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**ANÁLISE FILOGENÉTICA DE *RUSSULA PERS.*
(RUSSULACEAE, RUSSULALES: AGARICOMYCETES)**

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**“Análise filogenética de *Russula* Pers.
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por

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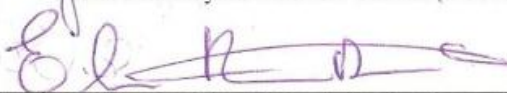
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RESUMO

Os macrofungos são parte integrante de todos os sistemas florestais, eles estão envolvidos em processos básicos como a decomposição de matéria orgânica, e a ciclagem, e absorção de nutrientes e água. Muitas espécies de árvores desenvolveram uma relação de mutualismo altamente especializada com algumas espécies de macrofungos, denominada ectomicorriza, para promover os processos de absorção de nutrientes e água. Uma das principais famílias de fungos envolvida em associações ectomicorrizicas e Russulaceae. Atualmente a família compreende quatro gêneros: *Lactarius*, *Lactifluus*, *Multifurca* e *Russula*. Dados mundiais apontam que existam 650 espécies de *Lactarius*, 118 de *Lactifluus*, 05 de *Multifurca* e 750 de *Russula*. No presente estudo são avaliadas as relações filogenéticas de táxons de *Russula* utilizando os marcadores moleculares ITS e LSU, incluindo espécimes tropicais do Brasil e da Colômbia. Para isso foram feitas análises de Máxima Verossimilhança e Bayesiana a partir de sequências geradas durante o presente estudo e sequências provenientes da base de dados do GenBank. Três novas espécies são descritas e ilustradas: uma para o Brasil e duas para a Colômbia. Duas espécies anteriormente registradas para a Guyana Francesa, *Russula rubropunctatissima* e *R. violaceotunicata*, são registradas pela primeira vez para o Brasil. As três novas espécies para a ciência e outras 15 sem serem descritas sugerem uma alta diversidade do gênero por ser descrita na região neotropical.

Palavras chave: Agaricomycetes, Brasil, Colômbia, ITS, LSU, Russulaceae.

ABSTRACT

Macrofungi are an integral part of all forest ecosystems, they are closely involved in processes such as decomposition of organic matter and cycling, and absorption of nutrients and water. Many species of trees and fungi developed an ectomycorrhizal relationship to promote the processes of absorption and exchange of nutrients and water. An important group of ectomycorrhizal fungi is Russulaceae, a family that went through drastic changes in taxonomy and systematics in the last decades due to molecular systematic studies. Currently Russulaceae comprises four genera: *Lactarius*, *Lactifluus*, *Multifurca* and *Russula*. Worldwide data show that there are 650 species of *Lactarius*, 118 of *Lactifluus*, 05 of *Multifurca* and 750 of *Russula*. In the present study the phylogenetic relationships of *Russula* using the molecular markers ITS and LSU are evaluated including tropical specimens from Brazil and Colombia. Bayesian and Maximum Likelihood analyses were made using sequences generated during this study, and sequences from the GenBank database. Three new species are described, one from Brazil and two from Colombia. *Russula rubropunctatissima* and *R. violaceotunicata* are reported for the first time from Brazil, both species were known before only from French Guyana. The three new species to science and the 15 other unnamed species suggest a high diversity to be described in the neotropical region.

Key words: Agaricomycetes, Brazil, Colombia, ITS, LSU, Russulaceae.

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INTRODUÇÃO

Os macrofungos são parte integrante de todos os sistemas florestais, uma vez que estão intimamente envolvidos em processos básicos como a decomposição de matéria orgânica, e a ciclagem, e absorção de nutrientes e água (Mueller & Bills, 2004; Mueller *et al.*, 2006). Muitas espécies de árvores desenvolveram uma relação de mutualismo altamente especializada com algumas espécies de macrofungos, denominada ectomicorriza, para promover os processos de absorção de nutrientes e água. Adicionalmente, fungos são usados na alimentação, como fonte de medicamentos, para o processamento de alimentos e bebidas, além de processos biotecnológicos (Mueller *et al.*, 2006).

Quando comparados com outros grupos de organismos, como as plantas ou animais, os fungos são pouco conhecidos; muitos são efêmeros e crípticos, o que faz deles organismos difíceis de registrar, mas sabe-se que são um grupo extremamente diverso (Mueller & Bills, 2004; Schmit & Mueller, 2006; Blackwell, 2011). A diversidade do reino Fungi tem sido estimada de 1.5 até 5.1 milhões de espécies. Da estimativa de 1.5 milhões, só 7% foram descritas, das quais Ascomycota

e Basidiomycota compreendem a maior parte da diversidade (Hawksworth, 1991, 2001; O'Brien *et al.*, 2005; Blackwell, 2011).

As estimativas de diversidade e taxonomia de fungos têm sofrido alterações drásticas nas últimas décadas devido aos estudos de sistemática molecular (Blackwell, 2011). Entre estes estudos, uma hipótese de filogenia molecular de Russulaceae Lotsy (Buyck *et al.*, 2008), revelou que *Lactarius* Pers. e *Russula* Pers. não são suportados como grupos monofiléticos, resultando na divisão da família em quatro gêneros, *Lactarius* (abreviado como *L.*), *Lactifluus* (Pers.) Roussel (abreviado como *Lf.*), *Multifurca* Buyck & Hofstetter e *Russula* (Buyck *et al.*, 2008, 2010). Dados mundiais apontam que existam 650 espécies de *Lactarius*, 118 de *Lactifluus*, 05 de *Multifurca* e 750 de *Russula* (Kirk *et al.*, 2008; Verbeken *et al.*, 2011; Van de Putte, 2012).

Histórico de Russulaceae

Agaricomycetes é uma classe de fungos com cerca de 100 famílias, 1.147 gêneros e 20.951 espécies descritas (Kirk *et al.*, 2008), incluindo as formas familiares como cogumelos, orelhas de pau, fungos

coraloides, gasteroides e ressupinados (Binder *et al.*, 2005). O conceito friesiano que define o aspecto morfológico macroscópico do basidioma dominou os sistemas de classificação nos Agaricomycetes até o final do século 20. Antes disso os fungos ressupinados, agaricáceos, coraloides e gasteroides eram relacionados de acordo com a morfologia do basidioma, e era inconcebível que táxons com distintas morfologias fossem classificados juntos (Miller *et al.*, 2006).

O sistema de classificação de Fries (1874) agrupava táxons com base nas características morfológicas do himenóforo. Posteriormente Donk (1964, 1971) dividiu as 6 famílias de Aphyllophorales, que incluem as orelhas de pau, do sistema de Fries em 23 famílias; Singer (1986) dividiu Agaricales, os típicos cogumelos, em 17 famílias; e Dring (1973) dividiu a antiga classe Gasteromycetes, dos fungos com himênio fechado, em 9 ordens com 23 famílias (Hibbett & Thorn, 2001). No entanto, a simplicidade anatômica, um registro fóssil escasso (com o fóssil mais antigo de um agaricomycete datando do Cretáceo, 200 ma), e a alta plasticidade fenotípica, tornam difícil separar homologias de homoplasias, tornando o estudo das relações evolutivas

em fungos complexo (Hibbett & Donoghue, 1995; Hibbett *et al.*, 1997; Larsson & Larsson, 2003).

Donk em 1971 foi o primeiro a discutir uma possível relação entre gêneros como *Hericium* Pers., *Echinodontium* Ellis & Everh., *Gloeocystidiellum* Donk, *Boidinia* Stalpers & Hjortstam, *Russula* e *Lactarius*. Todos esses gêneros possuem hifas gloeoíferas e basidiosporos com ornamentação amiloide, mas com características macromorfológicas muito diferentes. Esta hipótese foi expandida e desenvolvida por Oberwinkler (1977) que ratificou a proposta de um clado russuloide (Larsson & Larsson, 2003). Singer (1986) agrupou táxons com esporos com ornamentação amiloide, hifas sem fíbulas, trama himenoforal com esferocistos e/ou hifas laticíferas em Russulinae Sing., dentro de Agaricales, compreendendo nesta subordem duas famílias: Bondarsewiaceae, com *Bondarsewia* Singer e Russulaceae Lotsy com *Russula* e *Lactarius*. Posteriormente, baseado em estudos moleculares, Hibbett e Donoghue (1995), e Hibbett *et al.* (1997) argumentaram o reconhecimento de uma única linhagem russuloide, Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David, onde foram

incluídos os gêneros com hifas gloeóferas e basidiosporos com ornamentação amiloide.

Miller *et al.* (2001) explorou a filogenia molecular dos táxons agaricoides, gasteroides e pleurotoides em Russulales e sugeriram que a sinonímia de *Macowanites* Kalchbr, *Gymnomyces* Masee & Rodway, *Cystangium* Singer & A.H. Sm., *Martellia* Mattir em *Russula*, e a sinonímia de *Zelleromyces* Singer & A.H. Sm. e *Arcangelliella* Cavara em *Lactarius*, pode ser taxonomicamente justificável. Nesse estudo os gêneros gasteroides foram aninhados dentro de táxons estabelecidos em *Russula* e *Lactarius* sugerindo múltiplas origens da forma gasteroide. Adicionalmente, Larsson e Larsson (2003) forneceram um estudo detalhado de táxons de aphylophorales - russuloides, incluindo também representantes com a maioria das diferentes morfologias. Todas as espécies de Agaricomycetes com sistema de hifas gloeopleurais incluídas nas análises de Larsson e Larsson (2003) foram agrupadas no clado russuloide.

O monofiletismo de Russulales foi corroborado através da análise de sequência de DNA inúmeras vezes ao longo das últimas décadas (Hibbett & Donoghue, 1995; Hibbett *et al.*, 1997; Hibbett &

Binder, 2002; Larsson & Larsson, 2003; Binder *et al.*, 2005; Miller *et al.*, 2006) e a ordem é caracterizada pela presença de hifas oleíferas e laticíferas, a grande maioria das espécies com esporos com ornamentação amiloide, e um sistema heterômero constituído por hifas geralmente sem fibula e com presença de células globulares de paredes finas chamadas esferocistos. Russulales compreende 12 famílias, aproximadamente 80 gêneros e 4.000 espécies. Dentro de Russulales mais de um terço das espécies conhecidas são de gêneros pertencentes à Russulaceae Lotsy, família definida pela presença de esferocistos (células grandes, isodiamétricas) na trama do píleo, lamelas e estipe. Na família a grande maioria das espécies conhecidas é de táxons agaricoides pertencentes a *Russula* ou *Lactarius* (Miller *et al.*, 2006).

Até agora, todos os estudos filogenéticos recuperaram *Russulaceae* como monofilética, mas as relações internas entre *Russula* e *Lactarius* permaneceram fracamente suportadas ou não resolvidas (Buyck *et al.*, 2008). À luz dos dados morfológicos e moleculares apresentados por Buyck *et al.* (2008) o ponto de vista taxonômico sobre a família mudou drasticamente. Foi corroborado que *Lactarius* e *Russula* eram polifiléticos e foram divididos em quatro gêneros distintos

(Buyck *et al.*, 2008, 2010). *Lactarius furcatus* Coker e espécies anteriormente atribuídas a *Russula* subseção *Ochricompaetae* Bills & O.K. Mill. agora estão agrupadas no gênero *Multifurca* Buyck & Hofstetter. Os demais táxons de *Russula* formam um grupo monofiléticos. *Lactarius* foi dividido em dois gêneros: (1) *Lactarius*, que inclui os antigos subgêneros *Piperites* (Fr. ex J. Kickx f.) Kauffman, *Russularia* (Fr. Ex Burl.) Kauffman e *Plinthogalus* (Burl.) Hesler & A.H. Smith e (2) *Lactifluus* (Pers.) Roussel, compreendendo os antigos subgêneros *Gerardii* A.H. Sm. & Hesler, *Lactarius*, *Lactifluus* (Pers.) Hesler & A.H. Smith, *Lactariopsis* (Henn.) R. Heim, *Russulopsis* Verbekene a seção *Edules* Verbeken e seção *Panuoidei* Singer (Buyck *et al.*, 2010; Norvell, 2011; Hackel, 2014; Verbeken & Nuytinck, 2013). Embora as relações dentro da família tenham sido esclarecidas na última década, a classificação infragenérica, especialmente em *Lactifluus* e *Russula*, ainda é altamente inconsistente (Hackel, 2014).

Delimitação dos gêneros de Russulaceae

Ainda não existe uma classificação natural e abrangente de *Russula* apoiada por análise filogenética molecular. Infelizmente,

circunscrições anteriores de táxons infragenéricos em *Russula* na Europa, onde a maioria da classificação deste gênero se originou, não resultaram em um sistema de consenso utilizável (Miller & Buyck, 2002). Os principais sistemas de classificação em *Russula* são três: 1) Romagnesi dividiu *Russula* em dois subgêneros, *Compacta* (Fr.) Bom., que inclui as seções *Nigricatinae*, *Archaeinae* R. Heim ex Buyck & Sarnari e *Plorantinae* Romagn., e *Genuinae*. 2) Singer dividiu o gênero em 7 seções. O subgênero *Compacta* sensu Romagnesi foi dividido em 3 seções, *Archaeinae*, *Plorantes* e *Compactae*. 3) Sarnari (1998) dividiu em 17 seções e 28 subseções incluindo seis subgêneros (Miller & Buyck, 2002). Segundo Miller e Buyck (2002) aparentemente muitas das diferenças parecem ser nomenclaturais. A longa história taxonômica de *Russula* na Europa resultou em uma abundância de diferentes nomes para táxons infragenéricos similares (Miller e Buyck, 2002).

Hesler e Smith (1979) apresentam o principal tratamento taxonômico de *Lactarius* dividindo-o em seis subgêneros: *Lactarius*, *Plinthogalus*, *Lactifluus*, *Tristes*, *Piperite* e *Russularia*. Estes subgêneros foram posteriormente segregados em *Lactarius* (*Russularia*, *Piperites* e *Plinthogalus*) e *Lactifluus* (*Lactarius*, *Lactifluus* e

Lactariopsis e *L. sect. Edules*) (Buyck *et al.*, 2008; Verbeken & Nuytinck, 2013).

Segundo Buyck *et al.* (2008) a distinção formal entre *Russula* e *Lactarius* depende inteiramente de caracteres associados ao sistema de hifas laticíferas. Em *Russula* ele não é ramificado e não se estende no himênio. Em *Lactarius* os pseudocistídios do sistema laticífero ramificado se estendem no himênio. Adicionalmente, espécimes de *Lactarius* exudam látex quando manuseados ou cortados, enquanto espécimes de *Russula* não.

De acordo com Van de Putte (2012) nem *Lactifluus* nem *Lactarius* podem ser definidos por meio de caracteres sinapomórficos. Há, no entanto, algumas tendências morfológicas que poderiam ajudar na identificação dos gêneros. *Lactifluus* contém muitas espécies com véu, píleo velutino a tomentoso, assim como espécies com anel. Píleo zonado e viscoso a glutinoso, por outro lado, estão ausentes nos membros de *Lactifluus*, mas são comumente encontrados em *Lactarius*. Espécies pleurotoides foram registradas apenas em *Lactifluus*, enquanto que as espécies angiocárpicas só foram encontradas em *Lactarius*. Microscopicamente, esferocistos himeniais, cistídios himeniais de

parede engrossada (lamprocistídios), assim como elementos de parede engrossada na pileipelis são mais típicos de *Lactifluus*. Cistídios himeniais de parede fina com conteúdo granular e tipo agulha, chamados de macrocistídios, são comuns em *Lactarius* e típicos de *Russula* (Van de Putte, 2012).

Uma das diferenças mais marcantes entre *Lactarius* e *Lactifluus* é a distribuição geográfica (Van de Putte, 2012). Embora *Lactarius* apresente muitas espécies com distribuição tropical ou subtropical, é notável que compreenda espécies conhecidas para as regiões temperada e boreal. *Lactifluus*, por outro lado, tem poucas espécies que ocorrem em regiões temperadas, e a maioria dos táxons está nos trópicos e subtrópicos.

Multifurca pode ser reconhecido pela combinação de vários caracteres: píleo de cores claras e concentricamente zonado, lamelas regularmente bifurcadas de cor amarela a salmão no basidioma maduro, contexto zonado, esporada escura e esporos pequenos (no máximo $7,1 \times 5,9\mu\text{m}$) fracamente ornamentados. A distribuição conhecida atualmente para o gênero é sul da América do Norte, Costa Rica, Nova Caledônia,

Tailândia, Índia e China (Buyck *et al.*, 2008; Wang & Liu, 2010; Lebel *et al.*, 2013).

Russulaceae é muito bem conhecida na Europa, América do Norte e recentemente iniciaram-se mais estudos em regiões tropicais da América Central e África. Até agora existem registradas mundialmente 650 espécies de *Lactarius*, 118 de *Lactifluus*, 5 de *Multifurca* e 750 de *Russula* (Kirk *et al.*, 2008; Verbeken *et al.*, 2011; Van de Putte, 2012). Para o Brasil são conhecidos poucos registros de espécies nativas de Russulaceae (Jaeger, 2013). Com base em dados publicados na literatura, observação de materiais de herbários e descrição de novas espécies, Jaeger (2013) levantou um total de 27 espécies e 2 subespécies de *Russula*, 19 espécies de *Lactarius* e 10 de *Lactifluus* (incluindo os táxons exóticos) distribuídas nos estados do Rio Grande do Sul (RS), Paraná (PR), São Paulo (SP), Minas Gerais (MG), Espírito Santo (ES), Pernambuco (PE), Paraíba (PB), Rio Grande do Norte (RN), Pará (PA), Amazonas (AM) e Rondônia (RO).

A criação de um sistema de classificação mais estável em um clado grande e complexo como *Russulaceae* é fundamental para ajudar durante o processo de identificação. Um esquema de classificação sólido

também pode ajudar na identificação de caracteres que são mais adequados e úteis para a separação de entidades taxonômicas. Neste sentido, as análises filogenéticas baseadas em sequências de DNA de genes ribossomais proporcionam uma alternativa eficaz à morfologia na reconstrução das relações evolutivas e na avaliação de caracteres úteis na taxonomia (Miller & Buyck, 2002).

OBJETIVO GERAL

Avaliar as relações filogenéticas dentro de *Russula* (Russulaceae) utilizando os marcadores moleculares ITS e LSU, incluindo espécimes tropicais do Brasil e da Colômbia.

Objetivos Específicos

Extrair, amplificar e sequenciar as regiões ITS e 28S do rDNA nuclear de materiais coletados no Brasil e Colômbia.

Interpretar filogeneticamente as espécies do Brasil e Colômbia a partir das análises morfológicas e moleculares, e descrever as novidades científicas.

RESULTADOS

Os resultados desta dissertação estão organizados em um capítulo intitulado *RUSSULA: NEW AND INTERESTING NEOTROPICAL SPECIES BASED ON MORPHOLOGICAL AND MOLECULAR DATA*, a ser submetido para a revista *Mycologia*. Neste capítulo é apresentada uma filogenia molecular de *Russula* a partir do sequenciamento de 29 espécimes do Brasil e 27 da Colômbia coletados recentemente. Três novas espécies para a ciência são descritas e 2 espécies são registradas pela primeira vez no Brasil. Para a reconstrução filogenética foram usados os marcadores moleculares ITS e LSU através de análises de Máxima Verossimilhança e Bayesianas.

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CAPÍTULO 1

***Russula*: new and interesting Neotropical species based on morphological and molecular data**

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Abstract

Russula is a diverse ectomycorrhizal genus in the family Russulaceae and despite the high number of described species, very little is known about *Russula* diversity in neotropical countries such as Brazil and Colombia. Historically, there was extensive debate about diagnostic characters that delimit infrageneric groups within *Russula*. The combined analysis of molecular and morphological data can improve the current phylogenetic knowledge of *Russula*. Maximum likelihood and Bayesian analyses were carried out using sequences obtained in this

study and sequences taken from the Genbank database. One new species is described for science from the Brazilian Atlantic Forest and two from Colombian Amazon rain forest. *Russula rubropunctatissima* and *R. violaceotunicata* are reported for the first time for Brazil. This is the first study including Neotropical sequences in a worldwide phylogenetic analysis. The taxa included in the phylogeny helped us to understand how the Neotropical taxa of *Russula* related to the temperate, Pacific and Asian taxa.

Keywords: Agaricomycetes, Brazil, Colombia, ITS, LSU, Russulaceae.

INTRODUCTION

Russula Pers. includes 750 taxa reported worldwide (Kirk *et al.*, 2008). The genus is characterized by a chalky basidioma texture due to the presence of sphaerocysts, the lamellae are equal or intermixed, the basidiospores are ornamented with amyloid reaction on the ornamentations, the lactiferous system is not ramified and does not extend into the hymenium to form pseudocystidia (Singer, 1986; Romagnesi, 1996; Buyck *et al.*, 2008). The main taxonomic treatments

including an infrageneric classification system in *Russula* used today are Singer (1986), Romagnesi (1967, 1985, 1987), and Sarnari (1998). Current phylogenetic studies have shown that *Russula* is monophyletic (Miller & Buyck, 2002; Larsson & Larsson, 2003; Buyck *et al.*, 2008, 2010). The species within the genus present agaricoid, secotioid, gasteroid, or pleurotoid basidiomata (Buyck & Horak, 1999; Miller *et al.*, 2001; Lebel & Tonkin, 2007). *Russula* has a great ecological importance in forest ecosystems, forming ectomycorrhizal association with members of Dipterocarpaceae, Salicaceae, Betulaceae, Pinaceae, Fagaceae, Rosaceae, Fabaceae, Sapotaceae, Nyctaginaceae, Polygonaceae and Tiliaceae (Buyck & Halling, 2004; Lebel & Tonkin, 2007; Li *et al.*, 2015; Eberhardt, 2002). Additionally some species of *Russula* are of economic value because of their edible basidiomata (Li *et al.*, 2015) or its medical properties like antitumor activity (Zhang *et al.*, 2010).

The genus has a rich taxonomic history in the European mycological literature (Miller & Buyck, 2002) but it has never been thoroughly inventoried in the Neotropics, although new taxa have been described in the last two decades (Courtecuisse & Buyck, 1991; Gómez & Alfaro, 1996; Miller Jr. *et al.*, 2000; Miller *et al.*, 2012; Buyck &

Ovrebo, 2002; Buyck & Halling, 2004; Cheype & Campo, 2012). Despite the high number of described species, very little is known about *Russula* diversity in neotropical countries such as Brazil (Singer et al. 1983, Jaeger 2013; Sulzbacher et al., 2013; Sá et al., 2013; Oliveira et al., 2014) and Colombia (Franco-Molano & Uribe, 2000; Franco-Molano et al., 2000, 2010; Vasco-Palacio, 2013).

In Brazil 12 species and two subspecies have been reported for *igapó* (seasonally inundated forest) and *campinarana* forest (forests on white sand) in central Amazonia (Singer *et al.*, 1983, Singer & Araujo, 1986). Four species have been recorded from Rio Grande do Sul (Rick, 1961), two in Paraná (de Meijer, 2006), two in São Paulo (Grandi *et al.*, 1984; Capelari, 1989), and one species is known from Rondônia (Capelari & Maziero, 1988). In Colombia ten species have been reported from Andes region and one from the Pacific region (Franco-Molano & Uribe, 2000; Franco-Molano *et al.*, 2000, 2010).

Although recent classifications in *Russula* remain complex and largely incongruent (Miller *et al.*, 2001, Miller & Buyck, 2002; Lebel & Tonkin, 2007), some studies have provided solid basis to argue important taxonomic characters and evolutionary relationships through phylogenetic analysis (Miller & Buyck, 2002; Eberhardt, 2002;

Shimono *et al.*, 2004; Lebel & Tonkin, 2007; Buyck *et al.*, 2008). So far, phylogenetic studies of *Russula* have not included sequences from neotropical taxa (Buyck *et al.*, 2008; Eberhardt, 2002; Lebel & Tonkin, 2007; Miller & Buyck, 2002; *Park et al.*, 2013; *Park et al.*, 2014; Shimono *et al.*, 2004; Shimono *et al.*, 2014). Our main goal was to evaluate the strength of rDNA ITS and rDNA LSU sequences to elucidate the relationships of *Russula* including taxa from Brazil and Colombia. The molecular analyses include one undescribed species from the Brazilian Atlantic forest, two undescribed species from the Colombian Amazon, two new reports from Brazil, and 15 unnamed taxa from Brazil and Colombia. The new species for science and the new records from Brazil and Colombia are described and illustrated.

MATERIALS AND METHODS

Molecular technics

In the field a small portions of the basidiomata were preserved in silica gel, FTA card or CTAB for DNA extraction. The DNA extraction was made using one of the following protocols: Dentinger *et*

al. (2010), MasterPure™ Yeast DNA Purification kit (Epicenter, Madison, WI), Wizard Genomic DNA Purification kit (Promega Corp., Fitchburg, USA).

Two nuclear ribosomal DNA regions were amplified: the internal transcribed spacers and the 5.8S (ITS1-5.8S-ITS2) and the nuclear ribosomal large subunit (nuLSU). The pairs of primers ITS8-F + ITS6-R (Dentinger *et al.*, 2010) and ITS1f + ITS4 (White *et al.*, 1990; Gardes & Bruns 1993) were used to amplify the ITS region. The LSU region was amplified using the pair of primers LR0R + LR7 (Vilgalys lab.) and CTB6 + TW14 (designed by T. Bruns and T. White). The PCR reaction mix followed Dentinger *et al.* (2010), or was prepared using 14.3 µl purified water, 1 µl bovine serum albumin (10 mg/ml), 0.5 µl MgCl₂ (25 µM), 5 µl green GoTaq buffer (5x, Promega Corp., Fitchburg, Etats Unis), 0.5 µl of each primer (50 µM), dNTP (10mM) and GoTaq DNA polymerase (1 U/25 µl, Promega). The DNA was diluted 1/10 before the amplification. PCR amplification was performed using Eppendorf MasterCycler thermal cycler with the following parameters: two to three minutes of initial denaturation at 94 to 95°C, five denaturation cycles at 94°C for 30 to 45s, annealing at 55°C for 30

to 45 s and elongation at 72°C for one minute; the reaction continued for 25 to 40 cycles. A final elongation was performed at 72°C for 10 min and refrigerated at 4°C.

The amplification products were checked on agarose gel 1% with SightDNA (Euromedex, Souffelweiersheim). The PCR products were then sequenced following Dentinger *et al.* (2010) or were sent to GATC Biotech (Konstanz, Germany) or Macrogen (Lille, France). Sequence chromatograms were corrected in Geneious v. 6.1.8 (<http://www.geneious.com>, Kearse *et al.*, 2012), Sequencher 5.1 (Gene Codes Corp.) or DNAStart from SeqMan software (Swindell & Plasterer, 1997).

Phylogenetic analyses

Seventy taxa are included in the ITS + LSU combined analysis, and 216 taxa are in the ITS analysis. *Albatrellus flettii* Moser ex Pouzar and *Gloeocystidiellum aculeatum* Sheng H. Wu were used as outgroup taxa (Tab. 1). *Lactarius torminosus* (Schaeff.) Gray, *Lactifluus volemus* (Fr.: Fr.) Kuntze and *Multifurca ochricompacta* (Bills & O.K. Miller) Buyck & V. Hofst. sequences were included as Russulaceae representative taxa to test *Russula* monophyly. The alignment was made through the *E-INS-*

I strategy using MAFFT v.7 (Kato & Standley, 2013), available at mafft.cbrc.jp/alignment/server and was then refined manually with Mega 6 (Tamura *et al.*, 2013). The simple indel coding method was followed (SIC, Simmons & Ochoterena, 2000), as implemented in the SeqState software (Müller, 2005), to recode the indels present in the dataset as binary characters. The dataset was divided into five data partitions: ITS1, 5.8S, ITS2, LSU, and recoded indels for the combined analysis, and ITS1, 5.8S, ITS2, and recoded indels for the ITS analysis. The final alignment, as well as the resulting phylogenetic trees, will be deposited at TreeBASE (treebase.org/treebase/index.html) before the publication is submitted.

The best-fit model for nucleotide evolution to each partition (except the binary data) was obtained according to BIC (*Bayesian Information Criterion*), as implemented in the software jModelTest 2.1.4 (Guindon & Gascuel, 2003, Darriba *et al.*, 2012). The jModelTest 2.1.4 was used to estimate the base frequencies, the rates of nucleotide substitutions, gamma shape parameter and proportion of invariant sites (Tab. 2). A Maximum likelihood (ML) analysis was carried out as implemented in RAxML v.8.1.24 (Stamatakis, 2014), available in the

CIPRES science gateway (Miller et al 2010, <http://www.phylo.org/>). The ML analysis first involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree, under a GTRGAMMAI model, with all other parameters estimated by the software. To assess the reliability of the nodes, multi-parametric bootstrapping replicates under the same model were computed, allowing the program to halt bootstrapping automatically by the autoMRE option.

The Bayesian analysis (BI) was performed in the software MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003), as implemented on the CIPRES Science Gateway 3.1 (Miller *et al.*, 2010). Bayesian analysis was implemented by two independent runs, each one starting from random trees, with four simultaneous independent chains and performed 20,000,000 generations, keeping one tree every 1000th generation. Four rate categories were used to approximate the gamma distribution, and the nucleotide substitution rates were fixed to the estimated values. Of all trees sampled, 25% were discarded as burn-in and checked by the convergence criterion (frequencies of average standard deviation of split <0.01) in Tracer v.1.6 (Rambaut *et al.*, 2014), while the remaining ones were used to reconstruct a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPP) of the branches. A

branch was considered to be strongly supported if showed a BPP ≥ 0.95 and/or bootstrap support (BS) $\geq 90\%$, while moderate support was considered BPP ≥ 0.9 and/or BS $\geq 70\%$. The trees produced were refined with FigTree v1.4 (Rambaut, 2012).

Morphology

The specimens included in this work are mainly from the Atlantic forest in Florianópolis, southern Brazil, and from the Amazon rain forest at *Estación Biológica el Zafire* (ZBS) in southern Colombia. The specimens were collected, macroscopically described, and photographed in the field. The collections were macro- and microscopically studied following traditional mycological methods (Largent, 1986, Largent *et al.*, 1977; Mueller *et al.*, 2004). The color, shape, texture, characteristic from the hymenophore, and other aspects from the fresh specimens were recorded before they were air-dried in a food dehydrator for 8 h at approximately 40°C. Color codes follow Kramer (2004; oac758), and Kornerup & Wanscher (1978; 6E4). Microscopic features were studied from dried materials by mounting free-hand sections of the basidiomata in 5% KOH, Melzer's reagent, or

Congo red. Cresyl blue was used to verify the presence of ortho- or metachromatic reactions in cross sections. Basidiospores were observed and measured under Melzer's reagent. Basidiospore dimensions are given as length range×width range (n=30) and Q ratio range. Q value denotes the length/width ratio of the basidiospores excluding ornamentation. The specimens were deposited at Herbarium FLOR at Universidade Federal de Santa Catarina and at Herbarium HUA at Universidad de Antioquia. Herbarium acronyms follow Thiers (continuously updated).

Table 1. Taxa included in the phylogenetic analysis. *sequences generated for this study. TBP= to be provided. Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|------------------------------------|----------------|---------------------------|-------------|------------|
| <i>Albatrellus flettii</i> | BG Thesis | North America | NA AF518569 | AF518569 |
| <i>Gloeocystidiellum aculeatum</i> | Wu890714-52 | China | CN AF506433 | AF506433 |
| <i>Lactarius torminosus</i> | RW3183 | Czech Republic | CZ KF133281 | KF133314 |
| <i>Lactifluus volemus</i> | UE09.08.2004-5 | Sweden | SE DQ422008 | DQ422008 |
| <i>Multifurca orchricompacta</i> | BB02.107 | United States | US DQ421984 | DQ421984 |
| <i>R. acrifolia</i> | UE12.09.2003-3 | Europe | EU DQ421998 | DQ421998 |
| <i>R. adulterina</i> | 489RUS25 | Europe | EU AY061651 | |
| <i>R. adusta</i> | 547RUS27 | Europe | EU AY061652 | |
| <i>R. aeruginea</i> | n11292 (TUB) | Germany | DE AF418612 | |
| <i>R. aff. gelatinivelata*</i> | Vasco-P 1796 | Colombia | CO KT724178 | TBP |
| <i>R. aff. metachromatica*</i> | CATO40 | Brazil | BR TBP | |

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|---------------------------|----------------|---------------------------|-------------|------------|
| <i>R. aff. pluvialis</i> | TH9230 | French Guyana | GY KT339218 | KT339218 |
| <i>R. afrodelica</i> | M00/924 | Madagascar | MG JQ902095 | |
| <i>R. albobrunnea</i> | TL2136 | Australia | AU EU019933 | EU019933 |
| <i>R. albonigra</i> | AT2002064 | Sweden | SE DQ422029 | DQ422029 |
| <i>R. amethystina</i> | 929IF52 | Europe | EU AY061653 | |
| <i>R. amoenicolor</i> | 311IX76 | Europe | EU AY061655 | |
| <i>R. amoenipes</i> | 309IS77 | Europe | EU AY061656 | |
| <i>R. amoenolens</i> | nl27.9.95.6 | Europe | EU AF418615 | AF325295 |
| <i>R. aquosa</i> | 312RUF25 | Europe | EU AY061657 | |
| <i>R. archaea</i> | 987IS79 | Africa | AF AY061737 | |
| <i>R. atropurpurea</i> | hue178 | Germany | DE AF418618 | AF325296 |
| <i>R. aurantiaca</i> | 545IS80 | Europe | EU AY061658 | |
| <i>R. aurata</i> | 2-1120IS77 | Europe | EU AY061659 | |
| <i>R. azurea</i> | 487RUS25 | Europe | EU AY061660 | |
| <i>R. batistae</i> | MCA4007 | Guyana | GY KT339222 | KT339222 |
| <i>R. bernardii</i> | 836RUF37 | Madagascar | MG JQ902063 | |
| <i>R. betularum</i> | 216RU | Europe | EU AY061729 | |
| <i>R. bruneonigra</i> | H5813 | Australia | AU EU019945 | |
| <i>R. brunneoannulata</i> | 1174IS84 | Africa | AF JQ902081 | |
| <i>R. brunneorigida</i> | 992IR67 | Africa | AF JQ902060 | |
| <i>R. caerulea</i> | 504IS77 | Europe | EU AY061661 | |
| <i>R. camarophylla</i> | PAM01081108 | France | FR DQ421982 | DQ421982 |
| <i>R. cantharellicola</i> | UC1999420 | United States | US KF306036 | |
| <i>R. cascadenis</i> | UBC F23910 | Canada | CA KJ146726 | KJ146726 |
| <i>R. cavipes</i> | hue163 | Europe | EU AF418623 | AF325298 |
| <i>R. cessans</i> | 1-301IS55 | Europe | EU AY061730 | |
| <i>R. changbaiensis</i> | HMAS262369 | Cameroon | CM KC412162 | |
| <i>R. chloroides</i> | UBCF20353 | Canada | CA KC581331 | KC581331 |
| <i>R. clariana</i> | 492RUS26 | Europe | EU AY061664 | |
| <i>R. claroflava</i> | 224IS76 | Europe | EU AY061665 | |
| <i>R. compacta</i> | OSA-MY-1713 | Japan | JP AB291725 | AB154701 |
| <i>R. compressa</i> | 1001I113 | Madagascar | MG JQ902057 | |
| <i>R. congoana</i> | 897IS53 | Madagascar | MG JQ902058 | |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.

Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|--------------------------|-----------------|---------------------------|-------------|------------|
| <i>R. consobrina</i> | 1100IS72 | Europe | EU AY061666 | |
| <i>R. cuprea</i> | 2-1127IS77 | Europe | EU AY061667 | |
| <i>R. curtipes</i> | 1123IS77 | Europe | EU AY061668 | |
| <i>R. cyanoxantha</i> | 207RUS24 | Europe | EU AY061669 | |
| <i>R. delica</i> | hue22 | Europe | EU AF418605 | AF325303 |
| <i>R. densifolia</i> | ue116 (TUB) | Germany | DE AF418606 | |
| <i>R. disopus</i> | MM.00641 | Madagascar | MG JQ902055 | |
| <i>R. dissimulans</i> | OSA-MY-1727 | Japan | JP AB291731 | AB154717 |
| <i>R. drimeia</i> | 313IF57 | Europe | EU AY061672 | |
| <i>R. earlei</i> | WRW00-412 | United States | US DQ422025 | DQ422025 |
| <i>R. eccentrica</i> | HDT54344 | United States | US KF306039 | |
| <i>R. emetica</i> | UBCF20370 | Canada | CA KC581346 | KC581346 |
| <i>R. exalbicans</i> | n179/93 | Europe | EU AF418622 | AF325306 |
| <i>R. farinipes</i> | 484IS78 | Europe | EU AY061675 | |
| <i>R. fellea</i> | 316RUS25 | Europe | EU AY061676 | |
| <i>R. firmula</i> | AT2004142 | Sweden | SE DQ422017 | DQ422017 |
| <i>R. fistulosa</i> | BB99.529 | Europe | EU DQ421985 | DQ421985 |
| <i>R. foetens</i> | hue124 | Germany | DE AF418613 | AF325299 |
| <i>R. fuscorubroides</i> | hue168 (TUB) | Europe | EU AF418624 | |
| <i>R. galbana</i> | H4667 | Australia | AU EU019936 | EU019936 |
| <i>R. gelatinivelata</i> | TH8699 | French Guyana | GY KC155395 | KC155395 |
| <i>R. gelatinivelata</i> | SLM 10125 | French Guyana | GY JQ405655 | |
| <i>R. gracillima</i> | UE23.08.2004-14 | Sweden | SE DQ422004 | DQ422004 |
| <i>R. grisea</i> | UE2005.08.16-01 | Sweden | SE DQ422030 | DQ422030 |
| <i>R. helodes</i> | 497RUF26 | Europe | EU AY061680 | |
| <i>R. hemisilvae</i> | 1203IS83 | Cameroon | CM JQ902080 | |
| <i>R. heterophylla</i> | UE20.08.2004-2 | Sweden | SE DQ422006 | DQ422006 |
| <i>R. ilicis</i> | 563IC52 | Europe | EU AY061682 | |
| <i>R. illota</i> | UE26.07.2002-3 | Sweden | SE DQ422024 | DQ422024 |
| <i>R. inflata</i> | M00/344 | Madagascar | MG JQ902062 | |
| <i>R. ingwa</i> | MEL2101936 | Australia | AU EU019919 | EU019919 |
| <i>R. insignis</i> | 1223IS85 | Europe | EU AY061700 | |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.

Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|--|---------------------|---------------------------|-------------|------------|
| <i>R. integra</i> | 2-1126IS77 | Europe | EU AY061683 | |
| <i>R. integriformis</i> | 561IS78 | Europe | EU AY061684 | |
| <i>R. iterika</i> | JET1130 | Australia | AU EU019929 | EU019929 |
| <i>R. jilnensis</i> | HMAS 194253 | Cameroon | CM GU966632 | |
| <i>R. kanadii</i> | CUH AM087 | India | IN KM275230 | |
| <i>R. laricina</i> | 1112IS75 | Europe | EU AY061685 | |
| <i>R. laurocerasi</i> | 1087IS74 | Europe | EU AY061735 | |
| <i>R. leguminosarum</i> | MCA3958 | French Guyana | GY KT339221 | KT339221 |
| <i>R. leguminosarum</i> | TH7425 | French Guyana | GY KC155394 | |
| <i>R. lepida</i> | HJB9990 | Belgium | BE DQ422013 | DQ422013 |
| <i>R. lepida</i> | 218RUF24 | Europe | EU AY061686 | |
| <i>R. lepidicolor</i> | 493RUF26 | Europe | EU AY061687 | |
| <i>R. lilacea</i> | 707IC54 | Europe | EU AY061731 | |
| <i>R. littoralis</i> | 1222IS87 | United States | US AY061702 | |
| <i>R. luteomaculata</i> | 848IS45 | Africa | AF JQ902061 | |
| <i>R. maculata</i> | 524IC52 | Europe | EU AY061688 | |
| <i>R. mairei</i> | lw113 (TUB) | Germany | DE AF418620 | |
| <i>R. marangania</i> | MEL2293694 | Australia | AU EU019930 | EU019930 |
| <i>R. mariae</i> | SFC2012- 0922-07 | Korea | KR KF361777 | KF361827 |
| <i>R. melliolens</i> | 423IF57 | Europe | EU AY061690 | |
| <i>R. melzeri</i> | 1124IS77 | Europe | EU AY061691 | |
| <i>R. messapica</i> | 562IC52 | Europe | EU AY061692 | |
| <i>R. metachromatica</i> | TH7678 | French Guyana | GY KT339251 | |
| <i>R. metachromatica</i> | 951IS55 | French Guyana | GY JQ902096 | |
| <i>R. metachromatica</i> <i>subsp tarumaensis</i> | MCA1856 | French Guyana | GY JN168745 | JN168745 |
| <i>R. mustelina</i> | 503IS88 | Europe | EU AY061693 | |
| <i>R. myrmecobroma</i> | TH9145 | French Guyana | GY JN168752 | JN168752 |
| <i>R. nana</i> | 170RUS34 | Europe | EU AY061694 | |
| <i>R. nauseosa</i> | 735IF57 | Europe | EU AY061733 | |
| <i>R. neerimea</i> | MEL2101871 | Australia | AU EU019915 | EU019915 |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.
Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|----------------------------|-------------------|---------------------------|-------------|------------|
| <i>R. nigricans</i> | UE20.09.2004-07 | Sweden | SE DQ422010 | DQ422010 |
| <i>R. nitida</i> | 2-1148IS79 | Europe | EU AY061696 | |
| <i>R. ochraceorivulosa</i> | M99.130 | Madagascar | MG JQ902093 | |
| <i>R. ochroleuca</i> | 1101IS75 | Europe | EU AY061697 | |
| <i>R. ochrospora</i> | Donelli20.07.2004 | Italy | IT DQ412012 | DQ412012 |
| <i>R. odorata</i> | 1113IS75 | Europe | EU AY061698 | |
| <i>R. olivacea</i> | hue85 | Germany | DE AF418634 | AF325314 |
| <i>R. pallescens</i> | 146/2002 | Norway | NO DQ421987 | DQ421987 |
| <i>R. pallidospora</i> | JV02-218 | Europe | EU DQ422032 | DQ422032 |
| <i>R. paludosa</i> | 528RUS27 | Europe | EU AY061703 | |
| <i>R. parazurea</i> | MF01.10.2003 | Sweden | SE DQ422007 | DQ422007 |
| <i>R. pascua</i> | 1132IS76 | Europe | EU AY061705 | |
| <i>R. patouillardii</i> | 881IS47 | Africa | AF JQ902094 | |
| <i>R. paxilliformis</i> | SM-2012c | French Guyana | GY JQ405656 | |
| <i>R. pectinata</i> | 512IX77 | Europe | EU AY061706 | |
| <i>R. pectinatoides</i> | AT2001049 | Sweden | SE DQ422026 | DQ422026 |
| <i>R. persanguinea</i> | MEL2101880 | Australia | AU EU019916 | EU019916 |
| <i>R. persicina</i> | 499RUS26 | Europe | EU AY061707 | |
| <i>R. pilosella</i> | H5974 | Australia | AU EU019941 | |
| <i>R. polyphylla</i> | BB02.108 | Europe | EU DQ422027 | DQ422027 |
| <i>R. pseudocarmasina</i> | Buyck 5401 | Burundi | BI JQ902068 | |
| <i>R. pseudointegra</i> | 220RUS24 | Europe | EU AY061708 | |
| <i>R. puellaris</i> | 2-1099IS72 | Europe | EU AY061709 | |
| <i>R. puellula</i> | 2-1130IS76 | Europe | EU AY061710 | |
| <i>R. puiggarii</i> | TUB305-R | Ecuador | EC AY667425 | AY667425 |
| <i>R. puiggarii*</i> | Vasco-P 1224 | Colombia | CO KT724179 | |
| <i>R. puiggarii</i> | 1186IS85 | Cameroon | CM JQ902077 | |
| <i>R. puiggarii*</i> | JOH10 | Colombia | CO KT354746 | |
| <i>R. puiggarii*</i> | JOH11 | Colombia | CO KT354747 | |
| <i>R. puiggarii*</i> | Vasco-P 1995 | Colombia | CO KT354745 | |
| <i>R. puiggarii*</i> | Vasco-P 1813 | Colombia | CO TBP | |
| <i>R. puiggarii*</i> | Vasco-P 1555 | Colombia | CO TBP | |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.
Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | | ITS | LSU |
|--------------------------------|-----------------|---------------------------|----|------------|------------|
| <i>R. puiggarii</i> * | MAN 701 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | MJ 84 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | ACM 992 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | J. Duque 46 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | J. Duque 54 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | Vasco-P 1788 | Colombia | CO | TBP | TBP |
| <i>R. puiggarii</i> * | Vasco-P 1797 | Colombia | CO | TBP | TBP |
| <i>R. puiggarii</i> * | Vasco-P 1887 | Colombia | CO | TBP | TBP |
| <i>R. puiggarii</i> * | MJ 130 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | MAN 980 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | ACM 343 | Brazil | BR | TBP | TBP |
| <i>R. puiggarii</i> * | ACM 525 | Brazil | BR | TBP | |
| <i>R. puiggarii</i> * | MJ 111 | Brazil | BR | TBP | |
| <i>R. puiggarii</i> * | BZL26 | Brazil | BR | TBP | |
| <i>R. puiggarii</i> * | MAN 972 | Brazil | BR | TBP | |
| <i>R. puiggarii</i> * | Vasco-P 2096 | Colombia | CO | TBP | |
| <i>R. pulverolenta</i> | 4-1144IS79 | Europe | EU | AY061736 | |
| <i>R. pumicoidea</i> | Trappe 14771 | Australia | AU | EU019931 | EU019931 |
| <i>R. purpureoflava</i> | MEL2101866 | Australia | AU | EU019914 | EU019914 |
| <i>R. raoultii</i> | hue94 | Europe | EU | AF418621 | AF325317 |
| <i>R. reddelli</i> | H6172 | Australia | AU | EU019944 | |
| <i>R. risigallina</i> | UE03.07.2003-08 | Sweden | SE | DQ422022 | DQ422022 |
| <i>R. romellii</i> | 2-1119IS77 | Europe | EU | AY061714 | |
| <i>R. rosea</i> | 2-1121IC75 | Europe | EU | AY061715 | |
| <i>R. roseipes</i> | 482IS76 | Europe | EU | AY061716 | |
| <i>R. rostraticystidia</i> | H6165 | Australia | AU | EU019938 | EU019938 |
| <i>R. rubra</i> | 481IS75 | Europe | EU | AY061717 | |
| <i>R. rubrolutea</i> | Trappe 12610 | Australia | AU | EU019940 | EU019940 |
| <i>R. rubropunctatissima</i> * | MAN 1044 | Brazil | BR | TBP | TBP |
| <i>R. rubropunctatissima</i> * | MJ 46 | Brazil | BR | TBP | TBP |
| <i>R. rubropunctatissima</i> * | ACM 962 | Brazil | BR | TBP | TBP |
| <i>R. rubropunctatissima</i> * | ACM 961 | Brazil | BR | TBP | TBP |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.

Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | ITS | LSU |
|--------------------------------|----------------|---------------------------|-------------|------------|
| <i>R. rubropunctatissima</i> * | J. Duque 53 | Brazil | BR TBP | TBP |
| <i>R. rubropunctatissima</i> * | J. Duque 55 | Brazil | BR TBP | TBP |
| <i>R. rubropunctatissima</i> * | MJ 141 | Brazil | BR TBP | TBP |
| <i>R. rubropunctatissima</i> * | MJ 53 | Brazil | BR TBP | |
| <i>R. rubropunctatissima</i> * | MJ 113 | Brazil | BR TBP | |
| <i>R. sanguinea</i> | 314RUF31 | Europe | EU AY061718 | |
| <i>R. sardonía</i> | hue41 (TUB) | Europe | EU AF418626 | |
| <i>R. senecis</i> | CUH AM102 | India | IN KP142981 | |
| <i>R. sichuanensis</i> | HKAS53792 | China | CN JX391969 | JX391972 |
| <i>R. sinuata</i> | H4755 | Australia | AU EU019943 | |
| <i>R. solaris</i> | hue219 | Europe | EU AF418627 | AF325319 |
| <i>R. sphagnophila</i> | 3-1118IS76 | Europe | EU AY061719 | |
| <i>R. subnigricans</i> | OSA-MY-4266 | Japan | JP AB291747 | |
| <i>R. tapawera</i> | Trappe 12607 | New Zealand | NZ EU019935 | EU019935 |
| <i>R. tenuipilosa</i> | 839RUS37 | Madagascar | MG JQ902059 | |
| <i>R. tuberculosa</i> | 1438I119 | Cameroon | CM JQ902056 | |
| <i>R. turci</i> | 541RUS27 | Europe | EU AY061720 | |
| <i>R. variispora</i> | H5855 | Australia | AU EU019934 | EU019934 |
| <i>R. versicolor</i> | 320RUS25 | Europe | EU AY061722 | |
| <i>R. velenovskyi</i> | 526IS77 | Europe | EU AY061721 | |
| <i>R. venezueliana</i> | TH7874 | French Guyana | GY KT339269 | |
| <i>R. vesca</i> | AT2002091 | Sweden | SE DQ422018 | DQ422018 |
| <i>R. veternosa</i> | hue212 (TUB) | Europe | EU AF418630 | |
| <i>R. vinosa</i> | 500RUF26 | Europe | EU AY061724 | |
| <i>R. violácea</i> | 322IS55 | Europe | EU AY061725 | |
| <i>R. violaceotunicata</i> * | MJ 116 | Brazil | BR TBP | TBP |
| <i>R. violaceotunicata</i> * | ACM 960 | Brazil | BR TBP | TBP |
| <i>R. virescens</i> | HJB9989 | Belgium | BE DQ422014 | DQ422014 |
| <i>R. víscida</i> | ubc f16576 | Canada | CA FJ627039 | FJ627039 |
| <i>R. wollumbina</i> | MEL2238232 | Australia | AU EU019921 | EU019921 |
| <i>R. xerampelina</i> | UBC F23914 | Canada | CA KJ146730 | KJ146730 |
| <i>Russula</i> sp. 1* | Vasco-P 1915 | Colombia | CO TBP | TBP |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided. Taxa highlighted are the type specimens for the new species.

| Taxa | Voucher | Locality (abbrev.) | | ITS | LSU |
|-----------------------------------|----------------|---------------------------|----|------------|------------|
| <i>Russula</i> sp. 1* | Vasco-P 1886 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. 2* | Vasco-P 1996 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. 3* | Vasco-P 2209 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. 4* | Vasco-P 1796 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. 5* | JOH 20 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 6* | DLK 395 | Brazil | BR | TBP | |
| <i>Russula</i> sp. 7* | Vasco-P 1874 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 8* | Vasco-P 2093 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 9* | MAN 222 | Brazil | BR | TBP | |
| <i>Russula</i> sp. 10* | Vasco-P 1532 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. 10* | Vasco-P 1357 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 11* | Vasco-P 2200 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 12* | Vasco-P 2022 | Colombia | CO | TBP | |
| <i>Russula</i> sp. 13* | MAN 919 | Brazil | BR | TBP | |
| <i>Russula</i> sp. nov. 1* | MJ 114 | Brazil | BR | TBP | TBP |
| <i>Russula</i> sp. nov. 1* | MJ 121 | Brazil | BR | TBP | TBP |
| <i>Russula</i> sp. nov. 2* | Vasco-P 2001 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. nov. 2* | Vasco-P 2002 | Colombia | CO | TBP | |
| <i>Russula</i> sp. nov. 2* | Vasco-P 2202 | Colombia | CO | TBP | |
| <i>Russula</i> sp. nov. 3* | JOH 46 | Colombia | CO | TBP | TBP |
| <i>Russula</i> sp. nov. 3* | JOH 19 | Colombia | CO | KT354753 | |

Cont. Tab.1. *sequences generated for this study. TBP= to be provided.
Taxa highlighted are the type specimens for the new species.

RESULTS

Molecular Technics and Phylogenetic analyses

Three new species of *Russula* are described based on morphological and molecular analyses: *Russula* sp. nov. 1, from Brazil, and *Russula* sp. nov. 2 and *Russula* sp. nov. 3 from Colombia. Fifty-

seven ITS, and 31 LSU new sequences were generated for this study, while 161 ITS, and 59 LSU sequences were taken from GenBank database (Table 1). The rDNA amplification products ranged from 474 to 713 bp for ITS and 425 to 1230 bp for LSU. The combined ITS+LSU alignment included 1502 characters: 166 bp of ITS1, 162 bp of 5.8s, 212 bp of ITS2, 790 bp of LSU, and 172 of indel coding characters. The ITS alignment included 881 characters: 199 bp of ITS1, 160 bp of 5.8s, 219 bp of ITS2, and 303 of indel coding characters. The combined dataset for ITS+LSU analysis revealed 474 characters that were parsimony informative, and 680 conserved sites, while in the ITS analysis 355 were parsimony informative and 151 were conserved sites.

The topologies of the Maximum Likelihood analyses from ITS+LSU and ITS are presented (Fig. 1 and 2), with both BS and BPP values shown on the branches (BS/BPP). Four major groups at deeper branch level were recovered in the ITS+LSU analyses: 1) formed by *a*, *b* and *c clades* (BS=27, BPP=0.87), 2) formed by the *d-j clades* plus *R. iterika* Grgur. (BS=65, BPP=1), 3) *clade k* (BS=91, BPP=1), and 4) *clade l* (BS=64, BPP=0.99) (fig.1). On the other hand, the single ITS

analyses showed thirteen major clades with a moderate to strong BS and BPP branch support (fig. 2).

In the first major group (fig. 1), members of sect. *Plorantes* clustered in two separated groups, *clades a* and *c*, indicating that sect. *Plorantes* is not monophyletic. The *clade b* was formed by a member of sect. *Metachromaticae* (*R. methachromatica* subsp. *Tarumaensis*) and *Russula* sp. nov. 1.

In the second major group (*clades d-j* fig.1) clustered member of subgen. *Heterophyllidia*, subgen. *Ingratula*, subgen. *Amoenula*, subgen. *Compacta* and sect. *Rigidae*. In *clade d* (BS=99, BPP=1) clustered members from subsect. *Pluviales* Singer (subgen. *Heterophyllidia*, sect. *Pelliculariae*) and *Russula* sp. nov. 2. In *clade e* clustered members of the subsect. *Mimeticinae* Buyck (subgen. *Heterophyllidia*, sect. *Pelliculariae*). *Clade e* appeared as more closely related to *clade d* in the ML analysis (BS= 71), while in the BI analysis *clade e* appears as more closely related, but with a low posterior probability (BPP= 0.6, topology not shown), to members of the subgenus *Ingratula* (*clade f*). In the ML analysis *clade f* appears as more closely related to members of subgen. *Ingratula* and sect. *Pelliculariae*

(*clade g*). In *clade h* (BS=100, BPP=1) grouped several specimens of *R. puiggarii* (Speg.) Singer (subgen. *Heterophyllidia*). Member of subgen. *Amoenula* and sect. *Rigidae* clustered in *Clade i* (BS=100, BPP=1). In the *clade j* (BS=97, BPP=1) clustered only member of sect. *Heterophyllae*.

The third major group recovered was formed by members of subgen. *Russula* (BS=91, BPP=1; *clade k*), within this *clade* clustered *Russula* sp. nov. 3 with *R. gelatinivelata* S.L. Mill., Aime & T.W. Henkel as its more closely related species. The fourth major group (*clade l*) clustered members of sect. *Compacta* (BS=64, BPP=0.99).

Russula metachromatica sequences clustered in the same *clade* in the ITS analysis (fig. 2) but appeared as three different lineages. *Russula* sp. nov. 1 appeared as more closely related to *R. metachromatica* var. *tarumaensis* in the ITS+LSU analysis (BS=98, BPP=1), while *Russula* sp. nov. 1 appeared as more closely related to other two *R. metachromatica* sequences (TH7678 and 951IS55), but with low support (BS=46, BPP=0.82), in the ITS analysis.. The phylogenetic relationship of *R. pachycystis* and *Russula* sp. nov. 1 could not be tested because of the lack of sequences for *R. pachycystis*.

Russula sp. nov. 2 clustered in sect. *Pluvialis* and appeared as more closely related to *R. violaceotunicata* and *R. aff. pluvialis* in the ITS+LSU analysis (BS=99, BPP=1), while it was more closely related to *Russula* sp. 12 in the ITS analyses. *Russula* sp.nov. and *Russula* sp. 12 were closely related with the group formed by *R. rubropunctatissima*, *Russula* sp. 11 and *R. venezueliana*. *Russula* sp. nov. 3 appeared as a member of the subg. *Russula* forming a moderately to well supported clade with *R. gelatinivelata* in both, the single ITS and the ITS+LSU analyses (BS=100 and BPP=1 respectively).



Figure 1. Maximum Likelihood tree topology based on combined analyses of ITS and LSU sequences. Support values are shown on branches, Bootstrap Support (BS>70%) superior number, and Posterior Probability (BPP>90%) inferior number. Letters on right of the species name correspond to the sequence geographical origin (Tab. 1). Arrows indicate the new species described in the present study. Letters on the right side indicate the clade.

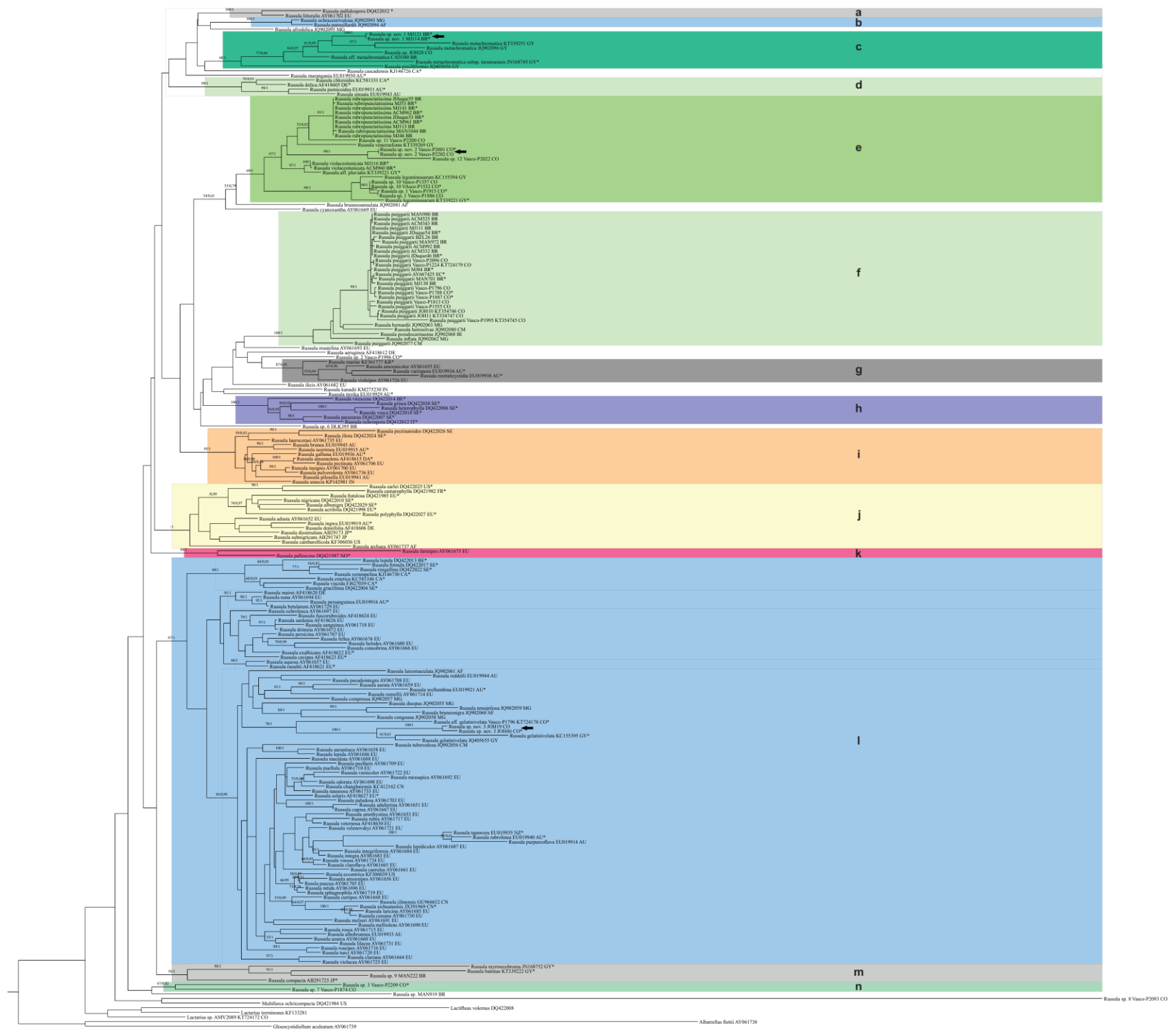


Figure 2. Maximum Likelihood tree topology based on ITS sequences analysis. Support values are shown on branches, Bootstrap Support (BS>70%) superior number and Posterior Probability (BPP>90%) inferior number. Arrows indicate the new species described in the present study. Letters on right of the species name correspond to the sequence geographical origin (Tab. 1). * Species included in the ITS+LSU analyses.

Taxonomy

***Russula* sp. nov. 1** (Figs. 3, 8A, 9A)

Type: BRAZIL, SANTA CATARINA, Florianópolis, Pântano do Sul, Lagoinha do Leste Trail, 04 January 2012, Jaeger MJ114.

Pileus parabolic or convex to plane, 24 to 34 mm diameter, surface off-white to cream (oac816 to oac857), stained irregularly of pale orange to ferruginous (oac758), finely squamose; margin regular to slightly crenate, straight to incurved. **Lamella** concolorous with pileus, off-white to cream, unchanging when bruised, adnexed to subdecurrent, narrow to moderately broad, subdistant to close; margin entire to finely eroded. Lamellula present. Spore print not obtained. **Stipe** 18-5 × 4-7 mm, off-white to cream, stained like the pileus, finely fibrous and longitudinally striate, slightly excentric, equal to tapering at the base, with thick rhizomorphs. **Context** whitish, unchanging, spongy and with 6 or more cavities. On soil.

Basidiospores 9-7 × 7-8.5 µm, globose to subglobose Q = 1.04, with amylaceous ornamentations, spines or conical warts to crests, with connecting lines forming an incomplete to complete reticulation, projecting 0.7 – 1.5 µm, ornamented amyloid plage, hilar appendix 1.5 -

2.5 μm . **Hymenophoral trama** with sphaerocysts in groups like rosettes, few dispersed, intermixed with few oleiferous hyphae, in edge-near portion sphaerocysts often smaller and scarce prostate and more flattened hyphae. **Pleurocystidia** abundant up to 90 μm , thick-walled, subcylindrical, often ventricose to sinuose, rounded-obtuse at the top, with droplets and needle-like contents, light brown, staining in sulphobenzaldehyde. **Cheilocystidia** abundant, clavate or filiform to cylindrical, apex rounded to obtuse or capitate. **Pileipellis** with 3 layers, suprapellis consisting of an ixotrichodermial layer composed of rounded to obtuse apex septate hypha, and pileocystidia; mediopellis is a gelatinized layer of scarce oleiferous hyphae intermixed with interwoven hyphae, some swollen in the middle; subpellis is a layer of prostate hyphae intermixed with scarce and large sphaerocysts.

Materials examined: BRAZIL. SANTA CATARINA: Florianópolis, Pântano do Sul, Lagoinha do Leste Trail, 27 January 2011, Jaeger MJ06 (FLOR), 04 January 2012, Jaeger MJ114 & Jaeger MJ121.

Additional materials examined: BRAZIL. AMAZONAS: Estrada Manaus-Caracará km 45, 25 June 1980, Singer B12123 (INPA,

HOLOTYPE of *Russula pachycystis*), Estrada Manaus- Caracaráí km 125, 21 May 1978, Singer B11051 (INPA, *Russula metachromatica* ssp. *metachromatica*).

Notes: Two species are similar with *Russula* sp. nov. 1, *R. metachromatica* ssp. *metachromatica* Singer and *R. pachycystis* Singer. The three species have off-white to white yellowish basidiomata that are macroscopically similar to *Russula* sp. nov. 1. When compared to *Russula* sp. nov. 1, *R. metachromatica* ssp. *metachromatica* presents a pileipellis with more abundant and longer thick walled pileocystidia; spores with similar dimensions but ornamentations that do not form a complete network. *Russula pachycystis* has less abundant pleurocystidia and spores are bigger ($8-11.7 \times 7.5-10.8 \mu\text{m}$) with a suprahilar amyloid spot, while the spores of *Russula* sp. nov. 1 are $9-7 \times 7-8.5 \mu\text{m}$ with an ornamented plage. The morphological analysis that we made suggest that *Russula* sp. nov. 1 fits better in the section *Metachromaticae* Singer than sect. *Pachycystides* Singer.

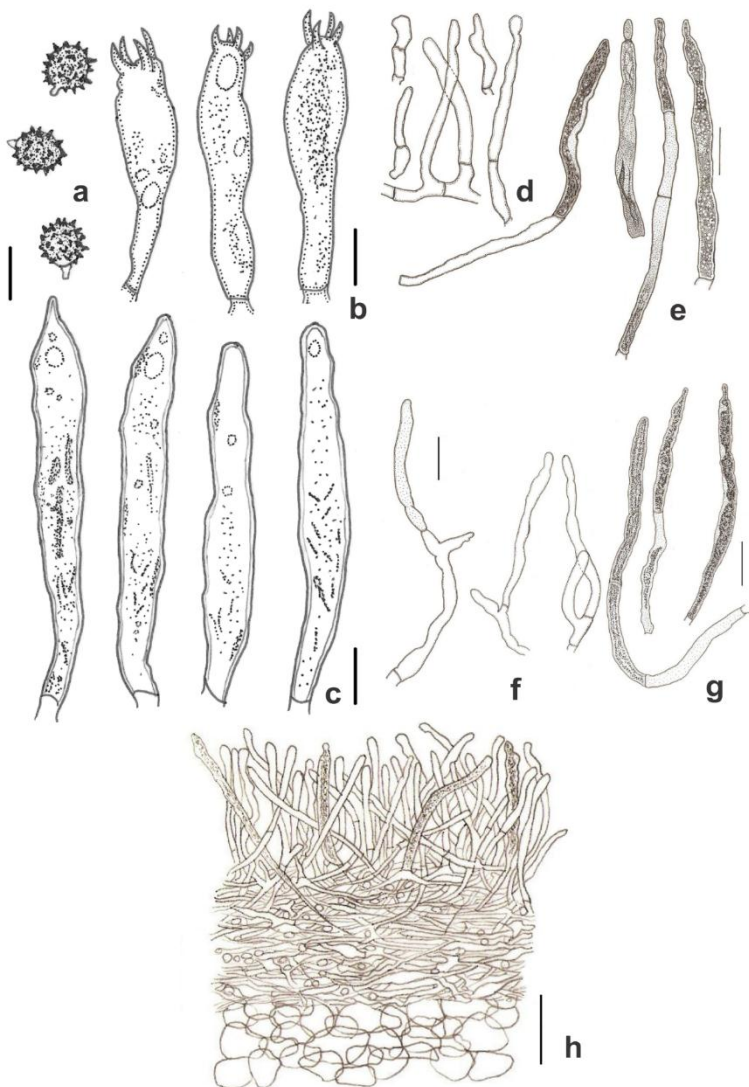


Figure 3. Microscopic structures of *Russula* sp. nov. 1. a) Basidiospores, b) Basidia, c) Pleurocystidia, d) terminal hyphae from the center of pileipellis, e) Pileocystidia from the center of pileipellis, f) terminal hyphae from the margin of pileipellis, g) Pileocystidia from the margin of pileipellis, h) cross-section of the pileipellis.

Pileocystidia from the margin of pileipellis, h) Pileipellis. Scale bar (a-g) 10 μm , (h) 50 μm .

***Russula* sp. nov. 2** (Figs. 4, 8C, 9B)

Type: COLOMBIA. AMAZONAS: Amazonas, El Zafire, Varillal, 09 January 2012, Vasco-P 2001 (HUA 196944).

Pileus up to 20 mm wide, convex when young to plane with a slightly depressed center at maturity, fleshy to membranous; surface pruinose, reddish brown, dark brown to greyish brown color (9F) at young to brown (6E4-6E5) at maturity; margin striate when young, wavy to areolate at maturity. **Context** white, up to 1mm width, spongy. **Lamellae** up to 1mm wide, adnate, anastomosing, serous; white color, blackish margin, some with reddish tonality (5A6). **Stipe** up to 20mm long, up to 2mm thick; central, equal, tapering toward the base; surface yellowish white (3A2) at the apex, brownish gray (8F2) toward the base, pruinose; hollow.

Basidiospores 6.4-8.8 \times 6.4-7.2 μm (Q=1.08), globose to subglobose, spines or conical warts, very few with connecting lines.

Basidia 25.6-32(-35) \times 7.2-8.8 μm , four-spored, sterigmata stout 3.2-4.8

× 0.8-1.6 µm, cylindrical to clavate. **Pleurocystidia** (43)51.6-89(100) × 7.2-13.6 µm, cylindrical to fusiform, with rounded apex, originating from the lamellar trama, with refringent granular homogeneous content. **Pileipellis** virescens-type, with three layers. Suprapellis of septate hyphae with 3-2 segments, basal segments globose, apical segments are ventricose, globose to slightly ellipsoid. Mediopellis with endocystidia of homogeneous granular content and interwoven hyphae. Subpellis of repent hyphae, some gloeopleural hyphae present.

Materials examined: COLOMBIA. AMAZONAS: Amazonas, El Zafire, Varillal, 09 January 2012, Vasco-P 2001 (HUA 196944), 18 march 2013 Vasco-P 2202 (HUA 196947), Vasco-P 2203 (HUA 196948).

Notes: *Russula* sp. nov. 2 is characterized by the almost reddish brown to dark brown basidiomata, striate pileus margin and anastomosing lamellae. *Russula* sp. nov. 2 is macromorphologically similar to *R. pluvialis* and *R. violaceotunicata* Buyck & Courtec (both from sect. *Pluviales*). The former species has reddish brown colors while the last two have purple colors. This three species have a virescens type pileipellis with pileocystidia well developed as similar

morphological characteristic. Other species with the same type of pileipellis is *Russula rubropunctatissima* (sect. *Pluviales*), however, the pileipellis present reddish incrustations in KOH and has a brighter reddish pileus with granular scales that can be easily seen with a hand lens. The morphological data presented here suggest the placement of *Russula* sp. nov. 2 in subsection *Pluviales*.

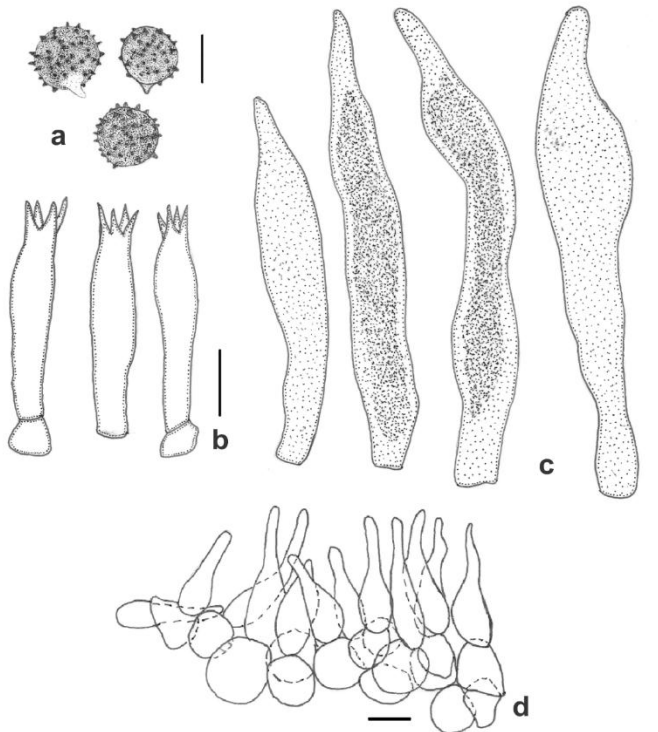


Figure 4. Microscopic structures of *Russula* sp. nov. 2. a) Basidiospores, b) Basidia, c) Pleurocystidia, d) Pileipellis. Scale bar a) 5 μ m, b-d) 10 μ m.

***Russula* sp. nov. 3** (Figs. 5, 8B, 9C)

Type: COLOMBIA. AMAZONAS: Amazonas, El Zafire, Varillal, 20 march 3013, JOH 46 (HUA 196958).

Pileus 14-59 mm wide, convex, plane to depressed; surface dry, smooth, radially zonate, scrobiculate, yellow (3A4) at center, light cream (2A2) toward the margin; margin sulcate, cuticle easily removable. **Context** 1- 2 mm width. **Lamellae** 2-3 mm wide, adnate, white, forking at center and margin of the pileus; margin serrate. **Lamellulae** absent. **Stipe** 24-61 mm long, 5-9 mm thick at the apex and 8-13 mm thick at the base, equal to clavate; surface dry, slightly striate, white color, changing to cream at touch. Context hollow to spongy. Basal mycelium scarce.

Basidiospores (7.2)8-9.6(11.2) \times 7.2-9.6 μ m (Q=1.052), globose to subglobose, ornamentation of mostly cylindrical warts of less than 0.8 μ m high, interconnected by fine lines in an incomplete reticulum, plage inamyloid. **Basidia** (32.8)36-42 \times 11.2-14.4 μ m, clavados, four-spored; sterigmata 4-8.8 \times 1.8-3.2 μ m. **Pleurocystidia**

(57)68-112(116) × (11)15.2-20 μm, fusiform to subclavate, obtuse to capitate, thin walled, with refringent vacuolar content, the longer ones arising deep within the lamellar trama, the shorter ones arising in the hymenium. **Subhymenium** palisadic, composed of relatively small, nearly isodiametrical to globose cells. **Lamellar trama** almost exclusively composed of sphaerocysts, with some refringent oily hyphae. **Pileipellis** one-layered, with repent to slightly erect hyaline hyphae, hyphae without content, cylindrical with rounded apex.

Materials examined: COLOMBIA. AMAZONAS: Amazonas, El Zafire, Varillal, 19 march 2013, JOH 19 (HUA 196957), 20 march 2013, JOH 46 (Type: HUA 196958).

Notes: The main diagnostic characteristic of *Russula* sp. nov. 3 is the white, radially zonate, and scrobiculate pileal surface. *Russula* sp. nov. 3 has morphological similarities with *R. gelatinivelata*, both species present forking lamellae and the lamellulae are absent, the size, form and origin of pleurocystidia are similar. *Russula gelatinivelata* present 75–130 × 15–18 μm pleurocystidia while those of *Russula* sp. nov. 3 are (57)68-112(116) × (11)15.2-20 μm. Both species have subclavate to cylindrical, obtuse to capitate, thin-walled pleurocystidia,

and in both species the pleurocystidia arise either deep in the lamellar trama or in the hymenium. The spores of *R. gelatinivelata* have isolated elements on the ornamentation while *Russula* sp. nov. 3 has spores with at least a partial reticulum. *Russula* sp. nov. 3 can be easily macromorphologically mistaken with *Russula* sp. (AMV 2209, fig, 6D, 7 and 8), an undescribed taxon from Colombian Amazon. However *Russula* sp. nov. 3 has fewer pleurocystidia, with a format completely different.

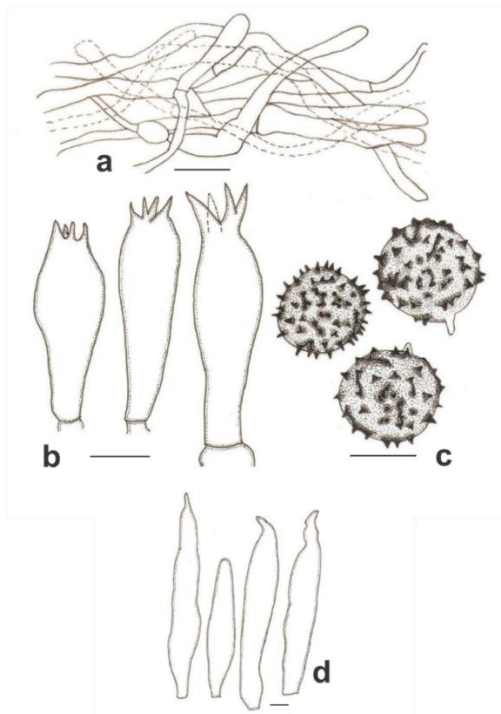


Figure 5. Microscopic structures of *Russula* sp. nov. 3. a) Pileipellis, b) Basidia, c) Basidiospores, d) Pleurocystidia. Scale bar (a,b and d) 10 μ m, (c) 5 μ m.

Russula rubropunctatissima J.L. Cheype & E. Campo, *Bull. Soc. mycol. Fr.* 128(1-2): 128 (2013) (Figs. 6, 8E, 9D)

Pileus 15-50 mm wide, convex to pulvinate when young and plane to depressed when mature; surface bright red (oac656 or oac621) when young, pinkish (oac667, oac652 or oac666) to ferruginous (oac658) in overall appearance when mature, when magnifying, the color background is cream with dark red (oac621) granular scales, the scales are bigger in the center and smaller toward the margin. Margin initially straight to decurved, becoming straight to uplifted, entire or shallowly striate when mature. **Lamellae** dark red in young specimens and cream (oac816), yellowish (oac812) when bruised, adnexed when young and decurrent in the mature, close to crowded; margin entire. **Lamellulae** intermixed, of different lengths, irregularly placed. Spore print not observed. **Stipe** 30-48 mm long, 4-6 mm thick, concolorous with pileus, central, equal or tapering downwards in the younger and slightly clavate when mature, texture firm. **Context** whitish, changing to

yellowish when injured, firm to spongy. Odor pleasant, taste a little acid. Basidiomata growing in groups, single or caespitose. On soil.

Basidiospores (5.5)6-6.5 × 5.5-6 μm, Q = 1.11, subglobose to globose, ornamentation amyloid, up to 0.6 μm high, composed of rounded spines, without connecting lines, suprahilar plage smooth or with few tiny ornamentations, slightly amyloid or inamyloid, hilar appendix 1.2-1.6 μm. **Basidia** 38-65 × 5-9 μm, clavate, four sterigmata. **Pleurocystidia** abundant, up to 110 μm, cylindric, clavate or obclavate, apex rounded or capitate, with refractive and granular contents, staining lightly brown in sulphobenzaldehyde, arisen from the hymenium. **Cheilocystidia** subclavate, with rounded or capitate apex, thin-walled. **Hymenophoral trama** near the edge of lamellae with numerous small to medium sized (7 × 8 μm) sphaerocysts, rounded to irregular, larger at half of the trama with large (15 × 20 μm) and irregular sphaerocysts intermixed with few laticiferous hyphae, not staining in sulphobenzaldehyde. **Pileipellis** consisting of three layers. Suprapellis subhymeniform, with dermatocystidia clavate, often with yellow content in KOH, raising from spherical or ampulaceous cells intermixed with laticiferous hyphae that project like pseudodermatocystidia, and large

sphaerocysts. Abundant red crystals above suprapellis elements, like incrustation, not dissolving in water or KOH. These same crystals appear above the hymenial elements in young specimens. Mediopellis of large spherical sphaerocysts intermixed with laticiferous hyphae. Subpellis of prostrated hyphae.

Materials examined: BRAZIL. SANTA CATARINA: Florianópolis, Pântano do Sul, Lagoinha do Leste Trail, 04 January 2012, Jaeger MJ113; 18 December 2011, Neves 1044; Lagoa da Conceição, Morro da Lagoa Trail, 16 March 2011, Jaeger MJ46; Jaeger MJ53 (FLOR).

Notes: *Russula rubropunctatissima* is characterized by the bright red color when young and pinkish overall appearance when mature. Microscopically it presents abundant red crystals above the suprapellis and the hymenial elements. There are no other species of *Russula* known from the neotropics that look like *R. rubropunctatissima*. Cheype and Campo (2012) placed *R. rubropunctatissima* in subsect. *Pluviales*, however they were doubtful about this classification emphasizing the need of molecular phylogenies to place this species in the right group.

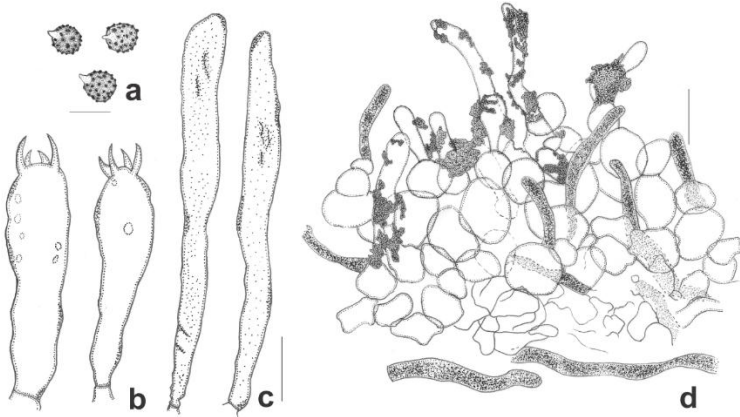


Figure 6. Microscopic structures of *Russula rubropunctatissima*. a) Basidiospores, b) Basidia, c) Pleurocystidia, d) Pileipellis. Scale bar 10 μm .

Russula violaceotunicata Buyck & Courtec. Courtecuisse & Buyck, *Mycol. helv.* 4(2): 216 (1991). (Figs. 7, 8F, 9E)

Pileus 13-20 mm wide, pulvinate in young specimens to plane-convex with depressed center when mature, purple (oac364/363) sometimes discolored at the center (oac428/430/427), often with a concentric zone near the margin, with acute margin that becomes sulcate and distinctly pectinate 3-6 mm; surface subvelutinous to punctate. **Context** white, unchanging when injured. Odor pleasing. **Lamellae** white to cream (oac816), adnate to decurrent, close to crowded,

intermixed with lamellulae, and regular to simple forked. **Stipe** usually entirely purple, paler than the pileus, violet punctate, equal to flexuous, 14-22 × 2-4 mm, with more than 10 cavities, basal mycelium absent. Solitary or in pairs on soil.

Basidiospores 7-7.5(8.0) × 6-7 μm, subglobose (Q = 1.10), heterotropic, with amylaceous echinulate ornamentation, spines or points, projecting up to 0.7 μm, without connecting lines, with a distinct plage, sometimes not totally smooth, with some little ornamentations. **Basidia** 35-40 × 7-10 μm, 4-spored, with droplets contents. **Pleurocystidia** abundant, up to 55 μm. **Cheilocystidia** clavate to cylindrical, rounded apex, with granular amorphous content, negative in sulphobenzaldehyde. **Lamellar trama** with sphaerocysts subangular (heteromerous trama). **Pileipellis** consisting of a suprapellis of dermatocystidia raising from sphaerocysts, normally from chains of rounded sphaerocysts, virescens-type structure, sometimes more ampullaceous cells, dermatocystidia medium sized 15-40 μm, in clusters, cylindrical, rounded at the apex, with granular content; subpellis consisting of horizontal hyphae, but more ascendant just below the suprapellis, with non-gelatinized matrix, accompanied by abundant

oleiferous hyphae. **Stipitipellis** consisting of interwoven hyphae in a slightly gelatinous layer, appressed and septate hyphae and oleiferous hyphae with thick-walled, more ascendant at the margin but not forming trichodermium.

Materials examined: BRAZIL. SANTA CATARINA: Florianópolis, Pântano do Sul, Lagoinha do Leste Trail, 04 January 2012, Jaeger MJ116 & Jaeger MJ117 (FLOR), Santo Amaro da Imperatriz, Plaza Caldas Da Imperatriz Resort & Spa Trail, 13 May 2012, Jaeger MJ140 (FLOR).

Additional materials examined: BRAZIL. AMAZONAS: Manaus, in secondary forest, 5 June 1977, Singer B 9933 (INPA, HOLOTYPUS of *Russula pluvialis*).

Notes: *Russula violaceotunicata* seems to be closely related to the Amazonian *R. pluvialis*, which has the same size and spore type but differs in the pileipellis structure that is three layered in *R. pluvialis* and two layered in *R. violaceotunicata*. The sphaerocysts in *R. violaceotunicata* are more abundant than regular hyphae, while *R. pluvialis* has fewer sphaerocysts.

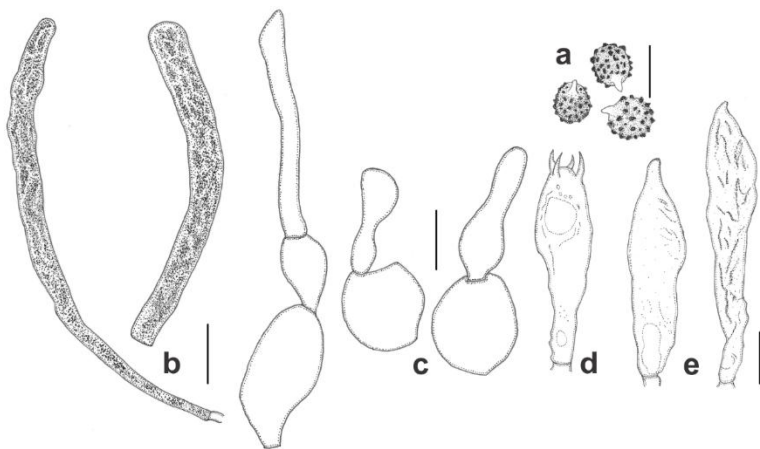


Figure 7. Microscopic structures of *Russula violaceotunicata*. a) Basidiospores, b) Pileocystidia, c) Terminal hyphae from pileipellis, d) Basidia, e) Pleurocystidia. Scale bar 10 μ m.

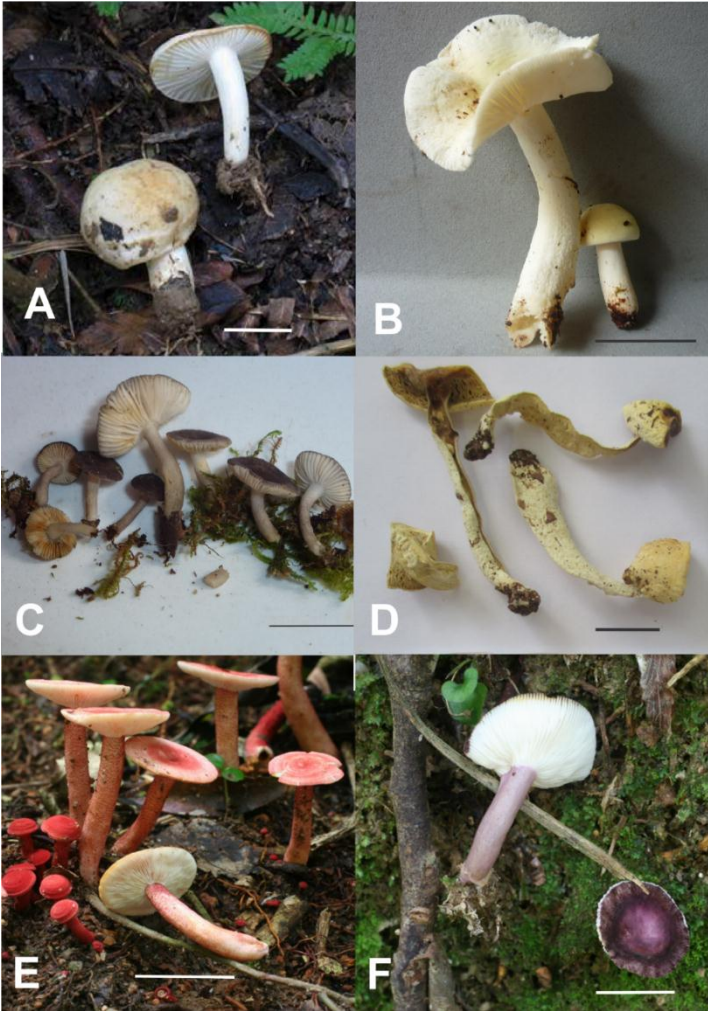


Figure 8. Basidiomata. A) *Russula* sp. nov. 1, B) *Russula* sp. nov. 3, C) *Russula* sp. nov. 2, D) *Russula* sp. 3 (AMV 2209), E) *R. rubropunctatissima*, F) *R. violaceotunicata*.

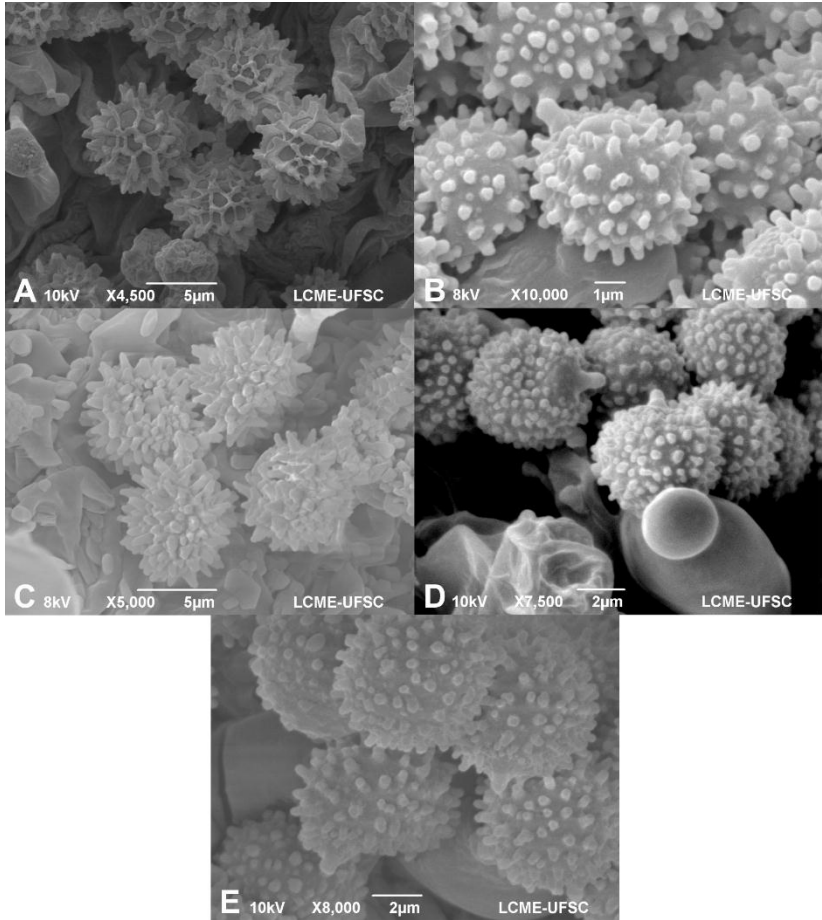


Figure 9. Basidiospores MEV. A) *Russula* sp. nov. 1, B) *Russula* sp. nov. 2, C) *Russula* sp. nov. 3, D) *R. rubropunctatissima*, E) *R. violaceotunicata*.

DISCUSSION

The phylogenetic analyses here presented agree with Buyck and Ovrebo (2002) in that there is no clear distinction between sect. *Plorantes* and sect. *Metachromaticae*. Both ITS and ITS+LSU analyses show that sections *Plorantes* and *Metachromaticae* together (*clades a, b and c*), might represent one higher-level clade at the subgeneric or sectional level. The ixotrichodermal structure of the pileipellis, spores ornamentation, and metachromatic pleurocystidia of *Russula* sp. nov. 1 and *R. metachromatica* show the morphological affinity between both species.

Based on morphology and molecular phylogenies *Russula* sp. nov. 2 fits well in *Russula* subsect. *Pluviales*, a neotropical subsection that includes species with basidiomata that are macroscopically characterized by lilac, purple, violet, pinkish, and reddish to reddish brown pileus. Microscopically the taxa in this group have ellipsoid spores with echinulate, spiny or punctuate ornamentation, pleurocystidia, well-developed dermatocystidia, and a virescens-type pileipellis. Two interesting species from subsect. *Pluviales* were found during this study that represent new records from Brazil, *R.*

violaceotunicata and *R. rubropunctatissima*. Both species were previously known only from French Guyana (Courtecuisse & Buyck, 1991; Cheype & Campo, 2012).

Singer et al. (1983) placed *R. leguminosarum* in subsect. *Pluviales*, while Buyck (1988; 1989) suggested that *R. leguminosarus* is a member of subsect. *Mimeticinae*. Our analyses suggest that *R. leguminosarum* can be assigned to a different clade of that of *Pluviales*. However, phylogenetic analyses including more members from subsect. *Mimeticinae* are needed to corroborate *R. leguminosarum* as a member of *Mimeticinae*. Two ITS sequences of *R. leguminosarum* from Guyana Francesa that formed two different lineages, suggest that more studies focused on *R. leguminosarum* are needed to have a better definition of the *sensu stricto* of this species. Between the two sequences of *R. leguminosarum* nested four sequences from Colombia (*Russula* sp. 1 and *Russula* sp. 10) that need more morphological studies to have a better knowledge of *clade e*. *Russula* sp. nov. 3 is phylogenetically closely related to *R. gelatinivelata*, both species have morphological affinities such as spores with isolated elements in the ornamentation and pleurocystidia with similar size and form. *Russula gelatinivelata* and

Russula sp. nov. 3 fall within the subgenus *Russula* forming a monophyletic clade together with another undescribed species from Colombian Amazon (*Russula* aff. *gelatinivelata* Vasco-P1796). Miller *et al.* (2012) pointed out the consistent relationship of *R. gelatinivelata* to other species from subgenus *Russula*, *R. nauseosa* (Pers.) Fr., *R. cessans* A. Pearson, *R. laricina* Velen., *R. aurea* Pers., and *R. romellii*. *Russula gelatinivelata*, *R. nauseosa* and *R. laricina*, have spores with isolated elements, while *Russula* sp. nov. 3, *R. cessans*, *R. aurea*, and *R. romellii* have spores with a partial reticulum. This group of species have dermatocystidia, uni- to multiseptate, incrustated or not that seems to be a good morphological character to test for a morphological definition of subgenus *Russula*.

Our analyses shows sect *Pelliculariae* as paraphyletic, what corroborate Buyck (1990, 1995) that considered sect. *Pelliculariae* as a highly heterogeneous artificial group. Singer *et al.* (1983) and Singer (1986) placed *R. puiggarii* in sect. *Pelliculariae* but as pointed out by Buyck and Obrevo (2002), the systematic placement of *R. puiggarii* is problematic. According to the ITS tree presented here, *R. puiggarii* is closely related to *R. pseudocarmesina* (subsect. *Pseudoepitheliosinae*

Buyck) and *R. inflata* (subsect. *Inflatinae* Buyck). It seems that the better solution would be to place *R. puiggarii* in either, subsect. *Inflatinae* or subsect. *Pseudoepitheliosinae*, as suggested in Buyck and Obrevo (2002). Additionally our ITS analyses suggest that *R. puiggarii* may represent several different lineages, one from Africa and at least two from South America.

The *clade j* found in the analyses presented here, correspond well to the clade 3b (sect. *Heterophyllidae*) identified by parsimony analysis in Miller and Buyck (2002). In the present study sect. *Compacta* is recovered as monophyletic as shown by Shimono *et al.* (2014), while subgen. *Compacta* is polyphyletic, as shown in the analyses presented by Lebel & Tonkin (2007). We present the subgenus *Ingratula* as polyphyletic, result that corroborate the results by Lebel & Tonkin (2007). It is noteworthy that the analyses here presented show the subgenus *Russula* as monophyletic, while others studies (Shimono *et al.*, 2004; Lebel & Tonkin, 2007) present subgen. *Russula* as polyphyletic.

The results presented here corroborate the vision of Singer (1986) that restricted sect. *Compactae* to the *R. nigricans* group and

established sect. *Plorantes* to include the species of the *R. delica* group. This concept was previously supported by morphological and anatomical analyses (Buyck, 1989), and by molecular analyses based on LSU rDNA (Miller *et al.*, 2001) and ITS regions (Miller and Buyck, 2002).

CONCLUSIONS

The present study represent the first to include phylogenetic analyses of several sequences of neotropical species. The three new species to science and the 15 other unnamed species (Tab. 1) suggest a high diversity to be described in the neotropical region. The complete understanding of infrageneric relationships within *Russula* is far to be complete. Taxonomic and phylogenetic studies are important to understand the real diversity of this important ectomycorrhizal genus. Research in the diversity of ectomycorrhizal genera such as *Russula* is important to a better understanding of the relationship of these taxa and its role in the ecosystems. This information can be applied to

reforestation and environment management, and conservation programs. This knowledge may also be an important contribution to the conservation community, in a time when firsthand knowledge for urgent decisions is required.

The sequences used in this work do not include all the sectional and sub-sectional levels that have been proposed for *Russula*. Thus, more representative sequences and molecular markers for each of the *Russula* sections are needed to have a better infrageneric resolution within the genus. It is of crucial importance for a better understanding of sect. *Metachromaticae* to know what is *R. metachromatica sensu stricto*, since the molecular data show this species as three different lineages. The phylogenetic relationship between *Russula* sp. nov. 1, *R. pachycystis* was not tested. Because the absence of sequences. Studies with focus in neotropical subsect. *Pluviales* and sect. *Pelliculariae* are needed to have a better understanding of the diversity of both sections in the neotropics. The inclusion of more sequences of species from subsect. *Mimeticinae* would give a better resolution to the relationship between subsect. *Pluviales* and subsect. *Mimeticinae*. A bigger effort in field trips to

collect fresh specimens and herbaria revision is necessary to better know the distribution of the species described in *Russula* from neotropics.

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CONSIDERAÇÕES FINAIS

A partir da revisão morfológica dos materiais de *Russula* provenientes do Brasil e da Colômbia, foi possível o reconhecimento de dois novos registros para o Brasil, *R. violaceotunicata* e *R. rubropunctatissima*, duas espécies que tinham registro só na Guiana Francesa. Uma espécie do Brasil e duas da Colômbia são descritas como novas para a ciência.

As análises morfológicas serviram de base para a interpretação das análises filogenéticas. Os resultados combinados oferecem uma nova perspectiva para a classificação do gênero *Russula*, o que possibilitou o reconhecimento da estrutura da pileipelis como uma característica taxonomicamente importante e filogeneticamente informativa. Um bom exemplo é observado na segregação das subseções *Mimeticinae* e *Pluvialis* em dois clados diferentes nas análises filogenéticas, resultado que corrobora as diferenças observadas na estrutura da pileipelis e que permite a distinção dos táxons com base morfológica e não só molecular.

Quinze espécimes que foram incluídos nas análises filogenéticas são táxons ainda não descritos, porém são coletas sem

material suficiente para que sejam propostas como novas espécies. É necessário uma maior amostragem que permita a delimitação adequada para propor novas espécies.

Duas espécies merecem especial atenção, *R. metachromatica* e *R. puiggarii*, ambas apresentam incongruências filogenéticas que sugerem a existência de mais de uma espécie. Estudos taxonômicos mais focados em sua delimitação *sensu stricto* são necessários. O claro entendimento destes dois táxons pode ajudar no esclarecimento dos clados nos quais ambas espécies se aninharam nos resultados obtidos nesse trabalho.

Propostas de classificação atualizadas e claras são cruciais, já que vários dos clados classicamente propostos aparecem como para ou polifiléticos. A inclusão de espécies neotropicais nas análises morfológicas e filogenéticas é crucial na construção de uma classificação natural para *Russula*. O entendimento do gênero não pode ser separado do conhecimento das relações de *Russula* com os outros gêneros dentro da família: *Lactifluus*, *Lactarius* e *Multifurca*.

Análises filogenéticas mais amplas, com ênfase em espécimes de *Lactifluus* do Brasil e da Colômbia, foram feitas em paralelo ao

desenvolvimento do presente estudo usando o marcador molecular ITS (anexo 1). Os resultados filogenéticos prévios incluindo táxons de *Lactarius*, *Lactifluus*, *Multifurca* e *Russula*, mostram *Russula* como um grupo monofilético e irmão de *Multifurca*, com suporte moderado. *Lactarius* e *Lactifluus* formaram grupos heterogêneos, com táxons de ambos os gêneros misturados nos grupos.

Os resultados com os táxons de *Lactifluus* não foram incluídos na dissertação porque serão analisados com maior profundidade durante a minha pesquisa de doutorado a ser iniciada em março do corrente ano no Programa de Pós-Graduação em Botânica na Escola Nacional de Botânica Tropical do Jardim Botânico de Rio de Janeiro, sob orientação do Dr. Aníbal Carvalho.



Anexo 1. Árvore de Máxima Verossimilhanças da família Russulaceae a partir do marcador molecular ITS. Sequências marcadas em vermelho são as sequências inéditas, as sequências em preto foram tiradas do GenBank.