepus</i></b>	<b>DLK 1854</b>	<b>Brazil</b>	<b>MT611966</b>	-
<i>Lf. longivelutinus</i>	XHW 1565	China	KR364114	-
<i>Lf. luteolus</i>	AV 05-253	North America	KR364016	KJ210067
<i>Lf. marmoratus</i>	LD 15-066	Martinique	MK046805	MK063907
<i>Lf. murinipes</i>	CL/MART06.019	Martinique	KP691417	-
<i>Lf. murinipes</i>	LD 15-054	Martinique	MK046828	MK063911
<i>Lf. murinipes</i>	LD 15-020	Martinique	MK046836	MK063918
<i>Lf. nebulosus</i>	LD 15-059	Martinique	MK046804	MK063905
<i>Lf. nebulosus</i>	PAM/Mart 05-091	Martinique	KP691415	KP691432
<i>Lf. nebulosus</i>	LD 15-061	Martinique	MK046833	MK063916
<i>Lf. nonpiscis</i>	BB 3171	Zambia	KR364030	-
<i>Lf. nonpiscis</i>	AV 11-137	Togo	KR364058	KR364317
<i>Lf. panuoides</i>	RC/Guy 10-024	French Guiana	MF038915	KP691428
<i>Lf. panuoides</i>	G182	French Guiana	KJ786647	-
<i>Lf. panuoides</i>	CL/GUAD06.045	Guadeloupe	MK046843	-

<i>Lf. panuoides</i>	D.N. Pegler 3133	Guadeloupe	MK046858	–
<i>Lf. panuoides</i>	RWG Dennis 109A	Trinidad and Tobago	MK046859	–
<i>Lf. aff. phlebonemus</i>	EDC 12-023	Cameroon	KR364062	KR364322
<b><i>Lf. piperogalactus</i></b>	<b>FW 22/2017</b>	<b>Brazil</b>	<b>MT672343</b>	<b>MT903964</b>
<b><i>Lf. piperogalactus</i></b>	<b>FW 110/2012</b>	<b>Brazil</b>	<b>MT902929</b>	–
<i>Lf. putidus</i>	LD 15-034	Martinique	MK046826	MK063910
<i>Lf. putidus</i>	LD 15-039	Martinique	MK046830	MK063913
<i>Lf. rubrobrunnescens</i>	EH 7194	Indonesia	KR364115	–
<i>Lf. aff. rubroviolascens</i>	EDC 12-051	Cameroon	KR364066	KR364465
<i>Lf. russilisorus</i>	REH 9398	Australia	KR364097	KR364307
<b><i>Lf. sphaatuliformis</i></b>	<b>DLK 1891</b>	<b>Brazil</b>	<b>MT672342</b>	<b>MT903962</b>
<b><i>Lf. sphaatuliformis</i></b>	<b>DLK 116</b>	<b>Brazil</b>	<b>MT611964</b>	<b>MT903961</b>
<b><i>Lf. sphaatuliformis</i></b>	<b>DLK 346</b>	<b>Brazil</b>	<b>MT611965</b>	–
<i>Lf. subclarkeae</i>	REH 9231	Australia	KR364095	KR364346
<i>Lf. tanzanicus</i>	TS 1277	Tanzania	KR364037	KR364351
<i>Lf. cf. tanzanicus</i>	AV 11-017	Tanzania	KR364053	KR364296
<i>Lactifluus sp.</i>	JLC 060310-011	French Guiana	KJ786714	–
<i>Lactifluus sp.</i>	PGK13-130	New Caledonia	KP691436	–
<i>Lactifluus sp.</i>	TH 7460	Guyana	KT339233	–
<i>Lactifluus sp.</i>	G3185	French Guiana	KJ786694	KP691434
<i>Lactifluus sp.</i>	EDC 12-046	Cameroon	KR364064	KR364257

## Figures

Figura 26 - **Fig. 1** Map showing the location of collection areas in Amazon, Atlantic Forest and Caatinga Biomes.

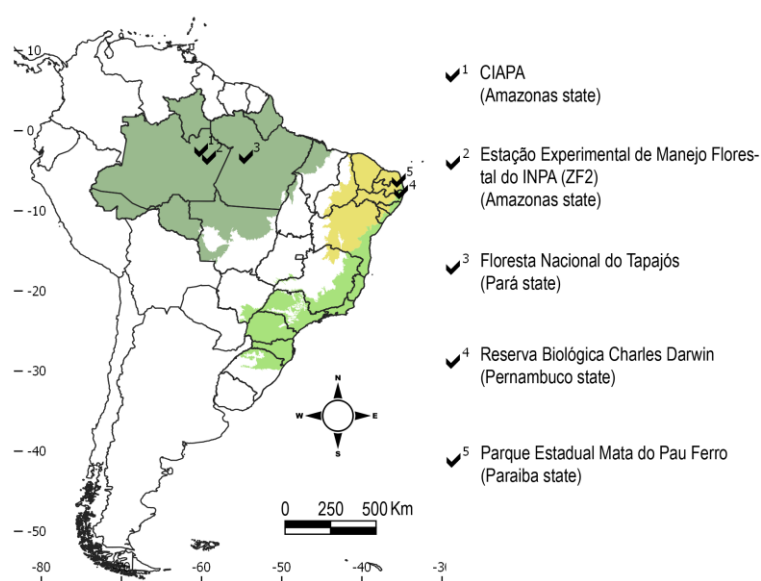


Figura 27 - **Fig. 2** ML phylogeny of representative collections of *Lactifluus* subg. *Gymnocarpi* based on complete nuc-ITS-rDNA spacer (ITS) and second largest subunit of the RNA polymerase II (*RPB2*). New species are noted in bold and the new section is highlighted in light brown. Thicker lines represent branches with maximum bootstrap values and posterior probabilities (100% BS / 1.0 BPM). The 'I S' represents branches of isolated species. Bootstrap values and Bayesian posterior probabilities are indicated if they exceed 0.95 and 70%, respectively. The scale bar represents the expected number of nucleotide changes per site.

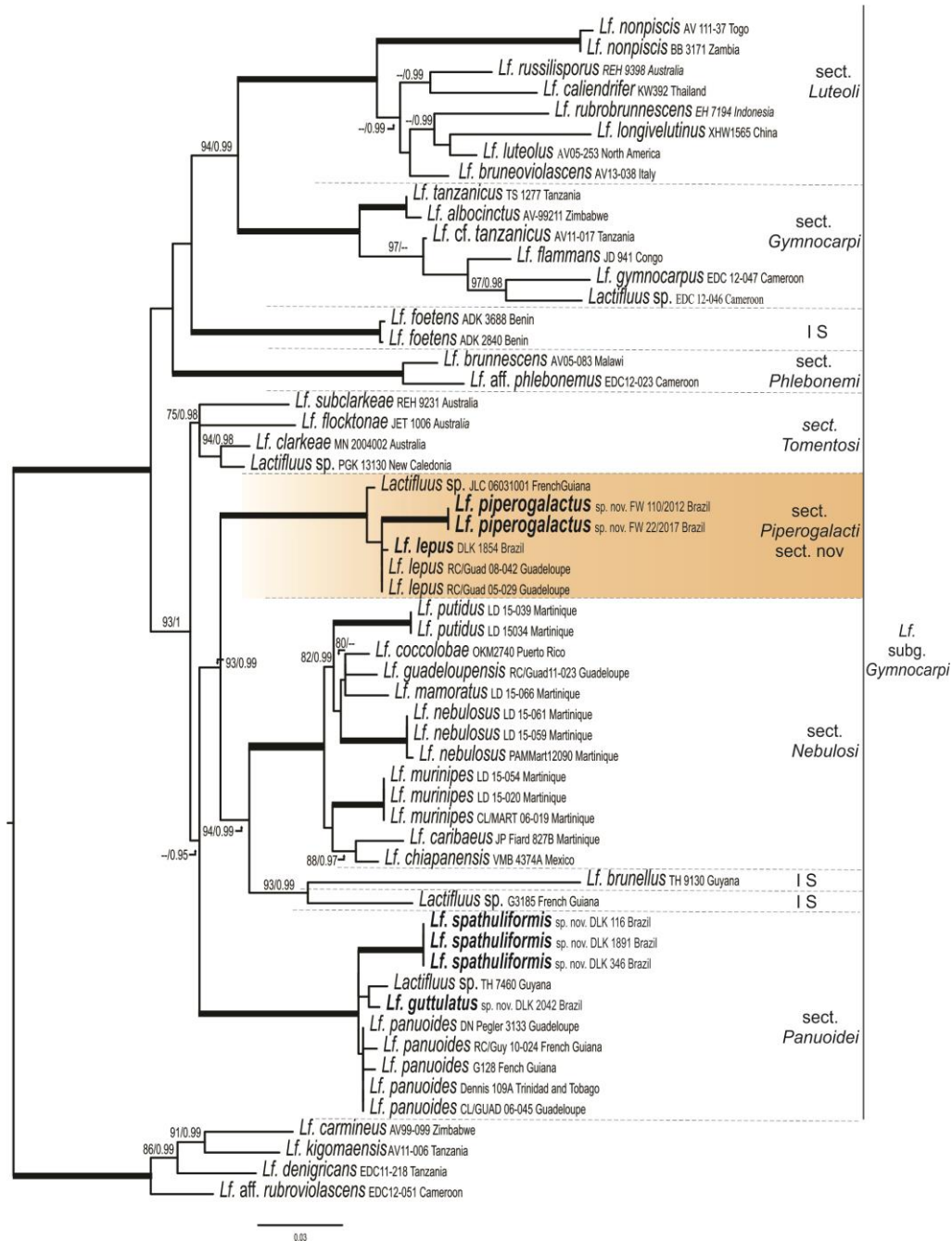


Figura 28 - **Fig. 3** Basidiomata of *Lactifluus guttulatus* (holotype D.L. Komura 2042). Scale bars = 10 mm. Photos: Dirce L. Komura.



Figura 29 - **Fig. 4** Micromorphological features of *Lactifluus guttulatus* (holotype D.L. Komura 2042). a Basidiospores. b Basidia. c Pleuropseudocystidia. d Pileipellis. e Marginal cells of the lamellar edge. f Cheilopseudocystidia. g Thick-walled elements of pileipellis. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

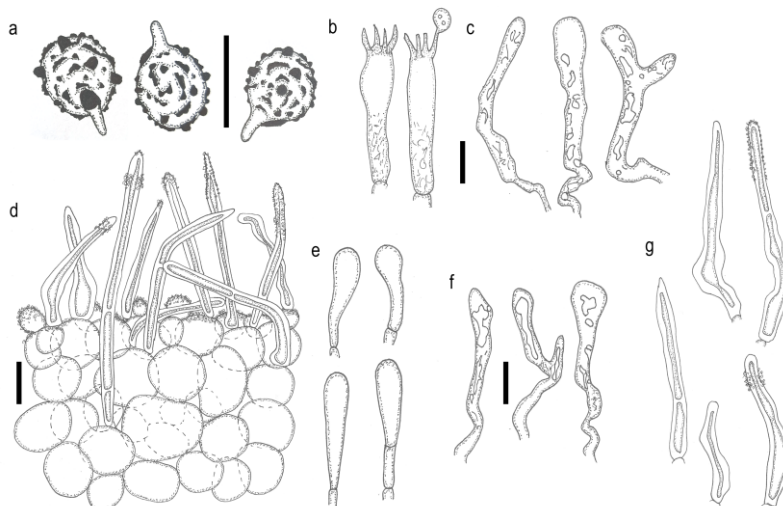


Figura 30 - **Fig. 5** Scanning electron micrographs of basidiospores: a *Lactifluus guttulatus* (holotype D.L. Komura 2042). b *Lactifluus spathuliformis* (holotype D.L. Komura 116). c-d *Lactifluus piperogalactus* (holotype F. Wartchow 22/2017). Scale bars = 2  $\mu$ m.

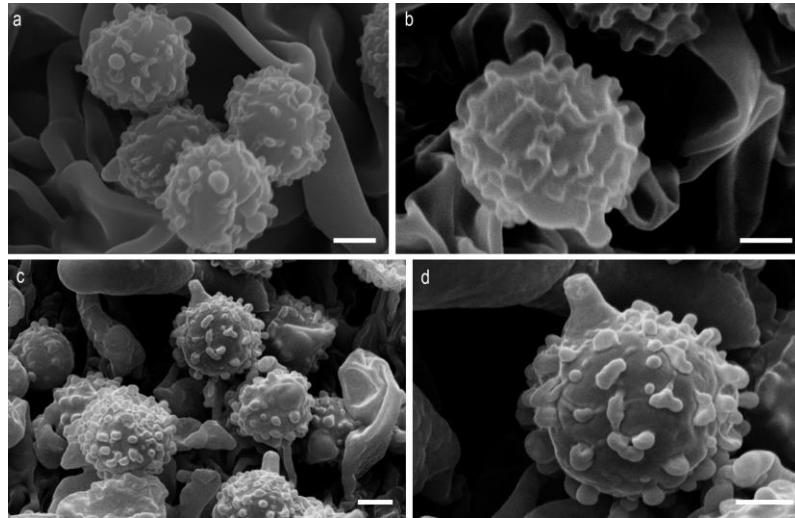


Figura 31 - **Fig. 6** Basidiomata of *Lactifluus spathuliformis*. a Holotype (D.L. Komura 116). b Paratype (D.L. Komura 1891). Scale bar = 10 mm. Photos: Dirce L. Komura.



Figura 32 - **Fig. 7** Micromorphological features of *Lactifluus spathuliformis* (holotype D.L. Komura 116). a Basidiospores. b Basidia. c Pleurospseudocystidia. d Pileipellis. e Marginal cells of lamellar edge. f Cheilopseudocystidia. g Thick-walled elements of pileipellis. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

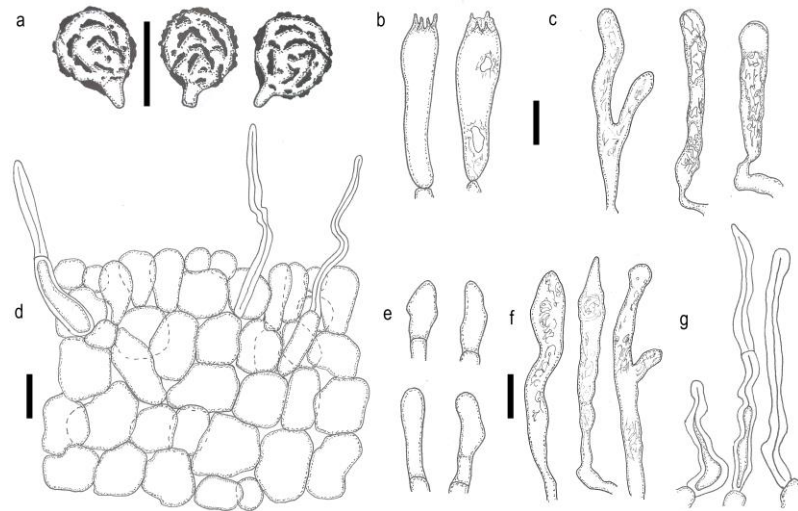


Figura 33 - **Fig. 8** Basidiomata of *Lactifluus lepus* (holotype D.L. Komura 1854). Scale bars = 10 mm. Photos: Dirce L. Komura.



Figura 34 - **Fig. 9** Micromorphological features of *Lactifluus lepus* (holotype D.L. Komura 1854). a Basidiospores. b Basidia. c Pleuropseudocystidia. d Pileipellis. e Thick-walled elements of pileipellis. f Cheilopseudocystidia. g Marginal cells of the lamellar edge. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

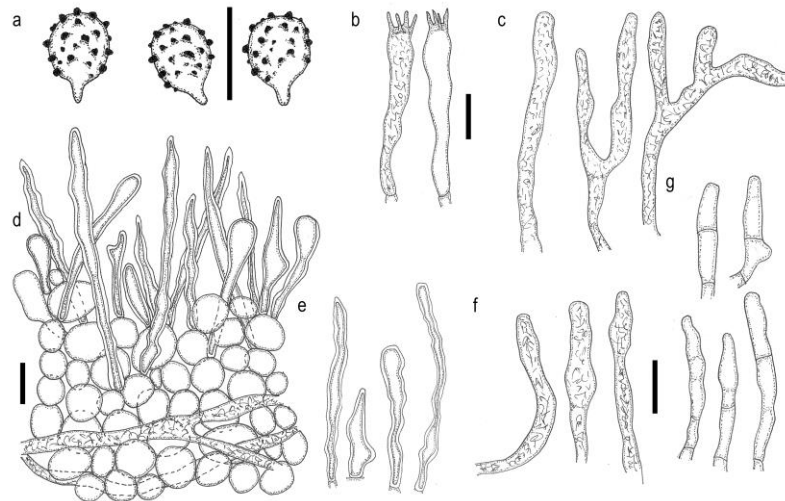
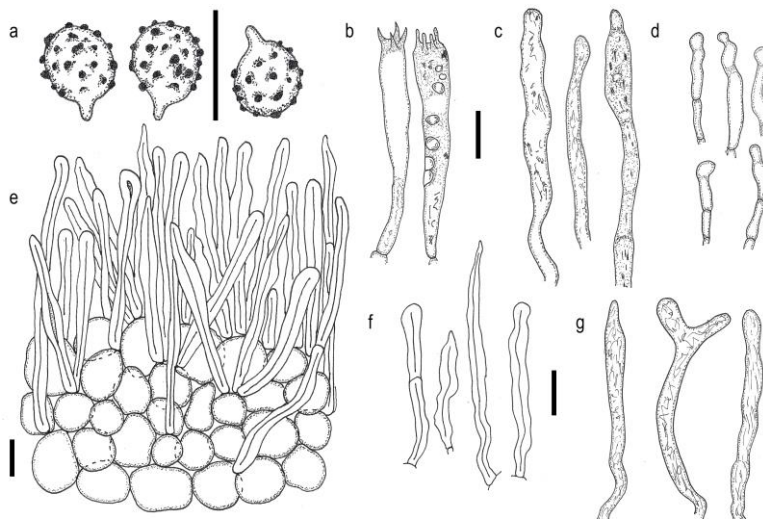


Figura 35 - **Fig. 10** Basidiomata of *Lactifluus piperogalactus*. a Holotype (F. Wartchow 22/2017). b Paratype (F. Wartchow 110/2012). Scale bars = 10 mm. Photos: Felipe Wartchow.



Figura 36 - **Fig. 11** Micromorphological features of *Lactifluus piperogalactus* (holotype F. Wartchow 22- 2017). a Basidiospores. b Basidia. c Pleuropseudocystidia. d Marginal cells of the lamellar edge. e Pileipellis. f Thick-walled elements of pileipellis. g Cheilopseudocystidia. Scale bars = 10  $\mu\text{m}$ . Drawings: Alexandre G. S. Silva-Filho.



**9 CAPÍTULO 4 - INTEGRATIVE TAXONOMY OF *LACTIFLUUS* SUBG. *LACTARIOPSIS* (RUSSULALES/BASIDIOMYCOTA) FROM BRAZILIAN TROPICAL FORESTS**

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**Abstract:** *Lactifluus* subg. *Lactariopsis* is a well-supported group of milkcaps occurring predominantly in tropical and subtropical Africa. The subgenus contain agaricoid and pleurotoid representatives and is the only in which species with a secondary velum occurs. An integrative taxonomy of *Lactifluus* subgenus *Lactariopsis* with collection from Brazilian tropical forests (Caatinga, Amazon and Atlantic Forests Biomes) are presented. Two new species are described: *Lf. aurantioaridus* into sect. *Neotropici*; and the pleurotoid *Lf. reniformis* into newly delimited sect. *Multiceps*. *Lactifluus mamorensis* and *Lf. dunensis* was confirmed in *Lf.* sect. *neotropici*. *Lactifluus neotropicus* and *Lf. mamorensis* have its distribution expanded to the Caatinga biome and a new sequence of *Lf. annulifer* was included. A combined dataset of nuclear rDNA internal transcribed spacer region (ITS) and nuclear large subunit (LSU) was analyzed and based on its phylogenetic hypothesis the two novel species and the new section are confirmed.

**Key words:** Russulales, biodiversity, ectomycorrhizal fungi, Neotropics, milkcaps

## Introduction

Recent molecular phylogenetic studies in Russulaceae proposed to share the two traditional genera, *Lactarius* Pers. (abbreviated as *L.*) and *Russula* Pers into four distinct genera: *Russula*, *Lactarius*, *Lactifluus* (Pers.) Roussel (abbreviated as *Lf.*) and *Multifurca* Buyck & V. Hofst. (Buyck et al. 2008). The last three comprise the ectomycorrhizal milkcap lineage with approximately 450, 200, and 10 spp. respectively (He et al. 2019).

*Lactariopsis* Henn was erected by Hennings (1901) to houses *Lactariopsis Zenkeri* Henn., a Cameroonian milkcap with ‘pseudoangiocarpic development’. After, Heim

(1938) considered *Lactariopsis* and *Lactarius* synonymous and proposed to decrease *Lactariopsis* to subgenus level. Singer (1942) and Singer et al. (1983) introduced the veiled milkcaps *L. neotropicus* Singer and *L. annulifer* Singer and group all veiled taxa in *Lactarius* sect. *Lactariopsidei* Singer. Verbeken (1988) proposed a large definition for *Lf.* subg. *Lactariopsis* and includes in the subgenus all milkcaps that belong to sections *Lactariopsis* Verbeken, *Chamaeleontini* (Verbeken) Verbeken and *Albati* (Bataille) Verbeken (Verbeken 1998, Verbeken et al. 2011).

A multi-gene phylogeny of *Lactifluus* proposed by De Crop et al. (2017) resulted in a new infrageneric classification. *Lactifluus* subg. *Lactariopsis* (Henn.) Verbeken has its monophyletic status confirmed. The subgenus has been formed by eleven well-supported clades and two isolated branches/species: the sections *Lactariopsis*, *Russulopsidei* and *Edules* (Verbeken) Verbeken are composed by African species; *Lf.* sect. *Albati* (Bataille) Verbeken has predominantly American species (De Crop et al. 2017; Delgat et al. 2020). Other three non-delimited clades were formed by species from Africa, one from Asia, two from Neotropics and one mixed with Asian and African taxa (De Crop et al. 2017). More recently, the section *Neotropici* J. Duque, L. Delgat, A. Verbeken, M. A. Neves & Anibal A. Carvalho Jr. was delimited and named from Brazilian collections (Barbosa et al. 2020).

With approximately 8.5 million km<sup>2</sup>, Brazil occupies approximately half of South America territory. The tropical and subtropical regions of Brazil are composed by a high diversity of ecosystems influenced in well-demarcated phytogeographical zones (IBGE 2004). The Amazon Forest is the largest humid tropical forest in the world with approximately 12,500 tree species (Hubbell et al. 2008). The Pantanal is a largest wetland in the center of South America, and its vegetation is referred to a complex of plant communities from tropical Amazonian rainforest, semiarid woodland, Brazilian

Cerrado and Chaco savannas (Hamilton 2002). The Caatinga biome is formed by semi-arid forests, with a high number of endemic species adapted to xeric climate (Oliveira et al. 2012). The Cerrado is the most diverse tropical savanna in the world with the richest vascular plant flora on the planet (Eiten 1994); The Atlantic forest extends along the Atlantic coast of Brazil and is characterized by rain forest with high biodiversity (Myers et al. 2000). The two last constitute important center of endemism, considered two of thirty-four recognized global hotspots (Forza et al. 2012).

Studies about the biodiversity in Brazilian biomes are unequal due their great territory, high population density in the coastal region and by difficulty access in some ecosystems. The genus *Lactifluus* is an example of this unequal inventory. *Lactifluus* species have been reported, mostly to Amazon and Atlantic rain forests (Singer et al. 1983; Singer 1984; Silva-Filho et al. 2019; Rick 1906, 1907, 1930; Buyck and de Meijer 1999; de Meijer 2006; Sá et al. 2013; Sá and Wartchow 2013; Barbosa et al. 2020; Silva-Filho et al. 2020). Few species have been reported to Caatinga (Wartchow et al. 2013; Wartchow and Cavalcanti 2010; Sá et al. 2019), while in Cerrado and Pantanal, there are no records. Despite this, *Lactifluus* has been receiving more taxonomic attention in Brazil and recent discoveries of new species, showed a high diversification within *Lf.* subgenus *Lactariopsis*, with four species described and confirmed, so far (Sá et al. 2019; Barbosa et al. 2020).

This Paper is part of ongoing effort to inventory milkcap mushrooms occurring in Brazilian tropical forests (Silva-Filho and Wartchow 2019; Silva-Filho et al. 2019, 2020). An integrative taxonomy was utilized to investigate the taxa collected in Amazon, Atlantic forests and Caatinga Biomes and also for delimit *Lf.* sect. *Multiceps*.

## **Materials and Methods**

**Collecting area:** Fresh specimens were collected in different areas in:

1) Amazon forest

a) ‘Igarapé do Mutum’ (2°0'56.57"S;59°54'42.19"W): This is a particular area in President Figueiredo municipality in Amazonas state. This region comprises an area of white-sand forests. This vegetation presents a set of unique features, as example, dominance of a few species, high endemism, and low species richness. The families with highest species richness are Fabaceae, Sapotaceae and Lauraceae (Demarchi et al. 2018).

b) ‘Itacoatiara-Mirim’ (0°9'15.00"S;67°0'19.75"W): This is an indigenous community located in municipality of São Gabriel da Cachoeira in the state of Amazonas. This Area is also composed by white-sand forests. Study in this area showed the predominance of species of Fabaceae Sapotaceae and Euphorbiaceae (Stropp et al. 2011).

2) Atlantic Forest

c) ‘Parque Estadual das Dunas de Natal’ (5°48'S–5°43'S;35°09'–35°12'W): This reserve is located at municipality of Natal, in the State of Rio Grande do Norte. The area contains elements of Atlantic Forest mixed with some species in common with Caatinga and Coastal Tableland, where species of Leguminosae, Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae are predominate (Freire 1990).

3) Caatinga

d) ‘Parque Estadual Mata do Pau Ferro’ (6°57'58.9"S; 35°44'55.15"W): This area situated in Areia municipality, in the Paraíba state is covered by a tropical wet mountain forest, known as "brejo de altitude". This humid forest is composed of approximately 300 angiosperm taxa, of which Rubiaceae, Malvaceae, Asteraceae, Convolvulaceae, Solanaceae and Fabaceae are the most diverse (Barbosa et al. 2004).

**Morphological analysis:** Macroscopic features were based on fresh material. Color names and codes follow Kornerup and Wanscher (1978). Studies were performed using

the methodology of Verbeken and Walley (2010). Basidiospores were measured excluding ornamentation, in lateral view using Melzer's reagent, the biometric measurements of the basidiospores we follow the methodology of Wartchow and Gamboa-Trujillo (2012). Other microscopic structures were measured and visualized in 5% KOH and Congo red. For scanning electron microscopy (SEM), dried lamellae were directly stuck on to an adhesive layer on a small clean coverslip, then coated with gold and observed under Jeol JSM-6360LV and JSM-6010LA. Specimens were deposited in the Herbaria JPB, INPA (Thiers 2020, continuously updated) and in the Mycological collection of the 'Universidade Federal do Rio Grande do Norte' (UFRN-Fungos).

**Molecular methods:** Total fungal DNA was extracted using the Qiagen DNeasy® Plant Mini Kit (Germantown, MD, USA), according to the manufacturer's instructions. Primer pairs ITS1-F/ITS4-B and LR0R/LR5 were used to amplify the nrITS and nrLSU region, respectively (White et al. 1990; Moncalvo et al. 2000), Sequencing reactions were conducted by MACROGEN (KOREA) and resulting sequences were assembled and edited in Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were deposited in Genbank public database (Table 1).

**Phylogenetic analyses:** Six new ITS and four novel LSU sequences were generated in this study. All of the retrieved sequences were downloaded from NCBI (Genbank) and represent species of *Lf.* subg. *Lactariopsis*. Four sequences of *Lf.* subg. *Pseudogymnocarpi* were used as outgroup: *Lf. pegleri* (Pacioni and Lalli) Delgat, *Lf. veraecrucis* (Singer) Verbeken and two undescribed species MR/Guy13-145 and RC/Guy 09-036. Our dataset was constructed with combined ITS + LSU with 87 sequences, which was aligned using MAFFT v.7 (Katoh 2013) under the E-INS-i criteria. Seaview v.4 (Gouy et al. 2010) was used to visualize and adjust the alignment. In order to compute the best fit model of nucleotide evolution the ITS was subdivided

into three data partitions, ITS1, 5.8S and ITS2. Maximum Likelihood analyses were performed in RAxML v8.2.X (Stamatakis 2006). The best nucleotide substitution model was selected with BIC (Bayesian Information Criterion) using jModelTest 2v.1.6. The evolutionary models selected for the dataset were: ITS1: TIM1ef+G, 5.8S: K80+I, ITS2: HKY+I+G and LSU TrNef+I+G. Bayesian inferences (BI) were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), performing  $2 \times 10^7$  MCMC generations, sampling one tree every  $1 \times 10^3$  generation. The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 software were implemented in the CIPRES Science Gateway 3.1 (Miller et al. 2010). The final alignment and the resulting topologies were deposited in TreeBASE, ID: xxxx.

## Results

### Phylogenetic results

The dataset included 87 sequences with 2011 characters (including introduced gaps). Both RAxML and Bayesian analyses yielded similar tree topologies and the ones generated in the RAxML analysis is shown, including the bootstrap and posterior probabilities. Our species are clustered in two separated branches that contain Neotropical taxa: the branch/section *Neotropici* houses *Lf. annulifer*, *Lf. dunensis* Sá & Wartchow, *Lf. neotropicus*, *Lf. mamorensis* and the novel species *Lf. aurantioceraceus*; the newly described section *Multiceps* has high support (100% BS, 1.0 BPP) and containing the new species *Lf. reniformis*, *Lf. multiceps* and one undescribed species (G3264).

### Taxonomy

***Lactifluus* sect. *Neotropici*** J. Duque, L. Delgat, A. Verbeken, M. A. Neves & Anibal A. Carvalho Jr.

*Lactifluus annulifer* (Singer) Nuytinck, in Verbeken, Nuytinck & Buyck, Mycotaxon 118: 450 (2011)

≡*Lactarius annulifer* Singer, in Singer, Araujo & Ivory, Beih. Nova Hedwigia 77: 290 (1983)

**Description and revision:** Singer et al. (1989) and Silva-Filho & Wartchow (2019).

**Materials examined:** BRAZIL, Amazonas, Presidente Figueiredo, Aldeia Mari-Mari, Próximo ao igarapé do Mutum, 22 Nov 2015, leg. Braga-Neto R1133 (INPA 271904).

**Ecology and distribution:** Solitary, on sand soil, in white-sand forest, under unknown host, in Amazonian growing in forest composed of potential ectomycorrhizal hosts including members of Fabaceae, and Sapotaceae (Demarchi et al. 2018). Known only from Brazil (Singer et al. 1983).

**Notes:** Our phylogeny confirms the position of *Lf. annulifer* in *Lf.* sect. *Neotropici*. This species forms a branch along with two sequences (TH9014 and TH7880) both from Guyana. *Lactifluus annulifer* is the only veiled milkcap occurring in the Amazon Forest. It was originally described by Singer et al. (1983) from white-sand forest and our sample represents the second occurrence of this species. Our collection was also collected in white-sand forest, approximately 95 km distance from type locality.

*Lactifluus aurantioceraceus* Silva-Filho & Wartchow, sp. nov.

Figs. 2-3

Mycobank: MB xxxxxx

**Type:** Brazil, Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, Trilha da Peroba, 05 Jul 2017, leg. Silva-Filho AGS879 (UFRN-fungos **Holotype!**).

Basidiomata agaricoid. Pileus 77–85 mm, convex deeply depressed becoming depressed to slightly umbilicated, light orange (5A4), greyish orange (5B4), orange (6A6), light

yellow (4A4, 4A5), brownish yellow (5C6), greyish red (7B5); surface slightly rugulose, rimose, hygrophanous, waxy, dry, dull; margin straight to slightly decurved, eroded, split, with rare brown (7E5) spots (Figs. 2A,C,D); context firm, 5mm at the disc, to very thin toward at margin, yellowish white (1A2), unchanging. Lamellae short decurrent, close, sometimes forked, yellowish white (1A2), pale yellow (3A2, 4A3), greyish yellow (5B4); edge entire to slightly crenate, concolorous; lamellulae frequent of four lengths. Stipe 43–45 × 9–11 mm, central, cylindrical, sometimes equal, sometimes slightly ventricose fusiform, sometimes tapering toward the base, yellowish white (3A2), pale yellow (3A3) light yellow (4A4) light Orange (5A4); surface slightly striated, sometimes slightly scrobiculated, dry, dull (Fig. A-D); context solid, fistulose, yellowish white (3A2), pale yellow (3A3) light yellow (4A4), unchanging. Veil absent. Latex white (1A1), unchanging (Fig. 2B). Smell and taste not determined.

Basidiospores (5.5–)6–7 × (4.5–)5–6(–6.5) μm; L = 6.3 μm, W = 5.5 μm; Q = 1.04–1.33, Qm = 1.15; subglobose, to broadly ellipsoid, predominantly subglobose, thin-walled, hyaline; ornamentation amyloid, up to 0.5 μm high, composed of irregular short ridges and warts connected by fine lines, not forming a complete reticulum; plage inamyloid; hilar appendix to 3 μm long (Fig. 3A). Basidia 36–50 × 7.5–12.5 μm, clavate and fusiform, predominantly fusiform, 4-spored, thin-walled, with refractive and oil contents; sterigmata up to 5.5 μm (Fig. 3B). Pleurocystidia absent. Pleuropseudocystidia scattered to frequent, 8.5–12.5 μm diam., cylindrical sometimes with swollen apex, sometimes slightly fusiform, thin-walled, with refractive contents (Fig. 3C); projecting up to 35 μm above to hymenium, arising deeply from hymenophoral trama. Lamellae edge sterile composed of common marginal cells, 10–24.5 × 3.5–7.5 μm, cylindrical, clavate, sometimes ventricose-rostrate, sometimes flexuous, thin-walled, hyaline (Fig. 3F). Hymenophoral trama composed of abundant

sphaerocytes up to 17.5  $\mu\text{m}$  diam., cylindrical hyphae up to 9  $\mu\text{m}$  diam, thin-walled, hyaline and scattered to frequent lactiferous hyphae up to 9  $\mu\text{m}$  diam. Subhymenium cellular. Pileipellis a lampropalisade of up to 75  $\mu\text{m}$  thick, consisting of a layer of isodiametric cells, thin-walled terminal cells and scattered hair-shaped, thick-walled elements (Fig. 3D): isodiametric cells up to 15.5  $\mu\text{m}$  diam., thin-walled; thin-walled terminal elements 34–71.5  $\times$  3–6  $\mu\text{m}$ , cylindrical, cylindrical-flexuous, ventricose-clavate, thin-walled, hyaline, vertically oriented; thick-walled terminal elements 18–38  $\times$  5.5–9.5  $\mu\text{m}$ , aciculate, ventricose-cylindrical, thick-walled (up to 1.5  $\mu\text{m}$  thick), hyaline vertically and diagonally oriented, arising deeply from isodiametric cells (Fig. 3E). Pileus trama composed of abundant sphaerocytes 9–24  $\mu\text{m}$  diam., and rare lactiferous hyphae 5–12  $\mu\text{m}$  diam. Stipitipellis a lamprotrichopalissade like to pileipellis. Clamp connection absent from all tissues examined.

**Diagnosis:** Distinguished from other species of *Lf.* sect. *Neotropici* by: Basidiomata lacking secondary vellum, with predominant orange tints. Pileus 77–85 mm, surface slightly rugulose, rimose, waxy. Lamellae close, waxy. Stipe 43–45  $\times$  9–11 mm, waxy. Basidiospores 6–7  $\times$  5–6  $\mu\text{m}$ , ornamentation up to 0.5  $\mu\text{m}$  high; pleuropseudocystidia scattered to frequent, cylindrical to clavate, 8.5–12.5  $\mu\text{m}$  diam.; pileipellis a lampropalisade with scattered aciculate, ventricose-cylindrical hair-shaped, thick-walled elements.

**Etymology:** From Latin (Borrer 1960): aurantio (orange), ceraceus (waxy), due to orange tints and waxy texture.

**Additional material examined:** Brazil, Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, Trilha da Peroba, 05 Jul 2017, leg. Silva-Filho AGS879 (UFRN-

fungos), *ibid*, 28 Jun 2017, leg. Silva-Filho AGS857 (UFRN-fungos) *ibid*, 28 Jun 2017, leg. Silva-Filho AGS859 (UFRN-fungos).

**Ecology and distribution:** Solitary on sand soil, in dune vegetation, under unknown host, in Atlantic Forest, growing surrounded by ectomycorrhizal tree species of Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae (Freire 1990). Known only from type locality.

**Notes:** In our Phylogeny, *Lf. aurantioceraceus* appears on isolated branch, close related to a small clade composed by *Lf. subiculatus* and other two not yet described specimens (AMV2003 and AMV2004). Despite of the low support node, *Lf. aurantioceraceus* presents distinct morphology compared with other species of the section *Neotropici*.

*Lactifluus neotropicus* and *Lactifluus annulifer* differ mainly by presence of secondary vellum, absent in *Lf. aurantioceraceus*. *Lactifluus subiculatus* S.L. Mill., Aime & T.W. Henkel grows on subiculum, has smaller pileus (up to 30 mm diam.) and stipe (4–8 × 8–15 mm) and a membranous pileus (Miller et al. 2012). *Lactifluus caatingae* Sá & Wartchow has smaller pileus (up to 37 mm diam.), smaller stipe (12–33 × 4–7 mm) and striated pileus margin (Sá et al. 2019). *Lactifluus mamorensis* Singer (Silva-Filho & Wartchow) differs in their smaller pileus (up to 51 mm diam.), conspicuous ribbed stipe and larger basidiospores (6.7–11 × 5.8–10 µm). *Lactifluus dunensis*, *Lf. marielleae* J. Duque & M.A. Neves and *Lf. venezuelanus* have more spaced lamellae and a palisade pileipellis (Dennis 1970; Sá et al. 2013; Barbosa et al. 2020). *Lactifluus paulensis* (Singer) Delgat and *Lf. rupestris* described from Brazil and not yet sequenced are probably member of *Lf. sect. Neotropici*. Both species have smaller pileus (up to 32 mm and 70 mm diam.) respectively and a palisade pileipellis structure (Wartchow and Cavalcati 2010; Singer et al. 1983).

*Lactifluus dunensis* Sá & Wartchow, in Sá, Baseia & Wartchow, *Mycosphere* 4(2): 263 (2013)

**Original description:** Sá et al. (2013).

**Material examined:** Brazil, Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, Trilha da Peroba, 21 Jun 2012, leg. J.C. Bezerra s/n (UFRN-Fungos 1882 Holotype); *ibid*, 05 Jul 2017, leg. Silva-Filho AGS 879 (UFRN fungos xxxx), Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, trilha da geologia, 31 Jun 2019, leg. Silva-Filho AGS 102-2019 (UFRN fungos xxxx); *ibid*, 31 Jun 2019, leg. Silva-Filho AGS 103-2019 (UFRN fungos xxxx).

**Ecology and distribution:** Solitary and gregarious on sand soil, in dune vegetation, under unknown host, in Atlantic Forest, growing surrounded by ectomycorrhizal tree species of Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae (Freire 1990) . Known from Brazil and French Guyana.

**Notes:** *Lactifluus dunensis* was described from morphological data (Sá et al. 2013). The original description was the only record of this species. Here we sequenced the type and confirm the placement in to sect. *Neotropici*, as well as bring novelties on its distribution. Our phylogeny showed *Lactifluus dunensis* clustered with other four sequences in a small branch: the sequences MR/Guy 13032 e MR/Guy 09/004 appears to form distinct lineages while the sequences G4819 and G4727 from French Guiana are co-specific to the type of *Lf. dunensis*. Thus, *Lf. dunensis* has their distribution expanded further north.

*Lactifluus mamorensis* Silva-Filho & Wartchow, *New Zealand Journal of Botany* 57 (4): 302 (2019)

≡*Lactarius mamorensis* (Singer) Singer, Beihefte zur Nova Hedwigia 77: 302 (1983)

**Description and review:** Singer et al. (1989) and Silva-Filho & Wartchow (2019).

**Material examined:** Brazil, Bahia, Abaíra, Catolés de Cima, Serra do Barbado, Mata do Tijuquinho, 9 Jan 2015, leg. Wartchow F. FW 3/2015 (JPB 61973; UFRN-Fungos 3029).

**Ecology and distribution:** Solitary, on soil, under unknown host, in ‘mata nebulosa’ of the Caatinga Biome, growing surrounded by ectomycorrhizal putative tree species of Fabaceae, Myrtaceae and Polygonaceae (Zappi et al. 2003).

**Notes:** Our samples clustered in the phylogeny together with sequences of *Lf. catarinensis*. *Lactifluus mamorensis* is macromorphologically characterized by a medium size basidiomata, orange tinted pileus close lamellae and an evidently ribbed stipe. The last cited feature is observed only in *Lf. mamorensis*, *Lf. catarinensis* and *Lf. paulensis*. However, *Lf. mamorensis* has a confused micromorphology or some variations regarding to the cystidia and pileipellis type. In the prologue, Singer et al. (1983) reported the occurrence of scattered ‘cystidia’ measuring  $26\text{--}37 \times 7.3\text{--}9 \mu\text{m}$ , this term corresponding, in their study, to pseudocystidia conception. In the reanalysis of paratypes ‘R. Singer B 12085’ and ‘R. Singer B12118A’, and in our new sample, were observed only scattered pleuropseudocystidia corroborating the original description and confirms our sample. The pileipellis of *Lf. mamorensis* was originally described as an ‘epithelium with dermatocystidia or hairs’ and the occurrence of thick-walled elements was not reported, understood that it corresponds to a palisade pileipellis structure. The analysis of the type and our new sample disagree with the original description, since was observed some scattered thick-walled terminal elements. Barbosa et al. (2020) reports the occurrences of scattered pleuromacrocystidia in *Lf. catarinensis* and

described the pileipellis structure as a palisade. Despite of morphological similarity and close phylogenetic relation, confirmation that these species are cospecifics will be done with the inclusion of the sequence of the *Lf.mamorensis paratype*.

*Lactifluus mamorensis* was originally described from Bolivia and Brazil in different ecosystems of Amazon Forest. From Brazil was firstly reported from North (Singer et al. 1983) and now we report it from Semi-arid region in Caatinga Biome in Northeast of Brazil.

*Lactifluus neotropicus* (Singer) Nuytinck, Mycotaxon 118: 450 (2011)

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

**Original description:** Singer (1952)

Materials examined: BRASIL. Paraíba: Areias, Parque Estadual Mata do Pau Ferro leg. Wartchow F. FW23/2010 (UFRN-fungos 2197, JPB); ibid 05 July 2017, Wartchow F. FW21/2017 (UFRN-fungos 2197, JPB), FW27/2017 (UFRN-fungos 2197, JPB). FW37/2017 (UFRN-fungos 2197, JPB).

**Ecology and distribution:** Scattered, on sand soil, in a tropical wet mountain forest, under unknown host in Caatinga Biome, growing surrounded by puntative ectomycorrhizal species of Fabaceae, Nyctaginaceae (species of *Guapira* Aubl.) and Polygonaceae (Barbosa et al., 2004). Known from Trinidad (Caribbean) and Brazil.

**Notes:** Our Phylogeny shows our sample of *Lf. neotropicus* clustered with the sequence of holotype RWG Denis 97. *Lactifluus neotropicus* has been reported from Trinidad and Tobago (Singer,1952; Pegler and Fiard,1979) and recently recorded in Atlantic forest in South and Northeast of Brasil (Barbosa et al. 2020). Our specimens were found in a wet montain forest in Brazilian semi-arid region, representing a new record from Caatinga.

***Lactifluus* section *Multiceps*** Silva-Filho & Wartchow, nom. prov.

Mycobank: MB XXXXXX

Diagnosis: Characterized in *Lf.* subg. *Lactariopsis* by a combination of the following features: Neotropical distribution. Basidiomata growing on a subiculum, small, yellow, orange to brownish tinted; pileus surface pubescent to hispidulous; lamellae distant. Basidiospore globose to subellipsoid, ornamentation composed of irregular short ridges and warts connected by fine lines, plage inamilod to centrally amyloid; true cystidia absent, and a pileipellis with hair-shaped thick-walled terminal elements with swollen base.

Type: *Lactifluus multiceps* (S.L. Mill., Aime & T.W. Henkel) De Crop

Etymology: Due to the epithet of first described species of the section: *Lactifluus multiceps*.

Remarks: This new section is morphologically delimited by its Neotropical distribution, small, yellow, orange to brownish basidiomata, growing on a subiculum, pubescent to hispidulous pileus surface, distant lamellae, globose to subellipsoid basidiospore, ornamentated by irregular short ridges and warts connected by fine lines, inamilod to centrally amyloid plage, absence of true cystidia and by the lampropalisade with hair-shaped thick-walled terminal elements with a swollen base. Neotropical species occur in other two sections of *Lactariopsis*. The section *Albati* harbors *Lactifluus hallingii* Delgat & De Wilde, originally described from Costa Rica, Panama, and Colombia (Delgat et al. 2019). This section is characterized by large white basidiomata, velutinous pileus, presence of macrocystidia and a pileipellis consisting of hyphae, without isodiametric cells. *Lactifluus* section *Neotropici* contain only Neotropical taxa with small to large basidiomata, close to subdistant lamellae, occasionally velled, central

stipe, occasionally true pleurocystidia and palisade to lampropalisade pileipellis type (Barbosa et al. 2020). *Lactifluus* sect. *Multiceps* is composed by *Lf. multiceps*, *Lf. reniformis* and an undescribed species (G3264) from French Guiana. A photo of the sample (G3264) is available in commons wikimedia<sup>11</sup> confirming the main morphological features of the section, as example, a small basidiomata, in which growing on a subiculum and a velutinous to hispidulous pileus surface.

***Lactifluus reniformis*** Silva-Filho, DL Komura & Wartchow, sp. nov. Figs. 4-5

Mycobank: MB xxxxxx

**Type:** Brazil, Amazonas, São Gabriel da Cachoeira, Itacoatiara-Mirim, 28 Mar 2013, leg. D. L. Komura, C. Domingos, J.A. Correia da Silva, C.E. Zartman & L. Keerada, DLK 1094 (INPA 283997).

Basidiome small, solitary, growing on subiculum. Pileus 15 mm diam., convex, orange white (5A2), to grayish orange (5B4); surface pubescent to velutinous, slightly moist; margin decurved to incurved, edge slightly irregularly sulcate, lobed (Figs. 4, 5A); context firm, up to 1 mm thick, white (1A1), unchanging. Lamellae narrowly adnate, distant, white (1A1) to orange white (5A2); edge entire, concolorous; lamellulae frequent of two lengths. Stipe 3.5 × 2.5 mm, eccentric, laterally reduced concolorous with the pileus; surface pubescent to velutinous with a white (1A1) hairy tomentum at the base (Figs. 4, 5A). Subiculum white (1A1), densely effused on plant branch (Figs. 4). Latex scant, white, unchanged. Smell and taste not determined.

Basidiospores (8–)8.5–10(–11) × 8–9.5(–10) μm; L = 9.1 μm, W = 8.8 μm; Q = 1–1.12(–1.18), Qm = 1.07; globose to subglobose, thin-walled, hyaline; ornamentation

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[https://commons.wikimedia.org/wiki/File:Lactifluus\\_sp\\_G3264\\_pleurotoid\\_French\\_Guiana\\_cropped.png](https://commons.wikimedia.org/wiki/File:Lactifluus_sp_G3264_pleurotoid_French_Guiana_cropped.png)

amyloid, up to 1.5  $\mu\text{m}$  high, composed of irregular short ridges and warts connected by fine lines, sometimes forming a complete reticulum; plage inamyloid (Fig. 5B); hilar appendix up to 4  $\mu\text{m}$  long. Basidia 32–63  $\times$  11–15  $\mu\text{m}$ , cylindrical, cylindrical-clavate, smaller near to edge, 2-4-spored, predominately 4-spored, thin-walled, hyaline, and with refractive contents (Fig. 5C); sterigmata up to 12  $\mu\text{m}$  long. Pleurocystidia absent. Pleuropseudocystidia scarce to frequent, 9–13  $\mu\text{m}$  diam., cylindrical, clavate, thin-walled, with dense refractive contents (Fig. 5D); not projecting above the hymenium; arising deeply from the hymenophoral trama. Lamellar edge fertile composed of scattered basidia and common terminal cells, 19–45  $\times$  3–5  $\mu\text{m}$ , cylindrical, flexuous, catenulate, thin-walled, hyaline (Fig. 5G). Hymenophoral trama composed of scattered sphaerocytes up to 25  $\mu\text{m}$  diam., frequent cylindrical hyphae, up to 6  $\mu\text{m}$  diam. and frequent lactiferous hyphae up to 9  $\mu\text{m}$  diam. Subhymenium cellular. Pileipellis a lampropalisade up to 80  $\mu\text{m}$ , consisting of a isodiametric cells layer, thin-walled terminal cells, and scattered thick-walled hair-shaped cells (Fig. 5E): isodiametric cells 12–59  $\mu\text{m}$  diam., thin-walled, hyaline: terminal cells 25–30  $\times$  11–17  $\mu\text{m}$ , clavate, thin-walled, hyaline; thick-walled terminal elements, 67–103  $\times$  4–6  $\mu\text{m}$ , cylindrical, filiform, mostly with swollen base, 8–11  $\mu\text{m}$  diam., thick-walled, (up to 1.5  $\mu\text{m}$  thick), hyaline (Fig. 5F). Pileus trama composed of abundant sphaerocytes up to 24  $\mu\text{m}$  diam., and some rare lactiferous hyphae up to 10  $\mu\text{m}$  diam. Stipitipellis a lampropalisade like to pileipellis. Clamp connection absent from all tissues.

**Diagnosis:** Distinguished from other species of *Lf.* sect. *Multiceps* by: Habit solitary. Lamellae concolorous with the pileus. Basidiospores globose to subglobose 8.5–10  $\times$  8–9.5  $\mu\text{m}$ , plage inamyloid, pleuropseudocystidia wider (9–13  $\mu\text{m}$  diam.), pileipellis with shorter hair-shaped thick-walled terminal elements (67–103  $\mu\text{m}$  long).

**Etymology:** ‘reniformis’ due to reniform format of the basidiome.

**Ecology and distribution:** Solitary growing on a subiculum of an unknown tree branch, in white-sand forests of Amazon forest.

**Notes:** In our phylogeny *Lf. reniformis* appears on a branch grouped with *Lf. multiceps* from Guyana and an undescribed species (G3264) from French Guiana. *Lactifluus multiceps* differs from *Lf. reniformis* by gregarious habit, discolored lamellae, subglobose to short ellipsoid basidiospores (6.4–8.0  $\mu\text{m}$  wide vs. 8–9.5  $\mu\text{m}$  wide), centrally amyloid plage, thinner pleuropseudocystidia (4.8–6.5  $\mu\text{m}$  diam. vs. 9–13  $\mu\text{m}$  diam.) and longer hair-shaped thick-walled terminal elements (44–220 vs. 67–103  $\mu\text{m}$  long., Miller et al. 2012).

## Discussion

This is our third studies on Brazilian *Lactifluus* diversity (Silva-Filho et al. 2019, Silva-Filho et al. 2020). Our research not only revealed new taxa, but also two sections: *Lf.* section *Piperogalacti* (cf. Silva-Filho et al. 2020) and *Lf.* section *Multiceps*. In this manuscript we bring six species collected in tropical forests of Brazil, among then, two are proposed as new species from the Amazon and Atlantic forests confirming the phylogenetic diversity of *Lactifluus* in these biomes

*Lactifluus* sect. *Multiceps* was delimited containing three species with small eccentric stipe, growing on subiculum. All species belong to this section have South American origin. *Lactifluus* sect. *neotropici* has eleven described species, up to now, and other nine sequences that correspond to seven undescribed species.

*Lactifluus venezuelanus* and *Lf. neotropicus* are the only occurring in Caribbean and South America, while all others have got South America distribution. *Lactifluus neotropicus* is the widest distributed species in Neotropical region. In Brazil it has been recorded in Atlantic Forest of South and Northeast (Barbosa et al. 2020). We collected

our samples in a tropical wet mountain forest an ecosystem in semiarid region of Brazil. This area is relative close to the Atlantic Forest reserve where one sample (ACM323) was collected (Barbosa et al. 2020). Both areas have similar environments with the presence of ectomycorrhizal members of the Nyctaginaceae and Polygonaceae (Barbosa et al. 2004). Thus, there is doubt about the distribution of *Lf. neotropicus* in Brazil, if it is restricted to the coastal region in Atlantic Forest and some ecosystems of the Caatinga, or further West in other biomes as in Brazilian Cerrado.

Additionally, we bring a new molecular data of a new collection of *Lf. annulifer* collected from the same type ecosystem in Brazilian Amazon. *Lactifluus annulifer* is in a branch with other two sequences (TH7880 and TH9014) from Guyana. However, there are no morphological data about these two samples. In our phylogeny we included the type sequence of *Lf. dunensis*, which matched with two sequences recovered from NCBI (Genbank). These sequences (G4727 and G4819) have origin from French Guiana, indicating a wider distribution of *Lf. dunensis*.

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Figura 37 - **Fig. 1** ML phylogeny of representative collections of *Lactifluus* subg. *Lactariopsis* based on complete nuc- rDNA spacers ITS and LSU. New species are noted in red and the sequences generated in this study are noted in blue. The new section is highlighted in reddish brown. Thicker lines represent branches with maximum bootstrap values and posterior probabilities (100% BS / 1.0 BPM). Bootstrap values and Bayesian posterior probabilities are indicated if they exceed 0.95 and 70%, respectively. The scale bar represents the expected number of nucleotide changes per site.

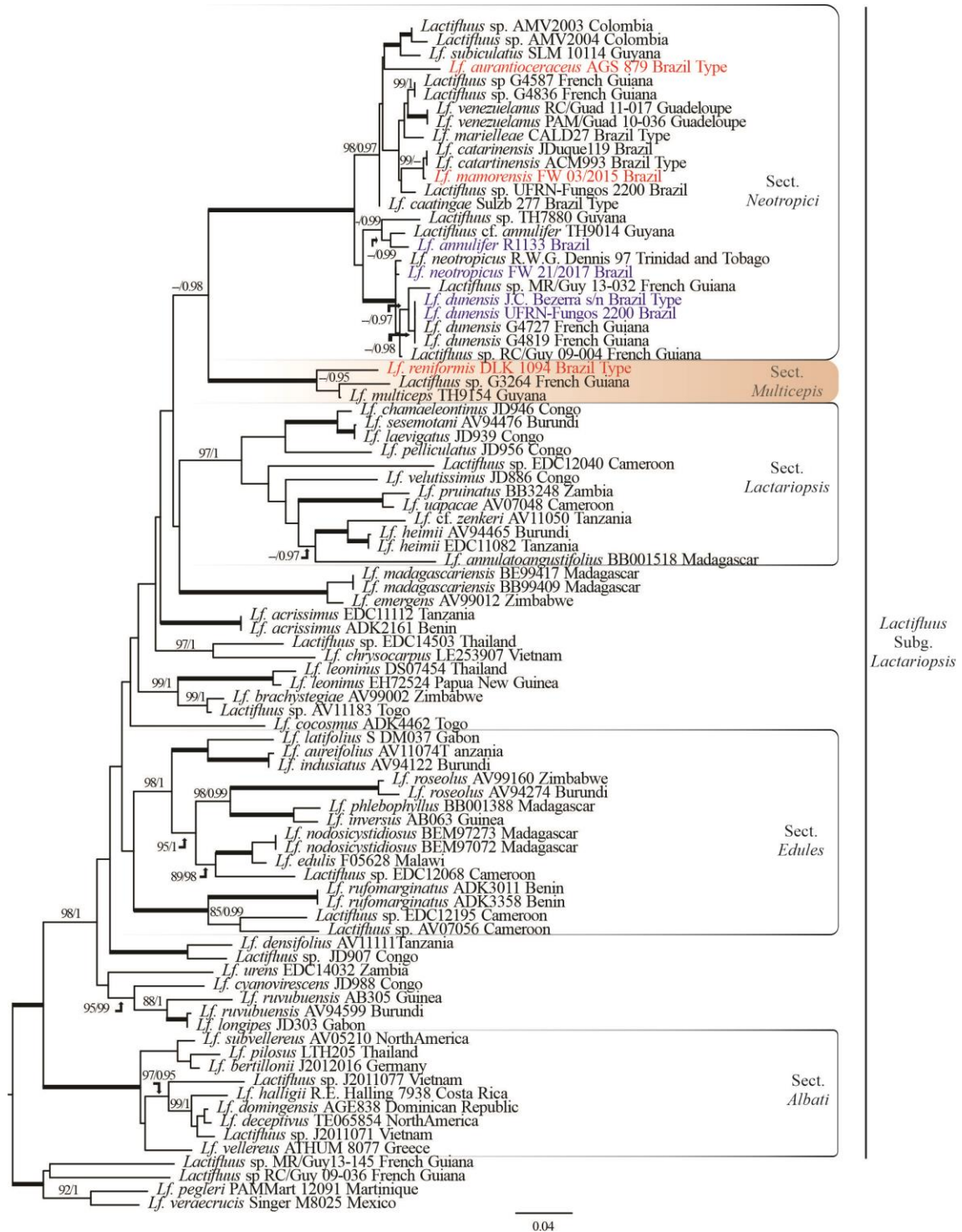


Figura 38 - **Fig. 2** Basidiomata of *Lactifluus aurantioceraceus*. A Holotype (AGS 879). B Paratype (AGS 859). C. Paratype (AGS 857). Scale bars = 10 mm. Photos: Alexandre G. S. Silva-Filho.



Figura 39 - **Fig. 3** Micromorphological features of *Lactifluus aurantioceraceus* (Holotype AGS 879). A Basidiospores. B Basidia. C Pleuropseudocystidia. D Pileipellis. E Thick-walled elements of pileipellis. E Marginal cells of the lamellar. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

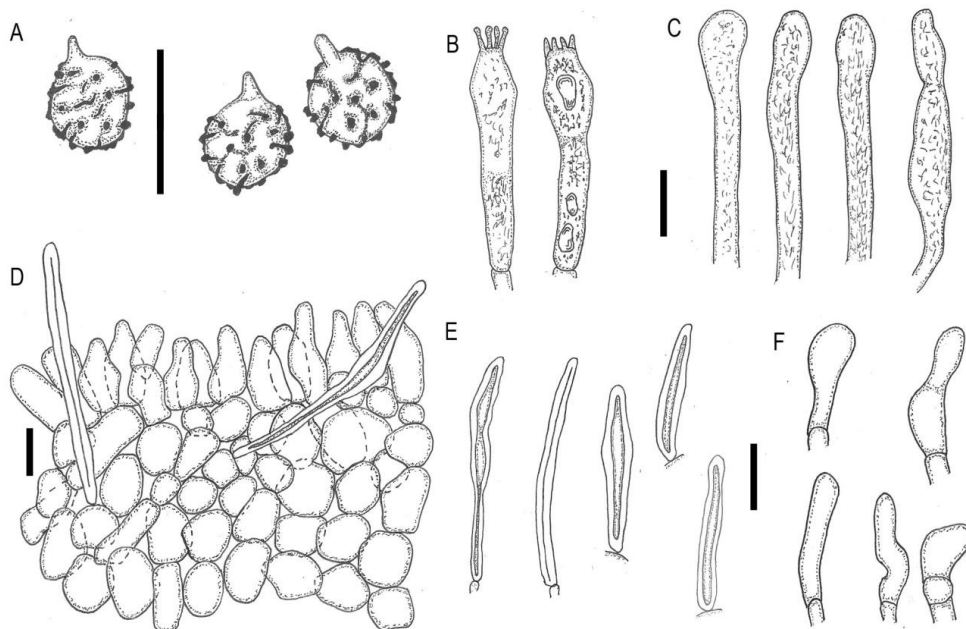


Figura 40 - **Fig. 4** Basidiome of *Lactifluus reniformis* (Holotype D.L. Komura 1094). Scale bars = 10 mm. Photos: Dirce Komura.



Figura 41 - **Fig. 5** Macro-and Micromorphological features of *Lactifluus reniformis* (Holotype 1094). A Basidiome. B Basidiospores. C Basidia. D Pleuropseudocystidia. E Pileipellis. F Thick-walled elements of pileipellis. G Marginal cells of the lamellar edge. Scale bars: A = 5mm. B-G= 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

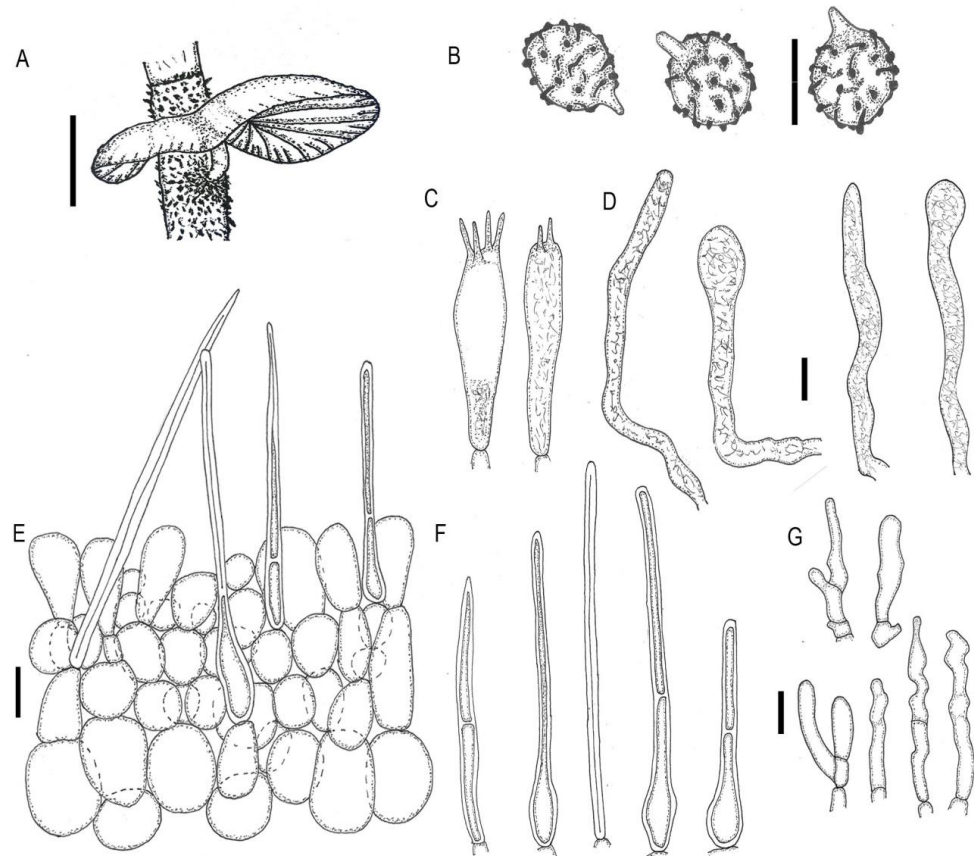


Tabela 6. **Table 1:** Species, voucher collection, country and GenBank accession numbers of ITS and LSU sequences used in molecular analyses. Accession numbers in bold were newly generated for this study. Sequences not yet deposited in Genbank are marked with ‘\*’.

Species	Voucher	Country	ITS	LSU
<i>Lactifluus acrisissimus</i>	ADK2161	Benin	KR364126	
<i>Lf. acrisissimus</i>	EDC 11-112	Tanzania	KR364041	KR364168
<i>Lf. annulatoangustifolius</i>	BB 00-1518	Madagascar	AY606981	KR364253
<b><i>Lf. annulifer</i></b>	<b>R1133</b>	<b>Brazil</b>	*	*
<i>Lf. cf. annulifer</i>	TH 9014	Guyana	KC155376	KC155376
<b><i>Lf. aurantioceraceus</i></b>	<b>AGS 879</b>	<b>Brazil</b>	*	*
<i>Lf. aureifolius</i>	AV 11-074	Tanzania	KR364056	KR364183
<i>Lf. bertillonii</i>	JN 2012-016	Germany	KR364087	KR364217
<i>Lf. brachystegiae</i>	AV 99-002	Zimbabwe	KR364018	KR364145
<i>Lf. caatingae</i>	Sulzb 277	Brazil	MG930781	
<i>Lf. chamaeleontinus</i>	JD 946	Congo	KR364079	KR364208
<i>Lf. chrysocarpus</i>	LE 253907	Vietnam	JX442761	JX442761
<i>Lf. cocosmus</i>	ADK 4462	Togo	KR364013	KR364141
<i>Lf. cyanovirescens</i>	JD 988	Congo	KR364082	KR364211
<i>Lf. deceptivus</i>	TENN 065854	Nort America	KR364101	
<i>Lf. densifolius</i>	AV 11-111	Tanzania	KR364057	KR364184
<i>Lf. dominigensis</i>	ANGE838	Costa Rica	MK931341	MN128990
<b><i>Lf. dunensis</i></b>	<b>UFRN-Fungos 2200</b>	<b>Brazil</b>	*	*
<i>Lf. dunensis</i>	G4727	French Guiana	KM073087	
<i>Lf. dunensis</i>	G4819	French Guiana	KM073086	
<b><i>Lf. dunensis</i></b>	<b>J.C.Bezerra s/n</b>	<b>Brazil</b>	*	*
<i>Lf. edulis</i>	FN 05-628	Malawi	KR364020	KR364147
<i>Lf. emergens</i>	AV 99-012	Zimbabwe	KR364021	KR364148

<i>Lf. halligii</i>	R.E. Halling 7983	Dominican Republic	MK931333	
<i>Lf. heimii</i>	EDC 11-082	Tanzania	KR364040	KR364167
<i>Lf. heimii</i>	AV 94-465	Burundi	KR364025	KR364152
<i>Lf. indusiatus</i>	AV 94-122	Burundi	KR364026	KR364153
<i>Lf. inversus</i>	AB 063	Guinea	AY606976	DQ421978
<i>Lf. laevigatus</i>	JD 939	Congo	KR364077	KR364206
<i>Lf. latifolius</i>	SDM 037	Gabon	KR364028	KR364155
<i>Lf. leoninus</i>	DS 07-454	Thailand	KF220055	JN388989
<i>Lf. leoninus</i>	EH 72-524	Papua New Guinea	KR364116	
<i>Lf. longipes</i>	JD 303	Gabon	KR364009	KR364137
<i>Lf. madagascariensis</i>	B-E 99-417	Madagascar	KR364120	KR364245
<i>Lf. madagascariensis</i>	BB 99-409	Madagascar	AY606977	DQ421975
<i>Lf. mamorensis</i>	ACM 993	Brazil	MK937538	MK937556
<b><i>Lf. mamorensis</i></b>	<b>FW 03/2015</b>	<b>Brazil</b>	*	
<i>Lf. mamorensis</i>	JDucke 119	Brazil	MK937537	
<i>Lf. marielleae</i>	CALD27	Brazil	MK937527	MK937547
<i>Lf. multiceps</i>	TH 9154A	Guyana	JN168731	
<b><i>Lf. neotropicus</i></b>	<b>FW 21/2017</b>	<b>Brazil</b>	*	*
<i>Lf. neotropicus</i>	R.W.G. Dennis 97	Trinidad and Tobago	MN102680	
<i>Lf. nodosicytidiosus</i>	BEM 97-273	Madagascar	KR364029	KR364156
<i>Lf. nodosicytidiosus</i>	BEM 97-072	Madagascar	AY606975	DQ421976
<i>Lf. pegleri</i>	PAM/Mart 12-091	Martinique	KP691416	KP691425
<i>Lf. pelliculatus</i>	JD 956	Congo	KR364080	KR364209
<i>Lf. phlebophyllus</i>	BB 00-1388	Madagascar	AY606974	DQ421979
<i>Lf. pilosus</i>	LTH 205	Thailand	KR364006	KR364134
<i>Lf. pruinatus</i>	BB 3248	Zambia	KR364031	KR364158
<i>Lf. ramipilosus</i>	EDC 14-503	Thailand	KR364128	

<i>Lf. roseolus</i>	AV 99-160	Zimbabwe	KR364032	KR364159
<i>Lf. roseolus</i>	AV 94-274	Burundi	KR364121	KR364242
<i>Lf. rufomarginatus</i>	ADK 3358	Benin	KR364033	KR364160
<i>Lf. rufomarginatus</i>	ADK 3011	Benin	KR364034	KR364161
<i>Lf. ruvubuensis</i>	AB 305	Guinea	KR364035	KR364162
<i>Lf. ruvubuensis</i>	AV 94-599	Burundi	KR364122	
<i>Lf. sesemotani</i>	AV 94-476	Burundi	KR364036	KR364163
<i>Lf. subiculatus</i>	SLM 10114	Guyana	JQ405654	
<i>Lf. subvellerus</i>	AV 05-210	North America	KR364010	KR364138
<b><i>Lf. reniformis</i></b>	<b>DLK 1094</b>	<b>Brazil</b>	*	
<i>Lf. uapacae</i>	AV 07-048	Caribbean	KR364007	KR364135
<i>Lf. urens</i>	EDC 14-032	Zambia	KR364124	KR364247
<i>Lf. vellerus</i>	ATHU-M 8077	Greece	KR364106	KR364237
<i>Lf. velutissimus</i>	JD 886	Congo	KR364075	KR364204
<i>Lf. venezuelanus</i>	PAM/Guad 10-036	Guadeloupe	MK046844	MK046794
<i>Lf. venezuelanus</i>	RC/Guad 11-017	Guadeloupe	KP691411	KP691420
<i>Lf. veraecrucis</i>	Singer M 8025	Mexico	KR364112	KR364241
<i>Lf. cf. zenkeri</i>	AV 11-050	Tanzania	KR364055	KR364182
<i>Lactifluus</i> sp.	12-040	Cameroon	KR364063	KR364192
<i>Lactifluus</i> sp.	AMV 2003	Colombia	KT354740	MK937561
<i>Lactifluus</i> sp.	AMV 2004	Colombia	KT354741	MK937560
<i>Lactifluus</i> sp.	AV 07-056	Cameroon	KR364136	KR364008
<i>Lactifluus</i> sp.	AV 11-183	Togo	KR364060	KR364189
<i>Lactifluus</i> sp.	EDC 12-068	Cameroon	KR364068	KR364197
<i>Lactifluus</i> sp.	EDC 12-195	Cameroon	KR364071	KR364200
<i>Lactifluus</i> sp.	G3264	French Guiana	KJ786706	KJ786620
<i>Lactifluus</i> sp.	G4587	French Guiana	KM073084	

<i>Lactifluus</i> sp.	G4836	French Guiana	KM073083	
<i>Lactifluus</i> sp.	JD 907	Congo	KR364076	KR364205
<i>Lactifluus</i> sp.	JN 2011-071	Vietnam	KR364043	KR364169
<i>Lactifluus</i> sp.	JN 2011-077	Vietnam	KR364044	KR364170
<i>Lactifluus</i> sp.	MR/Guy 13-032	French Guiana	KJ786686	KJ786581
<i>Lactifluus</i> sp.	MR/Guy 13-145	French Guiana	KJ786691	KJ786595
<i>Lactifluus</i> sp.	RC/Guy 09-004	French Guiana	KJ786643	KP691419
<i>Lactifluus</i> sp.	RC/Guy 09-036	French Guiana	KJ786645	KJ786550
<i>Lactifluus</i> sp.	TH7880	Guyana	KT339212	
<i>Lactifluus</i> sp.	UFRN-Fungos 2200	Brazil	*	

## **10 CAPÍTULO 5 - New diversity in *Lactifluus* sect.**

### ***Polysphaerosphori* (Russulaceae, Basidiomycota) from Brazilian tropical forests**

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## **New diversity in *Lactifluus* sect. *Polysphaerosphori* (Russulaceae, Basidiomycota) from Brazilian tropical forests**

Species diversity of *Lactifluus* in Brazil has been recently better investigated, revealed a high diversity in South American tropical forests. Based on morphological features and molecular phylogenetic analyses using ITS sequences, five new species are described from Brazil: *L. brunneoruber*, *L. candidus*, *L. flavuscaespitosus*, *L. perforatus* and *L. restingae*. Phylogenetic analyses indicated the placement of this species and of *L. amazonensis* in *Lactifluus* sect. *Polysphaerosphori*. Full descriptions, photo plates, illustrations and a phylogenetic tree are presented.

Key words: ITS, Basidiomycota, new taxon, phylogeny, Russulales

### **INTRODUCTION**

*Russulaceae* is composed by four genera of ectomycorrhizal fungi: *Russula* Pers. and the milkcap genera *Lactarius* Pers., *Lactifluus* (Pers.) Roussel and *Multifurca* Buyck & V. Hofstetter. *Lactifluus* containing approximately 210 species (He et al., 2019) distributed mainly in tropical and subtropical areas of Africa, Asia and America (De Crop et al., 2017). All genera, except *Multifurca*, have been recorded from Brazil: *Russula* is represented by 17 species, *Lactarius* by five related to Pinus plantations and *Lactifluus*, the common milkcap tropical genus, 29 species were reported from native forests of Brazil (Singer et al., 1983, Singer, 1984; Sulzbacher et al., 2013a; Sá & Wartchow, 2013; Sá et al., 2013b, 2019; Wartchow et al., 2013; Silva-Filho et al., 2018, 2019, 2020; Barbosa et al. 2020).

*Lactarius* sect. *Polysphaerosphori* Singer was erected to accommodate *Lactarius veraecrucis* Singer from Central America (Singer, 1973). After that, Singer et al. (1983) groups in the section *Lactarius amazonensis* Singer, *Lactarius brasiliensis* Singer, *Lactarius mamorensis* Singer, *Lactarius nebulosus* Pegler, *Lactarius subpallidipes* Singer, *Lactarius pallidipes* Singer, *Lactarius paulensis* Singer and *Lactarius venezuelanus* Dennis. Molecular studies do not recognize *Lactarius* sect. *Polysphaerosphori* sensu Singer (1973) as a monophyletic group

(De Crop et al., 2017). Despite this, the section was transferred and recognized in *Lactifluus* subg. *Pseudogymnocarpi* (Verbeken) De Crop based in a monophyletic clade contains the type species *L. veraecrucis*, *L. pegleri* (Pacioni & Lalli) Delgat and other three undescribed species from South America (De Crop et al., 2017). More recently, other three species were described in the section: *L. ceraceus* Delgat & M. Roy, *L. umbilicatus* Silva-Filho, D.L. Komura & Wartchow and *L. venosellus* Silva-Filho, Sá & Wartchow, all from South America (Crous et al., 2017; Silva-Filho et al., 2019).

This work proposes four new species in *Lactifluus* sect. *Polysphaerosphori* (Singer) Verbeken. All species are described based on morphological and molecular characters and together with *L. amazonensis* are confirmed in *Lf.* sect. *Polysphaerosphori*.

## MATERIALS AND METHODS

Collecting area: Fresh specimens were collected in two distinct areas of Atlantic Forest: (1) ‘Reserva Biológica Guaribas’, municipality of Mamanguape, (6°44'27.89" S 35°8'53.82" W) State of Paraíba, Northeast of Brazil. (2) ‘Parque Estadual das Dunas’ municipality of Natal (24°18'26"S and 53°54'29" W), in the East region of Rio Grande do Norte state, Northeast of Brazil. (3) ‘Parque Estadual Mata do Pau Ferro’ one area of tropical wet mountain forest in Caatinga Biome in Areia municipality (6°57'58.9"S and 35°44'55.15"W), Paraíba state, Northeast of Brazil; (3) and in ‘Balbina’ a area of white-sand-forest municipality of President Figueiredo (1°55'32.76"S and 59°29'30.15"W), in the state of Amazonas, North of Brazil.

Morphological analysis: Macroscopic features were based on fresh material. Color names and codes follow Kornerup & Wanscher (1978). Studies were performed using the methodology of Verbeken (1998) and Verbeken & Walley (2010). Forty basidiospores were measured excluding ornamentation, in lateral view using Melzer's reagent, and dimensions are reported as (<10%, minimum length) average minimum length – average maximum length (<10%,

maximum length) × (<10%, minimum width) average minimum width – average maximum width (<10%, maximum width). For biometric measurements of the basidiospores we follow the methodology of Wartchow & Gamboa-Trujillo (2012). Other microscopic structures were measured and visualized in 5% KOH and Congo red. Specimens were deposited in the Herbaria JPB, INPA (Thiers 2021) and in the Mycological collection at the 'Universidade Federal do Rio Grande do Norte' (UFRN-Fungos).

Molecular methods: DNA was extracted from fresh tissue blotted on FTA® cards or from dried basidioma using the Qiagen DNeasy® Plant Mini Kit (Germantown, MD, USA) according to the manufacturer's instructions.. The nuclear ribosomal internal transcribed spacer region (ITS) was amplified and sequenced using primers ITS1-F and ITS4-B (White et al., 1990). Sequencing reactions were conducted by MACROGEN (KOREA) the resulting sequences were assembled and edited in Sequencher™ v5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were deposited in Genbank public database (Table1).

Phylogenetic analyses: The newly generated ITS sequences from this study were used as BLAST queries to NCBI (GenBank) in order to retrieve similar sequences. Most of the retrieved sequences represent species of *L.* subg. *Pseudogymnocarpi* from De Crop et al. (2017). Four sequences of *Lf.* sect. *Albati* (Bataille) Verbeken were used as outgroup: *L. bertillonii* (Neuhoff) Verbeken), *L. pilosus* (Verbeken, H.T. Le & Lumyong) Verbeken *L. subvellereus* (Peck) Nuytinck and one undescribed species *Lactifuus* sp. (voucher JN 2011-071). Our dataset consisted of 41 ITS sequences in which six are new generated. The sequences were aligned using MAFFT v.7 (Katoh & Standley, 2013) under the E-INS-i criteria. Seaview v.4 (Gouy et al., 2010) was used to visualize and adjustment the alignment. Maximum Likelihood analyses were performed in RAxML v8.2.X (Stamatakis 2006). The best nucleotide substitution model, HKY+I+G was selected with AIC (Akaike Information

Criterion) using jModelTest 2v.1.6 (Darriba et al., 2012). Bayesian inferences (BI) were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 softwares were implemented in the CIPRES Science Gateway 3.1 (Miller et al., 2010). A node was considered significantly supported if it received a BPP  $\geq$  0.95 and/or BS  $\geq$  80%. The final alignment and the resulting topologies were deposited in TreeBASE.

## RESULTS

Our analyses place the five new species described and *L. amazonensis* in *L.* sect. *Polysphaerophori*. The section is composed by four clade/branch containing species from Neotropical region. The clade/branch one has high support (100% BS / 1.0 BPP) and harbors two new described species: *L. restingae* and *L. flavuscaespitosus*. The clade two clusters *L. venosellus*, the newly species *L. brunneoruber*, *L. candidus*, *L. perforatus* and other six sequences. The well-supported clade/branch three (99% BS / 1.0 BPP) groups the type species of the section, *Lf. veraecrucis*, *L. pegleri* and one undescribed species (MR/Gui 13-145). The basal clade/branch four has good support (100% BS / 1.0 BPP) and groups *L. amazonensis*, *L. ceraceus*, *L. umbilicatus* and other two sequences.

## Taxonomy

*Lactifluus amazonensis* (Singer) Silva-Filho & Wartchow, New Zealand Journal of Botany 57 (4): 291 (2019)

=*Lactarius amazonensis* Singer, Beihefte zur Nova Hedwigia 77: 295 (1983)

DESCRIPTION: Singer et al. (1983) and Silva-Filho & Wartchow (2018).

MATERIAL EXAMINED: BRAZIL, AMAZONIA: Balbina, Ramal da Morena, entrada km 13, 05 May 2019, leg. *Komura D.L., Costa F.M., Costa-Silva D., Mello A.T. Komura DL 2618* (INPA, JPB).

ECOLOGY AND DISTRIBUTION: Solitary, in sand soil, in white-sand forests of Amazon forest, growing surrounded by putative ectomycorrhizal species of Leguminosae. Known only from Brazil.

REMARKS: Our phylogeny confirms the position of *L. amazonensis* in *L.* sect. *Polysphaerosphori* in which is harbored in the clade four close related to *L. ceraceus* and *L. umbilicatus*. *Lactifluus amazonensis* was originally described by Singer et al. (1983) from white-sand forest and here is confirmed from morphological features, agreeing to protologue and to the type study performed by Silva-Filho & Wartchow (2019). In addition, our specimen was sampled in white-sand forest in Amazon Forest near to type locality.

*Lactifluus brunneoruber* Silva-Filho, D.L. Komura & Wartchow, nom. prov.

Mycobank: MB XXXXX

TYPE: BRAZIL, AMAZONIA: Balbina, Ramal da Morena, 05 May 2019, leg. *Costa F.M., Costa-Silva D., Mello A.T., Komura D.L. 2597* (INPA, JPB holotype!).

ETYMOLOGY: From latin brunneo = brown and ruber = red. In reference to reddish brown pileus.

DIAGNOSIS: Characterized among *L.* sect. *Polysphaerosphori* by a combination of the following: Pileus 5–20 mm diam., reddish brown. Basidiospores  $7.5\text{--}8.5 \times 7\text{--}7.5 \mu\text{m}$ , subglobose, ornamented with irregular warts measuring up to  $1.6 \mu\text{m}$  high. Pleuro and cheilolamprocistidia present. Pileipellis a lampropalisade with scattered thick-walled terminal elements and lacticifers.

## DESCRIPTION

Pileus 5–20 mm diam., convex at first becoming plane, slightly depressed at center, greyish red (8C5), reddish brown (8D5), reddish orange (7A6-7B7), brownish orange (7C5-7C7); surface rugulose, hygrophanous, moist, slightly waxy, slightly radially sulcate; margin broadly sulcate to plicate, incurved to decurved, edge crenate, sometimes pale discolorous, sometimes lobed (Fig. 10); context firm, up to 2 mm thick at the disc, concolorous with the pileus, unchanging. Lamellae adnate to short-decurrent, distant, sometimes forked, brownish orange (6C4-7C4), greyish orange (5B3), cream greyish yellow (4C3); edges entire and concolorous; lamellulae frequent, of two lengths (Fig. 10). Stipe 10–14 × 3–4.5 mm, central, cylindrical, equal to subequal, white (1A1) to yellowish white (7C4) greyish orange (6B3-5B3); surface slightly rugulose, waxy; context hollow, concolorous with the stipe. Subiculum white (1A1), densely effused on plant branch. Veil absent. Odor not determined. Taste not determined. Latex scanty, white (1A1), unchanging.

Basidiospores (7–)7.5–8.5(–9) × (6.5–)7–7.5(–8) μm; L = 8.2 μm, W = 7.2 μm; Q = (1–)1.03–1.21(–1.23), Qm = 1.13; subglobose to broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 1.6 μm high, composed of irregular warts; plage amyloid; hilar appendix to 5 μm long. Basidia 40–49.5 × 6.5–10 μm, cylindrical, cylindrical clavate to slightly fusiform, 1 and 4-spored, thin-walled, hyaline, sometimes with refringent content; sterigmata up to 10 μm long. Pleurolamprocystidia frequente to abundant, 51–86.5 × 3–6.5 μm, filiform to cylindrical, sometimes slightly flexuous, sometimes septed, thick-walled (up to 1 μm thick), hyaline; projecting up to 40 μm above the hymenium. Pleuropseudocystidia frequent, 5–10 μm diam., cylindrical, to slightly fusiform, sometimes with tapering to mucroned apex, sometimes with forked apex, thin-walled, with refractive content; not projecting above the hymenium. Lamellar edge fertile, composed of scattered basidia, frequent cheilolamprocystidia, frequent cheilopseudocystidia and marginal cells: cheilolamprocystidia

26–53 × 4.5–7 µm, cylindrical, sometimes with ventricose base, sometimes with mucroned apex, septed, thick-walled (up to 1 µm thick) projecting up to 20 µm above the hymenium; cheilopseudocystidia 4.5–7.5 µm diam., filiform, slightly fusiform, sometimes with mucroned apex, not projecting above the hymenium; marginal cells 13.5–28 × 5.5–7.5 µm, clavate, thin-walled, hyaline. Pileipellis a lamprolalisade up to 81 µm, consisting of a layer of isodiametric cells with scattered thick-walled elements and rarer to scattered lacticifers: isodiametric cells, 5.5–40 µm diam., slightly thick-walled (up to 0.5 µm); thick-walled terminal elements 14–21 × 4–7.5 µm, cylindrical to clavate, thick-walled, (up to 1 µm thick), hyaline; lacticifers 5–8 µm diam., cylindrical sometimes with mucroned apex, sometimes projecting up to 10 µm above the pileipellis, arising deeply from the pileus trama. Pileus trama composed of abundant sphaerocytes 8–30 µm diam., and frequent lacticifers, 9–15 µm diam, thin-walled. Stipitipellis a lamprolalisade similar to the pileipellis. Clamp connections absent from all tissues examined.

**ECOLOGY AND DISTRIBUTION:** Solitary growing on a subiculum of an unknown tree branch, in white-sand forests of Amazon forest. Known only from type locality.

**ADDITIONAL MATERIAL EXAMINED:** BRAZIL, AMAZONIA: Balbina, Trilha da Sussuarana, 04 May 2019, leg. *Komura D.L., Bento L.S., Bento M.S., Silva-Costa, D., Komura D.L.* 2585 (INPA, JPB Paratype)

**REMARKS:** *Lactifluus brunneoruber* groups in a major clade (clade 2) with *L. venosellus*, the new species *L. candidus* and *L. flavuscaespitosus*. It forms a small branch with one sequence from non identified *Lactifluus* from Colombia (AMV2209). *Lactifluus brunneoruber* exhibits distinct morphology with unique features. This is the first Neotropical species that produces pleuro and cheilolamprocystidia, previously reported in African species of *L. sect. Pseudogymnocarpi* (Verbeken) Verbeken and *L. sect. Rubroviolascetini* (Singer)

Verbeken (De Crop et al. 2017). Additionally, *L. brunneoruber* is the only species of the section that produces basidiospores ornamented with isolated warts.

*Lactifluus candidus* Silva-Filho, Sá & Wartchow, nom. prov.

Mycobank: MB XXXXX

TYPE: BRAZIL, RIO GRANDE DO NORTE: Parque Estadual Parque das Dunas, trilha da peroba, 26 march 2015, *JCB 145* (UFRN-Fungos 2659, holotype!).

ETYMOLOGY: From Latin candid = white. In reference to white basidiomata.

DIAGNOSIS: Characterized among *L.* sect. *Polysphaerosphori* by a combination of the following: Basidiomata whitish. Basidiospores  $7\text{--}8 \times 5\text{--}6 \mu\text{m}$ , subglobose to broadly ellipsoid, ornamentation of up to  $0.2 \mu\text{m}$  high, composed of irregular short ridges and warts. Pleuroleptocystidia present. Pileipellis a lampropalisade with abundant thick walled terminal elements

#### DESCRIPTION

Pileus 35–63 mm diam., plane-convex, becoming depressed when mature, white (1A1), yellowish white (1A2) to pale yellow (1A3) with light orange (5A5) dots at the center; surface slightly verrucose and wrinkled, dull; margin entire to slightly wrinkled, incurved at first becoming straight, lobed; context firm, up to 4 mm thick at the disc, concolorous with the pileus, unchanging. Lamellae short-decurrent, subdistant, white (1A1) to pale yellow (1A3), sometimes bifurcate near the margin; edges entire and concolorous; lamellulae frequent with diferente lengths. Stipe 23–25 × 15–19 mm, cylindrical, central, white (1A1) to yellowish white (1A2); surface smooth, dull; context hollow, concolorou with the stipe. Veil absent. Odor not determined. Taste not dtermined. Latex abundant, white (1A1), unchanging.

Basidiospores (6–)7–8 × 5–6(–6.5) μm; L = 6.9 μm, W = 5.7 μm; Q = 1.15–1.3, Qm = 1.20; subglobose to broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 0.2 μm high, composed of irregular short ridges and warts; hilar appendix to 3 μm long. Basidia 56–77 × 8–10 μm, clavate, cylindrical-clavate and fusiform, 4-spored, thin-walled, with refringent content; sterigmata up to 7 μm long. Pleuroleptocystidia scattered to frequent, 22–38 × 3–5 μm, cylindrical, flexuous sometimes with strangulated apex, thin-walled, hyaline. Pleuropseudocystidia frequent, 5–8 μm diam., cylindrical to cylindrical-clavate, thin walled, with oil and refractive content; not projecting above the hymenium. Lamellar edge sterium, composed of rare cheilomacrocystidia, scarce cheilopseudocystidia and marginal cells: cheilomacrocystidia 56–62 × 8–12 μm, cylindrical, ventricose-rostrate, thin-walled, with anamorphic content, not projecting above the hymenium; cheilopseudocystidia 5–8 μm diam., cylindrical to cylindrical-clavate, thin-walled, with oil and refractive content, not projecting above the hymenium; marginal cells 27–35 × 4.5–7 μm, cylindrical, cylindrical clavate, thin-walled, hyaline. Pileipellis a lampropalisade of up to 106 μm thick, consisting of a layer of isodiametric cells with abundant thick walled terminal elements: isodiametric cells 10–26 μm diam., thin-walled, hyaline; hair-like thick-walled terminal element, 18–45 × 3–9 μm, cylindrical, cylindrical slightly ventricose, cylindrical clavate, strangulated, flexuous, sometimes septed, anticlinal to oblique oriented, thick-walled (up to 1 μm thick), hyaline. Pileus trama composed of scattered sphaerocytes 11–35 μm diam., abundant cylindrical hyphae, up to 4–9 μm diam., smooth, thin-walled, hyaline. Stipitipellis a lampropalisade similar to the pileipellis. Clamp connections absent from all tissues examined.

ECOLOGY AND DISTRIBUTION: Solitary or scattered in Atlantic Forest, in sand soil.

REMARKS: *Lactifluus candidus* forms a small branch with the new species *L. perforatus* in the clade two. Despite this, *L. candidus* is easily distinguishable from the other in *L.* sect. *Polysphaerospori* by its whitish basidiomata and presence of pleuroleptocystidia.

*Lactifluus flavuscaespitosus* Silva-Filho & Wartchow, nom. prov.

Mycobank: MB XXXXX

TYPE: BRAZIL, PARAÍBA: Mamanguape, Reserva Biológica Guaribas, 05 July 2017, Wartchow F. FW 08/2017 (UFRN-fungos, JPB holotype!).

ETYMOLOGY: From latin: flav = yellow; caespitos = caespitose. Due to yellowish pileus and caespitose habitus.

DIAGNOSIS: Characterized among *L.* sect. *Polysphaerosphori* by a combination of the following: Pileus 14–80 mm, yellowish colored. Lamellae short-decurrent short-decurrent. Basidiospores  $7-9 \times 5-6.5 \mu\text{m}$ , subglobose to broadly ellipsoid, ornamentation of up to  $1 \mu\text{m}$  high, composed of irregular short ridges and warts connected by fine lines. True cystidia absent. Pleuropseudocystidia abundant  $5-10 \mu\text{m}$  diam., filiform, cylindrical and fusoid. Pileipellis a palisade with abundant thick walled terminal elements

#### DESCRIPTION

Pileus 14–80 mm, plano convex in young specimens then plane convex, slightly concave depressed, light orange (5A7) yellow orange (5B7) sometimes pale orange (5A3), surface wrinkled, rugulose, moist; margin smooth, waved straight to slightly decurved; context up to 8 mm, white (1A1) unchanging. Lamellae short-decurrent, distant, interveined, white (1A1); edges entire and concolorous; lamellulae of several lengths. Stipe  $20-40 \times 6-12$ , central, tapering toward the base, white (1A1), yellowish white (4A2) to pale yellow (4A3); surface smooth, glabrous, moist; context white (1A1) solid, unchanging. Veil absent. Odor not determined. Taste not determined. Latex scanty, exuding a watery latex.

Basidiospores  $7-9(-9.5) \times 5-6.5 \mu\text{m}$ ,  $L = 7.6 \mu\text{m}$ ,  $W = 5.6 \mu\text{m}$ ;  $Q = 1.16 - 1.5$ ,  $Q_m = 1.34$ , subglobose to broadly ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to  $1 \mu\text{m}$

high, composed of irregular short ridges and warts connected by fine lines; plage non-amyloid; hilar appendix to 5  $\mu\text{m}$  long. Basidia 54–79.5  $\times$  7–10  $\mu\text{m}$ , long clavate, 4 spored, thin-walled, with refractive and oil content; sterigmata up to 8  $\mu\text{m}$ . Pleurocystidia absent. Pleuropseudocystidia abundant, 5–10  $\mu\text{m}$  diam., filiform, cylindrical and fusoid, thin walled, with refractive content; often not projecting above to hymenium, sometimes projecting up to 34.5  $\mu\text{m}$  above. Lamellae edge sterile, composed of margin cells, 34–45  $\times$  5–8  $\mu\text{m}$ , cylindrical, clavate, fusoid, sometimes slightly flexuous, hyaline and with refractive content. Pileipellis a palisade of to up 83  $\mu\text{m}$  thick, consisting of a layer of isodiametric cells and inflated cylindrical hyphae and frequent to abundant terminal elements: isodiametric cells 7–20  $\mu\text{m}$  diam., thin-walled; inflated cylindrical hyphae, 9–12  $\mu\text{m}$  diam, hyaline, 13–35  $\times$  4–6  $\mu\text{m}$ , cylindrical, flexuous, sometimes slightly fusiform, sometimes with inflated base or apex, thin walled, hyaline, clinal and oblique oriented. Pileus trama composed of abundant sphaerocytes 15–32  $\mu\text{m}$  diam., thin-walled, hyphae 4–6  $\mu\text{m}$  diam thin walled, hyaline and scattered lacticifers 5–10  $\mu\text{m}$  diam., thin-walled. Stipitipellis a palisade similar to the pileipellis. Clamp connections absent from all tissues examined.

**ECOLOGY AND DISTRIBUTION:** Solitary or scattered in Atlantic Forest and in a tropical wet mountain forest in the Caatinga Domain, collected in July, under unknown host. Known only from Paraiba State, Northeastern Brazil.

**ADDITIONAL MATERIAL EXAMINED:** BRAZIL, PARAIBA: Areia, Parque Estadual Mata do Pau Ferro, Trilha do Cumbre, *Wartchow F. FW36/2017* (UFRN-fungos, JPB Paratype!).

**REMARKS:** *Lactifluus flavuscaespitosus* clusters together with the new species *L. restigae* in the clade one. Macroscopically *L. flavuscaespitosus* is distinguished to other species of the section *Polysphaerosphori* by its robust basidiomata, with pileus measuring 14–80 mm diam. and stipe 20–40  $\times$  6–12 mm, yellowish pileus and subdecurrent lamellae. Microscopically is

characterized by ornamentation of the spores measuring up to 1  $\mu\text{m}$  high and by absence of true cystidia.

*Lactifluus perforatus* Silva-Filho & Wartchow, nom. prov.

Mycobank: MB XXXXX

TYPE: BRAZIL, RIO GRANDE DO NORTE: Natal, Parque Estadual das Dunas, trilha da geologia, 20 August 2019, *Silva-Filho AGS 100-2019* (UFRN-fungos, JPB Holotype!)

ETYMOLOGY: '*perforatus*' in reference to cavity formed in the pileus of mature basidiome.

DIAGNOSIS: Characterized among *L.* sect. *Polysphaerosphori* by a combination of the following: Pileus 56–110 mm, orange colored, forming a cavity at the centre. 7–9  $\times$  5–6.5  $\mu\text{m}$ ; subglobose to ellipsoid, ornamentation of up to 0.5  $\mu\text{m}$  high, composed of irregular short ridges and warts connected by fine line. Pleuromacroscystidia scars 83–105  $\times$  4–6  $\mu\text{m}$  diam., cylindrical to long clavate. Pleuropseudocystidia absent. Pileipellis a lamprolalisade with frequent to abundant thick-walled elements.

#### DESCRIPTION

Pileus 56–110 mm diam., convex depressed to plane convex slightly depressed, becoming infubuliform umbilicate with a cavity at the centre, orange yellow (4B8), brownish orange (7C7), orange (6B7); surface rugose slightly scrobiculate, dull; margin smooth to slightly split, at first incurved becoming arched and then unlifted; context firm, up to 11 mm thick at the centre, yellowish white (4A2), pale yellow (4A3) to light yellow (4A5), unchanging. Lamellae adnate to short decurrent, distant, sometimes interveined, sometimes forked, brownish yellow (5C8), brownish orange (5C5), pale orange (6A3) to greyish orange (6B4); margin entire discoloured paler with the sides, orange white (6A2); lamellulae frequent with two leghs. Stipe 45–51  $\times$  15–29 (apex) 12–18 (base) mm, central, cylindrical, tapered at the

base, greyish orange (6B4) with brown (7E5) spots; surface smooth to slightly rugose, dull; context hollow, concolorous with the stipe. Veil absent. Odor not determined. Taste not determined. Latex not found

Basidiospores (6–)7–9 × 5–6.5 μm; L = 7.5 μm, W = 5.6 μm; Q = 1.16–1.60, Qm = 1.32; subglobose to ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 0.5 μm high, composed of irregular short ridges and warts connected by fine line; plage amyloid, hilar appendix up to 1 μm long. Basidia 51–72.5 × 7–10 μm, long clavate, 2 and 4-spored, thin-walled, hyaline and some with refringent content; sterigmata up to 6 μm high. Pleuromacrocystidia scarce 83–105 × 4–6 μm diam., cylindrical to long clavate, thin-walled, with oil content; not projecting above the hymenium. Pleuropseudocystidia absent. Lamellae edge sterile, composed of common margin cells, 29–40 × 4–7 μm, mostly cylindrical, sometime fusoid and fusiform, sometime septate and flexuous, thin walled, hyaline. Pileipellis a lamproparisade of up to 95 μm thick, consisting a layer of isodiametric and cylindrical inflated cells with frequent to abundant thick-walled elements: isodiametric cells 14–22 μm diam., inflated cylindrical cells, 13–20 μm diam., thin-walled, hyaline; thick-walled elements, 29–60 × 5–10 μm, aciculate, cylindrical, sometimes with swollen base, rare monofiliform pericline to oblique thick walled (up to 1 μm thick) hyaline. Pileus trama composed of abundant sphaerocytes 14–42 μm diam., thin-walled, cylindrical hyphae, up to 5–10 μm diam thin-walled. Stipitipellis a lamproparisade like the pileipellis. Clamp connections absent from all tissues examined.

ECOLOGY AND DISTRIBUTION: Solitary or scattered in Atlantic Forest, in sand soil, under unknown host.

ADDITIONAL MATERIALS EXAMINED: BRAZIL, RIO GRANDE DO NORTE: Natal, Parque Estadual das Dunas, Trilha da Peroba, 19 April 2008 Gurgel, F.E., *Baseia* I.G s/n

(UFRN Fungos 532; *ibid*, 06 May 2011, *Sulzbacher, M. A. Assis H.K.F., Sulzbacher 326* (UFRN-fungos 1871).

REMARKS: *Lactifluus perforatus* easily distinguishable from the other Brazilian species in *L. sect. Polysphaerosphori*. Macroscopically, it differs by the robust basidiomata with orange color and by cavity formed in the pileus of mature basidiome. Microscopically, is recognized by its lampropalisade pileipellis, absence of pleuropseudocystidia, and by cylindrical to long clavate pleuromacrocystidia measuring  $83\text{--}105 \times 4\text{--}6 \mu\text{m}$  diam.

*Lactifluus restingae* Silva-Filho & Wartchow nom. prov.

Mycobank: MB XXXXX

TYPE: BRAZIL, RIO GRANDE DO NORTE: Natal, Parque Estadual das Dunas, 20 August 2019, *Silva-Filho AGS 104-2019* (UFRN-fungos, JPB Holotype!)

ETYMOLOGY: '*restingae*' in reference to the Restinga, ecosystem where the new species was collected.

DIAGNOSIS: Characterized among *L. sect. Polysphaerosphori* by a combination of the following: Pileus approximately 8 mm, yellowish. Basidiospores  $7\text{--}9 \times 5\text{--}6 \mu\text{m}$ , subglobose to ellipsoid ornamentation of up to  $0.3 \mu\text{m}$  high, composed of irregular short ridges and warts. Pleuromacrocystidia  $58\text{--}104 \times 5\text{--}8 \mu\text{m}$  diam., filiform, cylindrical to slightly fusiform, sometimes with mucronate apex, sometimes with rounded apex. Pleuropseudocystidia  $7\text{--}9 \mu\text{m}$  diam., cylindrical, fusoid to slightly fusiform, sometimes with mucronate apex. cheilopseudocystidia,  $5\text{--}7 \mu\text{m}$  diam., cylindrical to fusoid. Pileipellis a palisade

Pileus 8 mm diam., infubiliiform umbonated at center, dull yellow (3B3), yellow (3B8), greyish yellow (4B4), light orange (5A4), brownish orange (5C5); surface rugose to scrobiculated, dull; margin slightly eroded, slightly decurved to straight lobed; context firm,

up to 2 mm thick at the disc, yellowish white (1A2) unchanging. Lamellae adnate, distant, veined, white (1A1), yellowish white (1A2); edges eroded and concolorous; lamellulae frequent, of two lengths. Stipe  $10 \times 4$  mm, central, cylindrical, equal to slightly tapered at the base dull yellow (3B3), yellow (3B8), greyish yellow (4B4), light orange (5A4), brownish orange (5C5); surface rugose to slightly scrobiculated, dull; context hollow, concolorous with the stipe. Veil absent. Odor not determined. Taste not determined. Latex scanty, white (1A1), unchanging.

Basidiospores  $7-9 \times 5-6$   $\mu\text{m}$ ;  $L = 7.8$   $\mu\text{m}$ ,  $W = 5.5$   $\mu\text{m}$ ;  $Q = (1.16)1.27-1.60$ ,  $Q_m = 1.40$ ; subglobose to ellipsoid, thin-walled, hyaline; ornamentation amyloid, up to 0.3  $\mu\text{m}$  high, composed of irregular short ridges and warts; plage non-amyloid; hilar appendix up to 2  $\mu\text{m}$  long. Basidia  $47-61 \times 6-9$   $\mu\text{m}$ , long long-clavate, to slightly fusiform, 4 spored, thin-walled, with refringent content; sterigmata up to 5  $\mu\text{m}$  high. Pleuromacrocistidia scattered to frequent  $58-104 \times 5-8$   $\mu\text{m}$  diam., filiform, cylindrical to slightly fusiform, sometimes with mucronate apex, sometimes with rounded apex, thin-walled, with refractive content; not projecting above the hymenium. Pleuropseudocystidia scattered to frequent, 7–9  $\mu\text{m}$  diam., cylindrical, fusoid to slightly fusiform, sometimes with mucronate apex, thin-walled, with refractive contents; projecting to 70  $\mu\text{m}$  above to hymenium. Lamellae edge fertile composed of scattered basidia, scattered cheilopseudocystidia and margin cells: cheilopseudocystidia, 5–7  $\mu\text{m}$  diam., cylindrical to fusoid, thin-walled, with refractive contents; common marginal cell,  $20-32 \times 3-10$   $\mu\text{m}$ , filiform, cylindrical to clavate, thin walled, hyaline. Pileipellis a palisade of up to 75  $\mu\text{m}$  thick, consisting a layer of isodiametric cells with frequent to abundant terminal elements and scattered laticifers: isodiametric cells 7–18  $\mu\text{m}$  diam., thin-walled, hyaline; terminal elements,  $12-24 \times 4-7$   $\mu\text{m}$ , cylindrical, slightly clavate, flexuous, rarer lageniform, anticline, pericline and oblique, mostly thin-walled, rarer slightly thick wall (up to 0.3  $\mu\text{m}$  thick) hyaline; laticifers 5–8  $\mu\text{m}$  diam., cylindrical, thin-walled, with refractive contents, not emergent,

arising deeply from pileus trama. Pileus trama composed of abundant sphaerocytes 6–20 µm diam., thin-walled, and scattered lacticifers 5–10 µm diam., thin-walled. Stipitipellis a palisade like the pileipellis. Clamp connections absent from all tissues examined.

**ECOLOGY AND DISTRIBUTION:** Solitary in Atlantic Forest, in sand soil, growing under unknown host. Known only from type locality.

Remarks – *Lactifluus restingae* is macroscopically characterized by its small basidiomata with yellowish pileus, whitish and adnate lamellae. Microscopically, easily distinguishable by its short basidiospore ornamentation (up to 0.3 µm), presence of true cystidia and by cylindrical, fusoid to slightly fusiform pseudocystidia.

## **DISCUSSION**

This study reveals a high diversification in *L.* section *Polysphaerosphori* in South America. The section is formed by four branches, one more than the one shown by Silva-Filho et al. (2019). Despite this, it is still unclear how these branches/clades are morphologically characterized, being necessary the inclusion of morphological data of the various specimens with sequences available and not yet described. Our five new described species are distributed in three of the four branches.

Clade one harbors the new described species *L. flavuscaespitosus* and *L. restingae*. Both species have a yellowish-orange pileus with a rugose to scrobiculated surface, whitish, veined and distant lamellae and produces basidiospores ornamented by irregular short ridges and warts connected by fine line, pleurocystidia and a palisade pileipellis. *Lactifluus restingae* differs in its smaller basidiomata, shorter spore ornamentation (up to 0.3 µm high) and in the presence of macropleurocystidia, absent in *L. flavuscaespitosus*.

Clade two is the highest represented, formed by 10 sequences, in which only four species were described, so far. *Lactifluus brunneoruber* and *L. candidus* have different

morphology from any other species in the section. *Lactifluus brunneoruber* produces lamprocystidia and isolated warts spores ornamentation, while *L. candidus* is the only with a whitish basidioma that produces pleuroleptocystidia. Despite of belong to the same clade, *L. perforatus* and *L. venosellus* are positioned on smaller distant branches. *L. perforatus* has an orange basidioma, the surface of the pileus is rough to slightly scrobiculated, in addition it produces true cystidia and a lampropalisade pileipellis, while *L. venosellus* has the basidioma brownish-red colored with velutinous pileus surface, produces pseudocystidia and a palisade pileipellis.

Clade four is composed of three species: *L. amazonensis* confirmed in this study, *L. ceraceus* and *L. umbilicatus*. The three species share common morphological features such as: orange pileus, whitish lamella, absence of pseudocystidia, presence of macrocystidia and the lampropalisada structure of the pileipellis.

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Figura 42 - **Fig. 1** ML phylogeny of representative collections of *Lactifluus* subg. *Pseudogymnocarpi* based on nuc- rDNA spacers ITS. New species are noted in blue. Thicker lines represent branches with maximum bootstrap values and posterior probabilities (100% BS / 1.0 BPM). Bootstrap values and bayesian posterior probabilities are indicated if they exceed 0.95 and 80%, respectively. The scale bar represents the expected number of nucleotide changes per site.

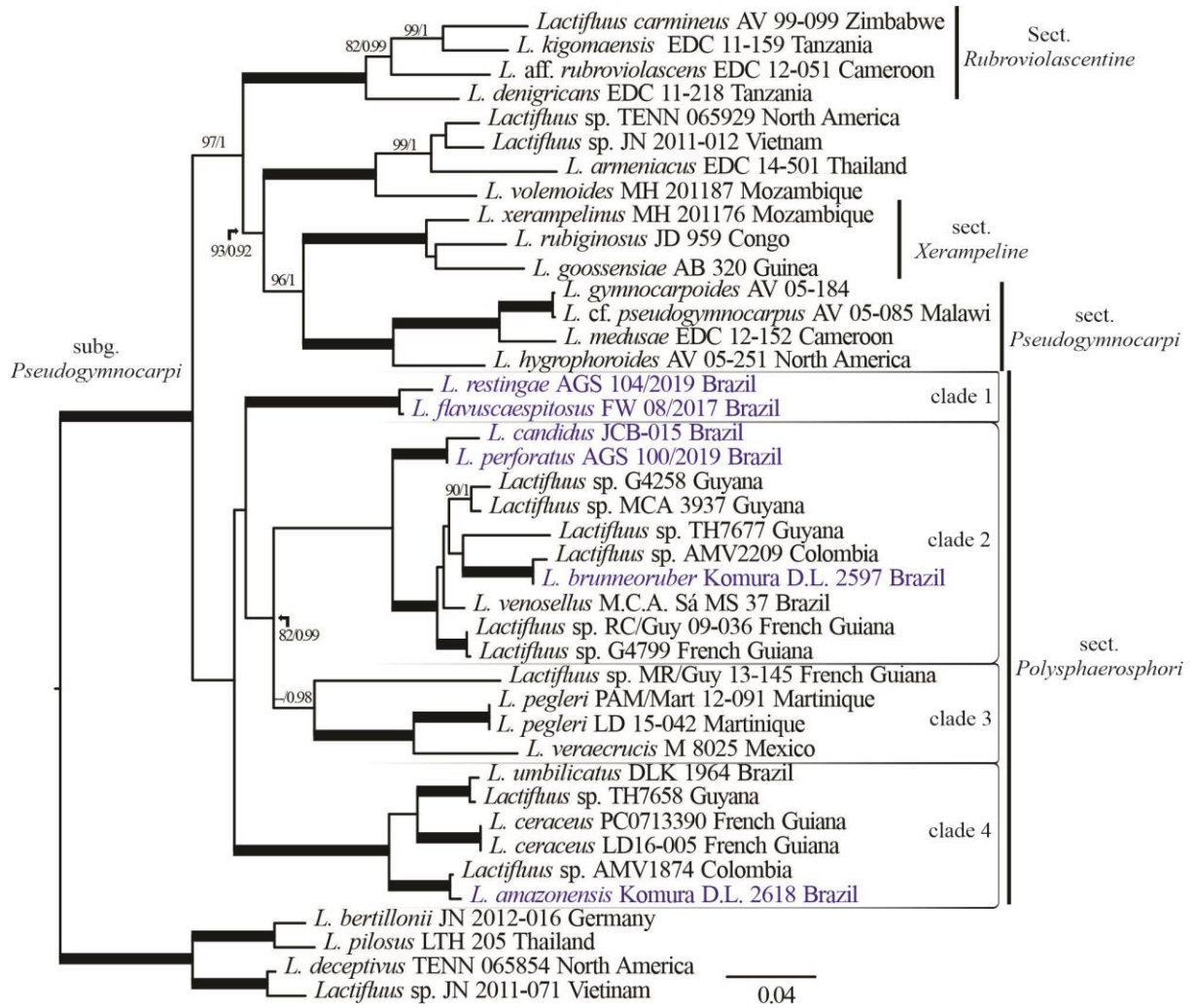


Figura 43 - **Fig. 2** Basidiomata of *L. brunneoruber*. A Holotype (Komura D.L. 2585). B Paratype (Komura D.L. 2585). Scale bars = 10 mm. Photos: Dirce D. Komura.



Figura 44 - **Fig. 3** Micromorphological features of *L. brunneoruber* (Holotype AGS 879). A Basidiospores. B Pleuropseudocystidia Basidia. C Pleurolamprocystidia. D Basidia. E Pileipellis. F Marginal cells of the lamellar. G Cheilolamprocystidia. H Cheilopseudocystidia. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

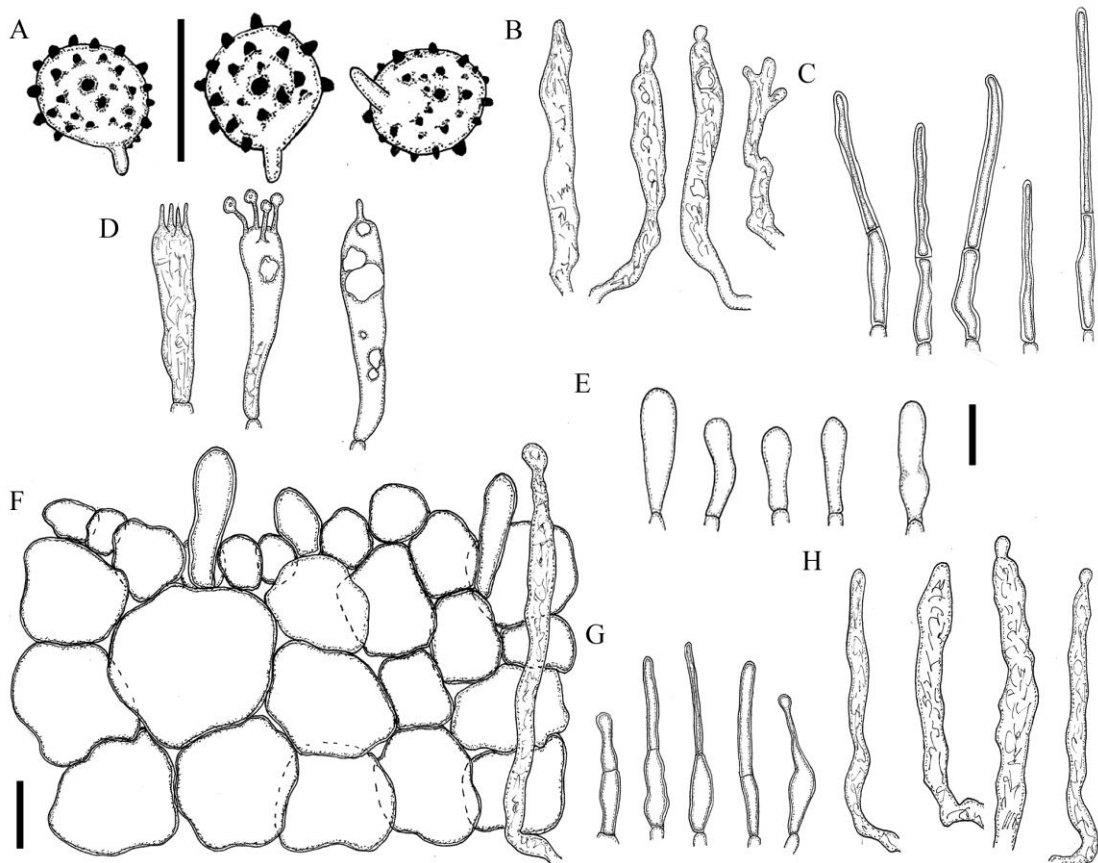


Figura 45 - **Fig. 4** Basidiomata of *L. candidus*. A,B Holotype (JCB145). Scale bars = 10 mm.



Figura 46 - **Fig. 5** Micromorphological features of *L. candidus* (Holotype JCB 145). A Basidiospores. B Basidia. C Pleuroleptocystidia. D Pleuropseudocystidia. E Pileipellis. F Thick-walled elements of pileipellis. G Cheilomacrocystidia. H Cheilopseudocystidia. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

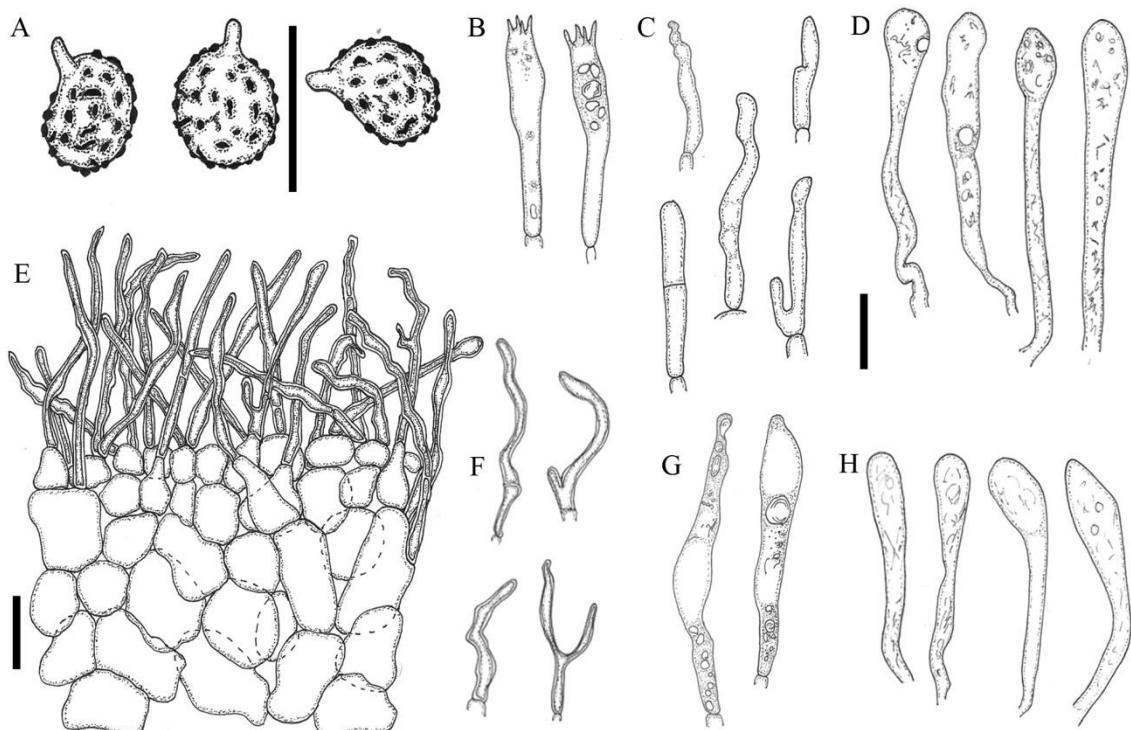


Figura 47 - **Fig. 6** Basidiomata of *L. flavuscaespitosu* Holotype (FW 08/2017). Scale bars = 10 mm. Photos: Felipe Wartchow.



Figura 48 - **Fig. 7** Micromorphological features of *L. flavuscaespitosu*. (Holotype FW 08/2017). A Basidiospores. B Basidia. C Pleuropseudocystidia. D Pileipellis. E Marginal cells of the lamellar. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

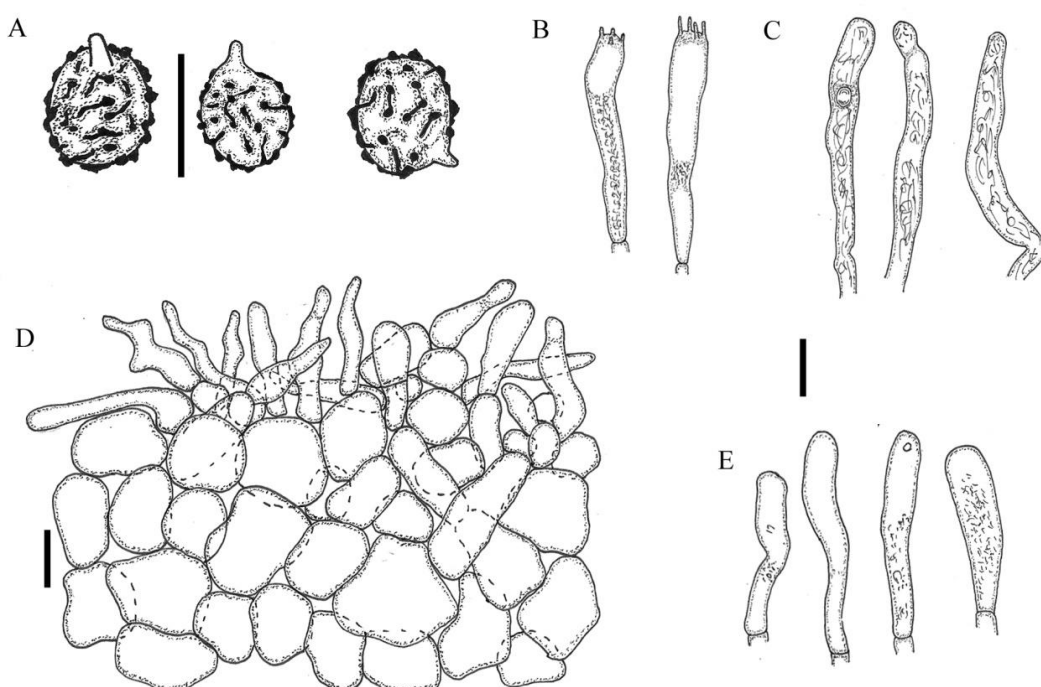


Figura 49 - **Fig. 8** Basidiomata of *L. perforatus*. A,B Holotype (AGS 100/2019). Scale bars = 10 mm. Photos: Alexandre G. S. Silva-Filho.



Figura 50 - **Fig. 9** Micromorphological features of *L. perforatus*. (Holotype AGS 100/2019). A Basidiospores. B Basidia. C Pleuropseudocystidia. D Pileipellis. E Marginal cells of the lamellar. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

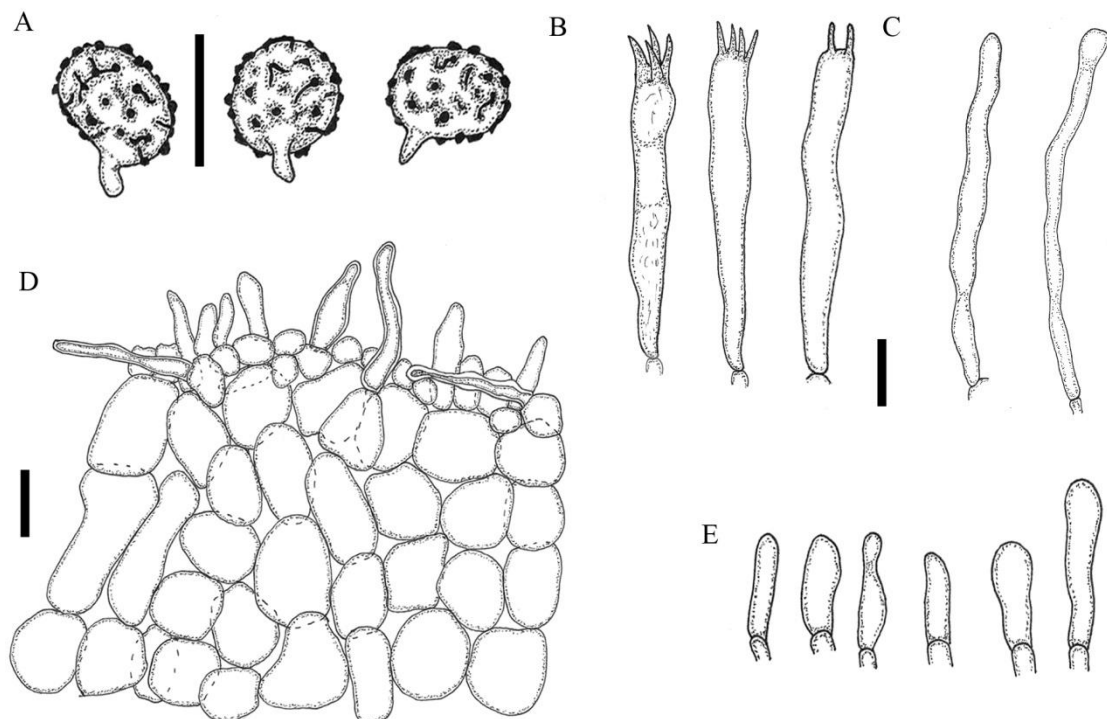


Figura 51 - **Fig. 10** Basidiomata of *L. restingae*. A-C Holotype (AGS 104/2019). Scale bars = 5 mm. Photos: Alexandre G. S. Silva-Filho.

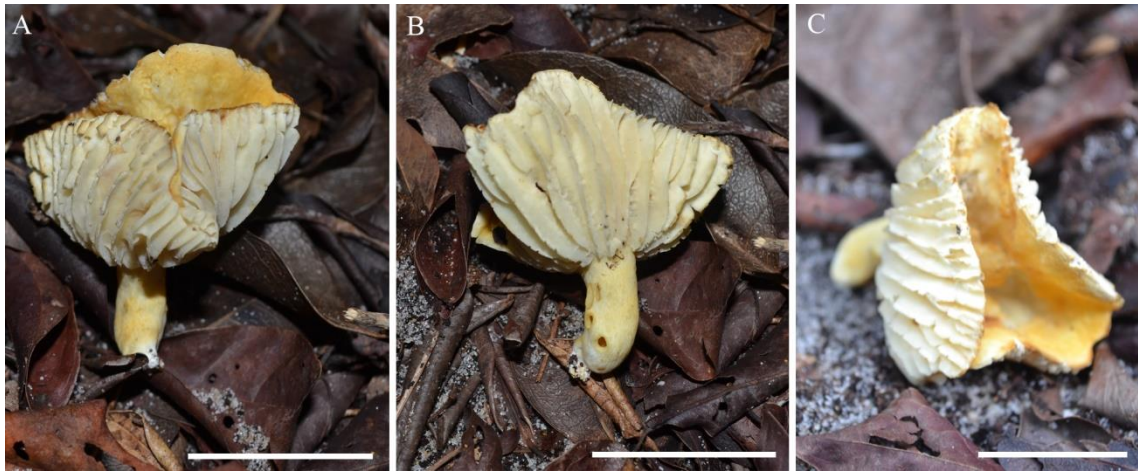


Figura 52 - **Fig. 11** Micromorphological features of *L. restingae*. (Holotype AGS 104/2019). A Basidiospores. B Basidia. C Pleuropseudocystidia. D Pleuromacrocytidia. E Pileipellis. F Cheilopseudocystidia. E Marginal cells of the lamellar. Scale bars = 10  $\mu$ m. Drawings: Alexandre G. S. Silva-Filho.

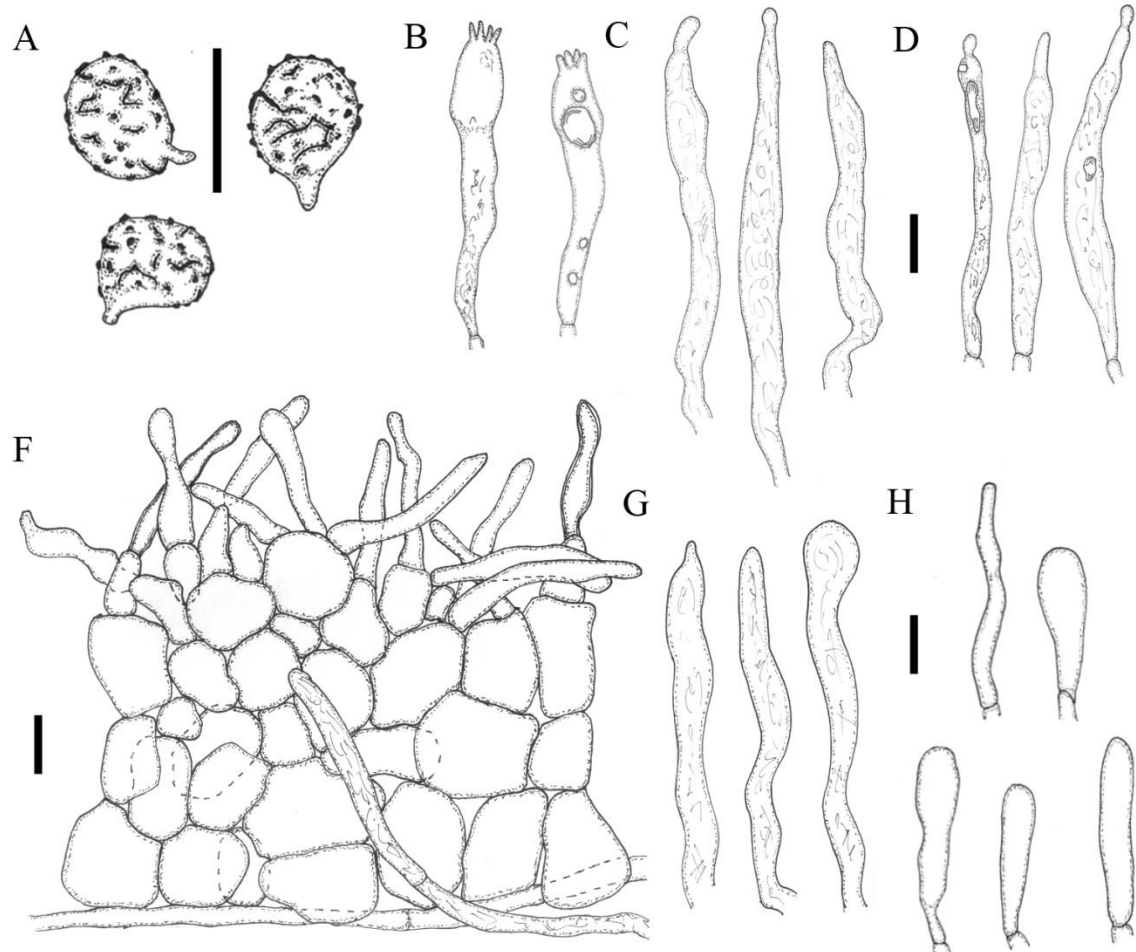


Tabela 7. **Table 1:** Species, voucher collection, country and GenBank accession numbers of ITS sequences used in molecular analyses. Accession numbers in bold were newly generated for this study. Sequences not yet deposited in genbank are marked with ‘\*’

Species	Voucher Collection	Country	ITS accession n°
<b><i>Lactifluus amazonensis</i></b>	<b>DLK2618</b>	<b>Brazil</b>	*
<i>L. armeniacus</i>	EDC 14-501	Thailand	KR364127
<i>L. bertillonii</i>	JN 2012-016	Germany	KR364087
<b><i>L. brunneoruber</i></b>	<b>DLK2597</b>	<b>Brazil</b>	*
<b><i>L. candidus</i></b>	<b>JCB145</b>	<b>Brazil</b>	*
<i>L. carmineus</i>	AV 99-099	Zimbabwe	KR364131
<i>L. ceraceus</i>	PC0713390	French Guiana	KY884996
<i>L. ceraceus</i>	LD16-005	French Guiana	KY884995
<i>L. deceptivus</i>	TENN 065854	North America	KR364101
<i>L. denigrans</i>	EDC 11-218	Tanzania	KR364051
<b><i>L. flavuscaespitosus</i></b>	<b>FW 08/2017</b>	<b>Brazil</b>	*
<i>L. goossensiae</i>	AB 320	Guinea	KR364132
<i>L. gymnocarpoides</i>	AV 05-184	Malawi	KR364024
<i>L. hygrophoroides</i>	AV 05-251	North America	HQ318285
<i>L. Kigomaensis</i>	EDC 11-159	Tanzania	KR364050
<i>L. medusae</i>	EDC 12-152	Cameroon	KR364069
<i>L. pegleri</i>	PAM/Mart 12-091	Martinique	KP691416
<i>L. pegleri</i>	LD 15-042	Martinique	MK046831
<b><i>L. perforatus</i></b>	<b>AGS 100-2019</b>	<b>Brazil</b>	*
<i>L. cf. pseudogymnocarpus</i>	AV 05-085	Malawi	KR364012
<i>L. pilosus</i>	LTH 205	Thailand	KR364006
<b><i>L. restingae</i></b>	<b>AGS 104-2019</b>	<b>Brazil</b>	*
<i>L. rubiginosus</i>	JD 959	Congo	KR364081
<i>L. aff. rubroviolascens</i>	EDC 12-051	Cameroon	KR364066
<i>Lactifluus</i> sp.	JN 2011-012	Vietnam	KR364045
<i>Lactifluus</i> sp.	TENN 065929	North America	KR364102
<i>Lactifluus</i> sp.	JN 2011-012	Vietnam	KR364045
<i>Lactifluus</i> sp.	TENN 065929	North America	KR364102
<i>Lactifluus</i> sp.	G4258	Guyana	KJ786715
<i>Lactifluus</i> sp.	TH7658	Guyana	KT339247
<i>Lactifluus</i> sp.	TH7677	Guyana	KT339245
<i>Lactifluus</i> sp.	G4799	French Guiana	KM073088
<i>Lactifluus</i> sp.	AMV2209	Colombia	KT354752
<i>Lactifluus</i> sp.	AMV1874	Colombia	KT354738
<i>Lactifluus</i> sp.	JN 2011-071	Vietnam	KR364043
<i>Lactifluus</i> sp.	RC/Guy 09-036	French Guiana	KJ786645
<i>Lactifluus</i> sp.	MR/Guy 13-145	French Guiana	KJ786691
<i>Lactifluus</i> sp.	MCA 3937	Guyana	KR364109
<i>L. veraecrucis</i>	M 8025	Mexico	KR364112
<i>L. volemoides</i>	MH 201187	Mozambique	KR364098

*L. xerampelinus*

MH 201176

Mozambique

KR364099

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## 11 CONSIDERAÇÕES FINAIS

A nossa pesquisa sobre *Lactifluus* começou em abril de 2017, quando apenas *Lf. annulifer*, *Lf. aurantiorugosus*, *Lf. batistae*, *Lf. dunensis*, *Lf. neotropicus* e *Lf. panuoides* eram referidos para o Brasil. À época, já se sabia que as espécies de *Lactarius* encontradas em florestas nativas e descritas por Rick 1906, 1907, 1930, 1938, Singer et al 1983, Singer (1984) e Wartchow & Cavalcanti (2010) pertenciam a *Lactifluus*. Mesmo assim, foi necessário efetuar uma revisão dos tipos para confirmar as espécies, já que uma espécie tratada por Singer et al. (1983) como *Lactarius* foi transferida para *Russula*: *Russula campinensis* (Singer) T.W. Henkel, Aime & S.L. Mill. teve seu *status* genérico confirmado com base em dados morfológicos e moleculares (Henkel et al. 2000).

O nosso estudo dos tipos resultou em três lectótipos designados e seis novas combinações em *Lactifluus*. Além disso, as exsicatas de *L. reticulatus* (exsiccata Singer B 10986), *L. subreticulatus*, e *L. igapoensis* (= *Lf. panuoides*) encontravam-se em péssimo estado de conservação. As exsicatas de *L. pallidipes* e *L. gigasporus* foram perdidas no herbário INPA, e espera-se a recoleta e a designação de um neótipo. Os tipos de *L. paulensis* e *L. subpallidipes* depositados no Field Museum Herbário não estavam disponíveis para empréstimo. *Lactarius steffenii* não existe exsicata disponível. Recentemente *L. paulensis* foi transferido para *Lactifluus*, e *L. subpallidipes*, para *Russula*. Ambas as espécies não foram revisadas tampouco confirmadas a partir de dados moleculares. *Russula subpallidipes* (Singer) Delgat, por exemplo, foi transferida por parecer com uma *Russula* (DE CROP et al. 2021).

Outras três espécies descritas por Singer et al. (1983) apresentam dúvidas taxonômicas e precisam ser brevemente comentadas. *Lactarius igapoensis* foi sinonimizada com *L. panuoides* por Henkel et al. (2000). *Lactifluus panuoides*, em um sentido mais amplo, é composto por espécimes do Caribe e da América do Sul. Após cuidadosa análise morfológica combinada com os estudos filogenéticos das espécies pleurotoides de *Lf. sect. Panuoides* foi observado que o tamanho e a forma do basidioma, o tipo de ornamentação dos esporos, a forma e o tamanho dos pseudocistídios e os elementos da camada do píleo são caracteres importantes para separar as espécies pleurotoides da seção *Panuoides*. Portanto, ao revisar os dados da literatura, pareceu provável que *L. igapoensis* seja uma espécie distinta de *Lf. panuoides* pelo seu basidioma sub-reniformes a petaloides (Singer 1952), seus pseudocistídios frequentemente bifurcados e com ápice de paredes espessas; e elementos terminais da superfície do píleo ocasionalmente bifurcados (Verbeke 1998). Tais caracteres são únicos entre as espécies pleurotoide de *Lf. seção Panuoides*.

*Lactifluus mamorensis* foi revisado e recoletado. Nossa amostra agrupou na filogenia juntamente com sequências de *Lf. catarinensis* (Capítulo 4). *Lactifluus mamorensis* é morfologicamente caracterizado pelo estipe evidentemente estriado. Esta característica é observada apenas em *Lf. mamorensis*, *Lf. catarinensis* e *Lf. paulensis* (Singer) Delgat. Todas essas espécies são macromorfologicamente similares, diferindo em poucos caracteres micromorfológicos. No entanto, *Lf. mamorensis* possui uma micromorfologia confusa ao comparar o protologue e a revisão dos tipos, como relatado no Capítulo 4, o que torna provável que *Lf. catarinensis* seja um sinônimo de *Lf. mamorensis*. Considerando essa variação morfológica e uma possível ampla distribuição de *Lf. mamorensis* na Bolívia e Brasil (Amazônia, Mata Atlântica e Caatinga), não me surpreenderia se *Lf. paulensis* também fosse um sinônimo de *Lf. mamorensis*. Para confirmar essa hipótese, seria necessário sequenciar o parátipo de *Lf. mamorensis* e reanalisar o tipo de *Lf. paulensis* e todos os tipos de *Lf. catarinensis*.

Além das seis espécies transferidas para *Lactifluus*, o nosso estudo revelou 11 novas espécies da Amazônia, Caatinga e Mata Atlântica, evidenciando uma alta diversidade filogenética de *Lactifluus* no Brasil. Apesar disso, é esperado ainda que se encontrem outras espécies, visto que: plantas ectomicorrízicas, como os gêneros *Coccoloba* e *Guapira*, ocorrem em todos os Biomas do Brasil; o Cerrado, Pampa e Pantanal ainda não foram inventariados em relação a *Lactifluus*.

As espécies apresentadas em nosso trabalho trazem novidades quanto à morfologia para o gênero *Lactifluus*. Algumas espécies produzem hifas lactíferas na superfície do píleo, caracter não reportado na literatura até o momento. Essas hifas lactíferas têm origem na trama do píleo e, na maioria das vezes, não emergem na camada do píleo. Tais hifas lactíferas foram observadas em quatro espécies da seção *Pseudogymnocarpi* e em *Lf. dunensis*, membro da seção *Neotropici*. Além disso, *Lactifluus brunneoruber* nom. prov. é o primeiro táxon neotropical da seção *Polysphaerosphori* que produz lamprocistídios e esporos ornamentados com verrugas.

As relações filogenéticas das espécies neotropicais de *Lactifluus* foram mais bem elucidadas. Aqui mostramos a delimitação de duas seções e a emenda da seção *Panuoidei*. No Capítulo 2, apresentamos uma árvore filogenética e evidenciamos os ramos que formam a seção *Polysphaerosphori*. A seção é formada por espécies Neotropicais, majoritariamente por espécies da América do Sul, e é formada por três ramos distintos. A filogenia apresentada no Capítulo 5 mostra uma melhor definição destes três ramos, com a formação de um novo ramo formado por duas novas espécies coletadas por nós: *Lf. flavuscaespitosus* nom. prov. e *Lf.*

*restingae* nom. prov. Ambas espécies supracitadas possuem píleo laranja-amarelado com superfície rugosa a escrobiculada, lamelas esbranquiçadas, com nervuras e distantes; e produzem esporos ornamentados por verrugas conectadas com cristas curtas irregulares; pleurocistídios; e a superfície do píleo do tipo paliçada. Outro clado composto por *Lf. amazonensis*, *Lf. ceraceus* e *Lf. umbilicatus*, compartilham características morfológicas comuns, como píleo alaranjado, lamela esbranquiçada, ausência de pseudocistídios, presença de macrocistídios e uma superfície do píleo do tipo lampropalisada. Apesar de existirem evidências morfológicas indicando que esses ramos são agrupamentos naturais, seria prematuro delimitá-los dado o grande número de espécies sequenciadas e ainda não descritas.

## Apêndice

Tabela 8. Espécie, voucher, país de origem e número de acesso do GenBank das espécies usadas na análise molecular do resultado. O símbolo \* representa sequências das espécies ainda não depositadas no GenBank.

Espécie	Voucher	País	Código de acesso do Gen Bank		
			ITS	LSU	<i>RPB2</i>
<i>Auriscalpium vulgare</i>	PBM 944	North America	DQ911613	DQ911614	*
<i>Bondarzewia montana</i>	AFTOL 452	sem dados	DQ200923	DQ234539	*
<i>Heterobasidion annosum</i>	AFTOL 470	sem dados	DQ206988	*	AY544206
<i>Lactarius fuliginosus</i>	MTB 97-24	Sweden	JQ446111	JQ446180	JQ446240
<i>L. hatsudake</i>	FH 12-052	Thailand	KR364085	KR364215	KR364285
<i>L. miniatescens</i>	AV 11-177	Togo	KR364059	KR364187	KR364315
<i>L. olympianus</i>	ED 08-018	North America	KR364089	KR364220	KR364320
<i>L. scrobiculatus</i>	JN 2001-058	Slovakia	KF432968	KR364219	KR364344
<i>L. tenellus</i>	ADK 3598	Benin	KF133280	KF133313	KF133345
<b><i>Lactifluus amazonensis</i></b>	<b>DLK2618</b>	<b>Brazil</b>	*	–	–
<b><i>Lf. aurantioceraceus</i> nom. prov.</b>	<b>AGS 879</b>	<b>Brazil</b>	*	*	–
<b><i>Lf. brunneoruber</i> nom. prov.</b>	<b>DLK 2585</b>	<b>Brazil</b>	*	–	*
<b><i>Lf. dunensis</i></b>	<b>UFRN 2200</b>	<b>Brazil</b>	*	*	–
<b><i>Lf. dunensis</i></b>	<b>JC Bezerra s/n</b>	<b>Brazil</b>	*	*	–
<i>Lf. acicularis</i>	KVP 08-002	Thailand	HQ318226	HQ318132	HQ328869
<i>Lf. acrisissimus</i>	EDC 11-112	Tanzania	KR364041	KR364168	KR364254
<i>Lf. acrisissimus</i>	ADK2161	Benin	KR364126		
<i>Lf. albocinctus</i>	AV 99-211	Zimbabwe	KR364117	KR364249	KR364258
<i>Lactifluus</i> sp.	EDC 12-046	Cameroon	KR364064	KR364193	KR364257
<i>Lf. allardii</i>	JN 2004-008	North America	KF220016	KF220125	KF220217
<i>Lf. ambicystidiatus</i>	HKAS J7008	China	KR364108	KR364239	KR364309

<i>Lf. annulatoangustifolius</i>	BB 00-1518	Madagascar	AY606981	KR364253	
<i>Lf. annulifer</i>	TH 9014	Guyana	KC155376	KC155376	
<b><i>Lf. annulifer</i></b>	<b>R1133</b>	<b>Brazil</b>	*	*	
<i>Lf. armeniacus</i>	EDC 14-501	Thailand	KR364127	–	–
<i>Lf. atrovelutinus</i>	DS 06-003	Malaysia	GU258231	GU265588	GU258325
<i>Lf. aurantiifolius</i>	AV 94-063	Burundi	KR364017	KR364144	
<i>Lf. aureifolius</i>	AV 11-074	Tanzania	KR364056	KR364183	KR364259
<i>Lf. bertillonii</i>	JN 2012-016	Germany	KR364087	KR364217	KR364261
<i>Lf. brachystegiae</i>	AV 99-002	Zimbabwe	KR364018	KR364145	KR364262
<i>Lf. brunellus</i>	TH 9130	Guyana	JN168728	–	–
<i>Lf. brunneoviolascens</i>	AV 13-038	Italy	KR364123	KR364246	KR364264
<i>Lf. brunnescens</i>	AV 05-083	Malawi	KR364019	KR364146	KR364263
<i>Lf. caatingae</i>	Sulz 277	Brazil	MG930781	–	–
<b><i>Lf. candidus nom. prov.</i></b>	<b>JCB 145</b>	<b>Brazil</b>	*	–	–
<i>Lf. caribaeus</i>	PAM/Mart 12-090	Martinique	KP691415	KP691424	KP691432
<i>Lf. carmineus</i>	AV 99-099	Zimbabwe	KR364131	KR364251	KR364265
<b><i>Lf. mamorensis</i></b>	<b>JPB 61973</b>	<b>Brazil</b>	*	–	–
<i>Lf. catarinensis</i>	JDuque 119	Brazil	MK937538	MK937556	
<i>Lf. catarinensis</i>	ACM 993	Brazil	MK937538	–	–
<i>Lf. cf. castaneibadius</i>	CL/MART06.019	Martinique	KP691417	KP691426	–
<i>Lf. chamaeleontinus</i>	JD 946	Congo	KR364079	KR364208	KR364267
<i>Lf. chiapanensis</i>	VMB 4374A	Mexico	GU258297	GU265580	GU258316
<i>Lf. chrysocarpus</i>	LE 253907	Vietnam	JX442761	JX442761	–
<i>Lf. clarkeae</i>	MN 2004002	Australia	KR364011	HQ318205	KR364268
<i>Lf. cocosmus</i>	ADK 4462	Togo	KR364013	KR364141	KR364269
<i>Lf. conchatulus</i>	LTH 457	Thailand	GU258296	GU265659	GU258399
<i>Lf. corrugis s.l.</i>	AV 05-392	North America	JQ753822	KR364143	JQ348127

<i>Lf. crocatus</i>	KVP 08-034	Thailand	HQ318243	HQ318151	HQ328888
<i>Lf. cyanovirescens</i>	JD 988	Congo	KR364082	KR364211	KR364270
<i>Lf. deceptivus</i>	TENN 065854	North America	KR364101	–	KR364271
<i>Lf. denigricans</i>	EDC 11-218	Tanzania	KR364051	KR364178	KR364272
<i>Lf. densifolius</i>	AV 11-111	Tanzania	KR364057	KR364184	KR364273
<i>Lf. dissitus</i>	AV-KD-KVP 09-134	India	JN388978	JN389026	JN375628
<i>Lf. distantifolius</i>	LTH 288	Thailand	HQ318274	HQ318193	KR364274
<i>Lf. dwaliensis</i>	LTH 55	Thailand	KF220111	KF220204	KF220278
<i>Lf. dwaliensis</i>	KD 612	India	KR364042	–	–
<i>Lf. edulis</i>	FN 05-628	Malawi	KR364020	KR364147	KR364275
<i>Lf. emergens</i>	AV 99-012	Zimbabwe	KR364021	KR364148	KR364276
<i>Lf. fazaoensis</i>	AV 11-178	Togo	HG426477	KR364188	KR364349
<i>Lf. flammans</i>	JD 941	Congo	KR364078	KR364207	KR364303
<b><i>Lf. flavuscaespitosus</i> nom. prov.</b>	<b>FW 08/2017</b>	<b>Brazil</b>	*	–	–
<i>Lf. flocktonae</i>	JET1006	Australia	JX266621	JX266637	–
<i>Lf. foetens</i>	ADK 3688	Benin	KR364022	KR364149	KR364278
<i>Lf. foetens</i>	ADK 2840	Benin	KR364023	KR364150	KR364279
<i>Lf. fuscomarginatus</i>	LM 4379	Mexico	HQ168367	HQ168367	–
<i>Lf. genevievae</i>	GG-DK 17-02-05	Australia	GU258294	GU265657	GU258397
<i>Lf. gerardii</i>	AV 05-375	North America	GU258254	GU265616	GU258353
<i>Lf. aff. gerardii</i>	LTH 270	Thailand	EF560685	GU265598	GU258335
<i>Lf. cf. gerardii</i> var. <i>fagicola</i>	JN 2007-029	Canada	GU258224	GU265582	GU258318
<i>Lf. glaucescens</i>	LGAM 2010-0132	Greece	KR364105	KR364236	KR364280
<i>Lf. aff. glaucescens</i>	AV 04-195	North America	KF220045	KF220146	KF220232
<i>Lf. aff. glaucescens</i>	AV 05-374	North America	KF220049	KF220150	KF220236
<i>Lf. aff. glaucescens</i>	JN 2011-014	Vietnam	KF220104	KF220199	KF220273
<i>Lf. aff. glaucescens</i>	LTH 274	Thailand	KR364107	KR364238	KR364325

<i>Lf. goossensiae</i>	AB 320	Guinea	KR364132	KR364252	KR364281
<b><i>Lf. guttulatus</i> sp. nov.</b>	<b>DLK 2042</b>	<b>Brazil</b>	<b>MT611966</b>	–	<b>MT903963</b>
<i>Lf. gymnocarpoides</i>	JD 885	Congo	KR364074	KR364203	KR364283
<i>Lf. gymnocarpoides</i>	AV 05-184	Malawi	KR364024	KR364151	KR364284
<i>Lf. gymnocarpus</i>	EDC 12-047	Cameroon	KR364065	KR364194	KR364282
<i>Lf. heimii</i>	EDC 11-082	Tanzania	KR364040	KR364167	KR364286
<i>Lf. heimii</i>	AV 94-465	Burundi	KR364025	KR364152	–
<i>Lf. hygrophoroides</i>	AV 05-251	North America	HQ318285	HQ318208	HQ328936
<i>Lf. igniculus</i>	LE 262983	Vietnam	JX442759	JX442759	–
<i>Lf. indusiatus</i>	AV 94-122	Burundi	KR364026	KR364153	KR364287
<i>Lf. inversus</i>	AB 063	Guinea	AY606976	DQ421978	DQ421917
<i>Lf. kigomaensis</i>	EDC 11-159	Tanzania	KR364050	KR364177	KR364295
<i>Lf. kivuensis</i>	JR Z 310	Congo	KR364027	KR364154	
<i>Lf. laevigatus</i>	JD 939	Congo	KR364077	KR364206	KR364290
		Papua new			
<i>Lf. lamprocystidiatus</i>	EH 72-195	Guinea	KR364015	–	–
<i>Lf. latifolius</i>	SDM 037	Gabon	KR364028	–	–
<i>Lf. leae</i>	FH 12-013	Thailand	KF432957	KR364213	KR364292
<i>Lf. leonardii</i>	GG 07-02-04	Australia	GU258308	GU265668	GU258408
<i>Lf. leoninus</i>	DS 07-454	Thailand	KF220055	JN388989	JN375592
		Papua New			
<i>Lf. leoninus</i>	EH 72-524	Guinea	KR364116		
<i>Lf. leptomerus</i>	AV-KD-KVP 09-131	India	JN388972	JN389023	JN375625
<b><i>Lf. lepus</i></b>	<b>DLK 1854</b>	<b>Brazil</b>	<b>MT611966</b>	*	–
<i>Lf. leucophaeus</i>	LTH 182	Thailand	KF220059	KF220157	KF220243
<i>Lf. limbatus</i>	DS 06-247	Malaysia	JN388955	JN388987	JN375590
<i>Lf. longipes</i>	JD 303	Gabon	KR364009	KR364137	KR364310

<i>Lf. longipilus</i>	LTH 184	Thailand	HQ318256	HQ318169	HQ328905
<i>Lf. longisporus</i>	AV 94-557	Burundi	KR364118	KR364244	–
<i>Lf. cf. longisporus</i>	AV 11-025	Tanzania	KR364054	KR364181	KR364311
<i>Lf. longivelutinus</i>	XHW 1565	China	KR364114	–	–
<i>Lf. luteolus</i>	AV 05-253	North America	KR364016	KR364142	KJ210067
<i>Lf. luteopus</i>	EDC 11-087	Tanzania	KR364049	KR364176	KR364312
<i>Lf. luteopus</i>	AV 94-463	Burundi	KR364119	–	KR364313
<i>Lf. madagascariensis</i>	BB 99-409	Madagascar	AY606977	DQ421975	DQ421914
<i>Lf. madagascariensis</i>	B-E 99-417	Madagascar	KR364120	KR364245	–
<i>Lf. marielleae</i>	Cald 27	Brazil	MK937527	MK937547	–
<i>Lf. medusae</i>	EDC 12-152	Cameroon	KR364069	KR364198	KR364314
<i>Lf. multiceps</i>	TH 9154A	Guyaba	JN168731	KJ786620	KP691435
<i>Lf. murinipes</i>	F.1890 Martinique	Martinique	KP691418	–	–
<i>Lf. nebulosus</i>	RC/Guad 11-023	Guadeloupe	KP691412	KP691421	KP691430
<b><i>Lf. neotropicus</i></b>	<b>FW 21/2017</b>	<b>Brazil</b>	*	*	–
<i>Lf. nodosicytidiosus</i>	BEM 97-273	Madagascar	KR364029	KR364156	KR364316
<i>Lf. nodosicytidiosus</i>	BEM 97-072	Madagascar	AY606975	DQ421976	DQ421915
<i>Lf. nonpiscis</i>	AV 11-137	Togo	KR364058	KR364185	KR364317
<i>Lf. nonpiscis</i>	BB 3171	Zambia	KR364030	KR364157	–
<i>Lf. cf. ochrogalactus</i>	AV-KD-KVP 09-120	India	KR364130	KR364248	KR364318
<i>Lf. oedematopus</i>	KVP 12-001	Germany	KR364100	KR364232	KR364319
<i>Lf. panuoides</i>	RC/Guy 10-024	French Guiana	KJ786647	KJ786551	KP691428
<i>Lf. pegleri</i>	PAM/Mart 12-091	Martinique	KP691416	KP691425	KP691433
<i>Lf. pelliculatus</i>	JD 956	Congo	KR364080	KR364209	KR364321
<b><i>Lf. perforatus nom. prov.</i></b>	<b>AGS 100/2019</b>	<b>Brazil</b>	*	–	*
<i>Lf. petersenii</i>	AV 05-300	North America	GU258281	GU265642	GU258382
<i>Lf. aff. phlebonemus</i>	EDC 12-023	Camerron	KR364062	KR364191	KR364322

<i>Lf. phlebophyllus</i>	BB 00-1388	Madagascar	AY606974	DQ421979	DQ421918
<i>Lf. pilosus</i>	LTH 205	Thailand	KR364006	KR364134	KR364323
<i>Lf. pinguis</i>	LTH117	Thailand	HQ318211	HG318111	HQ328858
<i>Lf. piperatus</i>	2001 08 19 68	France	KF220119	KF241840	KF241842
<i>Lf. aff. piperatus</i>	JN 2011-036	Vietnam	KF220105	KF220200	KF220274
<i>Lf. aff. piperatus</i>	JN 2011-072	Vietnam	KF220106	KF220201	KF220275
<i>Lf. aff. piperatus</i>	TENN 064342	North America	KR364103	KR364234	KR364324
<b><i>Lf. piperogalactus</i> sp. nov.</b>	<b>FW 22/2017</b>	<b>Brazil</b>	<b>MT672343</b>	–	<b>MT903964</b>
<b><i>Lf. piperogalactus</i> sp. nov.</b>	<b>FW 110/2012</b>	<b>Brazil</b>	<b>MT902929</b>	–	–
<i>Lf. pruinatus</i>	BB 3248	Zambia	KR364031	KR364158	KR364328
<i>Lf. pseudoluteopus</i>	FH 12-026	Thailand	KR364084	KR364214	KR364331
<i>Lf. cf. pseudogymnocarpus</i>	AV 05-085	Malawi	KR364012	KR364139	KR364329
<i>Lf. cf. pseudovolemus</i>	ADK 2927	Benin	KR364113	KR364243	KR364330
<i>Lf. cf. pumilus</i>	EDC 12-066	Cameroon	KR364067	KR364196	KR364332
<i>Lf. putidus</i>	PAM/Mart 11-013	Martinique	KP691413	KP691422	KP691431
<i>Lf. ramipilosus</i>	EDC 14-503	Thailand	KR364128	–	–
<b><i>Lf. reniformis</i></b>	<b>DLK 1094</b>	<b>Brazil</b>	*	–	–
<b><i>Lf. restingae</i> nom. prov.</b>	<b>AGS 104/2019</b>	<b>Brazil</b>	*	–	–
<i>Lf. reticulatovenosus</i>	EH 6472	Indonesia	GU258286	GU265649	GU258389
<i>Lf. roseolus</i>	AV 99-160	Zimbabwe	KR364032	KR364159	KR364333
<i>Lf. roseolus</i>	AV 94-274	Burundi	KR364121	KR364242	–
<i>Lf. roseophyllus</i>	JN 2011-076	Vietnam	KF220107	KF220202	KF220276
<i>Lf. rubiginosus</i>	JD 959	Congo	KR364081	KR364210	KR364304
<i>Lf. rubiginosus</i>	BB 3466	Zambia	KR364014	KR364250	–
<i>Lf. rubrobrunnescens</i>	EH 7194	Indonesia	KR364115	–	–
<i>Lf. aff. rubroviolascens</i>	EDC 12-051	Cameroon	KR364066	KR364195	KR364334
<i>Lf. rufomarginatus</i>	ADK 3358	Benin	KR364033	KR364160	KR364335

<i>Lf. rufomarginatus</i>	ADK 3011	Benin	KR364034	KR364161	KR364336
<i>Lf. rugatus</i>	EP 1212/7	Greece	KR364104	KR364235	KR364337
<i>Lf. ruvubuensis</i>	AB 305	Guinea	KR364035	KR364162	KR364343
<i>Lf. ruvubuensis</i>	AV 94-599	Burundi	KR364122	–	–
<i>Lf. sesemotani</i>	AV 94-476	Burundi	KR364036	KR364163	KR364345
<b><i>Lactifluus</i> sp.</b>	<b>FW 145</b>	<b>Brazil</b>	*	–	–
<i>Lactifluus</i> sp.	RC/Guad 08-042	Guadeloupe	KP691414	KP691423	KP752179
<i>Lactifluus</i> sp.	PGK13-130	New Caledonia	KP691436	KR605507	–
<i>Lactifluus</i> sp.	RC/Guy 09-036	French Guiana	KJ786645	KJ786550	KP752178
<i>Lactifluus</i> sp.	MR/Guy 13-145	French Guiana	KJ786691	KJ786595	KP752180
<i>Lactifluus</i> sp.	MCA 3937	Gyana	KR364109	KR364240	KR364350
<i>Lactifluus</i> sp.	EDC 12-040	Cameroon	KR364063	KR364192	KR364289
<i>Lactifluus</i> sp.	RC/Guy 09-004bis	French Guiana	KJ786643	KP691419	KP691427
<i>Lactifluus</i> sp.	G3264	French Guyana	KJ786706		
<i>Lactifluus</i> sp.	AV 11-183	Togo	KR364060	KR364189	KR364277
<i>Lactifluus</i> sp.	AV 07-056	Cameroon	KR364008	KR364136	KR364293
<i>Lactifluus</i> sp.	EDC 12-195	Camerron	KR364071	KR364200	KR364301
<i>Lactifluus</i> sp.	JD 907	Congo	KR364076	KR364205	KR364302
<i>Lactifluus</i> sp.	G3185	French Guiana	KJ786694	KJ786603	KP691434
<i>Lactifluus</i> sp.	KW 392	Thailand	KR364091	KR364222	KR364305
<i>Lactifluus</i> sp.	REH 9398	Australia	KR364097	KR364229	KR364307
<i>Lactifluus</i> sp.	EDC 12-001	Cameroon	KR364061	KR364190	KR364298
<i>Lactifluus</i> sp.	EDC 12-176	Cameroon	KR364070	KR364199	KR364300
<i>Lactifluus</i> sp.	JN 2011-012	Vietnam	KR364045	KR364171	KR364294
<i>Lactifluus</i> sp.	TENN 065929	North America	KR364102	KR364233	KR364308
<i>Lactifluus</i> sp.	EDC 12-068	Cameroon	KR364068	KR364197	KR364299
<i>Lactifluus</i> sp.	JN 2011-071	Vietnam	KR364043	KR364169	KR364255

<i>Lactifluus</i> sp.	JN 2011-077	Vietnam	KR364044	KR364170	KR364256
<i>Lactifluus</i> sp.	SA A12 L2	North America	KR364088	KR364218	KR364361
<i>Lactifluus</i> sp.	JN 2011-080	Vietnam	KR364048	KR364174	KR364359
<i>Lactifluus</i> sp.	AV 12-050	Thailand	KR364086	KR364216	KR364260
<i>Lactifluus</i> sp.	AV 12-070	Thailand	KR364090	KR364221	KR364326
<i>Lactifluus</i> sp.	TENN 051830	Nepal	KR364111	KR364140	
<i>Lactifluus</i> sp.	KW 304/FH 12-037	Thailand	KR364092	KR364223	KR364306
<i>Lactifluus</i> sp.	PUN 7046	India	KM658971	–	–
<b><i>Lf. sphauliformis</i> sp. nov.</b>	<b>DLK 1891</b>	<b>Brazil</b>	<b>MT672342</b>	–	<b>MT903962</b>
<b><i>Lf. sphauliformis</i> sp. nov.</b>	<b>DLK 116</b>	<b>Brazil</b>	<b>MT611964</b>	–	<b>MT903961</b>
<b><i>Lf. sphauliformis</i> sp. nov.</b>	<b>DLK 346</b>	<b>Brazil</b>	<b>MT611965</b>	–	–
<i>Lf. subclarkeae</i>	REH 9231	Australia	KR364095	KR364227	KR364346
<i>Lf. subgerardii</i>	AV 05-269	North America	GU258263	GU265625	GU258362
<i>Lf. subiculatus</i>	SLM 10114	Guyana	JQ405654	–	–
<i>Lf. subkigomaensis</i>	AV 11-006	Tanzania	KR364052	KR364179	KR364288
<i>Lf. subpruinosis</i>	JN 2011-061	Vietnam	KR364046	KR364172	KR364357
<i>Lf. subvellereus</i>	AV 05-210	North America	KR364010	KR364138	KR364347
<i>Lf. subvolemus</i>	KVP 08-048	Slovenia	JQ753927	JQ348379	KR364356
<i>Lf. sudanicus</i>	AV 11-174	Togo	HG426469	KR364186	KR364348
<i>Lf. tanzanicus</i>	TS 1277	Tanzania	KR364037	KR364164	KR364351
<i>Lf. cf. tanzanicus</i>	AV 11-017	Tanzania	KR364053	KR364180	KR364296
<i>Lf. aff. tenuicystidiatus</i>	JN 2011-074	Vietnam	KR364047	KR364173	KR364358
<i>Lf. uapacae</i>	AV 07-048	Cameroon	KR364007	KR364135	KR364352
<b><i>Lf. umbilicatus</i> sp. nov.</b>	<b>DLK 1964</b>	<b>Brazil</b>	<b>MK929291</b>	–	–
<i>Lf. urens</i>	EDC 14-032	Zambia	KR364124	KR364247	KR364353
<i>Lf. vellereus</i>	ATHU-M 8077	Greece	KR364106	KR364237	KR364354
<i>Lf. velutissimus</i>	JD 886	Congo	KR364075	KR364204	KR364355

<i>Lf. venezuelanus</i>	RC/Guad 11-017	Guadeloupe	KP691411	KP691420	KP691429
<b><i>Lf. venosellus sp. nov.</i></b>	<b>M.C.A. Sá MS 37</b>	<b>Brazil</b>	<b>MK929292</b>	–	–
<i>Lf. veraecrucis</i>	M 8025	Mexico	KR364112	KR364241	–
<i>Lf. versiformis</i>	AV-KD-KVP 09-045	India	JN388967	JN389031	JN375632
<i>Lf. vitellinus</i>	KVP 08-024	Thailand	HQ318236	HQ318144	HQ328881
<i>Lf. volemoides</i>	MH 201187	Mozambique	KR364098	KR364230	KR364363
<i>Lf. volemoides</i>	TS 0705	Tanzania	KR364038	KR364165	–
<i>Lf. volemus</i>	KVP 11-002	Belgium	JQ753948	KR364175	KR364360
<i>Lf. volemus s.l.</i>	AV-KD-KVP 09-121	India	JN388979	JN389014	JN375616
<i>Lf. volemus s.l.</i>	KVP 08-011	Thailand	HQ318232	HQ318139	HQ328876
<i>Lf. volemus s.l.</i>	KVP 08-031	Thailand	HQ318240	HQ318148	HQ328885
<i>Lf. volemus s.l.</i>	REH 9320	Australia	KR364096	KR364228	KR364362
<i>Lf. wirrabara s.l.</i>	PL 40509	New Zealand	GU258287	GU265650	GU258390
<i>Lf. wirrabara s.l.</i>	GG 24-01-04	Australia	GU258307	GU265667	GU258407
<i>Lf. xerampelinus</i>	MH 201176	Mozambique	KR364099	KR364231	KR364364
<i>Lf. xerampelinus</i>	TS 1116	Tanzania	KR364039	KR364166	
<i>Lf. cf. zenkeri</i>	AV 11-050	Tanzania	KR364055	KR364182	KR364297
<i>Multifurca furcata</i>	REH 7804	Costa Rica	DQ421995	DQ421995	DQ421928
<i>M. ochricompacta</i>	BB 02-107	North America	DQ421984	DQ421984	–
<i>M. stenophylla</i>	JET956	Australia	JX266631	JX266635	–
<i>M. zonaria</i>	FH 12-009	Thailand	KR364083	KR364212	–
<i>Multifurca sp.</i>	xp2-20120922-01	China	KR364125	–	–
<i>Russula cyanoxantha</i>	FH 12-201	Germany	KR364093	KR364225	KR364341
<i>R. delica</i>	FH 12-272	Belgium	KF432955	KR364224	KR364340
<i>R. gracillima</i>	FH 12-264	Germany	KR364094	KR364226	KR364342
<i>R. khanchanjungae</i>	AV-KD-KVP 09-106	India	KR364129	JN389004	JN375607
<i>Russula sp.</i>	EDC 12-063	Cameroon	KR364073	KR364202	KR364339

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<i>Russula</i> sp.	EDC 12-061	Cameroon	KR364072	KR364201	KR364338
<i>Stereum hirsutum</i>	AFTOL 492	sem dados	AY854063	AF393078	AY218520

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