UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
DEPARTAMENTO DE BOTÂNICA

TESE DE DOUTORADO

ESTUDOS TAXONÔMICOS E FILOGENÉTICOS DE FUNGOS BOLETOIDES (BOLETALES) NO BRASIL

ALTIELYS CASALE MAGNAGO

PORTO ALEGRE, RS
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Tese apresentada ao Programa de Pós-Graduação em Botânica, Área de Sistemática, Evolução e Ecologia de Algas, Plantas e Fungos, da Universidade Federal do Rio Grande do Sul (UFRGS), como requisito parcial para a obtenção do título de Doutor em Ciências (Botânica).

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RESUMO

Estudos taxonômicos e filogenéticos de fungos boletoides (Boletales) no Brasil

As regiões tropicais são consideradas importantes *hotspots* da biodiversidade do planeta. No entanto, o conhecimento da diversidade de fungos, incluindo os fungos boletoides nessas regiões ainda é muito limitado, e muitos estudos ainda devem ser realizados. Fungos boletoides são considerados um agrupamento morfológico não suportado filogeneticamente como monofilético. São representados por macrofungos com basidiomas epígeos, “carnudos” putrescentes, pileado-estipitados, com himenóforo tubular. Para este estudo foram utilizados materiais de herbário assim como novas coletas realizadas em várias localidades do Brasil nos últimos anos. As análises morfológicas e filogenéticas seguiram a metodologia tradicional para macrofungos. Duzentos e trinta e sete espécimes boletoides foram estudados e 104 sequências de DNA foram geradas, sendo 69 da região nrITS e 35 da região nrLSU. Cinquenta e duas espécies de fungos boletoides (representando 15 gêneros) têm registro nas florestas brasileiras. Duas espécies novas de *Tylopilus* foram descritas para o nordeste brasileiro; uma espécie nova de *Fistulinella* foi descrita para a Mata Atlântica e o primeiro registro de *Fistulinella campinaranae* var. *scrobiculata* foi feito para a Mata Atlântica; uma espécie nova de *Singerocomus* foi descrita para o Brasil e *Singerocomus rubriflavus* foi registrado pela primeira vez para o Brasil; uma espécie nova de *Gyroporus* foi descrita para o extremo sul da Mata Atlântica; uma espécie de *Boletellus* foi descrita para o nordeste brasileiro. Também foi proposta a sinonímia de *Phlebopus brasiliensis* com *Phlebopus beniensis*. Uma lista com dados geográficos e comentários dos fungos boletoides da Mata Atlântica brasileira é apresentada. O trabalho inclui chaves de identificação, fotografias coloridas e ilustrações da maioria das espécies apresentadas.

Palavras-chave: Agaricomycetes, Basidiomycota, Boletaceae, neotrópicos, taxonomia.

ABSTRACT

Taxonomic and phylogenetic studies of boletoid fungi (Boletales) in Brazil

Tropical regions are considered the greatest biodiversity hotspots of the planet. However, the knowledge about fungi diversity, including boletoid fungi, is still very limited. Boletoid fungi are considered a morphological grouping not supported phylogenetically as monophyletic. They are represented by pileate-stipitate macrofungi with tubular hymenophore, epigial basidiomata, and a putrescent context. For this study was used herbaria collections as well as collections from new field trips in many locations in Brazil in the last years. Morphological and phylogenetic analyses followed the traditional methodology for macrofungi. Two hundred and thirty-seven specimens were studied, and 104 DNA sequences were generated, 69 from the nrITS region and 35 from the nrLSU region. Fifty-two species of boletoid fungi (within 15 genera) occur in the Brazilian forests. Two new species of *Tylopilus* were described for the Brazilian Northeastern region, one new species of *Fistulinella* was described for the Atlantic Forest and the first record of *Fistulinella campinaranae* var. *scrobiculata* for the Atlantic Forest was registered; a new species of *Singerocomus* was described and the first record of *Singerocomus rubriflavus* for Brazil was made; a new species of *Gyroporus* was described for the south of the Atlantic Forest; a new species of *Boletellus* was described for the Brazilian Northeastern. The synonymia of *Phlebopus brasiliensis* with *Phlebopus beniensis* was proposed. A list with geographic data and comments of boletoid fungi registered in the Brazilian Atlantic Forest is provided. This work also includes identification keys, pictures and illustrations for the majority of the species.

Key-words: Agaricomycetes, Basidiomycota, Boletaceae, Neotropic, taxonomy.

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1. INTRODUÇÃO

O conhecimento sobre a diversidade de fungos nas regiões tropicais tem atraído cada vez mais taxonomistas ao redor do mundo, no entanto, nossa compreensão a respeito da diversidade e suas interações intra e interespecíficas ainda é bastante incompleta (Aime & Brearley 2012). Estimativas atuais indicam uma diversidade de fungos entre 2,2 e 3,8 milhões de espécies no planeta, mas atualmente temos descritas em torno de 120.000 espécies (Hawksworth & Lücking 2017). As regiões tropicais são áreas extremamente ricas em número de espécies, abrigando a maior parte da biodiversidade terrestre. Devido sua alta complexidade estrutural combinada com a grande diversidade de nichos e clima quente e úmido, ambiente propício para o desenvolvimento dos fungos, acredita-se que grande parte das espécies ainda por serem descritas estejam nas florestas tropicais (Hawksworth 2001, Hawksworth & Lücking 2017).

No Brasil, país considerado *hotspot* da biodiversidade mundial, os estudos envolvendo levantamento da micodiversidade têm aumentado na última década, principalmente devido à formação de novos taxonomistas e divulgação do reino Fungi à população em geral. No entanto, o número de espécies registradas para o Brasil ainda é considerado muito baixo. Lewinsohn & Prado (2005) apontam uma diversidade em torno de 14.510 espécies conhecidas para o Brasil. Maia et al. (2015) apresentam uma diversidade de 5.719 espécies, baseado no Catálogo de Plantas e Fungos do Brasil.

Devido à necessidade de conhecermos melhor a micota brasileira, ponto inicial para estudos como conservação e biotecnologia, este trabalho teve como objetivo principal ampliar o conhecimento sobre a diversidade das espécies de fungos boletoides que ocorrem em florestas brasileiras.

1.1. Fungos boletoides

O termo boletoide se refere a um agrupamento morfológico artificial e não a um grupo natural. Os fungos boletoides são macrofungos com hábito principalmente terrestre, com basidiomas epígeos, "carnudos" putrescentes, pileado-estipitados, com himenóforo tubular exposto (Singer et al. 1986). Muitos possuem ornamentações atraentes tanto no píleo quanto no estipe, além de muitas espécies apresentarem reações que causam mudança de coloração nas superfícies quando tocadas ou quando o contexto é exposto (Smith & Thiers 1971, Singer et al. 1986). Essa reação é resultado da oxidação de derivados do ácido pulvínico, como os ácidos variegático, xerocômico e o atrotomentínico (Nelsen 2010). Apesar de não definir grupos naturais as mudanças de coloração por testes de toque, exposição e macroquímicos ainda são características muito utilizadas na taxonomia (Figura 1).

Figura 1. Fungos boletoides: detalhe do himenóforo tubular e reações de oxidação ao toque nos poros. **A.** *Xerocomus basius* de Meijer, **B.** *Boletinellus rompelii* (Pat. & Rick) Watling, **C.** Boletaceae, **D.** Boletaceae, **E.** *Boletinellus exiguus* (Singer & Digilio) Watling, **F.** *Boletellus ananas* (M.A. Curtis) Murrill. (Fotos **A-F** Altielys C. Magnago)

Os fungos boletoides apresentam esporos geralmente pigmentados, com esporadas oliváceas, amarelas, marrons, avermelhadas, róseas ou negras. O tamanho e a forma dos esporos são muito variados, podem ser lisos como em *Boletus* L., *Fistulinella* Henn., *Tylopilus* P. Karst. entre outros, ou ornamentados como em *Afroboletus* Pegler & T.W.K Young, *Aureoboletus* Pouzar, *Austroboletus* (Corner)Wolfe, *Boletellus* Murril, *Heimioporus* E. Horak e *Strobilomyces* Berk. Os basídios são do tipo holobasídio, geralmente clavados, com no máximo quatro esterigmas. Os cistídios podem estar presentes ou ausentes tanto no tubo quanto na abertura dos poros, assim como na superfície do píleo e/ou estipe. A trama do tubo pode ser divergente ou paralela e as fíbulas podem estar presentes ou ausentes (Singer 1986).

Membros desse grupo atuam como decompositores favorecendo a ciclagem de nutrientes nos ecossistemas, mas mais de 90% dos representantes boletoides fazem parte de uma rede de interações com vários organismos (Agerer 1987-2012, Vellinga et al. 2009, Vasco-Palacios et al. 2018). Essas associações são conhecidas como micorrizas, interação entre fungos e as raízes das plantas, e são um dos fatores cruciais que possibilitam o melhor desenvolvimento das plantas e auxiliam na manutenção dos solos (Read 1999, Tedersoo et al. 2014). Os boletoides realizam associações do tipo ectomicorrízicas, e podem estar relacionados com mais de dez famílias de plantas, entre elas Betulaceae, Casaurinaceae, Dipterocarpaceae, Ericaceae, Fabaceae (Caesalpinoideae, Mimosoideae), Fagaceae, Myrtaceae, Nyctagenaceae, Pinaceae e Salicaceae (Smith & Pfister 2009; Becerra & Zak 2011; Henkel et al. 2012; Vasco-Palacios et al. 2018). As associações micorrízicas melhoram a capacidade de absorção de minerais e a tolerância ao estresse hídrico. Também aumentam a resistência da planta contra agentes patogênicos do solo e reduzem a sensibilidade às substâncias tóxicas (Brundrett 2002, Varma 2008).

Gastronomicamente, muito dos mais apreciados e caros cogumelos comestíveis pertencem ao grupo dos boletoides, que apresentam também alto valor nutricional. Possuem altas quantidades de antioxidantes; presença de selênio, mineral geralmente pouco encontrado em vegetais, e que auxilia nas funções enzimáticas do fígado e aumenta as resposta do sistema imunológico; presença de fibras, potássio, vitaminas, minerais e um baixo teor calórico auxiliando muito em uma dieta saudável (Feeney et al. 2014). São conhecidos principalmente nas regiões temperadas devido à cultura micofágica. No Brasil, os boletoides comestíveis que temos conhecimento se encontram em áreas de florestas introduzidas de pinheiros (*Pinus* spp.) e castanheiras portuguesas (*Castanea sativa* Mill). Entre eles estão o *porcini* (*Boletus edulis*

Bull.) e o *funghi secchi* (várias espécies de *Suillus* spp.) (Figura 2). Não existem dados de comestibilidade para as outras espécies boletoides encontradas no Brasil.

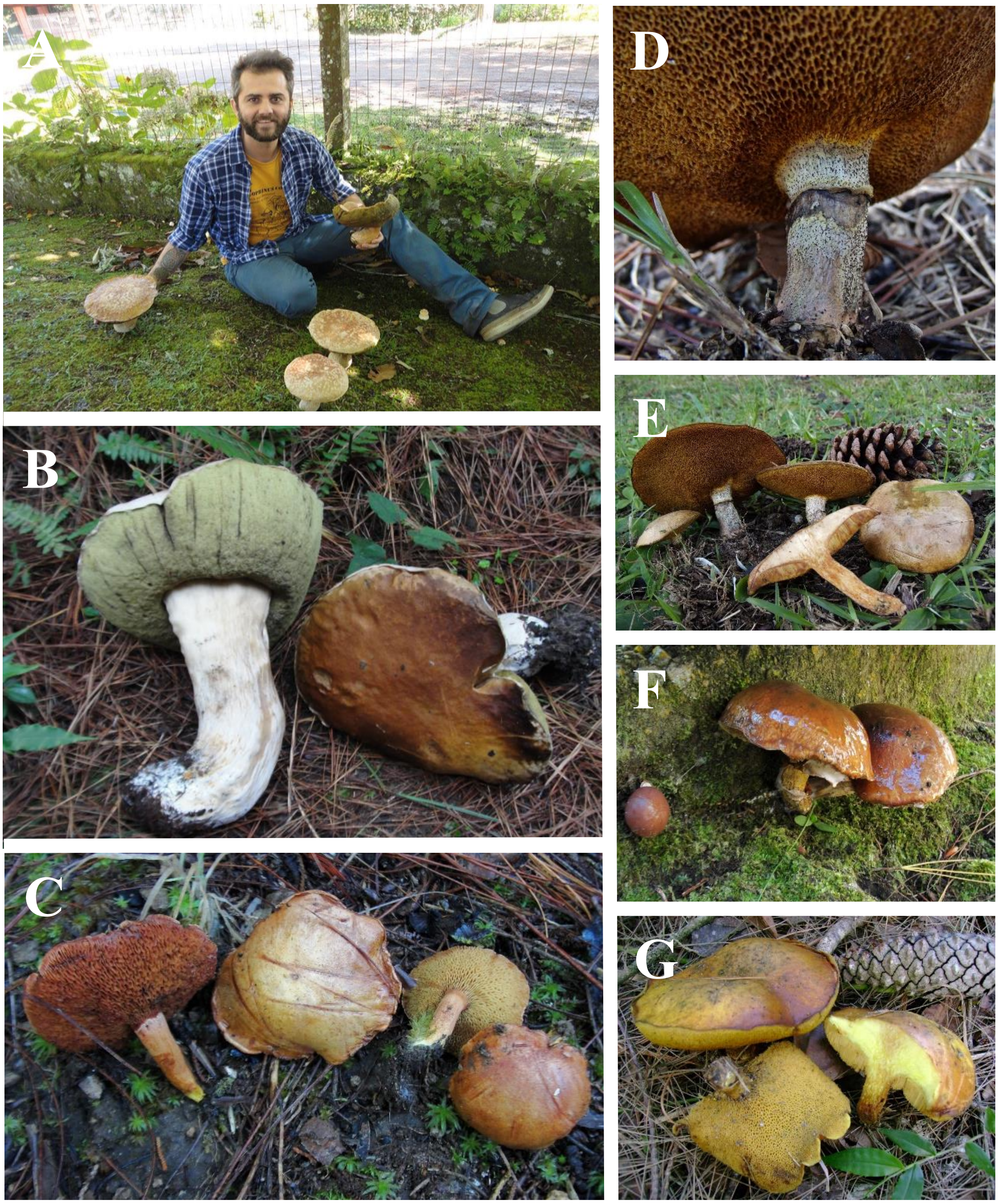

Figura 2. Boletoides coletados em áreas introduzidas com *Pinus* spp. e *Castanea sativa* Mill. **A-B.** *Boletus edulis* Bull., **C.** *Chalciporus piperatus* (Bull.) Bataille, **D.** Detalhe dos pontos glandulares enegrecidos no estipe característicos no gênero *Suillus*, **E.** *Suillus cothurnatus* Singer, **F.** *Suillus luteus* (L.) Roussel, **G.** *Suillus granulatus* (L.) Roussel. (Fotos **A-G** Altielys C. Magnago).

1.2. Histórico da classificação dos fungos boletoides

Durante as três publicações de *Systema Mycologicum* (1821-1832) o naturalista Elias Fries propôs o primeiro sistema de classificação para os fungos. O gênero *Agaricus* L. incluiu os tradicionais cogumelos, dentro da família Agaricaceae, ordem Agaricales. A fim de separar os cogumelos que apresentavam o himenóforo tubular dos que apresentavam himenóforo lamelar, Fries propôs dois gêneros: *Boletus* L. (onde os tubos estariam conectados entre si pelos dissepimentos) e *Fistulina* Bull. (com tubos livres entre si). Em 1926, Chevallier propôs a família Boletaceae (Agaricales) a fim de separar basidiomas com himenóforo tubular de Agaricaceae, que passava a apresentar apenas cogumelos com himenóforo lamelar. Boletaceae foi então subdividida em cinco gêneros: *Boletus* L., *Cladoporus* (Pers.) Chevall., *Fistulina* Bull., *Physisporus* Chevall. e *Polyporus* P. Micheli. Em 1931, Gilbert eleva a família Boletaceae Chavallier (Agaricales) a nível de ordem, criando Boletales. A partir daí foram incluídos também membros com basidiomas epígeos, "carnudos" putrescentes e com himenóforo tubular. Duas subordens foram estabelecidas, Boletineae, com representantes com esporos lisos, e Strobilomycetineae com esporos ornamentados. Smith & Thiers (1971) recircunscreveram Boletaceae Chevallier (Agaricales) incluindo basidiomas "carnosos" putrescentes, com himenóforo tubular e trama do tubo bilateral, e incluíram dez gêneros na família (*Boletus* L., *Boletellus* Murrill, *Fuscoboletinus* Pomerl. & A.H. Sm., *Gastroboletus* Lohwag, *Gyroporus* Quél., *Leccinum* Gray, *Pulveroboletus* Murrill, *Suillus* Gray, *Strobilomyces* Berk. e *Tylopilus* P. Karst.).

Nas séries de *Agaricales in Modern Taxonomy* (Singer 1949, 1962, 1975, 1986) várias atualizações em relação à classificação dos fungos boletoides foram apresentadas. Em 1986, a última edição publicada, Singer manteve a classificação com base na taxonomia morfológica e química e incluiu fungos lamelares ao grupo. Boletineae (Agaricales) foi subdividida em três famílias: Boletaceae (basidiomas com himenóforo tubular), incluindo os representantes de Strobilomycetineae, Gomphidiaceae (com himenóforo lamelar e esporada branca, creme ou marrom) e Paxillaceae (com himenóforo lamelar e esporada acinzentada, preta ou verde oliva). Boletaceae passa a ser subdividida em seis subfamílias: Boletoideae (*Austroboletus* (Corner) Wolfe, *Boletus* L., *Boletellus* Murrill, *Boletochaete* Singer, *Chalciporus* Bataille, *Fistulinella* Henn., *Leccinium* Gray, *Phylloboletellus* Singer, *Porphyrellus* E.-J. Gilbert, *Pulveroboletus* Murril, *Tylopilus* P. Karst., *Veloporphyrellus* L.D. Gómez & Singer e *Xanthoconium* Singer), Gyrodontoideae (*Gyrodon* Opat., *Meiorganum* Heim, *Paragyrodon* Singer (Singer) e *Phlebopus* (R. Heim) Singer), Gyroporoideae (*Gyroporus* Quél.), Suilloideae (*Boletinus*

Kalchbr., *Psiloboletinus* Singer e *Suillus* Gray), Strobilomycetoideae (*Strobilomyces* Berk.) e Xerocomoideae (*Phylloporus* Quél., *Tubosaeta* Horak e *Xerocomus* Quél.). Ao contrário de Singer, Pegler & Young (1981) reconheceram a ordem Boletales Gilbert incluindo não apenas fungos boletoides mas também os agaricoides e gasteroides, representados em seis famílias: Paxillaceae, Gyrodontaceae, Xerocomaceae, Boletaceae, Strobilomycetaceae e Gomphidiaceae, incluindo trinta e sete gêneros.

Nas últimas décadas, com o avanço dos estudos moleculares (Binder & Bresinsky 2002, Binder & Hibbett 2006, Drehmel et al. 2008, Nuhn et al. 2013, Wu et al. 2014, 2016), Boletales passa a ser representada por cinco clados principais, Boletineae (incluindo Paxillineae), Sclerodermatineae, Suillineae, Coniophorineae e Tapinellineae, agrupando uma grande variedade morfológica, incluindo fungos boletoides, gasteroides, secotioides, lamelares, ressupinados, crostosos e poliporoide (Figura 3). No entanto, os primeiros clados que divergiram em Boletales ainda necessitam ser melhor resolvidos. Atualmente sessenta e cinco gêneros boletoides são reconhecidos em Boletales (Anexo1).

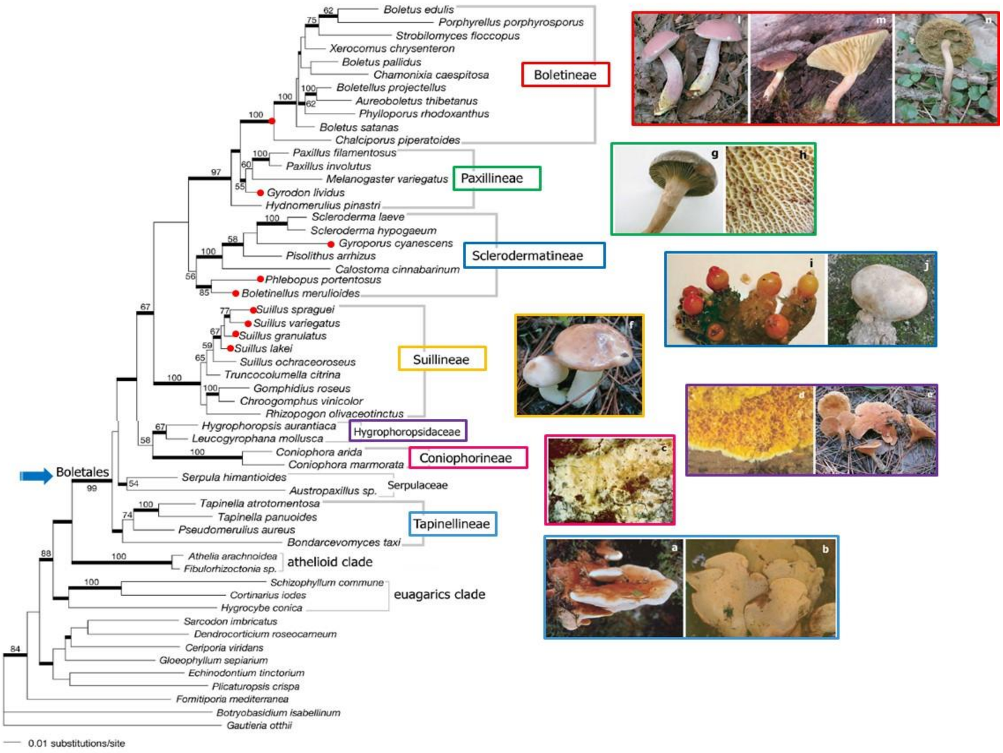

Figura 3. Filogenia de Boletales (Basidiomycota, Fungi) sensu Binder & Hibbett (2006). Pontos em vermelho indicam grupos boletoides. (Modificado de Binder & Hibbett 2006).

A morfologia boletoide surge em outros clados fora de Boletineae, onde se concentra a maioria dos boletoides conhecidos, como é o caso de *Boletinellus* Murril, *Gyroporus* Quél. e *Phlebopus* (R. Heim) Singer em Sclerodermatineae, *Gyrodon* Opat. em Paxillineae e *Suillus* Gray em Suillineae. Da mesma forma, morfologias gasteroides e secotioides também se misturam com os boletoides no clado Boletineae, como é o caso de *Chamonixia* Rolland e *Spongiforma* Dejardin, Manfr. Binder, Roekring & Flegel. Hibbett & Thorn (2001). Wilson et al. (2012) mostram que o caráter tubular do himenóforo, pode ter surgido várias vezes entre as linhagens de homobasidiomicetos ao longo de sua diversificação, e que somente as características morfológicas não seriam suficientes para delimitar os táxons.

1.3. Estudos de fungos boletoides no Brasil

Johannes Rick, jesuíta austríaco que se estabeleceu no Rio Grande do Sul no início do século XX, era um micólogo amador e foi considerado o pioneiro da micologia brasileira. Realizou as primeiras coletas de fungos no sul do Brasil, incluindo os primeiros boletoides. Quatro táxons de boletoides são citados e descritos em seus trabalhos (Rick 1907, 1960), *Boletinellus rompelii* (Pat. & Rick) Watling, *Boletus brasiliensis* Rick, *Boletus subtomentosus* L. e *Phlebopus tropicus* (Rick) Heinem. & Rammeloo. A maioria de suas coletas está na Coleção Rickiana depositada no Herbário PACA na Universidade do Vale do Rio dos Sinos. Outros exemplares, incluindo alguns holótipos estão no Herbário FH na Universidade de Harvard e no Herbário LIL na Fundação Miguel Lillo na Argentina. O alemão Rolf Singer junto a colaboradores, especialmente Antonio P. L. Digilio, teve grande importância nos estudos de boletoides principalmente na Amazônia brasileira entre as décadas de 50 e 80, onde descreveram várias espécies de macrofungos (Singer & Digilo 1957, 1960, Singer 1970, Singer et al. 1983). Suas coletas estão depositadas principalmente no Herbário INPA-Fungos, no Instituto Nacional de Pesquisas da Amazônia. Putzke et al. (1994) realizaram um levantamento dos fungos pertencentes à Boletaceae no Rio Grande do Sul, registrando nove táxons. Oliveira & Souza (1995, 1996, 2002) realizaram um levantamento de boletoides em fragmentos de Mata Atlântica na Universidade Federal da Paraíba. Suas amostras estão depositadas no Herbário JPB na própria Universidade. Watling & de Meijer (1997) e de Meijer (2008) realizaram estudos de macrofungos no estado do Paraná incluindo áreas de florestas mistas com presença de araucárias. Nestes trabalhos algumas combinações e ampliação geográfica de algumas espécies foram realizadas assim como descrição de *Xerocomus basius* de Meijer. Sobestiansky 2005 citou fungos boletoides para o estado do Rio Grande do Sul e Santa Catarina, em áreas nativas e áreas introduzidas com *Pinus* spp. Neves & Capelari 2007 apresentam uma lista de boletoides para o Brasil baseado em dados de literatura assim como Sulzbacher et al. 2013. Magnago & Neves (2014) apresentaram um novo registro de *Austroboletus festivus* (Singer) Wolfe para o estado de Santa Catarina. Barbosa-Silva et al. (2017) descreveram recentemente uma nova variedade, *Tylopilus aquarius* var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo em áreas de solo arenoso no nordeste brasileiro, e Barbosa-Silva & Wartchow (2017) descreveram *Boletellus cremeovelosus* Barbosa-Silva & Wartchow e citam o primeiro registro de *Boletellus ananas* var. *crassotunicatus* Singer para a América do Sul.

Neste trabalho estão incluídas descrições de novas espécies de boletoides. Novidades em relação à distribuição geográfica de táxons já conhecidos e uma ampliação da lista de espécies boletoides para o Brasil são apresentadas.

2. OBJETIVOS

Este trabalho teve como objetivo geral ampliar o conhecimento a respeito dos aspectos taxonômicos, geográficos e filogenéticos dos fungos boletoides no Brasil. Dentre os objetivos específicos estão:

- construir de chaves de identificação, descrições e ilustrações dos táxons;
- atualizar dos registros dos herbários consultados e ampliação do acervo dos herbários com o depósito dos materiais coletados e identificados;
- sequenciar regiões do DNA nuclear para fazer análises filogenéticas de boletoides.

3. MATERIAIS E MÉTODOS

3.1. Áreas de coleta

As excursões de coleta de macrofungos foram realizadas desde 2008, durante o curso de Biologia, seguindo durante o mestrado e doutorando, em várias localidades do Brasil, principalmente ao longo da Mata Atlântica com o auxílio de vários colaboradores. As licenças de coletas para as áreas, quando exigidas, foram obtidas junto aos órgãos competentes. Na Tabela 1 estão as localidades onde fungos boletoides foram coletados ao longo desses anos.

Tabela 1. Localidades onde espécimes de boletoides foram coletados.

RIO GRANDE DO SUL (RS)
Canoas (Parque Municipal Getúlio Vargas) Porto Alegre (Morro Santana e Parque Farroupilha) Santa Maria (Universidade Federal de Santa Maria, Parque Itaimbé) São Francisco de Paula (Floresta Nacional de São Francisco de Paula)
SANTA CATARINA (SC)
Blumenau (Universidade Regional de Blumenau) Florianópolis (Morro da Lagoa da Conceição, Parque Natural Municipal das Dunas da Lagoa da Conceição, Trilha da Costa do Santinho e Ilha do Campeche) Garopaba Santo Amaro da Imperatriz (Hotel Plaza Caldas da Imperatriz)
SÃO PAULO (SP)
Iporanga (Parque Estadual Turístico do Alto Ribeira, Núcleo Cablocos)
RIO DE JANEIRO (RJ)
Rio de Janeiro (Parque Nacional da Tijuca)
Teresópolis (Parque Nacional Serra dos Órgãos)
ESPÍRITO SANTO (ES)
Alfredo Chaves (Morro da Caixa D'água) Santa Teresa (Reserva Biológica Augusto Ruschi)
BAHIA (BA)
Itacaré (Ramal de acesso à Fazenda Marambaia) Serra Grande (Parque Estadual da Serra do Conduru)
PARAÍBA (PB)
João Pessoa (Universidade Federal da Paraíba e Jardim Botânico Benjamim Maranhão) Mamanguape (Reserva Biológica Guaribas)
RIO GRANDE DO NORTE (RN)
Natal (Parque Estadual das Dunas de Natal)
MATO GROSSO (MT)
Nova Ubiratã (Estação Ecológica Rio Ronuro)
GOIÁS (GO)
Anápolis (Colégio Estadual Jardim Salomão)
AMAZONAS (AM)
Manaus (Margens ao longo do Rio Cuieiras e Parque Nacional de Anavilhanas)

Outras localidades como o Parque Nacional do Iguaçu, Área de Proteção Ambiental de Guaraqueçaba, Parques Municipais de Curitiba e região de Guarapuava, no Paraná; Parque Estadual do Espinilho e Estação Ecológica do Taim, no Rio Grande do Sul, também foram incluídas na lista de expedições de coleta, no entanto nenhum boletoide foi encontrado.

3.2. Coleta e descrição de fungos boletoides

Uma boa coleção de fungos boletoides deve conter basidiomas em diferentes estágios de desenvolvimento. As características morfológicas podem variar significativamente entre o estágio jovem e o maduro, ou entre espécimes num mesmo estágio de maturação. Caso a espécie apresente hábito solitário, os espécimes devem ser recoletados sempre que possível para descrições mais completas incluindo as variações do táxon.

- Fotografias do material fresco em campo são importantes (Figuras 4,5), pois muitas características são perdidas com o manuseio, transporte e desidratação.
- Anotações ecológicas e do ambiente são importantes já que a maioria dos boletoides forma associações ectomicorrízicas e muitas espécies estão associadas a uma ou várias plantas simbiontes. É importante identificar quais plantas (família, gênero e se possível espécies) estão crescendo próximas aos fungos.
- A descrição macroscópica do material fresco complementa as fotografias e anotações de campo. A descrição deve incluir (Largent 1986, Singer 1986):
    - Píleo (tamanho, formato, cor, superfície, margem e reações de oxidação).
    - Himenóforo (forma, tamanho, cor e reações de oxidação dos poros e tubos).
    - Estipe (tamanho, formato, cor, superfície/ornamentação, presença de anel e reações de oxidação).
    - Contexto (coloração, textura e reações de oxidação).
    - Coloração da esporada
- Os códigos de cores utilizados nas descrições morfológicas (ex. OAC787), seguiram a tabela de cores *Online Auction Color Chart* (Kramer 2004).
- Hidróxido de amônio 10% (NH_4OH) é o reagente mais utilizado para testes macroquímicos em fungos boletoides. Uma pequena gota é pingada sobre a superfície dos basidiomas e no contexto para observar se ocorre mudança de coloração.

Figura 4. Macromorfologia dos basidiomas em campo. **A.** *Tylopilus* sp., **B.** *Tylopilus dunensis* A.C. Magnago & M.A. Neves, **C.** Boletaceae, **D.** *Fistulinella ruschii* A.C. Magnago, **E.** *Tylopilus aquarius* var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo, **F.** *Gyroporus autrobrasiliensis* A.C. Magnago. (Fotos **A-B** Maria Alice Neves, **C-F** Altielys C. Magnago).

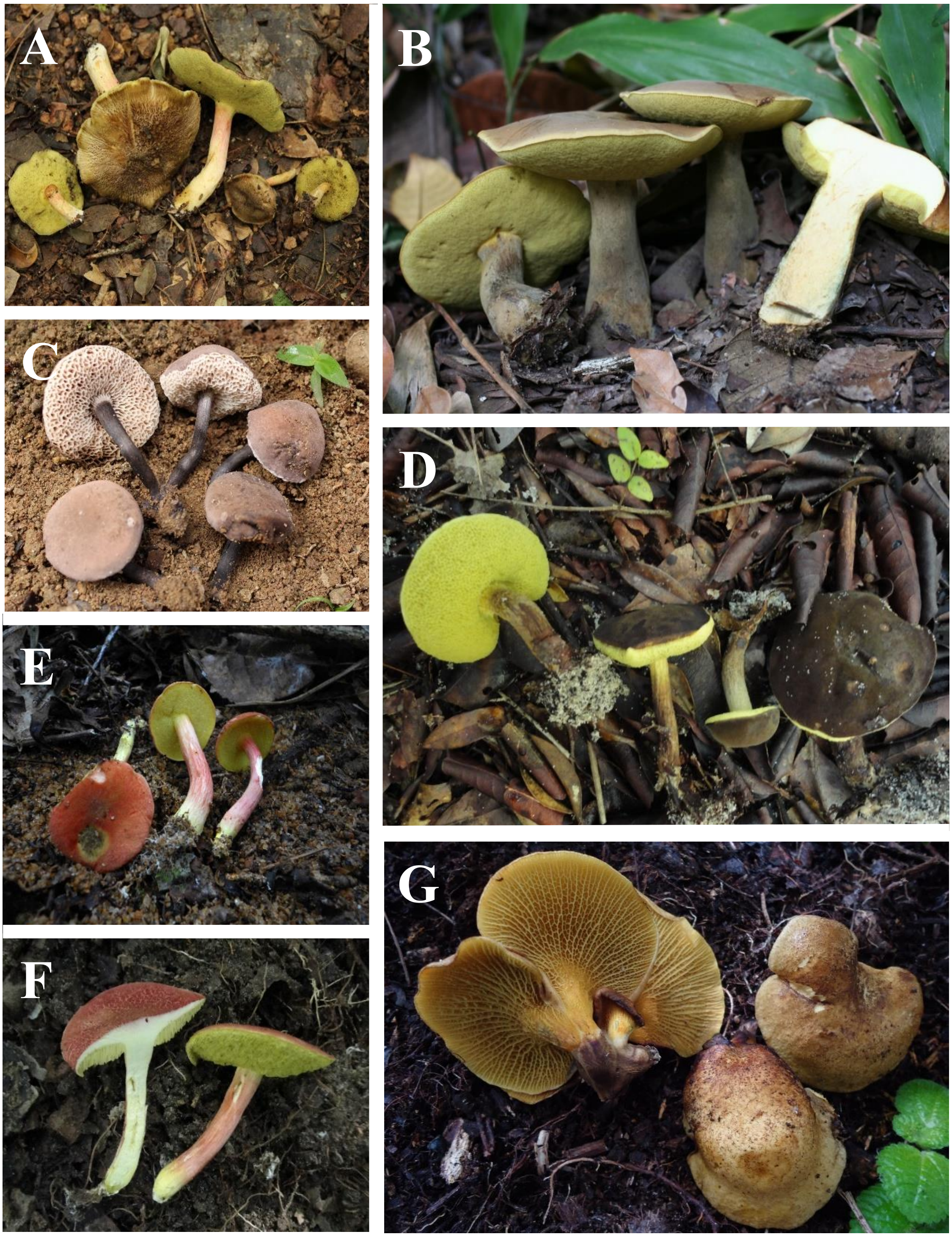

Figura 5. Macromorfologia dos basidiomas em campo. **A.** *Xerocomus basius* de Meijer, **B.** *Phlebopus beniensis* (Singer & Digilio) Heinem. & Rammeloo, **C.** Boletaceae, **D.** *Boletellus nordestinus* A.C. Magnago, **E.** *Singerocomus atlanticus* A.C. Magnago, **F.** *Singerocomus rubriflavus* T.W. Henkel & Husbands, **G.** *Boletinellus exiguus* (Singer & Digilio). (Fotos **A**,**C**,**E**-**G** Altielys C. Magnago, **B** Maria Alice Neves, **C** Eduardo Fazolino)

- Após coleta, os basidiomas foram transportados para o laboratório ou alojamento, onde foram lentamente desidratados em baixa temperatura (± 40ºC) em um desidratador de frutas elétrico (*Total Chef TCFD-05 Deluxe*), durante aproximadamente 24 horas, ou até estarem completamente desidratados. No caso de basidiomas grandes e robustos, foi necessário fatiar as amostras para melhor desidratação. Após este processo, os espécimes foram acondicionados em sacos plásticos (tipo *Ziplock*®), identificados com seu número de coleta e temporariamente armazenados para posterior depósito nos herbários.
- Para a descrição microscópica foi necessário realizar cortes histológicos tanto no contexto quanto na superfície do píleo e estipe, assim como nos tubos. Características relacionadas a tamanho, forma, superfície/ornamentação, cor e reação de Melzer dos esporos, basídios, cistídios e hifas foram observadas seguindo Largent et al. (1977) e Singer (1986).

3.3. Caracterização molecular e filogenética

Os procedimentos de extração e amplificação foram realizados nos Laboratórios de Sistemática Molecular, Biologia Molecular (Micologia) e Fisiologia do Departamento de Botânica da Universidade Federal do Rio Grande do Sul e Laboratório de biologia Molecular do Departamento de Botânica da Universidade Federal de Santa Catarina. As extrações de DNA foram realizadas a partir de porções dos basidiomas preservados em sílica ou fragmentos de basidiomas desidratados já depositados em herbários, seguindo o protocolo de Doyle & Doyle (1987) adaptado para fungos por Góes-Neto et al. (2005). Os *primers* ITS8-F /ITS6-R e LR7/LR0R foram utilizados no estudo para amplificar os seguintes marcadores nucleares: espaçador transcrito interno do DNA nuclear ribossomal (ITS1, 5.8S, ITS2), região codificadora da subunidade maior do DNA nuclear ribossomal (LSU), seguindo Dentinger et al. (2010) e Vilgalys & Hester (1990) respectivamente. Para os procedimentos de PCR (reação de polimerase em cadeia), purificação dos produtos de PCR e reações de sequenciamento, foram utilizados os protocolos disponibilizados pelo Hibbett Lab (http://www.clarku.edu/faculty/dhibbett). Para a etapa do sequenciamento, as amostras purificadas foram enviadas para a Macrogen na Coréia. Após o sequenciamento, as sequências *forward* e *reverse* de cada amostra foram combinadas (*assembly*) para gerar uma única sequência consenso (*contig*), utilizando o programa Geneious 6.1.8 (Kearse et al. 2012). O teste de similaridade entre os táxons foi feito utilizando a ferramenta BLAST (*Basic Local Alignment Search Tool*) através do *National Center for Biotechnology Information* (NCBI) (http://www.ncbi.nlm.nih.gov/blast/). As novas sequências geradas e utilizadas nos manuscritos foram depositadas no banco de dados do GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Para as análises filogenéticas foram utilizadas as sequências geradas durante esse trabalho e sequências obtidas no GenBank referenciadas em artigos relacionados. As matrizes geradas foram alinhadas utilizando o programa MAFFT v. 7 (Katoh & Standley 2013), e então manualmente inspecionadas e ajustadas quando necessário utilizando o programa MEGA 6 (Tamura et al. 2013). As análises filogenéticas foram feitas utilizando-se critérios de máxima verossimilhança e inferência bayesiana, utilizando os seguintes programas: RAxML v. 8.2.4 (Stamatakis 2014) e MrBayes v. 3.2.6 (Ronquist et al. 2013) respectivamente, disponíveis na plataforma CIPRES (http://www.phylo.org/) (Miller et al. 2010). As regiões de DNA foram analisadas separadamente, e quando não foram observadas diferenças significativas entre as topologias, os dados foram concatenados para as análises combinadas. A edição das árvores foi feita no programa FigTree (Morariu et al. 2009).

4. RESULTADOS GERAIS

4.1. Espécimes coletados e revisões de herbários

Duzentos e trinta e sete espécimes foram analisados neste trabalho: 148 espécimes coletados pelo autor e colaboradores em 21 localidades, principalmente ao longo da Mata Atlântica, e 89 espécimes provenientes dos herbários PACA, INPA, SP, UFRN e NY. Os materiais coletados durante esse trabalho foram depositados nos herbários da Universidade Federal do Rio Grande do Sul (ICN), Universidade Federal de Santa Catarina (FLOR), Universidade Federal do Espírito Santo (VIES) e Universidade Federal do Rio Grande do Norte (UFRN-Fungos).

Analisando conjuntamente os dados resultantes das análises dos materiais coletados, exsicatas de herbários e revisão de literatura foi possível constatar que 52 espécies de fungos boletoides (representando 15 gêneros), ocorrem nas florestas brasileiras. A lista das espécies e sua distribuição geográfica são apresentadas no item 4.6.

4.2. Épocas de coleta

Nas áreas de solo arenoso como no Parque Estadual das Dunas de Natal (Rio Grande do Norte) e Reserva Biológicas Guaribas (Paraíba) houve maior abundância de basidiomas entre os meses de abril e junho (outono). No Parque Municipal das Dunas da Lagoa da Conceição em Florianópolis (Santa Catarina) os materiais foram mais abundantes entre fevereiro e março (fim do verão). Em regiões de Mata Atlântica na Serra Estadual do Conduru (Bahia) e na Reserva Biológica Augusto Ruschi (Espírito Santo), os basidiomas estavam presentes principalmente no mês de dezembro (início do verão), *Fistulinella ruschii* foi recoletada durante os três anos de campo, na mesma localidade. Na região sul, nas áreas de Mata Atlântica próximas a Florianópolis e Porto Alegre, a maior abundância de basidiomas ocorreu entre os meses de janeiro e março (verão). Frequentemente a quantidade de basidiomas coletados durante as expedições não foi significativa e os táxons apresentaram na maioria hábito solitário ou em pequenos grupos (dois a seis basidiomas).

4.3. Resultados de sequenciamento

Dois marcadores moleculares (nrITS e nrLSU) foram utilizados e 104 sequências de DNA nuclear de fungos boletoides foram geradas neste trabalho, sendo 69 nrITS e 35 nrLSU. Foram obtidas sequências de 73 espécimes, distribuídos em 27 táxons. Os números de acesso do GenBank estão citados na Tabela 2.

Foi possível sequenciar a região ITS de três holótipos *Austroboletus rionegrensis* (Singer & I.J.A. Aguiar) Singer, INPA78693; *Xerocomus amazonicus* Singer, INPA 77399; e *Xerocomus globuliger* Singer, INPA 77280. Os demais materiais sequenciados correspondem a basidiomas de coletas realizadas durante esse trabalho.

Tabela 2. Lista dos espécimes e números de acesso do GenBank das sequências geradas durante esse trabalho. Números de coletor seguido do símbolo * indicam holótipos.

Espécies	**No. Coletor**	**Localidade**	**GenBank No.**	
			ITS	**LSU**
Austroboletus festivus	ACM575	Brasil (SC)	KY886203	KY888000
Austroboletus festivus	ACM573	Brasil (SC)	KY886202	KY888001
Austroboletus festivus	CHC245	Brasil (SC)	KY887998	–
Asutroboletus festivus	ACM564	Brasil (SC)	KY887999	–
Austroboletus festivus	ACM574	Brasil (SC)	KY888002	–
Austroboletus rionegrensis	INPA78693*	Brasil (AM)	KY886201	–
Boletellus nordestinus	UFRN2726*	Brasil (RN)	MG760443	MG760444
Boletellus nordestinus	UFRN2725	Brasil (RN)	MG760442	–
Boletinellus exiguus	CHC053	Brasil (SC)	Submetido	–
Espécies	**No. Coletor**	**Localidade**	**GenBank No.**	

			ITS	LSU
Boletinellus exiguus	ACM988	Brasil	–	Submetido
Boletinellus exiguus	ACM336	Brasil (SC)	Submetido	–
Boletinellus rompelii	AH01	Brasil (SC)	Submetido	–
Boletinellus rompelii	MAN1064	Brasil (SC)	Submetido	–
Boletinellus rompelii	MAN1091	Brasil	Submetido	–
Boletus edulis	ACM1082	Brasil (RS)	–	Submetido
Boletus sp.	ACM1018	Brasil (SC)	Submetido	Submetido
Chalciporus piperatus	ACM1074	Brasil (RS)	Submetido	–
Chalciporus cf. *amarellus*	ACM350	Brasil (SC)	Submetido	–
Chalciporus cf. *persicinus*	CATO107	Brasil (SC)	Submetido	Submetido
Chalciporus cf. *amarellus*	ACM492	Brasil (ES)	Submetido	–
Chalciporus cf. *amarellus*	ACM528	Brasil (ES)	Submetido	Submetido
Chalciporus cf. *amarellus*	ACM529	Brasil (ES)	Submetido	–
Fistulinella campinaranae var. *scrobiculata*	ACM484	Brasil (BA)	KY886204	KY888003
Fistulinella ruschii	ACM526*	Brasil (ES)	KY886206	KY888006
Fistulinella ruschii	ACM491	Brasil (ES)	KY886207	–
Fistulinella ruschii	ACM1317	Brasil (ES)	KY886208	KY888005
Fistulinella ruschii	ACM485	Brasil (BA)	KY886205	KY888004
Fistulinella ruschii	CATO105	Brasil (SC)	KY886209	–
Fistulinella ruschii	MAN391	Brasil (PB)	KY886210	–
Gyroporus austrobrasiliensis	ACM1136*	Brasil (RS)	MF436999	MF437014
Gyroporus austrobrasiliensis	ACM1144	Brasil (RS)	MF437000	MF437015
Gyroporus austrobrasiliensis	ACM1150	Brasil (RS)	MF437001	–
Phlebopus beniensis	MAN357	Brasil (PB)	Submetido	–
Phlebopus beniensis	ACM267	Brasil (PB)	Submetido	–
Phlebopus beniensis	ACM288	Brasil (PB)	Submetido	–
Phlebopus beniensis	ACM285	Brasil (PB)	Submetido	–
Phlebopus beniensis	ACM265	Brasil (PB)	Submetido	–
Phlebopus beniensis	DS1056	Brasil (MT)	Submetido	–
Phlebopus beniensis	DLK1024	Brasil (AM)	Submetido	–
Espécies	**No. Coletor**	**Localidade**	**GenBank No.**	

			ITS	LSU
Phlebopus beniensis	ACM1027	Brasil (RS)	—	Submetido
Singerocomus atlanticus	ACM527*	Brasil (ES)	KY907177	KY926776
Singerocomus atlanticus	CATO106	Brasil (SC)	KY907179	KY926778
Singerocomus atlanticus	ACM1275	Brasil (SC)	KY907178	KY926777
Singerocomus atlanticus	BZL69	Brasil (SC)	KY907180	—
Singerocomus atlanticus	MJ105	Brasil (SC)	KY907181	—
Singerocomus rubriflavus	GAS900	Brasil (BA)	KY907182	KY926779
Tylopilus aquarius var. *megistus*	ACM297	Brasil (PB)	MF113422	MF113430
Tylopilus aquarius var. *megistus*	MAN460	Brasil (PB)	MF113423	MF113431
Tylopilus aquarius var. *megistus*	ACM1297	Brasil (ES)	Submetido	Submetido
Tylopilus dunensis	MAN216	Brasil (RN)	MF113418	—
Tylopilus dunensis	MAN218*	Brasil (RN)	MF113419	MF113428
Tylopilus dunensis	MAN281	Brasil (RN)	MF113420	—
Tylopilus pygmaeus	ACM486*	Brasil (BA)	MF113421	MF113429
Tylopilus sp. 1	MAN217	Brasil (RN)	MF113424	MF113432
Tylopilus sp. 1	MAN282	Brasil (RN)	MF113425	—
Tylopilus sp. 2	MAN288	Brasil (RN)	MF113426	—
Tylopilus sp. 3	MAN215	Brasil (RN)	MF113427	—
Xerocomus amazonicus	INPA77399*	Brasil (AM)	Submetido	—
Xerocomus basius	ACM1257	Brasil (RS)	Submetido	Submetido
Xerocomus basius	ACM1345	Brasil (RS)	Submetido	Submetido
Xerocomus basius	ACM1154	Brasil (RS)	Submetido	Submetido
Xerocomus globuliger	INPA77280*	Brasil (AM)	Submetido	—
Boletaceae sp. 1	ACM1330	Brasil (ES)	Submetido	Submetido
Boletaceae sp. 1	ACM1331	Brasil (ES)	Submetido	—
Boletaceae sp. 2	ACM1310	Brasil (ES)	Submetido	Submetido
Boletaceae sp. 2	ACM1298	Brasil (ES)	Submetido	Submetido
Boletaceae sp. 2	MJ01	Brasil (SC)	Submetido	—
Espécies	**No. Coletor**	**Localidade**	**GenBank No.**	

			ITS	**LSU**
Boletaceae sp. 2	ACM1016	Brasil (SC)	Submetido	–
Boletaceae sp. 2	ACM1017	Brasil (SC)	Submetido	Submetido
Boletaceae sp. 3	MJ123	Brasil (SC)	Submetido	–
Boletaceae sp. 3	ACM1000	Brasil (SC)	Submetido	Submetido
Boletaceae sp. 3	ACM1152	Brasil (RS)	Submetido	Submetido
Boletaceae sp. 3	ACM1138	Brasil (RS)	–	Submetido

4.4. Chave para os gêneros boletoides citados para o Brasil

1. Fíbulas presentes (Boletinellaceae, Gyroporaceae) **2**

1’. Fíbulas ausentes (Boletaceae) **4**

2. Himenóforo decurrente, poros angulares radialmente orientados, ocasionalmente sublamelares. Estipe excêntrico a lateral .... ***Boletinellus***

2’. Himenóforo adnexo a sublivre, com poros pequenos e arredondados. Estipe central **3**

3. Himenóforo branco a amarelo claro. Estipe oco ou apresentando cavidades no contexto, hifas organizadas circunferencialmente. Esporada amarelo claro .... ***Gyroporus***

3’. Himenóforo amarelo a esverdeado. Estipe sem cavidades no contexto, hifas organizadas longitudinalmente. Esporada verde oliva .... ***Phlebopus***

4. - Esporos com superfície ornamentada em microscopia óptica. Margem do píleo geralmente apendiculada **5**

4’. Esporos com superfície lisa em microscopia óptica. Píleo com margem inteira **7**

5. Píleo com escamas piramidais enegrecidas. Esporos globosos, reticulados ou equinados. Esporada preta .... ***Strobilomyces***

5’. Píleo com cores variadas. Esporos com outras ornamentações. Esporada rosada ou esverdeada **6**

6. Himenóforo rosado. Esporos amidaliformes a alongado fusoides, verrucosos, reticulados ou enrugados. Esporada rosada .... ***Austroboletus***

6’. Himenóforo com tubos amarelados. Esporos elipsoides a subglobosos, estriados longitudinalmente. Esporada verde oliva .... ***Boletellus***

7. Himenóforo adnato a decurrente, avermelhado. Esporada marrom avermelhada .... ***Chalciporus***

7’. Himenóforo adnexo, sublivre, rosado ou amarelo **8**

8. Himenóforo e esporada rosados **9**

8’. Himenóforo amarelo e esporada esverdeada a amarronzada........ **11**

9. Presença de anel remanescente do véu parcial .... ***Pulveroboletus***

9’. Ausência de anel remanescente de véu parcial **10**

10. Basidiósporos fusoides a alongados. Cistídios hialinos, sem conteúdo pigmentado .... ***Fistulinella***

10’. Basidiósporos elipsoides a faseioliformes. Cistídios com conteúdo pigmentado ***Tylopilus***

11. Píleo geralmente viscoso. Presença de pontuações glandulares enegrecidas no estipe (resultado de aglomerações de caulocistídios pigmentados). Esporada amarronzada. Ectomicorrízico obrigatório com Pinaceae .... ***Suillus***

11’. Píleo geralmente seco. Ausência de pontuações glandulares no estipe. Esporada esverdeada. Ectomicorrízico com outros simbiontes, lignícola, ou saprotrófico **12**

12. Píleo tomentoso, vermelho rosado. Base do estipe amarela .... ***Singerocomus***

12’. Píleo glabro a subtomentoso com cores variadas **13**

13. Lignícola, geralmente em troncos de Pinaceae ....***Buchwaldoboletus***

13’. Ectomicorrízico ou saprotrófico..**14**

14. Esporos lisos em microscopia eletrônica de varredura, trama do tubo boletoide (divergente) ....***Boletus***

14’. Esporo com superfície bacilar em microscopia eletrônica de varredura, trama do tubo *phylloporus-type* (pararela) .... ***Xerocomus***

4.5. Descrição e distribuição para os gêneros boletoides citados para o Brasil

Este levantamento foi realizado baseado em literatura e coletas realizadas recentemente pelo autor e colaboradores.

Austroboletus (Corner) Wolfe, Bibliotheca Mycologica 69: 64 (1980).

Espécie tipo: ***Austroboletus dictyotus*** (Boedijn) Wolfe.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo hemisférico a convexo, superfície subtomentosa a glabra, seco a gelatinoso, margem geralmente se estendendo até o estipe cobrindo o himenóforo em basidiomas jovens, se rompendo quando maduro formando uma margem apendiculada; contexto esbranquiçado não alterando sua coloração quando exposto. Himenóforo depresso, poros rosados, angulares, geralmente tornando-se amarronzados sob pressão. Estipe central, pruinoso a reticulado; micélio basal branco a rosado.

Esporada rosa amarronzada. Esporos alongados a amidaliformes, ornamentados com verrugas, cristas reticuladas, depressões, ou irregularmente enrugados. Pleuro e queilocistídios abundante. Pileipelis subrepente a tricodermal, às vezes gelatinizada. Fíbulas ausentes.

Distribuição no Brasil: *Austroboletus festivus* (Singer) Wolfe – Paraná, Pernambuco e Santa Catarina (Singer 1970, Watling & de Meijer 1997, Magnago & Neves 2014); *Austroboletus graciliaffinis* Singer – Amazonas (Singer 1989); *Austroboletus olivaceus* Singer – Amazonas (Singer et al. 1983); *Austroboletus rionegrensis* (Singer & Araujo) Singer – Amazonas (Singer & Araujo 1979, Singer et al. 1983).

Boletellus Murrill, Mycologia 1: 9 (1909).

Espécie tipo: ***Boletellus ananas*** (M.A. Curtis) Murrill.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a amplamente convexo, superfície glabra, aveludada, tomentosa ou fibrilosa, seca, podendo apresentar margem apendiculada; contexto amarelado, podendo se tornar azulado quando exposto. Himenóforo adnexo, adnato a sinuado, poros amarelos, podendo se tornar azul ao toque. Estipe central, glabro a pruinoso; micélio basal branco. Esporada verde oliva. Esporos longitudinalmente estriados ou alados, subfusiformes. Pleuro e queilocistídios presentes. Pileipelis tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Boletellus ananas* var. *ananas* (M.A. Curtis) Murril – Amazonas (Singer et al. 1983, Magnago 2018e), *Boletellus ananas* var. *crassotunicatus* Singer – Amazonas (Barbosa-Silva & Wartchow 2017); *Boletellus cremeovelosus* Barbosa-Silva & Wartchow – Pernambuco (Barbosa-Silva & Wartchow 2017), *Boletellus nordestinus* A.C. Magnago – Paraíba e Rio Grande do Norte (Oliveira & Sousa 1995, como *B. lepidospora* e *B. pustulatus*, Magnago et al. 2018c, no prelo).

Boletinellus Murrill, Mycologia 1 (1): 7 (1909).

Espécie tipo: ***Boletinellus merulioides*** (Schwein.) Murrill.

Descrição: Basidioma pileado-estipitado, himenóforo tubular do tipo boletineloide. Píleo convexo a aplanado, superfície glabra a levemente tomentosa, seca; contexto amarelado, podendo reagir azulado quando exposto. Himenóforo decurrente, poros angulares radialmente orientado, ocasionalmente sublamelares, amarelo, geralmente tornando-se azul ao toque. Estipe lateral a excêntrico, raramente central, superfície seca e glabra. Esclerócio geralmente presente. Esporada verde amarronzada. Esporos ovoides a subgloboso, liso. Cistídios inconspícuos, geralmente ausente nos poros. Fíbulas presentes.

Distribuição no Brasil: *Boletinellus exiguus* – Bahia, Paraná, Santa Catarina, Rio de Janeiro e Rio Grande do Sul (Putzke et al. 1994, como *Gyrodon exiguus* Singer & Digilio, de Meijer 2001, Watling & de Meijer 1997, Magnago et al. 2018d, no prelo); *Boletinellus proximus* (Singer) Murril – Paraíba (Oliveira & Sousa 1996, como *Gyrodon proximus* Singer); *Boleteinellus rompelii* (Pat. & Rick) Watling – Paraná, Santa Catarina e Rio Grande do Sul (Singer & Digilio 1957, como *Gyrodon rompelii* (Pat. & Rick) Singer, Putzke et al. 1994, Watling & de Meijer 1997, de Meijer 2001, como *Gyrodon rompelii* , Sobestiansky 2005, Magnago et al. 2018d, no prelo).

Boletus L. Species Plantarum: 1176 (1753).

Espécie tipo: ***Boletus edulis*** Bull..

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a amplamente convexo, superfície glabra, tomentosa ou fibrilosa, seca a subvíscida; contexto esbranquiçado. Himenóforo adnato a adnexo, poros esbranquiçados e preenchidos quando jovens a amarelo esverdeados quando maduros. Estipe central, glabro, subpruinoso ou reticulado; micélio basal branco. Esporada verde oliva. Esporos lisos, fusoides. Cistídios presentes. Fíbulas ausentes. Análises filogenéticas sugerem o monofiletismo do gênero restrito ao grupo "*porcini*".

Distribuição no Brasil: *Boletus cinnamomeus* Rick – Rio Grande do Sul (Singer 1953, Rick 1960); *Boletus edulis* Bull. – Rio Grande do Sul (Sobestiansky 2005); *Boletus mutabilis* var. *austroamericanus* Rick – Rio Grande do Sul (1960); *Boletus neo-olivaceus* Rick – Rio Grande do Sul (Rick 1937, Singer 1953), *Boletus panormitanus* Inzenga – Rio Grande do Sul (1960); *Boletus spadiceus* Schaeff. – Rio Grande do Sul (Singer 1953).

Boletus cinnamomeus, B. mutabilis var. *austroamericanus, B. panormitanus* e *B. spadiceus*, todos citados para o Rio Grande do Sul, são representados apenas pelo holótipo, e segundo os herbários onde estão depositados, essas coleções não foram encontradas.

Buchwaldoboletus Pila´t, Friesia 9: 217 (1969).

Espécie tipo: ***Buchwaldoboletus lignicola*** (Kallenb.) Pila´t..

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a aplanado, superfície subtomentosa ou pulverulenta, seca, geralmente margem encurvada; contexto amarelado, tornando azul quando exposto. Himenóforo levemente decurrente a sinuado, poros amarelo claro a amarelo ocre, tornando-se azul escuro sob pressão. Estipe central, subcilíndrico. Esporada verde ocre. Esporos lisos, subfusiformes. Pleuro e queilocistídios subfusiformes, ventricosos ou clavados com ápice alongado. Pileipelis repente com hifas entrelaçadas. Fíbulas ausentes. Lignícola, crescendo geralmente sobre Pinaceae.

Distribuição no Brasil: *Buchwaldoboletus duckeanus* Singer – Amazonas (Singer et al. 1983, como *Pulveroboletus duckeanus* Singer).

Chalciporus Bataille, Bulletin de la Société d'Histoire Naturelle du Doubs 15: 39 (1908).

Espécie tipo**:** ***Chalciporus piperatus*** (Bull.) Bataille.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a amplamente convexo, superfície glabra a levemente subtomentosa, seca, raramente víscida; contexto amarelado, raramente tornando azulado quando exposto. Himenóforo adnato a decurrente, poros avermelhados, raramente tornando azulado sob pressão. Esporada marrom avermelhada. Esporos lisos, subfusiformes. Pileipelis tricodermal com hifas entrelaçadas. Fíbulas ausentes.

Distribuição no Brasil: *Chalciporus trinitensis* var. *trinitenis* (Heinem) Singer – Amazonas (Singer et al. 1983); *Chalciporus trinitensis* var. *amazonicus* L.D Gómez – Amazonas (Singer et al. 1983, Gómez 1996) e Espírito Santo (Magnago 2018e, no prelo); *Chalciporus piperatus* (Bull.) Bataille – Paraíba, Paraná, Pernambuco e Santa Catarina (Watling & de Meijer 1997, Giachini et al. 2000, de Meijer 2001, Oliveira & Sousa 2002, Karstedt & Stürmer 2008).

Fistulinella Henn., Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie 30: 43 (1901).

Espécie tipo**:** ***Fistulinella staudii*** Henn..

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo hemisférico a convexo aplanado, superfície glabra, tomentosa ou fibrilosa, geralmente escrobiculada, seca ou víscida, contexto esbranquiçado, não reagindo à exposição. Himenóforo geralmente depresso ao redor do estipe, poros rosados, angulares. Estipe central, glabro ou pruinoso, seco ou víscido. Esporada rosa amarronzada. Esporos fusoides, lisos. Pleuro e queilocistídios geralmente presentes. Pileipelis tricodermal, geralmente gelatinizada. Fíbulas ausentes.

Distribuição no Brasil: *Fistulinella campinaranae* Singer – Amazonas e Bahia (Singer et al. 1983, Magnago et al. 2017b); *Fistulinella ruschii* A.C. Magnago – Bahia, Espírito Santo, Paraíba e Santa Catarina (Oliveira & Sousa 2002, como *Fistulinella violaceipora* (Stevens.) Pegler & Young, Magnago et al. 2017b).

Gyroporus Quél., Enchiridion Fungorum in Europa media et praesertim in Gallia Vigentium: 161 (1886).

Espécie tipo: ***Gyroporus cyanescens*** (Bull.) Quél.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo, superfície glabra, fibrilosa a subescamosa, seca; contexto esbranquiçado a amarelado, podendo reagir azulado ou amarronzado quando exposto. Himenóforo adnexo a sublivre, poros arredondados, esbranquiçados a amarelo claro quando maduros, podendo reagir azulado ou amarronzado sob pressão. Estipe central, glabro ou fibriloso, seco, contexto oco ou sólido com cavidades, composto por hifas arranjadas circunferencialmente e não longitudinalmente como nos outros gêneros. Esporada amarelo claro. Esporos elipsoides, lisos. Pleuro e queilocistídios presentes. Pileipelis tricodermal. Fíbulas presentes.

Distribuição no Brasil: *Gyroporus castaneus* (Bull. Ex Fr.) Quel. – Amazonas (Singer et al. 1983); *Gyroporus austrobrasiliensis* A.C. Magnago – Rio Grande do Sul (Magnago et al. 2018b, no prelo).

Phlebopus (R. Heim) Singer, Annales Mycologici 34: 326 (1936).

Espécie tipo: ***Phlebopus colossus*** (R.Heim) Singer.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a aplanado, superfície glabra, seca a subvíscida, contexto amarelado, tornando-se azulado em algumas espécies. Himenóforo adnexo a adnato, poros amarelados, arredondados e pequenos, podendo ficar azulado sob pressão. Estipe glabro a aveludado, seco. Esporada verde oliva. Esporos elipsoides a subglobosos. Cistídios inconspícuos. Pileipelis tricodermal. Fíbulas presentes.

Distribuição no Brasil: *Phlebopus beniensis* (Singer & Digilio) Heinem. & Rammeloo – Amazonas (as *P. brasiliensis* Singer) Bahia, Espírito Santo, Goiás, Paraíba (as *P. hearley* Heinem. & Rammeloo), Paraná, Rio Grande do Sul (Singer et al.1983, Oliveira & Sousa 1996, Watling & de Meijer 1997, de Meijer 2001, Calaça et al. 2018, Magnago et al. 2018d, no prelo); *Phlebopus braunii* (Bres.) Heinem. – Rio de Janeiro, Rio Grande do Sul, Santa Catarina (Singer & Digilo 1960, Singer et al. 1983, Putzke et al. 1994); *Phlebopus tropicus* (Rick) Heinem. & Rammeloo – Paraná, Rio Grande do Sul e Santa Catarina (Putzke et al. 1994, Singer 1950, como *Boletus tropicus* Rick), Singer & Digilio 1957, como *Phaeogyroporus tropicus* (Rick) Singer).

Pulveroboletus Murrill, Mycologia 1: 9 (1909).

Espécie tipo: ***Pulveroboletus ravenelii*** (Berk. & M.A. Curtis) Murril.

Basidioma pileado-estipitado, himenóforo tubular. Píleo hemisférico, convexo a aplanado, superfície pulverulenta, raramente escamulosa, seca, margem algodonosa estendendo até o estipe quando jovem, margem algodonosa apendiculada quando maduro; contexto amarelado, tornando-se azulado quando exposto. Himenóforo geralmente adnexo, poros creme a amarelados esverdeado quando maduros, tornando-se azulados rapidamente sob pressão. Estipe central, amarelo, superfície pulverulenta; micélio basal creme. Esporada verde oliva. Esporos ovoides a subfusiformes, lisos. Pleuro e queilocistídios subfusiforme-ventricosos. Pileipelis entrelaçada, cristais extracelulares presentes. Fíbulas ausentes.

Distribuição no Brasil: *Pulveroboletus rosemarieae* Singer – Amazonas (Singer et al. 1983).

Singerocomus T.W. Henkel & M.E. Smith, Mycologia 108: 167 (2016).

Espécie tipo: ***Singerocomus inundabilis*** (Singer) T.W. Henkel.

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo convexo a convexo aplanado, rosa avermelhado, superfície tomentosa; contexto amarelado, não reagindo quando exposto. Himenóforo depresso ao redor do estipe, poros amarelos, subangulares, não alterando a coloração sob pressão. Estipe central, glabro ou com escâmulas, base amarela. Esporada verde oliva. Esporos lisos, elipsoides. Pleuro e queilocistídios presentes. Pileipelis tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Singerocomus atlanticus* A.C. Magnago – Espírito Santo e Santa Catarina (Magnago et al. 2018a); *Singerocomus inundabilis* (Singer) T.W. Henkel – Amazonas (Singer et al. 1983, como *Xerocomus inundabilis* Singer); *Singerocomus rubriflavus* T.W. Henkel – Bahia (Magnago et al. 2018a).

Strobilomyces Berk., Hooker's Journal of Botany and Kew Garden Miscellany 3: 78 (1851).

Espécie tipo: ***Strobilomyces strobilaceus*** (Scop.) Berk.

Descrição: Basidioma estipitado-pileado, himenóforo tubular. Píleo hemisférico, convexo ou aplanado, superfície escamosa ou escamulosa, geralmente marrom escura ou enegrecida, seco, margem geralmente apendiculada; contexto esbranquiçado a acinzentado, rapidamente se tornando avermelhado e enegrecido quando exposto. Himenóforo adnexo ou adnato, poros esbranquiçados, acinzentados a vináceos, tornando-se avermelhado a enegrecido sob pressão. Estipe central, superfície algodonosa ou escamosa fibrilosa, ápice geralmente reticulado; véu parcial geralmente presente, micélio basal esbranquiçado a enegrecido. Esporada negra. Esporos subglobosos a elipsoides, equinulados, reticulados ou longitudinalmente estriados.

Pleuro e queilocistídios subfusiformes, ventricosos ou clavados. Pileipelis tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Strobilomyces pauper* Singer – Amazonas (Singer et al. 1983).

Suillus Gray, A natural arrangement of British plants 1: 646 (1821).

Espécie tipo: ***Suillus luteus*** (L.) Roussel (1821).

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo víscido e glabro ou seco e escamuloso; contexto esbranquiçado a amarelado, podendo reagir avermelhado quando exposto. Himenóforo adnato a adnexo, poros amarelados a marrom avermelhados. Estipe central, seco, anel pode estar presente, pontos glandulares avermelhados a enegrecidos presentes. Esporada marrom avermelhada. Esporos lisos, fusoides. Pleurocistídios geralmente formando aglomerados com pigmentação escura assim como nos caulocistídios. Pileipelis ixotricodermal a tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Suillus cothurnatus* Singer – Paraná, Santa Catarina e Rio Grande do Sul (Watling & de Meijer 1997, Giachini et al. 2000, de Meijer 2001, Sobestiansky 2005); *Suillus granulatus* (L.) Kuntze – Paraná, Santa Catarina e Rio Grande do Sul (Putzke et al. 1994, Watling & de Meijer 1997, Giachini et al. 2000, de Meijer 2001); *Suillus luteus* (L.) Roussel – Paraná e Rio Grande do Sul (Putzke et al. 1994, Watling & de Meijer 1997, Giachini et al. 2000, de Meijer 2001); *Suillus subaureus* (Peck) Snell – Rio Grande do Sul (Putzke et al. 1994). Espécies consideradas exóticas, encontradas em plantações de *Pinus. elliottii* Engelm., *Pinus patula* Schiede ex Schltdl. & Cham. e *Pinus taeda* L..

Tylopilus P. Karst., Revue Mycologique Toulouse 3 (9): 16 (1881).

Espécie tipo: ***Tylopilus felleus*** (Bull.) P. Karst.,

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo hemisférico a aplanado, superfície subtomentosa a glabra, seca a víscida; contexto esbranquiçado, maioria com sabor amargo. Himenóforo depresso ao redor do estipe, poros esbranquiçados a rosa amarronzado, angulares a arredondados. Estipe central, glabro a reticulado; micélio basal branco. Esporada rosa amarronzada. Esporos elipsoides a oblongos. Pleuro e queilocistídios presente. Pileipelis subrepente a tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Tylopilus dunensis* A.C Magnago & M.A. Neves – Rio Grande do Norte (Magnago et al. 2017); *Tylopilus aquarius* var. *aquarius* (Singer) Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo – Amazonas (Singer et al. 1983, como *Tylopilus potamogeton* var. *aquarius*); *Tylopilus aquarius* var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo – Espírito Santo, Paraíba e Rio Grande do Norte (Barbosa & Silva 2017, Magnago et al. 2017a, Magnago et al. 2018e, no prelo), *Tylopilus potamogeton* var. *potamogeton* Singer – Amazonas (Singer et al. 1983); *Tylopilus pygmaeus* A.C. Magnago & R.M. Silveira – Bahia (Magnago et al. 2017a).

Xerocomus Que´l., Fl. Vosges, Champ.: 477 (1887).

Espécie tipo: ***Xerocomus subtomentosus*** (L.: Fr.) Que´l..

Descrição: Basidioma pileado-estipitado, himenóforo tubular. Píleo hemisférico, convexo a aplanado, superfície glabra ou subtomentosa, amarronzada; contexto esbranquiçado a amarelado, reagindo azulado ou não quando exposto. Himenóforo adnato ou sinuado, poros amarelos, geralmente tornando-se azulados sob pressão, angulares, compostos. Estipe central, flexível comparando aos outros gêneros; micélio basal esbranquiçado a amarelado. Esporada verde oliva. Esporos subfusiformes, superfície lisa sob microscopia óptica e geralmente bacilar sob microscopia eletrônica de varredura. Pleuro e queilocistídios subfusiforme-ventricosos ou clavados. Pileipelis tricodermal. Fíbulas ausentes.

Distribuição no Brasil: *Xerocomus amazonicus* var. *amazonicus* Singer – Amazonas (Singer et al. 1983); *Xerocomus amazonicus* var. *obscuratus* Singer & Araujo – Amazonas e Paraíba (Singer et al. 1983, Oliveira & Sousa 2002); *Xerocomus basius* de Meijer & Watling – Paraná (Watling & de Meijer 1997, como *Xerocomus coccolobae* Pegler, de Meijer 2008); *Xerocomus brasiliensis* (Rick) Singer – Rio Grande do Sul (Singer & Digilo 1957); *Xerocomus campinaranae* Singer – Amazonas (Singer et al. 1983); *Xerocomus chapinii* Singer – Amazonas (Singer et al. 1983); *Xerocomus globuliger* Singer – Amazonas (Singer et al. 1983); *Xerocomus hypoxanthus* Singer – Pernambuco e Rio de Janeiro (Singer & Digilo 1960); *Xerocomus* *radicicola* Singer & I.J. Araújo – Amazonas (Singer et al. 1983); *Xerocomus scrobiculatus* Singer – Amazonas (Singer et al. 1983).

4.6. Manuscritos

Os resultados mais relevantes deste trabalho estão apresentados a seguir na forma de artigos científicos divididos de acordo com os gêneros. Os manuscritos estão em inglês e formatados de acordo com as normas das revistas (Manuscritos I–VII). A proposição de duas novas espécies de *Tylopilus* para o nordeste brasileiro (Manuscrito I); uma nova espécie de *Fistulinella* da Mata Atlântica e o primeiro registro de *Fistulinella campinaranae* var. *scrobiculata* para a Mata Atlântica (Manuscrito II); uma nova espécie de *Singerocomus* e o primeiro registro de *Singerocomus rubriflavus* para o Brasil em área de Mata Atlântica (Manuscrito III); uma nova espécie de *Gyroporus* da Mata Atlântica (Manuscrito IV); uma nova espécie de *Boletellus* para o nordeste brasileiro (Manuscrito V); a disponibilização de dados moleculares para as espécies de *Boletinellus* registradas no Brasil e a sinonímia de *Phlebopus brasiliensis* Singer com *Phlebopus beniensis* (Manuscrito VI). Uma lista com dados geográficos e comentários sobre os fungos boletoides da Mata Atlântica é apresentada (Manuscrito VII).

ARTIGO I

Two new *Tylopilus* species (Boletaceae) from Northeastern Atlantic Forest, Brazil

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Tylopilus dunensis A.C. Magnago & M.A. Neves (Foto: Neves, M.A.)

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Two new *Tylopilus* species (Boletaceae) from Northeastern Atlantic Forest, Brazil

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Abstract

The Atlantic Forest of Brazil harbors a great diversity of boletoid fungi, many of which remain to be documented and described. Two distinct taxa of *Tylopilus* from Northeastern Atlantic Forest, *T. dunensis* and *T. pygmaeus*, are proposed as new based on evidence from both morphological and molecular data. We provide detailed macro- and microscopic descriptions of each species including scanning electron micrographs of the basidiospores.

Keywords: Boletales, ITS, LSU, Neotropics, Taxonomy

Introduction

Tylopilus P. Karst (1881: 16) is one of the largest and most widespread ectomycorrhizal genera in the Boletaceae. It is known from Africa, Australia, Asia, Europe and the Americas. About twenty species have been described in the Neotropics, from Colombia (Halling 1989), Belize (Halling *et al.* 2008), Costa Rica (Singer *et al.* 1983, Wolfe & Bougher 1993, Amtoft *et al.* 2002, Halling *et al.* 2008, Osmundson & Halling 2010), Guyana (Henkel 1999, 2001, Fulgenzi *et al.* 2007), Honduras, Mexico, Nicaragua (Singer *et al.* 1983), and Venezuela (Singer & Digilo 1960). Four species and two varieties of *Tylopilus* are known for Brazil: *T. acutesquamosus* Singer (1983: 117), *T. arenarius* Singer (1978: 423), *T. potamogeton* Singer (1978: 433), *Tylopilus aquarius* var. *aquarius* (Singer) Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo from Amazon Forest, and *Tylopilus aquarius* var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo from Atlantic Forest (Singer 1978, Singer *et al.* 1983, Barbosa-Silva *et al.* 2017).

The traditional morphological concept of *Tylopilus* s. l. includes boletoid fungi with a dry, glabrous to subtomentose pileus, pore surface that is usually whitish when young becoming pinkish to pinkish brown at maturity, and a solid, glabrous, pruinose or reticulate stipe that lacks a partial veil or glandular dots. The spores are pink, pinkish brown to vinaceous in mass (never with olivaceous hues), smooth, and fusoid to ovoid-phaseoliform. The hymenial cystidia are usually present as pseudocystidia, and clamp connections are absent (Karsten 1881, Singer 1986, Smith & Thiers 1971).

However, several recent molecular phylogenetic analyses have shown that the taxonomy of boletoid fungi is still elusive and generic circumscriptions are in a state of flux (Binder & Hibbett 2006, Dentinger *et al.* 2010a, Nunh *et al.* 2013, Wu *et al.* 2014). *Tylopilus* s. l. is considered to be a polyphyletic genus (Dentinger *et al.* 2010a, Nuhn *et al.* 2013, Wu *et al.* 2014) and many species that were traditionally classified in *Tylopilus* have been recently accommodated into new genera such as *Zangia* Yan C. Li et Zhu L. Yang (2011:129) (Li *et al.* 2011), *Australopilus* Halling & Fechner (2012: 422) and *Harrya* Halling, Nuhn & Osmundson (2012: 422) (Halling *et al.* 2012).

Recent fieldwork in the Northeastern Atlantic Forest of Brazil resulted in the collection of several tylopiloid boletes with combinations of features not reported before. Here we provide morphological and molecular phylogenetic evidence that these specimens represent two new species best classified in *Tylopilus* s. l. Molecular data for the recently described *T. aquarius* var. *megistus* are also provided.

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Material and Methods

Collection sites and Morphology

Collections were made between 2008 and 2013 during the rainy season in the following Brazilian Northeastern Atlantic Forest localities: Reserva Biológica Guaribas, Paraíba; Parque Estadual Dunas do Natal, Rio Grande do Norte; and Parque Estadual da Serra do Conduru, Bahia.

Macroscopic features were described from fresh basidiomes. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Micromorphological features were examined with an Olympus CX21 microscope and the use of descriptive terms followed Largent *et al.* (1977). Fungal tissue was rehydrated and mounted in water, 3% KOH, Melzer's solution, or Congo Red. At least twenty micro structures of each structure type were measured for each collection examined. Qm refers to the mean length/width ratio of basidiospores. For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomes, mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a scanning electron microscope (JEOL JSM-6390LV) operating at 10KeV at *Laboratório Central de Microscopia Eletrônica* of *Universidade Federal de Santa Catarina* (LCME-UFSC). Line drawings were traced from digital photographs. Voucher specimens are deposited at FLOR and HUEFS (Thiers, continuously updated).

DNA extraction, PCR amplification and sequencing

DNA extraction from dried basidiomes follows Góes-Neto *et al.* (2005). The primer pairs ITS6-R/ITS8-F and LR0R/LR7 were used to amplify the nuclear ribosomal internal transcribed spacers (ITS; ITS1-5.8S-ITS2) and the nuclear ribosomal large subunit (LSU, 28S) regions following Dentinger *et al.* (2010b) and Vilgalys & Hester (1990), respectively. PCR products were purified using PEG (polyethylene glycol) (Sambrook *et al.* 1989). Sequencing was performed with a BigDye Terminator v3.1 Cycle Sequencing Kit following the manufacturer's procedure, using the same primers cited above. Some specimens were processed from fresh tissue blotted in FTA Plant Cards as described in Dentinger *et al.* (2010b). Sequence chromatograms were manually checked and edited in Geneious 6.1.8 (Kearse *et al.* 2012).

Sequence alignment and phylogenetic analysis

Newly generated DNA sequences were combined with 23 ITS and 55 LSU sequences belonging to *Tylopilus* s. l. downloaded from GenBank, with sequences of *Bothia castanella* (Peck) Halling, T.J. Baroni & Manfr. Binder (2007: 311) used as an outgroup (Table 1). Alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), following the Q-INS–i and G-INS-i criteria (for ITS and LSU, respectively), and then manually inspected and adjusted, as necessary, with MEGA 6 (Tamura *et al*. 2013). The indels present in both datasets were recoded as binary characters according to the 'simple indel coding method' (SIC, Simmons and Ochoterena 2000) as implemented in SeqState (Müller 2005). The resulting binary characters were joined as distinct partitions to the final matrices. The final alignments (as well the final topologies) are deposited in TreeBASE (http://www.treebase.org/treebase/index.html) under ID S21078. Maximum Likelihood (ML) and Bayesian Inference (BI) criteria were applied to the datasets, which were divided in three partitions to the ITS (ITS1+ITS2, 5.8S and indels) and two to the LSU (LSU and indels).

For ML analysis, the best fit model of nucleotide evolution to each partition was obtained according to BIC (Bayesian Information Criterion), as implemented in the software jModelTest 2.1.6 (Guindon & Gascuel 2003, Darriba *et al.* 2012). ML analysis was carried out with RAxML v. 8.2.4 (Stamatakis, 2014), available in the CIPRES science gateway (Miller *et al.* 2010, http://www.phylo.org/), using GTRGAMMA as the model of evolution (Stamatakis 2006), with branch support estimated using nonparametric bootstrapping (BS) by implementing the rapid bootstrap option in RAxML (command–f a) with a random starting tree and auto bootstopping using MRE.

BI was performed using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller *et al.* 2010), with four parallel MCMC chains run for 10 million generations, sampling every 1000 generations. Four rate categories were used to approximate the gamma distribution parameter. Of all trees sampled, 20% were discarded as burn-in and checked by the convergence criterion (average standard deviation of split frequencies <0.01) with Tracer v.1.6 (Rambaut *et al.* 2014), while the remaining were used to reconstruct a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPP) of the branches. A branch was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or BS ≥ 90%, while moderate support was considered BPP ≥ 0.90 and/or BS ≥ 70%. *Bothia castanella* was defined as outgroup, based on previous papers (Nunh *et al.* 2013, Wu *et al*. 2014).

TABLE 1. Taxa, vouchers and Genbank accession numbers used in the molecular analyses.

Species	Collection No.	Origin	GenBank accesion No.	
			ITS	**LSU**
Bothia castellanea	MB03-53	USA	DQ867110	DQ867117
Tylopilus aff. balloui	HKAS59700	China	□	KF112458
Tylopilus aff. rigens	HKAS53388	China	□	KF112405
Tylopilus aquarius* var. *megistus	**ACM297**	**Brazil**	**MF113422**	**MF113430**
Tylopilus aquarius* var. *megistus	**MAN460**	**Brazil**	**MF113423**	**MF113431**
Tylopilus alboater	TH6941	USA	□	AY612832
Tylopilus atronicotianus	Both s.n.	USA	EU685114	EU685110
Tylopilus atronicotianus	snWN	USA	□	KF030293
Tylopilus badiceps	173/97	USA	□	DQ534628
Tylopilus badiceps	MB03-52	USA	□	KF030336
Tylopilus badiceps	78206	USA	□	KF030335
Tylopilus badiceps	NCJ20	USA	□	AY612833
Tylopilus balloui	2D7	Japan	AB973758	□
Tylopilus balloui	2D6	Japan	AB973757	□
Tylopilus balloui	TH8409	Guyana	□	HQ161873
Tylopilus balloui	TH8593	Guyana	□	HQ161872
Tylopilus balloui	TWO1121	Australia	□	EU430743
Tylopilus balloui	TWO1117	Australia	□	EU430741
Tylopilus balloui	TWO1122	Australia	□	EU430742
Tylopilus balloui	TWO1198	Thaliand	□	EU430740
Tylopilus balloui	TWO1132	Australia	□	EU430739
Tylopilus balloui	TWO1105	Australia	□	EU430738
Tylopilus balloui	TWO1030	USA	□	EU430737
Tylopilus balloui	REH8526	Belize	□	EU430736
Tylopilus balloui	REH8521	Belize	□	EU430735
Tylopilus balloui	REH8292	USA	□	EU430734
Tylopilus balloui	REH9467	Australia	□	JX889676
Tylopilus balloui	TH6385	Guyana	□	AY612823
Tylopilus balloui	FMNH1073250	Mexico	□	EU430733
Tylopilus dunensis	**MAN216**	**Brazil**	**MF113418**	□
Tylopilus dunensis	**MAN218**	**Brazil**	**MF113419**	**MF113428**
Tylopilus dunensis	**MAN281**	**Brazil**	**MF113420**	□
Tylopilus exiguus	TH9549	Guyana	KT339205	KT339205
Tylopilus exiguus	TH8929	Guyana	JN168776	□
Tylopilus felleus	JMP0093	USA	EU819449	□
Tylopilus felleus	17516	Italy	JF908787	□
Tylopilus felleus	HA33	Latvia	KR019864	□
Tylopilus felleus	KHL8542	Sweden	□	AY586723
Tylopilus felleus	HKAS54926	Germany	□	KF112411
Tylopilus felleus	Tf1	Germany	□	AF139710
Tylopilus felleus	HKAS55832	China	□	HQ326934
Tylopilus ferrugineus	210-97	USA	□	AF139711
Tylopilus indecisus	98/98	USA	□	AF456820
Tylopilus intermedius	BD277	USA	□	HQ161875
Tylopilus leucomycelinus	18463	Guatemala	JF908789	□
Tylopilus microsporus	HMAS:84730	China	KM975485	KM975494
Tylopilus microsporus	HKAS59661	China	□	KF112450
Tylopilus neofelleus	YT20090720	Japan	KM975489	KM975497
Tylopilus neofelleus	MG475a	China	KM975486	□
Tylopilus neofelleus	YT20121007	Japan	□	KM975496
Tylopilus neofelleus	YT20120811	Japan	□	KM975495
Tylopilus neofelleus	HKAS50319	China	□	HQ326936
Tylopilus oradivensis	REH8187	Costa Rica	□	EU430732
Tylopilus oradivensis	REH8087	Costa Rica	□	EU430731
Tylopilus orsonianus	TH8926	Guyana	JN168777	□
Tylopilus otsuensis	HKAS53401	China	□	KF112449

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TABLE 1. (Continued)

Species	**Collection No.**	**Origin**	**GenBank accesion No.**	
			ITS	**LSU**
Tylopilus pakaraimensis	TH8965	Guyana	JN168778	□
Tylopilus pernanus	8061	Indonesia	□	JX889644
Tylopilus pernanus	8066	Indonesia	□	JX889645
Tylopilus plumbeoviolaceoides	GDGM:32630	China	□	□
Tylopilus plumbeoviolaceoides	CHU25	China	DQ407261	□
Tylopilus plumbeoviolaceoides	GDGM:42624	China	□	KM975498
Tylopilus plumbeoviolaceoides	HKAS50210	China	□	HQ326937
Tylopilus porphyrosporus	GO-2009-237	Mexico	KC152268	□
Tylopilus porphyrosporus	17898	Italy	JF908788	□
Tylopilus porphyrosporus	HKAS76671	China	□	KF112482
Tylopilus potamogeton var. irengensis	TH8801	Guyana	JN168779	JN168779
Tylopilus pygmaeus	**ACM486**	**Brazil**	**MF113421**	**MF113429**
Tylopilus rhoadsiae	RV98-261	USA	□	AY612836
Tylopilus rubrobrunneus	1504-Q-6072	Canada	KM248939	□
Tylopilus rubrobrunneus	BD329	USA	□	HQ161876
Tylopilus rubrobrunneus	152/98	USA	□	DQ534629
Tylopilus rufonigricans	TH8925	Guyana	KC155380	□
Tylopilus rufonigricans	TH6376	Guyana	□	AY612835
Tylopilus sp	**MAN217**	**Brazil**	**MF113424**	**MF113432**
Tylopilus sp	**MAN282**	**Brazil**	**MF113425**	□
Tylopilus sp	**MAN288**	**Brazil**	**MF113426**	□
Tylopilus sp	**MAN215**	**Brazil**	**MF113427**	□
Tylopilus sp	TH9198	Guyana	KT339204	KT339204
Tylopilus tabacinus	HN2295	USA	□	AY612837
Tylopilus variobrunneus	snHor02	USA	□	KF030316
Tylopilus variobrunneus	9306tv	USA	□	KF030315
Tylopilus vinaceipallidus	TH8859	Guyana	JN168780	□
Tylopilus violatinctus	HKAS50208	China	□	KF112472
Tylopilus violatinctus	HKAS50279	China	□	HQ326935

Results

Molecular analysis

Fifteen new sequences of *Tylopilus* s. l. (10 ITS, 5 LSU) were generated during this study. The ITS dataset resulted in an aligned matrix of 862 bp (including gaps). For this dataset, the best fit models of nucleotide substitution estimated for each partition were TPM1uf+I+G (ITS1, ITS2) and TrNef+G (5.8S). The LSU dataset resulted in an aligned matrix of 933 bp (including gaps). The TrN+I+G model was chosen as the best fit model of nucleotide substitution implemented in MrBayes. For the ITS dataset, automatic bootstopping terminated after 300 pseudoreplicates. Both ML and Bayesian analyses resulted in very similar topologies, either for the ITS and LSU. The respective ML trees showing BS and BPP values on branches are shown in Figs. 1 and 2.

In the ITS analysis, the sequence of *Tylopilus pygmaeus* sp. nov. is closely related to an unidentified *Tylopilus* (TH9198) from Guyana (BS=80, BPP=0.94), and three sequences of *Tylopilus dunensis* sp. nov. clustered in a well-supported clade (BS=100, BPP=1), close to an unidentified (and possibly yet undescribed) *Tylopilus* species from Brazil (MAN215) (Fig. 1). In the LSU analysis, *T. pygmaeus* is closely related (BS=90, BPP=1) to an undescribed *Tylopilus* species (MAN217), together forming a clade sister to *T. dunensis* (Fig. 2).

Two sequences representing *Tylopilus aquarius* var. *megistus* clustered in the ITS analysis in a well-supported clade (BS=100, BPP=1), sister to a sequence identified as *T. potamogeton* var. *irengensis* T.W. Henkel (1999: 656) (TH8801) from Guyana. This clade (BS=94, BPP=1), represents sect. Potamogetones sensu Singer, including species with pileus and stipe more or less tomentose, not reticulate, short basidiospores, a bluing NH_4OH reaction, and bitter taste (Singer *et al.* 1983). In the LSU analyses, the two sequences of *T. aquarius* var. *megistus* clustered in a well-supported clade (BS=100, BPP=1), embedded within a poorly supported clade composed of several species of *Tylopilus* from the Paleotropics and Neotropics (Fig. 2).

The molecular phylogenies presented in Figs 1-2 provide strong evidence that, alongwith our morphological studies, indicate *T. dunensis* and *T. pygmaeus* are distinct taxa that should be recognized at the species level.

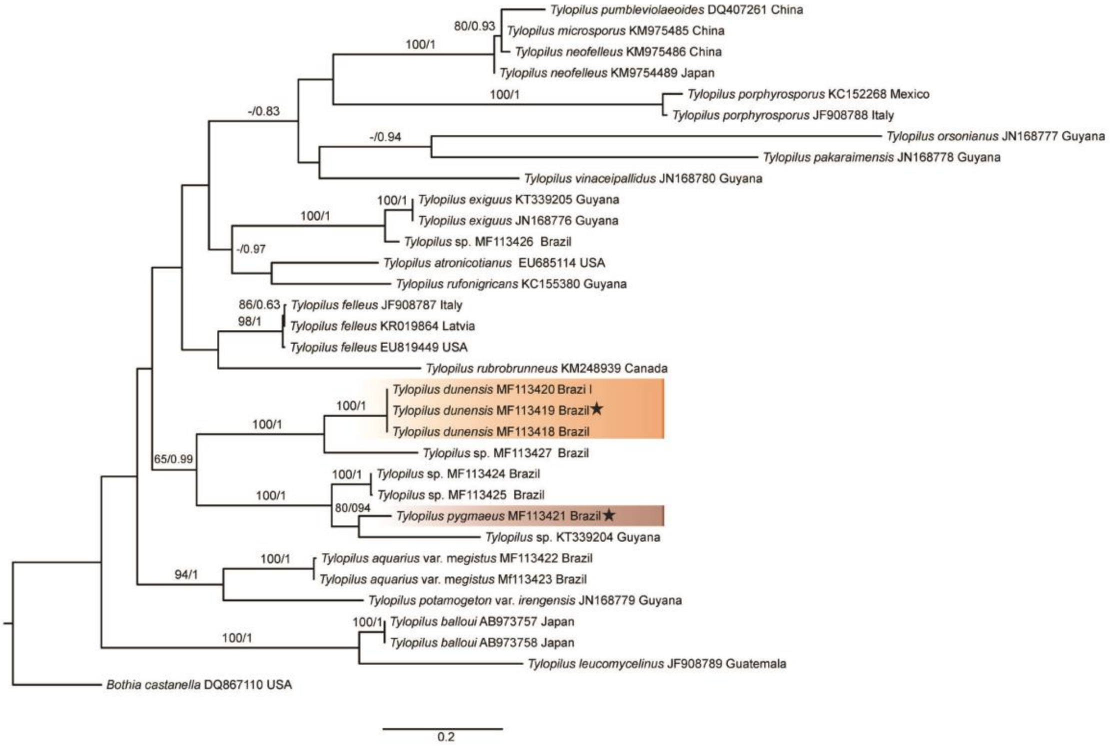

FIGURE 1. Maximum likelihood (ML) tree of *Tylopilus* from dataset of 33 ITS sequences rooted with the outgroup (*Bothia castanella*). Bayesian posterior probability above 0.7 and nonparametric bootstrap values above 50% are shown.

FIGURE 2. Maximum likelihood (ML) tree of *Tylopilus* from dataset of 63 LSU sequences rooted with the outgroup (*Bothia castanella*). Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown.

Taxonomy

Tylopilus dunensis A.C. Magnago & M.A. Neves, *sp. nov.* Fig. 3.

Mycobank: MB 819523

Type:—BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas do Natal, Trilha da Geografia, growing on white sand soil on dunes, 24 April 2008, *Neves MA 218* (holotype: HUEFS138281!; isotype: FLOR51718!) GenBank accession: ITS = MF113419, 28S = MF113428.

Etymology:—from the Latin *dunensis* = referring to the habitat (white sand dune area) where the new species was collected.

Pileus 25−115 mm broad, at first convex, with age becoming plano-convex to plano-depressed, yellow orange (OAC 789), to bright orange (OAC 644) with some red tones (OAC 670), finely velutinous under lens, dry, margin slightly inrolled and entire; context 5−14 mm centrally, whitish to yellowish (OAC 815), unchanging when exposed. *Tubes* 4−13 mm long, whitish to cream (OAC 816), slightly decurrent; pores 1−2 mm broad, whitish, staining light orange brown upon pressure. *Stipe* 30−65 × 8−28 mm, central to eccentrical, subequal, whitish to cream yellow (OAC 814); smooth, context cream (OAC 815), unchanging; extreme base with white mycelium. *Macrochemical reactions*: NH_4OH unchanging on pileus and stipe surfaces.. *Spore print* pinkish.

Basidiospores 6−9 × 3−4 µm (Qm=1.91), ellipsoid to elongate in frontal view, the inner side applanate to bean shaped (phaseoliform) in side-view, in mass light yellow, inamyloid to weakly dextrinoid, smooth, thin walled; hilar appendage 0.5−1 µm long. *Basidia* 27−40 × 6−10 µm, clavate, thin walled, hyaline, many with granular contents; 4-sterigmate, 4−6 µm long. *Cystidia* abundant both on pores (cheilocystidia) and tubes (pleurocystidia), similar in size and shape, 32−82 x 6−13 µm, fusoid, ventricose, some clavate, hyaline, but also many with golden contents, dextrinoid. *Hymenophoral trama* boletoid, mediostratum of subparallel to interwoven hyphae, 3−5 µm wide, lateral stratum hyphae 5−8 µm wide, divergent, inamyloid. *Pileipellis* a trichodermium, interwoven in a gelatinized matrix, hyphae 2−5 µm wide, light brown with golden brown contents, dextrinoid, hyphae regularly septate. *Pileus trama* interwoven to subparallel, partly gelatinized, light yellow, with dextrinoid contents. *Stipitipellis* in two layers, external layer hymeniform, with terminal cells 5−13 µm wide, hyaline, some with golden contents; presence of caulobasidia and caulocystidia in variables shapes, including clavate, cylindrical, fusoid, ventricose, and capitate; lower layer with narrow hyphae with golden brown contents as observed in H_2O, 2−4 µm wide, interwoven vertically arranged in a gelatinized matrix. *Stipe trama* parallel to subparallel, hyphae 4−15 µm broad, hyaline, inamyloid, smooth and thin walled. *Clamp connection* absent.

Habit and habitat:—Solitary to scattered, sometimes caespitose, growing on white sand dunes in *restinga* vegetation in the far north of costal Atlantic Forest.

Specimens examined (paratypes):—BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas do Natal, 5°51'S, 35°11'W, 24 April 2008, 216 (HUEFS138279!, FLOR51716!) GenBank accession: ITS = MF113418; 29 April 2008, *Neves MA* 255 (HUEFS138318!), 256 (HUEFS138319!), 258 (HUEFS138321!), 24 May 2008, *Neves MA* 281 (HUEFS138368!) GenBank accession: ITS = MF113420.

Additional specimens examined:—AUSTRALIA, Queensland, *Tylopilus balloui* Fraser Island, road from Central Station to Eurong. 25°29'6"S, 153°5'18"E, 11 February 2009, *Halling RE* 9053 (NY!). BELIZE, Cayo District, *Tylopilus balloui* Mountain Pine Ridge: Douglas Da Silva, British Military Swamp. 16°58'9"N, 88°59'38"E, 6 October 2003, *Halling RE* 8526 (NY!). COSTA RICA, Cartago, *Tylopilus oradivensis*, Guarco, Palo Verde. +/_ 4.5 km E of km 31 of Interamerican Highway. 9°46'34"N, 83°56'42"W, 1 June 2001, *Halling RE* 8087 (NY!).

Comments:—*Tylopilus dunensis* is morphologically similar to the North American *Tylopilus balloui* Peck (1912: 157). Both have a yellow-orange to orange-red pileus, white to cream hymenophore, cream to pale yellow stipe and do not change color when in contact with ammonium; basidiospores are shorter than 10.5 µm long and the pileipellis is trichodermium. *Tylopillus balloui*, however, does not have a gelatinized pileipellis, the trama has gloeopleurous hyphae, the pseudocystidia are fusoid ventricose to broadly-ventricose to ventricose-mammilate and abundant, the cheilocystidia are spheropedunculate to broadly spheropedunculate, and the caulocystidia are clavate to fusoid ventricose with staining contents restricted to the cytoplasm and interrupted by hyaline vacuoles, as observed by Wolfe (1981) in the type studies of *Tylopilus* described by Charles H. Peck.

Osmundson & Halling (2010) described *Tylopilus oradivensis* Osmundson & Halling (2010: 476) from Costa Rica as part of the *balloui* complex based on morphological and molecular data. This species differs from *T. dunensis* by having a reddish orange to red pileus and stipe and larger (8.2−12 × 3−4 µm) subfusiform to fusiform basidiospores.

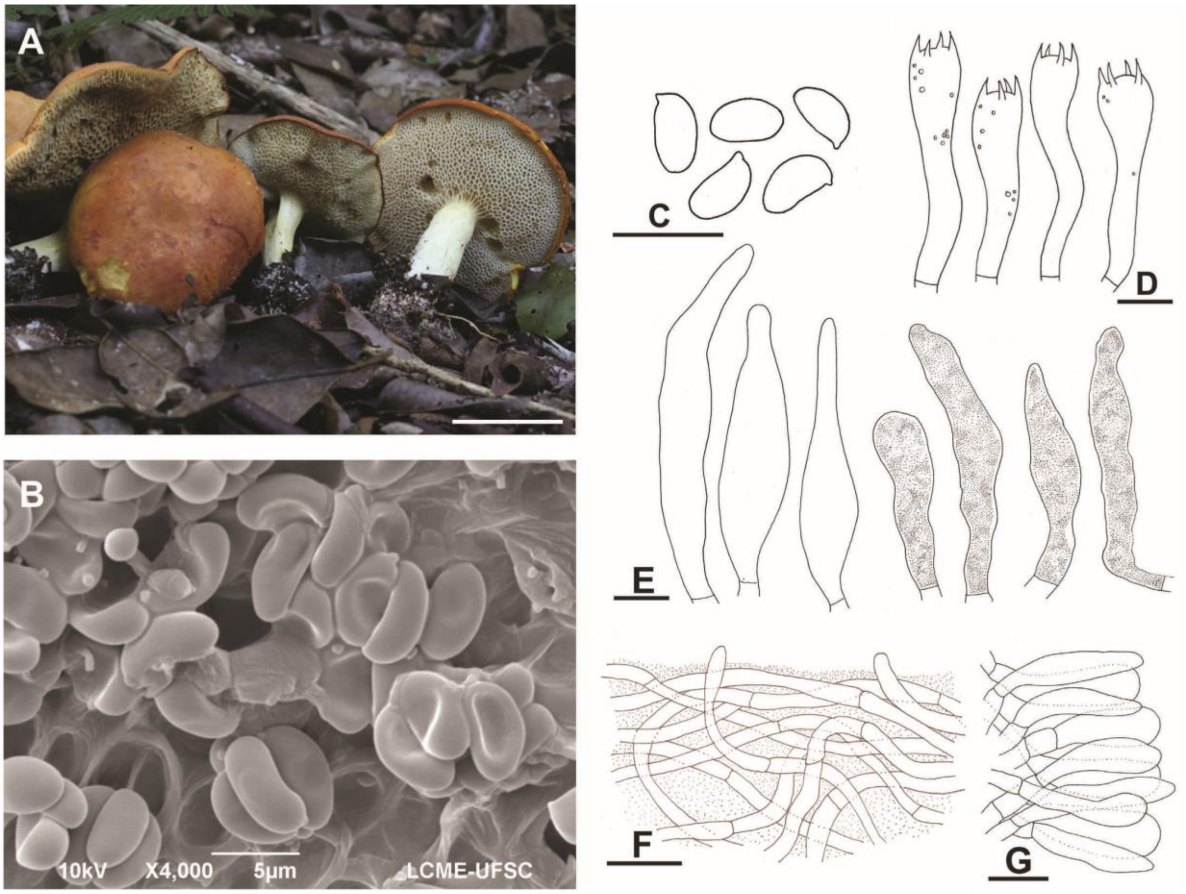

FIGURE 3. *Tylopilus dunensis*. A—Photographs of fresh basidiomes in the field. B-C—Basidiospores; D—Basidia; E—Cystidia; F—Pileipellis; G—Stipitipellis. Bar 10 µm.

Tylopilus pygmaeus A.C. Magnago & R.M. Silveira *sp. nov.* Fig. 4.

Mycobank: MB 819524

Type:—BRAZIL, Bahia, Itacaré, Parque Estadual da Serra do Conduru, 30 November 2012, *Col. Rezende DHC & Montoya CAS*, *Magnago AC* 486 (holotype: FLOR51612!) GenBank accession: ITS = MF113421, 28S = MF113429.

Etymology:—from the Latin *pygmaeus* = small, short; referring to the small size of the basidiomes.

Pileus 11−26 mm broad, at first parabolic, becoming plano-convex with age, brown (OAC 638) to tannish brown (OAC 721), velutinous, dry, becoming dark brown when bruised, margin slightly inrolled and entire when young; context 3−7 mm, cream (OAC 683). *Tubes* 3−6 mm long, adnate, slightly depressed around stipe, whitish then pale pinkish; pores 2−4 per mm, angular, staining light brown under pressure. *Stipe* 22−35 × 4−8 mm, central, sub-equal, glabrous to velutinous, cream to light pinkish brown; context cream (OAC 683); extreme base with white mycelium. *Basidiospores* 7−9 × 4−5 µm (Qm=1.69), ellipsoid in frontal view, the inner side applanate to phaseoliform in side-view, hyaline, inamyloid, smooth, thin walled, hilar appendage 0.5−1 µm long. *Basidia* 25−35 × 8−10 µm, clavate, thin walled, hyaline, inamyloid; 4-sterigmate, 3−4 µm long. *Cystidia* abundant on pores (cheilocystidia) and tubes (pleurocystidia), not differentiated from each other, projecting slightly or not, 24−39 × 8−11 µm, ventricose-rostrate to lageniform, the majority with golden brown contents, strongly dextrinoid but some hyaline and without contents. *Hymenophoral trama* boletoid in a gelatinized matrix, mediostratum of many narrow parallel to interwoven hyphae, 3−5 µm wide, these yellow to light yellow, lateral stratum hyphae 3−11 µm wide, hyaline, strongly divergent. *Pileipellis* a trichodermium consisting of erect to sub-erect terminal hyphae, cylindrical to fusoid, 28−73 × 8−10 µm, differentiated like pileocystidia, with golden brown contents and dextrinoid. *Pileus trama* interwoven; hyphae 4−6 µm wide, light yellow, some with granular dextrinoid contents. *Stipitipellis* hymeniform, terminal hyphae 23−42 × 7−11 µm, clavate to ventricose, with golden brown contents, strongly dextrinoid; caulobasidia present. *Stipe trama* subparallel to interwoven hyphae, vertically arranged, hyphae 3−10 µm wide, light yellow. *Clamp connections* absent. *Macrochemical reactions*: not observed. *Spore print* pinkish.

Habit and habitat:—Gregarious on sandy soil under broadleaf trees in Northeastern Atlantic Forest.

Additional specimens examined:—BRAZIL, Amazonas, *Tylopilus arenarius* Sing. Estrada Manaus-Caracaraí, km 45, 3 February 1978, *Singer B10590* (INPA-*type*!); *Tylopilus potamogeton* Sing. Rio Negro, 20 km ca. de São Gabriel da Cachoeira, 20 January 1978, *Araujo, I.* 938 (INPA!); *Tylopilus aquarius* var. *aquarius*, Igarapé do Tarumãzinho, 14 December 1978, *Singer B* 11433 (INPA-*type*!).

Comments:—*Tylopilus potamogeton* is morphologically similar to *T. pygmaeus* by having small basidiomes, a velutinous brownish pileus, and a whitish to pinkish hymenophore. *Tylopilus potamogeton* differs mainly by its cinnamon to fuscous umber stipe that is densely fibrillose and tomentose at the base, and the hymenophore does not turn brown when bruised. Microscopically the arrangement and appearance of cystidia, pileipellis and stipitipellis are similar, however, *T. potamogeton* has longer basidiospores (9−12 × 6−8 µm), cystidia that are hyaline, fusoid and mucronate. *Tylopilus aquarius* var. *aquarius* can be differed by the dimorphic basidiospores (8−11.5 × 5−7.5 and 11−16 × 5−6 µm), and versiforme cystidia. *Tylopilus arenarius* differs by the whitish to slightly lilac pileus, reticulation on the upper third of the stipe, larger basidiospores (7−9 × 4−5 µm) and cystidia that are fusoid to ampullaceous (Singer *et al.* 1983, Barbosa-Silva *et al.* 2017).

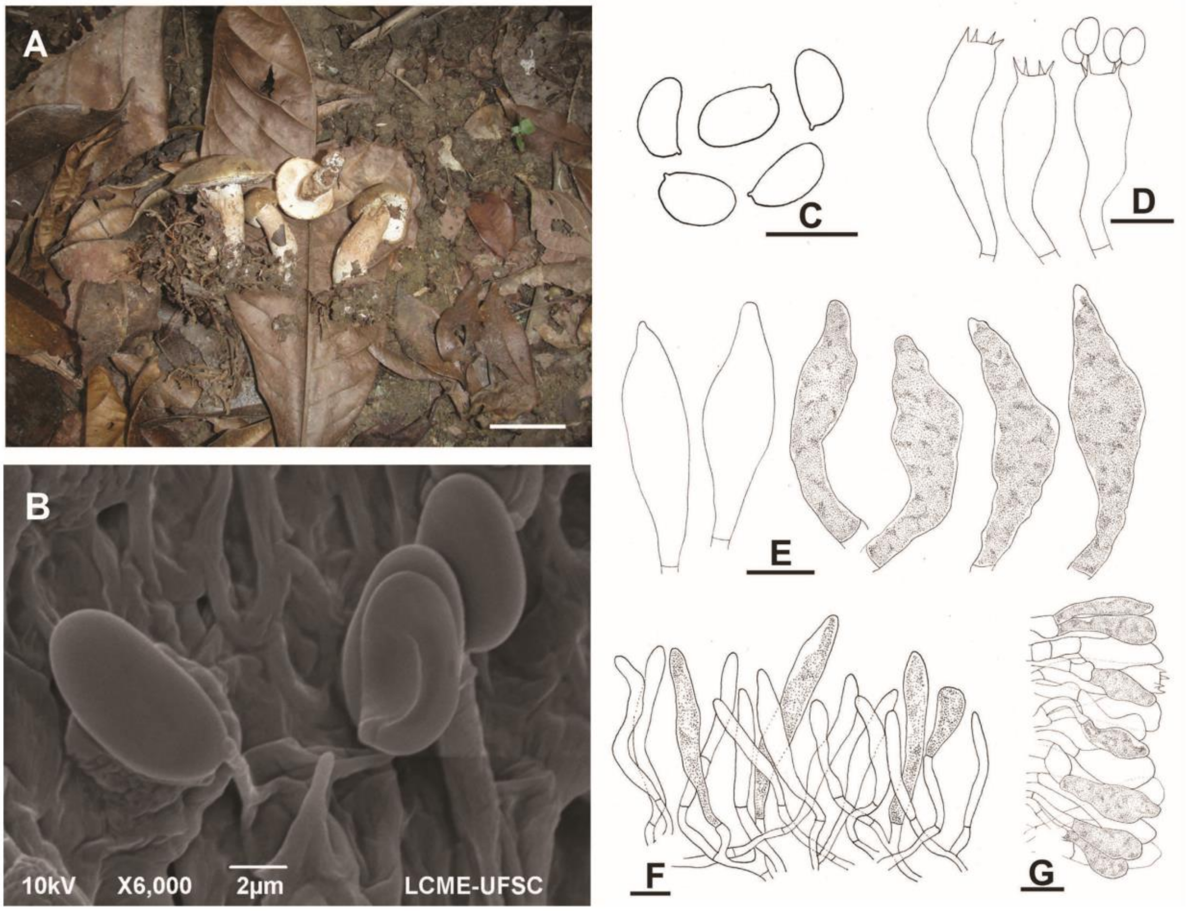

FIGURE 4. *Tylopilus pygmaeus.* A—Photographs of fresh basidiomes in the field. B-C—Basidiospores; D—Basidia; E—Cystidia; F—Pileipellis; G—Stipitipellis. Bar 10 µm.

Discussion

The Northeastern Atlantic Forest of Brazil is a habitat well-known for its unique flora but its fungal diversity has been thus far largely neglected. Our discoveries illustrate that these forests harbor a rich diversity of macrofungi that have never been documented. White sand dune environments, in particular, may be a reservoir for a specialized community of ectomycorrhizal species that have evolved in these distinct soils, as suggested by Singer *et al.* (1983), Sulzbacher *et al.* (2013) and Roy *et al.* (2016). For example, many other putatively ectomycorrhizal macrofungi have been recorded by us at Parque Estadual das Dunas, including other species belonging to the well-known ectomycorrhizal families Boletaceae, Amanitaceae, Cantharellaceae, Russulaceae and Sclerodermataceae. The woody vegetation in the park is mainly composed of species belonging to the Fabaceae, Myrtacaceae and Sapotaceae, which are candidates for ectotrophic mutualists (Sulzbacher *et al.* 2013). Further survey work is needed to better document both the ectotrophic status of the native woody vegetation and further document the extensive diversity of ectomycorrhizal fungi that remain unknown.

Tylopilus s. str. was recovered as monophyletic by Nuhn *et al.* (2013) and Wu *et al.* (2014) including the type species, *T. felleus*, using three molecular markers (LSU, tef1, and rpb1). Although the phylogenetic analyses shown here (Figs 1-2) are based on two markers, which are not sufficient for resolving persistent infrageneric ambiguities in the Boletaceae, they do allow us to confirm the distinctiveness of two new taxa among related *Tylopilus* s.l. using a molecular phylogenetic approach.

Halling *et al.* (2008) indicated that the current circumscription of the type species of *Tylopilus balloui* represents a species complex rather than a single species. Our phylogenetic reconstruction based on LSU supports this view since specimens identified as *T. balloui* cluster in two distinct, unrelated clades. The first clade includes specimens from Australia, China, Thailand, Mexico, and Belize. The second includes specimens from the USA (type locality), Australia, and Guyana. In the second clade are included specimens initially identified as *T. balloui*, suggesting that other specimens originating outside of the USA that are currently assigned to *T. balloui* should also be recognized as distinct taxa at the species rank.

These results suggest that there is a hidden diversity of species under the name *T. balloui* and reinforce the need for a comprehensive taxonomic review of the complex. Other unique sequences included in the molecular phylogenetic datasets presented here are from *Tylopilus* specimens collected in Northeastern Brazil (MAN215, MAN217, MAN282, MAN288), that probably also represent new species. However, for the time being, we do not possess enough material of good quality to definitively assess them. This highlights the fact that the Atlantic Forest of Brazil harbors many species of fungi yet to be described.

Acknowledgments

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ARTIGO II

Fistulinella ruschii sp. nov., and a new record of *Fistulinella campinaranae* var. *scrobiculata* for the Atlantic Forest, Brazil

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Fistulinella ruschii A.C. Magnago (Foto: Magnago, A.C.)

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Fistulinella ruschii, sp. nov., and a new record of *Fistulinella campinaranae* var. *scrobiculata* for the Atlantic Forest, Brazil

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ABSTRACT

Fistulinella is a small genus of boletoid fungi in the subfamily Austroboletoideae in the order Boletales. In this paper, *F. ruschii* from the Atlantic Forest is proposed as new to science and *F. campinaranae* var. *scrobiculata*, known from the Brazilian Amazon forest, is recorded for the first time in the Atlantic Forest. Macro- and microscopic descriptions, molecular data (nuc rDNA ITS1-5.8S-ITS2 and nuc 28S rDNA), photographs of the basidiomata, and scanning electron microscopy images of basidiospores are provided for both species. Based on sampling of six taxa, New World *Fistulinella* is found to be a strongly supported monophyletic group, but the genus at large is nonmonophyletic.

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INTRODUCTION

Fistulinella Henn. comprises 25 species, mainly with a pantropical distribution, including Australia, Cameroon, Colombia, Costa Rica, Jamaica, Guyana, Malaysia, Martinique, Mexico, New Zealand, and Venezuela (Guzmán 1974; Singer 1978, 1986; Wolfe 1979, 1982; Pegler and Young 1981; Pegler 1983; Singer et al. 1983, 1991; Watling and Gregory 1989; Ortiz-Santana et al. 2007; Kirk et al. 2008; Watling 2008; Fulgenzi et al. 2010; Vasco-Palacios et al. 2014). *Fistulinella staudtii* Henn. from Cameroon is the type species (Hennings 1901).

Fistulinella is included in Austroboletoideae G. Wu & Zhu L. Yang (Boletaceae, Boletales) together with *Veloporphyrellus* L.D. Gómez & Singer, *Mucilopilus* Wolfe, and *Austroboletus* (Corner) Wolfe (Wu et al. 2014). *Fistulinella* is characterized by a whitish tubular hymenophore that becomes pink at maturity, a pinkish to pink-brown spore print, and microscopically by the smooth, elongate-fusoid, weakly to distinctly dextrinoid basidiospores and presence of gelatinized tissues in the pileipellis and hymenophoral trama (Singer 1986).

Most of the boletoid species known from Brazil are from Amazonian forest (Singer and Digilo 1957, 1960; Singer et al. 1983; Singer and Araujo 1986; Neves and Capelari 2007). However, a high diversity of boletes has also been observed in the coastal Atlantic Forest (Rick 1960; Oliveira and Sousa 1995, 1996, 2002; Watling and de Meijer 1997; Magnago and Neves 2014; Barbosa-Silva et al. 2017; Magnago et al. 2017, 2018). The Atlantic Forest is considered an important global biodiversity hot spot, includes many endemic species, and environmentally is highly variable along the coast and inland areas of Brazil, including coastal forests, araucaria mixed forests, deciduous and semideciduous forests, mangrove swamps, marshlands, and oceanic islands. Originally, it covered 1 300 000 km^2 (~15% of Brazil). However, after decades of deforestation due to harvest of valuable lumber, agroindustry, and urbanization (Dean 1932 1994), less than 8% of the original area remains preserved (SOS Mata Atlântica 2013).

The two varieties of *F. campinaranae* Singer are the only taxa in the genus known from Brazil (Singer et al. 1983). In this study, *F. campinaranae* var. *scrobiculata* Singer is recorded for the first time from the Atlantic Forest and *Fistulinella ruschii* is described as new.

MATERIALS AND METHODS

Field work. Field expeditions were carried out in the following areas: Reserva Biológica Augusto Ruschi, municipality of Santa Teresa, Espírito Santo (19°54′19.60″S, 40°34′8.20″W); the Universidade Federal da Paraíba, João Pessoa, Paraíba (7°08′18″S, 34°50′38″W); Parque Estadual da Serra do Conduru, municipality of

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Serra Grande, Bahia (14°26′49.5″S, 39°05′37.1″W); and the Unidade de Conservação Ambiental Desterro, Florianópolis, Santa Catarina (27°31′51.6″S, 48°30′44.1″ W). The vegetation type in these areas is Atlantic Forest (dense ombrophilous forest). The climate is tropical humid with high annual rainfall (2800 mm). The mean annual temperature varies between 20 and 31 C.

Morphology. Macro- and microscopic analyses followed established methods used for basidiomycetes (Largent et al. 1977; Largent 1986). For basidiospore quotient (Q), Qr indicates the range of Q values; Qm indicates the average of Qr; n, the number of basidiospores measured; and s, the number of specimens examined. Color codes (e.g., OAC 663) were based on the Online Auction Color Chart (Kramer 2004). Basidiomata were dried with a food dehydrator (Total Chef TCFD-05) at about 40 C.

For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomata, mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a scanning electron microscope (JEOL JSM-6390LV; xx, xx), operating at 10KeV, at the Laboratório Central de Microscopia Eletrônica of Universidade Federal de Santa Catarina (LCME-UFSC). Line drawings were traced from digital photographs. Voucher material was deposited at FLOR and ICN. Duplicates are at VIES (Thiers, continuously updated).

Sequencing and phylogenetic analyses. DNA extraction from dried basidiomata followed Góes-Neto et al. (2005). The primer pairs ITS6-R/ITS8-F and LR0R/LR7 were used to amplify the nuc rDNA ITS1-5.8S-ITS2 (internal transcribed spacer [ITS]) and nuc 28S rDNA (28S) regions following Dentinger et al. (2010). Sequencing was performed by Macrogen Korea (xx, xx) using the polymerase chain reaction (PCR) primers. Sequence chromatograms were manually checked, and contigs were assembled and edited in Geneious 6.1.8 (Kearse et al. 2012). Alignments were generated using MAFFT 7 (Katoh and Standley 2013), using Q-INS-i and G-INS-i algorithms for ITS and 28S, respectively. The aligned sequences were cleaned using Gblocks, allowing smaller final blocks and gap positions within the final blocks (Talavera and Castresana 2007). The resulting alignments were deposited in the TreeBASE (http://www.treebase.org/treebase/index.html) under accession number 21027.

Phylogenetic trees were reconstructed under two criteria, maximum likelihood (ML) and Bayesian inference (BI), from the concatenated ITS+28S alignment. The data set was subdivided into four partitions: ITS1, 5.8S, ITS2, 28S. ML was carried out with RAxML-HPC 8 (Stamatakis 2014) in the CIPRES Science Gateway (Miller et al. 2010; http://www.phylo.org/) using GTRGAMMA as the model of evolution (Stamatakis 2006) and 1000 bootstrap replications. BI was conducted in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) also on the CIPRES Science Gateway using four parallel Markov chain Monte Carlo (MCMC) chains, which were allowed to run for 20 million generations, sampling trees and parameters every 1000 generations. Best-fit substitution models were set to each partition calculated by MrModeltest 2.3 (Nylander 2004). The Akaike information criterion (AIC) was chosen to select the most appropriate model of DNA substitution for each data set or data partition used in the analyses. The convergence diagnostic was calculated every 100 generations, and its critical value was set in order to automatically stop the analysis when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes stopval = 0.01). In all analyses, the first 25% of trees from each run were discarded as burn-in. Resulting trees from the two independent runs were then pooled to produce one 50% majority-rule consensus tree, and Bayesian posterior probabilities (BPPs) were generated for the resulting tree. A node was considered well supported if it showed a BPP ≥0.96 and/or bootstrap (BS) ≥80%. The ingroup included sequences of *Fistulinella, Austroboletus, Veloporphyrellus*, and *Mucilopilus. Bothia* and *Solioccasus* were used as outgroups since they are the sister group to Austroboletoideae (Wu et al. 2014). All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

RESULTS

Molecular analysis. Eleven new sequences of *Fistulinella* (seven ITS and four 28S) were generated during this study. ITS BLASTn queries of the Brazilian specimens of *Fistulinella* indicate the highest matches with species of *Fistulinella* and a sequence labeled *Boletellus*, but none of the searches resulted in more than 95% similarity. In the 28S BLASTn queries, the most similar taxa were sequences labeled *Fistulinella, Tylopilus*, and *Xerocomus*, but none resulted in more than 94% similarity. In GenBank, only four specimens named *Fistulinella* were represented by ITS sequences and five by 28S sequences.

Additional sequences from Brazilian *Austroboletus* species were generated for the analysis in order to provide additional molecular data regarding boletoide fungi from the Neotropics. These included three ITS and five 28S sequences of *A. festivus* from the Atlantic Forest studied morphologically by Magnago and Neves (2014) and one ITS sequence from the holotype (INPA78693) of *A. rionegrensis* from Amazonia (Singer et al. 1983). All sequences analyzed in this study are included in TABLE 1.

Phylogenetic analyses. Sixty-seven sequences were combined into a single matrix (33 ITS, 34 28S) from 47 specimens representing 28 taxa, both newly generated in this study or downloaded from GenBank. The final concatenated ITS+28S data set resulted in a matrix of 1341 sites. The best models of nucleotide substitution estimated for each partition in the data sets were the following: SYM+G for ITS1, K80+I for 5.8S, TPM1uf+G for ITS2, and TIM1+1+G for 28S. The ML tree, with BS and BPP values on branches, is shown in FIG. 1. Both phylogenetic analyses showed that specimens of *Fistulinella* sampled from the New World grouped into one strongly supported clade (BS = 100%, BPP = 1.0). The analysis showed a robust position for *F. ruschii* (BS = 100%, BPP = 1) in the New World *Fistulinella* group and as the sister group to a well-supported clade including other New World *Fistulinella* sequences used in the analyses. In addition, the Brazilian specimen of *F. campinaranae* var.

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species	Collection no.	Origin	GenBank acession No.	
			ITS	LSU
Austroboletus amazonicus	AMV2032*	Colombia	KF937309	KF714510
Austroboletus amazonicus	AMV1914	Colombia	KF937308	KF714509
Austroboletus amazonicus	AMV1839	Colombia	KF937307	KF714508
Austroboletus festivus	TH8732	Guyana	KT339224	
Austroboletus festivus	ACM575	Brazil	KY886203	KY888000
Austroboletus festivus	ACM573	Brazil	KY886202	KY888001
Austroboletus festivus	CHC245	Brazil		KY887998
Austroboletus festivus	ACM564	Brazil		KY887999
Austroboletus festivus	ACM574	Brazil		KY888002
Austroboletus fusisporus	HKAS75207	China	JX889719	JX889720
Austroboletus gracilis	N. Arnold 112/96	USA		DQ534624
Austroboletus lacunosus	PDD 83019	New Zealand	KP191804	
Austroboletus lacunosus	MEL2265009	Australia	KC552015	KC552057
Austroboletus niveus	PDD 81219	New Zealand	KP191802	
Austroboletus niveus	MEL2053830	Australia	KC552016	KC552058
Austroboletus niveus	PDD 105246	New Zealand	KP191801	KP191673
Austroboletus novaezelandiae	PDD 105213	New Zealand	KP191800	KP191672
Austroboletus novaezelandiae	PDD 105097	New Zealand	KP191803	KP191671
Austroboletus occidentalis	MEL2300518	Australia	KC552017	KC552059
Austroboletus rionegrensis	I. Araujo 937*	Brazil	KY886201	
Austroboletus rostrupii	TH8189	Guyana	JN168683	
Austroboletus sp.	KD12-250	India	KM597479	
Bothia castanella	MB03-053*	USA	DQ867110	DQ867117
Bothia fujianensis	HKAS82694	China	KM269195	KM269193
Fistulinella campinaranae var. *scrobiculata*	ACM484	Brazil	KY886204	KY888003
Fistulinella campinaranae var. *scrobiculata*	AMV1980	Guyana		KF714520
Fistulinella campinaranae var. *scrobiculata*	AMV1783	Guyana		KJ195892
Fistulinella cinereoalba	TH8471*	Guyana	KT339237	GQ477439
Fistulinella gloeocarpa	KM162946	Guyana	GQ981503	
Fistulinella prunicolor	REH9502	Australia		JX889648
Fistulinella ruschii	ACM526*	Brazil	KY886206	KY888006
Fistulinella ruschii	ACM491	Brazil	KY886207	
Fistulinella ruschii	MAN391	Brazil	KY886210	
Fistulinella ruschii	ACM485	Brazil	KY886205	KY888004
Fistulinella ruschii	ACM1317	Brazil	KY886208	KY888005
Fistulinella ruschii	CATO105	Brazil	KY886209	
Fistulinella sp.	AMV511	Colombia	KF878352	
Fistulinella sp.	MEL2382828	Australia	KP012703	
Fistulinella viscida	238	New Zealand		AF456826
Mucilopilus castaneiceps	HKAS75045	China		KF112382
Mucilopilus castaneiceps	Tsukuba 227	China	AB289669	
Veloporphyrellus pseudovelatus	HKAS52673	China		JX984541
Veloporphyrellus alpinus	HKAS57490	China		JX984537
Veloporphyrellus pantoleucus	Gomez 21232	Costa Rica		JX984547
Veloporphyrellus velatus	HKAS63668	China		JX984546
Veloporphyrellus conicus	BZ1670	Belize		JX984543
Solioccasus polychromus	Trappe 15399	Australia	JX888459	JQ287643

Note. Collections followed by an asterisk are holotypes.

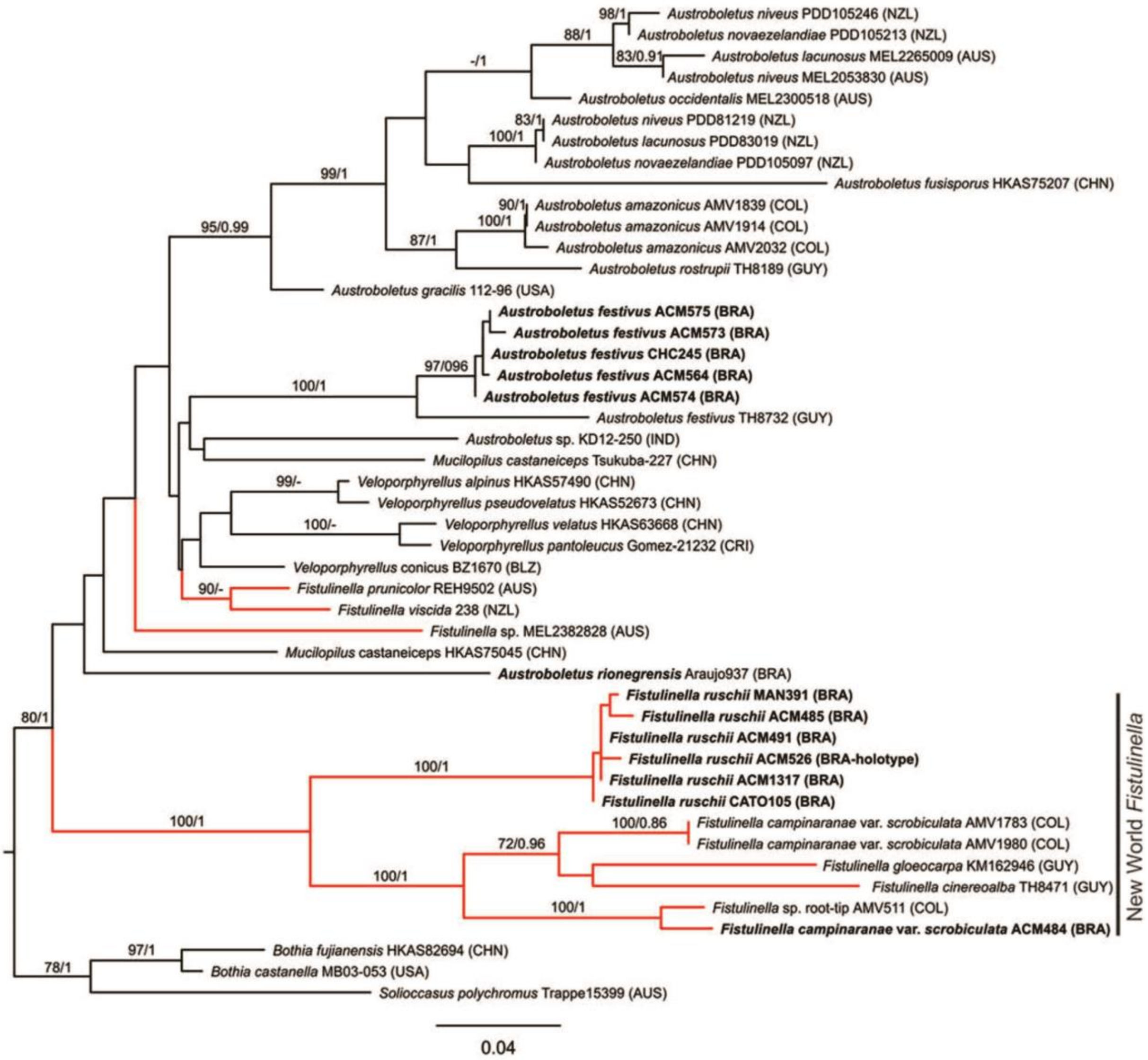

Figure 1. ML tree based on ITS and 28S sequences of selected Austroboletoideae. BS >70% and BPPs >0.90 are shown. Taxa in bold represent sequences generated in this study. Red bold lines represent *Fistulinella* specimens.

scrobiculata (ACM484) is most closely related to an ectomycorhizal (EcM) morphotype root tip isolated from the tree host *Pseudomonotes tropenbosii* (Dipterocarpaceae) from Colombia (BS = 100%, BPP = 1). Sequences from four specimens of *Fistulinella* from Colombia and Guyana clustered together (BS = 72%, BPP = 0.96) apart from the Colombian root tip and Brazilian sample. The name *F. campinaranae* var. *scrobiculata* has been applied to two of the samples from Colombia, but these do not cluster with branch labeled as such from Brazil. Three Old World samples from Australasia labeled in GenBank as *F. viscida* and *F. prunicolor* clustered outside the clade containing New World *Fistulinella* but with poor measures of branch support in the Austroboletoideae at large. *Fistulinella* is thus found to be a nonmonophyletic genus; however, the type (*F. staudtii*, described from Cameroon) has yet to be sequenced.

TAXONOMY

Fistulinella campinaranae var. *scrobiculata* Singer, Beih. Nova Hedwigia 77:148. 1983. FIGS. 2, 4A C

Pileus 16 27 mm broad, parabolic when young, becoming convex when mature; grayish brown (OAC 733) with a whitish margin; surface finely fibrillose,

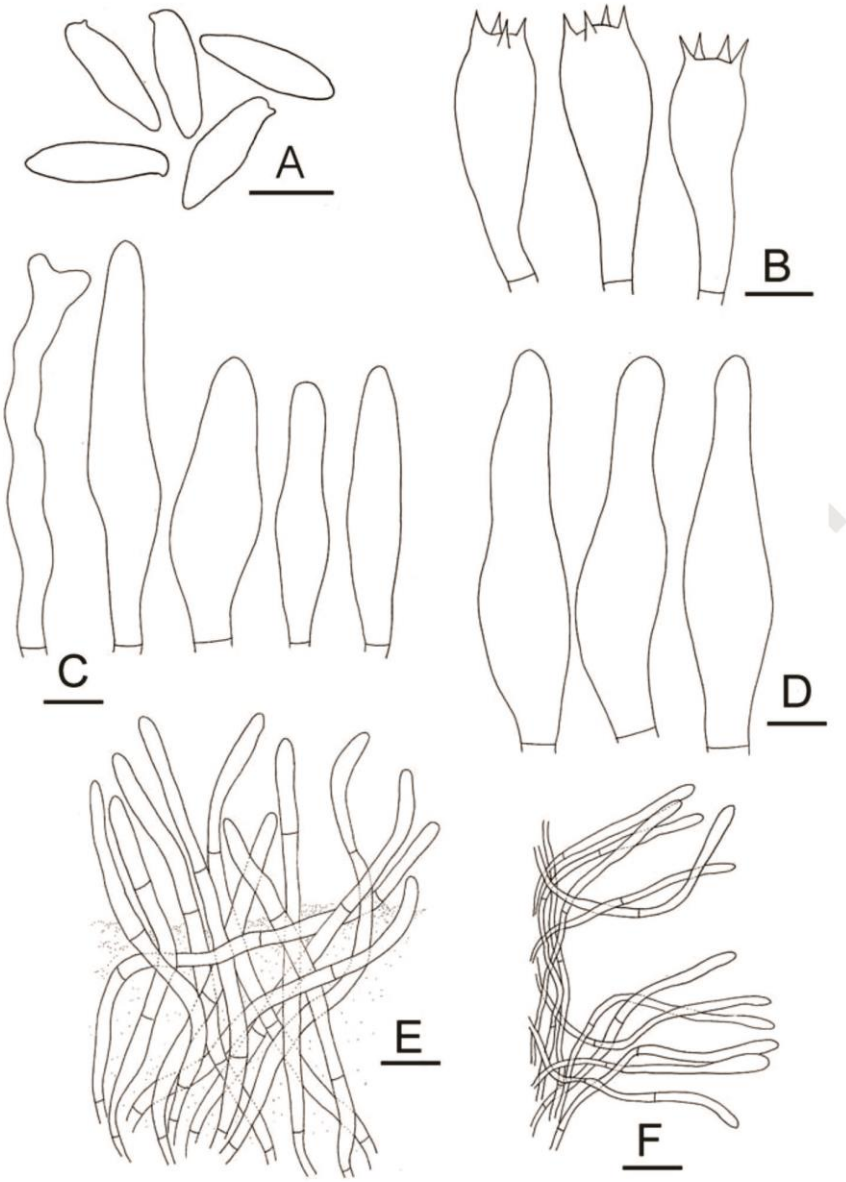

Figure 2. Anatomical features of *F. campinaranae* var. *scrobiculata* (ACM484). A. Basidiospores. B. Basidia. C. Cheilocystidia. D. Pleurocystidia. E. Upper layer of pileipellis. F. Stipitipellis. Bars: A D = 10 μm; E F = 25 μm.

scrobiculate over whitish ground, margin entire, slightly viscid to almost dry; context white, unchanging. Tubes 3 6 mm long, depressed around the stipe; two pores per mm, isodiametric, whitish gray when young, pinkish gray when mature (OAC 753). Stipe 60 71 mm long, 2 3 mm wide at the apex, 3 4 mm wide at the base, central, clavate and enlarged towards the base; surface glabrous, slightly viscid whitish but browning where touched; context white, unchanging. Spore print pinkish brown. Odor and Taste not observed.

Basidiospores 14 18 × 4 6 μm (Qr = 3.0 3.5; Qm = 3.23; n/s = 30/1), subfusiform to fusiform, hyaline to light yellow, weakly dextrinoid, multiguttulate with elongate to circular oil drops, smooth, thin-walled. Basidia 31 37 × 10 12 μm, narrowly clavate, thin-walled, hyaline, inamyloid, 4-spored, sterigmata 4 5 μm long. Pleurocystidia 43 76 × 10 17 μm, subventricose, lageniform or nearly fusiform, hyaline, inamyloid, smooth, thin-walled. Cheilocystidia with variable shapes: cylindrical, 24 42 × 3 4 μm, some with bifurcate apices; otherwise fusiform, 46 52 × 7 8 μm; or elongate cylindrical septate, 48 73 × 2 4 μm wide, these more frequent in young basidiomata. Hymenophoral trama boletoide, strongly divergent, mediostratum of many narrow parallel hyphae, these 2 4 μm broad, light yellow; lateral stratum lighter in color, gelatinized, hyphae 2 6 µm wide. Pileipellis an ixotrichoderm, some hyphae with ochre content in H_2O, dextrinoid, 5 7 µm wide, terminal cells with a rounded apex, regularly septate. Pileus trama strongly interwoven, hyphae 6 9 µm wide, light yellow. Stipitipellis in two layers, outer layer with clusters of slightly interwoven projecting cylindrical hyphae, hyphae 4 6 µm wide, septate, with light yellow intracellular content in H_2O; lower layer with thin hyaline hyphae, subparallel to loosely woven, immersed in a gelatinized matrix, no caulocystidia or caulobasidia observed. Stipe trama composed of vertically arranged hyphae, 6 10 µm wide. Clamp connections absent in all tissues.

Habit, habitat, and distribution: solitary or gregarious. In Brazil, known from Amazonas in the Amazon forest on the ground in humus-sandy or decayed wood (Singer et al. 1983) and from Bahia in the Atlantic Forest (present study) on the ground close to leguminous trees (Fabaceae). Also, recorded from Colombia, frequently in the Amazon Forest area dominated by *Pseudomonotes tropenbosii* (Vasco-Palacios et al. 2014).

Material examined: BRAZIL. BAHIA: Serra Grande, Parque Estadual da Serra do Conduru, 30 Nov 2012, *A. C. Magnago 484* (FLOR 51608); AMAZONAS: Estrada Manaus-Caracaraí, km 45, 25 Apr 1980, *R. Singer B12131* (**paratype** INPA 102043); AMAZONAS: Estrada Manaus-Caracaraí, km 45, 12 Feb 1979, *R. Singer B11491* (**paratype** INPA 106001).

Additional specimens examined: GUYANA. REGION 8 POTARO-SIPARUNI: *Fistulinella cinereoalba*, Pakaraima Mountains, Upper Potaro River Basin, vicinity of Potaro base camp, 17 Jun 2002, *Henkel 8471* (**isotype** NY 1193857).

Notes: Singer et al. (1983) separated *Fistulinella campinaranae* into two varieties according to the surface texture of the pileus: var. *scrobiculata* with a scrobiculate pileus and var. *campinaranae* with a glabrous pileus. Other minor characteristics were also observed in var. *scrobiculata*; however, Singer et al. (1983) questioned how reliable these were given they only had a few specimens and that both varieties occurred in the same vegetation types (*campinarana* and *campina*). In descriptions by Singer et al., the lignicolous habit was observed for var. *campinaranae*, but occurrences on the ground on sandy humus or rotten wood for var. *scrobiculata*. Our collection from the Atlantic Forest matches best the description of var. *scrobiculata* due to the finely rugulose scrobiculate pileus surface and terricolous habit.

Fistulinella campinaranae var. *scrobiculata* is now known from the Brazilian Amazon forest, with an extension into the coastal Atlantic Forest. This variety is morphologically similar to the Neotropical species *F. cinereoalba* and *F. gloeocarpa*, but it differs from these by the presence of cylindrical to aciculate pleurocystidia in *F. cinereoalba* and a browning reaction on the hymenophore when pressed, a feature not seen in *F. campinaranae* var. *scrobiculata*. *Fistulinella gloeocarpa* can also be distinguished from the other taxa by the presence of ampulaceous pleurocystidia and a repent ixocutis comprising a thick glutinous layer, a feature absent in *F. campinaranae* var. *scrobiculata*.

Both paratype specimens of *F. campinaranae* var. *scrobiculata* deposited at INPA (INPA 106001, INPA 102043) are in poor condition and comprise just a few fragments, making it difficult to study the morphology and to extract DNA of sufficient quality for molecular analyses. The holotype of *F. campinaranae* var. *campinaranae* (Singer B10109, INPA) was requested for examination but could not be located.

The specimens of *F. campinaranae* var. *scrobiculata* from the Brazilian Atlantic Forest and from the Colombian Amazon morphologically fit the description of the species provided by Singer et al. (1983). However, based on the molecular results presented here, it is possible that there are cryptic species hidden under this name. More collections and DNA sequencing from both *F. campinaranae* var. *scrobiculata* and var. *campinaranae* are necessary to better understand the relationships among the specimens from the Atlantic Forest and the Amazon.

Fistulinella ruschii A.C. Magnago, sp. nov. FIGS. 3, 4D H

MycoBank MB820546

Typification: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra (19°54′19.60″S, 40°34′8.20″W), 5 Dec 2012, *A. C. Magnago 526* (**holotype** FLOR 51611). GenBank: ITS = KY886206; 28S = KY888006.

Etymology: (Latin) in honor of the Brazilian naturalist Augusto Ruschi.

Diagnosis: Differs from other known Neotropical *Fistulinella* by the presence of broadly cylindrical septate pleurocystida.

Pileus 10 40 mm broad, parabolic when young to convex or depressed, chestnut brown (OAC 657, 659) to orange-brown (OAC 645, 646); surface velutinous, dry, becoming slightly viscid, margin entire, fibrillose when mature, fibrils dark orange (OAC 629, 663) against a light orange background (OAC 652, 632); context whitish, unchanging. Tubes 4 7 mm long, strongly depressed to almost free around the stipe, whitish to cream pinkish (OAC 676, 620); pores white when young, light pink when mature (OAC 620), 1.5 2 per mm, isodiametric. Stipe 42 80 mm long, 5 7 mm wide at the apex, 10 15 mm at base, subclavate to fusiform,

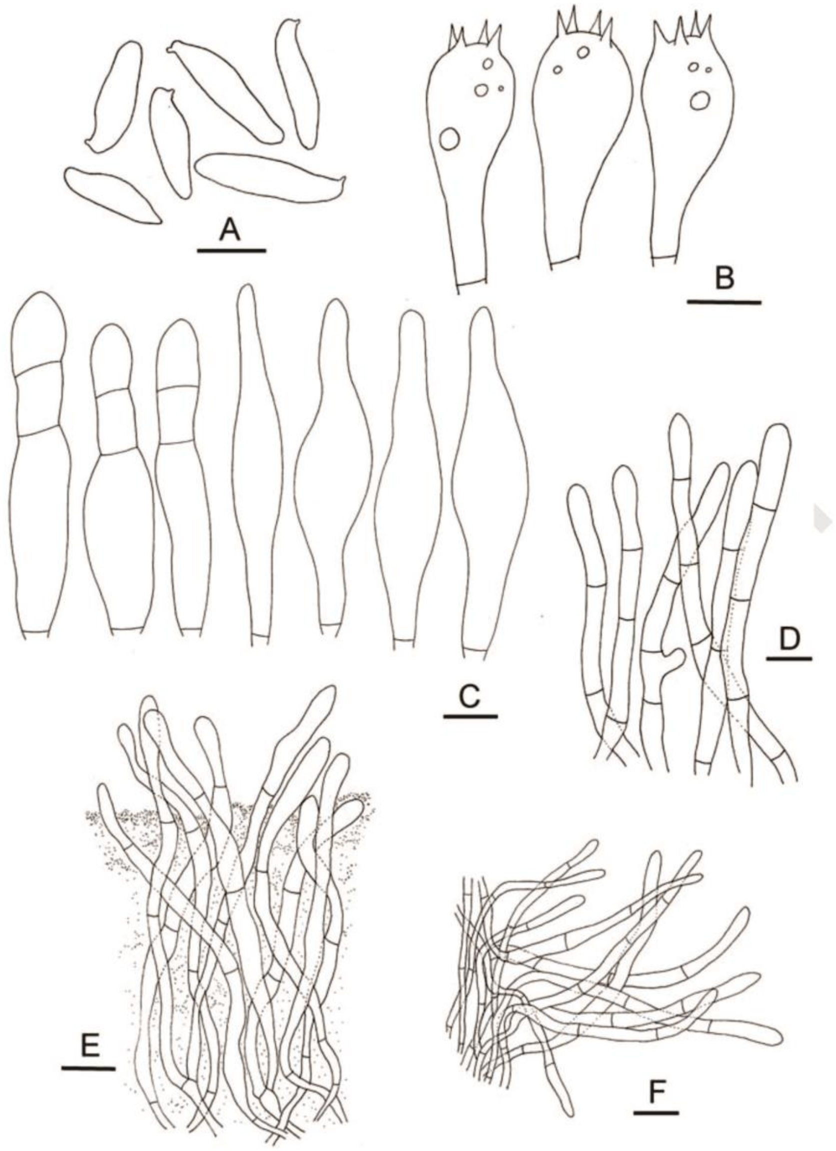

Figure 3. Anatomical features of *F. ruschii* (ACM526, holotype). A. Basidiospores. B. Basidia. C. Pleurocystidia of two types. D. Cheilocystidia. E. Upper layer of pileipellis. F. Stipetipellis. Bars: A D = 10 µm; E F = 25 µm.

surface slightly viscid, cream pinkish (OAC 655) covered by a whitish pruina, browning where touched, base with white rhizomorphs; context white, unchanging. Macrochemical reactions: NH_4OH 10% on pileus surface becoming reddish orange, on stipe surface becoming yellow. Spore print pinkish brown (OAC 605). Odor and Taste not observed.

Basidiospores 14 18(22) × 4 5 µm (Q = 3.44; Qr = 3.0 4.4; Qm = 3.44; n/s = 30/6), subfusiform, fusiform to slightly sigmoid, with suprahilar depression; hyaline to light pinkish, inamyloid, guttulate, smooth. Basidia 26 34 × 10 14 µm, broadly clavate, thin-walled, hyaline, inamyloid, 4-spored, sterigmata 2 3 µm long. Pleurocystidia of two kinds: lanceolate to ventricose-rostrate, 41 78 × 6 14 µm, hyaline, inamyloid, sometimes slightly wrinkled in the middle portion, very frequent; or broadly cylindrical, septate (1 3) on the upper two thirds, 48 70 × 7 12 µm, projecting 26 54 µm beyond hymenium, hyaline, inamyloid, scattered. Cheilocystidia cylindrical, septate, more frequent in young basidiomata, 4 6 µm wide, projecting 75 86 µm beyond hymenium. Hymenophoral trama boletoid, strongly divergent, mediostratum parallel, hyphae 3 5 µm wide, light yellow, inamyloid; lateral stratum divergent, immersed in a gelatinized matrix, hyphae 4 7 µm wide. Pileipellis in three layers immersed in a

Figure 4. Basidiomata and SEM images of basidiospores. A C. *Fistulinella campinaranae* var. *scrobiculata* (ACM484). D H. *Fistulinella ruschii*. D, E, F. Holotype (ACM526). G. Paratype (ACM491). H. Paratype (ACM1317). Bars = 10 mm.

gelatinized matrix: the suprapellis an ixotrichoderm or ixotrichodermal palisade, yellowish brown, inamyloid, regularly septate; terminal cells 4 11 µm wide with rounded apices; mediopellis interwoven to suberect, hyphae 2 4 µm wide, pale yellow; subpellis of repent hyphae 2 3 µm wide. Pileus trama interwoven to subparallel, hyphae 3 11 µm wide, light yellow. Stipitipellis in two layers, the outer layer with tufts of erect to suberect hyaline cylindrical elements, septate, 4 10 µm wide, light yellow, caulobasidia present; the lower layer with subparallel hyaline hyphae, 2 5 µm wide, immersed in a gelatinized matrix. Stipe trama parallel, hyphae 3 11 µm wide, hyaline. Clamp connections absent.

Other specimens examined: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra, 4 Dec 2012, A.C. Magnago 491 (FLOR 51610) (19°54 ′19.62″S, 40° 34′8.21″W), 14 Dec 2016, A.C. Magnago 1317 (ICN 192819); PARAÍBA: João Pessoa, Universidade Federal da Paraíba, Mata do Biotério (7°08′18″S, 34°50′38″W), 12 Dec 2009, *M.A. Neves 391* (ICN 192820); BAHIA:

Serra Grande, Parque Estadual da Serra do Conduru, 30 Nov 2012, *A.C. Magnago 485* (FLOR 51609); SANTA CATARINA: Florianópolis, Saco Grande, Unidade de Conservação do Desterro (27°31′51.6″S, 48°30′44.1″W), 15 Jan 2015, *C. Oliveira 105* (ICN 192818).

Habit, habitat, and distribution: Solitary or growing gregarious on the ground on soil near leguminous trees (Fabaceae). In Brazil, recorded in four areas ca. 1000 km from each other along the coastal Atlantic Forest.

Notes: Fistulinella ruschii is the only Neotropical species of the genus that has septate pleurocystida. *Fistulinella campinaranae* var. *campinaranae* differs by its grayish brown scrobiculate pileus and subventricose, lageniform, or fusiform pleurocystidia. *Fistulinella jamaicensis* is described with smaller basidiospores (9.5 12 × 4.8 5.2 μm). *Fistulinella mexicana* occurs on wood, has a stipe base that is disciform covered by a transparent glutinous layer, and has smaller basidiospores (11 14.2 × 4.3 5.3 μm) compared with *F. ruschii. Fistulinella venezuelae* has a pileus with an obtuse disc that is slightly umbonate and has much longer basidiospores (up to 21.5 μm) than *F. ruschii*. The pleurocystidia of *F. venzuelae* are described as elongate, fusoid, or ampullaceous.

Fistulinella mollis, which occurs widely in eucalyptus forests in Australia (trees also very abundant in Brazil), is morphologically similar to *F. ruschii* because of the orange-brown pileus and white to pink hymenophore, but it differs from *F. ruschii* by the viscid to glutinous pileus and the presence of nonseptate pleurocystidia. *Fistulinella ruschii* is also similar to the Australian *F. prunicolor*; however, the latter has a dark brown to reddish to plum pileus that is viscid when wet, soft-textured white context, vinaceous pink tubes, and a white stipe with yellow stains (Pegler and Young 1981; Watling and Li 1999).

DISCUSSION

The phylogenetic analyses performed during this study corroborate the morphological placement of the new species *F. ruschii* in *Fistulinella* However, the genus is not monophyletic, as New World samples cluster apart from Old World samples. The analyses also showed the relation of *F. campinaranae* var. *scrobiculata* with other Neotropical species such as *F. cinereoalba* and *F. gloeocarpa* from Guyana and a Colombian *F. campinaranae* var. *scrobiculata* as observed by Vasco-Palacios et al. (2014).

Most species of *Fistulinella* from the Neotropics are known from only the type specimen or few collections. In several cases, the specimens are poorly preserved, and the taxon should be re-collected to understand better the morphology and collection of molecular data. Unfortunately, the Austroboletoideae is not well represented in GenBank (Nuhn et al. 2013; Wu et al. 2014). Therefore, a reliable phylogenetic inference of *Fistulinella*, including combined analyses, is still difficult to obtain. No sequences of boletoid specimens from Brazil were available in GenBank, and there are only a few sequences from other Neotropical countries. Additional studies including more Neotropical boletoid sequences and type species should be carried out to assess the phylogenetic structure of these groups within Austroboletoideae.

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ARTIGO III

Singerocomus atlanticus sp. nov., and a first record of *Singerocomus rubriflavus* (Boletaceae, Boletales) for Brazil

Magnago, A.C., Henkel, T., Neves, M.A. & Silveira, R.M.B.

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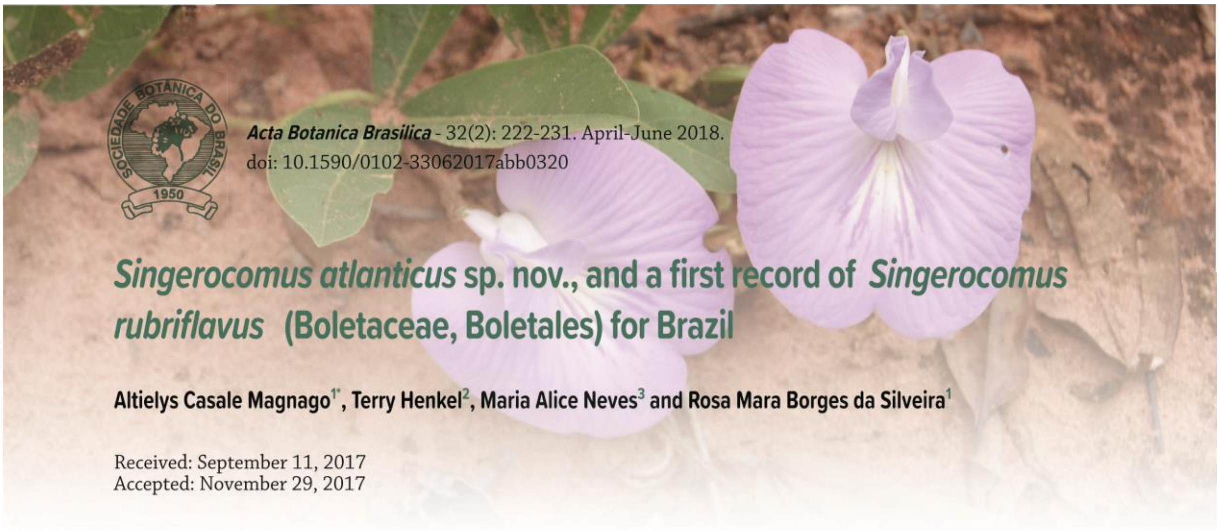
Singerocomus atlanticus A.C. Magnago (Foto: Magnago, A.C.)



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Singerocomus atlanticus sp. nov., and a first record of *Singerocomus rubriflavus* (Boletaceae, Boletales) for Brazil

Altielys Casale Magnago[1*], **Terry Henkel**[2], **Maria Alice Neves**[3] **and Rosa Mara Borges da Silveira**[1]

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ABSTRACT

Ongoing surveys of macrofungi in the Brazilian Atlantic Forest ecoregion continue to uncover a diverse assemblage of ectomycorrhizal fungi. A new species of Boletaceae, *Singerocomus atlanticus* sp. nov., is described. *Singerocomus rubriflavus*, previously known only from Guyana, is recorded for the first time from the Brazilian Atlantic Forest. Phylogenetic data, macro- and microscopic illustrations, and comments are presented for each species.

Keywords: Atlantic Forest, boletoid fungi, ITS and LSU sequences, Neotropics, taxonomy

Introduction

The Atlantic Forest ecoregion of Brazil is a globally important biodiversity hotspot, harboring many endemic species across multiple taxonomic groups (Olson & Dinerstein 2002; Paes *et al.* 2010; Joly *et al.* 2014). Ectomycorrhizal (ECM) fungi were traditionally poorly known from the Atlantic Forest, although this situation is changing (Meijer 2008; Maia *et al.* 2015; Barbosa-Silva *et al.* 2017; Magnago *et al.* 2017). In particular, macrofungi in ECM basidiomycete lineages of the Agaricales, Boletales, Russulales, and Cantharellales appear to be widespread in the Atlantic Forest region and new species and distribution records are being rapidly described (e.g. Sulzbacher *et al.* 2013a; b; Roy *et al.* 2017).

In Brazil new species of Boletaceae (Boletales, Agaricomycetes, Basidiomycota) have been described from the Amazon rainforest (e.g. Singer & Digilio 1957; 1960; Singer *et al.* 1983) as well as the Atlantic Forest (e.g. Rick 1960; Vinha 1988; Putzke *et al.* 1994; Oliveira & Sousa 1995; 1996; 2002; Watling & Meijer 1997; Neves & Capelari 2007; Meijer 2008; Magnago & Neves 2014; Barbosa-Silva *et al.* 2017; Magnago *et al.* 2017). Most of these new species were placed in traditional, morphology-defined genera of Boletaceae in the absence of corroborative DNA sequence data with the exception of the three last works cited.

The genus *Singerocomus* was proposed by Henkel *et al.* (2016) to accommodate a new species from Guyana, *Singerocomus rubriflavus*, and a new combination for *Xerocomus inundabilis*, originally described from the Brazilian Amazon (Singer *et al.* 1983). Diagnostic features for *Singerocomus* include a pileate-stipitate, epigeous basidioma, pinkish red to red, tomentose pileus, yellow, tubulose, non-cyanescent hymenophore depressed at the stipe, subangular pores, concolorous or lighter stipe with or without squamules, and olivaceous brown basidiospore deposit. The combination of smooth basidiospores, phylloporoid tube trama, trichodermial pileipellis, and lack of clamp connections characterize the genus micromorphologically. The new genus was justified by a multi-locus, taxon-dense phylogenetic analysis across the Boletaceae which demonstrated that *Singerocomus* species

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had no close relatives at the generic level within the family (Henkel *et al.* 2016).

As part of our ongoing macrofungal surveys in the Atlantic Forest, *Singerocomus atlanticus* sp. nov. is described, and *S. rubriflavus*, previously known only from Guyana, is recorded for the first time for Brazil.

Materials and methods

Collections

Collections of *Singerocomus* T.W. Henkel & M.E. Sm. were made between 2011 and 2016 at multiple sites in the Brazilian Atlantic Forest, including Reserva Biológica Augusto Ruschi in Espírito Santo, Parque Estadual da Serra do Conduru in Bahia, and Unidade de Conservação Ambiental do Desterro and Plaza Caldas da Imperatriz in Santa Catarina.

Macroscopic features were described from fresh basidiomata and specimens were dried with a food dehydrator (Total Chef TCFD-05 Deluxe) at about 40 ºC. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Macrochemical tests were performed according to Singer (1986). Microscopic structures were rehydrated in 3 % KOH and dyed with Congo Red. Melzer´s reagent was used for testing amyloidity. To observe the surface of the basidiospores in scanning electron microscopy (SEM), small fragments of the hymenophore were taken from dried specimens and mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a JEOL JSM-6390LV scanning electron microscope, operating at 10KeV, at the Centro de Microscopia e Microanálise of the Universidade Federal do Rio Grande do Sul. Line drawings of microscopic features were made using digital photographs from the specimens examined. Voucher materials were deposited at ICN and FLOR Herbaria and duplicates will be sent to VIES (Thiers 2017).

DNA extraction, amplification and sequencing

DNA was extracted from dried specimens using the Doyle & Doyle (1987) protocol adapted by Góes-Neto *et al.* (2005). Full nuclear ribosomal internal transcribed spacer nrITS (ITS1-5.8S-ITS2) and nuclear ribosomal large subunit (nrLSU) were PCR-amplified with primer pairs ITS6-R/ITS8-F, and the nuclear LSU rDNA (28S) were PCR-amplified with LR0R/LR7 following Dentinger *et al.* (2010) and Vilgalys & Hester (1990) respectively. Complementary unidirectional sequence reads were aligned and edited in Geneious 6.1.8 (Kearse *et al.* 2012) and deposited in GenBank (Tab. 1). The ITS ribosomal DNA sequence from the new taxon was initially subjected to a BLASTn query against GenBank to explore its putative phylogenetic relationships. A total dataset of 38 sequences (18 nrITS and 20 nrLSU) were used in the analyses, both newly generated in this study or downloaded from Genbank database. Five genera (*Butyriboletus*, *Lanmaoa*, *Rugiboletus*, *Rubroboletus*, *Singerocomus*) were included in the phylogenetic analyzes based on Henkel *et al.* (2016) with *Bothia* included as the outgroup.

Sequence alignment, and phylogenetic analysis

Each dataset was automatic alignment separately using MAFFT (Katoh & Standley 2013), following the L-INSi and G-INS-i criteria (for nrITS and nrLSU, respectively). Gblocks (Talavera & Castresana 2007) was used to exclude ambiguous portions of the alignment, producing a final aligned dataset of 775 bp for ITS and 841 bp for 28S. Alignments were deposited in TreeBASE (http://www.treebase.org/treebase/index.html) under accession no. ID 21441. Maximum likelihood (ML) analysis was performed separately on ITS and 28S to determine whether there were any well-supported phylogenetic incongruencies between these two loci. Because no supported incongruence was detected we conducted maximum likelihood and Bayesian inference (BI) analysis based on the concatenated ITS+28S dataset. The dataset was subdivided into four partitions: ITS1, 5.8S, ITS2, nrLSU. Maximum likelihood was carried out with RAxML-HPC v.8 (Stamatakis 2014), available in the CIPRES science gateway (Miller *et al.* 2010, http://www.phylo.org/), using GTRGAMMA as the model of evolution (Stamatakis 2006), choosing the rapid bootstrap analysis (command fa) with a random starting tree and 1000 maximum likelihood bootstrap replications. Bayesian inference was conducted on MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller *et al.* 2010), using four parallel MCMC chains, which were allowed to run for 20 million generations, with sampling every 1000 generations. The best-fitted substitution models were set to each partition calculated by MrModeltest 2.3 (Nylander 2004). The Akaike information criterion was chosen to select the most appropriate model of DNA substitution for each data set or data partition used in the analyses. The convergence diagnostic was calculated every 10^4 generations and its critical value was set in order to automatically stop the analysis when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes stopval = 0.01). In all analyses, the first 25 % trees from each run were discarded as burnin. Resulting trees from the two independent runs were then pooled to produce one 50 % majority-rule consensus tree and Bayesian posterior probabilities were generated for the resulting tree. A node was considered well supported if it showed a Bayesian Posterior Probabilities (BPP) ≥ 0.95 and/or Bootstrap (BS) ≥ 80 %, while moderate support was considered BS ≥ 70 %, and non-supported BPP < 0.95. All phylogenetic trees were visualized using FigTree (Morariu *et al.* 2009).

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species	Collection No.	Origin	GenBank accesion No.	
			ITS	LSU
Bothia castanella	MB03 067	USA	DQ867114	DQ867115
Butyriboletus fechtneri	AT2003097	-	KC584784	KF030270
Butyriboletus regius	MG 408a	-	KC584789	KC584790
Butyriboletus yicibus	Arora 9727	China	KC184474	KC184475
Lanmaoa asiatica	HKAS 54094	China	-	KF112353
Lanmaoa carminipes	MB06 001	USA	-	JQ327001
Rubroboletus latisporus	HKAS 80358	China	KJ951990	KP055023
Rubroboletus sinicus	HKAS 68620	China	KJ951991	KF112319
Rugiboletus brunneiporus	HKAS 83209	China	-	KM605134
Rugiboletus extremiorientalis	HKAS 563591	China	-	KM605136
Singerocomus atlanticus	ACM 527	Brazil	KY907177	KY926776
Singerocomus atlanticus	CATO 106	Brazil	KY907179	KY926778
Singerocomus atlanticus	ACM 1275	Brazil	KY907178	KY926777
Singerocomus atlanticus	BZ L69	Brazil	KY907181	-
Singerocomus atlanticus	MJ 105	Brazil	KY907181	-
Singerocomus inundabilis	TH8408	Guyana	JN021114	HQ161863
Singerocomus inundabilis	Henkel 9199	Guyana	LC043087	LC043087
Singerocomus inundabilis	Aime 4004	Guyana	LC043090	LC043090
Singerocomus inundabilis	TH10087	Guyana	KT380014	KT380016
Singerocomus inundabilis	TH10109	Guyana	KT380013	KT380015
Singerocomus rubriflavus	MCA 5472	Guyana	LC043093	-
Singerocomus rubriflavus	Henkel 9585	Guyana	-	KP941572
Singerocomus rubriflavus	GAS 900	Brazil	KY907182	KY926779

Results

BLASTn queries and phylogenetic analysis

Ten new sequences of *Singerocomus* from Brazil were generated (6 nrITS and 4 nrLSU). ITS BLASTn queries of each of the new taxa on GenBank indicated affinities with specimens in the genera *Xerocomus* and *Singerocomus*.LSU BLASTn queries indicated affinities with Boletaceae, but were uninformative at the genus level.

All rDNA markers were combined into a single matrix, including 38 sequences from 23 specimens representing 13 putative species, resulting in an aligned matrix of 1116 bp. The respective ML tree showing BS and BPP values on branches are shown in Figure 1. All phylogenetic analysis performed showed that specimens of *Singerocomus* grouped into one distinct, well supported clade (BS = 0.99, BPP = 1 %) that included the new species, *S. inundabilis* (Singer) T.W. Henkel, and *S. rubriflavus* T.W. Henkel & Husbands. The *S. inundabilis* clade contained only specimens from Guyana (BS = 1.0, BPP = 100 %). The Brazilian *S. rubriflavus* specimen clustered with others from Guyana in a well-supported clade (BS = 0.92, BPP = 98 %), although the variability in branch lengths among the specimens suggests intraspecific variability. The other specimens collected in the south of the Atlantic Forest clustered in a distinct well-supported clade (BS = 1.0, BPP = 100%) described below as the new species *Singerocomus atlanticus*.

Taxonomy

Singerocomus atlanticus A.C. Magnago **sp. nov.** (Figs. 2A-F, 3A-D)

MycoBank MB822576

Etymology. from the Latin *atlanticus* = referring to the Atlantic Forest where the new species was collected.

Diagnosis. Differs from other known *Singerocomus* species by the combination of the following characters: pileus red-orange to pink-red, velutinous with olivaceous yellow pruina, stipe glabrous to vertically rugulose, instantly bluing reaction on pileus surface and ferrugineous-orange on the stipe with NH_4OH, basidiospores broadly ellipsoid, 6–8 × 4–5 μm, cystidia in the tubes and pores fusoid to ventricose-rostrate.

Type. BRAZIL, Espírito Santo: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra, 19° 54 19.5 S; 40° 34 08.4 W, 05 December 2012, *A.C. Magnago 527* (ICN 188266!; GenBank accessions: ITS = KY907177, 28S = KY926776).

Description. *Pileus* 16–37 mm wide, parabolic at first to convex to planoconvex when mature, velutinous, red-orange to pink-red (OAC 629, 642), darkest centrally

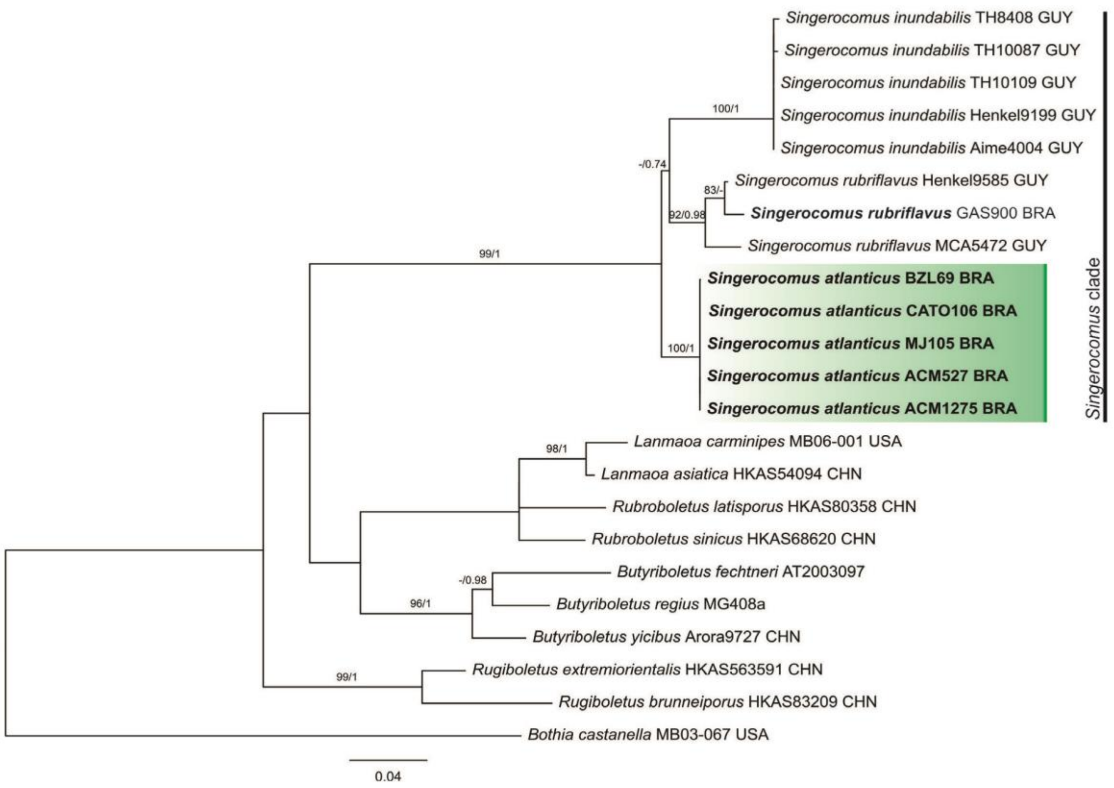

Figure 1. Maximum likelihood (ML) tree based on nrITS and nrLSU sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.9 are shown.

lighter progressively toward the margin; surface initially with olivaceous yellow (OAC 831, 838) pruina throughout (more visible under lens) with age becoming matted-velutinous and exposing a light pink to light yellow (OAC 855, 858) ground, dry; margin even; context whitish to cream yellow (OAC 812), solid, unchanging when exposed. *Tubes* 2–5 mm long centrally, sulphureus yellow (OAC 002, 895), depressed around stipe; pores mostly 2–3 per mm, subangular, unchanging under pressure. *Stipe* 26–54 mm × 3–5 mm centrally, subequal, glabrous to vertically rugulose to reticulate, off white to light yellow (OAC 003) over apical 1/5, red to pink (OAC 628, 629, 631) over central 3/5, and citrine yellow (OAC 010) over basal 1/5; *basal mycelium* white at extreme base; context cream yellow (OAC812), solid, unchanging when exposed. *Odor* and *Taste* not obtained. *Macrochemical reactions*: NH4OH instantly blue on pileus surface and ferrugineous-orange on the stipe; KOH on pileus and stipe surface becoming instantly yellow. *Spore print* olive brown (OAC 867), light deposit. *Basidiospores* 6–8 × 4–5 µm (Qm=1.60), broadly ellipsoid, olivaceous in H2O, paler in 3% KOH, inamyloid, smooth, thin walled; hilar appendage 0.5–1 µm long. *Basidia* 28–40 × 8–11 µm, narrowly clavate to clavate, thin-walled, hyaline, 4-sterigmate; sterigmata 2–5 µm long. *Pleurocystidia* and *cheilocystidia* similar in size and shape, 34–62 × 8–11 µm, fusoid to ventricose-rostrate, usually with a long neck, hyaline, inamyloid, smooth, thin-walled. *Hymenophoral trama* slightly divergent (phylloporoid); individual hyphae of mediostratum narrow, 3–6 µm wide, yellowish, inamyloid; oleiferous hyphae present; lateral stratum with individual hyphae more inflated, 6–10 µm wide, slightly divergent. *Pileipellis* a semi-erect to erect trichodermium; terminal elements 5–12 µm wide, cylindrical, rounded at apex, light brown to yellowish in H2O, inamyloid, slightly incrusted circumferentially under phase contrast. *Pileus trama* interwoven to subparallel; individual hyphae 4–14 µm wide, hyaline to light yellow in H2O. *Stipitipellis* repent with discontinuous clusters of suberect, clavate to cylindrical terminal elements, 24–28 × 6–12 µm, hyaline to pale yellow in H2O, inamyloid, thin walled. *Stipe trama* of longitudinally arranged hyphae; individual hyphae 5–7 µm wide, uninflated, hyaline to light yellow in H2O, inamyloid. *Clamp connections* absent.

Habit, habitat, and distribution Solitary to gregarious on soil in proximity to putative ECM host plants of the Nyctaginaceae and Fabaceae; known only from the Brazilian Atlantic Forest.

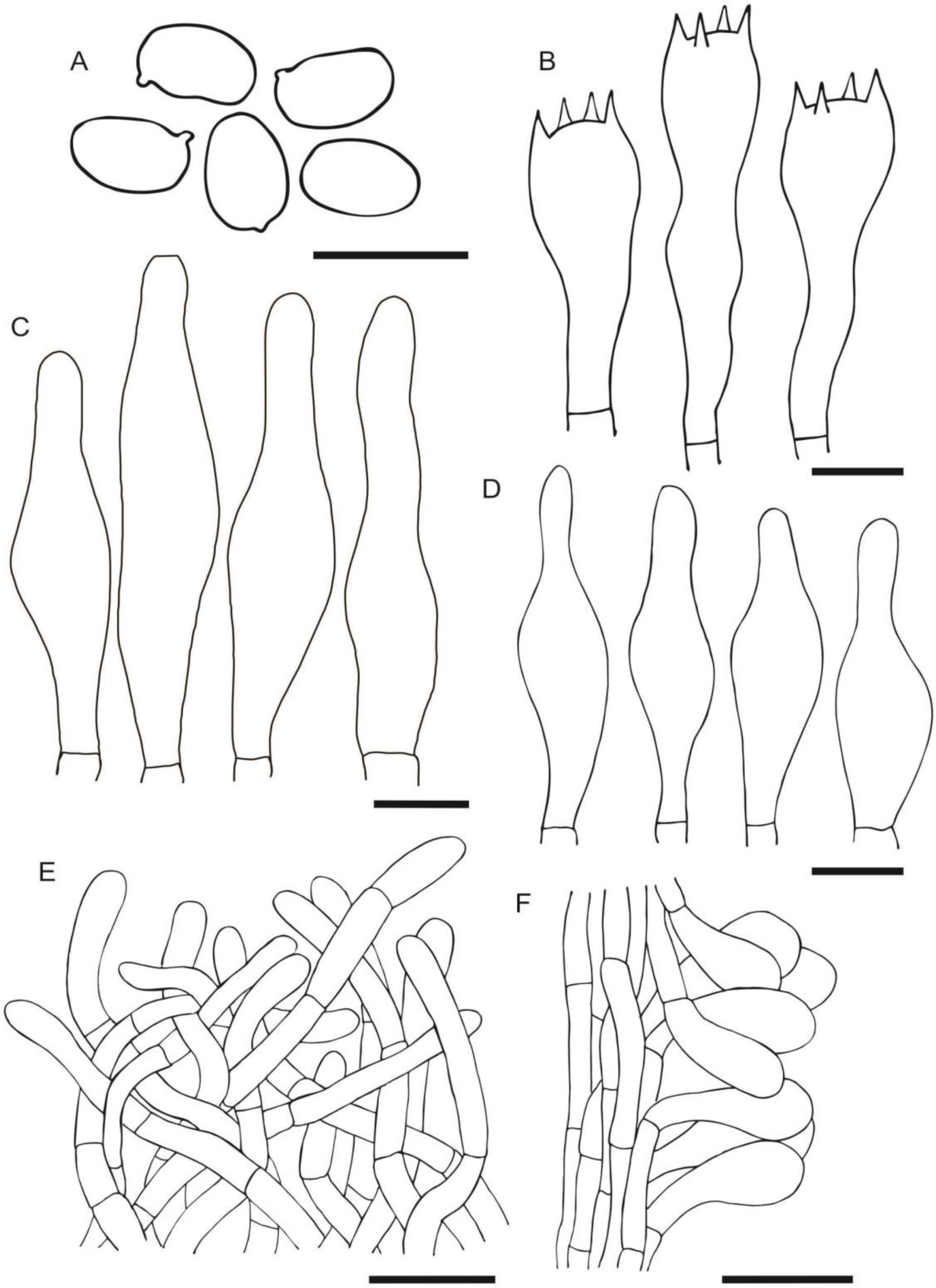

Figure 2. Microscopic features of *Singerocomus atlanticus* (holotype, *ACM 527*). **A.** Basidiospores. **B.** Four-sterigmate basidia. **C.** Pleurocystidia. **D.** Cheilocystidia. **E.** Pileipellis. **F.** Stipitipellis. Bars AD = 10 μm, EF = 25 μm.

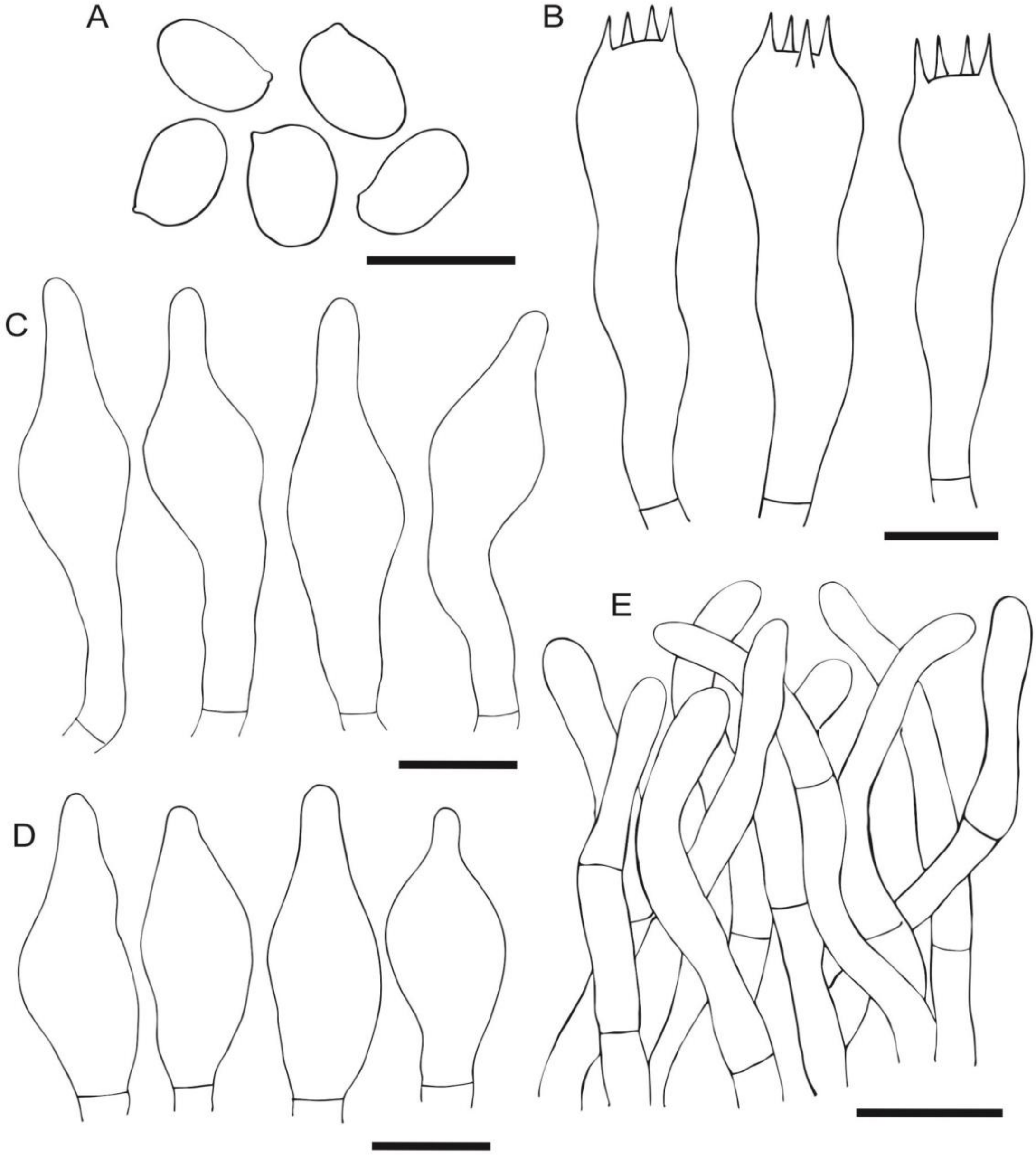

Figure 3. Microscopic features of *Singerocomus rubriflavus* (GAS 900). **A** Basidiospores. **B.** Four-sterigmate basidia. **C.** Pleurocystidia. **D.** Cheilocystidia. **E.** Pileipellis. Bars AD = 10 μm, E = 25 μm.

Additional specimens examined. BRAZIL, Santa Catarina: Florianópolis, Saco Grande, Unidade de Conservação Ambiental do Desterro, 27°3152.9S 48°3045.3W (Park headquarters coordinates) 20 December 2011, *M. Jaeger 105* (ICN 188267!; GenBank accession: ITS = KY907180); 21 December 2011, *B. Dentinger 69* (ICN 188268!; GenBank accession: ITS = KY907181); 15 January 2015, *C. Oliveira 106* (ICN 188269!; GenBank accession: ITS = KY907179, 28S = KY926778); 13 April 2017, Trilha do Pântano do Sul, *M.A. Neves 1216* (FLOR!); Santo Amaro da Imperatriz, Plaza Caldas da Imperatriz, 27°4402.3S; 48°4848.8W, 12 January 2016, *A.C. Magnago 1275* (ICN 188270!; GenBank accession: ITS = KY907178, 28S = KY926777). *Singerocomus inundabilis* BRAZIL.

Amazonas: Manaus, Igarapé do Turumãzinho, 09 October 1978, *Singer RB* B11432 (INPA-Fungos 82822 holotype!). *Xerocomus chapinii*. **Brazil:** Amazonas, Manaus, Igarapé do Turumãzinho, 12 March 1980, *Chapin 12027* (INPA-Fungos 101995 holotype!).

Commentary. *Singerocomus atlanticus* is morphologically similar to *S. inundabilis* (Tab. 2) but differs in its richer red pileus color and its NH4OH macrochemical reactions of instantly blue on the pileus and ferrugineous-orange on the stipe, features lacking in the other known species of the genus (Singer *et al.* 1983; Henkel *et al.* 2016). From a molecular standpoint *Singerocomus atlanticus* is also clearly different at the species level from *S. inundabilis* (Fig. 1). *Singerocomus atlanticus* has a broad distribution in the Atlantic Forest region, with conspecific specimens documented from the Espírito Santo type locality and from Santa Catarina ca. 1.700 km distant.

The combination of pink to red pileus and stipe colors and yellow hymenophore of *S. atlanticus* is reminiscent of several boletes described outside of the genus, including *Xerocomus chapinii*, *Hortiboletus campestris* and *H. rubellus*. *Xerocomus chapinii*, described from *igapó* vegetation in the Brazilian Amazon, differs from *S. atlanticus* in its mostly cylindrical, narrower basidiospores (6–7.8 × 2.7–3.2 μm), frequent ampullaceous hymenial cystidia with obtuse necks, and ixocutis pileipellis when young (Singer *et al.* 1983). The North American *H. campestris* and *H. rubellus* differ from *S. atlanticus* by their greenish-blue autoxidation reaction of exposed context and tubes, and elliptic to subfusoid basidiospores that are considerably longer (10–15 μm vs. 6–8 μm) (Smith & Thiers 1971; Vizzini 2015).

Singerocomus rubriflavus T.W. Henkel & Husbands Mycologia 108: 169. 2016 (Figs. 3E-F, 4A-E)

Description. *Pileus* 35 mm wide, convex to plane convex when mature, matted-tomentose, dark red (OAC 600, 656) over a pinkish (OAC 850) ground, dry, lighter towards the margin and darker in the center, margin entire, straight to slightly wavy. *Context* yellowish (OAC 812), solid, unchanging when exposed. *Tubes* 5–6 mm long centrally, olivaceous yellow (OAC 008, 019), depressed around stipe, sublamellate at stipe slightly decurrent; pores mostly 1–2 per mm, subangular, unchanging under pressure. *Stipe* 42 mm × 7–9 mm, central, subequal, pinkish (OAC 665, 672) covered by yellowish (OAC 811) depressed squamules. *Basal mycelium* a dense, bright yellow (OAC 001) tomentum, extreme base white. *Macrochemical reactions* not observed. *Spore print* olivaceous brown. *Basidiospores* 8–10 × 5–6 μm (Qm=1.69), ellipsoid, with a prominent suprahilar depression, hilar appendage 0.5–1 μm long, olivaceous brown in H2O, paler in 3 % KOH, inamyloid, smooth, thin walled. *Basidia* 29–40 × 9–11 μm, clavate, tapering toward the base, thin walled, hyaline, 4-sterigmate, 2–7 μm long. *Pleurocystidia* 33–66 × 11–12 μm, ventricose to ventricose-rostrate, hyaline, inamyloid, smooth, thin walled. *Cheilocystidia* 29–42 × 8–10 μm, broadly ventricose to obclavate, hyaline, inamyloid, smooth, thin walled. *Hymenophoral trama* subparallel to divergent (phylloporoid), mediostratum with narrow

Table 2. Comparison among taxa of *Singerocomus* (Singer *et al.* 1983; Henkel *et al.* 2016).

	S. atlanticus	*S. inundabilis*	*S. rubriflavus*
Pileus	1637 mm wide, convex to plane convex, velutinous to matted-velutinous, reddish to pinkish, with olive yellowish pruina all over	2533(47) mm wide, broadly convex to planate, areolate, occasionally with a low umbo, pinkish red	3777 mm broad, broadly convex, areolate to matted-tomentose, dark red
Hymenophore	Sulphureus yellow, unchanging with pressure	Olivaceous, unchanging with pressure	Olivaceous yellow, darkening slightly with pressure
Stipe	2654 mm × 35 mm, glabrous to vertically rugulose to reticulate, whitish to light yellow at the top, reddish to pink in the center, and citrine yellow in the base	3763 × 57 mm, pinkish red over upper two-thirds, lower third light yellow, glabrous, sligtly pulverulent and faintly longitudinally striate	3970 × 914 mm, light yellow squamules on concolorous ground
Basidiospores	68 × 45 μm (Qm=1.60), broadly ellipsoid, olivaceous	69 × 45.5(7) μm (Qm= 1.38), ellipsoid, with proemiment suprahilar depression, olivaceous brown	912 × 4.56.5 μm (Qm= 1.94), ellipsoid, with a proeminent suprahilar depression, olivaceous brown
Pleurocystidia	Similar in shape and size, 3462 × 811 μm, fusoid to ventricose-rostrate, usually with a long neck, conspicuous	34.561 × 7.510μm, narrowly to broadly ventricose rostrate, infrequent	3264 × 1015 μm, ventricose to ventricose-rostrate, infrequent to scattered
Cheilocystidia		Absent	29.539 × 6710 μm, broadly ventricose-rostrate or obclavate, infrequent
Reaction with NH_4OH	Bluing on pileus surface and ferrugineous-orange on the stipe	Slightly yellowing on pileus, unreactive elsewhere	Slightly yellowing or unreactive on all surfaces

Figure 4. Macromorphology and MEV of the basidiospores. **AD.** *Singerocomus atlanticus*. A,B,D (holotype, *ACM 527),* C (*ACM 1275*). **EF.** *Singerocomus rubriflavus* (*GAS 900*). Bar = 15 mm.

hyphae, individual hyphae 3–7 µm wide, yellow, inamyloid, oleiferous hyphae absent; lateral stratum with hyphae somewhat inflated, individual hyphae 6–13 µm wide, divergent. *Pileipellis* a trichodermium, in mass brownish in H2O, terminal individual hyphae 4–11 µm wide, round apex, with minute incrustations circumferentially, inamyloid. *Pileus trama* interwoven, individual hyphae 4–11 µm wide, hyaline to light yellow in H2O. *Stipitipellis* hymenidermous, sterile terminal elements clavate to cylindrical, 16–28 × 7–11 µm, clusters of caulobasidia present, hyaline to pale yellow in H2O, inamyloid, thin walled. *Stipe trama* slightly interwoven to vertically arranged hyphae, cylindrical, individual hyphae 4–8 μm wide, hyaline to light yellow in H20, inamyloid. *Clamp connection* absent.

Specimen examined. BRAZIL, Bahia: Uruçuca, Parque Estadual da Serra do Conduru, 14 2940.9S 39° 0801.8W, 19 September 2016, G *Alves-Silva 900* (ICN 188271!; GenBank accession: ITS = KY907182, 28S = KY926779).

Habit, habitat, and distribution. Solitary on soil, in the north region of the Atlantic Forest in Bahia. The species was previously known only from Guyana in association with ECM *Pakaraimaea dipterocarpacea* (Cistaceae).

Commentary. *Singerocomus rubriflavus* is a highly distinctive bolete recognized by its medium to large basidiomata with dark red, tomentose-pitted and centrally areolate pileus, and olivaceous yellow, non-cyanescent tubes. and unique squamulose surface of the concolorous, solid stipe. Micromorphologically the species combines ellipsoid basidiospores, a trichodermial pileipellis, and hymenidermous stipitipellis (Henkel *et al.* 2016).

Only one single-basidiome collection of *S. rubriflavus* has so far been made in Brazil, approximately 3.000 km from the type locality in Guyana. While the Brazilian specimen agrees well both macro- and micromorphologically with the Guyana type specimen, minor differences include the smaller stipe squamules and slightly shorter basidiospores (810 *μm* vs. 911(12) *μm*) of the Brazilian specimen. Although the Brazilian specimen and two specimens from Guyana formed a well-supported monophyletic group in our phylogenetic analysis (Fig. 1), the variability in branch lengths among the specimens suggests intraspecific variability. As noted by Henkel *et al.* (2016) similar genetic variability was found among multiple morphologically identical specimens from Guyana, and more research is needed to determine whether *S. rubriflavus* may encompass a species complex.

Discussion

Morphological and molecular analyses corroborate the placement of our boletoid specimens in *Singerocomus* Given that *Singerocomus* was first recognized from an Amazonian species described in *Xerocomus*, renewed taxonomic and phylogenetic scrutiny could be profitably applied to Neotropical Boletaceae. In this respect special attention should be given to the numerous Amazonian boletes described in *Xerocomus* by Rolf Singer (Singer & Digilo 1957; 1960; Singer *et al.* 1983). Most boletoid specimens deposited at INPA are poorly preserved, and many species have only the holotype, these were observed when INPA Herbarium was visited.. Recollections of these species will help to confirm the occurrence of *Xerocomus* species in Brazil and phylogenetic analysis may uncover more new genera.

While the Brazilian Atlantic Forest has long been recognized for its unique plants and animals, macrofungal surveys in the region continue to uncover a wealth of undescribed species. In the present study our discoveries of a new bolete species and a large range extension for another highlight the importance of continued mycological exploration in this unique region.

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ARTIGO IV

A new species of *Gyroporus* (Gyroporaceae, Boletales) from Atlantic Forest in Southern Brazil

Magnago, A.C., Alves-Silva, G., Neves, M.A. & Silveira, R.M.B.

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Gyroporus austrobrasiliensis A.C. Magnago (Foto: Magnago, A.C.)

Nova Hedwigia Vol. xxx (201x) Issue x–x, xxx–xxx **Article**

A new species of *Gyroporus* (Gyroporaceae, Boletales) from Atlantic Forest in Southern Brazil

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With 4 figures and 1 table

Abstract: Surveys of boletoid fungi in the Brazilian Atlantic Forest resulted in the discovery of a new species similar to Gyroporus castaneus named Gyroporus austrobrasiliensis. Macro- and microscopic descriptions, molecular data (nrDNA ITS and LSU sequences), photographs of the basidiomata, and scanning electron microscopy images of the basidiospores are provided.

Key words: boletoid fungi, chestnut bolete, diversity, Sclerodermatineae, taxonomy.

Introduction

Gyroporus Quél. typified by G cyanescens (Bull.) Quél., is a small genus of boletoid fungi in the monogeneric family Gyroporaceae in Sclerodermatineae (Boletales) comprising no more than twenty species (Kirk et al. 2008, Wu at al. 2014, Vizzini et al. 2015, Moreno et al. 2016, Das et al. 2017). Known originally from the temperate zone of north hemisphere, and found usually in coniferous and broadleaf forests, like pines (Pinus L.), oaks (Quercus L.), beech (Fagus L.), sweet chestnut (Castanea Mill.) or birch (Betula L.) as putative ectomycorrhizal host trees but possibly only saprobic, or merely facultatively mycorrhizal (Wilson et al. 2012). There are also reports of the genus in tropical Central and South Americas following the distribution of its possible hosts (Singer et al. 1983, Halling & Mueller 2005, Ortiz-Santana et al. 2007, Flores et al. 2010, Vizzini et al. 2015).

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The genus is perhaps one of the easiest genera of boletes to identify at genus level, characterized by basidiomata epigeous, fleshy, small to medium sized. Pileus surface dry, usually minutely velvety, subtomentose to floccose, firm and leathery, microscopically trichodermal or a cutis of appressed hyphae. Hymenophore tubular, free, with minute, simple and small pores, white then yellowish. Stipe surface villose to velvety, sometimes with horizontal fissures or cracks, context white then straw colored, unchanging or strongly turning blue to violaceus, cavernous or completely hollow at maturity, firm and brittle (breaking like chalk), due to the arrangement of the hyphae that are transversely instead of being vertically arranged like in the most mushrooms. Spore print pale yellow, spores shortly-ellipsoid, smooth, inamyloid, cheilocystidia and pleurocystidia present, clamp-connections usually present (Smith & Thiers 1971, Singer 1986, Watling 2008, Bessette et al. 2010).

Subsequent investigation on boletes diversity in the Brazilian Atlantic Forest revealed a taxon morphologically similar to G. castaneus, but differing in some morphological as well as molecular characters and is here proposed as a new species, G. austrobrasiliensis.

Material and methods

Collection sites and morphology: Collections were conducted during rainy summer season between December to March of 2015 and 2016 at Morro Santana, metropolitan area of Porto Alegre, Southern Region of Brazil. The collecting area is a remaining of seasonal semideciduous forest of about 321 ha, that reaches an altitude of 311m above sea level and is part of the northeast chain of granite hill of Serra do Sudeste, as part of the Atlantic Forest biome. The most representative arborescent families at Morro Santana are Rubiaceae, Myrtaceae, Lauraceae, Sapindaceae, Euphorbiaceae and Fabaceae (Vargas & Oliveira 2007, Silveira & Miotto 2013). Collections were also made at Campeche Island in Florianópolis, Santa Catarina state in March of 2014. The island is covered by Atlantic Forest vegetation and is 1.8 km far from Florianópolis. The first collection of Gyroporus from Southern Brazil in Rio Grande do Sul and the collection from the Amazon forest, both initially identified as G. castaneus, were also included in this study (Singer et al. 1983, Putzke et al. 1994).

Macroscopic features were described from fresh basidiomata. Color codes in brackets (e.g., OAC742) are based on the Online Auction Color Chart (Kramer 2004). Micromorphological features were examined with an Olympus CX21 microscope and descriptive terms follow Largent et al. (1977). For the basidiospores measurements and length/width ratios (Q) are presented here as (minimum)–mean minus standard deviation–**mean**–mean plus standard deviation–(maximum); n, the number of basidiospores measured per specimen; and s, the number of specimens studied. For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomata, mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a scanning electron microscope (operating at 10KeV) at Centro de Microscopia e Microanálise (CMM) at Universidade Federal do Rio Grande do Sul. Voucher materials were deposited at Herbarium ICN from the Universidade Federal do Rio Grande do Sul.

Sequencing and phylogenetic analysis: DNA extraction from dried basidiomata follows Góes-Neto et al. (2005). The primer pairs ITS6-R - ITS8-F and LR0R - LR7 were used to amplify the ITS (ITS1-5.8S-ITS2) and LSU (28S) regions following the cycling conditions in Dentinger et al. (2010). Sequencing was performed with BigDye Terminator 3.1 Cycle Sequencing Kit following the manufacturer's procedure, using the same primers cited above. Sequence chromatograms were manually checked and edited in Geneious 6.1.8 (Kearse et al. 2012) and deposited in GenBank with the accession numbers listed in Table 1.

Alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), following the L-INS–i and G-INS-i criteria (for ITS and LSU, respectively), and then cleaned using Gblocks v0.91b (Talavera &

Castresana 2007), allowing smaller final blocks and gap positions within the final blocks alignments were deposited in TreeBASE (http://www.treebase.org/treebase/index.html). Phylogenetic trees were reconstructed with two phylogenetic criteria, Maximum likelihood (ML) and Bayesian Inference (BI). ML was carried out with RAxML-HPC v.8 (Stamatakis 2014), available in the CIPRES science gateway (Miller et al. 2010, http://www.phylo.org/), using GTRGAMMA as evolutive model (Stamatakis 2006), choosing the rapid bootstrap analysis (command –f a) with a random starting tree and 1000 maximum likelihood bootstrap replications. BI was conducted on MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller et al. 2010), utilizing four parallel MCMC chains, which were allowed to run for 10 million generations, with sampling every 1000 generations. The best-fitted substitution model for for nrLSU gene marker was determined through jModeltest v2 (Nylander 2004) by using Akaike Information Criterion (AIC).

A node was considered well supported if it showed a a bootstrap (BS) ≥ 80% and/or Bayesian posterior probability (BPP) ≥ 0.96. Scleroderma verrucosum was defined as outgroup, based on Wu et al. (2014) and Vizzini et al. (2015). All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

Results

Molecular analysis

Five new sequences of Gyroporus (3 ITS, 2 LSU) were generated during this study, these are the first sequences of Gyroporus for South America. Both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses produced similar topology; however, only the ML trees with both bootstrapping (BS) and Bayesian posterior probability (BPP) values are shown (Figs 1, 2). The ITS data matrix comprised a total of 33 sequences (including 30 from GenBank and 3 new generated), resulted in an aligned matrix of 468 bp. The LSU matrix consisted of 39 sequences (including 37 from GenBank and 2 new generated), resulted in an aligned matrix of 910 bp. In the ITS analysis, the sequences of G. austrobrasiliensis form a clade of tropical taxa, together with Zimbabwe collection (BS = 94%, BPP = 0.8). In the LSU analysis G. austrobrasiliensis form like in the ITS analyses, a sister group with a clade including Gyroporus sp. from Zimbabwe and a collection identified as G. castaneus (BS = 89%, BPP = 1. The molecular phylogenies presented in Figs 1 and 2 provide strong evidence that, along with our morphological studies, indicate G. austrobrasiliensis are distinct taxa in Gyroporus.

Taxonomy

Gyroporus austrobrasiliensis A.C.Magnago Figs 3–4

MycoBank 821923

Holotype: BRAZIL. Rio Grande do Sul: Porto Alegre, Universidade Federal do Rio Grande do Sul, Morro Santana (30°03'48.0"S, 51°07'48.0"W), 12.I.2015, A.C.Magnago 1136 (ICN184399). GenBank accession: ITS = MF436999, LSU = MF437014.

Etymology: from the Latin austro = south, brasiliensis = Brazil. Referring to collection area in Southern Brazil.

Pileus 25–48 mm wide, convex to broadly plane convex, surface velvety to subtomentose, dry, orange-brown (OAC741, 748), margin straight, usually splitting

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis. Unavailable sequences for individual taxa are indicated by –.

Taxon	Voucher No.	Locality	GenBank acession No.	
			ITS	LSU
Gyroporus ammophilus	AH45814	Spain	KX869878	KX869892
Gyroporus ammophilus	AH45843	Spain	KX869877	KX869891
Gyroporus ammophilus	AH45842	Spain	KX869876	KX869890
Gyroporus austrobrasiliensis	ACM1136	Brazil	MF436999	MF437014
Gyroporus austrobrasiliensis	ACM1144	Brazil	MF437000	MF437015
Gyroporus austrobrasiliensis	ACM1150	Brazil	MF437001	–
Gyroporus aff. castaneus	REH8804	Thailand	EU718101	EU718137
Gyroporus aff. castaneus	E4600	–	–	EU718169
Gyroporus aff. castaneus	E843c	–	–	EU718170
Gyroporus castaneus	AH45841	Spain	KX869875	KX869889
Gyroporus castaneus	AH45844	Spain	KX869874	KX869888
Gyroporus castaneus	JMP0028	USA	EU819468	–
Gyroporus castaneus	SGT 2012	USA	JX030211	–
Gyroporus castaneus	PRL5664	USA	GQ166901	–
Gyroporus castaneus	PRL5872MAN	USA	GQ166884	–
Gyroporus castaneus	Gc1	Germany	EU718099	AF336252
Gyroporus castaneus	Arora 01 512	–	–	FJ710209
Gyroporus castaneus	HKAS76672	China	–	KF112478
Gyroporus castaneus	NCJ16	–	–	AY612808
Gyroporus castaneus	239-97	USA	–	AF336253
Gyroporus castaneus	Gc2	Germany	–	EU718168
Gyroporus castaneus	F1086418	–	–	EU718167
Gyroporus castaneus	F:PRL5664MAN	USA	GQ166887	–
Gyroporus castaneus	239/97	–	EU718100	–
Gyroporus castaneus	F:PRL5948MAN	USA	GQ166885	–
Gyroporus castaneus	3861	Canada	KM248947	–
Gyroporus aff. cyanescens	REH8821	Australia	EU718103	EU718139
Gyroporus aff. cyanescens	E486	Australia	–	EU718173
Gyroporus aff. cyanescens	E5685	Australia	–	EU718174
Gyroporus aff. cyanescens	REH8819	USA	–	EU718172
Gyroporus cyanescens	AH535	Spain	KX869879	KX869893
Gyroporus cyanescens	Gcy2	USA	–	AF336254
Gyroporus cyanescens	17184	Italy	JF908785	–
Gyroporus cyanescens	2837	Canada	KM248948	–
Gyroporus cyanescens	MB05-04	USA	EU718102	–
Gyroporus cyanescens	REH8758	Australia	–	EU718171
Gyroporus cyanescens	MCVE:28580	Italy	KT363684	KT363685
Gyroporus cyanescens	MB05-001	USA	–	EU718138
Gyroporus cyanescens	NAMA 190	USA	EU819495	–
Gyroporus lacteus	MCVE:28582	Italy	KT363682	KT363683
Gyroporus longicystidiatus	2B10	Japan	AB973746	–
Gyroporus paramjitii	KD 16-002	India	MF120284	MF120285

Gyroporus pseudocyanescens	OKM23719	Australia	–	EU718140
Gyroporus purpurinus	PRL3737	USA	EU718105	EU718141
Gyroporus sp.	REH8805	–	–	EU718175
Gyroporus sp.	REH8799	Thailand	EU718106	EU718142
Gyroporus sp.	Arora00-029	Zimbabwe	EU718107	EU718143
Gyroporus sp.	HKAS:63505	China	–	KF112476
Gyroporus sp.	HKAS:52520	China	–	KF112475
Gyroporus subalbellus	OKM25477	USA	EU718108	EU718144
Gyrporus sp.	E8155	Australia	–	EF561627
Gyrporus sp.	E4879cRBG Kew	–	–	FJ710208
Scleroderma verrucosum	K(M)54413	–	EU784416	–
Scleroderma verrucosum	Isolate 5	–	–	AF336271

in age. Tubes 3–4 mm long centrally, whitish to light yellow (OAC815, 816), free, depressed around stipe; pores 2–4 per mm, circular, unchanging under pressure. Stipe 22–36 mm × 3–4 mm, central, equal, brittle, developing several cavities or becoming hollow, surface like the pileus, paler especially near the apex. Context whitish (OAC 816), brittle, unchanging when exposed. Macrochemical reactions: $10\%NH_4OH$ on pileus staining pink-orange and on stipe surface orange. 5%KOH on pileus staining yellow-orange. Spore print pale yellow.

Basidiospores (6.0)–7.0–**7.5**–8.0 × 5.0–**5.5**–6.0 µm; Q = 1.2–**1.45**–1.6; n/s = 30; s = 11) ellipsoid to broad ellipsoid, hyaline in H_2O, inamyloid, smooth, thin walled. Basidia 21–27 × 11–12 µm, clavate, thin walled, hyaline, 4-sterigmate, 2–3 µm long. Pleurocystidia scattered or rarely present, usually not projecting over the hymenium, aculeate to fusoid, 26–31 × 10–12 µm, hyaline, inamyloid, smooth, thin walled. Cheilocystidia very abundant, 15–33 × 4–6 µm, fusoid, cylindrical to narrowly ventricose, hyaline, inamyloid, smooth, thin walled. Hymenophoral trama boletoid, mediostratum with narrow hyphae, individual hyphae 3–8 µm wide, slightly circumferentially incrusted; lateral stratum divergent, hyphae gelatinized. Pileipellis as a trichodermium composed of clusters of terminal elementes; these elements 28–56 × 9–15 µm, tapered to a subacute apex, most hyaline, however, some hyphae with yellowish contents, smooth, slightly thick-walled, inamyloid; inflated hyphae near the base of trichodermium. Pileus trama interwoven, individual hyphae 5–22 µm wide, hyaline. Stipitipellis a trichodermium composed by caulocystidia cylindrical, narrowed fusoid to clavate, like pileocystidia, 4–11 µm wide, hyaline, inamyloid. Stipe trama composed of hyphae circumferentially arranged (not longitudinal), more compacted near the stipitipellis and becoming loosely towards the center, hyaline, inamyloid. Clamp connection present, more visible in the trama.

Habit, habitat and distribution: the specimens examined were collected during rainy summer (Dec-Jan), growing gregarious to scattered on the ground, under broad-leaf trees (Myrtaceae and Lauraceae) in Southern Atlantic Forest in Rio Grande do Sul and Santa Catarina, and under leguminous trees in the Amazon forest (Singer et al. 1983).

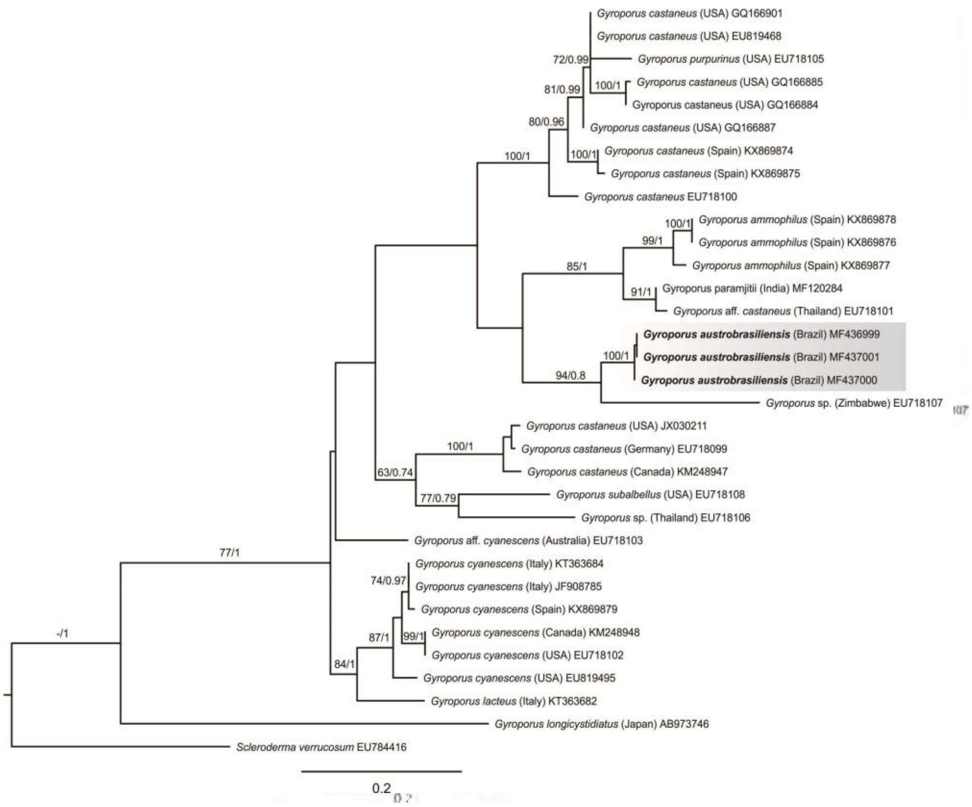
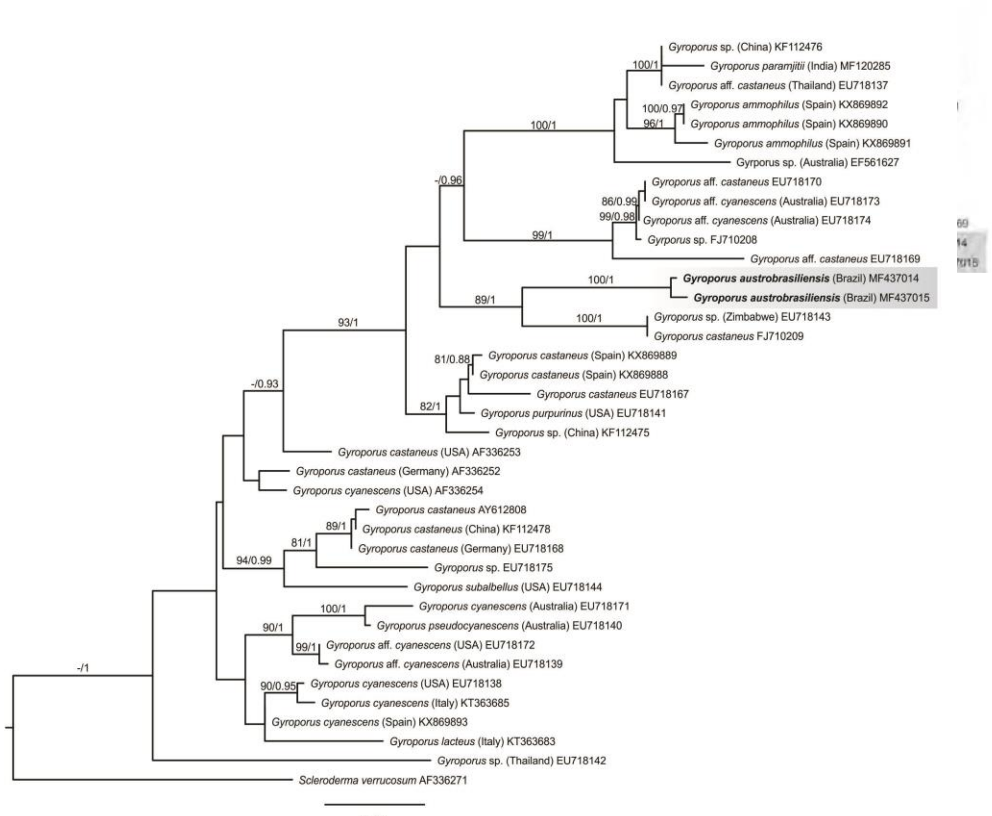


Fig. 1. Maximum likelihood tree of selected *Gyroporus* based on ITS sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.9 are shown.

Collections examined: BRAZIL. Rio Grande do Sul: Porto Alegre, Morro Santana, 16.I.2015, A.C.Magnago 1144 (ICN184400), 1148 (ICN184401), 1150 (ICN184402), 28.XII.2015, A.C.Magnago 1251 (ICN192821), 1252 (ICN192822), 1253 (ICN192823), 4.I.2016, A.C.Magnago 1260 (ICN192824), 9.I.2017, A.C. Magnago 1335 (ICN192826); Pelotas, Horto Botânico, 6.IV.1960, Edemar C.Santos 161, det. Singer B. 7.XI.1961 (SP60960); Santa Catarina: Florianópolis, Ilha do Campeche, (27°41'57.6"S, 48°28'01.6"W) 24 Mar 2014, A.C.Magnago 1021 (ICN192825).

Additional collection examined: BRAZIL. Amazonas: São Gabriel da Cachoeira, 23.I.1979, Singer R B11487 (INPA82388).

Commentary: Morphologically and phylogenetically *G. austrobrasiliensis* falls into *G. castaneus* complex, in the non-cyanescent group of *Gyroporus* species. Sharing the orange brown tones of the pileus and stipe, white to yellowish hymenophore, pale yellow spore print, and unchanging context. Using those macrocharacters usually specimens are identified widely as *G. castaneus*, and its distribution considered widespread. Microscopically *G. austrobrasiliensis* differs from *G. castaneus* as it circumscribed by the presence of smaller basidiospores, usually *G. castaneus* in the north hemisphere has basidiospores larger (8–13 × 5–6 µm) (Quélet 1886), and the



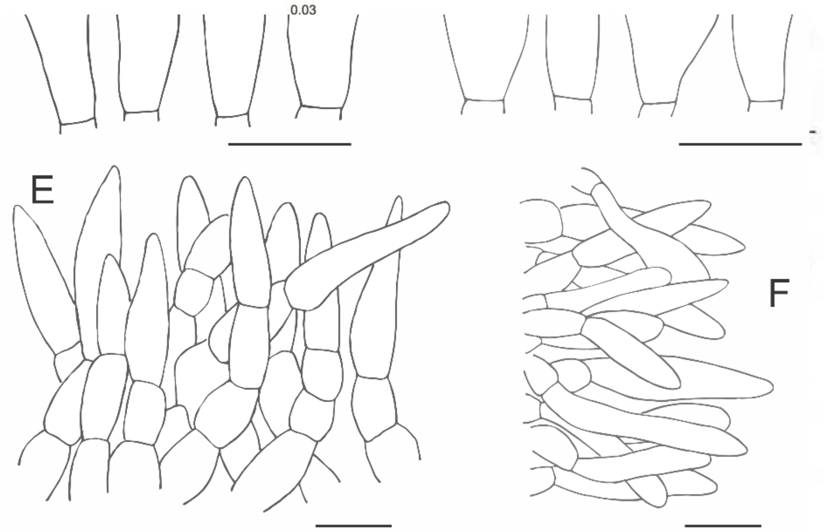
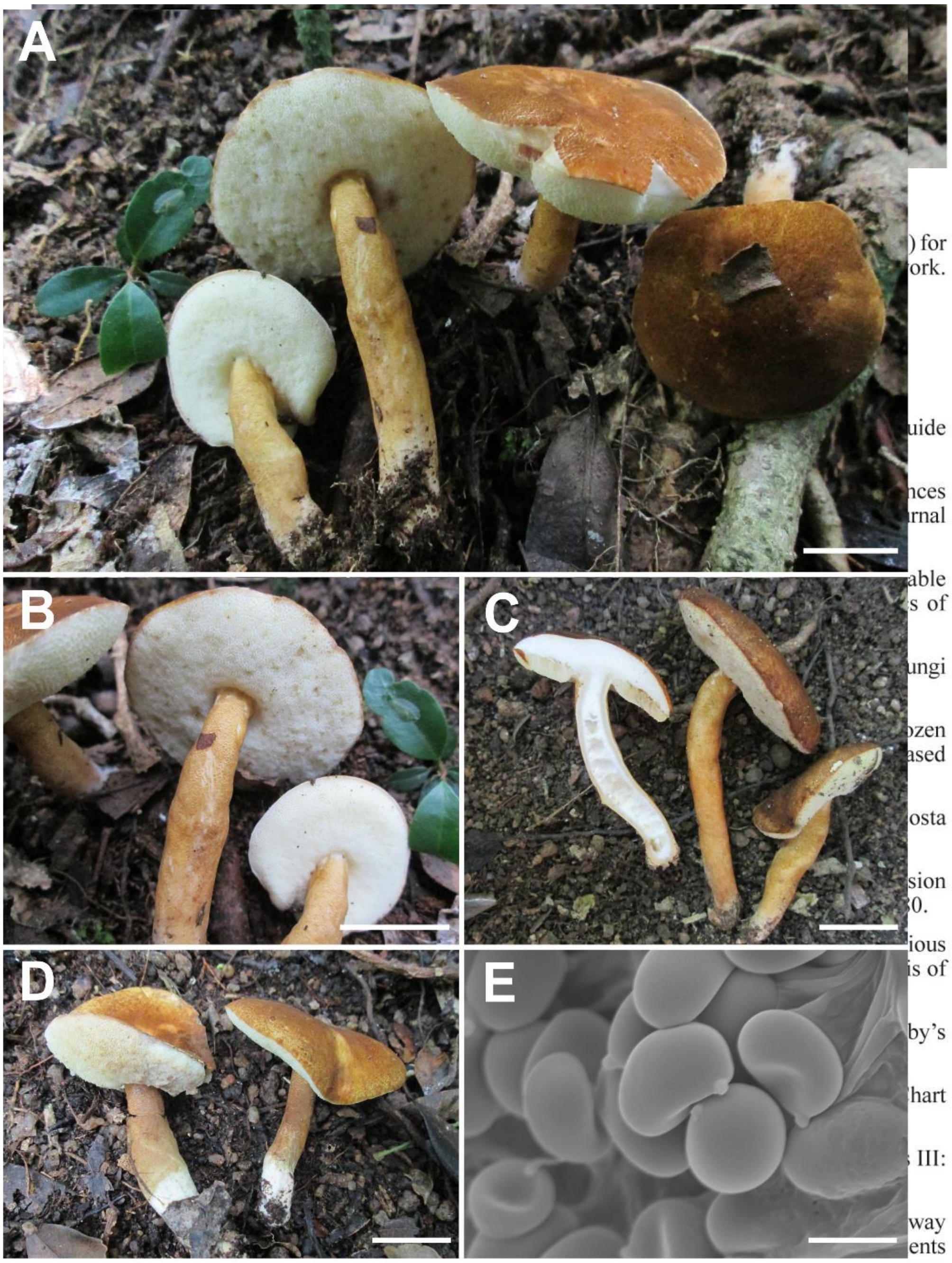


Fig. 3. **Gyroporus austrobrasiliensis**: micromorfological features. A. Basidiospores, B. Basidia, C. Pleurocystidia, D. Cheilocystidia, E. Pileipellis, F. Stipitipellis. Bar A–D = 10 µm, E–F = 25 µm.

Smith & Thiers (1971) commented that the range of colors and sizes presented in the collections of specimens identified as G. castaneus in the USA could cover more than one species. This idea was reinforced by molecular analyses by Wilson et al. (2012), Vizzini et al. (2015) and our studies, suggesting that the name G. castaneus as commonly ascribed to field and herbarium collections represents multiple cryptic taxa (complex of species), instead of a single species having a wide geographic distribution.



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ARTIGO V

Boletellus nordestinus (Boletaceae, Boletales), a new species from Northeastern Atlantic Forest, Brazil

Magnago, A.C., Neves, M.A. & Silveira, R.M.B.

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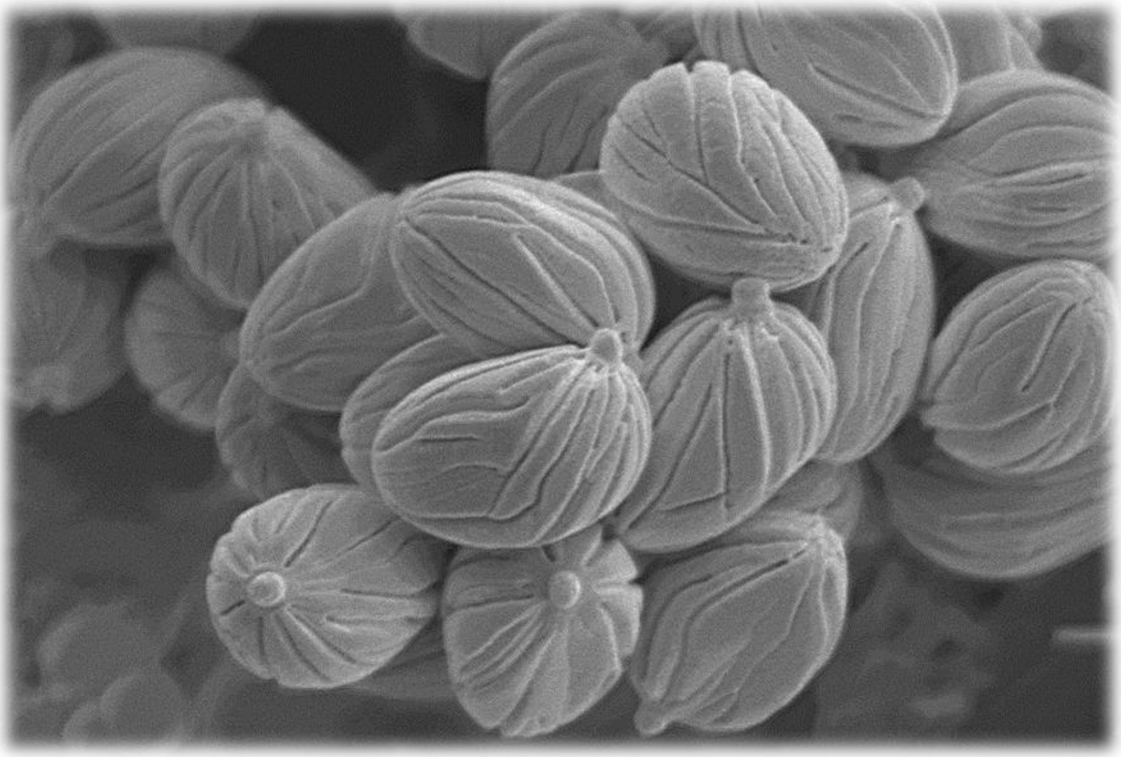

Esporos em MEV de *Boletellus nordestinus* A.C. Magnago (Foto: Magnago, A.C.)

Boletellus nordestinus (Boletaceae, Boletales), a new species from Northeastern Atlantic Forest, Brazil

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Magnago et al. (2018) *Boletellus nordestinus* (Boletaceae, Boletales), a new species from Northeastern Atlantic Forest, Brazil. – Sydowia XX (X): pp-pp.

Boletellus nordestinus is described as a new species from white sandy dunes in the Northeastern Brazilian Atlantic Forest. The area is known as a potential ectotrophic forest, since other ectomycorrhizal fungi had been found in the same area. The species is characterized by pileus dry, velutinous, olivaceus to chocolate brown, context and hymenophore non-cyanescent when exposed or pressured, basidiospores short, longitudinally ridged, cystidia fusiform to ventricose, and pileipellis trichodermal with disarticulated, acute terminal elements. Discussion, description, molecular data, drawings and photographs of the new species are provided.

Keywords: Agaricomycetes, boletoid fungi, Neotropics, taxonomy.

Boletellus Murrill (Boletaceae, Boletales, Basidiomycota) is a small group of ectomycorrhizal fungi forming mutualistic relationship with different host trees (Fugenzi *et al*. 2008, Tedersoo *et al.* 2010). Morphologically the genus encompasses 72 described species worldwide, the majority reported from tropical regions (Heinemann & Goossens-Fontana 1954, Snell & Dick 1970, Singer 1970, Smith & Thiers 1971, Corner 1972, Horak 1977, Singer 1986, Singer *et al.* 1992, Gómez 1996, Watling 2001, Halling & Mueller 2005, Ortiz-Santana *et al.* 2007, Fulgenzi *et al.* 2008, Halling & Ortiz-Santana 2009, Halling *et al.* 2015, Sato & Hattori 2015, Barbosa-Silva et al. 2017). The type species designated for the genus was the North American *B. ananas* (M.A.Curtis) Murrill (Murril 1909). According to authors, *Boletellus* has been variably defined, having in common: basidiospores olivaceous brown in deposit, yellow hymenophore becoming olivaceous with maturity and boletoid tube trama. Singer (1986) in the traditional morphological concept adhered a broad definition for *Boletellus* including seven sections based by the basidiospores ornamentation: smooth basidiospores (section *Mirabilis*); basidiospores with imbedded short spines or pits (section *Allospori*); reticulate basidiospores (section *Retispori*) and longitudinally winged or ridged basidiospores (sections *Boletellus*, *Chrysenteroidei*, *Ixocephali* and *Dictyopodes*) using morphological characters of pileus and stipe to separate sections. However, recent molecular phylogenetic studies indicate that such characters may not delimit clades in Boletaceae and *Boletellus* as morphologically circumscribed is polyphyletic (Dentinger *et al.* 2010, Nuhn *et al.* 2013, Wu *et al.* 2014, Halling *et al.* 2015). *Boletellus* s.str. may only include the two sections identified by Singer (1986), sect. *Boletellus* and sect. *Chrysenteroidei* Singer, with a length/width ratio of basidiospores over two. Many species that were traditionally classified in *Boletellus* s.l., especially those without longitudinally winged or ridged spores have been recently accommodated into new genera such as *Aureoboletus* Pouzar, *Heimioporus* E. Horak, and *Hemileccinum* Šutara.

For South America, few species are registered, and for Brazil only four *Boletellus* species are cited: *B. ananas* (M.A. Cutis) Murril for the Amazon Forest (Singer *et al.*1983), *B. lepidospora* E.-J.Gilbert ex Heinem., *B. pustulatus* (Beeli) E.-J.Gilbert and *Boletellus cremeovelosus* Barbosa-Silva & Wartchow for the Atlantic Forest (Oliveira & Sousa 1995, Barbosa-Silva & Wartchow 2017). Here we describe *Boletellus nordestinus* sp. nov. discovered in ectomycorrhizal white sandy dunes in the extreme north Atlantic Forest. In this same area other ectomycorryzal fungi (e.g. *Amanita* Pers., *Coltricia* Gray, *Lactifluus* (Pers.) Roussel, *Russula* Pers., *Scleroderma* Pers., *Tylopilus* P. Karst.) have been collected and described recently (Baltazar *et al.* 2010, Sá *et al.* 2013, Sulzbacher *et al.* 2013, Wartchow *et al.* 2013, Barbosa-Silva *et al.* 2017, Magnago *et al.* 2017).

Materials and Methods

Sampling

Collections were made in 2009 and 2016 in the Northeastern region of Brazil in the coastal Atlantic Forest, at “Reserva Biológica Guaribas”, municipally of Mamanguape, Paraíba State and at “Parque Estadual Dunas de Natal”, municipally of Natal, Rio Grande do Norte State. In both areas, the specimens were collected in white sandy soil. Macroscopic features were described from fresh basidiomata and dried with a food dehydrator (Total Chef TCFD-05 Deluxe) at about 40ºC. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Macrochemical tests were performed according to Singer (1986). Microscopic structures were rehydrated in 3% KOH and dyed with Congo Red (Largent et al. 1977). To observe the surface of the basidiospores in scanning electron microscopy (SEM), small fragments of the hymenophore were taken from dried specimens and mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a JEOL JSM-6390LV scanning electron microscope, operating at 10KeV, at the "Centro de Microscopia e Microanálise da Universidade Federal do Rio Grande do Sul". All microscopic features were line drawings by hand using digital photographs from the specimens examined. Voucher materials were deposited at UFRN, FLOR and ICN (Thiers, continuously updated).

Molecular data

DNA was extracted from dried specimens followed Doyle & Doyle (1987) protocol adapted by Góes-Neto et al. (2005). Nuclear ribosomal internal transcribed spacer nrITS (ITS1-5.8S-ITS2) and nuclear ribosomal large subunit (nrLSU) were PCR-amplified with primer pairs ITS6-R/ITS8-F (Dentinger et al. 2010) and LR0R/LR7 (Vilgalys & Hester 1990) respectively. Complementary unidirectional sequence reads were aligned and edited in Geneious 6.1.8 (Kearse et al. 2012) and deposited in GenBank with accession numbers listed in materials examined.

Taxonomy

Boletellus nordestinus A.C. Magnago **sp. nov.** – Figs. 1 - 2.

MycoBank no.: MB 823951

Diagnosis. – Differs from the other *Boletellus* non-cyanescent species with the combination of dry, velutinous, chocolate brown pileus, short basidiospores, pleurocystidia and cheilocystidia fusiform to ventricose, and pileipellis trichodermal with acute terminal elements.

Holotypus. – BRAZIL, Rio Grande do Norte State, Natal, Parque Estadual das Dunas de Natal, 5°50'40"S, 35°11'25,2"W, Trilha da Perobinha, 08 June 2016, Fazolino, E.P. & Assis, N.M. s/n (UFRN-Fungos 2726), GenBank accessions: ITS = MG760443, 28S = MG760444).

Description. – Pileus 38−66 mm wide, parabolic at first to convex when mature, velutinous to slightly fibrillose towards the margin, olivaceous to chocolate brown (OAC 733,735), dry; margin slightly uplifted when mature; context whitish to cream yellow (OAC 812), solid, unchanging when exposed. Hymenophore tubular, depressed around stipe, with decurrent tooth, tubes 5−8 mm long centrally, bright yellow (OAC 854, 895) when young maturing to olive yellow (OAC 10, 19), pores concolorours with tubes, not bluing under pressure, mostly 1−2 mm wide, subangular. Stipe 28−47 mm × 5−8 mm central to eccentric, equal, surface longitudinally slightly rugulose with short squamules, light yellow to pinkish (OAC 12,14), some specimens more brownish to vinaceous brown (OAC 636, 638), context solid, fibrillose, cream yellow (OAC 812) near the apex becoming vinaceous brown downwards (OAC 638), unchanging when exposed, basal mycelium white.

Basidiospores olivaceous-brown to dark olive-green (OAC 866, 867) in deposit, (7.5) 8−10 (11) × 6−7 (8) µm (Qm=1.40), broadly ellipsoid, longitudinally ridged, dichotomously forked, occasionally non-forked, terminating in unequal lengths near apex, ridges converging at hilar appendage, pale yellow in H_2O and KOH, inamyloid, thick-walled, hilar appendage 0.5−1 µm long. Basidia 27−42 × 12−14 µm, clavate, thin-walled, hyaline in H_2O, inamyloid; 4-sterigmate, 2−3 µm long. Pleurocystidia and Cheilocystidia not differentiated from each, 36−56 × 7−12 µm, fusiform to ventricose, hyaline in H_2O, inamyloid. Hymenophoral trama boletoid, hyphae 4−12 µm wide, hyaline in H_20, inamyloid, smooth and thin-walled. Pileipellis trichodermal, hyphae disarticulated, terminal hyphae acute, brownish in H_2O, inamyloid, terminal hyphae 8−15 µm wide; oileferous hyphae present. Pileus trama interwoven, hyphae 6−16 µm wide, light yellow in H_2O. Stipitipellis repent, with projecting tufts of caulobasidia and caulocistidia, clavate, fusiform to sphaeropedunculate, 28−38 x 13−15µm, hyaline in H_2O, inamyloid. Stipe trama parallel to subparallel, hyphae 5−12 µm wide, light yellow, in H_2O inamyloid. Clamp connections absent.

Etymology. – Referring to the Northeastern region (“Nordeste” in Portuguese) where the specimens were collected.

Habitat. – Solitary to scattered, growing on white sandy soil and dunes in *restinga* vegetation in the far north of coastal Atlantic Forest. The vegetation is dominated by species of Leguminosae, Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae (Freire 1990).

Geographic distribution. – Known from Northeastern Brazilian region in Paraíba and Rio Grande do Norte States.

Material examined (paratypes). BRAZIL, Paraíba State, Mamanguape, Reserva Biológica Guaribas, SEMA II, 6°44'14"S, 35°8'55"W, 15 August 2009, coll. Neves M.A. MAN481 (FLOR51603), 23 September 2009, coll. Neves, M.A., Barbora, M.R.V. & Thomas, W. MAN502 (FLOR51604); Rio Grande do Norte State, Natal, Parque Estadual das Dunas de Natal, 5°50'40"S, 35°11'25,2"W, Trilha da Geologia, 07 June 2016, coll. Fazolino, E.P. & Neto, J. F. F. s/n (UFRN-Fungos 2725) GenBank accessions: ITS = MG760442; 5°50'39"S, 35°11'25,4' Trilha da Perobinha, 08 June 2016, coll. Fazolino, E.P & Assis, N.M. s/n (UFRN-Fungos 2729).

Molecular data

Three new sequences of *Boletellus nordestinus* sp. nov. were generated (2 nrITS and 1 nrLSU). ITS BLASTn queries of the new taxon on GenBank indicated affinities with specimens in the genera *Boletellus* Murrill, *Boletus* L. and *Aureoboletus* Pouzar, with queries under 80%. LSU BLASTn queries indicated affinities with Boletaceae, but were uninformative at the genus level.

Discussion

Boletellus nordestinus is best disposed infragenerically in *Boletellus* section *Chrysenteroidei* Singer based on the longitudinally winged spore, perpendicular striations absent, pileus lacking red colors, veil absent, pileus and stipe not glutinous or viscid, stipe not lacunose-alveolate and clamp connections absent (Singer 1986). *Boletellus nordestinus* differs from the other Neotropical *Boletelus* accommodated in section *Chrysenteroidei* (Singer 1983). *Boletellus fibuliger* Singer from Venezuela has pileus brownish fibrillose over a yellowish ground, less fibrillose towards the margin, not or scarcely appendiculate margin, larger basidiospores (17−20 × 9.5−11 µm), cystidia ampullaceous, and clamp connections present. *Boletellus cubensis* (Berk. & M.A. Curt.) Singer from the eastern Caribbean, has squamulose pileus, red annular band on the stipe apex, bluing hymenophore when bruised, and the basidiospores are larger (20−22.5 × 7.5−10 µm). *Boletellus chrysenteroides* (Snell) Snell registered in Canada, USA and Honduras has larger basidiospores (9.5−18.2 × 5.3−9.8 µm) longitudinally rigded or winged, which are intervenose or themselves transversely striate, and is usually found growing under conifers. *Boletellus exiguus* T.W. Henkel and Fulgenzi from Guyana differs by the velutinous, finely squamulose and areolate pileus when mature, margin slightly inrolled when young, context and hymenophore bluing rapidly with exposure or pressure; pleurocystidia with golden incrustations in Melzer's, and a pileipellis of angular cells with tufts of erect inflated terminal elements (Fulgenzi *et al.* 2008). *Boletellus dicymbophilus* Fulgenzi and T.W. Henkel from Guyana has red-brown to yellow-tan, rugulose to rugose pileus, hymenophore instantly bluing on exposure or pressure, and larger basidiospores (12−15 × 8−11 µm) (Fulgenzi *et al.* 2008). Among the two Congolian *Boletellus* spp cited by Oliveira & Sousa (1995) for Atlantic Forest, Brazil, *Boletellus pustulatus* possesses ventricose-rostrate cystidia and a bluing reaction in the exposed context (Gilbert 1931). *Boletellus lepidospora* has rugulose brown to blackish pileus, stipe hollow towards the base, context yellow to light brown that becomes blue when exposed, basidiospores 11−12.5 × 6−7.4 µm, cystidia lanceolate (45−53 × 12−13 µm) and pileipellis composed by subglobose terminal elements (Heinemann & Goossens-Fontana 1954). These two species cited for the Atlantic Forest should be recollected in the area to confirm its identification, because in the description of the Brazilian species by Oliveira & Sousa (1995) the context when exposed are non-cyanescent, and this is a important character of both species cited by Gilbert (1931) and Heinemann & Goossens-Fontana (1954). *Boletellus cremeovelosus* differs by pileus beige, squamose, margin strongly appendiculate, context and hymenophore bluing when exposed and pressured, and basidiospores subfusoid, longitudinally ribbed (15.8–20.4 × 6.6–9.2).

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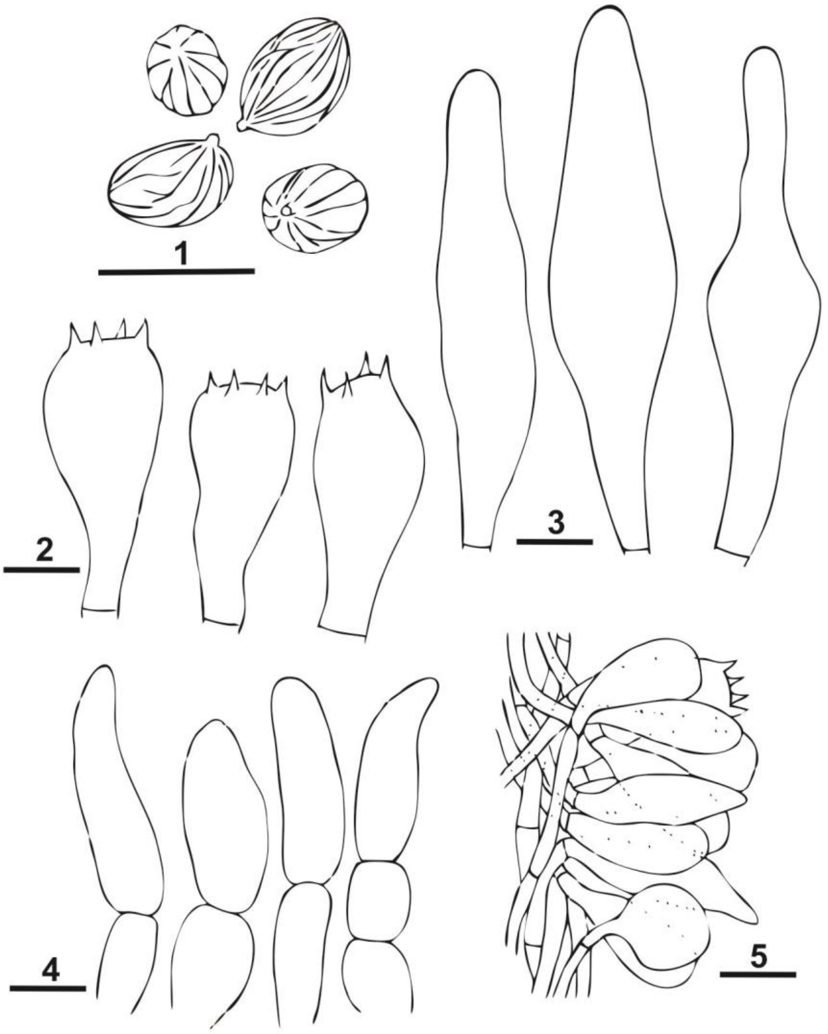
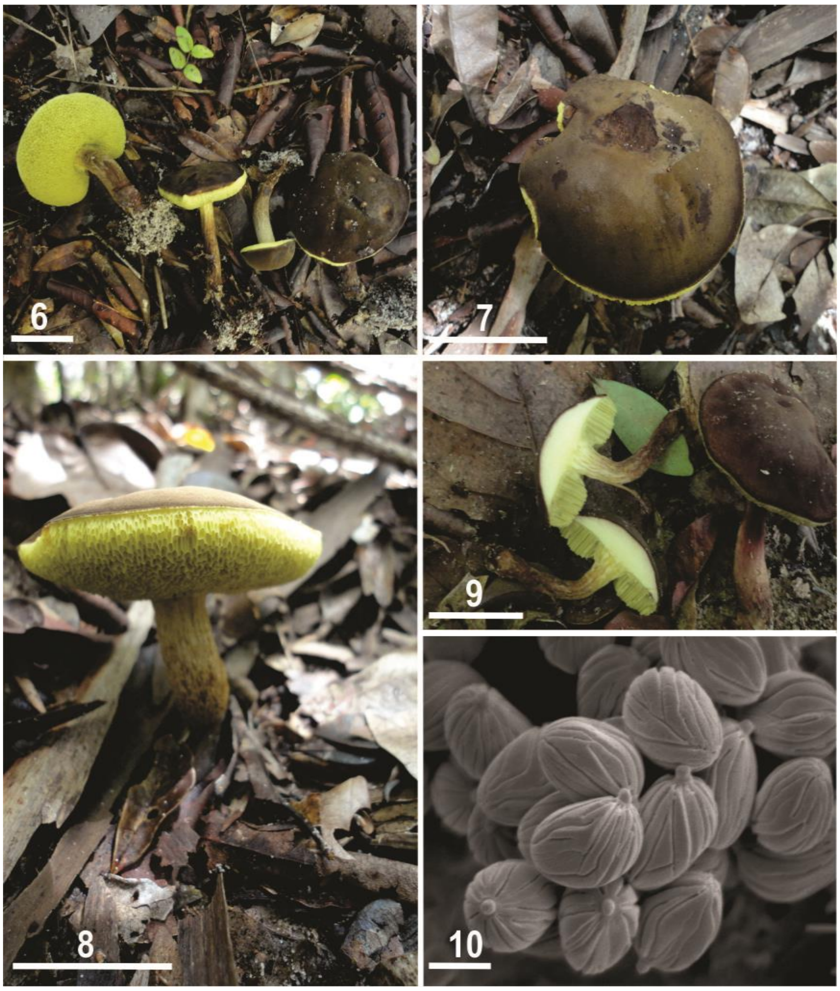


Figs. 1-5. Micromorphology of *Boletellus nordestinus* (holotype): **1.** Basidiospores. **2.** Basidia. **3.** Cystidia. **4.** Terminal cells of pileipellis from the pileus center. **5.** Stipitipellis, cluster of caulobasidia and caulocystidia. Bars 10 µm.



Figs. 6-10. Macromorphology in the field and SEM of the basidiospores of *Boletellus nordestinus*: **6.** Voucher UFRN-Fungos2721 (holotype). **7.** Detail of pileus surface (UFRN-Fungo 2725). **8.** Detail of the hymenophore (UFRN-Fungos 2725). **9.** Basidiomata in cross section (UFRN-Fungo 2729) (bar = 3 cm). **10.** SEM micrograph of basidiospores (FLOR51604) Bars 5 µm.

ARTIGO VI

Additional notes towards morphological and molecular data of *Phlebopus* and *Boletinellus* (Boletinellaceae, Boletales) from Brazil.

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Boletinellus exiguus (Singer & Digilio) Watling (Foto: Magnago, A.C.)

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Additional notes towards morphological and molecular data of *Phlebopus* and *Boletinellus* (Boletinellaceae, Boletales) from Brazil.

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ABSTRACT

Boletinellus and *Phlebopus* are boletoid genera in the Sclerodermatineae group with distribution mostly tropical. For Brazil three *Boletinellus* species are registered, and based on our recent collections, their geographic distribution in Brazil is enlarged. After analyzing morphologically and molecularly new collections of *Phlebopus* from many areas in Brazil, and reexamining the holotype of *P. brasiliensis,* we concluded that this species is a synonym of *P. beniensis*. Sequences of nuclear ribosomal DNA internal transcribed spacer regions were generated for comparisons with similar species. Descriptions, photographs, line drawings, identification key and commentaries are provided for each species.

KEY WORDS

Atlantic Forest, Boletoid fungi, Neotropic, Systematic, Taxonomy

1. INTRODUCTION

Boletinellus Murril and *Phlebopus* (R. Heim) Singer belong to Boletinellaceae P.M. Kirk, P.F. Cannon & J.C. David, and are considered an early-diverging boletoid lineage within the ectomycorrhizal suborder Sclerodermatineae Manfr. Binder & Bresinsky, which includes a few boletoid forms and a great diversity of gasteroid fungi (Binder & Bresinsky 2002; Binder & Hibbett 2006; Wilson et al. 2012).

Boletinellus was proposed by Murrill (1909), to accommodate *Daedalea merulioides* Schwein. The genus is characterized by basidomata with pileus circular to dimidiate, surface dry, minutely tomentose to floccose-tomentose, context white to yellowish, fleshy, tubes decurrent, pores large, elongate, shallow, radiating, yellow, not covered by a veil; stipe central, eccentric or lateral, solid, and fleshy; basidiospores short ellipsoid to subglobose, smooth, olivaceous brown, and the presence of clamp connections. The genus is considered mostly Neotropical, with distribution for Argentina, Bolivia, Brazil, Martinique, and United States of America (Singer & Digillo 1960; Pegler 1983; Singer et al. 1983). *Boletinellus merulioides* which is related to *Fraxinus* spp. was also registered in Japan, following the distribution of the host (Nagasawa 2001).

Phlebopus was proposed by Heim (1936) as a subgenus of *Boletus* accommodating *Boletus colossus* R. Heim., as the type species, and posteriorly elevated to genus by Singer (1936). The genus is considered pantropical, registered for Africa, Asia, Australia, and South America (McNabb 1968; Heinemann & Rammeloo 1982; Singer et al. 1983; Watling & Gregory 1988; Watling & Turnbull 1992; Li & Watling 1999; Pham et al. 2012; Baroni et al. 2015). *Phlebopus* is characterized by boletoid basidiomata with typically robust stature with a central, thick, non-hollow stipe, small round pores; short, smooth and olivaceous brown basidiospores, and abundant clamp connections (Singer et al. 1983; Singer 1986; Watling 2008).

The ectomycorrhizal roles of *Phlebopus* and *Boletinellus* are suspect. Studies showed a tripartite relationship for *Boletinellus merulioides* (Schwein.) Murrill involving *Fraxinus* spp. intermediated by aphids (Brundrett & Kendrick 1987; Tedersoo et al. 2009; Wilson et al. 2012), but it is also treated as facultative mycorryzal or just saprotrophic (Singer et al. 1983; Sulzbacher et al. 2013). For *Phlebopus,* studies have reported as saprotrophs as well as ectomycorrhizal fungi or only facultative mycorrhizal, and a few can form an unusual relationship with scale insects and plant root pathogens (Singer 1986; Thoen & Ducousso 1989; Sanmee et al. 2010; Pham et al. 2012; Sulzbacher et al. 2013; Baroni et al. 2015). Nouhra et al. 2008, after investigating the relation between *Phlebopus bruchii* (Speg.) Heinem. & Rammeloo and *Fagara coco* (Gillies ex Hook. f. & Arn.) Engl. (Rutaceae) no ectomycorrhizal (ECM) colonization was detected. The nature of the associations in Boletinellaceae is not discussed in this study, but needs further investigation.

The present paper is part of the investigation on diversity of boletoid fungi from Brazil (Magnago & Neves 2014; Magnago et al 2017a,b; Magnago 2018a,b). Molecular data of *Boletinellus exiguus* (Singer & Digilio) Watling and *Boletinellus rompelii* (Pat. & Rick) Watling are provided, as well as morphological description of recent collections from Brazil. Specimens of *Phlebopus* were collected from many localities in Brazil and based on new collections and molecular evidence we propose to synonymize *Phlebopus brasiliensis* Singer as *P. beniensis* (Singer & Digillo) Heinem. & Rammeloo, since the characters used to separate the species overlap between them and it is not corroborated by molecular analyses as separated species.

2. MATERIAL AND METHODS

2.1. Sampling

Collections were made between 2008 and 2017 in many localities of Brazil, including Amazon Forest, Atlantic Forest and *Cerrado* (Savanna vegetation) (Figure 1). Macroscopic features were described from fresh basidiomata and dried with a food dehydrator (Total Chef TCFD-05 Deluxe) at about 40ºC. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Microscopic structures were rehydrated in 3% KOH and dyed with Congo Red. All microscopic features were line drawings by hand using digital photographs from the specimens examined. Voucher materials were deposited at ICN herbaria (Thiers, continuously updated).

2.2. DNA isolation and amplification

Genomic DNA was extracted from dried specimens with CTAB method. We amplified nuc rDNA ITS1-5.8S-ITS2 (ITS) using primers ITS6-R/ITS8-F (Dentinger et al. 2010). Polymerase chain reaction (PCR) was performed in a total volume of 30 μL containing 1 unit Taq DNA polymerase, 3.0 μL of 10 × Taq polymerase reaction buffer (Applied Biological Material Inc., Vancouver, Canada), 3.0 μL of bovine serum albumin solution (Sigma-Aldrich, Missouri), 50 μM of dNTP mix, 0.75 μL of 10 μM each of the two primers, and 1–2 μL of total DNA. PCR amplification of ITS was performed with 2 min initial denaturation at 95 C, followed by 35 cycles of 30 s at 95 C, 1 min and 30 s at 57 °C, 30 s at 72 °C, with a final extension of 10 min at 72 C following the last cycle.

2.3. Alignment and phylogenetic reconstructions

The new sequences as well sequences download from GenBank, following cited literature, were assembled and manually corrected with Geneious 9.1.4 (Kearse et al. 2012). The ITS matrix from Wilson et al. (2012) corresponding to Sclerodermatineae was the backbone in our dataset. Automatically we aligned with MAFFT 7 (Katoh & Standley 2013) under the auto mode for strategy. The alignment was manually adjusted when necessary with MEGA 7.0.20 (Kumar et al. 2016). Two specimens of *Boletellus projectellus* (Murrill) Singer (AY789082) and *Aureoboletus thibetanus* (Pat.) Hongo & Nagas (DQ200917) were designated as outgroup. All materials and sequences used in this study are listed in Table I.

Phylogenetic analyses were all performed online on the CIPRES Science Gateway (Miller et al. 2010). We analyzed the dataset with maximum likelihood (ML) and Bayesian Inference (BI). Maximum likelihood analysis was carried out in RAxML 8.2.9 (Stamatakis, 2014). The analysis first involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree under a GTRGAMMAI model, with all parameters estimated by the software. We provided a partition file to force RAxML software to search for a separate evolution model for each dataset. To access the reliability of the nodes, rapid bootstrapping replicates under the same model were computed, allowing the program to halt bootstrapping automatically by the autoMRE option. Bootstrap (BS) values above 80 % were considered significant (high support), and above 70 % were considered moderate support. Bayesian Inference was performed in the MrBayes 3.2.6 (Ronquist et al. 2012) and evolutionary models for BI were estimated using the AIC (Akaike Information Criterion) for each partition, as implemented in MrModeltest 2.3 (Nylander 2004). The best-fit models for each partition were implemented as partition-specific models within partitioned mixed-model analyses (GTR+I+G for ITS1, SYM+I+G for 5.8S, and HKY+I+G for ITS2). We set simultaneously for 5 x 10^7 generations, sampling trees at every 1000th generation. The convergence diagnostic was calculated every 10^4 generation, and its critical value was set to stop the analysis automatically when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes, stopval = 0.01). In all analyses, the first 25 % of trees from each run were discarded as burn-in. Resulting trees from the two independent runs were then pooled to produce one 50 % majority-rule consensus tree, and Bayesian Posterior Probabilities (BPP) were generated for the resulting tree. A BPP value above 0.99 was considered significant (high support), and above 0.95 was considered moderate support. All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

3. RESULTS AND DISCUSSION

3.1. BLASTn queries and phylogenetic analysis

In this study we provided 13 new sequences of *Boletinellus* and *Phlebopus* from Brazil. The final aligned matrix of the ITS1–5.8S–ITS2 (ITS) region was 994 bp long and 90 taxa. The two Bayesian runs converged to stable likelihood values (-lnL 17283.62, -lnL 17287.32) after 560 000 generations. The average standard deviation of split frequencies was 0.009954 and 4 178 (about 75 % of the sampled trees) stationary trees from each analysis were used to compute a 50 % majority-rule consensus tree to calculate posterior probabilities. In the RAxML analysis, ITS alignment had 822 distinct patterns with a proportion of gaps and undetermined characteristics of 37.09 %, the bootstopping criteria indicated 402 replications being sufficient to access the internal branch support, and final ML Optimization Likelihood was -lnL 17730.654840. The best-scoring ML tree and 50 % majority-rule consensus tree did not show major conflicts in the tree topology and were mostly congruent, which allowed us to combine them (Figure 2). The topology of the final tree is similar to Wilson et al. (2012). A supported Sclerodermatineae clade divided into Core Sclerodermatineae, including the gasteroids *Astraeus* Morgan, *Calostoma* Desv., *Pisolithus* Alb. & Schwein, *Scleroderma* Pers. and the boletoid *Gyroporus* Quél.. The other clade, Boletinellaceae, includes the boletoid *Phlebopus* and *Boletinellus* genera.

3.2. Taxonomy

Boletinellus exiguus (Singer & Digillo) Watling, Edinburgh Journal of Botany 54: 243 (Figures 3A-C, 4A-C)

≡ *Gyrodon exiguus* Singer & Digillo, Lilloa 30: 154 (1960)

Pileus 12−73 mm wide, flabelliform, reniforme, rarely circular, convex to applanate, pale yellow (OAC 812) to light olivaceous yellow (OAC 846) covered with brownish (OAC 784) depressed fibrils, more concentrated in the center, dry, slightly viscid in wet weather, margin regular, wavy. *Hymenophore* boletinoid (lamellar-porose-anastomosed), subdecurrent to decurrent; tubes up to 4 mm long centrally, compound, yellow (OAC 854); pores mostly 1−2 mm wide, angular, elongate towards the margin, radiating, blueing when bruised. *Stipe* 4−13 mm × 3−8 mm, lateral, short, cylindrical, subequal, brownish (OAC 737), paler in the apex, dry, velvet. *Context* whitish to light yellow (OAC 858), blueing when exposed. *Spore print* olivaceous brown.

Basidiospores (8-)9−10(-11) x 5−6 µm (Qm = 1.72) (n/s = 30; s = 16), ellipsoid to elongate, pale yellow, slightly dextrinoid, smooth, thin-walled. *Basidia* 29−45 x 9−11 µm, clavate, thin walled, hyaline, (2-)4-sterigmate, 4−6 µm long. *Cystidia* 28−39 x 8−10 µm, ventricose to clavate, hyaline, thin-walled, scattered and inconspicuous, more visible in the pores. *Hymenophoral trama* boletoid, hyphae 4−7 µm wide, pale yellow, inamyloid. *Pileipellis* trichodermal, terminal hyphae 5−7 µm wide, cylindrical, hyaline to pale yellow, slightly incrusted, dextrinoid. *Pileus trama* interwoven, hyphae 7−13 µm wide, cylindrical, hyaline. *Stipitipellis* in two layers, the outer layer trichodermal similar to pileipellis, and lower layer subparallel to parallel, hyphae light yellow, inamyloid. *Stipe trama* vertically arranged, lacunose, hyphae 4−6 µm wide, cylindrical, hyaline. *Clamp connection* present.

Habit, habitat and distribution. The specimens examined were growing solitaries or gregarious on the base of living *Psychotria laciniata* Vell. (Rubiaceae), *Sebastiania brasiliensis*

Spreng. (Euphorbiaceae), *Dicksonia sellowiana* Hook. (Dicksoniaceae), as well as terricolous. The species is known in Brazil for Paraná, Rio Grande do Sul (Putzke et al. 1994, Watling & de Meijer 1997, Neves & Capelari 2007), Bahia, Rio de Janeiro, Santa Catarina, and São Paulo (present study). Also registered for Martinique (Pegler 1983), and Bolivia (Singer & Digillo 1960).

Recent materials collected. BRAZIL. Bahia: Uruçuca, Parque Estadual da Serra do Conduru, 14°29'40.9"S 39°08'01.8"W, 19-XI-2016, *Alves-Silva G.866*, *867* (ICN). Rio de Janeiro: Teresópolis, Parque Nacional Serra dos Órgãos, Trilha Mozart Catão, 12-X-2016, *Duque J.* 212 (RB). Rio Grande do Sul: Porto Alegre, Morro Santana, 30°03'42.9"S 51°07'40.0"W, 02-X-2014, *Magnago A.C 1100* (ICN); 28-IX- 2015, *Magnago A.C. 1204* (ICN); São Francisco de Paula, FLONA São Francisco de Paula, 29° 25' 25"S 50°23' 13"W, 02-IV-2012, *Magnago A.C. 1045* (ICN); 15-IV-2013, *Magnago A.C. 605* (ICN); 14-IV-2014, *Magnago A.C. 1066* (ICN), *1067* (ICN), 25-V-2015, *Magnago A.C. 1174* (ICN); São Francisco de Paula, Hotel Parque Veraneio Hample, 29°26'34.0"S 50°36'41.0"W, 13-I-2015, *Magnago A.C. 1105* (ICN); São Francisco de Paula, Centro de Pesquisas e Conservação da Natureza – Pró-Mata, 29°28'48.0"S 50°10'29.1"W, 26-V-2015, *Magnago A.C. 1182* (ICN), *1189* (ICN). Santa Catarina: Florianópolis, Morro da Lagoa, 27°35' 00"S 48°28'00"W, 11-V-2012, *Magnago A.C. 336* (ICN); 01-VII-2013, *Magnago A.C. 651* (ICN), *652* (ICN), *653* (ICN), 20-III-2014, *Magnago A.C. 988* (ICN), *989* (ICN), *999* (ICN), *1002* (ICN); Concordia, Parque Fritz Plaumann, 14-II-2013, *Heisecke C. 053* (ICN). São Paulo: Iporanga, Parque Estadual Turístico do Alto Ribeira, Núcleo Cablocos, 24°27'40.5"S 48°35'52.7"W, 08-II-2018, *Alves-Silva G. 1306* (ICN), *1307* (ICN).

Commentary. *Boletinellus exiguus* is easily identified by its small yellowish basidiomata, pleurotoid habit, eccentric to lateral short stipe, and yellow strongly boletinoid hymenophore that turns blue under pressure. The type specimen was described from Bolivia (Singer & Digillo

1960). *Boletinellus merulioides* is morphologically and molecularly similar (Figure 2) but it is always associated with *Fraxinus* spp, usually found in the North America following the distribution of the host.

Boletinellus rompelii (Pat. & Rick) Watling, Edinburgh Journal of Botany 54: 243 (Figures 3D-G, 4D-F)

≡ *Phylloporus rompelii* Pat. & Rick, Brotéria Série Botânica 6: 81

≡ *Gyrodon rompelii* (Pat. & Rick) Singer, Revue de Mycologie 3 (4-5): 171

Pileus 55−235 mm wide, dark brown (OAC 735) to reddish brown (OAC 635,636), velutinous to slightly tomentose and convex when young to pale brown (OAC 742,749), brownish (OAC 719,733), glabescent, plano-convex eventually with central depression when mature. *Hymenophore* boletinoid (lamellar-porose-anastomosed), subdecurrent to decurrent; tubes up to 6 mm long centrally, compound, yellow (OAC 853,854); pores mostly 1−2 mm wide, angular, more elongate towards the margin, radiating, blueing when bruised. *Stipe* 20−100 mm × 8−28 mm, central to slightly eccentric, cylindrical, pale yellow (OAC 856, 857) near the tubes, brownish downwards (OAC 736,737) with a pinkish red band in the middle portion, dry, velvet. *Context* white to yellowish (OAC 858), blueing when exposed. *Spore print* olivaceous brown.

Basidiospores 7−8(-9) x 5−6 μm (Qm = 1.48) (n/s = 30; s = 13), short ellipsoid to subglobose, pale yellow, dextrinoid, smooth, slightly tick walled. *Basidia* 22−37 x 8−10 μm, clavate, thin-walled, hyaline, (2-)4-sterigmate, 3−5 μm long. *Cystidia* 17−26 x 3−6 μm, filamentous nodulose to narrowly clavate, hyaline, inamyloid, thin walled, very scattered and inconspicuous, more common on the edges of the tubes. *Hymenophoral trama* boletoid, hyphae 4−11 μm wide, hyaline to pale yellow, inamyloid. *Pileipellis* trichodermal with more prostate hyphae in mature basidiomata, terminal hyphae clavate, fusoid, cilyndrical, 4−14 µm wide, pale yellow to orangey, dextrinoid, slightly gelatinized. *Pileus trama* interwoven, hyphae 3−17 µm wide, hyaline to light yellow, inamyloid, oleiferous hyphae present. *Stipitipellis* trichodermal similar to pileipellis. *Stipe trama* vertically arranged, subparallel, hyphae 5−18 µm wide, pale yellow, inamyloid *Clamp connection* present.

Habit, habitat and distribution. The specimens examined were growing on soil in the Atlantic Forest and open grassy areas, some basidiomata attached to buried roots. The species is known in Brazil for Paraná, Rio Grande do Sul, Santa Catarina (present study), and São Paulo (Putzke et al. 1994, Watling & de Meijer 1997, Neves & Capelari 2007). Also registered for Argentina (Singer & Digillo 1957) and Bolivia (Singer & Digillo 1960).

Recent materials collected. BRAZIL. Rio Grande do Sul: Porto Alegre, UFRGS Campus do Vale, 30°04'11" 51°07'13"W, 17-III-2014, *Fazolino, E.P. 400* (ICN); Porto Alegre, Morro Santana, 30°03'42.9"S 51°07'40.0W, 12-I-2015, *Magnago A.C. 1135* (ICN), 21-VIII-2015, *Magnago A.C. 1198* (ICN), 04-II-2016, *Magnago A.C. 1254* (ICN), 13-I-2017, *Magnago A.C. 1350* (ICN), *1351* (ICN). Santa Catarina: Blumenau, Campus I-FURB, 26°54'22"S 49°04'47"W, 15-V-2012, *Hodecher A. 01* (ICN); Florianópolis, Ilha do Campeche, 27°41'51"S 48°27'56"W, 13-XII-2013, *Magnago A.C. 855* (ICN), *856*(ICN), 24-III-2014, *Magnago, A.C. 1022* (ICN); Florianópolis, Parque Municipal do Córrego Grande, 27°35'48.3"S 48°30'36"W, 15-I-2012, *Neves, M.A. & Smith, N. 1064* (ICN); *Robson A. MAN 1091* (ICN); Florianópolis, Campeche, 20-III-2013, *Simon, J. 37* (ICN).

Commentary. *Boletinellus rompelii* was described by Patouillard & Rick (Rick 1907) for Rio Grande do Sul, Southern Brazil. The reddish pink to red zone near the base of the stipe, which becomes rusty to red brown in age is distinctive. *Boletinellus monticola* (Singer) Watling described from the montane region of Tucumán, Argentina (Singer & Digillo 1957), is similar but usually associated with *Alnus* spp (Becerra et al. 2005). Morphologically the basidiospores in *B. monticola* are more ellipsoid (5.5−9.2 x 3.3−4.5 µm), and the cystidia are more frequent (Singer & Digillo 1957). *Boletinellus proximus* (Singer) Murrill can be distinguished because its context and hymenophore do not stain blue when exposed or bruised.

Phlebopus beniensis (Singer & Digillo) Heinem. & Rammeloo, Mycotaxon 15: 390 (1982) (Figures 5A-E)

≡ *Phaeogyroporus beniensis* Singer & Digillo, Lilloa 30: 150 (1960)

= !*Phlebopus brasiliensis* Singer, Beihefte zur Nova Hedwigia 77: 43 (1983)

Pileus 65−146 mm diam., hemispheric at first to convex applanate when mature, slightly depressed in the center, smooth to slightly velutinous, viscid in wet weather, light brown, caramel to sepia yellow (OAC 840, 838); margin straight, irregular, sometimes splitting when mature. *Hymenophore* tubular, adnate to depressed around the stipe; tubes 5−12 mm long centrally, yellow (OAC 895, 888) to yellow olive (OAC 887, 859); pores mostly 2−3 per mm, round to subisodiametric, concolorous with the tubes, becoming darker under pressure. *Stipe* 30−96 mm × 11−23 mm, central to slightly eccentric, subequal, tapering upwards, bulbous downwards, dark grayish brown (OAC 866, 868) yellowish near the apex, glabrous to velutinous, solid. *Context* fleshy, pale yellow (OAC 899), unchanging or blueing when exposed. *Spore print* olive brown.

Basidiospores (5-)5.6–7.4 × 4.5–5(-5.5) µm (Qm = 1.40) (n/s = 30; s = 17), ellipsoid, yellow brown, inamyloid, guttulate, smooth, thick walled. *Basidia* 16–26 × 7–10 µm, clavate, thin walled, hyaline, guttulate, 4-sterigmate, 3−5 µm long. *Cystidia* 20−38 × 6−10 µm, ventricose, subclavate, obclavate to cylindrical, obtuse to round apex, hyaline, inamyloid, smooth, thin walled, inconspicuous and more visible in the pores. *Hymenophoral trama* boletoid, gelatinized mediostratum with narrow hyphae 3−5 µm wide, hyaline, inamyloid, oleiferous hyphae present in some basidiomata; medium stratum with irregular to inflated hyphae 6−10 µm wide, divergent. *Pileipellis* trichodermal, terminal elements 4−6 µm wide, round apex, hyaline, inamyloid, smooth, thin walled. *Pileus trama* parallel to subparallel, hyphae 2−10 µm wide, hyaline. *Stipitipellis* composed by clavate to cylindrical terminal elements, 3−14 µm, hyaline, inamyloid, thin walled, caulobasidia and caulocystidia similar to the hymenium present. *Stipe trama* of vertically arranged hyphae, cylindrical, hyphae 4−10 µm wide, hyaline, inamyloid. *Clamp connection* present.

Habit, habitat and distribution. Growing solitary or in small groups, in forests or open grassy areas. In Brazil is registered from the Amazon Forest, Atlantic Forest, and *Cerrado* (Savanna vegetation). Originally was described from Bolivia, but it is also reported from Argentina, Brazil, Costa Rica, Ecuador, Martinique, Puerto Rico and Venezuela (Heinemann & Rammeloo 1982, Ovrebo 1983, Pegler 1983, Singer et al. 1983, Miller et al. 2000, Guzmán et al. 2007).

Recent materials collected. BRAZIL. Amazonas: Manaus, Campus INPA, 3°05'40"S 59°59'17"W, 04-X-2012, *Komura DL 1024* (ICN). Espírito Santo: Alfredo Chaves, Morro da Caixa D`água, 20°38'03.0"S 40°45'17.1"W, 10-XII-2016, *Magnago A.C. 1284* (ICN), *1285* (ICN). Mato Grosso: Chapada dos Guimarães, Parque Estadual da Chapada dos Guimarães, 15°19'04.8"S 55°52'36.8"W, 26-III-2013, *Dreschler-Santos E.R; Costa-Rezende, D.H., Mafalda-Freire, F. DS 1056* (ICN); Nova Ubiratã, Estação Ecológica Rio Ronuro, 13°06'19.4"S 54°27'33.3"W, 22-II-2017, *Magnago A.C. 1403* (ICN), *1404* (ICN). Paraíba: João Pessoa, Universidade Federal da Paraíba, Mata do Biotério, 7°08'18.9"S 34°50'40.1"W, 7-II-2009, *Neves M.A. 357* (ICN); 11-III-2011, *Magnago A.C. 265* (ICN), *267* (ICN), 20-VI-2011, *Magnago A.C. 285* (ICN), *288* (ICN). Rio Grande do Sul: Porto Alegre, Parque Farroupilha, 30°02'02.6"S 51°12'57.7"W, 30-III-2014, *Magnago A.C. 1027* (ICN); Santa Maria, Parque Itaimbé, 29°41'08.3"S 53°48'10.8"W, 11-III-2008, *Cortez V.G 047/08* (ICN)*;* Canoas, Parque Municipal Getúlio Vargas, 29°54'57.5"S 51°10'07.0"W, 26-XII-2015, *Perez E.F. 605* (ICN), *608* (ICN), *609* (ICN). Santa Catarina: Florianópolis, Lagoa do Peri, 27°44'41.8"S 48°31'10.7"W, 07-I-2017, *Farias, F. ACM 1026* (ICN).

Additional collections examined. AMAZONAS: Manaus, Campus do INPA, 11-IV-1980, Singer, R. B12098 (INPA-Fungos 102022) *Phlebopus brasiliensis* (holotype).

Commentary. *Phlebopus beniensis* was described by Singer & Digillo (1960) as *Phaeogyroporus beniensis* Singer & Digillo from Amazon in Departamento del Beni, Bolivia, and later combined into *Phlebopus* by Singer et al. (1983). *Phlebopus beniensis* was characterized by light brown pileus, without any olive tinge, 53−123 mm wide, small pores, short spores, and when fresh and humid the context becomes blue or unchanging in dry weather, terrestrial in the Amazon Forest. *Phlebopus brasiliensis* Singer was described based on collections from Amazonas and Paraíba, found under mixed trees in disturbed areas (Singer et al. 1983). Differing from *P. beniensis*, *P. brasiliensis* is characterized by olivaceous pileus, 30−65 mm wide, and context unchanging when exposed. The two species have many similarities: yellowish color and small size of the pores that become brownish under pressure; stipe subequal, with usually larger base, cream yellow colored near the apex, becoming brownish to fuliginous towards the base; basidiospores inamyloid, smooth, and short (5.8–7.5 × 4.2–6.2 µm in *P. beniensis* and 5.8–6.8 × 4.6–5.3 µm in *P. brasiliensis*); the cystidia are not numerous, and it is more visible in the pores; and in both species the pileipellis is a trichodermium.

Based on these characteristics, *P. brasiliensis* used to differs morphologically from *P. beniensis* in the non blueing context when exposed, and smaller and olivaceous pileus. However, these characteristics were not constant in the recent collections. Some collections fit better in the description of *P. beniensis* (Singer & Digillo 1960), other in the description of *P. brasiliensis* (Singer et al. 1983), and some collections have mixed characters of both species in the same basidioma. In the phylogenetic analyses, the recent collections mixing all these characters cluster in the same clade, showing that the characters used to distinguish these two species were not so clear. Based on the morphological and molecular data we proposed to synonymize *P. brasiliensis* to *P. beniensis,* expanding its geographic distribution in South America.

3.3. Key to Boletinellaceae species registered in Brazil

1. Hymenophore decurrent, with a radial boletinoid orientation, pores angular to elongate, occasionally sublamellate .... (*Boletinellus*) 2

1'. Hymenophore subdecurrent to depressed, pores small (1–2 mm), circular to subangular...(*Phlebopus*) 4

2. Basidioma robust and large, stipe central eccentric, usually with a pinkish red band in the middle portion .... *Boletinellus rompelii*

2'. Basidioma medium to small size, stipe lateral to absent.. 3

3. Basidioma with pink reddish tones, context and hymenophore unchanging when bruised or exposed...*Boletinellus proximus*

3'. Basidioma yellowish to olivaceous, context and hymenophore turning blue under pressure and when is exposed..*Boletinellus exiguus*

4. Pores wide (1 mm or more in diam.), subgyrose or boletinoid ....*Phlebopus tropicus*

4'. Pores small and subisodiametric 5

5. Basidiospores large, majority larger than 7.5 µm, pleurocystidia absent or inconspicuous .... *Phlebopus braunii*

5'. Basidiospores small, majority smaller than 7.5 µm when mature, pleurocystidia present.. *Phlebopus beniensis*

4. AUTHOR CONTRIBUTION STATEMENT

ACM, GAS and MAN participated in the field collections. ACM analyzed morphologically and performed the molecular procedures in the collections. ACM and GAS analyzed the molecular and phylogenetic data. All the four authors contributed in the writing, as well as in the construction of the entire manuscript.

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Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species	**Collection No.**	**Origin**	**ncITS GenBank No.**
Boletinellus exiguus	M.A. Fungi 494011	Brazil	AJ419183
Boletinellus exiguus	M.A. Fungi 476771	Brazil	AJ419185
Boletinellus exiguus	CHC053	Brazil	MG996738
Boletinellus exiguus	ACM336	Brazil	MG996737
Boletinellus merulioides	AFTOL-575	_	DQ200922
Boletinellus merulioides	2630a	France	KM248952
Boletinellus rompelii	M.A. Fungi 494951	Brazil	AJ419192
Boletinellus rompelii	MAN1064	Brazil	MG996740
Boletinellus rompelii	MAN1091	Brazil	MG996739
Boletinellus rompelii	AH01	Brazil	MG996741
Gyroporus austrobrasiliensis	ACM1144	Brazil	MF437000
Gyroporus austrobrasiliensis	ACM1136	Brazil	MF436999
Phlebopus beniensis	MAN357	Brazil	MG996747
Phlebopus beniensis	DS1056	Brazil	MG996748
Phlebopus beniensis	ACM265	Brazil	MG996742
Phlebopus beniensis	ACM267	Brazil	MG996743
Phlebopus beniensis	ACM285	Brazil	MG996744
Phlebopus beniensis	ACM288	Brazil	MG996745
Phlebopus beniensis	DLK1024	Brazil	MG996746
Phlebopus marginatus	REH8883	Australia	EU718109
Phlebopus mexicanus	Cappello-233	Mexico	KM675999
Phlebopus mexicanus	Cappello-2507	Mexico	KM676000
Phlebopus sp.	REH8795	Thailand	EU718111
Phlebopus spongiosus	CMUB39824	Thailand	KX575660
Phlebopus spongiosus	CMUB39826	Thailand	KX575661
Phlebopus portentosus	CMU55-271-1	Thailand	KF768407
Phlebopus portentosus	CY-421	China	KJ439037
Phlebopus portentosus	CY-331	China	KJ439034
Phlebopus portentosus	TT10003	China	JX576702
Phlebopus portentosus	GKVK 13	India	MF403066
Phlebopus portentosus	php1	Africa	EU718110

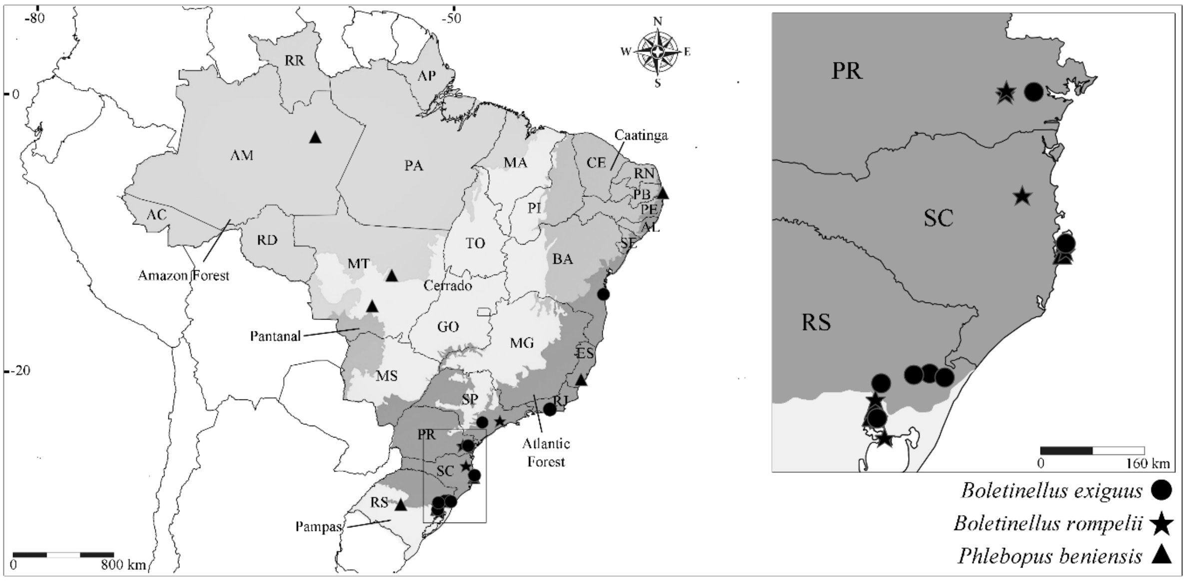

Figure 1: Geographic distribution of *Boletinellus exiguus, B. rompelii* and *Phlebopus beniensis* from Brazil.

Figure 2. Maximum likelihood (ML) tree based on nrITS sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.95 are shown. Symbols black square: pileus smaller than 100 mm; empty square: larger than 100 mm; black pentagon: context blueing when exposed; empty pentagon: context not blueing when exposed; black circle: pileus olivaceous; empty circle: pileus brownish.

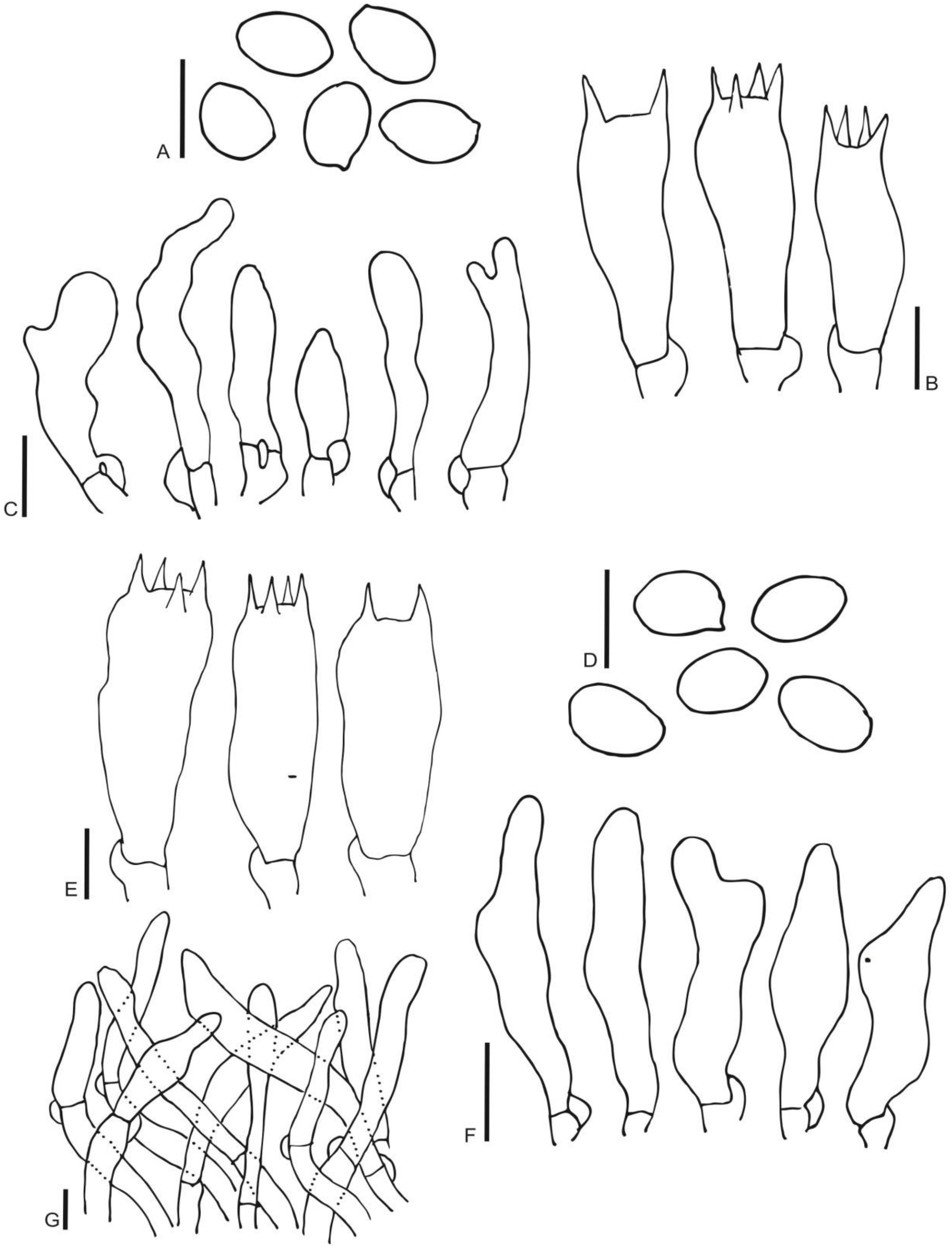

Figure 3. Microscopic features of *Boletinellus exiguus.* A. Basidiospores. B. Basidia. C. Cheilocystidia. *Boletinellus rompelii.* D. Basidiospores. E. Basidia. F. Cheilocystidia. G. Pileipellis. Bars A-F = 10 µm.

Figure 4. Macromorphology. A–C *Boletinellus exiguus*. D–F *Boletinellus rompelii.* Bars A-F = 15 mm.

Figure 5. Macro and microscopic features of *Phlebopus beniensis* (*MAN 357*). A. Basidomata in the field. B. Basidiospores. C. Basidia. D. Cheilocystidia. E. Pileipellis. Bars A = 15 mm; B-E = 10 μm.

ARTIGO VII

Checklist of boletoide fungi from the Brazilian Atlantic Forest.

Magnago, A.C. Neves, M.A. & Silveira, R.M.B.

Check List – a ser submetido

Trilha da Cachoeira, Reserva Biológica Augusto Ruschi, Santa Teresa, Espírito Santo (Foto: Magnago, A.C.)

ALS

Magnago, Neves, Silveira | Boletales from Brazil

Checklist of boletoide fungi from the Brazilian Atlantic Forest.

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Abstract

Based on literature and new collections, an annotated list of boletoid fungi registered from the Atlantic Forest is presented. Thirty-two species are cited, distributed in eleven genera. *Chalciporus trinitensis* var. *amazonicus* is registered for the first time for the Atlantic Forest, and the distribution of *Tylopilus aquarius* var. *megistus* is extended towards the south of the Atlantic Forest. Comments, photographs, geographic distribution, and an identification key is presented.

Key words

Boletales, Boletaceae, Boletinellaceae, Gyroporaceae, Neotropics, diversity, taxonomy.

Introduction

The knowledge about boletoid fungi diversity in the tropics has attracted more and more taxonomists around the world. Due to its high structural complexity combined with the great diversity of niches and hot humid climate, the tropical regions are extremely rich in number of fungi species, but many still to be described (Hawksworth 2001, Maia *et al.* 2015, Hawksworth and Lücking 2017).

The number of bolete species recorded for Brazil is still considered very low. Father Johannes Rick, the pioneer of the mycology studies in Brazil, was the first to record boletoid fungi in Southern Brazil in the beginning of twenty century (Rick 1907, 1926, 1937, 1960, 1961). Rolf Singer registered the presence of boletoid fungi in Amazonian lowland forest, describing several new taxa (Singer and Digilio 1957, 1960, Singer 1964, Singer 1970, Singer *et al.* 1983). Recent publications on boletes have added to the knowledge of this group in Brazil (Putzke *et al.* 1994, Oliveira and Souza 1995, 1996, 2002, Watling and de Meijer 1997, Giachinni *et al.* 2000, de Meijer 2008, Magnago and Neves 2014, Magnago *et al.* 2017, Barbosa-Silva *et al.* 2017, Barbosa-Silva and Wartchow 2017, Magnago *et al.* 2018a, b, c, d, e).

Continuing on the investigation on boletes from Brazil, recent surveys in the Atlantic Forest Domain, some taxa were recent collected, expanding its geographic distribution in Brazil. This work presents a list of boletoid fungi known from the Atlantic Forest including commentaries, photographs, geographic distribution, and an identification key.

Methods

The studied area includes the Atlantic Forest Domain, a highly diverse biome with large environmental variation distributed along the Brazilian’s Atlantic coast and inland areas, including coastal forests, araucaria mixed forests, deciduous and semi-deciduous forests, mangrove swamps, marshlands, and oceanic islands. Originally, it covered 1 300 000 km2 (~15% of Brazil). However, after decades of deforestation due to harvest of valuable lumber, agroindustry, and urbanization (Dean 1932–1994), less than 8% of the original area remains preserved (SOS Mata Atlântica 2017).

The list of the species presented here was compiled from recent fieldwork, herbaria information and formally published literature. Macro- and microscopic analyses followed established methods used for basidiomycetes (Largent *et al.* 1977; Largent 1986). Color codes in brackets (e.g., OAC 663) are based on the Online Auction Color Chart (Kramer 2004). Recent collections were deposited at FLOR, ICN and VIES. Herbaria acronyms follow Thiers (continuously update).

Results

Thirty-two boletoid fungi are reported for the Atlantic Forest Domain in Brazil distributed in eleven genera, *Austroboletus* (Corner) Wolfe, *Boletellus* Murril, *Boletinellus* Murril, *Boletus* L., *Chalciporus* Bataille, *Fistulinella* Henn., *Gyroporus* Quél., *Phlebopus* (R. Heim) Singer, *Singerocomus* T.W. Henkel & M.E. Sm., *Tylopilus* P. Karst., and *Xerocomus* Quél.. *Chalciporus trinitensis* var. *trinitensis* is registered for the first time for the Atlantic Forest. New collection of *Tylopilus aquarius* var. *megistus* is registered for the first time for Espírito Santo state, 2.000 km far from the type locality. Twenty-three species are listed below, including notes, illustrations, geographic distribution and an identification key are provided. The other eight remaining taxa are commented in the excluded taxa section.

Boletaceae Chevall. Flore Générale des Environs de Paris 1: 248 (1828)

Austroboletus festivus (Singer) Wolfe, Bibliotheca Mycologica 69: 92 (1980) – Fig 1A.

Description and illustrations: Singer 1961, Watling and Meijer 1997, Magnago and Neves 2014.

Distribution in Brazil: Pernambuco (Singer 1961, 1970), Paraná (Watling and Meijer 1997), and Santa Catarina (Magnago and Neves 2014).

Comments: *Austroboletus festivus* is easily identified by its reddish brown densely matted-fibrillose pileus, whitish to sand dust colored hymenophore, a multicolorful context when exposed, and pinkish basal mycelium. Basidiospores fusiform to subfusiform, slightly roughened, pleurocystidia frequent, aciculate to aculeate, with golden yellow contents, trichodermal pileipellis granularly incrusted (Singer 1961, Watling and Meijer 1997, Magnago and Neves 2014). This is the only species in the genus cited for the Atlantic Forest.

Boletellus cremeovelosus A. Barbosa-Silva & F. Wartchow, Current Research in Environmental & Applied Mycology 7: 388 (2017)

Description and illustrations: Barbosa-Silva and Wartchow (2017).

Distribution in Brazil: Pernambuco (Barbosa-Silva and Wartchow 2017).

Comments: The species differs from all other taxa of *Boletellus* section *Boletellus* Singer found in the Neotropics, by the lack of pink, red, lavender or purple tints on the basidiomata being pale beige with shade of cream, and the pileus surface covered by pyramidal to erect squamules.

Boletellus nordestinus A.C. Magnago, Sydowia (2018) – Fig 1B.

Description and illustrations: Magnago *et al.* (2018d).

Distribution in Brazil: Paraíba, Rio Grande do Norte (Magnago *et al.* 2018d, Oliveira and Sousa 1995 as *B. pustulatus* and *B. lepidospora*).

Comments: Differs from the other *Boletellus* non-cyanescent species with the combination of dry, velutinous, chocolate brown pileus, short basidiospores, pleurocystidia and cheilocystidia fusiform to ventricose, and pileipellis trichodermal with acute terminal elements. The only species in the genus cited for the Atlantic Forest (Magnago *et al.* 2018d)

Boletus neo-olivaceus Rick, Brotéria Série Trimestral: Ciências Naturais 6: 167 (1937)

Description: Singer (1953), Rick (1937).

Distribution in Brazil: Rio Grande do Sul (Rick 1960).

Comments: The *typus* of *Boletus neo-olivaceus* is deposited at Herbarium Farlowiano (FH) sub *Boletus olivaceus* Rick (Rick 1960). In the circumscription of the species, few characters are provided, basidioma olivaceous, pileus with brownish tints, reaching 1cm diam., small pores, short stipe, 0.5 cm diam., and terricolous (Rick 1937, 1960). There is no register that the species has been recollected.

Chalciporus trinitensis* var. *amazonicus L.D. Gómez, Revista de Biologia Tropical 44(4): 80 (1997) – Fig 1C.

Pileus 15–45 mm broad, convex to plano-convex, to plano-depressed when mature, surface dry becoming subviscid, velvet to finely fibrillose, yellow brown to cinnamon (OAC 683, OAC 650), with red tones; margin entire, enrolled sometimes; context 2–4 mm, unchanging. *Tubes* 3–5 mm long, pinkish (OAC 655) to yellow (OAC 805), adnate to depressed around stipe; pores 1–1.5 mm broad, irregular, angular to elongate near the margin, not staining under pressure. *Stipe* 21–58 mm × 3–5 mm, subequal, light brown (OAC 717) to orange red (OAC 642), smooth to finely fibrillose; context light yellow (OAC 815), unchanging. *Macrochemical reactions*: NH_4OH and KOH 3% on surfaces becoming yellow to unchanging. *Spore print* reddish brown. *Basidiospores* 11–13 × 4–5 µm (Q=2.54), subfusiform, with suprahilar depression, pale yellow, slightly dextrinoid in accumulation, smooth, thin walled. *Basidia* 25–32 × 8–10 µm, clavate, thin walled, hyaline, inamyloid; 4-sterigmate, 3–4 µm long. *Cystidia* on the edges and sides not differentiated from each other, 48–84 × 8–13 µm, ventricose-rostrate to lanceotate, few pleurocystidia with 1–2 septa, hyaline, inamyloid, frequent. *Hymenophoral trama* divergent to subparalel in a gelatinized matrix, hyphae 7–10 µm wide, hyaline, inamyloid, thin walled. *Pileipellis* repent to subtrichodermal, reddish brown, inamyloid, terminal hyphae 5–8 µm wide. *Pileus trama* interwoven to subparallel, hyphae 4–12 µm wide, light yellow. *Stipitipellis* repent, hyphae 4–6 µm wide, amber yellow, inamyloid. *Caulocystidia* 21–27 × 9–10 µm, clavate, forming scattered clusters. *Stipe trama* parallel, hyphae 5–13 µm wide, light yellow, inamyloid. *Clamp connection* absent.

Recent specimens collected: Brazil: Espírito Santo: Santa Teresa, Reserva Biológica Augusto Ruschi, Casa da Pedra, 19°54' 19.60"S, 40°34'8.20"W, 04 Dez 2012, *Magnago, A.C. 492* (FLOR 51607); 05 Dez 2012, *Magnago, A.C. 528* (FLOR 51605), *529* (FLOR 51606).

Additional specimens examined: Brazil: Amazonas: *Chalciporus trinitensis* var. *amazonicus*, Igarapé do Tarumãnzinho, 14 Dec 1978, *R.Singer, B 11434* (INPA-Fungos 82360).

Distribution in Brazil: Amazonas (Singer *et al.* 1983, Goméz 1996), Espírito Santo (present study).

Commentary: These collections constitute the first record of *Chalciporus trinitensis* var. *amazonicus* for the Atlantic Forest. The description here presented agrees with the description of *Chalciporus trinitensis* by Singer *et al.* (1983), differing only on the size of the cystidia that are shorter in the Amazonian specimen (25–54 µm), and by the absence of any septate pleurocystidia in the description. The *typus* of *Chaciporus trinitensis* was collected by Heinemann in Trinidad in 1953 and it is characterized by having incrusted cystidia and elongated filiform cheilocystidia (Singer *et al.* 1983). In Brazil *Chalciporus trinitensis* was collected in Amazon Forest by Singer in 1978. Later on, Goméz (1996) created the variety *amazonicus* for the Brazilian specimen, considering the presence of shorter less incrusted cheilocystidia, basidiospores with suprahilar depression, and the depressed hymenophore around the stipe.

Fistulinella campinaranae* var. *scrobiculata Singer, Beihefte zur Nova Hedwigia 77: 148 (1983)

Description and illustrations: Singer *et al.* (1983), Magnago *et al.* (2018a).

Distribution in Brazil: Amazonas (Singer *et al.* 1983), Bahia (Magnago *et al.* 2018a).

Comments: Singer *et al.* (1983) separated *F. campinaranae* into var. *scrobiculata* Singer and var. *campinaranae* Singer mainly by pileus ornamentation, but at the same time affirms that both varieties occur in the same area and it is difficult to state whether any of these characteristics are constantly present in specimens with scrobiculate pileus. *Fistulinella campinaranae* is morphologically and molecularly similar to *F. cinereoalba* Fulgenzi and T.W. Henkel, described from Guyana, differing by browning of the hymenophore when bruised and cylindrical to aciculate cystidia in *F. cinereoalba* (Fulgenzi *et al.* 2010, Vasco-Palacios *et al.* 2014).

Fistulinella ruschii A.C. Magnago, Mycologia (2018) – Fig 1D.

Description and illustrations: Magnago et al. (2018a).

Distribution in Brazil: Paraíba, Bahia, Espírito Santo, Santa Catarina (Oliveira and Souza 2002, as *F. violaceipora*, Magnago *et al.* 2018a).

Comments: *Fistulinella ruschii* is widely distributed along the Atlantic Forest. It is the only species within the genus with septate pleurocystida (Magnago *et al.* 2018a). *Fistulinella campinaranae* var. *scrobiculata* differs by its grayish brown, scrobiculate pileus, and subventricose, lageniform to fusiform pleurocystidia (43–76 × 10–17 μm).

Singerocomus atlanticus A.C. Magnago, Acta Botanica Brasilica 32: 224 (2018) – Fig 1E.

Description and illustrations: Magnago *et al.* (2018b).

Distribution in Brazil: Espírito Santo and Santa Catarina (Magnago *et al.* 2018b).

Comments: *Singerocomus atlanticus* is similar to *S. inundabilis* (Singer) T.W. Henkel & Huasbands but differs in its richer red pileus color and its NH_4OH macrochemical reactions of instantly blue on the pileus and ferrugineous-orange on the stipe, features lacking in the other known species of the genus (Singer *et al.* 1983, Henkel *et al.* 2016, Magnago *et al.* 2018b).

Singerocomus rubriflavus T.W. Henkel & Husbands, Mycologia 108: 169 (2016)

Description and illustrations: Henkel *et al.* (2016), Magnago *et al.* (2018b).

Distribution in Brazil: Bahia (Magnago *et al.* 2018b).

Comments: *Singerocomus rubriflavus* is a distinctive bolete recognized by its medium to large basidioma with dark red, tomentose-pitted and centrally areolate pileus, and olivaceous yellow, non-cyanescent tubes and unique squamulose surface of the concolorous, solid stipe. Micromorphologically the species combines ellipsoid basidiospores, a trichodermial pileipellis, and hymenidermous stipitipellis (Henkel *et al.* 2016).

Tylopilus aquarius **var. *megistus*** Wartchow, A. Barbosa-Silva, B. Ortiz & Ovrebo, Sydowia 69: 116 (2017) – Fig 2A.

Description and illustrations: Barbosa-Silva *et al.* (2017).

Distribution in Brazil: Paraíba and Rio Grande do Norte (Barbosa-Silva *et al.* 2017, Magnago *et al.* 2017), Espírito Santo (present study).

Recent specimen collected: Brazil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi, 13 December 2016, A.C. Magnago 1297 (ICN, VIES).

Comments: This collection expand the geographic distribution of the species southwards the Atlantic Forest, before the species was registered from Rio Grande do Norte and Paraíba. Characterized by large basidiomata, pileus brownish and velutinous, up to 120 mm wide, stipe longitudinally fibrillose, sometimes more reticulate at apex, unchanging context. Short amygdaliform to elliptic basidiospores (7.5–9.7 × 3.8–6), cistidia versiform from ventricose, subventricose, fusoid, subfusoid, sometimes clavate to subclavate, with appendages on apex and sometimes with knobs or outgrowths appearing diverticulate (Barbosa-Silva *et al.* 2017).

Tylopilus dunenis A.C. Magnago & M.A. Neves, Phytotaxa 316: 255 (2017) – Fig. 1F.

Description and illustrations: Magnago *et al.* (2017).

Distribution in Brazil: Paraíba and Rio Grande do Norte (Magnago et al. 2017).

Comments: *Tylopilus dunensis* is very similar to *Tylopilus balloui* Peck. Both have a yellow-orange to orange-red pileus, white to cream hymenophore, cream to pale yellow stipe and do not change color when in contact with ammonium; basidiospores are shorter than 10.5 μm long and the pileipellis is trichodermium. However, *T. dunensis* differ from *T. balloui* principally by the gelatinized pileipellis, and the cystidia in the tubes and pores similar in size and shape, fusoid, ventricose, some clavate.

Tylopilus pygmaeus A.C. Magnago & R.M. Silveira, Phytotaxa 316: 256 (2017)

Description and illustrations: Magnago *et al.* (2017).

Distribution in Brazil: Bahia (Magnago *et al.* 2017).

Comments: Tylopilus pygmaeus is morphologically similar to *T. potamogeton* var. *potamogeton* Singer by having small basidiomata, pileus velutinous, brownish, and a whitish to pinkish hymenophore. *Tylopilus potamogeton* differs mainly by its cinnamon to fuscous umber stipe that is densely fibrillose and tomentose at the base, and the hymenophore does not turn brown when bruised. Microscopically the arrangement and appearance of cystidia, pileipellis and stipitipellis are similar, however, *T. potamogeton* has longer basidiospores (9−12 × 6−8 µm), cystidia that are hyaline, fusoid and mucronate (Singer *et al.* 1983, Magnago *et al.* 2017).

Xerocomus amazonicus Singer, Persoonia 9 (4): 429

Description and illustrations: Singer *et al.* (1983), Oliveira and Souza (1995).

Distribution in Brazil: Amazonas (Singer *et al.* 1983), Paraíba (Oliveira and Souza 1995).

Comments: *Xerocomus amazonicus* is distinguish within the neotropical *Xerocomus* by its basidiospores mostly over 8.5 µm long, rich positive bluing reaction with NH_4OH on pileus surface, apex of stipe reticulated, and bright yellow basal mycelium. Singer *et al.* (1983) divided *X. amazonicus* in var. *amazonicus* Singer and var. *obscuratus* Singer considering differences on pileus coloration, however, at the same time suggest that the var. *obscuratus* may only be a form of the type variety since both varieties appears in some collections.

Xerocomus basius de Meijer, Macrofungos notáveis das Florestas de Pinheiro-do-Paraná, 125 (2008) – Fig 2B.

Description and illustrations: Watling and Meijer (1997 as *Xerocomus* cf. *coccolobae* Pegler), de Meijer (2008).

Distribution in Brazil: Paraná (de Meijer 2008), Rio Grande do Sul (present study).

Recent material collected: Brazil, Rio Grande do Sul, Porto Alegre, Morro Santana, 16 January 2015, A.C. Magnago 1154, 6 December 2015, A.C. Magnago 1218, 1219, 4 January 2016, A.C.Magnago 1255, 1256, 1257, 1258, 1259, 13 January 2017, A.C. Magnago 1345.

Comments: *Xerocomus basius* is morphologically related to *X. coccolobae* Pegler and *X. hypoxanthus* Singer, the first was described for the Lesser Antilles associated to *Coccoloba* spp., and differs by pileus and stipe coloration and broader basidiospores (Pegler 1983). *Xerocomus hypoxanthus* differs by smaller basidiomata, longer basidiospores, and yellow basal mycelium (de Meijer 2008).

Xerocomus brasiliensis (Rick) Singer, Farlowia 2: 298 (1946)

Description: Singer and Digilio (1957), Singer *et al.* (1983).

Distribution in Brazil: Paraíba (Singer et al. 1983), Rio Grande do Sul (Singer and Digilio 1957), and São Paulo (Neves and Capelari 2007).

Comments: *Xerocomus brasiliensis* fits in section *Brasilienses* Singer (1986), due its tomentose and often viscid pileus, unchanging context when exposed, and basidiospores small (6–9.3 × 2.8–4.2 µm). The pale yellowish pileus, olivaceous yellow slightly depressed hymenophore, inconspicuous cystidia, and an undifferentiated pileipellis are other characteristics for the species (Singer and Digilio 1957). *Xerocomus radicicola* Sing. & Araujo differs from *X. brasiliensis* by its extremally small basidiomata (about 10 mm), subolivaceous-fuscous pileus, citrine yellow hymenophore and ivory distinctly pubescent stipe, and the presence of veil in young basidiomata (Singer *et al.* 1983).

Xerocomus hypoxanthus Singer, Farlowia 2: 289

Description: Singer and Digilio (1960).

Distribution in Brazil: Rio de Janeiro (Singer and Digilio 1960).

Comments: The species is characterized by its chestnut to reddish brown velvety to fibrillose-granular pileus; citrine yellow hymenophore staning blue or not; pale yellow stipe covered with a brownish pruina, and vibrant yellow basal mycelium (Singer and Digilio 1960, Pegler 1983). *Xerocomus hypoxanthus* is native to southeastern of United States, and it was collected in the Botanical Garden of Rio de Janeiro, and probably was introduced with foreign plant material (Singer *et al.* 1983).

Boletinellaceae Manfr. Binder & Bresinsky, Mycologia 94 (1): 93 (2002)

Boletinellus exiguus (Singer & Digilio) Watling, Edinburgh Journal of Botany 54: 243 (1997) – Fig 2C.

Description: Singer and Digilio (1960), Magnago *et al*. (2018e).

Distribution in Brazil: Paraná (Watling and Meijer 1997), Bahia, Rio de Janeiro, Rio Grande do Sul and Santa Catarina (Magnago *et al*. 2018e).

Comments: *Boletinellus exiguus* was described by Singer and Digilio (1960) for Bolivia. The species is characterized by small basidiomata, yellowish pileus covered by brownish depressed fibril, context and hymenophore bluing under pressure or exposed, and lateral stipe. Morphologically *B. exiguus* is very similar to *B. merulioides* (Schweinitz) Murill, however *B. merulioides* is often associated to *Fraxinus* L. (Oleaceae).

Boletinellus proximus (Singer) Murrill, Lloydia 9: 329 (1946)

Description and illustrations: Singer (1945), Singer and Digilio (1960), Oliveira and Sousa (1996).

Distribution in Brazil: Paraíba (Oliveira and Sousa 1996).

Comments: Characterized by medium to small basidiomata, with pink reddish tones, context and hymenophore unchanging when bruised or exposed. *Boletinellus exiguus* is similar but its basidioma is yellowish to olivaceous, the context and hymenophore turn blue under pressure or when is exposed (Singer and Digilio 1960).

Boletinellus rompelii (Pat. & Rick) Watling, Edinburgh Journal of Botany 54: 243 (1997) – Fig 2D.

Description: Singer (1964), Singer and Digilio (1957), Magnago et al. (2018d).

Distribution in Brazil: Paraíba (Oliveira and Sousa 1996 as *Gyrodon proximus*), Paraná (Watling and Meijer 1997), São Paulo (Singer 1945), Rio Grande do Sul (Singer and Digilio 1957, Magnago et al. 2018d), Santa Catarina (Magnago et al. 2018d).

Comments: Characterized by the brownish olive to caramel color of the pileus, yellow boletineloid decurrent hymenophore, brownish stipe with pinkish red to red zone near the base, which becomes rusty red to red brown in age is distinctive, and bluing context and hymenophore under pressure and exposure. It is similar to *B. proximus* and *B. monticola* (Singer) Watling. *Boletinellus proximus* can be distinguished because its basidiomata do not stain blue when bruised and *B. monticola* has shorter basidiospores (5–9 × 3–4.5 µm) when compared to *B. rompelii* (7–10 × 5.5–6.5 µm) and is associated with *Alnus* Mill. (Betulaceae).

Phlebopus beniensis (Singer & Digilio) Heinem. & Rammeloo, Mycotaxon 15: 390 (1982) – Fig 2E.

Description: Singer and Digilio (1960), Heinemann and Rammeloo (1982).

Distribution in Brazil: Amazonas, Espírito Santo, Goiás, Mato Grosso, Rio Grande do Sul, Santa Catarina (Magnago *et al.* 2018e), Paraná (Watling and Meijer 1997).

Comments: Characterized by light brown to olivaceous pileus, 53−123 mm wide, small pores, when fresh and humid the context becomes blue or unchanging in dry weather, the short basidiospores are short up to 7.5 µm long**.**

Phlebopus braunii (Bres.) Heinem., Bulletin du Jardin Botanique de l'État à Bruxelles 21: 243 (1951)

Description and illustration: Singer and Digilio (1960).

Distribution in Brazil: Rio de Janeiro, Santa Catarina (Singer and Digilio 1960), Rio Grande do Sul (Putzke *et al.* 1994).

Comments: Species characterized by the grayish olivaceous to chestnut pileus, hymenophore depressed around stipe, bluing under pressure, and basidiospores larger (6.5–9.5 × 5–7 µm) compare to the other species with short basidiospores like *P. beniensis* (5.5–7.5 × 4–5 µm) (Singer and Digilio 1960).

Phlebopus tropicus (Rick) Heinem. & Rammeloo, Mycotaxon 15: 389 (1982)

Description: Singer and Digilio (1957), Heinemann and Rammeloo (1982).

Distribution in Brazil: Rio Grande do Sul (Singer and Digilio 1957).

Comments: *Phlebopus tropicus* is the only species within the genus with large pores (1 mm or more in diam.), occasionally subgyrose to boletinoid.

Gyroporaceae (Singer) Manfr. Binder & Bresinsky, Mycologia 94 (1): 93 (2002) – Fig 2F.

Gyroporus autrobrasiliensis A.C. Magnago, Nova Hedwigia (2018)

Description and illustrations: Magnago *et al.* (2018c).

Distribution in Brazil: Rio Grande do Sul (Magnago *et al.* 2018c).

Comments: Gyroporus austrobrasiliensis is very similar to *G. castaneus* by the orange brown tones of the pileus and stipe, white to yellowish hymenophore, light yellow spore print, and unchanging context. *Gyroporus austrobrasiliensis* has smaller basidiospores (7−8 × 5−6 µm) than in *G. castaneus* (8−13 × 5−6 µm), and the presence of aculeate to fusoid pleurocystidia in *G. austrobrasiliensis* (Singer 1945, Magnago *et al.* 2018c).

Excluded taxa

Boletus cinnamomeus Rick, *B. mutabilis* var. *austroamericanus* Rick, *B. panormitanus* Inzenga, *B. spadiceus* Karst., all cited for Southern Brazil in Rio Grande do Sul, are represented only by the *typus*. These species were not included here after Herbaria denote a missing type specimen. In exotic plantation of *Pinus* spp and *Castanea sativa* within the Atlantic Forest Domain six boletoid species are also registered: *Boletus edulis* Bull., *Chalciporus piperatus* (Bull.) Bataille, *Suillus cothurnatus* Singer, *S. granulatus* (L.) Roussel, *S. subaureus* (Peck) Snell and *S. luteus* (L.) Rousse (Putzke et al. 1994), but are not treated in this paper.

Key to boletoid fungi from the Atlantic Forest

1. Clamp connections present .... (Boletinellaceae, Gyroporaceae) 2

1’. Clamp connections absent ....(Boletaceae) 8

2. Hymenophore decurrent, with a radial boletinoid orientation, pores angular to elongate, occasionally sublamellate 3

2’. Hymenophore subdecurrent to depressed, pores small (1–2 mm), circular to subangular .. 5

3. Basidioma robust and large, stipe central to eccentric, usually with a pinkish red band in the middle portion .... *Boletinellus rompelii*

3’. Basidioma medium to small size, stipe lateral or absent 4

4. Basidioma with pink reddish tones, context and hymenophore unchanging when bruised or exposed.. *Boletinellus proximus*

4’. Basidioma yellowish to olivaceous, context and hymenophore turning blue under pressure and when is expossed .... *Boletinellus exiguus*

5. Pores wide (1 mm or more in diam.), subgyrose or boletinoid................ *Phlebopus tropicus*

5’. Pores small and subisodiametric 6

6. Stipe hollow or with cavities, spore print light yellow to cream.. .... *Gyroporus austrobrasiliensis*

6’. Stipe not hollow or with cavities, spore print green olive... 7

7. Basidiospores large, majority larger than 7.5 μm, pleurocystidia absent or inconspicuous..... .... *Phlebopus braunii*

7’. Basidiospores small, majority smaller than 7.5 μm when mature, pleurocystidia present .... *Phlebopus beniensis*

8. Basidiospores surface ornamented 9

8’. Basidiospores surface smooth... 11

9. Basidiospores finely rugullose, context when exposed changing to multiple colors .... *Austroboletus festivus*

9’. Basidiospores longitudinally ribed or striated, context when exposed unchanging............10

10. Pileus covered with pyramidal to erect squamules, beige to cream color, margin appendiculate .... *Boletellus cremeovelosus*

10’ Pileus smooth to velvet, chocolate to olivaceous brown, margin not appendiculate .... *Boletellus nordestinus*

11. Spore print pinkish to pink brown..12

11’. Spore print reddish brown or olivaceous 16

12. Cystidia hyaline, basidiospores fusoid...13

12’. Cystidia usually with golden contents, basidisopores short ellipsoid................................14

13. Pileus grayish brown, matted-fibrillose, pleurocystidia not septate .... *Fistulinella campinaranae* var. *scrobiculata*

13’. Pileus chestnut orange, presence of pleurocystidia septate .... *Fistulinella ruschii*

14. Pileus orange red.. *Tylopilus dunensis*

14’. Pileus brownish..15

15. Basidiomata robust and large... *Tylopilus aquarius* var. *megistus*

15’. Basidomata small (up to 30 mm)... *Tylopilus pygmaeus*

16. Spore print reddish brown...................................... *Chalciporus trinitensis* var. *amazonicus*

16’. Spore print olivaceous 17

17. Pileus pinkish red to red, stipe with yellow base 18

17’. Pileus and stipe with different colors...19

18. Stipe with yellow squamules over the apex that transition downward into recurved brownish red scales, NH_4OH reaction on surfaces unchanging .... *Singerocomus rubriflavus*

18’. Stipe surface smooth, NH_4OH reaction on pileus surface bluing.. *Singerocomus atlanticus*

19. Basidioma arising from bright yellow mycelium 20

19’. Basidioma arising from white and scanty mycelium..21

20. Pileus ochre to tawny, cystidia lanceolate, numerous..................... *Xerocomus hypoxanthus*

20’. Pileus reddish brown, cystidia fusoid, inconspicuous …………. *Xerocomus amazonicus* var. *obscuratus*

21. Context and hymenophore cyanescent under pressure or exposed ...*Xerocomus brasiliensis*

21’. Context and hymenophore non-cyanescent under pressure or exposed...*Xerocomus basius*

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Authors’ Contributions

ACM collected and described the recent specimens. ACM, MAN and RMBS collected the data, filled the database and wrote the text.

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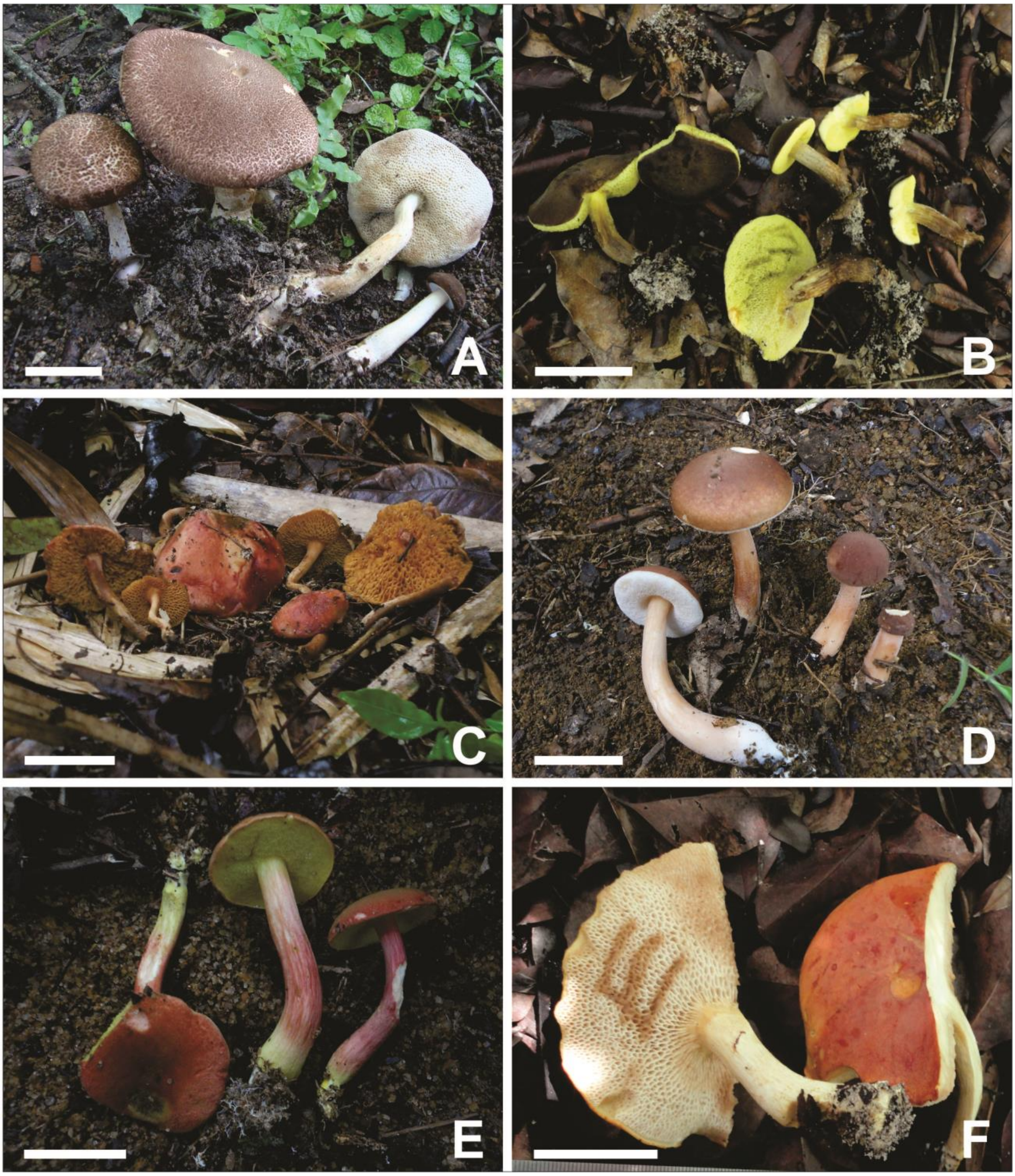

Figure 1. Macromorphology in the field. **A.** *Austroboletus festivus*. **B.** *Boletellus nordestinus.* **C.** *Chalciporus trinitensis* var. *amazonicus.* **D.** *Fistulinella ruschii.* **E.** *Singerocomus atlanticus.* **F.** *Tylopilus dunensis*. Bar A–B = 15 mm. (Photographs A and F by E.F. Perez).

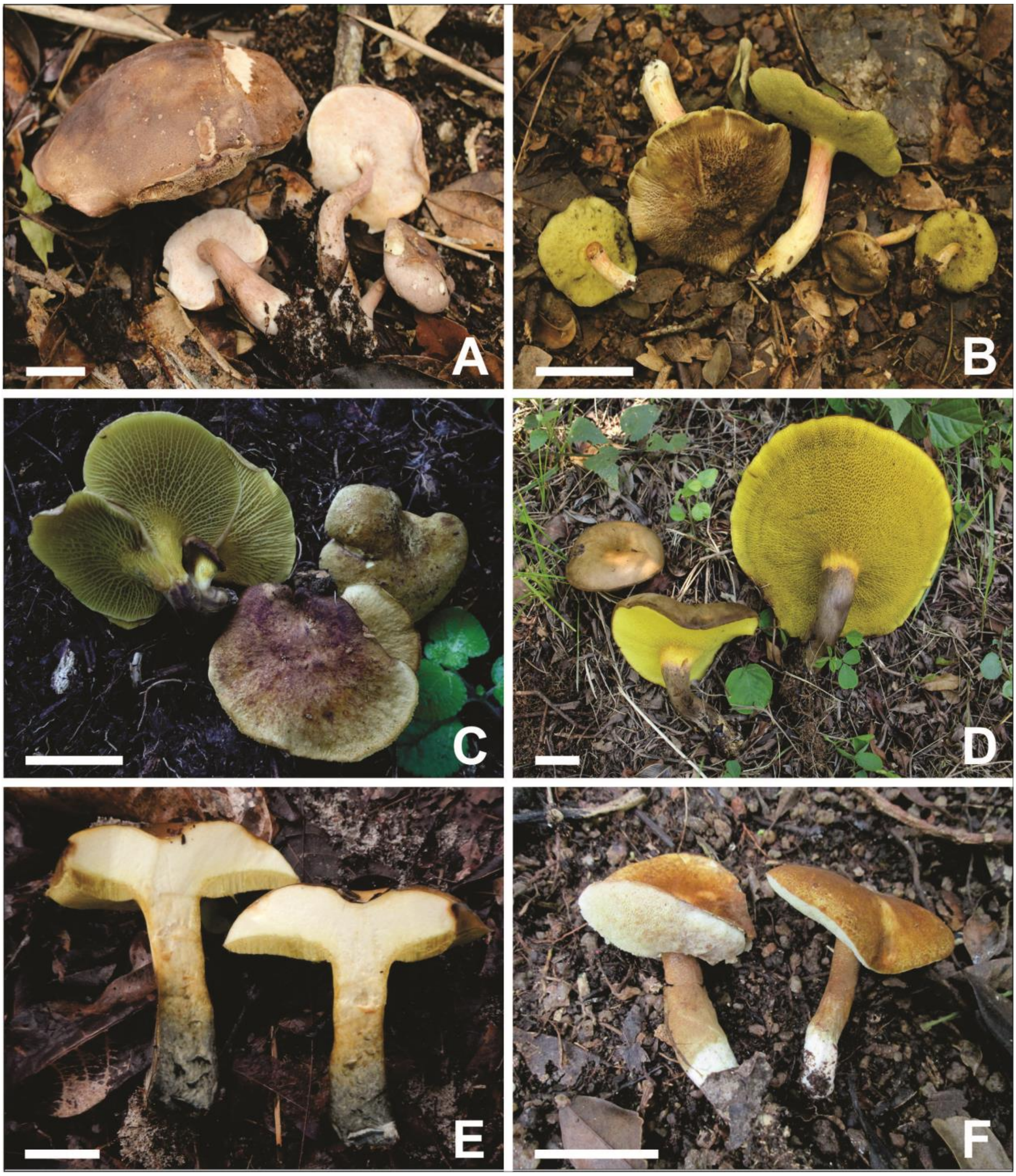

Figure 2. Macromorphology in the field. **A.** *Tylopilus aquarius* var. megistus. **B.** *Xerocomus basius.* **C.** *Boletinellus exiguus.* **D.** *Boletinellus rompelii.* **E.** *Phlebopus beniensis.* **F.** *Gyroporus austrobrasiliensi.* Bar A–B = 15 mm. (Photograph E by E.F. Perez).

5. CONSIDERAÇÕES FINAIS

Como continuidade aos estudos sobre a diversidade de macrofungos para o Brasil iniciadas durante o mestrado na Universidade Federal de Santa Catarina, este trabalho amplia o conhecimento taxonômico e filogenético, assim como a distribuição geográfica dos fungos boletoides registrados para o Brasil.

Cinquenta e duas espécies de fungos boletoides são citadas para o Brasil, sendo a maioria para a Floresta Amazônica. Dentre as 52 espécies, 12 espécies foram descritas detalhadamente neste trabalho, incluindo dados moleculares e distribuídas ao longo dos manuscritos.

Seis novas espécies foram propostas neste trabalho *Boletellus nordestinus, Fistulinella ruschii, Gyroporus austrobrasilienis, Singerocomus atlanticus, Tylopilus dunensis* e *Tylopilus pygmaeus*, todas para a Mata Atlântica.

Singerocomus rubriflavus foi registrado pela primeira vez para o Brasil, no estado da Bahia. O gênero apresenta apenas três espécies, incluindo a nova espécie proposta neste trabalho, sendo difícil a separação das espécies morfologicamente, pois muitos caracteres se sobrepõem, no entanto foi possível observar clados bem suportados entre as espécies na filogenia.

Fistulinella campinaranae var. *scrobiculata* antes registrada para a Amazônia e florestas de *Pseudomonotes tropenbosii* na Colômbia, foi registrada pela primeira vez para Mata Atlântica no estado da Bahia. Assim como *Chalciporus trinitensis* var. *amazonicus,* antes registrado apenas para a Amazônia também foi registrada pela primeira vez para a Mata Atlântica no estado do Espírito Santo.

Tylopilus aquarius var. *megistus* citada anteriormente apenas para o nordeste brasileiro em áreas de dunas, foi registrada no estado do Espírito Santo, ampliando em direção ao sul sua distribuição.

Em relação a *Boletinellus*, gênero comumente encontrado no sul do Brasil, tivemos novos registros de *Boletinellus exiguus* para a Bahia, Rio de Janeiro, São Paulo e Santa Catarina, de *Boletinellus rompelii* para o estado de Santa Catarina, incluindo a disponibilização de dados moleculares de ambas espécies.

Phlebopus é um gênero onde ocorre muita dificuldade na delimitação das espécies devido à grande sobreposição de caracteres. *Phlebopus brasiliensis* foi proposto como sinônimo de

Phlebopus beniensis baseado em dados morfológicos e moleculares, onde as características morfológicas que eram utilizadas para separar as espécies não foram corroboradas na filogenia.

Apesar de ter tido grande colaboração em envios de materiais e imagens de boletoides de outras regiões, a Mata Atlântica foi a área mais explorada neste trabalho principalmente por questões financeiras e de logística. Desta forma conseguimos ter um melhor entendimento das espécies deste bioma em relação aos outros, como por exemplo a Amazônia. A maioria das espécies de boletoides da Amazônia foram descritas por Rolf Singer, onde muitas espécies apresentam apenas o holótipo e a maioria está em estado precário de conservação nos herbários. Desta forma, torna-se necessário um estudo aprofundado dos boletáceos especialmente da região Amazônica, incluindo novas coletas nas áreas onde foram coletados os tipos para que novas coleções de referência dos táxons amazônicos possam ser comparadas e incorporadas ao herbário. Devido a esses fatores o Manuscrito VII apresenta dados geográficos e chave de identificação apenas das espécies registradas na Mata Atlântica.

Onze espécimes coletados durante este trabalho em áreas de Mata Atlântica não foram identificados a nível específico. Esses materiais foram apenas separados em três morfotipos pertencentes à Boletaceae, listados na Tabela 2 como Boletaceae 1, 2 e 3. Taxonomicamente existe a indicação de possíveis três novos gêneros a serem descritos. No entanto, a proposição desses novos gêneros não foi abordada neste trabalho pois houve a necessidade do sequenciamento de outros marcadores para um melhor suporte do monofiletismo desses grupos. Etapa que está sendo executada como continuidade aos estudos com fungos boletoides no Brasil.

Desta forma, os resultados apresentados corroboram afirmativas de vários autores (Henkel 1999, 2001; Henkel et al. 2002; Halling 2001; Halling et al. 2008; Brown 2014, Hawksworth & Lücking 2017) de que os fungos boletoides assim como outros grupos ectomicorrízicos são altamente diversos nas regiões tropicais, onde a mudança de paradigma vem sendo reformulada na última década, onde se acreditava que essa diversidade era maior nas regiões temperadas. Consequentemente, acreditamos que a partir do incremento do número de expedições de coleta e da ampliação das áreas de coleta, muitas novas espécies boletoides ainda poderão ser propostas.

Uma das grandes demandas no país a respeito dos fungos boletoides e de outros grupos de fungos, continua sendo suas relações ectomicorrízicas nas florestas brasileiras nativas. Uma vez que aproximadamente 90% das espécies de plantas realizam esse tipo de associação tais informações são pouco documentadas na literatura. A coleta de material e a ampliação do conhecimento no que diz respeito aos simbiontes é muito importante para conhecermos melhor o equilíbrio das comunidades vegetais, o que ajudaria também nos processos de reflorestamento e conservação dos biomas.

Apesar das dificuldades, acredita-se que o presente trabalho apresenta contribuições relevantes à taxonomia, distribuição, ecologia e filogenia de boletoides para o Brasil. Espera-se que os resultados aqui apresentados sejam úteis para um melhor entendimento destes fungos, servindo de base para estudos de identificação, projetos de conservação e biotecnologia, assim como de utilidade para a comunidade científica em geral e micólogos amadores, estimulando-os a desvendar cada vez mais o mundo micológico.

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ANEXO I

Lista de gêneros com morfologia boletoides reconhecidos em *Boletales* nas subordens: Boletineae, Sclerodermatineae e Suillineae (Binder & Hibbett 2006, Nunh et al. 2013, Wu et al 2014, Wu et al. 2016).

Boletineae

1. ***Afroboletus*** Pegler & T.W.K. Young, Transactions of the British Mycological Society 76 (1): 130 (1981)

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2. ***Alessioporus*** Gelardi, Vizzini & Simonini, Mycologia 106 (6): 1171 (2014)

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3. ***Aureoboletus*** Pouzar, Ceská Mykologie 11 (1): 48 (1957)

   Pouzar, Z. 1957. Nova genera macromycetum I. Ceská Mykologie. 11(1):48-50.

4. ***Australopilus*** Halling & Fechner, Australian Systematic Botany 25 (6): 426 (2012)

   Halling, R.E.; Nuhn, M.; Osmundson, T.; Fechner, N.; Trappe, J.m.; Soytong, K.; Arora, D.; Hibbett, D.; Binder, M. 2012. Affinities of the *Boletus chromapes* group to *Royoungia* and the description of two new genera, *Harrya* and *Australopilus*. Australian Systematic Botany. 25(6):418-431.

5. ***Austroboletus*** (Corner) Wolfe, Bibliotheca Mycologica 69: 64 (1980)

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6. ***Baorangia*** G. Wu & Zhu L. Yang, Fungal Diversity 81: 2 (2015)

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