MARIANA DE PAULA DREWINSKI

AGARICUS NO BRASIL: CONTRIBUIÇÕES À SISTEMÁTICA DO GÊNERO

Dissertação submetida ao Programa de pós-graduação em Biologia de Fungos, Algas e Plantas da Universidade Federal de Santa Catarina para obtenção do Grau de Mestre em Biologia de Fungos, Algas e Plantas.

Orientadora: Profa. Dra. Maria Alice Neves

Coorientador: Prof. Dr. Nelson Menolli Junior

FLORIANÓPOLIS
2017

"*Agaricus* no Brasil: contribuições à sistemática do gênero"

por

Mariana de Paula Drewinski

Dissertação julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (Port. 08/PPGFAP/2017) do Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas - UFSC, composta pelos Professores Doutores:

Banca Examinadora:

Maria Neves

Profa. Dra. Maria Alice Neves

Marcelo Sulzbacher

Profa. Dra. Marcelo Sulzbacher

Prof. Dr. Elisandro Ricardo Drechsler dos Santos

Admir Giachini

Prof. Dr. Admir Giachini

Prof. Dr. Elisandro Ricardo Drechsler dos Santos
Coordenador do Programa de Pós-Graduação
em Biologia de Fungos, Algas e Plantas

Florianópolis, **24 de março de 2017**.

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Drewinski, Mariana de Paula
Agaricus no Brasil: contribuições à sistemática do gênero / Mariana de Paula Drewinski ; orientadora, Maria Alice Neves, coorientador, Nelson Menolli Junior, 2017.
90 p.

Dissertação (mestrado) - Universidade Federal de Santa Catarina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Florianópolis, 2017.

Inclui referências.

1. Biologia de Fungos, Algas e Plantas. 2. Micologia. I. Neves, Maria Alice . II. Menolli Junior, Nelson. III. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas. IV. Título.

Dedico às trabalhadoras e aos trabalhadores que financiaram este estudo. **Fora Temer!**

AGRADECIMENTOS

Gratidão! À minha família. À minha mãe e ao meu pai, Dalmice e Carlão por proporcionar tudo que tenho e sou, hoje e sempre. Obrigada pelo amor e compreensão, pela força e pelos exemplos. À Cecília e à Ísis, por tornarem minha vida colorida e renovarem meu ar. Ao Dudu e à Helo, pela paciência e carinho. Aos de Paula e aos Drewinski pelo amor, pela força, boas energias, mensagens, visitas, rolês e churrascos. Às minhas amigas e amigos, comadres e compadres de Gorpa Hell e outras partes do Brasil e do mundo, sem vocês a vida seria vazia. Amo vocês!

Aos Fungos, por cruzar meu caminho com o caminho de tantas pessoas especiais. À Maria Alice, pela oportunidade, por acreditar, confiar, orientar e desafiar. Pela paciência e força. Valeu galera do MICOLAB! Contemporâneos ou não. Obrigada coletoras e coletores de *Agaricus*! Por todas as horas felizes(!), almoços, cafés, chimas, ônibus errado, conversas, coletas, risadas, bares, rolês, praias, festas, reuniões... Por todos os momentos especiais. Pela paciência, ensinamentos e amizade. Vocês estão no meu coração! À toda galera do Instituto de Botânica, estudantes, funcionárias e funcionários, professoras e professores. Ao Nelson, pelas risadas, almoços e discussões. Por orientar, acreditar e inspirar. Ao Luiz, pela companhia, risadas, jantinhas, ajudas, pranchas lindas, discussões e rolês.

À galera do PPGFAP e do Departamento de Botânica, alunas e alunos, professoras e professores, funcionárias e funcionários, obrigada pela ótima convivência e por toda a ajuda. Ao grupo do rolê e à turma 2015, pelo companheirismo, aulas, discussões, bares, almoços, coletas e festas. Conseguimos!

Aos que conheci em Floripa. Às Mítias e Marias Bonitas, maravilhosas, libertárias, fortes e lutadoras. Quero ser como vocês! À juventude do Levante, pelo sonho. Tamo junto! À galera da Bio, por ser a galera da Bio.

À Renata da Fiocruz Minas e toda a equipe, pelo sequenciamento das amostras. À Eliana e toda equipe do Laboratório Central de Microscopia Eletrônica. À CAPES, pela bolsa.

À todas as pessoas que direta ou indiretamente me ajudaram na realização deste trabalho: Gratidão!

A cura para tudo é sempre água salgada: o suor, as lágrimas ou o mar.

Karen von Blixen-Finecke

RESUMO

O gênero *Agaricus* é monofilético e possui ampla distribuição com mais de 400 espécies descritas. Os cogumelos desse gênero são caracterizados pelas lamelas livres, inicialmente de coloração branca, depois rosada e finalmente marrom com a maturação dos esporos, presença de anel e crescimento no solo. Embora o gênero seja facilmente reconhecido em campo, a identificação de espécies é difícil devido à falta de caracteres morfológicos e às variações intraespecíficas. Foram analisadas sequências de DNA das regiões ITS, LSU e *tef-1α* de 73 espécimes coletados no domínio Mata Atlântica, nos estados de Paraíba, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina e Rio Grande do Sul. As análises moleculares e taxonômicas demonstraram a existência de dois novos clados e cinco espécies novas. Mais de 70% das sequências analisadas foram agrupadas em *Agaricus* subg. *Minores* nas seções *Minores* e *Laeticolores,* e *Agaricus* subg. *Flavoagaricus* na seção *Arvenses*. O restante das sequências foram agrupadas em outras quatro seções: *Agaricus*; *Rarolentes* e *Subrutilescentes*; e *Xanthodermatei*, representantes dos outros três subgêneros: *Agaricus*, *Spissicaules* e *Pseudochitonia*, respectivamente. A inclusão de novas sequências de espécimes subtropicais, as diferentes relações filogenéticas encontradas entre as seções e os subgêneros, além da descrição de novas espécies reforçam a potencialidade de estudo do gênero *Agaricus* no Brasil.

Palavras chave: Agaricaceae. Filogenia. ITS. LSU. Mata Atlântica. *tef-1α*.

ABSTRACT

The genus *Agaricus* is monophyletic and has a worldwide distribution with more than 400 described species. Mushrooms of the genus are characterized by free lamellae, initially white, then pinkish and finally brown with spore maturation, presence of annulus and growth in the soil. Although the genus is easily recognized in the field, species identification is difficult because the lack of characters and intraspecific variability. ITS, LSU and *tef-1α* DNA sequences from 73 specimens collected in the Atlantic Rain Forest, in the states of Paraíba, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul were analyzed. Molecular and taxonomic analyzes demonstrated the existence of two new clades and five new species. More than 70% of the analyzed sequences clustered in *Agaricus* subg. *Minores* into sections *Minores* and *Laeticolores*, and *Agaricus* subg. *Flavoagaricus* into section *Arvenses*. The other specimens clustered into four other sections: *Agaricus*; *Rarolentes* and *Subrutilescentes*; *Xanthodermatei*, representatives of the other three subgenera: *Agaricus*, *Spissicaules* and *Pseudochitonia*, respectively. The inclusion of new sequences of subtropical specimens, the different phylogenetic relationships found between the sections and the subgenera, and the description of new species reinforce the potentiality of study of the genus *Agaricus* in Brazil.

Keywords: Agaricaceae. Atlantic Rain Forest. ITS. LSU. Phylogeny. tef-1α.

SUMÁRIO

Apresentação..13

Introdução..13

Objetivos...17

Objetivo geral...17

Objetivos específicos...17

Material e Métodos...19

Amostragem e identificação morfológica..19

Análises moleculares..20

Resultados e Discussão...33

Capítulo 1 – *Agaricus* sp1: a new neotropical species with pleurocystidia in *Agaricus* section *Laeticolores*.................................45

Capítulo 2 – New section of *Agaricus* subgenus *Flavoagaricus* from America..63

Considerações finais e recomendações para trabalhos futuros........95

Referências Bibliográficas...97

APRESENTAÇÃO

Este trabalho está dividido em resultados gerais e em dois capítulos apresentados em formato de artigo. No primeiro capítulo é descrita uma espécie nova de *Agaricus*, e no segundo capítulo são descritas uma nova seção para o gênero e quatro espécies novas.

INTRODUÇÃO

O gênero *Agaricus* L. é cosmopolita e contém aproximadamente 400 espécies (Karunarathna et al., 2016). Linnaeus descreveu o gênero em 1753, incluindo todos os cogumelos que possuíam lamelas e estipe (Linnaeus, 1753). O gênero é caracterizado por apresentar basidiomas geralmente grandes e carnosos, com píleo branco, creme, amarelo, pardo e, às vezes, marrom ou púrpura; as lamelas são próximas e livres, brancas quando jovens, mudando para rosa com a produção de esporos e finalmente tornando-se marrom devido à maturação destes; o anel é sempre presente, geralmente membranoso e branco, podendo conter escamas na superfície inferior, algumas vezes com formação de anel duplo (Heinemann, 1956; Singer, 1986; Zhao et al., 2016). Microscopicamente, *Agaricus* é caracterizado por apresentar pileipelis em forma de cútis, trama do himenóforo regular a levemente entrelaçada, hifas sem fíbulas, queilocistídios geralmente presentes e basidiósporos geralmente marrons, de parede lisa e engrossada (Zhao et al., 2016). As características do gênero são facilmente reconhecidas em campo, porém, a delimitação das espécies é difícil. Fatores ambientais, variabilidades intraespecíficas e falta de caracteres morfológicos para diferenciar espécies são alguns problemas encontrados na taxonomia do grupo (Zhao et al., 2011).

Os métodos de taxonomia para análise dos espécimes de *Agaricus* incluem características macroscópicas do píleo, estipe, contexto e anel; odor; características microscópicas como tamanho dos esporos e presença ou ausência de queilocistídios; além de testes macroquímicos com KOH 3% e reação de Schäffer (Largent, 1986; Singer, 1986; Heinemann, 1956). A mudança de coloração do basidioma, assim como a reação de Schäffer, é melhor observada quando o cogumelo está fresco, o que dificulta a identificação após desidratação (Freeman, 1979). Como os caracteres macroscópicos são importantes na diferenciação das espécies, os estudos baseados em materiais desidratados devem ser feitos com cautela, pois podem fornecer informações parciais (Albertó, 1998).

O gênero *Agaricus* apresenta um grande histórico de proposições taxonômicas. A classificação infragenérica de *Agaricus sensu* Heinemann (1956) e Singer (1986) é dividida em várias seções relacionadas ao habitat e mudança na coloração do contexto quando exposto. Heinemann (1956) dividiu o gênero em três subgêneros e onze seções, agrupando representantes cosmopolitas no subgênero *Agaricus*, enquanto que nos subgêneros *Lanagaricus* Heinem. e *Conioagaricus* Heinem. estariam os representantes com ocorrência tropical.

O uso da biologia molecular e o aperfeiçoamento dos estudos filogenéticos têm ajudado na delimitação de espécies e compreensão das relações entre os táxons de diferentes regiões. Bunyard et al. (1996) realizaram as primeiras análises moleculares do gênero *Agaricus* utilizando enzimas de restrição e demonstraram que a classificação das seções baseada somente em características morfológicas pode representar uma classificação artificial. Zhao et al. (2011), pesquisando espécimes do gênero *Agaricus* coletadas em regiões de climas temperado e tropical (incluindo três espécimes brasileiros), observaram que apenas um terço das espécies tropicais se enquadravam nas seções historicamente propostas por pesquisadores europeus e norte-americanos, e que a diversidade de espécies em regiões tropicais e subtropicais pode ser tão grande quanto em áreas temperadas.

Zhao et al. (2016) propuseram uma revisão do sistema de classificação infragenérica do gênero *Agaricus* considerando o tempo de divergência das espécies para estabelecer os níveis taxonômicos. Para isso, os autores utilizaram como critérios para reconhecimento de subgêneros e seções: i) a monofilia e o suporte estatístico das análises multigênicas; ii) tempo de divergência das espécies, sendo a idade estimada dos subgêneros mais antiga que a das seções; e iii) possíveis características fenotípicas. Neste estudo, foram utilizadas matrizes combinadas da região ITS e dos genes LSU, *tef-1α* e *rpb2* de 745 espécimes, incluindo 64 tipos, de regiões tropicais e temperadas da Ásia (China, Tailândia e Malásia), Europa (França e Espanha) e América do Norte (Canadá e Estados Unidos). Dessa forma, a proposta de classificação de Zhao et al. (2016) segregou o gênero em cinco subgêneros e 20 seções (Tabela 1).

Tabela 1. Classificação proposta por Zhao et al. (2016) baseada em análises moleculares, considerando o tempo de divergência das espécies.

	Subgêneros				
	Agaricus	*Flavoagaricus*	*Minores*	*Pseudochitonia*	*Spissicaules*
Seções	*Agaricus*	*Arvenses*	*Minores*	*Bivelares*	*Amoeni*
			Laeticolores	*Bohusia*	*Rarolentes*
				Brunneopicti	*Spissicaules*
				Chitonioides	*Subrutilescentes*
				Crassispori	
				Flocculenti	
				Hondenses	
				Nigrobrunnescentes	
				Rubricosi	
				Sanguinolenti	
				Trisulphurati	
				Xanthodermatei	

Os cogumelos do gênero *Agaricus* são conhecidos por possuírem alto valor nutricional e por suas propriedades farmacológicas, pois representam uma fonte importante de polissacarídeos e complexos proteicos com atividade anticancerígena e imunoestimulante (Wasser, 2011). A espécie mais cultivada e consumida no mundo é *Agaricus bisporus* (J.E. Lange) Imbach, o *champignon* (Royse, 2014). Apesar da maioria das espécies de *Agaricus* ser comestível, algumas apresentam compostos tóxicos, principalmente os representantes de *Agaricus* seção *Xanthodermatei* (Petrova et al., 2007).

A constante publicação de espécies novas, principalmente por pesquisadores das regiões tropicais da Ásia, demonstra que o gênero possui uma grande diversidade ainda desconhecida em regiões tropicais e subtropicais (Chen et al., 2015; Gui et al., 2015; Liu et al., 2015; Zhou et al, 2016; Dai et al., 2016; Karunarathna et al., 2016; Zhao et al., 2016). Dessa forma, são necessárias coletas e revisões de materiais que ocorrem nas regiões não amostradas para maior conhecimento das espécies e das relações infragenéricas no grupo.

OBJETIVOS

Objetivo geral:

Contribuir para o conhecimento das relações filogenéticas e da ocorrência do gênero *Agaricus* no Brasil.

Objetivos específicos:

Identificar as espécies de *Agaricus* ocorrentes na Mata Atlântica;

Analisar as relações filogenéticas dos táxons coletados na Mata Atlântica brasileira com espécies ocorrentes em clima temperado e tropical.

MATERIAL E MÉTODOS

Amostragem e identificação morfológica

As coletas foram realizadas em áreas do domínio Mata Atlântica, e em áreas urbanas como jardins, gramados e canteiros, nos estados de São Paulo, Paraná, Santa Catarina e Rio Grande do Sul. Também foram incluídos espécimes coletados, por colegas, nos estados da Paraíba, Espírito Santo e Rio de Janeiro e depositados no herbário FLOR da Universidade Federal de Santa Catarina (UFSC).

Os basidiomas foram coletados com auxílio de uma colher e acondicionados em caixas plásticas com divisórias ou em sacos de papel. Ainda em campo, os espécimes foram fotografados e analisados macroscopicamente. Um fragmento do píleo do basidioma ainda fresco foi retirado, separado em papel manteiga e desidratado em sílica ou em microtubo com solução de CTAB 2% conservado na geladeira, para futuras análises moleculares. Os espécimes foram desidratados em secadora de frutas (*Total Chef Food Dehydrator*) com temperatura aproximada de 40 °C por cerca de 24 horas ou até a desidratação completa dos basidiomas. Depois de desidratados, os basidiomas foram acondicionados em sacos plásticos tipo *ZipLock* e etiquetados com o número de coletor. Posteriormente foram levados ao freezer por um período de sete dias, para eliminar possíveis larvas e ovos de insetos que possam ter resistido à desidratação, para então serem depositados no herbário FLOR do Departamento de Botânica da UFSC.

As análises macroscópicas foram realizadas no Laboratório de Micologia (Departamento de Botânica da UFSC) ou em campo, e as microscópicas no Laboratório Multiusuário de Microscopia Anatômica (Departamento de Botânica da UFSC) e no Núcleo de Micologia do Instituto de Botânica de São Paulo. As descrições morfológicas dos basidiomas, macro e microscópicas, foram realizadas seguindo o glossário de Vellinga (1988). Foram observadas possíveis alterações na coloração do contexto e foram realizados testes com KOH 3% e NaOH 10% na superfície do píleo e no contexto. Os códigos de cores foram baseados no *Online Auction Color Chart* (Kramer, 2004). Foram realizados cortes longitudinais do himenóforo para observação de características microscópicas da trama, basidiósporos, basídios e cistídios; da superfície e do contexto do píleo e da superfície e contexto do estipe. Os cortes foram montados em lâminas com água, KOH 3%, Melzer e Vermelho Congo, separadamente ou em combinação adequada, para visualização das características em diferentes corantes e reagentes. As microestruturas foram observadas em microscópio óptico (*OLYMPUS* CX21 e Zeiss) e medidas com o auxílio de uma lente ocular com régua micrométrica acoplada. Foram obtidas 20 medidas para cada estrutura. O quociente comprimento/largura, indicado por "Q" nas descrições, foi calculado para facilitar a descrição da forma dos basidiósporos, seguindo a terminologia de Bas (1969).

Análises moleculares

Foram realizadas extrações de DNA total dos espécimes coletados (Tabela 2) segundo o protocolo de Doyle & Doyle (1987) adaptado por Góes-Neto et al. (2005). Três regiões do DNA foram amplificadas por meio de reações em cadeia da polimerase (PCR): ITS, região intergênica *barcoding* para fungos, LSU e *tef-1α* com os *primers* ITS8-F e ITS6-R (Dentinger et al., 2010), LROR e LR5 (Vilgalys & Hester, 1990) e EF1-983F e EF1-1567R (Morehouse et al., 2003), respectivamente. Para PCR, o *mix* foi preparado contendo 4µL de DNA (1:10), 10µL de água, 10µL de Master Mix (Promega) e 0,5µL de cada *primer* (10pmol/L). Os programas da PCR utilizados foram: para ITS, segundo Zhao et al. (2011): 5 min. a 95 °C; 35 ciclos (1 min. a 94 °C, 1,5 min. a 55 °C, 1,5 min. a 72 °C); 5 min. a 72 °C; para LSU, Segundo Vilgalys & Hester (1990): 1 min. a 72 °C; 30 ciclos (1 min. a 94 °C, 45 seg. a 50 °C, 1 min. a 72 °C); 7 min. a 72 °C; para *tef-1α*, segundo Morehouse et al. (2003): 3 min. a 94 °C; 35 ciclos (1 min. a 94 °C, 30 seg. a 50 °C, 1 min. a 72 °C); 7 min a 72 °C. Os produtos da PCR foram purificados com PEG 20% e enviados para sequenciamento no Centro de Pesquisas René Rachou (Fiocruz Minas, Brasil), utilizando os mesmos pares de *primers*. Os procedimentos de extração, amplificação e purificação foram realizados no Laboratório de Biologia Molecular do Departamento de Botânica da UFSC.

Os cromatogramas gerados a partir do sequenciamento foram inspecionados e corrigidos manualmente no programa *Geneious* v.8.1 (Kearse et al., 2012). As sequências finais foram então usadas para montar uma matriz individual para cada marcador, juntamente com sequências adicionais obtidas no banco de dados *Genbank* (Benson et al., 2005). A matriz de dados foi alinhada utilizando o *software MAFFT* v.7 (Katoh, 2013) com estratégia Q-INS-I e posteriormente editadas manualmente por meio do programa *MEGA* v.7 (Tamura et al., 2013). Para concatenar as sequências foi utilizado o programa *FASconCAT* v1.0 (Kück & Meusemann, 2010). O modelo de evolução molecular utilizado nas análises foi avaliado no programa *jModelTest* v.2.1.6, (Guindon et al., 2010; Darriba et al., 2012) com o critério AIC (*Akaike Information Criterion*). A região ITS foi particionada em ITS1, 5.8S e ITS2; e *tef-1α* separada em íntrons e os éxons em códons. Para reconstrução filogenética foram utilizados os métodos de máxima verossimilhança (MV) e Inferência Bayesiana (IB). A análise de Máxima Verossimilhança foi realizada no software *RAxML* v.8.2.8 (Stamatakis, 2014) utilizando o modelo GTRGAMMA com valores de *bootstrap* com 1.000 pseudoréplicas para sustentação dos ramos. A análise de Inferência Bayesiana foi realizada com o programa *Mr.Bayes* v.3.2.6 (Ronquist & Huelsenbeck, 2003) utilizando os modelos de substituição estimados anteriormente. As análises foram feitas com duas corridas independentes, cada uma com quatro cadeias em 20 milhões de gerações com frequência de amostragem a cada mil gerações. Os programas *jModelTest*, *RAxML* e *Mr.Bayes* foram utilizados por meio da Plataforma CIPRES (Miller et al., 2010). Os resultados das análises foram plotados no *FigTree* v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) e editados no *InkScape* (https://inkscape.org/en/). Ao final do trabalho, as sequências serão depositadas no *GenBank*.

Tabela 2. Espécimes utilizados nas análises moleculares. As sequências geradas neste trabalho estão em **negrito**. * representa as sequências que serão depositadas no GenBank; - dados faltantes.

Subgênero	Seção	Coletor	Espécie	ITS	LSU	*Tef-1a*	Localidade	Referência
Minores	*Minores*	AH-44509	*Agaricus kerriganii*	KF447893	-	-	León, Espanha	Parra (2013)
		LAPAG808	*Agaricus kerriganii*	KT951306	KT951442	KT951589	Madrid, Espanha	Zhao et al. (2016)
		LAPAG412	*Agaricus edmondoi*	KT951326	KT951481	KT951590	Segovia, Espanha	Zhao et al. (2016)
		AH-44506	*Agaricus matrum*	KF447896	-	-	La Rioja, Espanha	Parra (2013)
		LAPAG855	*Agaricus matrum*	KT951310	KT951443	KT951598	Asturias, Espanha	Zhao et al. (2016)
		LAPAG592	*Agaricus friesianus*	KT951316	-	KT951594	Pyrénées Atlantiques, França	Zhao et al. (2016)
		TRgmb01309	*Agaricus purpurellus*	KF447903	-	-	Itália	Parra (2013)
		AH-44510	*Agaricus gemlii*	KF447891	-	-	Canarias, Espanha	Parra (2013)
		LAPAG724	*Agaricus comtulus*	KT951332	KT951448	KT951593	Burgos, Espanha	Zhao et al. (2016)
		LAPAG810	*Agaricus arrillagarum*	KF447900	-	KT951592	Asturias, Espanha	Parra (2013)
		LAPAG926	*Agaricus pallens*	KT951315	-	KT951591	Lapland, Suécia	Zhao et al. (2016)
		ZRL2012576	*Agaricus* sp.	KT951372	KT951499	KT951596	Tibet, China	Zhao et al. (2016)
		ZRLWXH3067	*Agaricus* sp.	KT951387	KT951497	KT951611	Jiangxi, China	Zhao et al. (2016)
		ZRLWXH3076	*Agaricus* sp.	KT951388	KT951458	KT951612	Fujian, China	Zhao et al. (2016)
		ZRLWXH3150	*Agaricus* sp.	KT951390	KT951447	KT951609	Guangdong, China	Zhao et al. (2016)
		LAPAG454	*Agaricus pseudolutosus*	KT951329	KT951453	KT951602	Burgos, Espanha	Zhao et al. (2016)
		AH-42929	*Agaricus callacii*	KF447899	-	-	Canarias, Espanha	Parra (2013)
		ZRL2012199	*Agaricus* sp.	KT951367	KT951470	KT951595	Yunnan, China	Zhao et al. (2016)
		NT055	*Agaricus* sp.	JF727846	-	-	Tailândia	Zhao et al. (2011)
		ZRL2012357	*Agaricus* sp.	KT951369	KT951496	KT951610	Yunnan, China	Zhao et al. (2016)
		CA846	*Agaricus* sp.	JF727865	KT951452	KT951601	Tailândia	Zhao et al. (2011)

		ZRL2011039	*Agaricus* sp.	KT951351	KT951449	KT951606	Yunnan, China	Zhao et al. (2016)
		ZRL2012714	*Agaricus* sp.	KT951381	KT951476	KT951607	Tibet, China	Zhao et al. (2016)
		MPD30	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		CA848	*Agaricus* sp.	JF727864	KT951445	KT951605	Tailândia	Zhao et al. (2011)
		CA921	*Agaricus* sp.	KT951323	-	-		Zhao et al. (2016)
		ZRL2011156	*Agaricus* sp.	KT951352	KT951480	KT951603	Yunnan, China	Zhao et al. (2016)
		ZRLLD013	*Agaricus* sp.	KT951384	KT951516	KT951604	Tailândia	Zhao et al. (2016)
		LAPAG589	*Agaricus aridicola*	KT951331	-	-	Castellón, Espanha	Zhao et al. (2016)
		TL2013a	*Agaricus* sp.	JX984565	-	-	Austrália	Lebel & Syme et al. (2012)
		MEL2063323	*Agaricus wariatodes*	JF495054	-	-		Lebel & Syme et al. (2012)
		ZRL2012004	*Agaricus* sp.	KT951355	KT951457	KT951608	Yunnan, China	Zhao et al. (2016)
		LAPAG639	*Agaricus huijsmanii*	KF447889	KT951444	KT951571	Navarra, Espanha	Parra (2013)
		ZRL2012012	*Agaricus* sp.	KT951359	KT951494	KT951597	Yunnan, China	Zhao et al. (2016)
Minores	*Clado a*	**MPD107**	***Agaricus* sp.**	*	*	*	**São Paulo, Brasil**	
		MPD108	***Agaricus* sp.**	*	*	*	**São Paulo, Brasil**	
		MPD109	***Agaricus* sp.**	*	*	*	**São Paulo, Brasil**	
		MPD110	***Agaricus* sp.**	*	*	*	**São Paulo, Brasil**	
		FTF194	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MAR1274	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		JDuque78	***Agaricus* sp.**	*	*	-	**Santa Catarina, Brasil**	
		MAN1178	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD134	***Agaricus* sp.**	*	*	*	**Rio de Janeiro, Brasil**	
		MPD115	***Agaricus* sp.**	*	*	*	**Paraná, Brasil**	
		ACM473	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	

		MAN686	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
Minores	*Incertae sedis*	**MAN1183**	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		ZRLWXH3161	*Agaricus* sp.	KT951391	KT951526	KT951615	Guangdong, China	Zhao et al. (2016)
		LAPAM14	*Agaricus* sp.	KT951312	-	KT951613	Sosúa, República Dominicana	Zhao et al. (2016)
		LD2012129	*Agaricus candidolutescens*	KT951335	KT951525	KT951616	Tailândia	Zhao et al. (2016)
		ACM250	***Agaricus* sp.**	*	*	*	**Paraíba, Brasil**	
Minores	*Laeticolores*	**ACM513**	***Agaricus* sp.**	*	*	*	**Espírito Santo, Brasil**	
		LAPAM1	*Agaricus* aff. *aufoaurantiacus*	JF797183	-	-	Venezuela	Zhao et al. (2011)
		MPD16	***Agaricus* sp.**	*	*	*	**Rio Grande do Sul, Brasil**	
		FTF140	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		ANMF530	***Agaricus* sp.**	-	*	*	**Santa Catarina, Brasil**	
		MPD13	***Agaricus* sp.**	*	*	*	**Rio Grande do Sul, Brasil**	
		MPD14	***Agaricus* sp.**	-	*	*	**Rio Grande do Sul, Brasil**	
		MPD15	***Agaricus* sp.**	*	*	*	**Rio Grande do Sul, Brasil**	
		MPD69	***Agaricus* sp.**	-	-	*	**Paraná, Brasil**	
		MPD70	***Agaricus* sp.**	*	*	*	**Paraná, Brasil**	
		CHC172	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD10	***Agaricus* sp.**	*	*	*	**Rio Grande do Sul, Brasil**	
		JDuque70	***Agaricus* sp.**	*	-	*	**Santa Catarina, Brasil**	
		ACM260	***Agaricus* sp.**	*	*	*	**Paraíba, Brasil**	
		LAPAM15	*Agaricus rufoaurantiacus*	KT951313	-	KT951641	Sosúa, República Dominicana	Zhao et al. (2016)
		JDuque130	***Agaricus* sp.**	*	*	*	**Rio de Janeiro, Brasil**	
		MAN545	***Agaricus* sp.**	*	-	-	**Paraíba, Brasil**	
		EC31	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina,**	

							Brasil	
		MATA816	*Agaricus* sp*1*	JF727870	-	-	Veracruz, México	Zhao et al. (2011)
		MPD02	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina, Brasil**	
		MPD03	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina, Brasil**	
		MPD04	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina, Brasil**	
		MPD128	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina, Brasil**	
		MPD19	**_Agaricus_ sp_1_**	*	*	-	**Santa Catarina, Brasil**	
		MPD29	**_Agaricus_ sp_1_**	*	*	*	**Santa Catarina, Brasil**	
		MPD71	**_Agaricus_ sp_1_**	*	*	-	**Paraná, Brasil**	
		MPD118	**_Agaricus_ sp.**	*	*	*	**Paraná, Brasil**	
		HAI0386	*Agaricus comtulus*	AJ884624	-	-	Durham, EUA	Didukh (2005)
		JH1	*Agaricus floridanus*	KM349609	-	-	New York, EUA	Kerrigan (2016)
		MPD105	**_Agaricus_ sp.**	*	-	-	**São Paulo, Brasil**	
		MPD111	**_Agaricus_ sp.**	*	*	*	**São Paulo, Brasil**	
		MAN1060	**_Agaricus_ sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD17	**_Agaricus_ sp.**	*	*	-	**Rio Grande do Sul, Brasil**	
		ACM204	**_Agaricus_ sp.**	*	*	*	**Paraíba, Brasil**	
		F2815	*Agaricus martinicensis*	JF727855	-	-	Martinique, França	Zhao et al. (2011)
Pseudochitonia	*Sanguinolenti*	ZRL2012010	*Agaricus dilutibrunneus*	KT951358	KT951512	KT951569	Yunnan, China	Zhao et al. (2016)
Pseudochitonia	*Sanguinolenti*	ZRL2012013	*Agaricus sylvaticus*	KT951360	KT951500	KT951570	Tailândia	Zhao et al. (2016)
Flavoagaricus	*Arvenses*	ZRL2012030	*Agaricus* sp.	KT951364	KT951455	KT951628		Zhao et al. (2016)
		ZRL2012630	*Agaricus* sp.	KT951379	KT951495	KT951621	Tibet, China	Zhao et al. (2016)
		MAFungi80999	*Agaricus arvensis*	KF114474	-	-	Burgos, Espanha	Parra (2013)
		ZRL2012002	*Agaricus* sp.	KT951354	KT951462	-	Yunnan, China	Zhao et al. (2016)
		ZRL2012026	*Agaricus* cf. *essettei*	KT951363	KT951450	KT951630	Yunnan, China	Zhao et al.

								(2016)
		ZRL2012599	*Agaricus essettei*	KT951374	KT951514	KT951625	Tibet, China	Zhao et al. (2016)
		ZRL2012608	*Agaricus* sp.	KT951377	KT951498	KT951627	Tibet, China	Zhao et al. (2016)
		ZJL2016i	*Agaricus* sp.	KT951380	-	-	China	Zhao et al. (2016)
		SHY2011073117	*Agaricus* sp.	KT951407	KT951459	KT951622	Yunnan, China	Zhao et al. (2016)
		ZRL2012604	*Agaricus* sp.	KT951375	KT951515	KT951620	Tibet, China	Zhao et al. (2016)
		ZRL2012005	*Agaricus abruptibulbus*	KT951356	KT951460	KT951626	Yunnan, China	Zhao et al. (2016)
		ZRL2012598	*Agaricus augustus*	KT951373	KT951477	KT951629	Tibet, China	Zhao et al. (2016)
		JM03-193	*Agaricus flocculosipes*	KT951325	-	KT951617	Tailândia	Zhao et al. (2016)
		ZRL2012105	*Agaricus flocculosipes*	KT951365	KT951463	KT951618	Yunnan, China	Zhao et al. (2016)
		CA276	*Agaricus subrufescens*	KT951317	KT951461	KT951631		Zhao et al. (2016)
		FLOR48215	***Agaricus subrufescens***	*****	*****	*****	**Surrey, Reino Unido**	
		ACM138	***Agaricus* sp.**	**-**	*****	*****	**Espírito Santo, Brasil**	
		ZRL2012722	*Agaricus subrufescens*	KT951383	KT951451	KT951632	Yunnan, China	Zhao et al. (2016)
Flavoagaricus	*Clado novo*	**MAN1150**	***Agaricus* sp2**	*****	*****	*****	**Santa Catarina, Brasil**	
		MAN1152	***Agaricus* sp2**	*****	*****	**-**	**Santa Catarina, Brasil**	
		MAN651	***Agaricus* sp2**	*****	*****	*****	**Santa Catarina, Brasil**	
		MPD131	***Agaricus* sp2**	*****	*****	*****	**Santa Catarina, Brasil**	
		DCD2013	*Agaricus* sp.	KJ010887	-	-		Dias (2013)
		JDuque131	***Agaricus* sp3**	*****	*****	*****	**Rio de Janeiro, Brasil**	
		AGP08	***Agaricus* sp4**	*****	**-**	*****	**Santa Catarina, Brasil**	
		ACM376	***Agaricus* sp5**	*****	*****	**-**	**Santa Catarina, Brasil**	

		MK01	***Agaricus* sp5**	*	*	*	**Paraná, Brasil**	
Spissicaules	*Spissicaules*	ZRL2011250	*Agaricus planipileus*	KT951398	-	-	Yunnan, China	Zhao et al. (2016)
		ZRL2012151	*Agaricus lanipedisimilis*	KT951366	KT951484	KT951578	Yunnan, China	Zhao et al. (2016)
		ZRL2011249	*Agaricus litoraloides*	KT951353	KT951523	KT951580	Yunnan, China	Zhao et al. (2016)
		ZRLQHY43	*Agaricus* sp.	KT951386	KT951485	KT951579	Shandong, China	Zhao et al. (2016)
		LAPAG420	*Agaricus litoralis*	KT951327	KT951483	KT951572	Burgos, Espanha	Zhao et al. (2016)
		FLOR48245	***Agaricus* sp.**	*	*	*	**Surrey, Reino Unido**	
Spissicaules	*Rarolentes*	**MPD127**	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD133	***Agaricus* sp.**	*	*	*	**Rio de Janeiro, Brasil**	
		DKSG14	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		F2301	*Agaricus* sp.	JF727854	-	-	-	Zhao et al. (2011)
		MAN1182	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MAN1184	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD113	***Agaricus* sp.**	*	*	*	**Paraná, Brasil**	
		MPD114	***Agaricus* sp.**	*	*	*	**Paraná, Brasil**	
		AW145	*Agaricus* sp.	KT951308	-	KT951637	Awenda, Canada	Zhao et al. (2016)
		JH105	*Agaricus* sp.	KT951324	KT951490	-	Jokers Hill, ON, Canada	Zhao et al. (2016)
		RWK2015	*Agaricus butyreburneus*	KC135887	-	-		Kerrigan (2016)
		MPD55	***Agaricus* sp.**	*	*	*	**Paraná, Brasil**	
		LD201214	*Agaricus leucolepidotus*	KT951336	KT951519	KT951635	Tailândia	Zhao et al. (2016)
		LD2012192	*Agaricus albosquamosus*	KT951394	KT951520	KT951636	Tailândia	Zhao et al. (2016)
		SK2012	*Agaricus hanthanaensis*	KT951424	-	-	-	Zhao et al. (2016)
Spissicaules	*Amoeni*	LD2012100	*Agaricus suthepensis*	KT951409	-	-	Tailândia	Zhao et al. (2016)

		ZRL2010072	*Agaricus amoenus*	KT951348	KT951524	KT951638	Yunnan, China	Zhao et al. (2016)
		Rammeloo5756	*Agaricus kivuensis*	JF514541	-	-	-	Zhao et al. (2011)
		ZRL3093	*Agaricus* sp.	JF691548	KT951488	-	Tailândia	Zhao et al. (2011)
Spissicaules	*Subrutilescentes*	ZRL2011027	*Agaricus parasubrutilescens*	KT951350	KT951521	KT951583	Yunnan, China	Zhao et al. (2016)
		ZRL2012025	*Agaricus parasubrutilescens*	KT951362	KT951487	KT951584	Yunnan, China	Zhao et al. (2016)
		SHY2012070604	*Agaricus* sp.	KT951341	KT951475	KT951581	Yunnan, China	Zhao et al. (2016)
		ZRL2012618	*Agaricus linzhiensis*	KT951378	KT951503	KT951582	Tibet, China	Zhao et al. (2016)
		ZRLWXH3276	*Agaricus subrutilescens*	KT951392	KT951522	KT951585	China	Zhao et al. (2016)
		ZRL160	*Agaricus* sp.	KT951345	KT951486	KT951586	Tailândia	Zhao et al. (2016)
		ZRLLD014	*Agaricus inthanonensis*	KT951422	-	-	Tailândia	Zhao et al. (2016)
		ZRL2012115	*Agaricus brunneopileatus*	KT951404	KT951489	KT951587	Yunnan, China	Zhao et al. (2016)
		MAN1110	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD135	***Agaricus* sp.**	*	*	*	**Rio de Janeiro, Brasil**	
Agaricus	*Agaricus*	F2272	*Agaricus* sp.	JF727850	-	-	Martinique, França	Zhao et al. (2011)
		FTF193	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		JS75	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MPD126	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		MAN1153	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		ZRL2010010	*Agaricus* sp.	KT951347	KT951511	KT951639	Tailândia	Zhao et al. (2016)
		ZRLLD026	*Agaricus* sp.	KT951385	KT951513	KT951640	Tailândia	Zhao et al. (2016)
		MPD11	***Agaricus* sp.**	*	*	*	**Rio Grande do Sul, Brasil**	
		LAPAG370	*Agaricus campestris*	KM657927	KR006607	KR006636	Madrid, Espanha	Zhou et al.

								(2016)
		MAN1151	***Agaricus* sp.**	*****	*****	**-**	**Santa Catarina, Brasil**	
		CA637	*Agaricus campestris*	KT951322	KT951468	KT951633		Zhao et al. (2016)
		FLOR48207	***Agaricus porphyrocephalus***	*****	*****	**-**	**East Sussex, Reino Unido**	
		ZRL2012006	*Agaricus* sp.	KT951357	KT951466	KT951634	Yunnan, China	Zhao et al. (2016)
Pseudochitonia	*Rubricosi*	ZRL2012015	*Agaricus kunmingensis*	KT951361	KT951506	KT951642	Yunnan, China	Zhao et al. (2016)
		ZRLWXH3078	*Agaricus* sp.	KT951464	KT951464	KT951643	Fujian, China	Zhao et al. (2016)
		ZRL2012715	*Agaricus dolichopus*	KT951382	KT951502	KT951573	Tibet, China	Zhao et al. (2016)
		ZRL2014120	*Agaricus dolichopus*	KT951433	-	-		Zhao et al. (2016)
Pseudochitonia	*Nigrobrunnescentes*	LAPAG687	*Agaricus biberi*	KM657919	KR006614	KR006642	Hungria	Zhou et al. (2016)
		WXR2012903	*Agaricus padanus*	KM657903	KR006616	KR006644	Xingjiang, China	Zhou et al. (2016)
		WZR2012907	*Agaricus desjardinii*	KM657901	KT951474	KT951644	Xinjiang, China	Zhou et al. (2016)
Pseudochitonia	*Sanguinolenti*	LAPAG382	*Agaricus sylvaticus*	KM657929	KR006608	KR006637	Burgos, Espanha	Zhou et al. (2016)
		ZRL2012568	*Agaricus sylvaticus*	KT951371	KT951501	KT951568	Tibet, China	Zhao et al. (2016)
		ALG07_213	*Agaricus sylvaticus*	KT951307	-	KT951567	Algonquin,ON, Canadá	Zhao et al. (2016)
Pseudochitonia	*Flocculenti*	LD2012165	*Agaricus erectosquamosus*	KT951338	KT951509	KT951565	Tailândia	Zhao et al. (2016)
		ZRL2012358	*Agaricus pallidobrunneus*	KT951370	KT951471	KT951566	Yunnan, China	Zhao et al. (2016)
Pseudochitonia	*Bohusia*	LAPAG562	*Agaricus bohusii*	KM657928	KR006613	KR006641	Madrid, Espanha	Zhou et al. (2016)
		ZRL2012607	*Agaricus crassisquamosus*	KT951376	KT951510	KT951645	Tibet, China	Zhao et al. (2016)
Pseudochitonia	*Brunneopicti*	SWK014	*Agaricus* sp.	KT951342	KT951482	KT951654	Lanjak-Entimau, Malásia	Zhao et al. (2016)
		ZRL2012267	*Agaricus* sp.	KT951368	KT951504	KT951655	Yunnan, China	Zhao et al. (2016)
		ZRL133	*Agaricus* sp.	KT951344	KT951505	KT951656	Tailândia	Zhao et al. (2016)

		ADK2564	*Agaricus brunneopictus*	JF514518	-	-	Plateau Atlantique, Benin	Zhao et al. (2011)
Pseudochitonia	*Xanthodermatei*	**ACM429**	***Agaricus* sp.**	*	*	-	**Santa Catarina, Brasil**	
		TENN61603	*Agaricus pocillator*	FJ596845	-	-	EUA	Hughes et al. (2009)
		MVL21	***Agaricus* sp.**	*	*	-	**Santa Catarina, Brasil**	
		RP32	*Agaricus* sp.	KT951340	-	KT951652	Rogers Park, Canadá	Zhao et al. (2016)
		ZRL2012585	*Agaricus tibetensis*	KM657895	KR006633	KR006658	Tibet, China	Zhou et al. (2016)
		LAPAG387	*Agaricus xanthodermus*	KM657923	KR006609	KR006638	Soria, Espanha	Zhou et al. (2016)
		MAN1109	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		ZRL2012629	*Agaricus* sp.	KM657890	KR006627	KR006656	Tibet, China	Zhou et al. (2016)
		LD2012185	*Agaricus atrodiscus*	KT284912	KT951473	KT951653	Tailândia	Ariyawansa et al. (2015)
		MFLU121010	*Agaricus atrodiscus*	KT284912	-	-	Tailândia	Ariyawansa et al. (2015)
		ZRLWXH3077	*Agaricus tytthocarpus*	KM657889	KR006618	KR006645	Fujian, China	Zhou et al. (2016)
		XHWANG3077	*Agaricus tytthocarpus*	KM657889	-	-		Zhou et al. (2016)
		LD201271	*Agaricus microvolvatulus*	KJ575614	KT951508	KT951651	Tailândia	Thongklang et al. (2014)
		LD2012111	*Agaricus bisporiticus*	KJ575611	KT951507	KT951650	Tailândia	Thongklang et al. (2014)
		ACM328	***Agaricus* sp.**	*	*	*	**Santa Catarina, Brasil**	
		LAPAG20	*Agaricus pseudopratensis*	DQ182526	-	-		Kerrigan et al. (2015)
		ZRL2012014	*Agaricus nigrogracilis*	KM657882	KR006621	KR006647	Yunnan, China	Zhou et al. (2016)
Pseudochitonia	*Hondenses*	GB2011a	*Agaricus* sp.	JF896229	-	-	Itália	Parra (2013)
		ZRL2012611	*Agaricus grandiomyces*	KM657879	KR006624	KR006652	Tibet, China	Zhou et al. (2016)
Pseudochitonia	*Chitonioides*	LAPAG257	*Agaricus nevoi*	KM657922	KR006606	KR006635	Burgos, Espanha	Zhou et al. (2016)
		LAPAG535	*Agaricus nevoi*	KT951330	-	KT951574	Teruel, Espanha	Zhao et al. (2016)

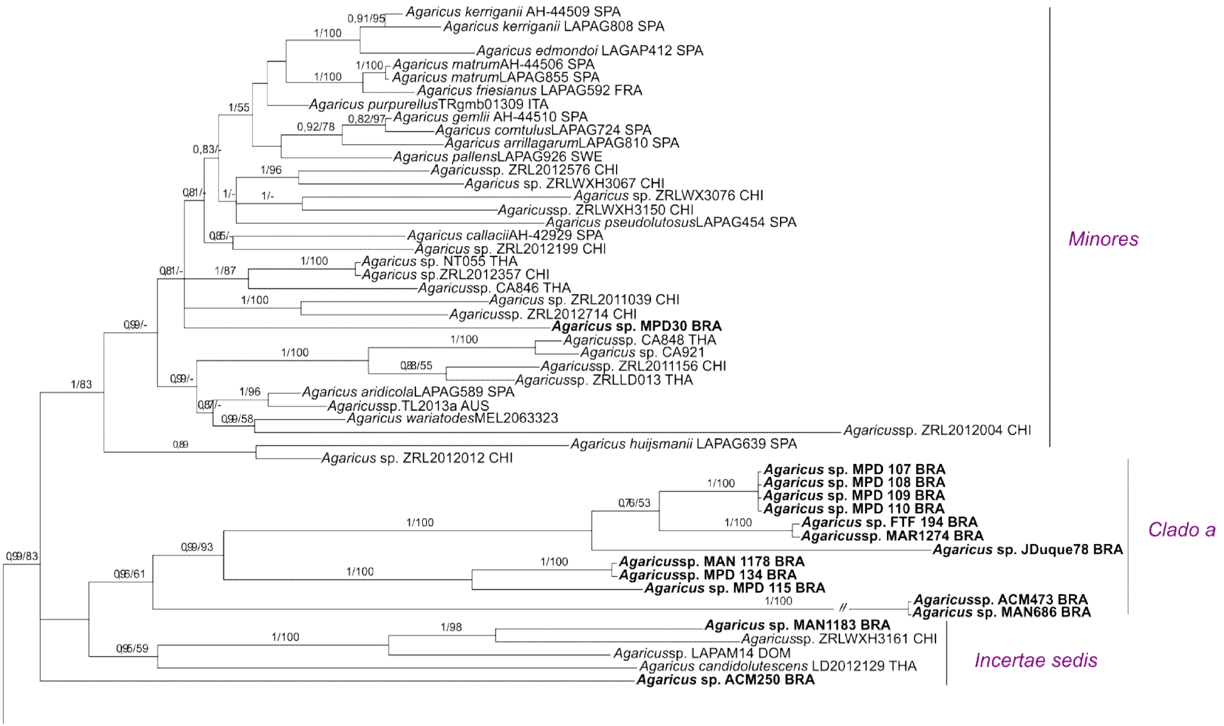
		CA339	*Agaricus gennadii*	KT951318	-	KT951575	-	Zhao et al. (2016)
		CA383	*Agaricus* cf. *bernardii*	KT951319	KT951469	KT951576	-	Zhao et al. (2016)
		FLOR48221	***Agaricus bernardii***	*****	*****	*****	**Surrrey, Reino Unido**	
		CA433	*Agaricus bernardiformis*	KT951321	KT951467	KT951577	-	Zhao et al. (2016)
Pseudochitonia	*Bivelares*	CA427	*Agaricus bitorquis*	KT951320	KT951491	KT951646	-	Zhao et al. (2016)
		WZR2012827	*Agaricus bitorquis*	KM657916	KT951492	KT951647	Xingjiang, China	Zhou et al. (2016)
		FLOR48246	***Agaricus subperonatus***	*****	*****	**-**	**Reino Unido**	
		WZR2012822	*Agaricus sinodeliciosus*	KM657907	KT951518	KT951648	Xingjiang, China	Zhou et al. (2016)
		LAPAG446	*Agaricus bisporus*	KM657920	KR006611	KR006640	Burgos, Espanha	Zhou et al. (2016)
		LAPAG889	*Agaricus cupressicola*	KT951334	KT951465	KT951649	Roma, Itália	Zhao et al. (2016)
Pseudochitonia	*Crassispori*	LD201234	*Agaricus variicystis*	KT951339	KT951517	KT951562	Tailândia	Zhao et al. (2016)
		ZRL3099	*Agaricus lamellidistans*	JF691556	-	-	Tailândia	Zhao et al. (2011)
Pseudochitonia	*Trisulphurati*	LAPAF7	*Agaricus trisulphuratus*	KM657924	KR006605	KR006634	Plateaux, Togo	Zhou et al. (2016)
		SWK079	*Agaricus trisulphuratus*	KT951343	KT951472	KT951561	Lanjak-Entimau, Malásia	Zhao et al. (2016)
		ZRL185	*Heinemannomyces*	KT951346	KT951527	KT951657	Tailândia	Zhao et al. (2016)

RESULTADOS E DISCUSSÃO

A matriz final contém sequências de 218 espécimes, incluindo 70 sequências de ITS, 71 sequências de LSU e 62 sequências de *tef-1α* de coletas brasileiras. Além disso, foram geradas 38 sequências de *rpb2* que não foram incorporadas nas análises filogenéticas. O melhor modelo de substituição de nucleotídeos para cada partição foi: TVM+G para ITS1; JC para 5.8S; TIM3+G para ITS2; TrN+I+G para LSU; TIM3ef+I+G para *tef-1α* éxon códon 1; SYM+I+G para *tef-1α* éxon códon 2; TIM2ef+I+G para *tef-1α* éxon códon 3; TVM+I+G para *tef-1α* íntron. As árvores de Inferência Bayesiana e Máxima Verossimilhança apresentaram topologia similar. A árvore de Inferência Bayesiana é apresentada na Figura 1 com valores de probabilidade posterior (PP) e bootstrap (BS) mostrados acima dos ramos.

Em *Agaricus* subgênero *Pseudochitonia,* apenas quatro sequências (ACM429, MVL21, MAN1109 e ACM328) foram agrupadas em *Agaricus* seção *Xanthodermatei*, sendo a única seção do subgênero que incluiu sequências de espécimes brasileiros. *Agaricus bernardii* Quél. (FLOR48221) e *Agaricus subperonatus* (J.E. Lange) Singer (FLOR48246), espécimes coletados no Reino Unido, agruparam em *Agaricus* seção *Chitoniodes* e *Bivelares*, respectivamente.

Em *Agaricus* subgênero *Agaricus* (Figura 2) foram agrupadas sequências de seis espécimes (JS75, FTF193, MPD126, MAN1153, MPD11, MAN1151) coletados em região de Mata Atlântica nos estados de Paraná, Santa Catarina e Rio Grande do Sul. A sequência do espécime MAN1151 aparece próxima a duas sequências de *Agaricus campestris* L. (LAPAG370 e CA637), espécie tipo do gênero. *Agaricus porphyrocephalus* F.H. Møller (FLOR48207), coletado no Reino Unido, também agrupou em *Agaricus* seção *Agaricus*.


0,91/95
Agaricus kerriganii AH-44509 SPA
Agaricus kerriganii LAPAG808 SPA
1/100
Agaricus edmondoi LAGAP412 SPA
1/100
Agaricus matrumAH-44506 SPA
1/100
Agaricus matrumLAPAG855 SPA
Agaricus friesianus LAPAG592 FRA
1/55
Agaricus purpurellusTRgmb01309 ITA
0,82/97
Agaricus gemlii AH-44510 SPA
0,92/78
Agaricus comtulusLAPAG724 SPA
Agaricus arrillagarumLAPAG810 SPA
0,83/-
Agaricus pallensLAPAG926 SWE
1/96
Agaricussp. ZRL2012576 CHI
Agaricus sp. ZRLWXH3067 CHI
0,81/-
1/-
1/-
Agaricus sp. ZRLWX3076 CHI
Agaricussp. ZRLWXH3150 CHI
Agaricus pseudolutosusLAPAG454 SPA
0,85/-
Agaricus callaciiAH-42929 SPA
Agaricus sp. ZRL2012199 CHI
0,81/-
1/87
1/100
Agaricus sp. NT055 THA
Agaricus sp.ZRL2012357 CHI
Agaricussp. CA846 THA
1/100
Agaricus sp. ZRL2011039 CHI
Agaricussp. ZRL2012714 CHI
0,99/-
Agaricus sp. MPD30 BRA
1/100
Agaricussp. CA848 THA
1/100
Agaricus sp. CA921
0,88/55
Agaricussp. ZRL2011156 CHI
0,99/-
Agaricussp. ZRLLD013 THA
1/83
1/96
Agaricus aridicolaLAPAG589 SPA
0,87/-
Agaricussp.TL2013a AUS
0,99/58
Agaricus wariatodesMEL2063323
Agaricussp. ZRL2012004 CHI
0,89
Agaricus huijsmanii LAPAG639 SPA
Agaricus sp. ZRL2012012 CHI
Minores
1/100
Agaricus sp. MPD 107 BRA
Agaricus sp. MPD 108 BRA
Agaricus sp. MPD 109 BRA
0,76/53
Agaricus sp. MPD 110 BRA
1/100
1/100
Agaricus sp. FTF 194 BRA
Agaricussp. MAR1274 BRA
0,99/93
Agaricus sp. JDuque78 BRA
Clado a
0,99/83
0,96/61
1/100
1/100
Agaricussp. MAN 1178 BRA
Agaricussp. MPD 134 BRA
Agaricus sp. MPD 115 BRA
1/100
//
Agaricussp. ACM473 BRA
Agaricus sp. MAN686 BRA
1/98
Agaricus sp. MAN1183 BRA
1/100
Agaricussp. ZRLWXH3161 CHI
0,96/59
Agaricussp. LAPAM14 DOM
Agaricus candidolutescens LD2012129 THA
Incertae sedis
Agaricus sp. ACM250 BRA

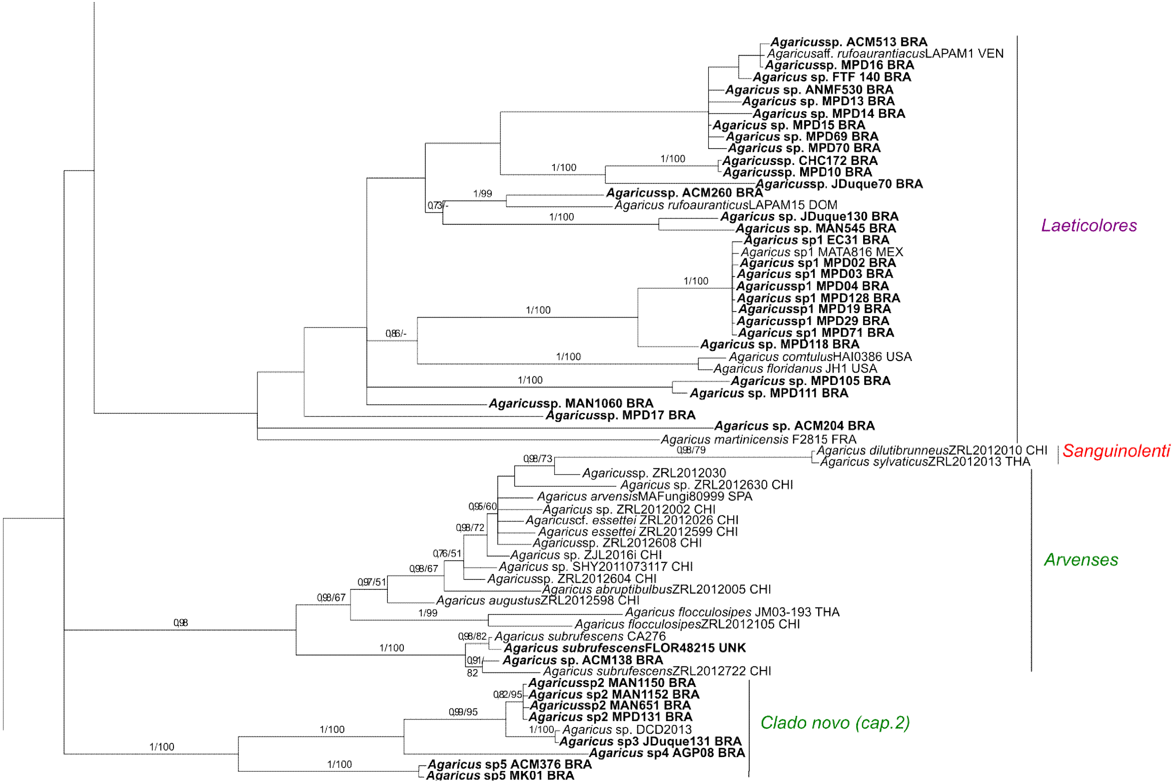


Figura 1a. Árvore de Inferência Bayesiana do gênero *Agaricus* baseada em ITS, LSU e *tef-1α* enraizada com *Heinemanomyces* sp. (ZRL185). As sequências geradas neste trabalho estão em **negrito**. Os valores de Probabilidade Posterior (PP) maiores que 0,7 e *Bootstrap* (BS) maiores que 50% são mostrados acima dos ramos (PP/BS). BRA: Brasil; USA: Estados Unidos da América; CHI: China; SPA: Espanha; SWE: Suécia; FRA: França; THA: Tailândia; DOM: República Dominicana; CAN: Canadá; MAL: Malásia; TOG: Togo; ITA: Itália; UNK: Reino Unido; VEN: Venezuela; MEX: México; HUN: Hungria. Em cores os subgêneros: Vermelho – *Pseudochitonia*, Azul – *Agaricus*, Amarelo – *Spissicaules*, Verde – *Flavoagaricus*, Lilás – *Minores*.

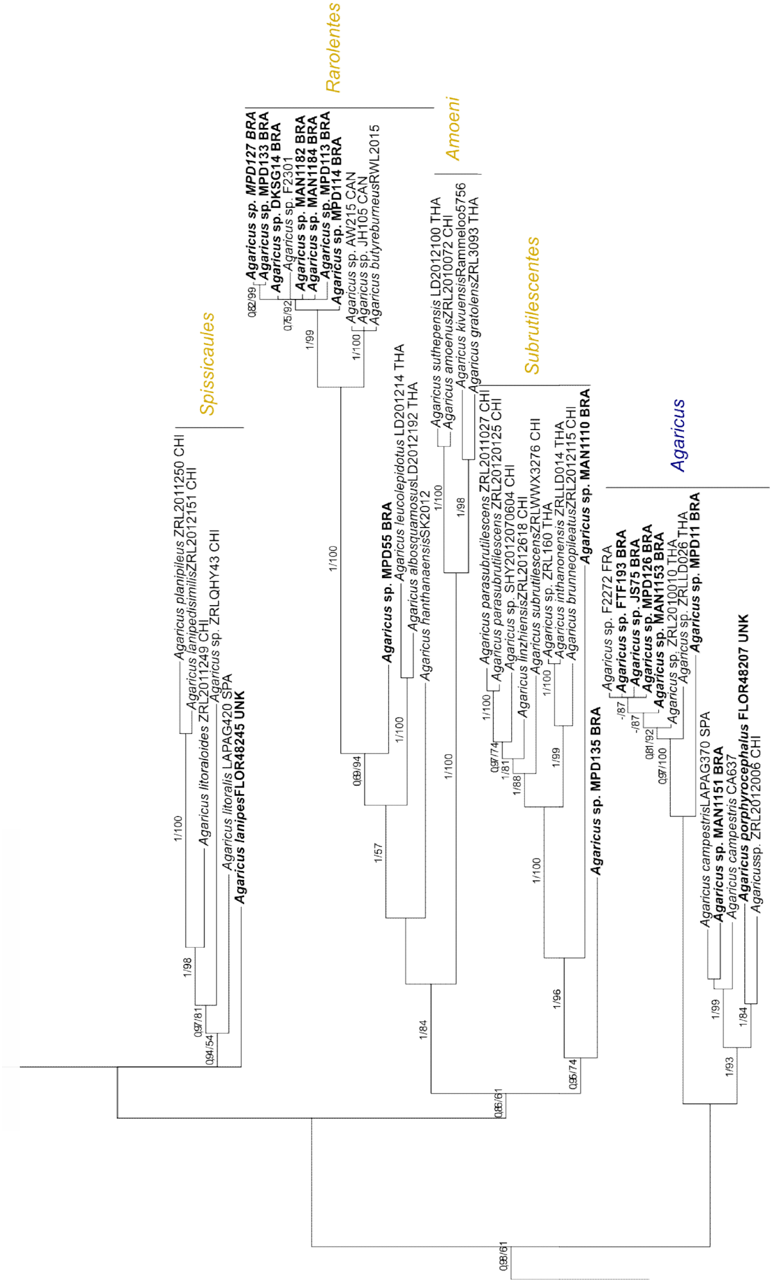

Spissicaules
Agaricus planipileus ZRL2011250 CHI
Agaricus lanipedisimilisZRL2012151 CHI
Agaricus litoraloides ZRL2011249 CHI
Agaricus sp. ZRLQHY43 CHI
Agaricus litoralis LAPAG420 SPA
Agaricus lanipesFLOR48245 UNK
1/100
1/98
0,97/81
0,94/54
Rarolentes
Agaricus sp. MPD127 BRA
Agaricus sp. MPD133 BRA
Agaricus sp. DKSG14 BRA
Agaricus sp. F2301
Agaricus sp. MAN1182 BRA
Agaricus sp. MAN1184 BRA
Agaricus sp. MPD113 BRA
Agaricus sp. MPD114 BRA
Agaricus sp. AW215 CAN
Agaricus sp. JH105 CAN
Agaricus butyreburneusRWL2015
0,82/99
0,75/92
1/99
1/100
1/100
Agaricus sp. MPD55 BRA
Agaricus leucolepidotus LD201214 THA
Agaricus albosquamosusLD2012192 THA
Agaricus hanthanaensisSK2012
0,89/94
1/100
1/57
Amoeni
Agaricus suthepensis LD2012100 THA
Agaricus amoenusZRL2010072 CHI
Agaricus kivuensisRammeloo5756
Agaricus gratolensZRL3093 THA
1/100
1/98
1/100
1/84
Subrutilescentes
Agaricus parasubrutilescens ZRL2011027 CHI
Agaricus parasubrutilescens ZRL20120125 CHI
Agaricus sp. SHY2012070604 CHI
Agaricus linzhiensisZRL2012618 CHI
Agaricus subrutilescensZRLWWX3276 CHI
Agaricus sp. ZRL160 THA
Agaricus inthanonensis ZRLLD014 THA
Agaricus brunneopileatusZRL2012115 CHI
Agaricus sp. MAN1110 BRA
1/100
0,97/74
1/81
1/88
1/100
1/99
1/100
Agaricus sp. MPD135 BRA
1/96
0,95/74
0,86/61
Agaricus
Agaricus sp. F2272 FRA
Agaricus sp. FTF193 BRA
Agaricus sp. JS75 BRA
Agaricus sp. MPD126 BRA
Agaricus sp. MAN1153 BRA
Agaricus sp. ZRL2010010 THA
Agaricus sp. ZRLLD026 THA
Agaricus sp. MPD11 BRA
-/87
-/87
0,81/92
0,97/100
Agaricus campestrisLAPAG370 SPA
Agaricus sp. MAN1151 BRA
Agaricus campestris CA637
Agaricus porphyrocephalus FLOR48207 UNK
Agaricussp. ZRL2012006 CHI
1/99
1/84
1/93
0,98/61

Figura 1b. Árvore de Inferência Bayesiana do gênero *Agaricus* baseada em ITS, LSU e *tef-1α* enraizada com *Heinemanomyces* sp. (ZRL185). As sequências geradas neste trabalho estão em **negrito**. Os valores de Probabilidade Posterior (PP) maiores que 0,7 e *Bootstrap* (BS) maiores que 50% são mostrados acima dos ramos (PP/BS). BRA: Brasil; USA: Estados Unidos da América; CHI: China; SPA: Espanha; SWE: Suécia; FRA: França; THA: Tailândia; DOM: República Dominicana; CAN: Canadá; MAL: Malásia; TOG: Togo; ITA: Itália; UNK: Reino Unido; VEN: Venezuela; MEX: México; HUN: Hungria. Em cores os subgêneros: Vermelho – *Pseudochitonia*, Azul – *Agaricus*, Amarelo – *Spissicaules*, Verde – *Flavoagaricus*, Lilás – *Minores*.

O subgênero *Spissicaules* aparece como parafilético nas análises. Sequências de oito espécimes (Figura 3) foram agrupadas em *Agaricus* seção *Rarolentes* (MPD127, MPD133, DKSG14, MAN1182, MAN1184, MPD113, MPD114 e MPD55), e duas (MAN1110 e MPD135) em *Agaricus* seção *Subrutilescentes*. As seções *Amoeni* e *Rarolentes* aparecem como clado-irmão da seção *Subrutilescentes*. Apesar do parafiletismo do subgênero, as seções apresentaram monofiletismo.

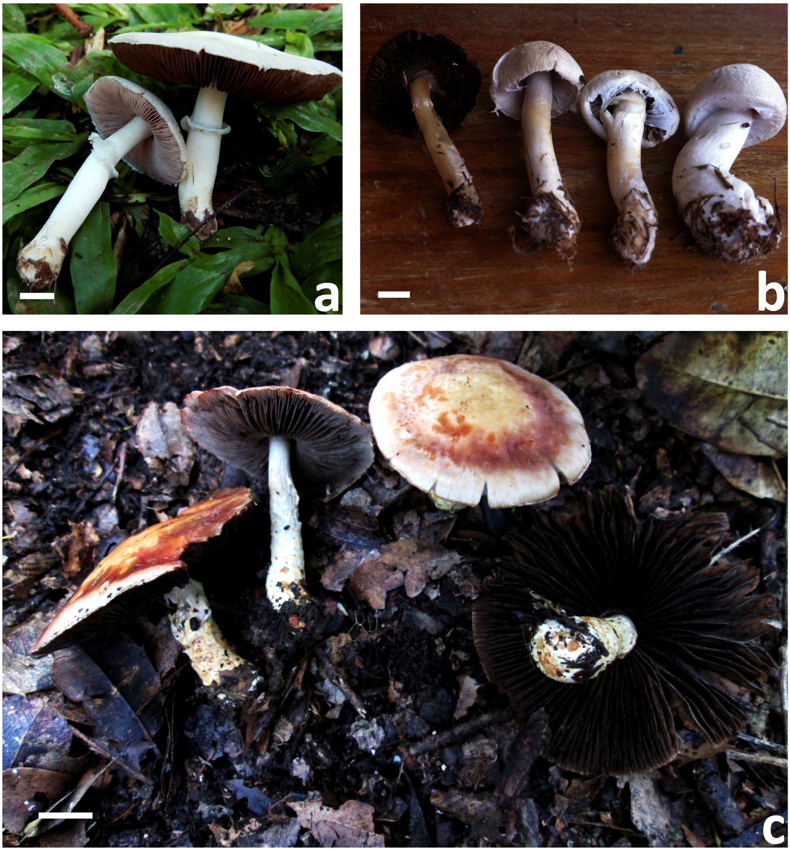


Figura 2. Basidiomas de *Agaricus* subgênero *Agaricus*. a MPD126; b. MAN1151; c.MPD11. Escalas a,b: 1cm; c:2cm. Fotos: a. Ariadne Nóbrega MarinhoFurtado; b. Maria Alice Neves; c. Mariana Drewinski.

Figura 3. a-c, e. Basidiomas de *Agaricus* subgênero *Spissicaules* seção *Rarolentes*. a-b MPD113; c. MPD127; e.DKSG14. d. Espécime ACM138 *Agaricus* cf. *subrutilescentes* *Agaricus* subgênero *Flavoagaricus* seção *Arvenses*. Escalas a-b,d-e: 1cm; c:2cm. Fotos: a-c, e. Mariana Drewinski; d. Altielys Casale Magnago.

No subgênero *Flavoagaricus* foi observada a formação de um clado composto apenas por materiais brasileiros, sem nenhuma sequência representante das seções e subgêneros já descritos. As sequências de oito espécimes coletados no Rio de Janeiro (JDuque 131), Santa Catarina (MAN1150, MAN1152, MAN651, MPD131, AGP08, ACM376) e Paraná (MK01) agruparam em uma nova seção, grupo-irmão de *Agaricus* seção *Arvenses*. As quatro espécies desta nova seção são descritas no capítulo 2.

A sequência do espécime ACM138 (Figura 3), coletado no Espírito Santo, foi agrupada em *Agaricus* seção *Arvenses*, juntamente com outras três sequências de *Agaricus subrufescens* Peck (CA276) de outros países como China (ZRL2012722) e Reino Unido (FLOR48215).

Em *Agaricus* subgênero *Minores*, apenas uma sequência (MPD30) agrupou em *Agaricus* seção *Minores*. Duas sequências (MAN1183 e ACM250) agruparam no clado *Incertae sedis* juntamente com outras sequências estudadas por Zhao et al. (2016). Doze sequências (MPD107, MPD108, MPD109, MPD110, FTF194, MAR1274, JDuque78, MAN1178, MPD134, MPD115, ACM473, MAN686), representando possíveis seis espécies, agruparam num clado exclusivo de representantes brasileiros, o *clado a*, com espécimes coletados no Rio de Janeiro, São Paulo, Santa Catarina e Paraná (Figura 4). A maioria das sequências geradas neste estudo foi agrupada em *Agaricus* seção *Laeticolores* (Figura 5), com um total de 29 espécimes, incluindo as sequências de *Agaricus* sp1, a espécie nova com pleurocistídio proposta no capítulo 1 (EC31, MPD02, MPD03, MPD04, MPD19, MPD29, MPD71 e MPD128). *Agaricus* seção *Laeticolores* aparece como grupo irmão dos clados *Minores*, *Incertae sedis* e *clado a*, representando o subgênero *Minores*.

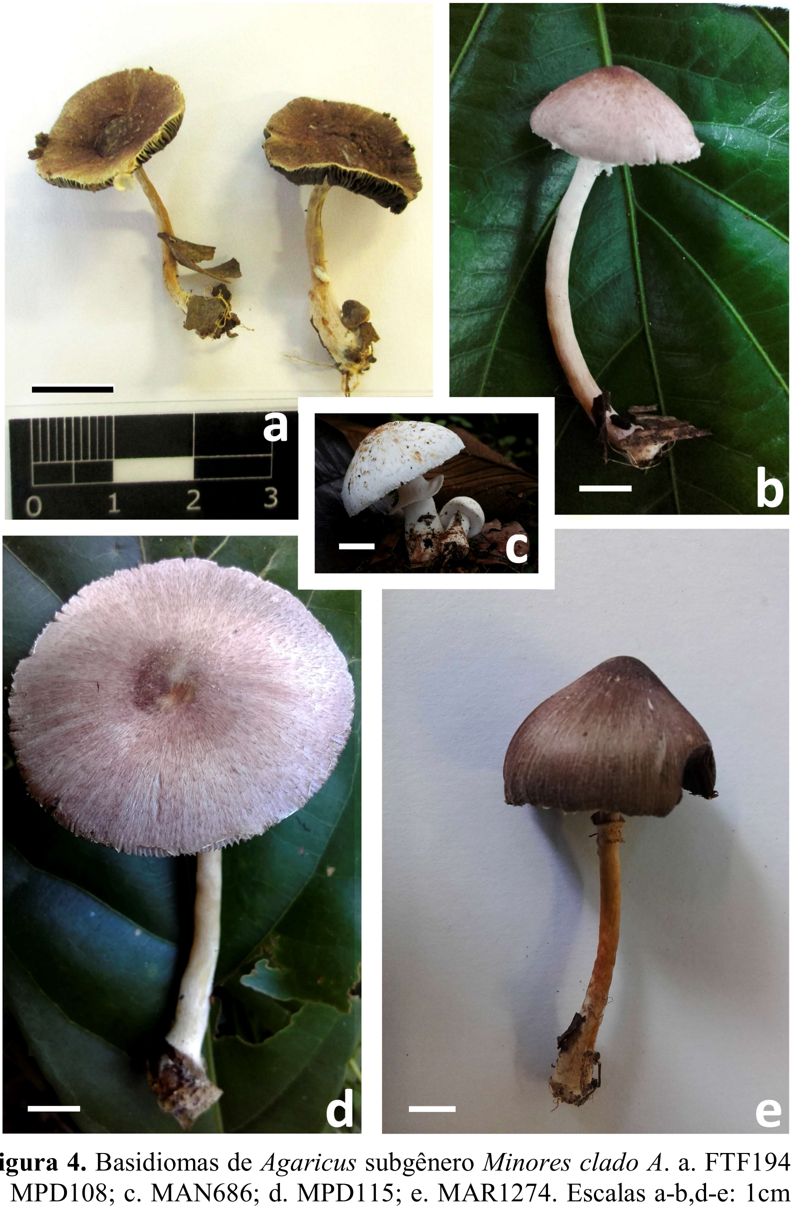


Figura 4. Basidiomas de *Agaricus* subgênero *Minores clado A*. a. FTF194; b. MPD108; c. MAN686; d. MPD115; e. MAR1274. Escalas a-b,d-e: 1cm; c:2cm. Fotos: a-b,d-e. Mariana Drewinski; c. Maria Alice Neves.

Figura 5. Basidiomas de *Agaricus* subgênero *Minores* seção *Laeticolores*. a. MPD118; b. MPD15; c. MAN1060; d.ANMF530; e. MPD17; f. ACM204. Escalas a-f: 1cm. Fotos: a-b,e. Mariana Drewinski; c. Maria Alice Neves; d. Ariadne Nóbrega MarinhoFurtado; f. Altielys Casale Magnago.

Das sequências dos 53 espécimes brasileiros, aproximadamente 70% do total dos espécimes analisados foram agrupadas nos subgêneros *Minores* e *Flavoagaricus*, distribuídas nas seções já conhecidas para os subgêneros e em mais dois clados novos. A relação filogenética entre os subgêneros *Agaricus* e *Pseudochitonia* encontrada nos resultados deste estudo não corrobora com os resultados obtidos no trabalho de Zhao et al. (2016). Apesar do baixo valor de sustentação dos ramos, o clado *Pseudochitonia* aparece aqui como sendo o mais externo da árvore e os subgêneros *Spissicaules* e *Pseudochitonia* aparecem parafiléticos. Zhao et al. (2011) reportaram a polifilia de *Spissicaulis*. As relações entre os subgêneros *Minores, Flavoagaricus* e *Spissicaules* corroboram com a encontrada por Zhao et al. (2016).

Agaricus seção *Arvenses* e o clado novo descrito no capítulo 2 aparecem como grupo irmão dos clados *Minores*+*Incertae sedis*+*clado a*+*Laeticolores*. Nas nossas análises, o clado *Incertae sedis* aparece como grupo irmão do *clado a*, mais próximos de *Agaricus* seção *Minores*, diferente de Zhao et al. (2016) onde aparece como grupo irmão de *Agaricus* seção *Laeticolores*.

Heinemann (1956) definiu *Agaricus trisulphuratus* Berk. como espécie tipo do subgênero *Lanagaricus* (seção *Trisulphurati*). Zhao et al. (2011) estudaram um espécime de *A. trisulphuratus* e observaram que o espécime foi agrupado no clado TRb no subgênero *Pseudochitonia*. Dessa forma, no trabalho de Zhao et al. (2016), o subgênero *Lanagaricus* foi considerado sinônimo heterotípico do subgênero *Pseudochitonia*. *Agaricus* seção *Laeticolores* foi proposta por Heinemann (1961) dentro do subgênero *Lanagaricus*, baseada em espécimes coletados na África e na América Central. Zhao et al. (2016) classificou a seção *Laeticolores* no subgênero *Minores*.

As sequências geradas neste estudo foram agrupadas em todos os subgêneros de *Agaricus*, em pelo menos uma de suas seções, sendo nos subgêneros *Agaricus, Flavoagaricus* e *Minores* incluídas em todas as suas seções. Já nas seções *Spissicaules, Amoeni* e *Rubricosi* de *Agaricus* subgênero *Spissicaules*; e nas seções *Nigrobrunnescentes, Sanguinolenti, Flocculenti, Bohusia, Brunneopicti, Hondenses, Chitonioides, Bivelares, Crassispori* e *Trisulphurati* de *Agaricus* subgênero *Pseudochitonia*, nenhuma sequências dos espécimes coletado no Brasil foi agrupada.

CAPÍTULO 1 − *Agaricus* sp1: a new neotropical species with pleurocystidia in *Agaricus* section *Laeticolores*

Agaricus sp1: a new neotropical species with pleurocystidia in *Agaricus* section *Laeticolores*

Abstract

Agaricus is a monophyletic genus with worldwide occurrence and more than 400 species described. The genus is composed by representatives growing on soil and can be easily recognized by the macroscopic characters that include the presence of annulus on stipe and free and dark brown lamellae when mature. Although the genus is easily recognized in the field because its macroscopic characters, the identification at the species level is difficult. Based on specimens collected in the states of Paraná and Santa Catarina in Brazil we propose a new species, *Agaricus* sp1. The new taxon is distinguished mainly by the presence of pleurocystidia, a rare condition in the genus. The molecular analyses based on nuc rITS1-5.8-ITS2 (ITS) barcode sequences show that *A.* sp1 belongs to *Agaricus* section *Laeticolores*.

Keywords = Agaricaceae, Brazil, ITS, Phylogeny, Systematic.

Introduction

The genus *Agaricus* L. is monophyletic (Vellinga, 2004) and comprises approximately 400 species worldwide (Karunarathna et al., 2016). Linnaeus (1753) was the first to describe the genus, in a very broad way, including all mushrooms with lamellae and stipe. Due to this fact, many species of mushroom-forming fungi were firstly described as *Agaricus* and has this genus name as basionym. The representatives of *Agaricus* are characterized by usually large and fleshy basidioma, with white, yellow, brown and sometimes blackish or purple pileus, which is easily separable from the stipe; the lamellae are close and free, white when young, changing to pink and finally becoming brown to dark brown when the basidiospores are mature; the annulus is always present and it is usually membranous and white, sometimes double (Singer, 1986; Mitchell & Bresinsky, 1999; Zhao et al., 2016). Macroscopic characters can be easily recognized in the field but the species delimitation is very complex. Environmental factors, intraspecific variability and lack of characters to differentiate species are some problems found in the taxonomy of the genus (Zhao et al., 2011). *Agaricus* is microscopically characterized by the pileipellis as a cutis, hymenophoral trama regular to slightly interwoven, hyphae without clamp connections, basidiospores brown without a true apical pore, absence of pleurocystidia and presence or absence of cheilocystidia (Heinemann, 1956; Singer, 1986; Zhao et al., 2016). In addition to macro and microscopic features, the Schäeffer reaction and organoleptic character as odor is also useful to identify some species (Heinemann, 1956).

The use of molecular data and the improvement of phylogenetic studies have helped to clarify the relationship among *Agaricus* taxa in the last decades. Bunyard et al. (1996) produced the first phylogenetic analysis of the genus, based on 26S rDNA and the IGR-1/5S rDNA, and showed that the classification based only on macrochemical and morphological characters are mostly artificial. Recently, Zhao et al. (2016) analyzed morphological and molecular data from more than 700 samples and proposed a reconstruction of the *Agaricus* systematic based on divergence time to define taxonomic ranks. They produced a multi-gene phylogeny combining ITS, LSU, *tef-1α* and *rpb2* sequences from samples from tropical and temperate areas, and segregated the genus into five subgenera and twenty sections.

Fries (1874) was the first to propose *Minores* to name a group in *Agaricus*. Heinemann (1956) considered *Minores* as a subsection in subgenus *Agaricus* section *Arvenses*. Recent studies have shown that *Minores* is a monophyletic subgenus, sister group of *Agaricus* subgenus *Flavoagaricus* (Zhao et al., 2016). Zhao et al. (2016) delimited *Agaricus* subgenus *Minores* with basidiomata often small to medium size; KOH reaction positive and Schäffer reaction usually positive; pileus surface and context often turning yellow; odour of anise; annulus superous, simple or double with floccose or squamose lower surface; and cheilocystidia usually present. *Agaricus* subgenus *Minores* includes *Agaricus* sections *Minores* and *Laeticolores*. Section *Laeticolores* was established by Heinemann (1961) based on samples from Africa and Central America in subgenus *Lanagaricus*, which included tropical species. The characters of *Agaricus* section *Laeticolores* are those of subgenus *Minores*, with Schäffer and KOH reaction positive, annulus lower surface smooth, fibrillose or squamose, with remnants of universal veil often present on the pileus (Zhao et al., 2016).

Pegler (1990) revised some species of agaricoid fungi from Brazil collected by J. P. F. C. Montagne in 1843. Pegler (1990) described 28 *Agaricus* species with holotypes deposited in the Herbarium of the Muséum National d'Histoire Naturelle in France. Heinemann (1993) studied the *Agariceae* in Brazil and described a new species, *Agaricus meijeri* Heinem., collected in Paraná. Meijer (2008) described another *Agaricus* from Paraná, *Agaricus stijvei* de Meijer. Unfortunately, there are not available sequences of these specimens. Collection and review of materials from subtropical regions is important for a better knowledge of the species occurrence and the infrageneric relationships in the group (Zhao et al., 2011). In this paper a new species of *Agaricus* section *Laeticolores* is described based on morphological and molecular data.

Materials and methods

Sampling and morphological study

The specimens herein studied were collected in the states of Paraná and Santa Catarina, South Brazil, and were described macroscopically following Largent (1986) and Vellinga (1988). The chemical reactions were performed with KOH 3% (yellow if positive) and NaOH 10% (orange-red if positive) on the pileus and stipe surface of fresh or dried basidiomata. The color code is based on the *Online Auction Color Chart* (Kramer, 2004). Micromorphological features were observed from dried specimens following Largent et al. (1977) and Vellinga (1988). The notation [a/b/c] at the beginning of a set of basidiospores represents: (a) basidiospores were measured from (b) basidiomata taken from (c) collections. Basidiospore measurements were based on at least 20 structures per specimen, including Q, quotient of length to width, Q_m, the mean of Q-value, L_m, the mean of length and W_m the mean of width. Basidiospore shape was based on Q-value (Bas, 1969).

DNA extraction, amplification and sequencing

DNA was extracted from dried pileus and lamellae fragments, following a modified CTAB extraction method (Doyle & Doyle, 1987 adapted por Góes-Neto et al., 2005). The nuc rITS1-5.8S-ITS2 (ITS) sequences were generated using the primer pair ITS8F and ITS6R (Dentinger et al., 2010). The PCR mix was prepared containing 4 μL genomic DNA

(1:10), 10 µL water, 10 µL Master mix (Promega) and 0.5 µL each primer (10pmol/L). The PCR program was according to Zhao et al. (2011): 5 min at 95 °C; 35 cycles (1 min. at 94 °C, 1.5 min. at 55 °C, 1.5 min. at 72 °C); 5 min at 72 °C. The PCR products were purified with PEG 20% and sequenced at Centro de Pesquisas René Rachou (Fiocruz Minas, Brazil) using the same primer pair.

Phylogenetic analyses

The generated sequences were manually checked and edited with Geneious v.8.1 (Kearse et al., 2012). Our dataset is composed of sequences from eight newly collected *Agaricus* specimens from the states of Paraná and Santa Catarina, South Brazil, plus 34 sequences of *Agaricus* sect. *Minores* taxa retrieved from GenBank. Sequence of *Agaricus linzhiensis* R.L. Zhao (ZRL2012618) – *Agaricus* section *Subrutilescentes* – was used as outgroup. Newly obtained sequences were deposited in GenBank and accession numbers and country of origin are given in Table 1. Sequences were aligned in MAFFT v7 (Katoh, 2013) following the Q-INS-I criteria and then manually corrected using MEGA v7 (Tamura et al., 2013). The final ITS alignment had a total of 716 characters (gaps included). To identify the best nucleotide evolution model, we used the AIC criterion (Akaike Information Criterion) in jModelTest v2.1.6 (Darriba et al., 2012; Guindon et al., 2003). The ITS region was partitioned in ITS 1, 5.8S and ITS2; estimating evolution model for each partition. Maximum Likelihood (ML) analysis was performed in RAxML v8.2.8 (Stamatakis, 2014) using GTRGAMMA model with a rapid bootstrap analysis with 1,000 replicates and search for best-scoring ML tree, with other parameters estimated by the software. Bayesian Inference (BI) analysis was performed with Mr.Bayes v3.2.6 (Ronquist & Huelsenbeck, 2003) using three partitions (ITS1, 5.8S, ITS2), with two independent runs, four simultaneous independent chains and 20,000,000 generations with sample frequency every 1,000 generation. The jModelTest, RAxML and Mr.Bayes were used in CIPRES Science Gateway 3.1 (Miller et al., 2010). The outputs of the analyses were displayed with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table1. Specimens used in the molecular analyses. In **bold** are the sequences generated during this work. * Sequences to be deposited in GenBank.

Subgenus	Section	Taxon	Collection number	ITS	Location	Reference
Minores	*Minores*	*Agaricus kerriganii*	LAPAG808	KT951306	Madrid, Spain	Zhao et al. (2016)
		Agaricus kerriganii	AH-44509	KF447893	León, Spain	Parra (2013)
		Agaricus edmondoi	LAPAG412	KT951326	Segovia, Spain	Zhao et al. (2016)
		Agaricus matrum	LAPAG855	KT951310	Asturias, Spain	Zhao et al. (2016)
		Agaricus matrum	AH-44506	KF447896	La Rioja, Spain	Parra (2013)
		Agaricus friesianus	LAPAG592	KT951316	Pyrénées Atlantiques, France	Zhao et al. (2016)
		Agaricus sp.	CA848	JF727864	Thailand	Zhao et al. (2011)
		Agaricus sp.	CA921	KT951323	-	Zhao et al. (2016)
		Agaricus sp.	ZRL2011156	KT951352	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRLLD013	KT951384	Thailand	Zhao et al. (2016)
		Agaricus sp.	ZRLWXH3076	KT951388	Fujian, China	Zhao et al. (2016)
		Agaricus sp.	ZRLWXH3150	KT951390	Guangdong, China	Zhao et al. (2016)
		Agaricus comtulus	LAPAG724	KT951332	Burgos, Spain	Zhao et al. (2016)
		Agaricus gemlii	AH-44510	KF447891	Canarias, Spain	Parra (2013)
		Agaricus pallens	LAPAG926	KT951315	Lapland, Sweden	Zhao et al. (2016)
		Agaricus sp.	CA846	JF727865	Thailand	Zhao et al. (2011)
		Agaricus sp.	ZRL2012357	KT951369	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRLWXH3067	KT951387	Jiangxi, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012576	KT951372	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012714	KT951381	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2011039	KT951351	Yunnan, China	Zhao et al. (2016)
		Agaricus aridicola	LAPAG589	KT951331	Castellón, Spain	Zhao et al. (2016)
		Agaricus pseudolutosus	LAPAG454	KT951329	Burgos, Spain	Zhao et al. (2016)
		Agaricus sp.	ZRL2012012	KT951359	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012199	KT951367	Yunnan, China	Zhao et al. (2016)
		Agaricus callacii	AH-42929	KF447899	Canarias, Spain	Parra (2013)
	Laeticolores	***Agaricus* sp1**	**MPD02**	*	Santa Catarina	

		***Agaricus* sp1**	**MPD03**	*	Santa Catarina, Brazil	
		***Agaricus* sp1**	**MPD04**	*	Santa Catarina, Brazil	
		***Agaricus* sp1**	**MPD29**	*	Santa Catarina, Brazil	
		***Agaricus* sp1**	**MPD128**	*	Santa Catarina, Brazil	
		***Agaricus* sp1**	**MPD19**	*	Santa Catarina, Brazil	
		***Agaricus* sp1**	**MPD71**	*	Paraná, Brazil	
		***Agaricus* sp1**	**EC31**	*	Santa Catarina, Brazil	
		Agaricus sp1	MATA816	JF727870	Veracruz, Mexico	Zhao et al. (2011)
		Agaricus sp.	RMC-1256	KM349611	Arizona, USA	Kerrigan (2016)
		Agaricus sp.	RMC-1257	KM349612	Arizona, USA	Kerrigan (2016)
		Agaricus rufoaurantiacus	LAPAM15	KT951313	Sosúa, Dominican Republic	Zhao et al. (2016)
Incertae sedis		*Agaricus* sp.	ZRLWXH3161	KT951391	Guangdong, China	Zhao et al. (2016)
		Agaricus sp.	LAPAM14	KT951312	Sosúa, Dominican Republic	Zhao et al. (2016)
		Agaricus candidolutescens	LD2012129	KT951335	Thailand	Zhao et al. (2016)
Spissicaules	*Subrutilescentes*	*Agaricus linzhiensis*	ZRL2012618	KT951378	Tibet, China	Zhao et al. (2016)

Results and Discussion

The final dataset contains 41 ingroup ITS sequences, including eight generated in this study. The best models of nucleotide substitution estimated for each partition in the datasets were: TPM3uf+G to ITS1, JC to ITS 5.8S, and HKY+I+G to ITS 2. Both ML and BI tree topologies were the same. The BI obtained by the analysis is presented in Figure 1, with posterior probability and bootstrap values above branches.

Sequences of the new species clustered in a well-supported (1 PP/100 BS) clade with a sequence from Mexico (JF727870) and other sequences of *Agaricus* section *Laeticolores*. Zhao et al. (2011) produced a phylogeny including tropical and subtropical species of *Agaricus*. They analyzed ITS data sequence and observed that only about one-third of the species belong to the classical sections historically proposed by European researchers. The tropical species were distributed in eleven exclusively tropical clades, seven well supported, numbered as TR I to TRVII on their work. The sequence MATA816 (JF727870) clustered in TR II with other five specimens, but did not receive a taxonomic treatment and was not included in the analyzes of Zhao et al. (2016). Although it is necessary to revise the specimen MATA816, our results indicate that is the same species here described, therefore suggesting a neotropical distribution of *A.* sp1.

The sequences KT951391 (ZRLWXH3161), KT951312 (LAPAM14), and KT951335 (LD2012129) formed a separate clade, close to *Agaricus* sections *Minores* and *Laeticolores*, with an uncertain taxonomic position. In our analysis *Agaricus* section *Laeticolores* appear as sister group of *Agaricus* section *Minores*, and clade *Incertae sedis*, as well as in Zhao et al. (2016) study. Zhao et al. (2016) placed *Agaricus* section *Laeticolores* in subgenus *Minores*, including one species, *Agaricus rufoaurantiacus* (LAPAM15).

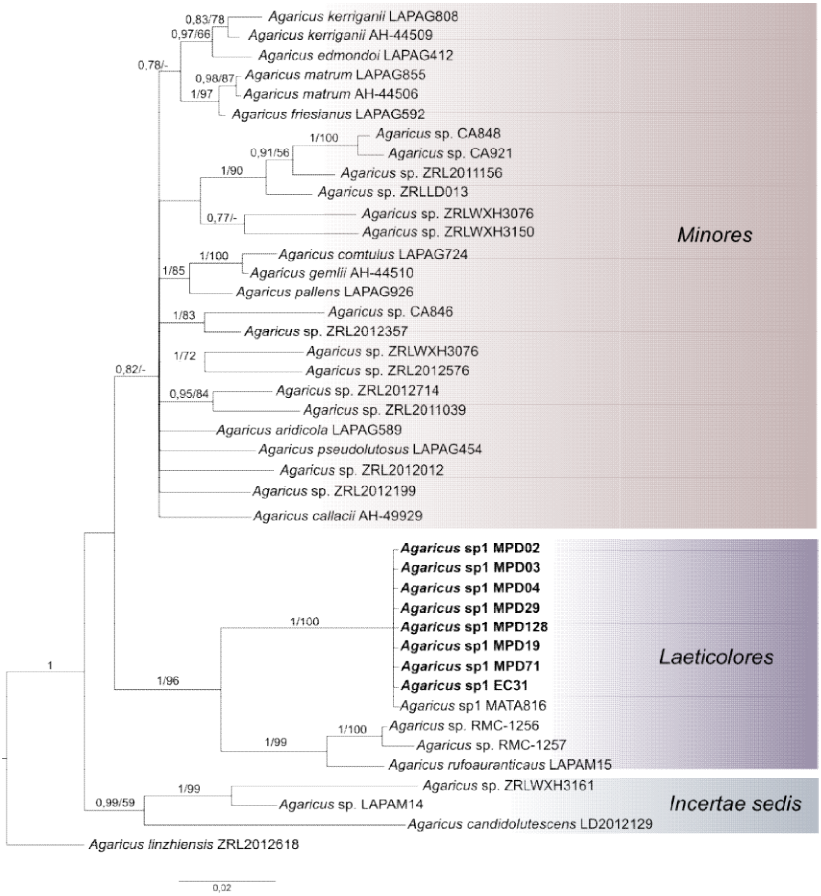


Figure 1. Bayesian Inference tree of *Agaricus* subgenus *Minores* based on ITS data and rooted with *A. linzhiensis*. The sequences generated in this work are in **bold**. Bayesian posterior probability (PP) above 0.7 and bootstrap values (BS) above 50% are shown above branches (PP/BS).

Taxonomy

Agaricus sp1 *nov.*

Fig 2, 3.

Diagnosis: Similar to *Agaricus pleurocystidiatus* by presence of pleurocystida but differing in the pileus surface covered by concentrically arranged purple scales, pileus margin regular, stipe with brown scales, slightly yellowish in some parts when exposed.

Etymology:

Holotype: Brazil. Santa Catarina: Florianópolis, Universidade Federal de Santa Catarina, next to the botanic department, 10-XII-2015, *M. P. Drewinski MPD29*.

Pileus 30−122 mm diam., at first parabolic, then truncately conical to convex and finally expanding to plane-convex, slightly depressed, covered by concentrically arranged purple (oac527) scales on beige (oac780) background, darker at center with concentrated scales and scattered towards the margin, margin regular. **Pileus context** approx. 5mm thick at center and 1mm at margin, whitish or slightly yellowish in some parts when exposed, fleshy. **Lamellae** free, white at first, then pinkish and finally brown when mature, margin regular, crowded, with lamellulae. **Stipe** 102−120 × 9−20 mm, central, tapering at base, fistulous, beige (oac767) pearly, smooth above annulus, below with brown scales, changing to yellow in some parts including the context of the basal region. **Annulus** superous, pendant, membranous, floccose at lower surface, whitish. **Basidiospores** [160/10/8] 5.0−6.2(−7.5) × (3.7−)5.0 µm, [Q= 1.25-1.66; Q_m= 1.42; L_m=4.77µm; W_m=3.26µm], broadly ellipsoid to ellipsoid, dark brown, smooth, thick-walled, apiculus conspicuous, without a germ pore (Fig 2.e). **Basidia** (16.2−)21−37(−40) × 6.2−10.0(−11.2) µm, narrowly utriform to clavate, hyaline, smooth, 4-spored (Fig 2.b). **Pleurocystidia** (25−)26−43 × (16.2−)18.7−23 µm, ovoid, subglobose to broadly clavate, hyaline, smooth, thick-walled (Fig 2.d). **Cheilocystidia** (11.2−)13.7−45(−47) × (7.5−)10−21(−23) µm, clavate, subglobose to obovoid, hyaline, thin-walled, usually covering all the lamellae edge (Fig 2.c). **Lamellar trama** regular, with abundant oleiferous hyphae, 6.5(−8.7) µm diam. **Pileipellis** a cutis composed of thin to rather thick-walled, cylindrical hyphae, (3.7−)5.0−11.2µm diam., branched, with purple pigments (Fig

2.a). **Pileus context** composed of thin-walled, cylindrical hyphae, (5.0−)6.2−12.5 µm diam., cylindrical, with abundant oleiferous hyphae. **Stipitipellis** a cutis, composed of thin-walled, cylindrical hyphae, 5.0-10.0 µm diam., inflated. **Macrochemical reactions** KOH 3% positive, yellow; NaOH 10% positive, yellow.

Habit and habitat: solitary to gregarious, terrestrial, growing on grassland in urban area and in Atlantic Rain Forest domain.

Materials examined: Brazil, Santa Catarina: Florianópolis, near to the botanic department, 30-VII-2015, *M.P. Drewinski MPD02*; 05-X-2015 *M.P. Drewinski MPD03*; 07-X-2015 *M.P. Drewinski MPD04*; 10-XII-2015 *M.P. Drewinski MPD29*; 16-III-2016 *M.P. Drewinski MPD128;* 09-II-2017 *M.P. Drewinski MPD145*; Parque Ecológico do Córrego Grande, *E.Copini EC31*; Cacupé, *Tim Brightwell MPD19*; Paraná: Guarapuava, Parque Municipal das Araucárias, 12-I-2016, *M.P. Drewinski MPD71*.

Notes: The presence of pleurocystidia is a rare condition and, although they have a large size which stands out from the other structures in the hymenium, they are not abundant and may go unnoticed if the material is not observed with attention (Heinemann, 1980). *Agaricus* sp1. is similar to *Agaricus pleurocystidiatus* Heinem. from Singapore mainly due to the presence of pleurocystidia, a rare condition in the genus. Additionally, the annulus described for both species are also similar: membranous, pendant, white and with flakes on lower side. However, *A. pleurocystidiatus* differs in the pileus surface which has dark brown scales on light brown background, pileus margin appendiculate and fibrillose light brown stipe. Furthermore, *A. pleurocystidiatus* change to reddish brown when cut and has a different distribution (Heinemann, 1980). *Agaricus sinodeliciosus* Z.R. Wang & R.L. Zhao from China is another *Agaricus* species characterized by the presence of pleurocystidia. However, *A. sinodeliciosus* differs by grows buried in sandy soil, semihypogeous habit, pileus surface covered with light brown or buff brown squamules on a dirty background, margin involute, smooth or fibrillose white stipe that becomes reddish-brown when bruised, the negative reactions in KOH and NaOH, the annulus inferior forming a broad sheath, and the context turns reddish-brown when cut (Wang et al., 2015).

The basidiomata of *Agaricus* sp1 collected in Brazil have a KOH and Schäffer reaction positive, discoloring in slightly yellow in some parts of context when exposed, and annulus superous and simple, which characterizes *Agaricus* sp1 morphologically into the *Agaricus* section *Laeticolores* (Zhao et al. (2016)). The other two species with pleurocystidia, *A. pleurocystidiatus* and *A. sinodeliciosus* present discoloring in reddish brown and belong to *Agaricus* sections *Sanguinolenti* and *Bivelares*, respectively (Heinemann, 1980; Wang et al., 2015).

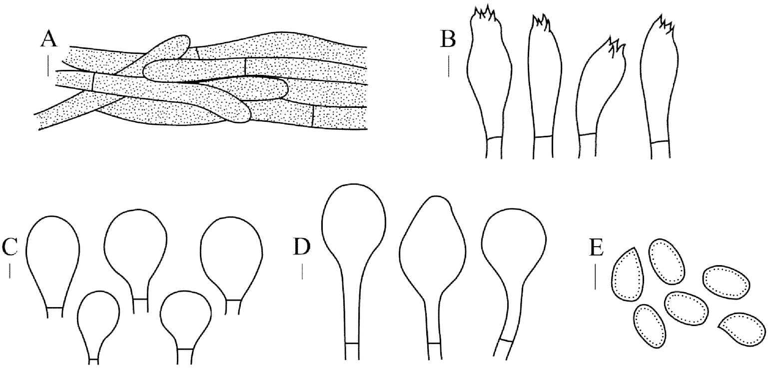


Figure 2. *Agaricus* sp1 (*MPD02, MPD03, MPD04, EC31, MPD71, MPD29*) a. Pileipellis with pigments; b. Basidia; c. Cheilocystidia; d. Pleurocystidia; e. Basidiospores. Scale 5 µm.

Figure 3. *Agaricus* sp1 a–b. Basidiomata; a.*MPD145*; b. *MPD29* (Holotype); c. Pileus surface (*MPD71*); d. Stipe with details to the scales and annulus with floccose lower surface (*MPD145*); e. Pleurocystidia (*MPD29*); f. Pleurocystia(*MPD04*).

Agaricus sp1 is a new species in subgenus *Laeticolores*, increasing the knowledge of the species within the section. The morphological and phylogenetic analyses support the classification of the taxa as new. The knowledge of *Agaricus* species that occur in

America is important to better understand the phylogenetic relationships and the biogeographic patterns in the genus.

References

Bas, C. (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella.* Persoonia 5: 285-579.

Bunyard, B. A.; Nicholson, M. S.; Royse, D. J. (1996) Phylogeny of the genus *Agaricus* inferred from Restriction Analysis of Enzymatically Amplified Ribosomal DNA. Fungal Genetics and Biology 20: 243-253.

Chen, J.; Zhao, R-L.; Parra, L. A.; Guelly, A. K.; Kesel, A.; Rapior, S.; Hyde, K. D.; Chukeatirote, E.; Callac, P. (2015)*Agaricus* section *Brunneopicti*: a phylogenetic reconstruction with descriptions of four new taxa. Phytotaxa 192(3): 145-168.

Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

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

Freeman, A.E. (1979) *Agaricus* in the Southeastern United States. Mycotaxon 8(1):50-118.

Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3):307-21.

Heinemann, P. (1956) Champignons récoltés au Congo Belge par madame M. Goossens-Fontana. II. *Agaricus* Fries s.s. Bulletin Du Jardin Botanique National de Belgique. 26(1):1-127

Heinemann, P. (1961) *Agaricus* of Trinidad. Kew Bulletin. 15(2): 231-248.

Heinemann P. (1980) Les genres *Agaricus* et *Micropsalliota* em Malaisie et em Indonésie. Bull Jard Bot Nat Belg 50: 3–68.

Heinemann, P. (1993) Agarici Austroamericani VIII. Agariceae des régions intertropicales d'Amérique du Sud. Bulletin du Jardin Botanique National de Belgique 62(1-4): 355-384.

Katoh, S. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30:772-780.

Karunarathna, S.C.; Chen, J.; Mortimer, P.E.; Xu, J.C.; Zhao, R.L.; Callac, P.; Hyde, K.D. (2016) Mycosphere Essay 8: A review of genus Agaricus in tropical and humid subtropical regions of Asia. Mycosphere 7(4):417-439.

Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Mentjies, P.; Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12): 1647-1649.

Kerrigan, R.W. (2016) *Agaricus* of North America. New York Botanical Garden Press, Bronx, (in press).

Kramer, E. (2004) The Online Auction Color Chart: The New Languageof Color. Online Auction Color Chart Company. pp 12.

Largent, D.L.; Thiers, H.D. (1977) How to Identify Mushrooms to Genus II: Field Identification of Genera. 3nd ed. Eureka, Mad River Press.

Largent D.; Johnson, D.; Watling, R. (1977) How to identify mushrooms to genus III: microscopic features. Eureka, Mad River Press.

Largent, D. (1986) How to identify mushrooms to genus III: macroscopic features. Eureka, Mad River Press.

Linnaeus, C. (1753) *Species plantarum* 2: 1171. Disponível em: < http://biodiversitylibrary.org/page/359192#page/613/mode/1up> Acesso em: 14 Jul. 2015.

Meijer, A.A.R. (2008) Macrofungos notáveis das Florestas de Pinheiro do Paraná. Colombo, PR: Embrapa Florestas.

Miller, M.A.; Pfeiffer, W.; Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In*

Proceedingsof the Gateway Computing Environments Workshop (GCE). New Orleans, Louisiana, pp. 1–8. http://dx.doi.org/10.1109/GCE.2010.5676129

Mitchell, A.D.; Bresinsky, A. (1999) Phylogenetic relationships of *Agaricus* species based on ITS-2 and 28S ribosomal DNA sequences. Mycologia. 91(5):811–9.

Parra, L.A. (2013) Fungi europaei, Volume 1A, *Agaricus* L.: *Allopsalliota*, Nauta & Bas (Parte II) Alassio: Candusso Edizioni.

Pegler, D. N. (1990) Agaricales of Brazil described by J. P. F. C. Montagne. Kew Bulletin 45(1): 161– 177.

Romano, E. & Brasileiro, A.C.M. (1998) Extração de DNA de plantas. *Biotecnologia: Ciência & Desenvolvimento* 2 (9): 40–43.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 572-574.

Singer R. (1951) The Agaricales (mushrooms) in modern taxonomy. Lilloa 22: 5-832.

Singer, R. (1986) The Agaricales in modern taxonomy, 4th ed. Koeltz, Königstein, Germany.

Sitotaw, R.; Li, Y.; Wei, T.Z.; Abate, D.; Yao, Y.J. (2015) Two new records of *Agaricus* spp. from Ethiopia. Mycotaxon 130(4): 1171-1183.

Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics Advance Access.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Thongklang, N.; Nawaz, R.; Khalid, A. N.; Chen, J.; Hyde, K. D.; Zhao, R.; Parra, L. A.; Hanif, M.; Moinard, M.; Callac, P. (2014). Morphological and molecular characterization of three *Agaricus* species from tropical Asia (Pakistan, Thailand) reveals a new group in section *Xanthodermatei*. Mycologia 106 (6): 1220-32.

Vellinga, E.C. Glossary. (1988) In: Bas, C.; Kuyper, T.W.; Noodelos, M.E.; Vellinga, E.C. (eds.) Flora Agaricina Neerlandica, v. 1. Amsterdam/Brookfield: A.A. Balkema Publishers. p 54–64.

Vellinga, E. C. (2004) Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. Mycol. Res. 108 (4): 354–377.

Wang, Z.; Parra, L.A.; Callac, P.; Zhou, J.; Fu, W.; Dui, S.; Hyde, K.D.; Zhao, R.L. (2015) Edible species of *Agaricus* (Agaricaceae) from Xinjiang Province (Western China). Phytotaxa 202(3):185-197.

Zhao, R.; Karunarathna, S.; Raspé, O.; Parra, L. A.; Guinberteau, J.; Moinard, M.; Kesel, A. D.; Barroso, G.; Courtecuisse, R.; Hyde, K. D.; Guelly, A. K.; Desjardin, D. E.; Callac, P. (2011). Major clades in tropical *Agaricus*. Fungal Diversity 51:279-296.

Zhao, R.; Zhou, J.; Chen, J.; Margaritescu, S.; Sánchez-Ramírez, S.; Hyde, K. D.; Callac, P.; Parra, L. A.; Li, G.; Moncalvo, J. (2016) Towards standardizing taxonomic ranks using divergence times - a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Diverity. DOI 10.1007/s13225-016-0357-x.

Zhou, J.; Su, S.; Su, H.; Wang, B.; Callac, P.; Guinberteau, J.; Hyde, K.D.; Zhao, R.L. (2016) A description of eleven new species of *Agaricus* sections *Xanthodermatei* and *Hondenses* collected from Tibet and the surrounding areas. Phytotaxa 257(2):099-121.

CAPÍTULO 2 − New section of *Agaricus* subgenus *Flavoagaricus* from the Americas

New section of *Agaricus* subgenus *Flavoagaricus* from the Americas

Abstract

Agaricus is an important and diverse genus with a worldwide distribution. Macroscopic characters of the genus can be easily recognized but its infrageneric identification is difficult. The use of molecular techniques have helped to understand the relationship within the group and the proposition of changes on the systematic arrangements. Our aim was to better understand the diversity of *Agaricus* in the Atlantic Rain Forest in Brazil and its phylogenetic relationships among the taxa including mainly neotropical specimens. The molecular analyses included nuc rlITS1-5.8S-ITS2 (ITS), nuclear large subunit rDNA (LSU) and translation elongation factor alpha (*tef-1α*) sequences from 15 Brazilian specimens collected in the states of Paraíba, Rio de Janeiro, São Paulo, Paraná and Santa Catarina. Most of the Brazilian collections represent a new section, sister to *Agaricus* section *Arvenses*, within *Agaricus* subgenus *Flavoagaricus*. Four new species belonging to a new section are described and six sequences are included in *Agaricus* section *Laeticolores*. The inclusion of sequences from neotropical collections can reveal a new internal relationships and new evolutionary lineages in the genus. This study helps to a better understanding of the phylogenetic relationships within the genus and increases the knowledge about species occurring in Brazil.

Keywords = *Arvenses*, Brazil, Diversity, *Laeticolores*, Phylogeny, Systematics.

Introduction

Agaricus L. is the genus type of Agaricaceae Chevall. and was proposed by Linnaeus (1753) to includ all mushrooms with lamellae and stipe. The genus is monophyletic (Vellinga, 2004) and has a worldwide occurrence with nearly 400 described species (Thongklang et al., 2014). Important edible and medicinal mushrooms belong to *Agaricus* and most of them are also known to have a high nutritional value and for their pharmacological properties as an important source of polysaccharides and protein complexes with anticancer and immunostimulating activity (Wasser, 2011). Among the most cultivated species *Agaricus bisporus* (J.E. Lange) Imbach, the button mushroom is the most consumed mushroom worldwide (Wisitrassameewong et al., 2012; Royse, 2014).The mushrooms of the genus are saprobic and generally humicolous, have brown and free lamellae, with regular trama; annulus present; dark brown to purple-brown spore print; brown, usually ellipsoid, smooth and thick-walled basidiospores (Mitchell & Bresinsky, 1999). Although the genus have some macroscopic characters which can be easily recognized in the field, infrageneric identification is difficult because of environmental effects, intraspecific variability and lack of characters to differentiate species (Zhao et al. (2011)). Besides macro and microscopic features, taxonomic methods for species recognition include reactions with KOH and with aniline and nitric acid, the Schäeffer reaction (Freeman, 1979; Singer, 1986).

The genus has a long history and is part of different taxonomic classifications. The classical concept of *Agaricus* (Heinemann, 1956; Singer, 1986) considers some morphological and macrochemical characters (e.g. context color change when exposed and Schäffer reaction) to define subgenera and sections. Bunyard et al. (1996) produced the first molecular analysis of the genus, based on 26S rDNA and the IGR-1/5S rDNA, and showed that the classification based only on macrochemical and morphological characters could be artificial. Recently, several species of *Agaricus* have been described, mainly from temperate and tropical areas of Asia, but the circumscriptions of the subgenera and sections still remain confuse (Calvo-Bado et al., 2000; Challen et al., 2003; Didukh et al., 2005; Kerrigan et al., 2008; Zhao et al., 2012; Gui et al., 2015; Chen et al.,2015). Zhao et al. (2011) studied data of ITS sequences of species from temperate and tropical areas and observed that only about one-third of the species belong to the classical sections historically proposed by European researchers, including. The use of molecular data and the improvement of phylogenetic studies have helped to clarify the relationships among taxa from different regions and to propose taxonomic changes. Zhao et al. (2016) proposed a recent reconstruction of *Agaricus* infrageneric systematic based on molecular and morphological data. They produced a multi-gene phylogeny combining ITS, LSU, *tef-1α* and *rpb2* sequences using species divergence time to redefine taxonomic ranks into five subgenera and twenty sections.

According to Zhao et al. (2016), *Agaricus* subgenus *Flavoagaricus* Wasser is monophyletic and has one recognized section, *Agaricus* sect. *Arvenses*. The characteristics that define the subgenus is the same as the section and include: KOH and Schäffer's reaction positive; pileus surface usually changing to yellow; context turning to yellow, reddish or without change; odor of anise; annulus superous, double with scales or flakes on the lower layer and cheilocystidia present, which are usually catenulate (Zhao et al., 2016). Gui et al. (2015) cited thirty-five species recognized in *Agaricus* section *Arvenses*, including *Agaricus subrufescens* Peck., a species that has raised discussions about its identity and nomenclature (Kerrigan, 2005). The occurrence of some *Agaricus* species in Brazil is known from few punctual works (Capelari, 1989; Pegler, 1990; Rosa & Capelari, 2009; Putzke et al., 2014) and more studies are needed to certify the species identification and to treat the taxa within the infrageneric classification. Thus, based on specimens recently collected in some Brazilian states, the goal of this work was to report new species occurring in Brazil and to understand how they are phylogenetically related to other tropical and temperate taxa from morphological and molecular data.

Materials and methods

Sampling and morphological study

The Brazilian specimens herein studied were collected in the states of Paraíba, Rio de Janeiro, São Paulo, Paraná and Santa Catarina and were described macroscopically following Largent (1986) and Vellinga (1988). The chemical reactions were performed with KOH 3% (yellow if positive) and NaOH 10% (orange-red if positive) on pileus and stipe surface of fresh or dried basidiomata. The color code is based on the *Online Auction Color Chart* (Kramer, 2004). Micromorphological features were observed from dried specimens and described following descriptive terms of Largent et al. (1977) and Vellinga (1988). The notation [a/b/c] at the beginning of a set of basidiospores represents: (a) basidiospores were measured from (b) basidiomata taken from (c) collections. Basidiospore measurements were based on at least 20 structures per basidioma, including Q value (quotient of length to width), Q_m (the mean of Q-value) and L_m/W_m (the mean of length/width). Basidiospores shape was based on Q-value according to Bas (1969).

DNA extraction, amplification and sequencing

DNA was extracted from dried pileus and lamellae fragments, following a modified CTAB extraction method (Doyle & Doyle, 1987 adapted por Góes-Neto et al., 2005). The nuc rITS1-5.8S-ITS2 (ITS), nuclear large subunit rDNA (LSU) and translation elongation factor alpha (tef-1α) sequences were generated using the primers pairs ITS 8F and ITS 6R (Dentinger et al., 2010), LR0R and LR5 (Moncalvo et al., 2000), EF1-983F and EF1-1567R (Morehouse et al., 2003), respectively. The PCR mix was prepared containing 4 µL DNA extract (1:10), 10 µL water, 10 µL Master mix (Promega) and 0.5 µL each primer (10pmol/L). The PCR program to ITS was according to Zhao et al. (2011): 5 min. at 95 °C; 35 cycles (1 min. at 94 °C, 1.5 min. at 55 °C, 1.5 min. at 72 °C); 5 min at 72 °C; to LSU was according to Vilgalys & Hester (1990): 1 min. at 72 °C; 30 cycles (1 min. at 94 °C, 45 sec. at 50 °C, 1 min. at 72 °C); 7 min. at 72 °C; and to tef-1α was according to Morehouse et al. (2003): 3 min. at 94 °C; 35 cycles (1 min. at 94 °C, 30 sec. at 50 °C, 1 min. at 72 °C); 7 min at 72 °C. The PCR products were purified with PEG 20% and sequenced at Centro de Pesquisas René Rachou (Fiocruz Minas, Brazil) using the same primer pairs.

Phylogenetic analyses

We performed a previous analysis with ITS and LSU data, including species of five recognized subgenera and twenty sections of *Agaricus* (data not shown). From this analysis we restrict to *Agaricus* subg. *Flavoagaricus*, *Agaricus* subg. *Minores* and *Agaricus* subg. *Spissicaules* to compose the dataset.

The generated sequences were manually checked and edited with Geneious v.8.1 (Kearse et al., 2012). Our dataset is composed of sequences from fifteen newly collected *Agaricus* specimens from five Brazilian states plus 63 sequences retrieved from GenBank including species of all section of *Agaricus* subg. *Flavoagaricus, Agaricus* subg. *Minores* and *Agaricus* subg. *Spissicaules*. A Sequence of *Agaricus campestris* L. (LAPAG370) – *Agaricus* section *Agaricus* – was used as outgroup (Table 1). Newly obtained sequences were deposited in GenBank and accession numbers and country of origin are given in Table 1. All sequences were aligned in MAFFT v7 (Katoh, 2013) following the Q-INS-I criteria and then manually corrected using MEGA v7 (Tamura et al., 2013). To concatenate the sequences, FASconCAT v1.0 was used (Kück & Meusemann, 2010). The final alignment had a total of 2093 characters (gaps included). To identify the best nucleotide evolution model, we used the AIC criterion (Akaike Information Criterion) in jModelTest v2.1.6 (Guindon et al., 2010; Darriba et al., 2012). The ITS region was partitioned in ITS 1, 5.8S and ITS2; and *tef-1α* in introns and exons into codons, estimating evolution model for each partition. Maximum Likelihood (ML) analysis was performed in RAxML v8.2.8 (Stamatakis, 2014) using GTRGAMMA model with a rapid bootstrap analysis with 1,000 replicates and search for best-scoring ML tree, with other parameters estimated by the software. Bayesian Inference (BI) analysis was performed with Mr.Bayes v3.2.6 (Ronquist & Huelsenbeck, 2003) using eight partitions (ITS1, 5.8S, ITS2, LSU, tef-1α exons codon 1, tef-1α exons codon 2, tef-1α exons codon 3, tef-1α introns), with two independent runs, four simultaneous independent chains and 20,000,000 generations with sample frequency every 1,000 generation. The jModelTest, RAxML and Mr.Bayes were used in CIPRES Science Gateway 3.1 (Miller et al., 2010). The outputs of analyses were displayed with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table1. Specimens used in the molecular analyses. The sequences generated in this work are in **bold**. * Sequences to be include in GenBank.

Subgenera	Section	Taxon	Collection number	ITS	LSU	tef-1α	Location	Reference
Flavoagaricus	***New***	***Agaricus* sp2**	**MPD131**	*	*	*	**Santa Catarina, Brazil**	
		***Agaricus* sp2**	**MAN1152**	*	*	*	**Santa Catarina, Brazil**	
		***Agaricus* sp2**	**MAN651**	*	*	*	**Santa Catarina, Brazil**	
		***Agaricus* sp2**	**MAN1150**	*	*	*	**Santa Catarina, Brazil**	
		***Agaricus* sp2**	**MC4632**	*	**-**	**-**	**São Paulo, Brazil**	
		***Agaricus* sp3**	**JDuque131**	*	*	*	**Rio de Janeiro, Brazil**	
		Agaricus sp.	B77	KR812271	-	-	Bahia, Brazil	Reis et al. (2015)
		***Agaricus* sp4**	**AGP08**	*	**-**	*	**Santa Catarina, Brazil**	
		Agaricus sp.	TR130	HQ608135	-	-	Texas, USA	Rodrigues et al. (2011)
		***Agaricus* sp5**	**MK01**	*	*	*	**Paraná, Brazil**	
		***Agaricus* sp5**	**ACM376**	*	*		**Santa Catarina, Brazil**	
		Agaricus sp.	LAPAM4	JF797185	-	-	Brazil	Zhao et al. (2011)
		Agaricus sp.	JSP 30-04 C 1.2	KR093941	-	-	São Paulo, Brazil	Pereira et al. (2016)
		Agaricus sp.	JSP 01-10 B 5.2	KR093888	-	-	São Paulo, Brazil	Pereira et al. (2016)
		Agaricus fiardii	DeMeijer1904	JF797196	-	-	Paraná, Brazil	Zhao et al. (2011)

		Agaricus deserticola	MSmithUC	HM488747	-	-	California, USA	Vellinga et al. (2011)
Flavoagaricus	*Arvenses*	*Agaricus* sp.	ZRL2012030	KT951364	KT951455	KT951628	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012630	KT951379	KT951495	KT951621	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012608	KT951377	KT951498	KT951627	Tibet, China	Zhao et al. (2016)
		Agaricus cf. *essettei*	ZRL2012026	KT951363	KT951450	KT951630	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012002	KT951354	KT951462		Yunnan, China	Zhao et al. (2016)
		Agaricus essettei	ZRL2012599	KT951374	KT951514	KT951625	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	ZJL-2016i	KT951380	KT951478	KT951623	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012604	KT951375	KT951515	KT951620	Tibet, China	Zhao et al. (2016)
		Agaricus sp.	SHY2011073117	KT951407	KT951459	KT951622	Yunnan, China	Zhao et al. (2016)
		Agaricus abruptibulbus	ZRL2012005	KT951356	KT951460	KT951626	Yunnan, China	Zhao et al. (2016)
		Agaricus augustus	ZRL2012598	KT951373	KT951477	KT951629	Tibet, China	Zhao et al. (2016)
		Agaricus flocculosipes	ZRL2012105	KT951365	KT951463	KT951618	Yunnan, China	Zhao et al. (2016)
		Agaricus subrufescens	CA276	KT951317	KT951461	KT951631	-	Zhao et al. (2016)
Minores	*Minores*	*Agaricus edmondoi*	LAPAG412	KT951326	KT951481	KT951590	Segovia, Spain	Zhao et al. (2016)
		Agaricus kerriganii	LAPAG808	KT951306	KT951442	KT951589	Madrid, Spain	Zhao et al. (2016)
		Agaricus pallens	LAPAG926	KT951315	-	KT951591	Lapland, Sweden	Zhao et al. (2016)
		Agaricus friesianus	LAPAG592	KT951316	-	KT951594	Pyrénées Atlantiques, France	Zhao et al. (2016)
		Agaricus matrum	LAPAG855	KT951310	KT951443	KT951598	Asturias, Spain	Zhao et al. (2016)

	Agaricus comtulus	LAPAG724	KT951332	KT951448	KT951593	Burgos, Spain	Zhao et al. (2016)
	Agaricus pseudolutosus	LAPAG454	KT951329	KT951453	KT951602	Burgos, Spain	Zhao et al. (2016)
	Agaricus sp.	ZRLLD013	KT951384	KT951516	KT951604	Thailand	Zhao et al. (2016)
	Agaricus sp.	ZRL2011156	KT951352	KT951480	KT951603	Yunnan, China	Zhao et al. (2016)
	Agaricus sp.	CA848	JF727864	KT951445	KT951605	Thailand	Zhao et al. (2011)
	Agaricus sp.	ZRL2012004	KT951355	KT951457	KT951608	Yunnan, China	Zhao et al. (2016)
	Agaricus sp.	ZRLWXH3150	KT951390	KT951447	KT951609	Guangdong, China	Zhao et al. (2016)
	Agaricus sp.	ZRL2012576	KT951372	KT951499	KT951596	Tibet, China	Zhao et al. (2016)
	Agaricus sp.	ZRLWXH3067	KT951387	KT951497	KT951611	Jiangxi, China	Zhao et al. (2016)
	Agaricus sp.	CA846	JF727865	KT951452	KT951601	Thailand	Zhao et al. (2011)
	Agaricus sp.	ZRL2012357	KT951369	KT951496	KT951610	Yunnan, China	Zhao et al. (2016)
	Agaricus sp.	ZRL2011039	KT951351	KT951449	KT951606	Yunnan, China	Zhao et al. (2016)
	Agaricus sp.	ZRL2012714	KT951381	KT951476	KT951607	Tibet, China	Zhao et al. (2016)
	Agaricus sp.	ZRLWXH3076	KT951388	KT951458	KT951612	Fujian, China	Zhao et al. (2016)
	Agaricus sp.	ZRL2012199	KT951367	KT951470	KT951595	Yunnan, China	Zhao et al. (2016)
	Agaricus sp.	ZRL2012012	KT951359	KT951494	KT951597	Yunnan, China	Zhao et al. (2016)
Incertae sedis	*Agaricus* sp.	LAPAM14	KT951312	-	KT951613	Sosúa, Dominican Republic	Zhao et al. (2016)
	Agaricus sp.	ZRLWXH3161	KT951391	KT951526	KT951615	Guangdong, China	Zhao et al. (2016)
	Agaricus candidolutescens	LD2012129	KT951335	KT951525	KT951616	Thailand	Zhao et al. (2016)

Minores	*Laeticolores*	***Agaricus* sp.**	**ACM260**	*	*	*	Paraíba, Brazil	
		Agaricus rufoaurantiacus	LAPAM15	KT951313	-	KT951641	Sosúa, Dominican Republic	Zhao et al. (2016)
		***Agaricus* sp.**	**JDuque130**	*	*	*	Rio de Janeiro, Brazil	
		***Agaricus* sp.**	**FTF140**	*	*	*	Santa Catarina, Brazil	
		***Agaricus* sp.**	**MPD03**	*	*	*	Santa Catarina, Brazil	
		***Agaricus* sp.**	**MPD04**	*	*	*	Santa Catarina, Brazil	
		***Agaricus* sp.**	**EC31**	*	*	*	Santa Catarina, Brazil	
Spissicaules	*Subrutilescentes*	*Agaricus parasubrutilescens*	ZRL2012025	KT951362	KT951487	KT951584	Yunnan, China	Zhao et al. (2016)
		Agaricus parasubrutilescens	ZRL2011027	KT951350	KT951521	KT951583	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	SHY2012070604	KT951341	KT951475	KT951581	Yunnan, China	Zhao et al. (2016)
		Agaricus sp.	ZRL2012618	KT951378	KT951503	KT951582	Tibet, China	Zhao et al. (2016)
		Agaricus subrutilescens	ZRLWXH3276	KT951392	KT951522	KT951585	China	Zhao et al. (2016)
		Agaricus sp.	ZRL160	KT951345	KT951486	KT951586	Thailand	Zhao et al. (2016)
		Agaricus brunneopileatus	ZRL2012115	KT951404	KT951489	KT951587	Yunnan, China	Zhao et al. (2016)
Spissicaules	*Amoeni*	*Agaricus* sp.	ZRL3093	JF691548	KT951488	-	Thailand	Zhao et al. (2011)
		Agaricus amoenomyces	ZRL2010072	KT951348	KT951524	KT951638	Yunnan, China	Zhao et al. (2016)
Spissicaules	*Rarolentes*	*Agaricus albosquamosus*	LD2012192	KT951394	KT951520	KT951636	Thailand	Zhao et al. (2016)
		Agaricus leucolepidotus	LD201214	KT951336	KT951519	KT951635	Thailand	Zhao et al. (2016)
		Agaricus sp.	AW145	KT951308	-	KT951637	Awenda, Canada	Zhao et al. (2016)

		Agaricus sp.	JH105	KT951324	KT951490	-	Jokers Hill, Canada	Zhao et al. (2016)
Spissicaules	*Spissicaules*	*Agaricus litoraloides*	ZRL2011249	KT951353	KT951523	KT951580	Yunnan, China	Zhao et al. (2016)
		Agaricus lanipedisimilis	ZRL2012151	KT951366	KT951484	KT951578	Yunnan, China	Zhao et al. (2016)
		Agaricus litoralis	LAPAG420	KT951327	KT951483	KT951572	Burgos, Spain	Zhao et al. (2016)
		Agaricus sp.	ZRLQHY43	KT951386	KT951485	KT951579	Shandong, China	Zhao et al. (2016)
Agaricus	*Agaricus*	*Agaricus campestris*	LAPAG370	KM657927	KR006607	KR006636	Madrid, Spain	Zhou et al. (2016)

Results and Discussion

Molecular phylogenetic analyses

The final dataset contains 77 ingroup specimens, including fifteen sequences of ITS, thirteen of LSU, and twelve of *tef-1α*. The best models of nucleotide substitution estimated for each partition in the datasets were: TPM3uf+G to ITS1, JC to ITS 5.8S, TrN+I+G to ITS 2, TIM2+I+G to LSU, TPM2uf+I+G to tef-1α first codon position, TVM+I+G to tef-1α second codon position, TIM2+G to tef-1α third codon position and TPM1uf+I+G to tef-1α intron. We performed a previous analysis with ITS and LSU data, including species of all recognized subgenera and sections (data not shown). From this analysis we choose the three most related subgenera: *Minores, Flavoagaricus* and *Spissicaules* to compose the dataset. The Bayesian Inference best tree obtained by analysis is presented in Figure 1 with posterior probability and bootstrap values above branches. Despite a difference in intern relationship of subgenus *Spissicaules*, Maximum Likelihood and Bayesian trees were similar.

Zhao et al. (2016) presented two different trees with *Agaricus* subg. *Spissicaules*. In the first one, a tree based on ITS, LSU, *tef-1α* and *rpb2* genes with the five subgenera proposed, the relationships between the sections corroborates with the tree presented in our study, with *Agaricus* sect. *Subrutilescentes* and *Agaricus* sect. *Amoeni* as sisters to *Agaricus* sect. *Rarolentes* and *Spissicaules*. In the second tree, based only on ITS data and exclusive of subg. *Spissicaules*, *Agaricus* sect. *Rarolentes* is sister to *Agaricus* sect. *Amoeni*, internal of *Agaricus* sect. *Subrutilescentes* and *Spissicaules*. Dai et al. (2016) found a different result to the relationship within *Agaricus* subg. *Spissicaules*, in which *Agaricus* sect. *Spissicaules* is sister to *Agaricus* sect. *Subrutilescentes*. The internal relationships of the subg. *Spissicaules* are still unclear.

The phylogenetic analyses provided strong support values for a new clade, corresponding to a new section in the subgenus *Flavoagaricus*, sister group of *Agaricus* section *Arvenses*, composed only by American species. *Agaricus deserticola* (MSmithUC) from California, USA, appears related with DeMeijer1904, specimen collected on dunes in Paraná, Brazil. Sequences of LAPAM4 and DeMeijer1904 are in Zhao et al. (2011) as tropical clade TRIV, but did not receive a taxonomic treatment and was not included in the analyzes of Zhao et al. (2016).

This new clade includes four new species (*Agaricus* sp2*, Agaricus* sp3*, Agaricus* sp4 and *Agaricus* sp5) collected in the Atlantic Rain Forest in Brazil. *Agaricus* species B77 (BRA), TR130 (USA), SP 30-04 C 1.2 (BRA), SP 01-10 B 5.2 (BRA) were isolated from ant nests and appear in both intern clades of the new section. The sequences KR093941, KR093888 and KR812271 (vouchers JSP 30-04 C 1.2, JSP 01-10 B 5.2 and B77), were isolated from *Atta sexdens rubropilosa* Forel, *Atta capiguara* Gonçalves and *Atta cephalotes* L. nests, respectively, from Brazil (Pereira et al., 2016; Reis et al., 2015). The sequence HQ608135 (TR130) was isolated from *Trachymyrmex septentrionalis* McCook nest, from Central Texas (Rodrigues et al., 2011). Ants from tribe *Attini* (Hymenoptera: Formicidae) interact with a lot of organisms, including filamentous fungi, yeasts and bacteria (Carreiro et al., 1997; Rodrigues et al., 2005). They cultivate fungi as the main food source for the colony, mainly the basidiomycetous fungus, *Leucoagaricus gongylophorus* (Möller) R. Heim, a domesticated mutualistic partner (Schultz & Brady 2008). The interactions in the nest are not restricted to ants. Fungi of the genus *Escovopsis* J.J. Muchovej & Della Lucia are an example of parasite that inhibits the growth of *L. gongylophorus* (Currie et al., 1999). *Agaricus* accounted for less than 3% of the fungi abundance and can be considered rare in these ant communities (Rodrigues et al., 2011; Reis et al., 2015; Pereira et al., 2016). Many of the fungi isolated from ants nests may not play a specific role in the ant colony (Reis et al., 2015). Pereira et al. (2016) indicate that community structure and fungal composition differ between gardens, and this difference is strongly influenced by the plant substratum foraged by ants. The most part of the microorganism present in the fungus garden are from the soil, integuments of workers, or from plant substratum used by the ants (Arcuri et al., 2014; Coblentz & Van Bael, 2013).

The *Laeticolores* clade includes six new sequences from four putative species (ACM260, JDuque130, FTF140, MPD03, MPD04, EC31), and besides not significantly supported, appear as sister group of *Agaricus* section *Minores*, clade *Incertae sedis* (Zhao et al. (2016))*, Agaricus* section *Arvenses* and the new clade. Subgenus *Spissicaules* showed different internal relationship in the analyses. In Bayesian

Inference analysis, *Agaricus* section *Amoeni* appears as sister group of *Subrutilescentes*, while in the Maximum Likelihood analysis appears as sister group of *Rarolentes*.

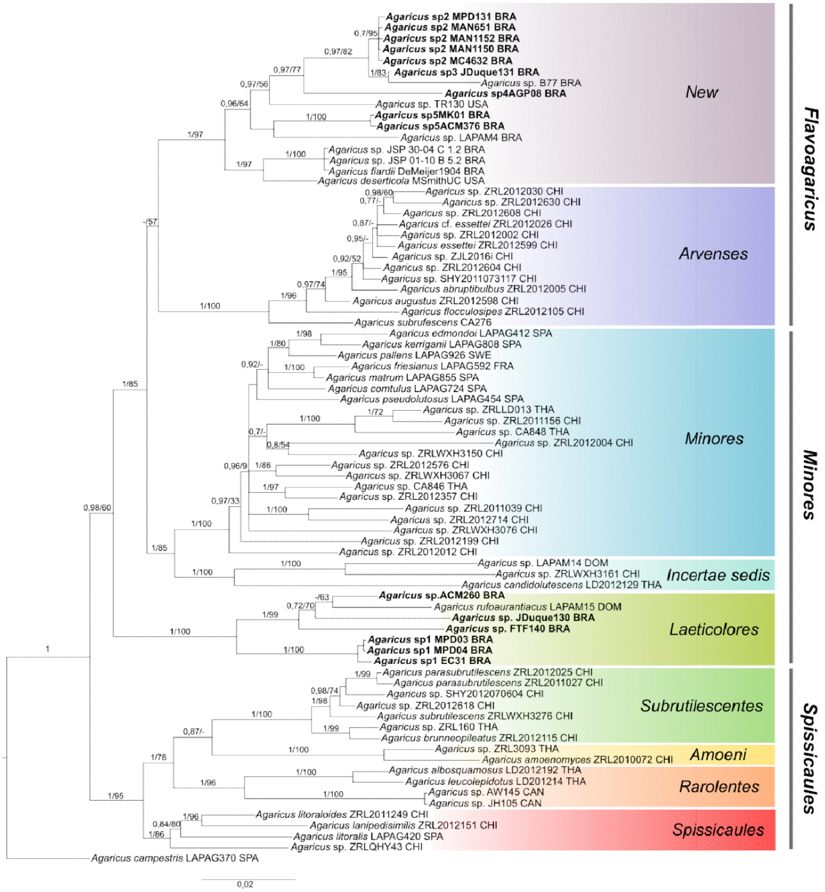


Figure 1. Bayesian Inference tree of *Agaricus* subgenera *Minores, Flavoagaricus* and *Arvenses* based on ITS, LSU and *tef-1α* data and rooted with *A. campestris*. The sequences generated in this work are in **bold**. Bayesian posterior probability (PP) above 0.7 and bootstrap values (BS) above 50% are shown above branches (PP/BS). BRA: Brazil; USA: United States of America; CHI: China; SPA: Spain; SWE: Sweden; FRA: France; THA: Thailand; DOM: Dominican Republic; CAN: Canada.

Taxonomy

Delimitation of *Agaricus* new section

Table 2, Figure 2

Basidiomata generally with pileus squamose with margin appendiculate. Annulus superous, simple, pendant or subperonate, membranous or floccose at lower surface. Context turning weakly yellow or not changing. Basidioles clavate, thin-walled, with brown pigment deposited in the base. Cheilocystidia thin-walled, clavate, obovoid to pyriform, hyaline. Abundant oleiferous hyphae in pileus context and lamellar trama. KOH positive and NaOH reaction negative.

Table 2. Morphological characters of *Agaricus* subgenera *Minores, Flavoagaricus* and *Spissicaules* (based on Zhao et al. (2016)).

Subgenus	**Section**	**KOH reaction**	**Schäffer's reaction**	**Discoloration**	**Annulus**	**Cheilocystidia**	**Others**
Minores	*Minores*	+	+	yellow	Superous, simple	Simple, clavate, pyriform or none	
Flavoagaricus	*Laeticolores*	+	+	None, yellow	Superous, simple or double	Variable in shape	
	Arvenses	+	+	Yellow, reddish or none	Superous, double	Generally catenulate	
	New	+	-	None, weakly yellow	Supeous, simple	Clavate, obovoid, pyriform	Presence of oleiferous hyphae and basidioles with brown pigment
Spissicaules	*Spissicaules*	-	-	Yellow, pink, reddish or none	Simple, superous	Cylindrical to clavate, some septate at base	
	Rarolentes	-	-	None or pink reddish	Superous	Various, cylindrical to clavate	
	Amoeni	- or +	- or +	None, weakly yellow or red	Superous, simple or double	Rare or abundant, variable	
	Subrutilescentes	Teal to olivaceous green	-	None, weakly pink or yellow	Superous, simple or double	Variable, some septa at the base	

Figure 2. Specimens of *Agaricus new section.* a−f. Basidiomata; a. *Agaricus* sp5 *ACM376* (holotype); b. *Agaricus* sp3 *JDuque131* (holotype); c-e. *Agaricus* sp2 *c. MAN1152*; d. *MAN1150*; e. *MPD131* (holotype); f. *Agaricus* sp4 *AGP08* (holotype). Photos by: a. Altielys Magnano; b. Jaime Duque; c-d. Maria Alice Neves; e. Mariana Drewinski; f. André Parise.

Agaricus sp2

Figure 2.c-e, 3

Diagnosis: Similar to *Agaricus meijeri* by pileus shape plane, whitish with brown scales but diferring by the presence of oleiferous hypahe, pigmented basidioles and context not discoloring.

Etymology:

Holotype: Brazil. Santa Catarina: Florianópolis, Universidade Federal de Santa Catarina, next to the phycology laboratory, 01-VI-2016, *M. P. Drewinski MPD131*.

Pileus 10−85 mm diam., at first sub-ovoid to parabolic, then pulvinate to convex and finally expanding to plane-convex, covered with concentrically arranged brown (oac751), appressed scales on white to pale-whitish (oac816) background, darker at center with concentrated scales and scattered towards the margin, margin usually appendiculate with white velar remnants. **Pileus context** approx. 5 mm thick at center and 2 mm at margin, whitish, not discoloring, fleshy. **Lamellae** free, white then pinkish and finally coffee-brown when mature, margin regular, crowded, with lamellulae. **Stipe** 20−104 × 9−12 mm, central, cylindrical to slightly broader at base, not bulbous, stuffed to narrowly fistulose, above annulus smooth, below annulus smooth to slightly fibrillose mainly towards the base, pale-whitish to pearly beige (aoc816), yellowing in some parts including the context at base, sometimes with short rhizomorphs. **Annulus** superous, simple, pendant or subperonate, membranous or floccose at lower surface, whitish. **Basidiospores** [120/6/5] 5.0−6.2(−8.7) × 3.7−5.0(−6.2)µm [Q=(1.00−)1.25−1.70;Q_m= 1.52; L_m = 5.7µm; W_m = 4.1µm], broadly ellipsoid to elongate, rarely globose, olive to brown, smooth, thick-walled, apiculus conspicuous, without germ pore. **Basidia** (10.0−)12.5−32 × 5.0−10.0(−12.5)µm, cylindrical to cylindrical-clavate, hyaline to light brown, conspicuous, 4-spored. **Basidioles** (9.0−)12.0-17.0 × (5.0−)6.0-8.0µm, clavate, with brown internal pigment deposited mainly in the base, smooth, thin-walled, sometimes broken. **Pleurocystidia** absent. **Cheilocystidia** (6.2−)15.0−32(−39) × (6.5−)7.5−18.7(−24) µm, clavate to obovoid, hyaline, inflated, thin-walled. **Lamellar edges** sterile. **Lamellar trama** regular to subregular composed of thin-walled hyphae, 3.7−15.0 µm diam., hyaline, interspersed with oleiferous hyphae, 3.7−8.7 µm diam. **Pileipellis** a cutis with few ascendant elements composed of thin- to thick-walled hyphae, 3.7−12.7µm diam., sometimes inflated, usually branched, hyaline or sometimes olive-green in KOH. **Pileus context** undifferentiated, composed of parallel chains of cylindrical, thin-walled hyphae, 5.0−21(−27) µm diam., sometimes inflated, interspersed with abundant oleiferous hyphae, 5.0−18.7 µm diam. **Stipitipellis** composed of parallel chains of cylindrical, thin- to thick-walled hyphae,(2.5−)3.7−18.7(−25) µm diam., sometimes inflated, usually branched, hyaline.

Macrochemical reactions: KOH reaction positive, NaOH negative.

Habit and habitat: solitary to scattered, terrestrial, growing over grass in urban area.

Materials examined: Brazil, Santa Catarina: Florianópolis, near to the Phycology laboratory, 01-VI-2016, *M. P. Drewinski MPD131*; *M. A. Neves MAN651*; *M. A. Neves MAN1150; M. A. Neves MAN1152.* São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, growing on grass in front of the student accommodation, 16-XII-2011, *M. Capelari MC 4632*.

Notes: *Agaricus* sp2 is similar to *A. meijeri* Heinem. from Paraná, mainly due to pileus shape plane and whitish with scales brown arranged concentrically in the center, margin with veil remnants; stipe fistulose and smooth above annulus; superous, simple and membranous annulus. However, *A. meijeri* differs by context changing to yellow citric when cut, NaOH reaction positive on stipe context and smaller basidiospores (4.2−5.5 × 3.3−4.1). Furthermore, *Agaricus* sp2 has oleiferous hyphae in lamellar trama and pileus context, and basidioles pigmented. *A. meijeri* was described by Heinemann (1993) in *Agaricus* sect. *Arvenses* subsect. *Augusti*.

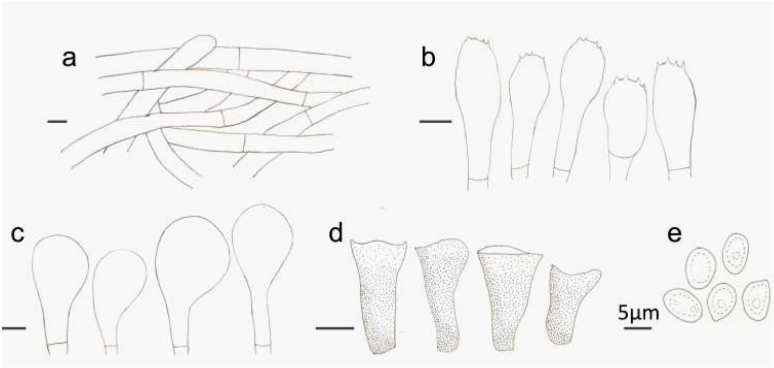


Figure 3. *Agaricus* sp2 (*MPD131* – holotype) a. Pileipellis; b. Basidia; c. Cheilocystidia; d. Basidioles with pigments; e. Basidiospores.

Agaricus **sp3**

Figures 2.b, 4

Diagnosis: Similar to *Agaricus toluenolens* by the pileus with browish gray squamules but differing in annulus without flakes and presence of oleiferous hyphae in lamellar trama and hipoderm and basidioles pigmented.

Etymology:

Holotype: Brazil. Rio de Janeiro: Rio de Janeiro, Jardim Botânico do Rio de Janeiro, 21-III-2016*, J. A. D. Barbosa JDuque 131*.

Pileus 85mm diam., plane-convex, covered with concentrically grayish scales on beige background, mainly at center and scattered to the margin, margin appendiculated with velar fragments. **Pileus context** approx. 7 mm thick at center, fleshy, whitish, without color changing. **Lamellae** free, initially white, then pinkish and finally brown when mature, margin regular, crowded, with lamellulae. **Stipe** 62 × 15 mm, central, cylindrical, fistulous, smooth above annulus, below fibrillose, beige, changing to yellow when touched. **Annulus** superous, descendent, pendant, membranous. **Basidiospores** [20/1/1] 5.0−6.2 × 3.7−5.0µm [Q=(1.25)1.33-1.66; Q_m=1.50; L_m=5.8µm; W_m=3.8µm] ellipsoid to elongate, rarely broadly ellipsoid, dark brown, smooth, thick-walled, apiculus conspicuous, without germ pore. **Basidia**

(16.2−)17.5−23(−27) × 6.2−7.5(−8.7)µm, cylindrical to cylindrical-clavate, hyaline, sometimes broken or collapsed, smooth, 4-spored. **Basidioles** (10.0−)14.0−26 × (5.0−)6.0−9.0µm, clavate, with brown pigment deposited mainly in the base, smooth, thin-walled, sometimes broken. **Pleurocystidia** absent. **Cheilocystidia** (15.0−)21−31(−37) × (10.0−)12.5−18.7(−21)µm, clavate, to obovoid, hyaline, thin-walled, generally covering all the lamellae edge,. **Lamellar trama** regular, with abundant sinuous oleiferous hyphae, (2.5−) 3.7−7.5µm diam. **Pileipellis** a cutis composed of hyphae (5.0−)6.2−12.5µm diam., thin- to rather thick-walled, cylindrical, branched, hyaline or sometimes olive-green in KOH. **Pileus context** hyphae 5.0−17.5 µm, cylindrical, inflate, thin-walled, with abundant oleiferous hyphae, sinuous to coiled. **Stipitipellis** hyphae (5.0−)6.2−13.7µm diam., cylindrical, thin to rather thick-walled, strictly parallel.

Macrochemical reactions: KOH reaction positive, NaOH negative.

Habit and habitat: solitary, growing on soil.

Material examined: Brazil. Rio de Janeiro: Rio de Janeiro, Jardim Botânico do Rio de Janeiro, 21-III-2016*, J. A. D. Barbosa JDuque 131.*

Notes: *Agaricus toluenolens* Callac, L.J.Chen & K.D. Hyde is morphologically similar to *Agaricus* sp3 by the plane-convex pileus, covered with brownish gray squamules concentred at center and scattered at margin; stipe surface smooth, changing to pale yellow when touched. The annulus of *A. toluenolens* is also superous, pendant and white, but differs by the presence of gray flakes on lower surface. The basidiospores and cheilocystidia of *A. toluenolens* are also longer than those of *Agaricus* sp3, and additionally the first species has pileipellis cells with vacuolar pigments. *Agaricus* sp3 has oleiferous hyphae in lamellar trama and hipoderm, and basidioles pigmented, different from *A. toluenolens*, which belongs to *Agaricus* subg. *Pseudochitonia* sect. *Brunneopicti* (Chen et al., 2015).

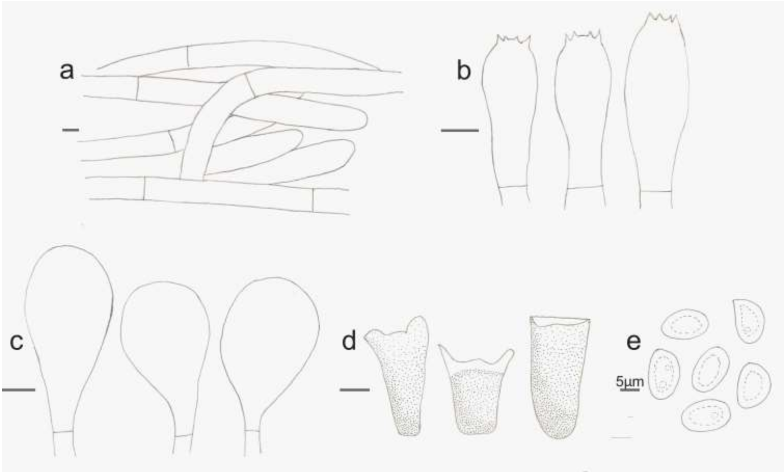


Figure 4. *Agaricus* sp3 (*JDuque131*) a. Pileipellis; b. Basidia; c. Cheilocystidia; d. Basidioles with pigments; e. Basidiospores.

Agaricus sp4

Figures 2.f, 5

Diagnosis: Similar to *Agaricus rufoaurantiacus* by the orange-rufous squamules in pileus surface but differing by the smooth stipe, presence of abundant oleiferous hyphae in lamellar trama and hypoderm, and basidioles with brown pigments.

Etymology:

Holotype: Brazil. Santa Catarina: Florianópolis, Sítio Pedras Vivas, 27-XII-2014, *A. Parise AGP08*.

Pileus 90−100mm diam., plane, covered with concentrically orange brown scales on beige background, mainly at center and scattered to the margin, margin appendiculated with white velar fragments. **Pileus context** approx. 7 mm thick at center, fleshy, whitish, no changing. **Lamellae** free, initially white, then pinkish and finally brown when mature, margin regular, crowded, with lamellulae. **Stipe** 57 × 10 mm, central, cylindrical, fistulous, smooth above annulus, below fibrillose, beige. **Annulus** superous, pendant, membranous. **Basidiospores**

[40/2/1] 5.0−6.2 × 3.7 µm [Q=1.33-1.66; Q_m=1.6; L_m=6.0; W_m=3.7 µm] ellipsoid to almost elongate, dark brown, smooth, thick-walled, without germ pore. **Basidia** (10.0−)12.5−18.7(−21) × (5.0−)6.2−8.7 µm, cylindrical to cylindrical-clavate, hyaline, sometimes deformed, 4-spored. **Basidioles** (7.0−)11.0−14.0 × 5.0−6.0µm, clavate, with brown pigment deposited mainly in the base, smooth, thin-walled, sometimes broken. **Pleurocystidia** absent. **Cheilocystidia** (12.5−)15.0−21(−27) × (7.5−)10.0−13.7 (−23)µm, clavate to obovoid, hyaline, thin-walled. **Lamellar trama** regular with abundant oleiferous hyphae 3.7−7.5µm diam. **Pileipellis** a cutis composed of hyphae 5.0−8.7µm diam., thin-walled, cylindrical, branched, hyaline. **Pileus context** hyphae (5.0−)7.5−12.5µm diam., with abundant sinuous oleiferous hyphae. **Stipitipellis** hyphae 3.7−6.2µm diam., cylindrical, thin-walled, parallel.

Macrochemical reactions: KOH reaction positive, NaOH negative.

Habit and habitat: Two basidiomata near, growing on soil.

Material examined: Brazil. Santa Catarina: Florianópolis, Sítio Pedras Vivas, 27-XII-2014, *A. Parise AGP08*.

Notes: *Agaricus* sp4 is similar to *A. rufoaurantiacus* Heinem., from Trinidad, due to the pileus surface with orange-rufous squamules which are concentrically arranged at center, margin appendiculate, shape and size of basidiospores. However, *A. rufoaurantiacus* differs in pileus morphology convex, slightly smaller, stipe with orange squamules below annulus, pileipellis frequently with orange droplets and annulus floccose and orange at margin. Furthermore, *Agaricus sp4* has abundant oleiferous hyphae in lamellar trama and hypoderm, and basidioles with brown pigments. Heinemann (1961) included *A. rufoaurantiacus* in *Agaricus* subg. *Lanagaricus*.

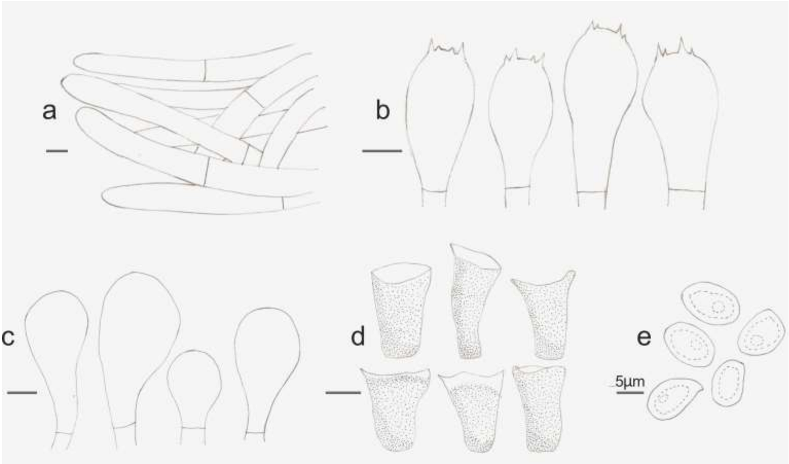


Figure 5. *Agaricus* sp4 (*AGP08*) a. Pileipellis; b. Basidia; c. Cheilocystidia; d. Basidioles with pigments; e. Basidiospores.

Agaricus sp5

Figures 2.a, 6

Diagnosis: Similar to *Agaricus osecanus* by the large pileus and floccose lower surface of annulus, but differing in the yellow scales in pileus surface and presence of basidia, basidioles and cheilocystidia pigmented.

Etymology:

Holotype: Brazil. Santa Catarina: Florianópolis, Universidade Federal de Santa Catarina, Centro de Filosofia e Ciências Humanas, 10-VII-2012, *A. C. Magnano ACM 376.*

Pileus 160 mm diam., at first convex and then expanding to plane-convex, covered with concentrically arranged yellow scales on beige background, mainly at center and scattered to the margin, margin apendiculated with white veil fragments. **Pileus context** approx. 30 mm thick at center, fleshy, whitish. **Lamellae** free, initially white, then pinkish and finally brown when mature, margin regular, crowded, with lamellulae. **Stipe** 98 × 35 mm, central, cylindrical, fusiform at base, solid, beige, slightly yellowish in some parts of stipe context when exposed. **Annulus** superous, descendent, pendant to subperonate, whitish, with brownish scales at lower surface. **Basidiospores** [40/1/2] (5.0−)6.2−7.5 × (3.7−)5.0 µm, [Q=(1.0−)1.25-1.50; Q_m=1.32; L_m=6.5µm; W_m=4.9µm] broadly ellipsoid to ellipsoid, dark brown, smooth, thick-walled, apiculus conspicuous, without germ pore. **Basidia** (12.5−)13.7−23(−28) × 6.2−7.5(−8.7) µm, cylindrical to cylindrical-clavate, hyaline, sometimes with brown internal pigment, smooth, 4-spored. **Basidioles** (12.0−)14.0−20 × (5.0−)6.0−8.0µm, clavate, with brown internal pigment deposited mainly in the base, smooth, thin-walled, sometimes broken. **Pleurocystidia** absent. **Cheilocystidia** (15.0−)18.7−26 × (10.0−)12.5−18.7µm, thin-walled, clavate to obovoid, with brown pigments, generally covering all the lamellae edge. **Lamellar trama** regular, composed of thin-walled hyphae, 3.7−7.5 (−8.7) µm diam., with abundant sinuous oleiferous hyphae. **Pileipellis** a cutis composed of thin- to rather thick-walled hyphae (3.7−)5.0−11.2µm diam., cylindrical, inflated, branched, hyaline. **Pileus context** hyphae (5.0−) 6.2−18.7 µm, cylindrical, inflate, thin-walled, with abundant oleiferous hyphae, sinuous to coiled. **Stipitipellis** hyphae (3.7−)5.0−8.7 µm diam., cylindrical, thin to rather thick-walled, strictly parallel.

Macrochemical reactions: KOH reaction positive, NaOH negative.

Habit and habitat: solitary, terrestrial, growing over grass in urban area.

Materials examined: Brazil. Paraná: Guarapuava, Seminário Diocesano Nossa Senhora de Belém, *M. Kurta MK01*.

Notes: *Agaricus* sp5 is similar to *Agaricus osecanus* Pilát because the large and robust pileus (70−150mm diam.), slightly yellowish on handling, base of stipe fusiform and shape and size of basidiospores. The annulus of *A. osecanus* is similar because of floccose lower surface, but differs in the color of the flakes. *Agaricus osecanus* also differs in pileus surface, the white scales and basidia slightly larger. *Agaricus arvensis* Schaeff. is also similar to *Agaricus* sp5 in the white pileus surface, which is later ochraceous and covered with squamules with size up to 110mm. *A. arvensis* differs by present a stipe floccose-squamulose below annulus with abrupt bulb, flattened underside, and larger basidiospores. *A. osecanus* and *A. arvensis* belong to *Agaricus* sect.

Arvenses. Moreover, *Agaricus* sp5 differs by present pigmented basidia, basidioles and cheilocystidia.

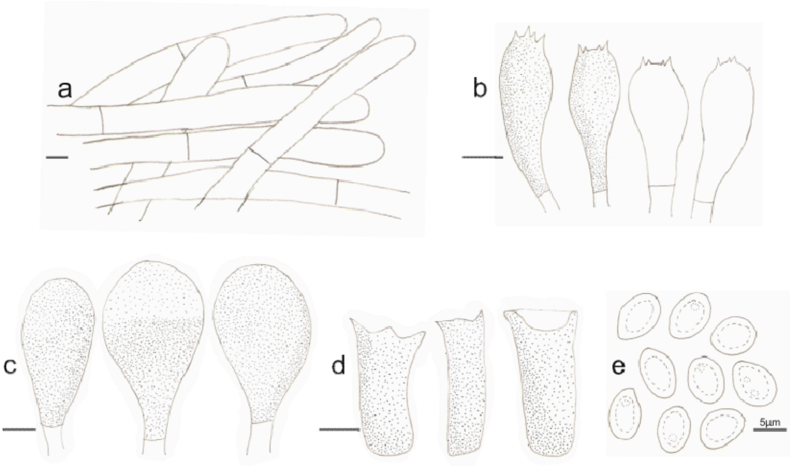


Figure 6. *Agaricus* sp5 (*ACM376, MK01*) a. Pileipellis; b. Basidia pigmented and hyaline; c. Cheilocystidia with pigments; d. Basidioles with pigments; e. Basidiospores.

Conclusions

There are some evidences that the geographical origin of genus *Agaricus* is paleotropical. Zhao et al. (2011) identified eleven clades composed only by tropical species, with a different phylogenetic distribution of American and African+Asian species indicating that species diversification probably occurred independently in paleotropic and in the Americas. This biogeographic fact indicates that species occurrence in America is different from those found in Africa or Asia. The present study introduces a new section in *Agaricus* subg. *Flavoagaricus* with four new species and six new sequences from specimens clustered in *Agaricus* section *Laeticolores*, showing that the diversity of species of *Agaricus* in America corresponds to different lineages and are not represented by recognized classical sections. The knowledge of *Agaricus* species occuring in Brazil and other neotropical countries is important to better understand the phylogenetic relationships in the genus and to better understand how this taxa are related to species that occur in different continents.

References

Arcuri, S.L.; Pagnocca, F.C.; Melo, W.G.; Nagamoto, N.S.; Komura, D.L.; Rodrigues, A. (2014) Yeasts found on an ephemeral reproductive caste of the leaf-cutting ant *Atta sexdens rubropilosa*. Antonie van Leeuwenhoek 106: 475-487.

Bas, C. (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella.* Persoonia 5: 285-579.

Bunyard, B. A.; Nicholson, M. S.; Royse, D. J. (1996) Phylogeny of the genus *Agaricus* inferred from Restriction Analysis of Enzymatically Amplified Ribosomal DNA. Fungal Genetics and Biology 20: 243-253.

Calvo-Bado, L.; Noble, R.; Challen, M.; Dobrovin-Pennington, A.; Elliott, T. (2000) Sexuality and genetic identity in the *Agaricus* section *Arvenses*. Applied and environmental microbiology. 66(2): 728-734.

Capelari, M. (1989) Agaricales do Parque Estadual da Ilha do Cardoso (exceto Tricholomataceae). Dissertação de Mestrado, Universidade de São Paulo, São Paulo.

Carreiro, S.C.; Pagnocca, F.C.; Bueno, O.C.; Bacci, M.Jr.; Hebling, M.J.A.; Silva, O.A. (1997) Yeasts associated with nests of the leaf-cutting ant *Atta sexdens rubropilosa* Forel, 1908. Antonie van Leeuwenhoek 71: 243–248.

Challen, M. P.; Kerrigan, R. W.; Callac, P. (2003) A phylogenetic reconstruction and emendation of *Agaricus* sections *Duploannulatae*. Mycologia, 95(1): 61-73.

Chen, J.; Zhao, R-L.; Parra, L. A.; Guelly, A. K.; Kesel, A.; Rapior, S.; Hyde, K. D.; Chukeatirote, E.; Callac, P. (2015)*Agaricus* section *Brunneopicti*: a phylogenetic reconstruction with descriptions of four new taxa. Phytotaxa 192(3): 145-168.

Coblentz, K.E.; Van Bael, S.A. (2013) Field colonies of leaf-cutting ants select plants materials containing low abundances of endophytic fungi. Ecosphere 4: art 66.

Currie, C.R.; Mueller, U.G.; Malloch, D. (1999) The agricultural pathology of ant fungus gardens. Proceedings of the National Academy of Sciences USA 96:7998-8002.

Dai, R.C.; Li, G.J; He, M.Q.; Liu, R.L.; Ling, Z.L.; Wu, J.R.; Zhao, R.L. (2016) Characterization of four species including one new species of Agaricus subgenus Spissicaules from Eastern China. Mycosphere 7(4):405-416.

Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

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

Didukh, M.; Vilgalys, R.; Wasser, S. P.; Isikhuemhen, O. S.; Nevo, E. (2005) Notes on *Agaricus* section *Duploannulati* using molecular and morphological data. Mycol. Res. 109(6): 729-740.

Freeman, A.E. (1979) *Agaricus* in the Southeastern United States. Mycotaxon 8(1):50-118.

Gui, Y.; Zhu, G-S.; Callac, P.; Hyde, K-D.; Parra, L-A.; Chen, J.; Yang, T-J.; Huang, W-B.; Gong, G-l.; Liu, Z-Y. (2015)*Agaricus* section *Arvenses*: three new species in highland subtropical Southwest China. Fungal Biology 119: 79-94.

Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3):307-21.

Heinemann, P. (1956) Champignons récoltés au Congo Belge par madame M. Goossens-Fontana. II. *Agaricus* Fries s.s. Bulletin du Jardin Botanique National de Belgique. 26(1):1-127

Heinemann, P. (1961) *Agaricus* of Trinidad. Kew Bulletin. 15(2): 231-248.

Heinemann, P. (1977) Essai d'une cle de determination des genres *Agaricus* et *Micropsalliota*. Sydowia 30:6-37.

Heinemann, P. (1990) Agarici Austroamericani VII. Agariceae des zones tempérées de l'Argentine et du Chili. Bulletin du Jardin botanique national de Belgique 60: 331-370.

Heinemann, P. (1993) Agarici Austroamericani VIII. Agariceae des regions intertropicales d'Amérique du Sud. Bulletin du Jardin Botanique National de Belgique, Meise, v. 62, p. 355-384.

Katoh, S. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30:772-780

Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Mentjies, P.; Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12): 1647-1649.

Kerrigan, R.W. (2005) *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. Mycologia. 97(1):12–24.

Kerrigan, R. W.; Callac, P.; Parra, L. A. (2008) New and rare taxa in *Agaricus* section *Bivelares* (*Duploannulati*). Mycologia, 100(6): 876-892.

Kramer, E. (2004) The Online Auction Color Chart: The New Language of Color. Online Auction Color Chart Company. pp 12.

Kück, P.; & Meusemann, K. (2010) FASconCAT: Convenient handling of data matices. Molecular Phylogenetics and Evolution 56(3):1115-1118.

Largent, D.L.; Thiers, H.D. (1977) How to Identify Mushrooms to Genus II: Field Identification of Genera. 3nd ed. Eureka, Mad River Press.

Largent D.; Johnson, D.; Watling, R. (1977) How to identify mushrooms to genus III: microscopic features. Eureka, Mad River Press.

Largent, D. (1986) How to identify mushrooms to genus III: macroscopic features. Eureka, Mad River Press.

Linnaeus, C. (1753) *Species plantarum* 2: 1171. Disponível em: < http://biodiversitylibrary.org/page/359192#page/613/mode/1up> Acesso em: 14 Jul. 2015.

Miller, M.A.; Pfeiffer, W.; Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, Louisiana, pp. 1–8. http://dx.doi.org/10.1109/GCE.2010.5676129

Mitchell, A.D.; Bresinsky, A. (1999) Phylogenetic relationships of *Agaricus* species based on ITS-2 and 28S ribosomal DNA sequences. Mycologia. 91(5):811–9.

Moncalvo, J-M.;Vilgalys, R.; Redhead, S. C.; Johnson, J. E.; James, T. Y.; Aime, M. C.; Hofstetter, V.; Verduin, S. J. W.; Larsson, E.; Baroni, T. J.; Thorn, R. G.; Jacobsson, S.; Clémençon, H.; Miller Jr., O. K. (2002) One hundred and seventeen clades of euagarics.Molecular Phylogenetics and Evolution 23: 357–400.

Morehouse, E.A.; James, T.Y.; Ganley, A.R.D.; Vilgalys, R.; Berger, L.; Murphy, P.J.; Longcore, J.E. (2003) Multilocus sequence typing suggests that the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403.

Pegler, D. N. (1990) Agaricales of Brazil described by J. P. F. C. Montague. Kew Bulletin 45(1): 161-177.

Pereira, J.S.; Costa, R.R.; Nagamoto, N.S.; Forti, L.C.; Pagnocca, F.C.; Rodrigues, A. (2016) Comparative analysis of fungal communities in colonies of two leaf-cutting ant species with different substratum preferences. Fungal ecology 21:68-75.

Putzke, J.; Putzke, M. T. L.; Köhler, A. (2014) Notas sobre os fungos Agaricaceae (Agaricales-Basidiomycota) comestíveis encontrados em áreas em regeneração natural em Santa Cruz do Sul - RS, Brasil. Caderno de Pesquisa, série Biologia, 25(3): 44-53.

Reis, B.M.S.; Silva, A.; Alvarez, M.R.; Oliveira, T.B.; Rodrigues, A.(2015) Fungal communities in graden of the leafcutter ant *Atta cephalotes* in forest and cabruca agrosystems of southern Bahia State (Brazil). Fungal biology 119:1170-1178.

Rodrigues, A.; Pagnocca, F.C.; Bacci, Jr. M.; Hebling, M.J.A.; Bueno, O.C.; Pfenning, L.H. (2005) Variability of non-mutualistic fungi associated with *Atta sexdens rubropilosa* nests. Folia Microbiologica 50: 421-425.

Rodrigues, A.; Mueller, U.G.; Ishak, H.D.; Bacci, Jr. M.; Pagnocca, F.C. (2011) Ecology of microfungal communities in gardens of fungus growing ants (Hymenoptera: Formicidae): a year-long survey of three species in Central Texas. FEMS Microbiology Ecology 78: 244-255.

Romano, E. & Brasileiro, A.C.M. (1998) Extração de DNA de plantas. *Biotecnologia: Ciência & Desenvolvimento* 2 (9): 40–43.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 572-574.

Rosa, L. H.; Capelari, M. (2009) Agaricales Fungi from Atlantic Rain Forest fragments in Minas Gerais, Brazil. Brazilian Journal of microbiology 40: 846-851.

Royse, D. J. (2014) A global perspective on the high five: *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia & Flammulina.* Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products.

Schultz, T.R.; Brady, S.G. (2008) Major evolutionary transitions in ant agriculture. Proceedings of the National Academy of Sciences USA 105: 5435-5441.

Singer, R. (1986) The Agaricales in modern taxonomy, 4th ed. Koeltz, Königstein, Germany.

Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics Advance Access.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Thongklang, N.; Nawaz, R.; Khalid, A. N.; Chen, J.; Hyde, K. D.; Zhao, R.; Parra, L. A.; Hanif, M.; Moinard, M.; Callac, P. (2014). Morphological and molecular characterization of three *Agaricus* species from tropical Asia (Pakistan, Thailand) reveals a new group in section *Xanthodermatei*. Mycologia 106 (6): 1220-32.

Vellinga, E.C. Glossary. (1988) In: Bas, C.; Kuyper, T.W.; Noodelos, M.E.; Vellinga, E.C. (eds.) Flora Agaricina Neerlandica, v. 1. Amsterdam/Brookfield: A.A. Balkema Publishers. p 54–64.

Vellinga, E. C. (2004) Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. Mycol. Res. 108 (4): 354–377.

Vellinga, E. C. (2004) Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. Mycol. Res. 108 (4): 354–377.

Vellinga, E.C.; Sysouphanthong, P.; Hyde, K.D. (2011) The family Agaricaceae: phylogenies and two new white-spored genera. Mycologia 103(3):494-509.

Vilgalys, R.; Hester, M. (1990) Rapid Genetic Identification and Mapping of Enzymatically Amplified Ribosomal DNA from Several *Cryptococcus* Species. Journal of bacteriology 172(8): 4238-4246.

Wartchow, F. (2005) A família Agaricaceae Fr. (Agaricales, Basidiomycota) em áreas de Mata Atlântica na região metropolitana de Recife - Pernambuco, Brasil. Dissertação de mestrado. Universidade Federal de Pernambuco, Pernambuco.

Wasser, S. P. (2011) Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl. Microbiol. Biotechnol 89:1323-1332.

Wisitrassameewong, K.; Karunarathna, S.C.; Thongklang, N.; Zhao, R.; Callac, P.; Moukha, S.; Férandon, C.; Chukeatirote, E.; Hyde, K.D. (2012) *Agaricus subrufescens*: A review. Saudi J Biol Sci. 19(2):131–46.

Zhao, R.; Karunarathna, S.; Raspé, O.; Parra, L. A.; Guinberteau, J.; Moinard, M.; Kesel, A. D.; Barroso, G.; Courtecuisse, R.; Hyde, K. D.; Guelly, A. K.; Desjardin, D. E.; Callac, P. (2011). Major clades in tropical *Agaricus*. Fungal Diversity 51:279-296.

Zhao, R.; Hyde, K. D.; Desjardin, D. E.; Raspé, O.; Soytong, K.; Guinberteau, J.; Karunarathna, S. C.; Callac, P. (2012)*Agaricus flocculosipes* sp. nov., a new potentially cultivatable species from the palaeotropics. Mycoscience 53:300-311.

Zhao, R.; Zhou, J.; Chen, J.; Margaritescu, S.; Sánchez-Ramírez, S.; Hyde, K. D.; Callac, P.; Parra, L. A.; Li, G.; Moncalvo, J. (2016) Towards standardizing taxonomic ranks using divergence times - a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Diverity. DOI 10.1007/s13225-016-0357-x.

Zhou, J.; Su, S.; Su, H.; Wang, B.; Callac, P.; Guinberteau, J.; Hyde, K.D.; Zhao, R.L. (2016) A description of eleven new species of *Agaricus* sections *Xanthodermatei* and *Hondenses* collected from Tibet and the surrounding areas. Phytotaxa 257(2):099-121.

CONSIDERAÇÕES FINAIS E RECOMENDAÇÕES PARA TRABALHOS FUTUROS

Este trabalho representa uma importante contribuição para o estudo do gênero *Agaricus* no Brasil. *Agaricus* possui uma ampla ocorrência e diversidade no Brasil, ocorrendo muitas vezes em áreas urbanas, crescendo em canteiros e jardins, e também no solo de florestas. Diferentes espécimes do gênero foram coletados e demonstraram pertencer aos cincos subgêneros conhecidos. O subgênero *Pseudochitonia* é o que apresenta maior número de seções, doze ao total, porém as sequências de espécimes brasileiros só agruparam em uma seção deste subgênero.

Nas análises filogenéticas foram apresentados dois novos clados, um deles descrito no capítulo 2, e cinco novas espécies (*Agaricus* sp1 descrita no capítulo 1 e *Agaricus* sp2*, Agaricus* sp3*, Agaricus* sp4 e *Agaricus* sp5 no capítulo 2). As diferentes relações filogenéticas encontradas entre as seções e os subgêneros, além da descrição de novas espécies reforçam a potencialidade de estudo do gênero *Agaricus* no Brasil e em outras regiões tropicais e neotropicais.

Zhao et al. (2011) analisaram a distribuição geográfica das espécies de *Agaricus* e encontraram algumas evidências sobre a possível origem paleotropical do gênero. Em seus resultados, as sequências de espécimes de áreas tropicais da África+Ásia se posicionaram nos ramos mais internos das árvores, antes dos clados com representantes Americanos. Este fato biogeográfico indica que algumas das espécies que ocorrem nas Américas são diferentes das encontradas na África e na Ásia, e que o número de espécies tropicais e subtropicais pode ser maior que o já conhecido para espécies de áreas temperadas.

Os trabalhos desenvolvidos por Zhao et al. (2011 e 2016) foram de extrema importância para a sistemática do grupo, tanto por incluir sequências de espécimes de regiões pouco amostradas, quanto pela proposição de uma nova classificação para o gênero. Neste trabalho, a inclusão de novas sequências de espécimes subtropicais é importante também para a continuidade do conhecimento da sistemática do gênero e possível descrição de novas seções e subgêneros.

As características macroscópicas são importantes para a identificação das espécies do gênero. Boas fotografias e anotações após a coleta do basidioma podem auxiliar na caracterização das espécies. Fotos e anotações nunca são demais. Devido às dificuldades na identificação taxonômica, técnicas moleculares são fundamentais. A reação de Schäffer, importante para o reconhecimento taxonômico das espécies não foi realizada neste trabalho. A reação pode ser feita no basidioma desidratado, mas é melhor observada quando fresco. É feita em forma de cruz, utilizando anilina pura ou a 10% de um lado e ácido nítrico a 50% de outro. Se positiva, o local onde as linhas se cruzam, apresenta coloração vermelha ou laranja (Heinemann, 1956; 1961). Não foi possível observar esta mudança ou não na coloração. A anilina utilizada, quando diluída apresentou coloração azul e deixou uma marca também azul no basidioma.

Agaricus, apesar de apresentar dificuldades na taxonomia e classificação das espécies, é um gênero diverso e de ampla ocorrência, com grande potencial medicinal, alimentar e de descrição de novas espécies.

REFERÊNCIAS BIBLIOGRÁFICAS

Albertó, E. (1998) *Agaricus santacatalinensis* a new species from Argentina. Mycotaxon 66: 205-213.

Ariyawansa, H. A. et al. (2015) Fungal diversity notes 111-252 - taxonomic and phylogenetic contribution to fungal taxa. Fungal Diversity 75:27–274.

Bas, C. (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella.* Persoonia 5: 285-579.

Benson, D. A.; Karsch-Mizrachi, I.; Lipman, D. J.; Ostell, J.; Wheeler, D. L. (2005) GenBank. Nucleic Acids Research, 33.

Bunyard, B. A.; Nicholson, M. S.; Royse, D. J. (1996) Phylogeny of the genus *Agaricus* inferred from Restriction Analysis of Enzymatically Amplified Ribosomal DNA. Fungal Genetics and Biology 20: 243-253.

Chen, J.; Zhao, R-L.; Parra, L. A.; Guelly, A. K.; Kesel, A.; Rapior, S.; Hyde, K. D.; Chukeatirote, E.; Callac, P. (2015)*Agaricus* section *Brunneopicti*: a phylogenetic reconstruction with descriptions of four new taxa. Phytotaxa 192(3): 145-168.

Dai, R.C.; Li, G.J; He, M.Q.; Liu, R.L.; Ling, Z.L.; Wu, J.R.; Zhao, R.L. (2016) Characterization of four species including one new species of Agaricus subgenus Spissicaules from Eastern China. Mycosphere 7(4):405-416.

Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

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

Dias,D.C.; de Oliveira,A.C.C.; Dias,P.F.; Valle,J.S.; Ribeiro,R.A.; Hungria,M.; Colauto,N.B.; Barcellos,F.G. (2013) Genetic diversity, taxonomy, and phylogeny of Basidiomycetes.

Didukh, M.; Vilgalys, R.; Wasser, S. P.; Isikhuemhen, O. S.; Nevo, E. (2005) Notes on *Agaricus* section *Duploannulati* using molecular and morphological data. Mycol. Res. 109(6): 729-740.

Doyle, J.J. & Doyle, J. L. (1987) A rapid isolat ion procedure for small quantitites of fresh tissue. Phytochemical Bulletim 19:. 11-15.

Freeman, A.E. (1979) *Agaricus* in the Southeastern United States. Mycotaxon 8(1):50-118.

Góes-Neto, A.; Loguercio-Leite, C.; Guerrero, R. T. (2005) DNA extraction from frozen field collected and dehydrated herbarium fungal basidiomata: performance of SDS and CTAB based methods. Biotemas 18(2): 19-32.

Gui, Y.; Zhu, G-S.; Callac, P.; Hyde, K-D.; Parra, L-A.; Chen, J.; Yang, T-J.; Huang, W-B.; Gong, G-l.; Liu, Z-Y. (2015) *Agaricus* section *Arvenses*: three new species in highland subtropical Southwest China. Fungal Biology 119: 79-94.

Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3):307-21.

Heinemann, P. (1956) Champignons récoltés au Congo Belge par madame M. Goossens-Fontana. II. *Agaricus* Fries s.s. Bulletin du Jardin Botanique National de Belgique. 26(1):1-127

Heinemann, P. (1961) *Agaricus* of Trinidad. Kew Bulletin. 15(2): 231-248.

Hughes, K.W.; Petersen, R.H.; Lickey, E.B.; Lodge, D.J.; Ovrebo, C.; Horak, E.; Matheny, B.; Methven, A.S.; McCleneghan, S.C. (2009) Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species delimitation across basidiomycete fungi. New Phytol. 182(4):795-8.

Karunarathna, S.C.; Chen, J.; Mortimer, P.E.; Xu, J.C.; Zhao, R.L.; Callac, P.; Hyde, K.D. (2016) Mycosphere Essay 8: A review of genus Agaricus in tropical and humid subtropical regions of Asia. Mycosphere 7(4):417-439.

Katoh, S. (2013) MAFFT multiple sequence alignment software version

7: improvements in performance and usability. Molecular Biology and Evolution 30:772-780

Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T.; Ashton, B.; Mentjies, P.; Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12): 1647-1649.

Kerrigan, R.W.; Callac, P.; Guinberteau, J.; Challen, M.P.; Parra, L.A. (2015) *Agaricus* section *Xanthodermatei*: a phylogenetic reconstruction with commentary on taxa. Mycologia 97(6): 1292-1315.

Kerrigan, R.W. (2016) *Agaricus* of North America. New York Botanical Garden Press, Bronx, (in press).

Kramer, E. (2004) The Online Auction Color Chart: The New Language of Color. Online Auction Color Chart Company. pp 12.

Kück, P.; & Meusemann, K. (2010) FASconCAT: Convenient handling of data matices. Molecular Phylogenetics and Evolution 56(3):1115-1118.

Largent D.; Johnson, D.; Watling, R. (1977) How to identify mushrooms to genus III: microscopic features. Eureka, Mad River Press.

Largent, D. (1986) How to identify mushrooms to genus III: macroscopic features. Eureka, Mad River Press.

Lebel, T.; Syme, A. (2012) Sequestrate species of Agaricus and Macrolepiota from Australia: new species and combinations and their position in a calibrated phylogeny. Mycologia (104)2: 496-520.

Linnaeus, C. (1753) *Species plantarum* 2: 1171. Disponível em: < http://biodiversitylibrary.org/page/359192#page/613/mode/1up> Acesso em: 14 Jul. 2015.

Liu, J. K. et al. (2015) Fungal diversity notes 1-100:taxonomic and phylogenetic contributions to fungal species. Fungal Diversity 72:1–197.

Miller, M.A.; Pfeiffer, W.; Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In Proceedings of the Gateway Computing Environments Workshop*

(GCE). New Orleans, Louisiana, pp. 1–8. http://dx.doi.org/10.1109/GCE.2010.5676129

Morehouse, E.A.; James, T.Y.; Ganley, A.R.D.; Vilgalys, R.; Berger, L.; Murphy, P.J.; Longcore, J.E. (2003) Multilocus sequence typing suggests that the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403.

Parra, L.A. (2013) Fungi europaei, Volume 1A, *Agaricus* L.: *Allopsalliota*, Nauta & Bas (Parte II) Alassio: Candusso Edizioni.

Petrova, A.; Alipieva, K.; Kostadinova, E.; Antonova, D.; Lacheva, M.; Gjosheva, M.; Popov, S.; Bankova, V. (2007) GC-MS studies of the chemical composition of two inedible mushrooms of the genus *Agaricus.* Chemistry Central Journal 1: 33–33. http://dx.doi.org/10.1186/1752-153X-1-33

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 572-574.

Royse, D. J. (2014) A global perspective on the high five: *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia* & *Flammulina.* Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products.

Singer, R. (1986) The Agaricales in modern taxonomy, 4th ed. Koeltz, Königstein, Germany.

Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics Advance Access.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Thongklang, N.; Nawaz, R.; Khalid, A. N.; Chen, J.; Hyde, K. D.; Zhao, R.; Parra, L. A.; Hanif, M.; Moinard, M.; Callac, P. (2014). Morphological and molecular characterization of three *Agaricus* species from tropical Asia (Pakistan, Thailand) reveals a new group in section *Xanthodermatei*. Mycologia 106 (6): 1220-32.

Vellinga, E.C. Glossary. (1988) In: Bas, C.; Kuyper, T.W.; Noodelos, M.E.; Vellinga, E.C. (eds.) Flora Agaricina Neerlandica, v. 1. Amsterdam/Brookfield: A.A. Balkema Publishers. p 54–64.

Vilgalys, R.; Hester, M. (1990) Rapid Genetic Identification and Mapping of Enzymatically Amplified Ribosomal DNA from Several *Cryptococcus* Species. Journal of bacteriology 172(8): 4238-4246.

Wasser, S. P. (2011) Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl. Microbiol. Biotechnol 89:1323-1332.

Zhao, R.; Karunarathna, S.; Raspé, O.; Parra, L. A.; Guinberteau, J.; Moinard, M.; Kesel, A. D.; Barroso, G.; Courtecuisse, R.; Hyde, K. D.; Guelly, A. K.; Desjardin, D. E.; Callac, P. (2011). Major clades in tropical *Agaricus*. Fungal Diversity 51:279-296.

Zhao, R.; Zhou, J.; Chen, J.; Margaritescu, S.; Sánchez-Ramírez, S.; Hyde, K. D.; Callac, P.; Parra, L. A.; Li, G.; Moncalvo, J. (2016) Towards standardizing taxonomic ranks using divergence times - a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Diverity. DOI 10.1007/s13225-016-0357-x.

Zhou, J.; Su, S.; Su, H.; Wang, B.; Callac, P.; Guinberteau, J.; Hyde, K.D.; Zhao, R.L. (2016) A description of eleven new species of *Agaricus* sections *Xanthodermatei* and *Hondenses* collected from Tibet and the surrounding areas. Phytotaxa 257(2):099-121.